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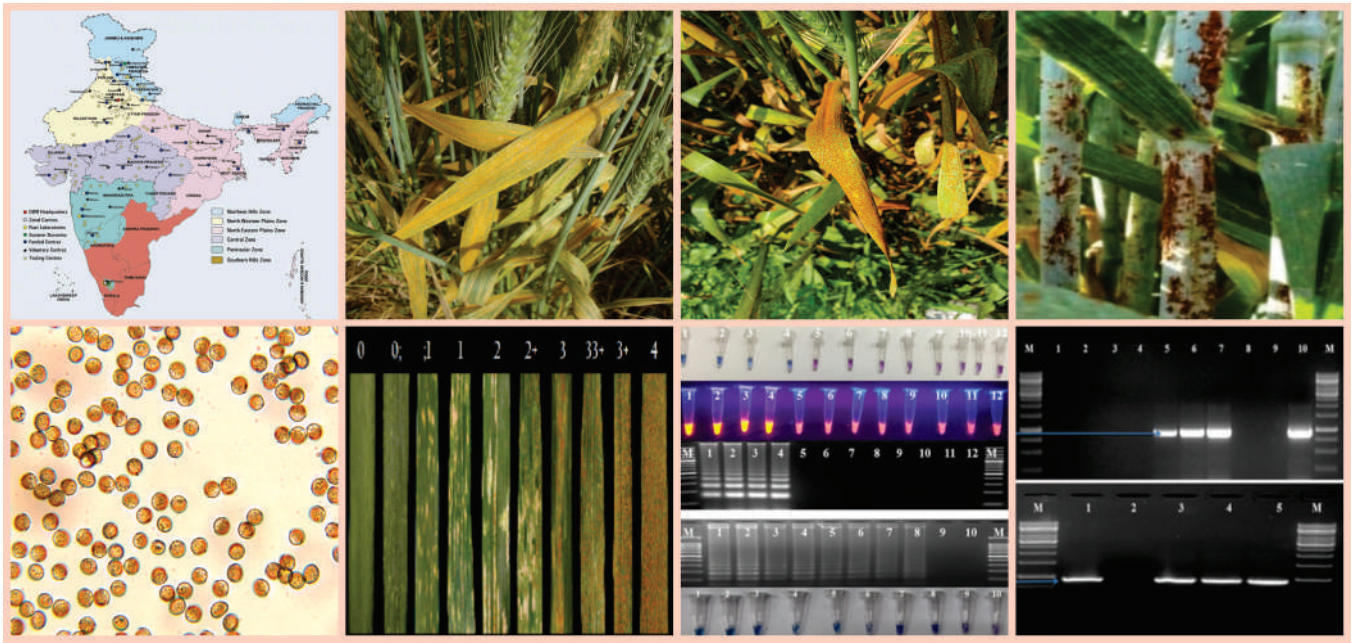
NATIONAL AGRICULTURAL HIGHER EDUCATION PROJECT
Centre for Advanced Agricultural Science & Technology (CAAST) on
"Genomics Assisted Crop Improvement and Management"

"Shaping human resources to harness the global gene pool for sustainable agriculture"

Training Manual

"Pathophenotyping and Genome guided Characterization of Rust fungi infecting Wheat and other Cereals"

January 22 - February 01, 2020



Division of Plant Pathology and Division of Genetics
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Centre for Advanced Agricultural Science & Technology (CAAST) sponsored

Short Term Training Programme

on

Pathophenotyping and Genome guided Characterization of Rust fungi infecting Wheat and other Cereals

January 22 - February 01, 2020

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About NAHEP-CAAST at ICAR-IARI, New Delhi

Centre for Advanced Agricultural Science and Technology (CAAST) is a new initiative and student centric subcomponent of World Bank sponsored **National Agricultural Higher Education Project (NAHEP)** granted to The Indian Council of Agricultural Research, New Delhi to provide a platform for strengthening educational and research activities of post graduate and doctoral students. The ICAR-Indian Agricultural Research Institute, New Delhi was selected by the NAHEP-CAAST programme. NAHEP sanctioned Rs 19.99 crores for the project on "**Genomic assisted crop improvement and management**" under CAAST programme. The project at IARI specifically aims at inculcating genomics education and skills among the students and enhancing the expertise of the faculty of IARI in the area of genomics.

Objectives:

1. **To develop online teaching facility and online courses for enhancing the teaching and learning efficiency, and scientific communications skills**
2. **To develop and/or strengthen state-of-the art next-generation genomics and phenomics facilities for producing quality PG and Ph.D. students**
3. **To develop collaborative research programmes with institutes of international repute and industries in the area of genomics and phenomics**
4. **To enhance the skills of faculty and PG students of IARI and NARES**
5. **To generate and analyze big data in genomics and phenomics of crops, microbes and pests for genomics augmentation of crop improvement and management**

IARI's CAAST project is unique as it aimed at providing funding and training support to the M.Sc. and Ph.D students from different disciplines who are working in the area of genomics. It will organize lectures and training programmes, and send IARI students and covering students from several disciplines. It will provide opportunities to the students and faculty to gain international exposure. Further, the project envisages developing a modern lab named as **Discovery Centre** that will serve as a common facility for students' research at IARI.

Core-Team Members:

S.No.	Name of the Faculty	Discipline	Institute
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अधिष्ठाता एवं संयुक्त निदेशक (शिक्षा) - कार्यकारी

Dr. (Mrs.) Rashmi Aggarwal
Dean and Jt. Director (Education) - Acting

Foreword

ICAR-Indian Agricultural Research Institute (IARI) has made significant contributions in the development of crop protection & production technologies, diagnostic protocols, integrated management technologies for agricultural pests and pathogens of national importance. The institute has core strength in the area of phenomics and genomics and modern research facilities for conducting advanced phenotyping and genotyping research programmes on plants and pathogens. The institute maintained its leadership position in agricultural research, education and extension in India. To address the needs of farmers, ICAR-IARI has developed mega varieties of different crops which are playing important role in country's food & nutritional security, agricultural exports and farm profit.

During green revolution, ICAR-IARI has played leadership role in improving the wheat production and making the country self-sufficient on the food front. The stability in wheat production has been achieved through the sustained efforts made in bringing about varietal diversity for resistance to the biotic and abiotic stresses, particularly the rust pathogens. Wheat improvement programme of the Institute has employed cutting edge approaches for developing durable rust resistance, high yielding and quality wheat varieties for different agro-climatic zones of the country. These wheat varieties have been popular among farmers and widely adopted in their recommended areas/agro-climatic zones of the country.

Wheat Pathology Programme of the Institute has contributed significantly for keeping vigil on wheat rust pathogens and other biotic stresses, pathogenic variability, identification of resistant sources, phenotyping for different diseases under artificially inoculated conditions and genomics aspects like genomics-assisted molecular diagnosis, characterization and sequencing of major wheat pathogens. The wheat production has increased manifold from 6.60 million tonnes at the time of independence to 102.19 million tonnes in 2018-19. But climate change can affect wheat production in Asia in near future due to threat of new pathogens and emergence of new rusts pathotypes. To keep pace with the advances, there is a need to train the students in the areas of the advanced techniques to understand dynamicity of wheat rusts so that sustainable management to curtail losses can be achieved. With this background, the Centre for Advanced Agricultural Science and Technology (CAAST) under National Agricultural Higher Education Project (NAHEP) is organizing a short term training programme on "Pathophenotyping and Genome guided Characterization of Rust fungi infecting Wheat and other Cereals" in the Division of Plant Pathology, ICAR-IARI, New Delhi during January 22 - February 01, 2020 for the benefit of students of SAU's, ICAR institutes and other universities involved in agricultural research, education and extension.

I am sure that the training programme will greatly benefit the students in understanding the basics of pathophenotyping and genome guided evolution and phylogeny of rapidly evolving rust pathotypes affecting wheat and other cereals.

January 15, 2020

(Rashmi Aggarwal)

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Preface

Rust fungi are pathogens of major importance to agriculture, and dominant factor limiting yield potential in wheat and other cereal crops worldwide. These obligate biotrophic fungi are functionally highly specialized and unveil significant variation in the pathogen population. Rusts are known historically as the most economically destructive diseases of the cereal crops worldwide. The economic losses due to rusts have been reported up to Rs. 60 million annually and estimated losses of Rs. 392 million in India. Furthermore, in the last decade new rust races have emerged that are adapted to warmer temperatures, have expanded virulence profiles, and are more aggressive than previously characterized races leading to wide scale epidemics. Targeted breeding for rust resistance, based on information from rust survey and monitoring of occurrence and movement, early diagnosis and pathotype identification, have been effective. Nevertheless, breakdown of resistance occurs frequently and continued efforts are needed to understand how these fungi overcome resistance and to determine the range of available resistance genes. This requires new, innovative, data-driven, pathophenotyping, diagnostic and genomics tools. Reliable molecular diagnosis and early detection methods are still one of the main and most effective actions to develop control strategies for rust pathogens.

The development of genomic resources for these fungi and their comparison has released a torrent of new ideas and approaches to use this information to assist pathologists and agriculture in general. Recently, considerable progress has been made to develop tools with high specificity and low detection limits for use in the detection of these plant pathogens. Advances in sequencing technologies and bioinformatics pipelines have permitted the development of new in-field pathogenomics methods which have been tested and validated for rust. The sequencing of gene transcripts and the analysis of proteins from haustoria has yielded candidate virulence factors among which could be defense-triggering avirulence genes. Genome-wide computational analyses, including genetic mapping and transcript analyses by RNA sequencing of many fungal isolates, will predict many more candidates. This will allow the screening of wheat germplasm for novel resistance genes for breeding. Comparative analyses have also revealed fungal virulence genes, providing fungal targets for disease control in host-produced RNAi approaches. Recently, wheat pathology group at Division of Plant Pathology, ICAR-Indian Agricultural Research Institute (IARI) has been instrumental in successfully demonstrating the genomics-assisted molecular diagnosis, characterization and genome sequencing.

The main objective of this training programme is to train young students and research scholars on the advance application of phenotyping and genomic tools for characterization with special relevance to gene postulation, host-pathogen interaction, pathogenic variability, molecular diagnostic and pathogenomics. The recent advances on phenotyping and genomics will facilitate students to update their skill on pathogen characterization and management. The proposed training course would, therefore, be an opportunity for students/research scholar on a national level to have active interactions and experiences to refine their skills in the area of phenotyping & genome guided characterization. Hands-on training in this topic will be imparted in addition to lectures by eminent experts so that the participants could apply the same in their research programmes.

January 15, 2020

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Acknowledgments

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Contents

Global Importance of Wheat & Barley with Special Emphasis on Research and Development in India

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Wheat

Wheat (*Triticum* species) is a crop of global significance not only because it is usually accorded a premier place among cereals, but also due to its high nutritive value and huge acreage devoted to its cultivation. It is grown in diversified environments. It is a staple food of millions of people worldwide. Approximately one-sixth of the total arable land in the world is cultivated with wheat covering all the continents of the world. It supplies about 20% of the food calories for the world's growing population. While wheat is grown in over a wide range of latitudes covering considerable diversity of conditions, the largest quantities of the best wheats are produced in countries favoured with cool, moist weather during a fairly long growing period followed by dry and warm weather to enable the grain to ripen properly. Wheat cultivation is so widely distributed geographically that the crop is being harvested in one country or the other all the year round. Wheat is perhaps the oldest domesticated plant and was already an important crop when history was first recorded. Thus accurate information on the time and place of origin is exactly not known. The distribution of the wild wheats and grasses, believed to be the progenitors of the cultivated wheats, supports the belief that wheat originated in south-eastern Asia. According to paleobotanists and archaeologists, the modern domesticated form of this cereal grain originated in South-eastern Anatolia, around the region of Diyarbakir Province in present-day Turkey, around 8500 BC. Many cultures in the region had developed a semi-dependence on wild grains that are the progenitors of modern wheat plants. Over a period of hundreds, and later thousands of years, farmers and proto-agronomists developed more and more fruitful and reliable specimens and bred them selectively. Some species were cultivated in Greece, Persia, and Egypt in prehistoric times while the cultivation of other species may be of more recent origin. In India, evidences from Mohen-Jo-Daro excavations, indicate that wheat was cultivated there more than 5000 years ago. In India wheat is generally consumed in the form of unleavened flat breads commonly known as *chapatis/rotis.* It is also an important part of the daily diet of many millions of people. Only rice challenges wheat for the title of most important food grain in the world. Distribution of the acreage under wheat in the world, the total production and the productivity is here.

Globally wheat is cultivated in an area about 225 million hectares with production of more than 750.0 million tonnes of grain. China is the major producer of wheat India, Russia and USA. The average wheat yield in major wheat-growing countries is significantly low, and only China has maximum yield (~5.5 tonnes/ha) followed by Ukraine, India and the USA. Despite India's productivity being on par with the world average, the per day productivity is relatively high (20 kg/day) in comparison to other countries, *viz.* the USA, Uzbekistan, Hungary, Poland, Italy, Bulgaria and Romania, which predominantly cultivates winter wheat with crop cycle hovering around 275 days.

World Wheat Production, Consumption, and Stocks
Local Marketing Years, Thousand Metric Tons

	2015/16	2016/17	2017/18	2018/19	2019/20 Nov
Production					
Argentina	11,300	18,400	18,500	19,500	20,000
Australia	22,275	31,819	20,941	17,298	17,200
Brazil	5,540	6,730	4,264	5,428	5,300
Canada	27,647	32,140	30,377	32,201	33,000
China	132,639	133,271	134,334	131,430	132,000
Egypt	8,100	8,100	8,450	8,450	8,770
European Union	160,480	145,369	151,125	136,863	153,000
India	86,527	87,000	98,510	99,870	102,190
Iran	14,500	14,500	14,000	14,500	16,800
Kazakhstan	13,748	14,985	14,802	13,947	11,500
Pakistan	25,086	25,633	26,600	25,100	25,600
Russia	61,044	72,529	85,167	71,685	74,000
Turkey	19,500	17,250	21,000	19,000	19,000
Ukraine	27,274	26,791	26,981	25,057	29,000
Uzbekistan	6,965	6,940	6,941	6,000	6,800
Others	59,402	51,987	53,512	53,713	59,133
Subtotal	682,027	693,444	715,504	680,042	713,293
United States	56,117	62,832	47,380	51,306	52,258
World Total	738,144	756,276	762,884	731,348	765,551

https://apps.fas.usda.gov › psdonline › circulars › grain-wheat

Barley

Barley (*Hordeum vulgare* L.) is a member of the grass family. It is a self-pollinating, diploid species with 14 chromosomes. It is an important cereal crop in the world ranking next to maize, wheat and rice. It is one of the earliest domesticated food crops since the start of civilization. Barley is believed to originate in western Asia or Ethiopia dating back to the Stone Age. Archaeological evidence indicates that the earliest signs of barley cultivation date back to Neolithic times. Several finding from around 7000 B.C. show that the first crops under domestication were wheat, hulled two-rowed barley, peas, and lentils. The original area of cultivation is assumed to be the Fertile Crescent in the Near East, from present day Israel and Jordan via Syria and southern Anatolia to the Zagros Mountain area in western Iran. Before Hel-beak's (1959, 1964, 1965, 1966 & 1970) works appeared, most of the older archaeological remains of barley were of six-rowed type. Hel-baek's finding together with those of others showed that most of the remains dating from 7000 to 6000 B.C. constitute two-rowed forms, while six-rowed forms do not become common until after 6000 B.C. The theory of Fertile Crescent as the cradle for cultivation and the time schedule for the evolution of many of our common crop plants has been generally accepted. Barley is grown on nearly sixty million hectares of land worldwide, resulting in the production of approximately 140 million metric tons of grain.

The top ten barley producing countries include Russia, Germany, Canada, Ukraine, France, Australia, the United Kingdom, Turkey, United States and Denmark. Barley producers select the appropriate varieties to be grown and crop management schemes to produce grain well suited to a particular end use. Specific producer considerations vary widely, but include the choice of variety to plant, the timing of planting and grain harvesting, and agricultural inputs such as fertilizers, herbicides, fungicides, and insecticides. In India, barley is an important cereal in winter after wheat in both area and production. Due to its very hardy nature, barley is successfully cultivated in adverse agro-environments like drought, salinity, alkalinity etc. in varied topographical conditions like plains and hilly areas under rainfed and irrigated conditions. Barley is generally considered as a poor man's crop because of its input requirement and better adaptability to harsh environments. Area under this crop is mainly concentrated in the states of UP, Punjab, Rajasthan, Haryana, MP, Bihar and also in the plains of HP, UK and J&K.

The area, production and productivity of barley in India is given below.

Year	Production (1000 mt)	Year	Production (1000 mt)
2009	1689	2014	1831
2010	1355	2015	1613
2011	1633	2016	1440
2012	1619	2017	1750
2013	1752	2018	1790

Source: USDA

Wheat Research in India

In India wheat is the second most important food crop, after rice, both in area and production. Overall India is the second largest producer of wheat after China. The wheat growing regions in India represent diverse agro-climatic conditions and a range of soils. The success story of wheat in India during last 40-45 years is historical in nature. The era of semi-dwarf wheats which started in 1960's became an instant success and these genotypes revolutionized the wheat production in coming years- leading to "Green Revolution". The wheat production in India, ever since has increased many folds from 6.4 million tons in 1950 to 101.20 million tons in 2018-19. The country has also witnessed an increase in area from 10 million hectares to around 30 million hectares in 2018-19. The major wheat growing states in India are Punjab, Haryana, UP, MP, Rajasthan and Bihar. These states account for up to 90 percent of India's wheat produce.

The area, production and productivity of wheat in India during last few years are given in table below:

Year	Area (mha)	Production (mt)	Productivity (t/ha)	Year	Area (mha)	Production (mt)	Productivity (t/ha)
2000-01	25.7	69.7	2.70	2010-11	29.2	85.9	2.90
2001-02	26.3	72.8	2.80	2011-12	29.9	93.9	3.14
2002-03	25.2	65.8	2.60	2012-13	30.0	93.5	3.12
2003-04	26.6	72.1	2.70	2013-14	30.5	95.9	3.15
2004-05	26.4	68.6	2.60	2014-15	31.5	86.5	2.75
2005-06	26.7	69.4	2.60	2015-16	30.4	92.3	3.03
2006-07	27.9	74.9	2.60	2016-17	30.7	98.4	3.20
2007-08	28.1	78.6	2.80	2017-18	29.7	98.6	3.32
2008-09	27.8	80.7	2.90	2018-19	29.6	101.2	3.42

Some work in wheat improvement was carried out in India during the last quarter of the nineteenth century by Howard and Howard, but wheat-improvement work on systematic lines commenced when the Agricultural Research Institute (Now Indian Agriculture Research Institute, New Delhi) was founded at Pusa, in Bihar. Albert Howard and his wife, Gabrielle, commenced at Pusa, in 1906, their pioneering work in this field which resulted in the production of the early 'Pusa wheats', such as Pusa 4, Pusa 6, Pusa 12, Pusa 52 and Pusa 80-5*; these gained reputations worldwide. Among the other early wheat breeders in India, mention may be made of T. Milne in the Punjab, H.M. Leake and Ram Prasad in Uttar Pradesh and Geoffrey Evans in Madhya Pradesh. Wheat is grown in India mostly during the *rabi* (winter) season which extends from October to May, but as one goes from the northern hills down south through the northern plains to peninsular India, the seasons becomes shorter, and so is the case as one goes from the west to the east in the northern plains. In habit, Indian wheats correspond to the spring wheats of Europe and America. Prior to the commencement of wheat improvement work in India, the wheat crop in general consisted of a mixture of different varieties. These mixtures, usually known as 'sorts', where classified on the basis grain characters, such as red or amber colour, hard of soft grain, etc., irrespective of their other botanical characters. It was not uncommon for a cultivator to grow different botanically distinct varieties together in his filed as long as they were not different from each other in grain characters. Prominent among these 'sorts' were the *sharbati, dara, saphed, pissi, chandausi,* Karachi Choice Wheat, Hard Red Calcutta, *lal kanak, lal pissi,* etc. in the *aestivum* group*; haura, bansi, kathia, jalalia, jamali, gangajally, khandwa, malvi*, etc., in the *durum* and *khapli, popatia,* and *sambha* in the *dicoccum* group. *Sharbati* wheat with its uniformly amber-coloured and hard grains was the choice wheat in the market, fetching a premium over the others 'sorts'. This was considered to be very good for *chapatee* making. The *sharbati* wheat and other white-grained types which were exported to foreign countries from Karachi port was commercially known as 'Karachi Choice White'. The wheats sent from Uttar Pradesh to the markets in Calcutta were referred to as *chandausi.* These wheats were often mixtures and used to fetch very low prices in the world markets. Yet, certain areas in the country were known for the production of wheats of good grain quality. As a result of wheat-breeding work carried out during the past six decades there has been a marked improvement in respect of the uniformity and good quality of the wheats grown commercially.

Abiotic stress, especially heat stress, induces complex morpho physiological phenomenon in plants which decrease the yield and quality of wheat grain. Furthermore, current estimates indicate that in India alone, more than 13.5 million ha of wheat growing area is heat stressed. Both the proximity to the equator and the popular rice-wheat cropping system, which involve late sowing of wheat, are the major causes of exposure of wheat in India and other neighbouring countries to high temperatures during grain filling. The current trends in India indicate that the 'cool period' for the wheat crop is shrinking, while the threat of terminal heat stress is increasing. High temperature may cause changes in phenological, physiological, biochemical, and morpho-anatomical behaviour in wheat thereby affecting its growth and development. Heat stress was reported to reduce the grain filling duration and change the starch composition and activity profiles of starch biosynthesis enzymes. In India, wheat is cultivated during the winter season from October to April. The Indian wheat germplasm is not only adapted to cooler areas of the north but also to the warmer area of central and peninsular parts. The productivity of wheat in the country is higher in the north than down south, due to temperature difference as well as longer duration and availability of water for irrigation in the northern region. The early heat stress at tillering and terminal heat stress at grain filling are a major concern for wheat productivity in the country. Early heat stress is mainly experienced is central and peninsular India, but terminal heat stress is noticed to affect crop area by 10% for every 1°C rise in temperature.

Wheat with its high protein content is an important source of plant protein in the human diet. Among the cereals, the flour of bread wheat has a superior capability of forming leavened bread. Majorly wheat is consumed in India in the form of unleavened bread called chapati. However, with growing industrialization, the demand for bread, biscuit, and related products is growing throughout the world including India. In today's scenario, works on both quantity and quality of the wheat are required. The quality requirements of wheat for this product differ, i.e., hard wheat with strong gluten (>60 ml sedimentation value), >12.0% protein, 5 þ 10 high molecular weight glutenin subunit with a 9 or 10 Glu-1 score is required for making good bread. This is due to the structure and composition of its seed storage proteins, which upon hydration can interact to form gluten, an insoluble, but highly hydrated, viscoelastic aggregate that endows the wheat dough with its unique properties. So the HMW glutenins are necessary to create the strong dough, which is essential for making high-quality bread. HMW-glutenin proteins are majorly responsible for high dough strength for bread-making traits. Different alleles of HMW glutenin proteins, LMW-glutenin proteins, albumins, and gliadins have been exploited for their impact on bread and chapati-making quality in near isogenic lines of wheat variety HD2329. Many Indian varieties have been characterized for various end products such as C306, WH147, UP262, Sujata, and PBW226 for chapati; HI977, K9107, HD2285, GW120, GW190, DWR195, and NI5439 for bread; Sonalika for biscuit, and durum varieties PDW233, WH896, Raj1555, HI8498, GW1139, HI8381 and MACS2846 for pasta products.

The impact of climate change on wheat diseases is most devastating in a developing country like India because it can influence the growth of the crop, host–pathogen interactions and alter the susceptibility window. Temperature rises above normal and increased humidity and provides a potent way for pathogen action on the crop. Wheat crop is one of the victims, in this case, one such threat observed in Punjab state change in temperature and humidity will reduce the importance of yellow rust (*P. striiformis*) and Karnal bunt (*T. indica*); the importance of leaf rust, foliar blights, *Fusarium* head blight, and stem rust may increase in the future, particularly in the absence of resistance in wheat cultivars. Wheat cultivars that have remained resistant for a long time, which carry durable or race-nonspecific resistance, are known to occur. Resistance from such genotypes can be transferred in a planned manner to the susceptible cultivars, either through the selected bulk approach or single backcross breeding approach: these are the two efficient selection methodologies currently used by CIMMYT that allow the simultaneous accumulation of the desired number of slow rusting genes concurrently with increased grain yield potential and other traits. An aggressive strategy to promote these resistant cultivars in farmers' fields through largescale quality seed production is the only viable option as the resource for poor farmers in most of South Asian country cannot afford to use chemical control.

Barley Research in India

The barley research in India has been progressed with development of varieties for different purposes such as feed, malt, fodder and hulless barley for varied agro-climatic conditions. However, major emphasis of barley breeding programme has been made on development of feed, malt and dual-purpose varieties with high and stable yield, resistance to biotic stresses (yellow rust, leaf blight, aphids and cereal cyst nematode) and abiotic stresses (drought, salinity, alkalinity, rainfed, brackish water and diara lands). In addition, breeding for early maturity, bold and plump seeds and adaptation to specific environments has also assumed importance. A substantial progress in enhancing yield with reducing the losses from biotic stresses, increasing the seed size, lodging resistance and tolerance to salinity stress has been made by adopting the appropriate breeding approaches such as pure line selection, pedigree method, bulk method, backcross method, single seed descent method, mutation and biotechnological tools for molecular profiling. The research programme for improvement of this crop was initiated in India, sometimes during 1916 with research activities such as:

1. Introduction of exotic barley germplasm and improvement through selection
2. Collection of land races of barley and improvement through selection
3. Development and popularizing package of practices to encourage its cultivation
4. To recommend well tested varieties both hulled and hull less for cultivation.

Prior to inception of the AICBIP, numerous varieties were selected from land races and developed the improved barley varieties with high yield along with other desirable traits. As a result of selection from land races a variety C 251 was developed during 1928. It combined high yield potential along with excellent malting quality and tolerance to saline/ alkaline conditions of soil. Other varieties developed and released were Type 4, Type 5, C 84, C 50, NP 100, barley local, BR 21, BR 22, BR 32, CN 292, CN 294, K 12, Balia barley, K 14, RS 17, KB 71, Ratna and PR 502. All these varieties were recommended on the basis of performance in their regional tests. Four hulless varieties namely CN 292, CN 294, Sindhu and Nurboo were directly selected from local materials. Varieties CN 292 and CN 294 were suitable for plain zone, whereas Sindhu and Nurboo were adopted for summer cultivation in Leh and Laddakh. Barley grain is used as feed for animals, malt for industrial uses and for human food. Barley straw is used as animal fodder in many developing countries including India. Barley straw is also used for animal bedding and as cover material for hut roofs. Barley is also used for green forage and either directly fed to the animal or used for silage. It also has immense potential as quality cereal especially for nutritional and medicinal point of view. In developed countries barley is considered as a functional food and used in many bakery products and recipes. In India, its utilization as food crop (mainly hull less type) is restricted to the tribal areas of hills.
The barley products like "Sattu" (in summers because of its cooling effects on human body) and Missi Roti have been traditionally used in India. However, barley is predominantly consumed as food crop in the semi-arid regions of Africa (Morocco, Algeria, Libya and Tunisia), Middle East (Saudi Arabia, Iran, Iraq and Syria), highlands of Nepal, Ethiopia and Tibet, Andean countries of South America (Peru and Chile) and in some Asian countries (China and North Korea). Malt is the second largest use of barley and malting barley is grown as a cash crop in a number of developed and developing countries including India.

Barley cultivation in India was facing stiff challenges in terms of area with wheat in winter season, as the crop was considered less remunerative due to less MSP and market price, resulting in drastic reduction in area and production. Recent changes in situation triggered by the industrial demand for malting and brewing has given it a new lease of life and it is becoming a commercial crop.

Coordinated Efforts for Development of Rust Resistant Wheat & Barley Varieties

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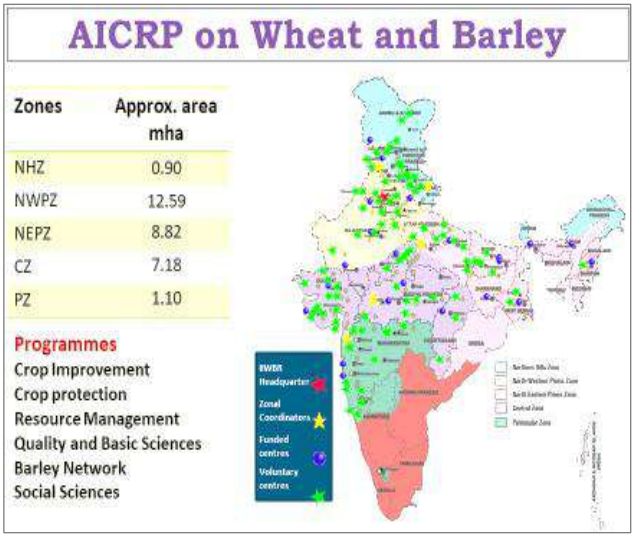
Wheat & barley are important cereal crops in world and contributes significantly in the nutrition of human beings. Barley is also used for malt industries and animal feed. Three types of wheat are grown commercially. The most popular wheat is 'Bread wheat' (*Triticum aestivum*) used for making bread, roti, cookies and pastries. It accounts for nearly 90% of global wheat production. Its grain is semi hard in nature and thus suitable for flour production. The hexaploid wheat generated through natural crossing between tetraploid wheat and grass (AABBDD). Wheat crop is attacked by fungi, bacteria, viruses and nematodes. The fungal diseases are known to cause epidemics in wheat. Wheat and barley are known to be affected due to rust diseases and there had been epidemics of these in some countries. Being a compound interest disease, it is difficult to manage in field in situations of susceptible cultivars, favorable weather and virulent pathotypes. The deployment of resistant varieties against rusts is most practical, simple, effective, ecofriendly and economic method to keep away the rusts.

In India, wheat research started in an organized manner exactly hundred years ago during British period after joining of Sir Howards as the Imperial Botanist at Pusa (Bihar) in 1905. Later on with the establishment of Indian Council of Agricultural Research (ICAR) in 1935, it became the main funding agency and promoter of wheat research in India and Indian Agricultural Research Institute (IARI) played an important role for wheat Research related activities. An important milestone in this process was the establishment of All India Coordinated Wheat Improvement Project (AICWIP) in 1965 by ICAR. AICWIP was elevated to the status of Directorate of Wheat Research in 1978 and in 1990 it moved from IARI, New Delhi, to its present location at Karnal, 130 km north of Delhi along with two regional stations located at Flowerdale, Shimla and Dalang Maidan (Lahaul valley). In 2014, it became an institute, ICAR-Indian Institute of Wheat & Barley Research.

The institute is having six major disciplines namely, crop improvement, crop protection, resource management, quality and basic sciences, barley network and social sciences. In addition, five zonal coordinators represent each wheat growing zone and also help in prioritizing area and need based researchable issues. In India wheat is grown on an area of about 30 million hectares and the cultivation extends from 9^0N (Palni hills) to above 35^0N (Srinagar valley of J & K), thus the wheat crop is exposed to a wide range of agro-climatic changes such as humidity, temperature, photoperiod during crop season, soil types, altitudes, latitudes and cropping systems. Considering these agro-climatic conditions, extension of wheat cultivation in nontraditional areas and based on land use planning, the country is divided into following five major zones; i) Northern Hills Zone (NHZ), ii) North Western Plains Zone (NWPZ), iii) North Eastern Plains Zone (NEPZ), iv) Central Zone (CZ) and v) Peninsular Zone (PZ) as described below:

Zone	Area covered
NHZ	Western Himalayan regions of J&K (except Jammu & Kathua); HP (except Una & Paonta Valley); Uttarakhand (except Tarai area); Sikkim & hills of WB and NE States
NWPZ	Punjab, Haryana, Delhi, Rajasthan (except Kota & Udaipur), Western UP (except Jhansi), parts of J&K (Jammu & Kathua), parts of HP (Una & Paonta valley) and Uttarakhand (Tarai region)
NEPZ	Eastern UP, Bihar, Jharkhand, Orissa, WB, Assam & plains of NE States
CZ	MP, Chhattisgarh, Gujarat, Rajasthan (Kota & Udaipur) and UP (Jhansi)
PZ	Maharashtra, Karnataka, AP, Goa and plains of Tamil Nadu



Through coordinated research efforts more than 460 wheat varieties suited to different agro-ecological conditions and growing situations have been released so far. These genotypes were very successful in increasing wheat production from a mere 12.5 million tons in 1964 to 101.2 million tons during 2018-19. Wheat crop in India is attacked by a number of diseases. It is estimated that, on an average, more than 10% of the crop is lost due to these diseases annually. The three wheat rusts: black or stem rust (*Puccima graminis tritici*), brown or leaf rust (*P. recondita*) and yellow or stripe rust (*P. striiformis*) are the most important diseases. The stinking smuts/bunts (include smooth-spored *Tilletia foetida* & rought-spored *T. caries*) are found only in hills of northern India. Powdery mildew (*Blumeria graminis tritici*) is also found in hills of both northern hills as well southern hills of India. Karnal bunt (*Neovossia indica*) and Flag smut (*Urocystis tritici*) are found to attack wheat crop in southern Punjab, northern Rajasthan, Delhi and Western UP. Leaf spot (*Helmithosporium* and *Alternaria* spp.) are responsible for considerable damage in the states of Bihar, West Bengal, Maharashtra and Gujarat. Foot rots (*Helminthosporium*) and some other fungi have been frequently reported from Rajasthan, Madhya Pradesh, Maharashtra and some other states. Tundu disease, in which a bacterium (*Corynebacterium tritici*) and a nematode (*Anguina tritici*) are associated, also takes a toll of wheat crop in western UP, Delhi, southern Punjab and northern Rajasthan.

Rusts

All the three rusts attack the wheat crop in hills & plains of northern India and hills of southern India. It is therefore, necessary to breed varieties for these areas combining resistance to all of them (Dastur and Pal, 1947). In Central & Peninsular India (MP, Maharashtra, Gujarat, AP & Mysore), black rust predominates but the brown rust also appears sporadically. In the states of West Bengal, Orrisa and parts of Bihar, both black & brown rusts are equally important. Knowledge concerning the annual recurrence of wheat rust and physiologic races of the three rusts occurring in India first became available in the early thirties of the present century as a result of the pioneering work of Late Dr. K.C. Mehta of Agra University. Till to date, 13 races and 3 biotypes of black rusts, 12 races of brown rust and 10 races of yellow rust have been reported. Thus, at present, the number of physiologic races which the wheat breeder in India has to contend with is relatively small, although it is possible that newer races and biotypes may be isolated in future as a result of intensification of the race-analysis work. It may be mentioned in this connection that as the area under rust-resistant varieties in the country increases, the picture regarding the preponderance of certain races or biotypes over the others is likely to change and new races and biotypes may be picked up. Thus, in India, as elsewhere, the fight against the rust is perpetual one.

The establishment of Wheat-Breeding Substation in 1935 at Shimla in northern hills, under IARI and financed by ICAR, was the first step taken in India in the direction of breeding of rust resistance wheat varieties. It was felt that, since the rust inoculums infecting the crop in the northern wheat belt came from the northern hills to a great extent, minimize the hazards of the rusts in the northern plains. It was subsequently appreciated, however, that a step may not be the full answer to the question for the following reasons : (1) even if fully rust resistant varieties became owing to the difficult terrain and lack of inadequate communications; hence, self-sown volunteer plants of the local susceptible varieties could always serve as source of inoculums; (2) on barley and certain other grasses act as collateral hosts; and (3) certain areas in the northern hills, such as Nepal, are outside the political boundary of India and the growth of susceptible varieties there could always serve as a source of inoculums.

It was, therefore, considered necessary to enlarge the scope of rust control programme. A wheat-breeding station was established at Powarkheda in Madhya Pradesh in 1941 for breeding black rust resistant varieties; for carrying out the ancillary plant pathological work, a wheat rust research station was set up in 1942 at Mahableshwar in Bombay States (Now in Maharashtra State). Both these stations, as also the breeding scheme commenced in 1943 at Niphad in Bombay States (Now in Maharashtra), were jointly sponsored by the Indian Council of Agricultural Research, on the one hand and the respective states Governments on the other. In 1949, the Indian Council of Agricultural Research sponsored the Coordinated Wheat Rust Control Scheme for further augmenting the wheat rust programme. Under this scheme, the breeding and mycological work at Indian Agricultural Research Institute was further strengthened. For the breeding work, in addition to the station at Shimla, five other centers were established under the Institute at New Delhi, Pusa (Bihar), Indore (Madhya Pradesh), Wellington (Nilgiri Hills, Madras States) and subsequently, Bhowali (UP hills). The control of Rust Research laboratory at Shimla was transferred to the Institute in 1951 and another center for mycological work was located at Wellington. The breeding and mycological work at the Institute has now been placed on a permanent basis under the auspices of the Government of India. In the states, breeding stations were set up under this scheme, under the sponsorship of Indian Council of Agricultural Research, at Gurdaspur in the Punjab, Durgapura in Rajasthan, Kanpur in Uttar Pradesh, Badanpur in Hyderabad (Now in Maharashtra) and Kalyani in West Bengal. In addition to those preciously located at Powarkheda, Niphad and Mahableshwar.

Keeping the diseases at bay: Work done in Crop protection discipline has significantly contributed in insulating wheat cultivars against the menace of rust diseases in the country since over last four decades. Survey & surveillance activity has helped to monitor the dynamics of important wheat diseases particularly the rusts. With this mechanism, occurrence/evolution of new races is made known before crossing the threshold limit of disease infestation, in the meantime, genetic resistance is created against the new virulence in form of resistant varieties. Large number of donor lines carrying 'R-genes' conferring resistance against different rust races have been identified for utilization in breeding programmes. Crop protection technology/strategy adopted under AICWIP has successfully avoided the onslaught of a new virulence of yellow rust popularly known as *Yr*9 virulence which had caused havoc in Syria, Turkey, Iran, Afghanistan and Pakistan, but it could not cause any damage in Indian wheat fields due to deployment of a resistant wheat variety PBW 343 in the year of 1996. The challenge posed by another pathotype of yellow rust, 78S84, which knocked down PBW 343 was successfully encountered in 2007 by releasing two resistant varieties DBW 17 and PBW 550 to replace PBW 343. Similarly, the threat caused by a new stem rust race-Ug99 has effectively been pre-empted anticipatory resistance breeding undertaken in collaboration of BGRI-CIMMYT in Kenya, since Ug99 has fortunately not been detected so far in India. More than a dozen of improved Indian wheat varieties are already in seed production chain and grown as commercial varieties.

All India Coordinated Wheat & Barley Improvement Programme (AICW&BIP) caters to the needs of cooperating centres by streamlining the research efforts and facilitating the evaluation and screening of the breeding materials. Every year 10-12 nurseries are supplied to different centres across various wheat growing zones with an aim of screening the lines at hotspots and assessing the resistances across locations and environments. Care is taken to keep every economically important disease and other biotic stresses in one or the other nursery. Crop Protection Programme is having a mandate to minimize the losses caused by biotic stresses (Diseases, insect pests and nematodes) so that maximum yield and quality potentials of wheat varieties may be harnessed. The crop health of wheat is monitored by keeping vigil on new pathotypes of rusts and other diseases, any exotic diseases, survey and surveillance of rusts. Details of the activities taken up are discussed below:

Survey, surveillance and crop health monitoring: Crop health is rigorously monitored during the crop season. Major focus is on the occurrence of rusts and surveillance for wheat blast. Status of other diseases is also monitored during these survey trips. The extensive surveys are conducted by the wheat crop protection scientists of different cooperating centers including ICAR-IIWBR Karnal. Special teams of scientists are also constituted during annual Wheat & Barley Workers' Meet held every year during the month of August. Advisory for stripe rust management is issued during December-March regularly. Information on wheat crop health is disseminated through the *"Wheat Crop Health Newsletter",* which is issued during the crop season. This is also put on ICAR-IIWBR website (www.iiwbr.org).

Seedling resistance tests and gene postulation: For identification of rust resistant lines of wheat & barley and to characterize R-genes, seedling resistance tests are done using an array of pathotypes of black (*Puccinia graminis tritici*- Pgt), brown (*P. triticina*- Pt) and yellow rust (*P. striiformis tritici*- Pst) having varying avirulence/virulence structures. These studies are conducted under climate-controlled conditions. Race analysis is normally done at ICAR-IIWBR, Regional Station, Flowerdale, Shimla. This centre caters to the need of wheat & barley breeders by providing uredospores inoculum of the desired strains of rusts and also by screening the advanced breeding lines for resistance and gene characterization.

Host resistance: For providing support to wheat breeding programme, evaluation of disease/pest screening nurseries is undertaken at various hot-spot locations and under artificially inoculated conditions across the country. The major nurseries are:

1. Initial Plant Pathological Nursery (IPPSN)
2. Plant Pathological Nursery (PPSN)
3. Elite PPSN
4. Initial Barley Disease Screening Nursery (IBDSN)
5. National Barley Disease Screening Nursery (NBDSN)
6. Elite Barley Disease Screening Nursery (EBDSN)
7. SAARC Wheat Disease Monitoring Nursery (WDMN)
8. Multiple Disease Screening Nursery (MDSN)
9. Multiple Pest Screening Nursery (MPSN)
10. Loose smut screening nursery (LSSN)
11. Powdery Mildew Screening Nursery (PMSN)
12. Leaf Blight Screening Nursery (LBSN)
13. Karnal Bunt Screening Nursery (KBSN)
14. Other minor disease specific nurseries

Multi-disciplinary approach of variety testing in AICRP on wheat & barley: Initially, the system of varietal evaluation was confined to the specific zones including initial varietal trials and advance varietal trials for different production conditions. Under this system flow material from one zone to another zone was not done and the adaptability of genotypes was limited to specific zones. Realizing this problem and widening the testing environments and to have free flow material of material across zones, the system of testing was re-structured with the incorporation of National Initial Varietal Trials (NIVTs) and their details along with production conditions and zones are presented below.

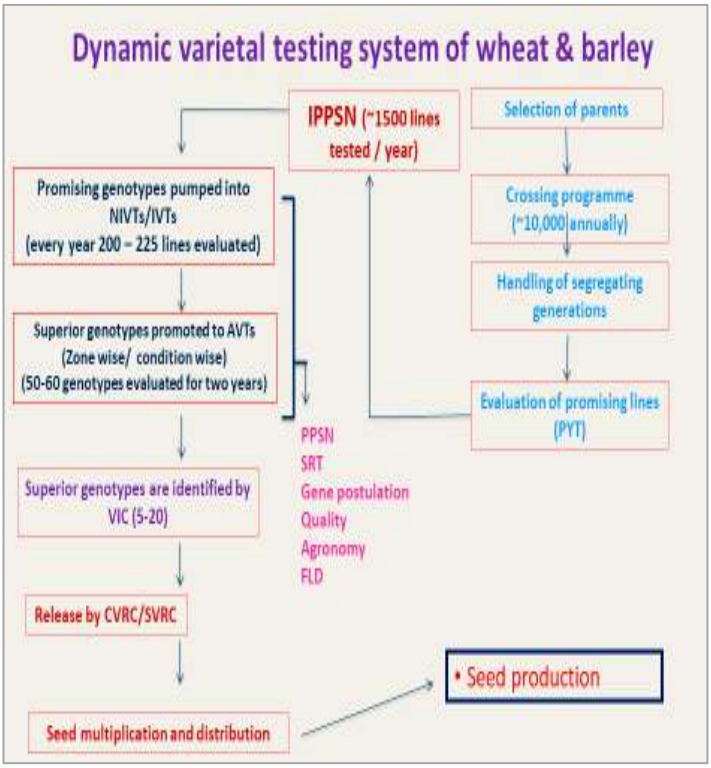
National Initial Varietal Trials (NIVTs)

NIVT	Cultural conditions	Zones
NIVT-1A	Timely sown irrigated high fertility condition (*T. aestivum*)	NWPZ & NEPZ
NIVT-1B	Timely sown irrigated high fertility condition (*T. aestivum*)	NWPZ & NEPZ
NIVT-2	Timely sown irrigated high fertility condition (*T. aestivum*)	CZ & PZ
NIVT-3A	Late sown irrigated medium fertility condition (*T. aestivum*)	NWPZ, NEPZ
NIVT-3B	Late sown irrigated medium fertility condition (*T. aestivum*)	CZ, PZ
NIVT-4	Timely sown irrigated high fertility condition (*T. durum*)	CZ and PZ
NIVT-5A	Timely sown restricted irrigation condition (*T. aestivum*)	NWPZ, NEPZ
NIVT-5B	Timely sown restricted irrigation condition (*T. aestivum & T. durum*)	CZ and PZ

However, in NHZ separate zone-specific Initial Variety Trials in place of NIVTs are conducted. This way, the Indian wheat programme is unique regarding the multi-location testing of new genotypes through different trials. The procedure of evaluation system was re-structured in such a way that the materials from different centres are pooled and tested at different levels namely, station trials, national initial varietal trials (NIVT) and advance varietal trials (AVTs) to sort out superior germplasm with respect to yield, disease resistance and quality in the following manner.

Procedure of testing of new wheat materials in the coordinated trials

Materials are evaluated for one year in Station Trials for yield potential and for disease reactions in IPPSN	
↓	
One Year Inter Zonal Test	
Trial Series	**Criteria of promotion / retention**
Respective NIVT (NIVT-1A, NIVT-1B, NIVT-2, NIVT-3A, NIVT-3B, NIVT-4, NIVT-5A, NIVT-5B	Yield potential, disease reactions and quality parameters are taken into account for promoting materials into various zonal level AVTs.
↓	
AVT-I (First year)	
One Year Zonal Test	
AVT-IR-TS-TAS/TAD/TDM AVT-IR-LS-TAS/TAD AVT-RIR-TS-TAD	Yield potential, disease reactions and quality parameters are taken into account for retaining materials in AVT-II.
↓	
AVT-II (Final year)	
One Year Zonal Test	
AVT-IR-TS-TAS/TAD/TDM AVT-IR-LS-TAS/TAD AVT-RIR-TS-TAD	Yield potential, disease reactions, quality parameters & agronomical evaluations are performed on final year entries.



Varietal evaluation system leading to development and deployment of rust resistant cultivars

After one year of test in IVTs, deserving genotypes are promoted to AVTs in NHZ at zonal level. However, in case of remaining four zones, deserving genotypes come from NIVTs for advance testing in each of four AVTs of NWPZ, NEPZ, CZ and PZ.

The importance of this system of evaluation in different trials and nurseries in India's wheat improvement programme is reflected in the effective management of wheat & barley rusts in India through the deployment of diverse rust resistant wheat varieties based on the pathotype distribution in different areas. The breeding lines are screened prior to national yield evaluation and hence only resistant genotypes are promoted to evaluation. Also, throughout the varietal development process the national nurseries are constituted based on the promoted lines and specific benchmarks based of rust resistance are used at each stage. This system has been very effective for development and deployment of rust resistant wheat and barley varieties and further strengthening the wheat and barley improvement programme of the country.

Suggested Readings

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Overview of The Cereal Rust Fungi

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Rust fungi (Order: Uredinales or Pucciniales, Phylum: Basidiomycota) are the most speciose and complex group of plant pathogenic fungi, referring to the reddish-brown colour of some of the spores. The order comprises of more than 7000 species distributed among over 100 genera (Cummins & Hiratsuka, 2003). Rusts are functionally highly specialized obligate biotrophic fungi and known historically as the most economically destructive diseases of the cereal crops worldwide. Rust diseases, the greatest enemy of the cereals have a great economic impact as they have caused serious epiphytotics damaging cereals and had a great influence on human civilizations.

The Cereal Rusts in History

There has been great historical importance of cereal rusts. Early records testify that Pliny, a prodigious Roman compiler/author of miscellaneous data on natural history in the 1st century AD described rust of wheat as "the greatest pest of the crops" (Hanson *et al.,* 1982). Aristotle (384-322 BC) wrote of rust being produced by the "warm vapours" and mentioned the devastation of rust and years when rust epidemic occurred. Theophrastus (371-286 BC) reported that rust was more severe on cereals than legumes (Roelfs *et al*., 1992). There are Biblical allusions to wheat rusts. The earliest reference of this kind is the caution of Moses to the Israelis during their entry into Canaan (Deut 28.22) around 1360 BC. Moses had warned the people of Israel that if they failed to observe the commandments of Jahova, Lord would punish them with smut and rust". A similar reference of Bible in the prayer attributed to Solomon (I King 8: 37; II Chron 6:28) offered at the dedication of the temple, is the testimony of the existence of rust around 950 BC. The exhortation of astrologer Haggai (500 BC) to the people," I smote you with smut and with rust and with hail in the labour of your hand, yet turned not to me", is another allusion of the prevalence of this disease in ancient period (Levine, 1919). Excavations in Israel have revealed uredospores of stem rust that have been dated at about 1300 BC (Kislev, 1982). Among the diseases known to the Romans, the rust of wheat & barley was one of the most destructive. The Roman wheat farmers plagued by rust at that time, created a god 'Robigus' (the god of rust) and believed that the rust god 'Robigus' had destroyed grain crops as a punishment for the antics of a 12 year old boy who caught a fox robbing his father's henroost, tied straw around the animal and burnt it before releasing the fox. In order to appease the Roman god 'Robigus', a religious festival known as 'Robigalia' was celebrated in the spring, on 25th April of each year from about 700 BC until the decline of the Roman Empire. "The opening prayer stern Robigus spare the herbage of the cereals; withhold, we pray, the roughening hand…" was followed by the sacrifice of reddish coloured animals, such dogs or another animal of a similar colour alongwith other ceremonies. Historical accounts of the epidemics in India have been provided by Nagarajan and Joshi (1975) and Joshi *et al.* (1975).

Taxonomy and Classification

The cereal rusts are diseases characterized by coloured pustules resulting from uredial development of fungi classified into the Phylum: Basidiomycota, Class: Uredioniomycetes, Order: Uredinales or Pucciniales and Family: Pucciniaceae, which contains 17 genera and approximately 4121 species, of which the majority are in the Genus: *Puccinia* (Kirk *et al.,* 2001). The characteristics factures of the Family: Pucciniaceae is the presence of stalked teliospores. These may be free from each other or variously united but never in the form of layers or crusts. Puccinia (after T. Puccini, Italian anatomist) is the largest genus with about 3000-4000 species, parasitizing angiospermic plants throughout the world. The teliospores are 2-celled though in some species the spores are 1-celled (mesospores). The species may be heteroecious or autoecious with a macrocyclic or microcyclic life cycle. Historically and economically they are responsible for causing serious diseases limiting yield potential in cereals and millets worldwide. Some of the important diseases and their pathogens are given in Table 1.

Table 1: Important rust diseases of cereals & millets caused by *Puccinia* species

Cereal & Millet Host	Disease	Pathogen
Wheat (*Triticum aestivum* L.)	Stripe or yellow rust	*P. striiformis* Westend. f.sp. *tritici* Erikss.
	Leaf or brown rust	*P. triticina* (*P. recondita* Rob. ex. Desm. f.sp. *tritici* Eriks. & Henn. = *P. rubigovera*)
	Stem or black rust	*Puccinia graminis* Pers. f.sp. *tritici* Eriks & Henn.
Barley (*Hordeum vulgare* L.)	Leaf rust	*P. hordei* Otth
	Crown rust	*P. coronate* var. *hordei* Jin & Steff.
	Stem rust	*P. graminis* Pers. f.sp. *tritici* Erikss. & Henning (wheat stem rust) *P. graminis* Pers. f.sp. *secalis* Erikss. & Henning (rye stem rust)
	Stripe rust	*P. striiformis* Westend. f.sp. *tritici* Erikss.
Oat (*Avena sativa* L.)	Crown rust	*P. coronata* f.sp. *avenae* P. Syd. & Syd.
	Stem rust	*P. graminis* Pers. f.sp. *avenae* Erikss. & Henning
Rye (*Secalis cereale* L.)	Leaf rust	*P. recondita* f.sp. *secalis* Roberge
	Crown rust	*P. coronata* var. *hordei* Jin & Steff.
	Stem rust	*P. graminis* f.sp. *tritici* secalis Erikss. & Henning
Maize (*Zea mays* L.)	Rust	*P. sorghi* *P. polyspora*
Pearl Millet / Bajra (*Pennisetum typhoides* L.)	Rust	*P. penniseti*
Sorghum / Jowar (*Sorghum vulgare* L.)	Rust	*P. purpurea*

Typical Life Cycle of Rust Fungi

The rusts have a pleomorphic life cycle and have more than one independent form or spore stage in the life cycle. Although they are morphologically quite simple, some rusts may have a complex life cycle. Some of them have a great degree of specificity for hosts of a single species and sometimes of a particular genetic strain. Rusts are functionally obligate biotrophs and typically macrocyclic heteroecious fungi, and requires two exclusive and unrelated host plant taxa for completion of their life cycle, as demonstrated in the well-documented example of wheat stem rust on species of *Berberis* (barberry) and *Triticum* (wheat). The life cycle can comprise up to five distinct spore stages (frequently numbered 0-IV) differing in morphology and cytologic characters (Cummins & Hiratsuka, 2003). For completion of life cycle, Pycnial or Spermogonial stage (stage 0) and Aecidial stage (stage I) occur during sexual reproduction on alternate hosts (*Berberis* / Thalictruum), while, the other three stages *i.e.* Uredial (stage II), Telial (stage III) and Basidiosporal (stage IV) occur on during asexual reproduction on wheat (Fig. 1).

The uredospores are dikaryotic (2n), disseminated by the wind and germinate on wheat plant by producing a germ tube. The uredospores propagate the fungus asexually through repeated cycles and the uredial stage is referred to as the repeating stage. Uredospore production is followed by teliospore development within uredia in separate telial sori at the end of the crop season. These spores are black, binucleate, 2-celled and have thick smooth walls. The teliospores are the resting spores and they remain attached to the host material. A period of freezing weather is required before the teliospores of stem and leaf rust fungi can germinate. Prior to germination, the two nuclei in each cell fuse to produce a true diploid condition (2n). The diploid nucleus formed in teliospores undergoes meiosis and germinates into four-celled promycelium (basidium) with uninuceate haploid sporidia (basidiospore). The basidiospores are of two different strains, namely + and - each being self-incompatible. These sporidia are carried by wind, get deposited on alternate host and infect young leaves, producing yellow orange pycnia on upper leaf surface. Within the pycnium uninucleate, hyaline pycniospores (spermatium) are produced which are functional sex-gametes. In addition to pycniospres the pycnia also produce receptive or flexous hyphae. Pycnia arise by rupturing the epidermis of the leaf within 3-4 days. Receptive hyphae extend beyond the opening of pycnia. Insects, rain water and other agents transfer pycniospores from one pycnium onto the receptive hyphae of another pycnium. The pycniospore contents along with nucleus pass into the receptive hyphae through a pore by dissolving the wall at the place of contact. The nucleus divides into the compatible receptive hyphae. The

extra nucleus migrates into an adjacent cell where the process is repeated and eventually the mycelium of pycnium becomes dikaryotized. The dikaryotic mycelium thus formed thereafter proliferates and forms aecial cups rupturing the epidermis on lower surface of leaves of alternate host. From the base of the aecium numerous rows of aeciospores are produced in chains. The dikaryotic spores are yellow, hexagonal, fairly thick walled and echinulate, having six germ pores. Aeciospores are incapable of infecting *Berberis* / *Thalictrum*. These get blown off by the wind to wheat where they produce infections that in turn produce uredospore, and the life cycle is completed (Fig. 1).

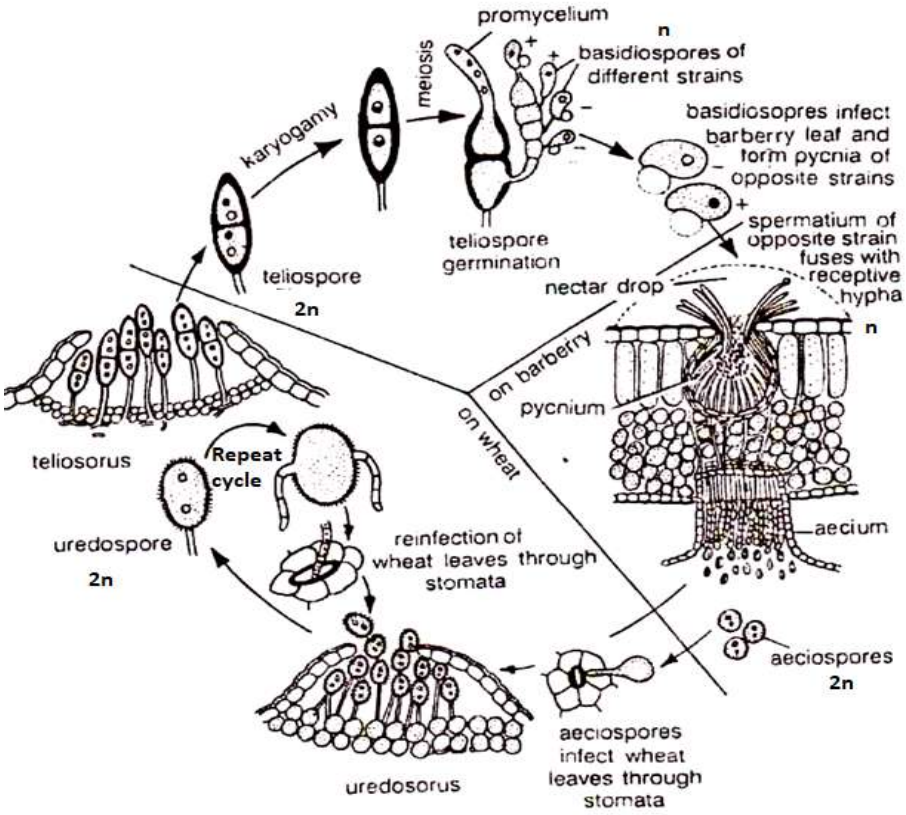

Fig. 1: Typical life cycle of Rust fungi (Black/Stem rust fungi of wheat- Puccinia graminis tritici)

Specialization of Parasitism (Biological Specialization)

The rusts exhibit an extreme degree of specialization of parasitism and this is well exemplified by *Puccinia graminis*. At least seven specialized forms (biological subspecies) of *P. graminis* are known. The biological subspecies differ only slightly in their morphology, but greatly in their ability to attack various grass hosts and are specialized to attack only certain grass hosts. This phenomenon is known as biological specialization, physiological specialization or parasitic specialization. To differentiate these subspecies (or formae speciales), a third name has been added to the binomial *Puccinia graminis*, as below:

Puccinia graminis tritici on wheat
P. graminis hordei on barley
P. graminis avenae on oats
P. graminis secalis on rye
P. graminis phleipratensis on timothy
P. graminis agrostidis on Redtop and other species of Agrostis
P. graminis poae on *Poa pratensis*

The subspecies *tritici, avenae* and *secalis* are further composed of a number of physiologic races (earlier known as biological forms). A race/pathotype is defined as 'one of a group of forms like in morphology but unlike in certain cultural, physiological, biochemical, pathological or other characters. Although there may be slight morphological differences among races of each variety, the most important differences are in pathogenicity in certain varieties of wheat, oat and rye respectively. To crop varieties designated as differential varieties are then used to distinguish races within the *tritici* and *avenae*. The races are further subdivided into biotypes. A biotype is a population of individuals which are identical genetically.

RUST DISEASES OF WHEAT

Wheat (*Triticum* spp.) is important cereals provided daily sustenance to the large proportion of world's population for millennia. Approximately 681 mt of wheat are produced annually grown over an area of 225 mha with productivity of 3.02 t/ha (Singh *et al.,* 2011). India is a privileged country to attain and retain the status of second largest producer of wheat in the world next to China. During 2018-19, India registered historic production of 102.19 million tonnes with productivity of 3.26 t/ha. This achievement in India's wheat production is remarkable but India continues to face formidable problem of ever-increasing population and future wheat demand will be projected to 140 million tons by 2050. Therefore, there is an urgent need for constant improvement of crop yield on one hand and stabilizing the achieved yields on the other hand. Wheat crop is suffered by several biotic and abiotic stresses, but the fungal diseases appear to cause the most predominant biotic stresses as compared to other incitants. Among the fungal diseases, the three different rust fungi, causing stripe or yellow rust (*Puccinia striiformis tritici*), leaf or brown rust (*Puccinia recondita*) and stem or black rust (*Puccinia graminis tritici*) are historically known devastating diseases posing a potential threat to global wheat production. In India all the three wheat rusts are prevalent but their importance is region based.

Stripe Rust

Stripe (yellow) rust, is one of the most destructive disease and dominant factor limiting yield potential in wheat worldwide (Chen, 2005). Yellow rust prefers low temperatures for infection and symptom expression, and more destructive than stem and leaf rusts. It appears in the form of yellow stripe on leaves, causes substantial losses in yield through damaging its photosynthetic system, kills foliar parts, makes growth of plant stunted, most importantly reduced grain weight and affecting its quality (Line, 2002; Chen, 2005). Grain losses caused by this devastating pathogen have been reported from 10-70%. In severe stripe rust epidemics, the grain damage scales up to 100% (Chen, 2005). The ability of yellow rust to mutate, multiply rapidly and spread over large areas has led to wide spread epiphytotics in India (Nagarajan and Joshi, 1975). In India, stripe rust has gained importance in recent past particularly in North-Western Plain Zone (NWPZ) as well as Northern Hills Zone (NHZ) due to favourable weather conditions (low temperature and high humidity) and poses a potential threat to wheat belt of these areas (Prashar *et al.,* 2007; Saharan *et al.,* 2010, 2013). During 2013, stripe rust appeared on most popular wheat varieties planted in large scale in plains of Jammu & Kashmir, parts of Haryana, foot hills Punjab and Himachal Pradesh, plains of western Uttar Pradesh and tarai regions of Uttarakhand (Saharan *et al.,* 2013).

Symptoms: The uredo-pustules develop in the form of narrow, yellow, linear stripes mainly on leaves and spikelets. When earheads are infected, the uredia appear on the inner surface of the glumes and lemmas, occasionally invading the developing grains. The pathogen on infecting leaf blade remains partially systemic and the stripes keep on enlarging. Later on, these stripes turn black when teliosori are formed. The teliosori are dull black in colour, also arranged in long stripes and covered by epidermis.

Causal Organism: Stripe rust is caused by a heteroecious fungus, *P. striiformis* Westend. f.sp. *tritici* Erikss. Uredial and telial stages occurring on wheat. The uredospores are yellow to orange in colour, more or less spherical to oval with some angular walls. However, it is difficult to identify the leaf and stripe rust fungi by uredospores characters if they are observed separately. The teliospores are dark brown to black and are two celled with thick walls and are interspersed with brown unicellular paraphyses. They are more or less similar in size and shape to those of *P. recondita*, except that the cap or crown is flattened, not rounded. *P. striiformis* prefers cooler environmental conditions for its normal growth. The uredospores initiate germination at 9-13˚C with free water. The germtube growth takes place at 10-15˚C. An optimal temperature 8-13˚C favours formation of appressorium and sub-stomatal vesicle under humid conditions. Physiological races of *P. striiformis* recorded in India are 13, 14, 14A, 19, 20, 20A, 24, 31, 38, 38A, 57, A, G, G-1, I, K, L, M, N, P, Q, T, U, C I, C II, C III and 46S119, 78S84 & 110S119. Among these, race 46S119 & 110S119 are the most predominant race of *Pst* in NWPZ & NHZ.

Disease Cycle: Stripe rust can exist independent of an alternate host. The pathogen over-summers in the inner valleys of Himalayas on wheat and volunteer plants in uredial stage under cool climate. If temperatures are very low, infection results in extended latency. Sporulating urediospore can survive to a temperature of minus 4˚C and incipient infections can prevail as long as the host leaf survives. When favourable temperature returns, the soris of rust bust into uredia with abundant uredospores and the inoculum moves towards foothills of northern India. By December / January when the crop is about a month

old, the inoculum then spreads by katabatic winds to sub-mountainous parts of NWPZ. Primary infection foci occur in this region along the mouth of rivers, *viz.* Tavi, Ravi, Beas, Satlaj and Jamuna. Later on, the Katabatic winds carry the uredospores to adjoining plains and cause infection. The pathogen on infecting a leaf remains partially systematic within that leaf and thereafter, several uredo cycles on infected plants are completed resulting in high disease severity. At maturity of the crops, the temperature begins to rise and it is not congenial for uredospore production. Spread of rust is checked above 25˚C. With the ceasing of sporulation the telial stage develops. Teliospores apparently serve no function in absence of alternate host and recurrence of disease takes place through air-borne uredospores from primary foci of infection.

Leaf Rust

Leaf (brown) rust is the most important rust, widely distributed and most frequently occurring disease of wheat in the Indian sub-continent. It is the most common rust prevalent in North-western and North-eastern, central and peninsular region of the country. In South India, it is found on the crop grown both in the Nilgiris and Pulney hills and in the plains including Tamil Nadu, Karnataka and Andhra Pradesh. It is economically most important disease because of its capacity to cause epidemics in almost all parts of the country. In 1972-73 leaf rust appeared in epidemic form severely affecting extensively grown wheat cultivar Kalyansona in Punjab, Haryana and western Uttar Pradesh. The crop suffered a loss of nearly 1.5 million tonnes of wheat (Joshi, 1975). The "Sonalika epidemic" of leaf rust which swept over the entire Uttar Pradesh and part of Bihar in 1980 caused a loss of one million tonnes (Joshi *et al*., 1985).

Symptoms: Disease appears as small, circular, brown to orange brown pustules scattered mainly on upper surface of leaf blade and sometime on leaf sheath, peduncles, internodes and ear heads also. The pustules break through the epidermis, but do not cause loose epidermal tissue at the margins as is the characteristic of stem rust uredia. The telia develop during the later stage of plant development in uredo-sori on leaf sheaths and on both surfaces of leaf blades. They are small, oval or linear and dull black. The teliospores remain in the leaf tissues and are covered by the epidermis.

Causal Organism: The leaf rust is caused by a heteroecious, fungus, *Puccinia recondita* Rob. ex. Desm. f.sp. *tritici* Eriks. & Henn. (*P. triticina* = *P. rubigovera*). The uredial and telial stages appear on wheat and collateral hosts and the pycnial and aecial stages on alternate host, *Thalictrum* and *Isopyrum* species. The pycniospores and aeciospores are not produced in India, so the survival and perpetual of the fungus is through uredospores. Uredospores initiate germination within 30 minutes after contact with free water at temperatures varying from 15-25^{o} C. The germ tube grows along the moist leaf surface until it reaches a stoma and forms an appresorium. The appresorium formation and development of penetration peg and substomatal vesicle take place at 20^{0}C temperature and high humidity. Telia usually formed on under surface of the leaf, are deeply embedded in host tissues. The Teliospores are dark brown; two celled with thick walls, and are rounded or flattened at the apex, arranged in groups separated by paraphyses. Thirty nine races of the fungus, namely 10, 11, 12, 12-1, 12-2, 12-3, 12-4, 12A, 17, 20, 26, 61, 63, 70, 77, 77-1, 77-2, 77-3, 77-4, 77-5, 77-6, 77-7, 77A, 77A-1, 77B, 104, 104-1, 104-2, 104-3, 104A, 104B, 106, 107, 107-1, 108, 131, 162, 162A and162B have been recorded in the country from time to time. The race 77-5 is most predominant in the country.

Disease Cycle: Leaf rust of wheat is a macrocyclic rust. In the absence of alternate hosts the fungus perpetuates in the form of uredial stage. The uredospores like that of stem rust, survives on wheat, self sown plants and collateral hosts (grasses) in South Indian hills and Himalayas throughout the year. The rust inoculum in the form of uredospores spreads from hilly region into plains where they causes infection on regular wheat crop. These spores are repeatedly reproduced asexually on wheat plants during crop season. It is because of this reason, the uredospores are also called repeating spores. In this way the pathogen completes its disease cycle with the uredospores only.

Stem Rust

Stem (black) rust, prevalent in central, peninsular and south zones of the country. Basically, stem rust prefers warmer climate and is generally predominant in Nilgiris, foot hills of Nigiris and Pulney hills, Karnataka, Maharashtra and Madhya Pradesh. The uredospores of stem rust from central zone never arrive in time so the disease appears late in this part and usually does not cause much damage. The epidemic of the disease in 1946-47 in Madhya Pradesh and Maharashtra destroyed over two million tons of wheat grain (Asthana, 1948). In 1956-57, rust created almost famine conditions in West Bengal, Bihar and eastern parts

of Uttar Pradesh (Prasada, 1965). An isolated but severe epidemic of stem and leaf rusts was recorded on wheat in Jalore district of Rajasthan in 1973. It is estimated that nearly one-third of the entire crop (approximately 8000 hectares) was completely destroyed and not even harvested in this area. In 1978-79, a large area of the Narmada valley in Madhya Pradesh was hit badly by stem and leaf rusts causing 60-75% losses to local wheat varieties Pissi and Malvi local (Joshi *et al.,* 1980).

Symptoms: It appears on all the above-ground plant parts. Disease symptoms most commonly develop on the stem and leaf sheaths, but leaf blade and spikes may also become infected. The first symptom of stem rust is characterized by the appearance of small chlorotic spots or flecks on both sides of affected parts of the plant. These flecks after passing through incubation period give rise to dark brown, large elongated pustules or uredosori. In the beginning, the pustules are scattered but later they may coalesce. Millions of uredospores are produced in pustules that rupture, throwing up large fragments of the epidermis and exposing masses of reddish-brown spores. Remnants of the epidermis are visible on the margins of the pustule, giving it a ragged appearance. As the infection advances, the uredospores are gradually replaced by black teliospores in the same pustule and telia are formed. They are conspicuous, linear, oblong, dark brown to black and often merging with one another to cause linear patches of black lesions.

Causal Organism: The causal agent of stem rust of wheat is macrocyclic, heteroecious fungus, *Puccinia graminis* Pers. f.sp. *tritici* Eriks & Henn. Two stages of the fungus i.e. uredial and telial stages are produced on wheat and other two stages, *viz.* pycnial and aecial are developed on alternate hosts like *Berberis* and *Mohonia. B. vulgaris* is the most common alternate host of *P. graminis tritici.* Since under Indian conditions alternate hosts are not functional, the uredospores, therefore, play major role in the survival and perpetuation of the fungus. When the uredospores are lodged on the host surface, they start germination under suitable climatic conditions. The germ tubes emerge from uredospores through germpores. In order to facilitate penetration, the tip of germ tube swells and produce an elongated appresorium from which infection pegs are produced. Then entry of fungus in the host is through stomatal opening. The infection peg emerging from appresorium enters the sub-stomatal cavity where a sub stomatal vesicle is formed, from which hyphae strands arise. These hyphae spread intercellularly, sending haustoria into cells to absorb nutrients. When fully established, the fungus produces the uredosori which erupt and release powdery mass of brick red coloured uredospores. The production of uredospores is favoured by suitable temperature and availability of sufficient moisture. The spore germination initiates when dew for 2 hours or more is available on the host surface. Temperature ranging from > 5-30^{o} C (optimum 15-24^{0}C) at this stage promotes the germination process. During germ tube growth and appresorium formation, the germinating spores require dew for 10 hours and optimum temperature varying from 16-27^{0}C. The penetration of infection pegs through stomata is rapid under film of free water. Light intensity above 3.2 x 10^{3} lux hampers germination of uredospores, which ceases at 11 x 10^{3} lux.

Over 35 physiological races of *P. gramins tritici* have been recorded in India. They are 11, 11A, 14, 17, 21, 21-1, 21-A-1, 21A-2, 24, 24A, 34, 34-1, 40, 40A, 40-1, 42, 42B, 42B-1, 42B-2, 53, 75, 117A, 117A-1, 117-1, 117-2, 117-3, 117-4, 117-5, 117-6, 122, 184, 194, 222 and 295. At present, race 40A (62 G 29) occur in the most virulent form in certain parts of the country (Bhardwaj *et al.,* 2006).

Disease Cycle: *P. gramins tritici* is an obligate parasite, being unable to complete its life cycle in the absence of living host. It has developed a very complex life cycle involving 5 spore types alternating between two host species i.e. wheat and *Berberis/Mahonia.* In a number of temperate countries *Berberis* species serve as alternate host and help to perpetuate the disease from one season to the next. Teliospores of rust are produced in autumn before harvest and remain dormant till the spring. In the early spring, the teliospores germinate to produce four basidiospores. These are carried by wind to infect young leaves of *Berberis*, producing pycnia and pycniospores. The pycniospores are functional sex gametes, which after fertilization give rise to aeciospores in aecial cups. The aeciospores re-infect the young wheat plants and produce uredospores in uredia. Uredospores are capable to repeat reproduction asexually.

Under Indian conditions where alternate hosts are not functional, no pycnial and aecial stages of the fungus have been discovered. The teliospores are not capable of immediate germination under hot and dry conditions prevailing in summer months. The basidiospores are not formed to infect any known plant species (Mehta, 1940). Therefore, survival of the rust in the Indian sub-continent is through uredospores produced on collateral hosts at high altitudes on the hills. In hilly areas, besides summer wheat crop and self-sown wheat plants, some graminaceous hosts (grasses) are found throughout the year, which allows the rust to survive in the area in the form of uredospores.

Epidemiology of Wheat Rusts in India

Epidemiological studies on rusts of wheat was first taken up in India by late Dr. K.C. Mehta. Mehta (1929, 1933, 1940) recorded that the alternate host (*Berberis* spp. & *Thalictrum*) occurring in the hills of India are non-functional in the annual recurrence of wheat rusts in the plains. He showed that due to intense summer heat the inoculum of wheat rusts in any form is completely destroyed in the Northern Plains during the summer months. But the rust survived in the hill of North (Himalayan hills) and South hills (Nilgiri & Pulney hills) on stray or self-sown wheat plants and late & off-season wheat crops.

Recent work identified different foci of infection and has shown that primary source of black & brown rust lie mainly in the Southern hills & Northern hills contributes very little, if at all. Yellow rust, on other hand, comes mainly from Northern hills while leaf rust is comes from both by Southern & Northern hills.

South India gets black rust infection much earlier than the North India. During November, when there is a month-old wheat crop in Central India, tropical cyclone that cross Tamilnadu or Andhra Pradesh and dissipate over Central India transport large quantities of uredospores. These uredospores carried from the Southern source are rain scrubbed over Central India and, condition being congenial disease epidemics develops before mid-March in plains of Northern India.

In case of brown rust of wheat, there are clearly two foci of infection, the Himalayas in the North hills and Nilgiri & Pulney hills in the South. The functioning alternate host *Isopyrum fumarioides* does not occur in India. Thus, the observation of Mehta with regards to the movement of foci of infection of brown rust of wheat are still valid.

Yellow rust of wheat is restricted in its distribution in epidemic form from only in Northern Western Plain Zones, foot of Northern Hill Zones, plains of Uttar Pradesh, Bihar, Nilgiri & Pulney hills in South of India. According to Mehta, the chief foci of infection/ yellow rust are the northern Himalayas. Its absence in south India is attributed due to the presence of high temperature during crop season.

Puccinia Path in India

The existence of Puccinia Path in India was demonstrated by Nagarajan and Joshi (1980). In India, the dispersal route of uredospores from South hills (Nilgiri & Pulney hills) are carried through cyclonic wind (in the Bay of Bengal) to North India crossing thousands of miles, through a fixed geographical track called the 'Puccinia Path in India'.

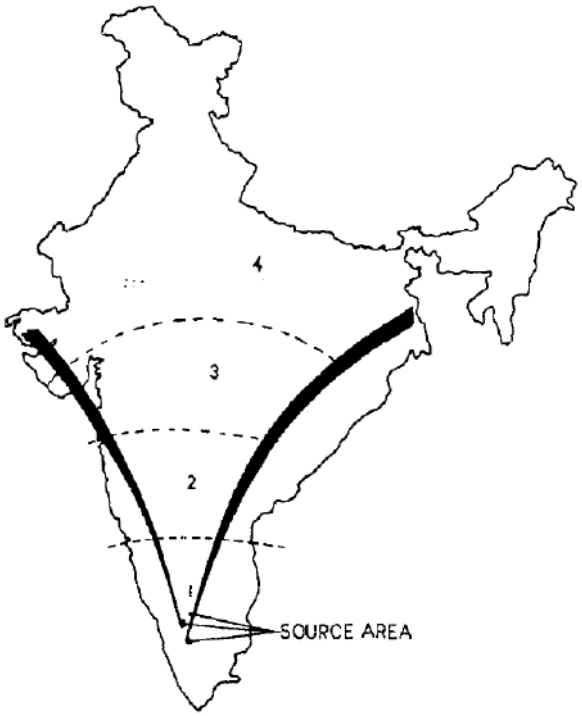

Fig. 2. The 'Puccinia Path' as it occurs in India between Southern hills (darkly shaded) to Central India and its sub-epidemiological zones (1-4).

Nagarajan and Joshi considered the whole Indian sub-continent to be a single epidemiological zone for *Puccinia graminis tritici* which can be split into three sub-zones. They have also defined the "Puccinia Path" i.e. the usual dissemination pattern of the spread of inoculum from foci of infection (Fig. 2). According to them sub-zone 1, by virtue of its proximity to the source-area, gets inoculum by means other than rain such as katabatic wind current. This sub-zone, however, is not of much economic significance as the wheat acrage in this area is negligible. In sub-zone 2, the inoculum arrives either by wind current or with rain around November while in sub-zone 3 the inoculum is transported by cyclonic disturbances and deposited by rain du¡ late October to November. This zone is epidemiologically important because the upper air transportation and rain deposition of uredospores can create an epidemic. This zone can also serve as the main secondary focus for zone 4. If there is quick and early built up of inoculum in this zone sometime in November or early December it can provide adequate inoculum to the eastern regions of sub-zone 4. Sub-zone 4 remains almost free from stem rust in the absence of an appropriate tropical cyclone. This basic information on dissemination of rust can be utilized in erecting suitable genetic barriers in "Puccinia Path" by effective gene deployment to mitigate the rust menace. It should be possible, to delay the spread of the rust disease in the major wheat area by obstructing the "Puccinia Path" by growing genetically different resistant material in different zones.

Management of Wheat Rusts

Cultural practices: Cultural practices like cultivation of wheat with other non-cereal crops, mixed cropping, early maturing varieties and judicious application of nitrogen fertilizers are helpful in checking the disease.

Gene deployment: The epidemiology of rust has an important bearing on their management in India. Nagarajan and Joshi (1980) recognized that Puccinia Path consisting of four sub-epidemiological zones exists in India, therefore they explored the possibility of obstructing the path through deployment of horizontal resistance slow rusting varieties in South and North Indian hills and vertical resistance against prevalent pathotypes in other zones to minimize the threatening rust epidemics or pandemics. Horizontal resistance appears to be useful at foci of rust infection because it is evenly spread against all races of the pathogen while in slow rusting rust develop slowly and never reaches a high degree of severity. As a result, damage is slight. The stability of slow rusting is an important question. There is now ample evidence that minor genes for resistance can affect rust development at various stages, for example: receptivity, length of latent period, pustule size and spore production. Each gene has a relatively small effect but when several of them are combined, satisfactory resistance can result (Knott, 1988). Kulkarni and Chopra (1980, 1982) have noticed environment as the cause of differential interaction between slow rusting cultivars and pathogenic races. In comparison to horizontal resistance, the deployment of vertical resistance which is effective against some races seems to be useful in Zones 2, 3 & 4. Nagarajan *et al.,* (1985) and Bahadur and Nagarajan (1985) suggested that gene combination *Lr2a* + *Lr15* and *Sr9b* + *Sr36* in Zone 2; combination *Lr15* + *Lr20* and *Sr11* + *Sr13* in Zone 3 and *Lr9* + *Lr10* and *Sr6* + *Sr8* in Zone 4 would be useful for deployment along the Puccinia Path. Recently, a revised strategy for managing stem and leaf rusts of wheat in six different ecological areas of the country has been proposed (Bahadur *et al.,* 1994*)*. In areas 1 and 6, use of horizontal resistance / slow rusting varieties has been re-advocated as recommended earlier (Sharma and Pal, 1993). In addition to earlier recommended combinations, an addition of *Lr13* and *Sr2* in area 2; *Lr19* and *Lr23* and *Sr2* and *Sr26* in area 3; *Sr2* and *Sr31* and *Lr34* and *Lr26* in area 4 and *Lr24* and *Sr2* and *Sr24* in area 5 is considered to be useful in increasing the productive life of wheat cultivars. However, Bhardwaj *et al.* (2005) recorded that *Lr19* resistance in wheat has become susceptible to *P. recondite* in India.

Resistance: Among the various approaches to combat with rusts, growing resistant cultivars is the most efficient, economical and environmentally safe approach to eliminate the use of fungicides and reduce the threat of rusts. But virulent races of the rusts appear quiet frequently and varieties become susceptible. Therefore, need for lasting resistance is always felt. In recent times, alien sources have been utilized for rust resistance. Cytological observations of the presence of 1B/1R carrying *Sr31* / *Lr26* / *Yr9* indicate that several wheat varieties in India carry 1B / 1R translocation (Nayar *et al*., 1993). 1B / 1R substitution from rye to wheat has not only contributed for rust resistance but also revolutionized the production. Stem rust resistance gene *Sr26* is known to confer resistance for the last many years (Luig, 1983). Wheat varieties carrying *Sr24* produce low infection types to most of the stem rust pathotypes. Resistance gene *Sr2*, in addition to other unknown minor genes derived from cultivars Hope and H44 provides durable resistance. For example, variety Sonalika released in1960 in the Indian sub-continent has remained resistant, carry *Sr2* gene. *Sr5* is known to produce resistant type of infection to *P. graminis tritici* pathotypes. Many wheat varieties throughout the world have resistance gene *Sr31* which was introgressed into wheat on translocated chromosomal fragment from rye. This gene has provided highly effective resistance for many years. Among 320 lines of Indian wheat, 56 lines have been found to possess resistance gene *Sr31*. *Sr2* has been identified in 22 lines. Of these DL 803-3, DWR 195, GW 190, HUW 318, MACS 2496 carrying *Sr2* + *Sr31* are suitable for cultivation in stem rust prone areas in central and peninsular India. Wheat varieties GW 273, HW 1085, JW 515, NIAW 34, and RAJ 3675 having *Sr5* are recommended for central zone (Prashar *et al*., 2004)

In 1999, a new variant of *P. graminis tritici Ug99* on gene *Sr31* was picked up from Uganda in Africa and subsequently it was detected from Kenya, Ethopia, Yemen and Sudan (Singh *et al.,* 2011). It is feared that the spores of *Ug99* may migrate and threaten wheat production in the Middle East and central Asia. The value of *Sr31* is still rated high in Indian Wheat programme as this gene shows enhanced resistance to stem rust in combination with *Agropyron elongtum* derived gene *Sr24* and *Sr25* in the background of many bread wheat varieties. Therefore, use of stem rust resistance genes *Sr24*, *Sr25* and *Sr26* in Indian wheat cultivars is recommended as a pre-emptive measure to face the challenge of *Ug99* (DWR, 2006). Some Indian wheat varieties like DSW17 and PBW 550 are reported resistant to this race.

The exploitation of genes for leaf rust resistance has led to the development of several wheat varieties carrying genes *Lr9, Lr10, Lr13, Lr14, Lr23, Lr24, Lr26* and *Lr28.* The diversification of varieties and their strategic deployment in different agro-climatic zones has been instrumental in arresting the spread of rust to major wheat growing areas of the country. *Lr3* in mid-thirties, *Lr23* in early seventies and *Lr26* in eighties

were used in breeding for leaf rust resistance. However, races with matching virulences for these resistance genes were selected during 1980-2004. In view of these reports, search for new resistance genes should continue and marker assisted selection be exploited for improving leaf rust resistance. Saini (2002) has identified two new genes *Lr48* and *Lr49* which are available for incorporation. According to Singh (2004) and Parashar *et al.* (2008) resistance genes *Lr34* and *Lr46* and other minor genes hold promise for durable resistance to leaf rust. *Lr34* gene is linked to *Yr18.* The presence of *Lr34* can be indicated by the presence of leaf tip necrosis in adult plants. Combination of these genes results in adequate resistance levels in most environments. Similarly, slow rusting *Yr29* is completely linked to gene *Lr46* which confers moderate resistance to leaf rust (William *et al.,* 2003). Pyramiding of *Lr34* has been attempted through molecular assisted selection (Rao *et al.,* 2007). The genetic basis of seedling and adult plant resistance has been analysed in wheat lines CS 2A/ 2M 4/ 2 and CS 2A/ 2M/ 318 by Bansal *et al.* (2008) which showed that leaf rust resistance is conferred by *Lr28* gene.

In mid-1960's, Kalyansona and Sonalika, the most popular wheat cultivar in India exhibited high degree of resistance to stripe rust, presumably due to the presence of *Yr2* gene. The extensive cultivation of Kalyansona exposed the resistance to new virulence *Yr2 (Ks)* in 1971-72, causing stripe rust epidemic in the Indo-Gangetic plains. Subsequently, Kalyansona was replaced by Mexican genotypes having chromosomal translocation 1B / 1R that carries *Yr9*. A new virulence o *P. striiformis* matching *Yr9*, which was first recorded from Eastern African highlands in 1991, has been picked up from Iran, Afghanistan, Pakistan, Nepal, China and India (Singh, 2004). In recent years, some Veery # 5 selections like Pak 81, Pirsabak 85 Rohtas 90, Annapurna I and PBW 343 have shown susceptibility to *Yr9* virulence in Indian sub-continent (Nagarajan *et al*., 2006). Therefore, the breakdown of *Yr9* underlines the need for cultivars having durable resistance genes such as *Yr18* (Singh, 1992; McIntosh, 1992). According to Tomar *et al.* (2004), the use of APR genes with few other genes like *Yr5*, *Yr10* and *Yr15* shall be useful for achieving long term rust control. Two independent dominant genes of wheat variety HP 1731 responsible for adult plant resistance have been isolated (Datta *et al.* 2007). This type of single gene of APR can be useful in breeding program for leaf rust resistance. Nayar (2008) has also reported some resistance genes effective in India against three rusts of wheat (Table 1). The resistance sources in wheat against rusts have been identified through multilocation tests (Sharma *et al.,* 2002). The genetic stocks FLW 10, FLW 16, FLW 17, FLW 18 and FLW 24 carrying *Yr5, Yr10* and *Yr32* are being pursued at ICAR-IIWBR, Karnal.

Table 1: Rust resistance gene effective in India

Rust	Gene
Stem rust (*P. graminis tritici*)	Sr25, Sr26, Sr27, Sr31, Sr32, Sr39
Leaf rust (*P. recondita*)	Lr24, Lr25, Lr28, Lr32, Lr39, Lr45
Stripe rust (*P. striiformis*)	Yr10, Yr11, Yr12, Yr13, Yr14, Yr16

Table 2: Recommended rust resistant wheat varieties effective in India

Rust	Varieties
Stripe rust (*P. striiformis*)	HD 3086, HD 2967, HD 3059, DPW 621-50, PBW 644, PBW 550, PBW 502 recommended for North Western Plain Zone (NWPZ) and VL 829, VL 832, VL 907, HS 490, HS 365, HPW 155 for Northern Hill Zone (NHZ)
Leaf rust (*P. recondita*)	HD 2985, HD 2967, HD 3086, HD 2733, HD 2864, HD 3090, HD 3043, HD 3086, HD 3059, HD 2888, DL 784-3, Raj 3765 and PBW 373
Stem rust (*P. graminis tritici*)	HD 3043, HD 2833, HD 2933, HD 2888, HD 2987, HD 2967, HD 3090, WH 1081, PBW 502 and PBW 550

Use of fungicides: Several chemicals have been tested and recommended for controlling wheat rusts. An appropriate spray of systemic fungicides such as Propiconazole / Tebuconazole / Triademefon @ 0.1% is found to be effective in controlling the disease. However, the success of chemical control will depend, to a great extent on disease forecasting system.

Survey, surveillance and monitoring of rusts: Control of rusts is more critical for achieving the higher yields. India in particular has not faced any rust epidemic since last three and half decades because of proper deployment of rust resistance genes in wheat breeding programmes. Wheat and wheat rusts have evolved hand in hand for centuries. With the domestication of wheat, new rust resistance genes were introgressed. However, rust pathogens are also evolving consequentially. The rust pathogens are highly variable. The evolution of new pathotypes occurs in each rust render a resistant variety susceptible**.** Monitoring of rusts is being done every year through extensive surveys and planting trap plot nurseries at hot spot locations. This has helped in identifying rust pathotypes and also for effective rusts management.

RUST DISEASES OF BARLEY

Barley (*Hordeum vulgare* L.) is an important coarse cereal crop in the world ranking next to maize, wheat and rice. It is one of the earliest domesticated food crops since the start of civilization. Barley is grown on nearly sixty million hectares of land worldwide, resulting in the production of approximately 140 million metric tons of grain. Barley is used for multiple purposes like food, feed and malting. It is low input crop can also be grown in marginal lands. Area under this crop is mainly concentrated in the states of UP, Punjab, Rajasthan, Haryana, MP, Bihar and also in the plains of HP, UK and J&K. During 2018-19, the crop occupied nearly 6.62 lakh ha area with the production of 1.75 m t grains, where the highest production was reported from Rajasthan (50 %) followed by Uttar Pradesh (23%) and Madhya Pradesh (17%). A number of biotic and abiotic factors pose a challenge to increase barley production. Stripe rust / yellow rust (*Puccinia striiformis* f.sp. *hordei*) and leaf rust/ brown rust (*Puccinia hordei*) are major problems in NWPZ), while in NEPZ, leaf rust and leaf blights are common. In NHZ, stripe rust and powdery mildew are serious problems.

Stripe (Yellow) Rust

Barley stripe rust caused by *Puccinia striiformis* f.sp. *hordei* and is predominant diseases in the areas under NWPZ and NHZ. This disease mostly appears in cooler climates (2-15°C), where the leaves are wet for prolonged periods (8-10 hrs) and provide ideal conditions for infection. Generally, disease appears in the Month of January and February but sometimes its appearance is also reported in December. Infection produce linear, orange-yellow pustules appear on leaves and leaf sheath. As the disease progresses, pustules coalesce to form long stripes between leaf veins.

Leaf Rust

Leaf rust caused by *P. hordei* is characterized by small orange brown circular spore masses surrounded by a bleached or yellow halo. Pustules are mainly confined to the upper surface and irregularly distributed on the leaves. A temperature ranging from 20-25°C and prolonged wet weather are pre-requisite for faster disease development and spread.

Black Rust

Black rust caused by *P. graminis* f.sp. *hordei*, is primarily a disease of CZ and PZ of India. It often infects the crop late in the season and, therefore, the losses are minimal. Dark res brown spore masses on the stem and leaf sheath. In severe case, spores can form on leaf blade, glumes and awns. Optimal conditions for infection are a temperature range of 15-28°C and 6-8 hours of free moisture on the leaf surface.

Management of Barley Rusts

- Cultural practices like cultivation of barley with other non-cereal crops, mixed cropping, early maturing varieties and judicious application of nitrogen fertilizers are helpful in checking the disease
- Use newly released varieties recommended for the zone. For NWPZ grow DWRB 160, PL 891, RD 2917, DWRB 137, DWRB 123, PL 784 and for CZ grow RD 2899 etc.
- Grows diverse stripe rust tolerant varieties avoiding single variety over large areas.
- Always use balanced and recommended quantity of fertilisers (avoid high dose of nitrogen).
- Keep strict watch on appearance of disease & immediate spraying of affected areas with recommended fungicides, *viz.* Propiconazole @ 0.1% to avoid its further spread from initial infection foci.

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Biotic Constraints in Barley Production with Special Emphasis on Rust Fungi

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Barley is an important coarse cereal and is used for multiple purposes like food, feed and malting. It is low input crop can also be grown in marginal lands. During 2018-19, the crop occupied nearly 6.62 lakh ha area with the production of 1.75 m t grains, where the highest production was reported from Rajasthan (50 %) followed by Uttar Pradesh (23%) and Madhya Pradesh (17%). Barley is cultivated in Rajasthan, Uttar Pradesh, Madhya Pradesh, Bihar, Punjab, Haryana, Himachal Pradesh and Jammu & Kashmir. A number of biotic and abiotic factors pose a challenge to increase production of barley. It is attacked by various diseases and insect pests causing heavy losses in yield and quality. Barley diseases prominently rusts, net blotch, spot blotch, Smuts, Septoria leaf blotch, powdery mildew, stripe disease, barley yellow dwarf and molya disease are the major biotic constraints in enhancing the barley grain production. Though barley is attacked by many pathogens but few are economically important in India. Stripe rust / yellow rust (*Puccinia striiformis* f.sp. *hordeii*) and leaf rust/ brown rust (*Puccinia hordei*) are major problems in North Western Plain Zone (NWPZ), while in North Eastern Plain Zone (NEPZ) leaf rust and leaf blights are common. In Hill Zone (NH), stripe rust and powdery mildew are serious problems.

Stripe or Yellow Rust

It is caused by *Puccinia striiformis* f.sp. *hordei* and is predominant in the areas of North Western Plane Zone (NWPZ) and Northern Hill Zone (NHZ). Yellow rust is principally a disease of barley in cooler climates (2-15°C), where the leaves are wet for prolonged periods (8-10 hours) and provide optimum conditions for infection. Generally, disease appears in the Month of January and February but sometimes its appearance is also reported in December. Infection produce linear, orange-yellow pustules appear on leaves and leaf sheath. As the disease progresses, pustules coalesce to form long stripes between leaf veins. Spores rub off easily on fingers.

Leaf Rust

It is caused by *Puccinia hordei*. It is characterized by small orange brown circular spore masses surrounded by a bleached or yellow halo. The pustules are mainly confined to the upper surface and irregularly distributed on the leaves. A temperature ranging from 20- 25 ℃ and prolonged wet weather are pre-requisite for faster spread of the disease.

Black Rust

It is caused by *Puccinia graminis* f.sp. *hordei* and is primarily a disease of the Central and Peninsular India. It often infects the crop late in the season and, therefore, the losses are minimal. Dark res brown spore masses on the stem and leaf sheath. In severe case, spores can form on leaf blade, glumes and awns. Optimal conditions for infection are a temperature range of 15-28°C and 6-8 hours of free moisture on the leaf surface.

Rust Phenotyping

Creation of rust epiphytotic conditions is important for effective phenotyping against rust resistance. First create disease under polyhouse and then inoculate on the field:

The main methods of inoculation include:

- Dusting or brushing with dry spores, with or without a carrier such as talc,
- Plant tissue injection using water-based suspensions: Hypodermic injections of elongating stems with aqueous uredospore suspensions provide reliable contamination-free infections without need for dew formation. The technique is suitable for the greenhouse or field. This method is the most labour intensive, although it is very efficient with minimal contamination during infection and exogenous moisture is not necessary for successful infection,
- Spraying with water: Inoculation of field-grown plants with water with 0.2% Tween 20 of urediospores in late afternoon in anticipation of overnight dew.

Field epidemics are commonly initiated by injection of susceptible plants, placement of infected potted plants at intervals throughout the target nursery, or by dusting or spraying on days prior to overnight dew development. Pots of healthy susceptible plants positioned in field plots prior to inoculation, and returned to a greenhouse after the anticipated dew treatment, are a convenient and rapid means of determining the outcome of the inoculation.

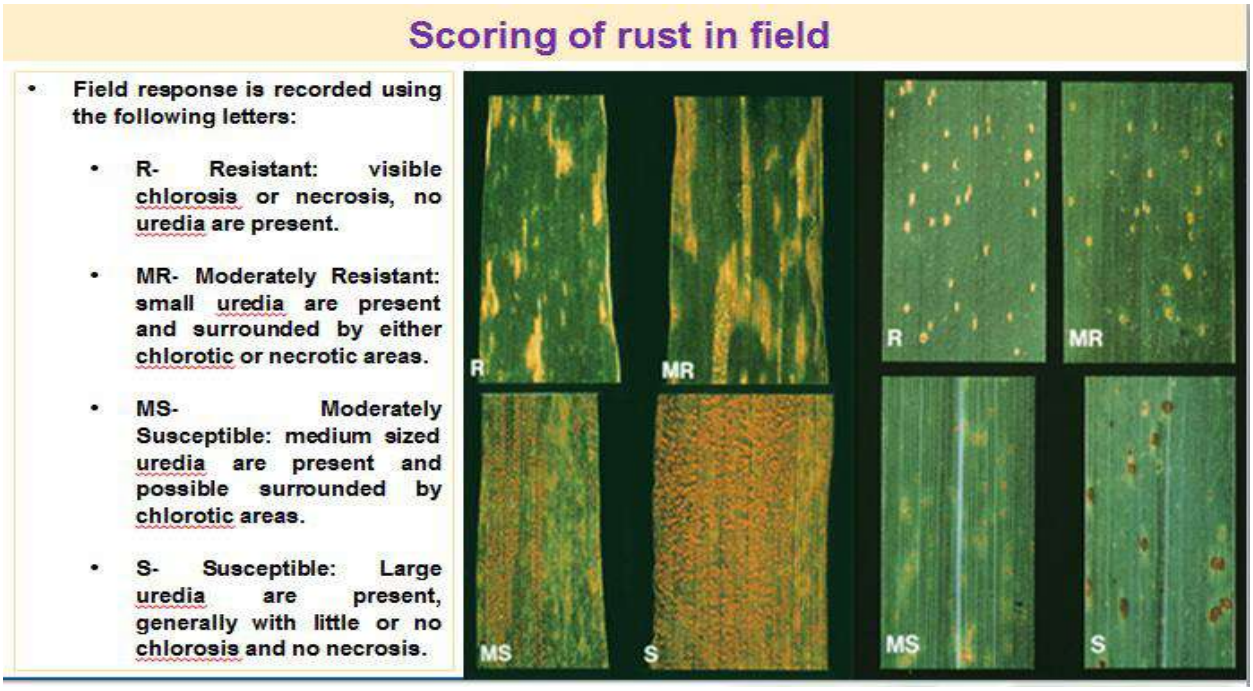
Scoring

There are various rust scoring scales, but a more common approach under field conditions is to use the modified Cobb scale as a quantitative measure of disease. Alternatively, a coefficient of infection (CI) which weights the modified Cobb scale rating by the disease response (R, MR, MS, S) has been used.

Severity is recorded as a percentage, according to the modifed Cobb scale. This recording process relies upon visual observations, and it is common to use the following intervals: Trace / 5 / 10 / 20 / 40 / 60 / 80/ 100 percent infection.

Field response is recorded using the following letters:

R- Resistant: visible chlorosis or necrosis, no uredia are present.
MR- Moderately Resistant: small uredia are present and surrounded by either chlorotic or necrotic areas.
MS- Moderately Susceptible: medium sized uredia are present & possible surrounded by chlorotic areas.
S- Susceptible: Large uredia are present, generally with little or no chlorosis and no necrosis.



Management of Rusts:

- Grow newly released varieties recommended for the zone. For NWPZ grow DWRB 160, PL 891, RD 2917, DWRB 137, DWRB 123, PL 784 for CZ grow RD 2899 etc.
- To avoid large scale spread, grows diverse stripe rust tolerant varieties avoiding single variety over large areas.
- Use balanced and recommended quantity of fertilisers – avoid high dose of nitrogen.
- Keep strict watch on appearance of disease & immediate spraying of affected areas with recommended fungicides, *viz.,* Propiconazole @ 0.1% to avoid its further spread from initial infection foci.

Foliar blight or Spot Blotch

The disease is caused by *Bipolaris sorokiniana.* Foliar blight is the main problem in humid and warmer areas especially in North Eastern Plains Zone (NEPZ). On leaves, dark brown round or elongated spots that may join into larger irregular patches. Both spots and patches surrounded by yellow halo. Severely affected leaves die and dry up, leaving the characteristic brown lesions visible.

Net Blotch

It is caused by *Pyrenophora teres.* A characteristic "netting" of the dark, chocolate-coloured blotches on leaves, sheaths and glumes. The fungus can over season as mycelium and pseudothecia on host stubble and then produce conidia and/or ascospores that can infect the next season's crop. Moreover, infected volunteer plants or wild *Hordeum* species may also serve as sources of primary inoculum for newly sown crops.

Management of Blight and Blotch:

- Grow resistant or tolerant varieties.
- Apply the balanced fertilizers as per recommendations.

Powdery Mildew

It is caused by *Blumeria graminis* f.sp. *hordei.* The disease is most prevalent in early sown crops with good canopy cover. White to buff or gray powdery masses of spores scattered on or completely covering the leaf blade. All above ground parts of the plant can be affected. Late in the growing season, the black, globose-shaped cleistothecia (the structure containing the sexual spores) form within the cottony masses of mycelium and conidia. The powdery mildew fungus is a biotrophic pathogen and unique in that it can infect barley without the presence of free moisture. In general, the disease is favoured by cool (15°C -25°C) and humid weather but can also occur in warmer, semiarid environments.

Management:

- Use recommended quantity of seed, avoid excessively dense stands.
- For the control of powdery mildew in disease prone areas, one need-based spray of Propiconazole (Tilt 25 EC @ 0.1%) can be given at appearance of disease.

Loose smut

Loose smut is caused by *Ustilago nuda.* It is characterised by masses of olive brown smut spores replace the entire head of plants with little development of floral bracts and awns. Spores are dislodged and scattered by wind when the delicate membranes surrounded them break. The fungus infects open flowers and becomes established in the embryo of the seed.

Covered smut

It is caused by *Ustilago hordei.* Masses of dark brown smut spores replaced the entire head of plants. Floral bracts and awns at least partially develop and spores are contained in a membrane until plant maturity when they are dislodged by threshing and infect the seed.

Management of Loose and Covered Smuts:

- Grow resistant or tolerant varieties.
- Use disease free seed.
- Seed treatment with Carbendazim 50% WP or Carboxin 75% WP @ 2g/kg seed.
- Apply the balanced fertilizers as per recommendations.
- Rogue the loose and covered smut infected plant and burn.

Barley Yellow Dwarf Virus

The virus is transmitted by several species of aphids, and symptoms can occur in patches. Bright yellow tips and margins of older leaves. Stunting, small seed and sterility.

Termite

Termite damage the crop soon after sowing and near maturity. The damaged plants dry up completely and are easily pulled out. The plants damaged at later stages give rise to white ears. Treat the seed at the rate of Chlorpyriphos 20 EC @ 4 ml per kg seed. In standing crop, one liter Chlorpyriphos 20 EC dilute in 2-3 liter water and mix in 20 kg sand and broadcast in the one hector field.

Aphid

Barley crop is affected by aphid which causes heavy loss to the crop as well as the grain quality in susceptible variety. Spraying with Thiamethoxam 25WG @ 20 g ai/ ha or Imidacloprid @ 20 g ai/ ha or Clothianidin @ 15 g ai/ha in 100 liters of water. In case of heavy incidence, the second spray can be made at an interval of 15 days.

Molya Disease

It is caused by *Heterodera avenae* and common in sandy areas of north eastern Rajasthan and adjoining Haryana. Aboveground, the plants are severely stunted, usually with a patchy distribution and show symptoms of nutrient deficiency. This causes heavy losses in crop by drastically reducing tillering and earhead formation. Juveniles gain entry to the root, and females set up a feeding site in the vascular system of the root. The females become swollen, produce eggs and are transformed into cysts, which protrude through the roots. These cysts are white when young and then turn brown. The eggs are formed within the cyst and the cysts can survive for long periods of time and overwinter. The use of resistant varieties like RD2052 and RD2035 and RD2592 should be encouraged for such areas.

Integrated Disease Management

- Grow resistant or tolerant varieties.
- Use disease free seed.
- Crop rotation with a non-host crop will reduce the inoculum load.
- Seed treatment with Carbendazim 50% WP or Carboxin 75% WP @ 2g/kg seed.
- If termite infested field treat the seed with Chlorpyriphos 20 EC @ 4 ml per kg seed.
- Apply the balanced fertilizers as per recommendations.
- Rogue the loose and covered smut infected plant and burn.
- If any diseases or pest appears in crop spray suitable pesticide.

Crop Health Monitoring and Surveillance of Wheat Diseases

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Introduction

Disease surveillance is the systematic monitoring of biotic factors of the crop ecosystem in order to predict the disease outbreak. By the surveillance programmes, the disease dynamics under field conditions can be known which in turn helps in devising the appropriate management strategies. Disease surveying is basic to all effective disease control and research programs. These surveys are equally important throughout research work as a means of assessing the effectiveness of control measures. Surveys are required to maintain claims of "disease-free" status of an area and to detect new populations of quarantine pathogens. Disease surveys are also an integral part of control and eradication programs. Surveys are helpful to know how a disease is multiplying in an area and when it is expected.

Realizing the importance of crop health monitoring, regular wheat disease surveys were initiated during 1967 under 'All India Co-ordinated Wheat Improvement Programme (AICWIP)'. These surveys were carried out through mobile units and trap nurseries which generated considerable information on the appearance of rusts in different parts of the country. This also helped in epidemiological studies. These surveys further confirmed the findings of Dr. K. C. Mehta on the movement and occurrence of rusts in the country. The information generated was published in the form of bulletins that were issued by the Division of Mycology and Plant Pathology, IARI, New Delhi from time to time. The survey and surveillance programme was strengthened during 1995 through an AP-cess fund project on survey and surveillance for pests and diseases with IIWBR as the nodal centre and four other zonal centres viz., Ludhiana (NWPZ), Kanpur (NEPZ), Powarkheda (CZ) and Pune (PZ). Extensive surveys were conducted and pest profile was prepared. During 1995, a 'Wheat Crop Health Newsletter' was published from DWR, Karnal on monthly basis during the crop season and is still continuing. The latest information on crop health status is disseminated quickly through this newsletter. All issues of it are available on ICAR-IIWBR website (https://www.iiwbr.org) since the year 2000.

Crop health surveillance, apart from providing a decision-making tool for taking up the plant protection measures, also provides information on the occurrence and spread of the diseases and other pests, already occurring in an area or the ones newly introduced to the area due to the change in cropping sequences and cultural practices, introduction of newer and resistant genotypes, and similar factors. Monitoring of diseases of wheat crop was systematically conducted under the AICWIP during late sixties to early eighties in the post-green revolution era, when new high yielding varieties were introduced in the country. This helped in enlisting and prioritizing the health problems of wheat for developing relevant crop protection strategy. Now, under the changing cropping sequences, like introduction of Rice-Wheat rotation, more area coming under irrigation, and the increase in cropping intensity, etc. there is every likelihood of a changed disease scenario too. In other words, the pests of less importance during yester years, may become the pests of major concern tomorrow.

Crop health monitoring and surveillance is being done with following objectives:

(i) To monitor the occurrence and spread of the diseases/pests.

(ii) To monitor the inoculum in the environment for purpose of prediction or forewarning.

(iii) To monitor or keep vigil on the entry of new pests or their races/pathotypes, etc.

For achieving these goals or objectives, the following course of action in envisaged:

(i) Trap Plot Nurseries

(ii) Mobile or roving surveys

Rapid progress has been made towards the goal of establishing a Global Cereal Rust Monitoring System. The system has reached the point where it can now be regarded as a fully operational global disease monitoring system. New technologies are playing an increasingly important role in rust tracking. Through ICAR-BGRI collaboration, a robust and functional data management system - the Wheat Rust Toolbox - is

now in place in India. This includes extensive rust surveillance and race databases. New web resources are providing access to a wealth of information regarding rust surveillance and monitoring in ways not previously possible.

Wheat Diseases

Overall India is the second largest producer of wheat after China. Wheat is the next most important food crop in the country following rice, both in area, production and consumption. Wheat growing regions in India represent a diverse agro-climatic condition. The major wheat growing states of India are Punjab, Haryana, Uttar Pradesh, Madhya Pradesh, Rajasthan and Bihar. These states account for about 90% of India's total wheat produce. The success story of enhanced wheat production during last 40-45 years by keeping the rusts at its lowest ebb demonstrates the strength of not only of the consolidated efforts of the technology developed by the scientists across the country but the government's policies for making them adaptable by the farmers through different countrywide programmes. The era of cultivation of semi-dwarf wheat which began in 1960's, ushered a phenomenal success as these genotypes along with spurt in inputs revolutionized the wheat production in the following years, leading to "Green Revolution". The wheat production in India, ever since has increased many folds from 6.58 mt in 1946-47 to 102.19 mt during 2018-19 with productivity to the tune of 3.57 t/ha (Source: Ministry of Agri. and Farmers Welfare, GOI). Sustaining this level of productivity is a big challenge and efforts are on to break the yield barriers.

A host of biotic stresses affect wheat crop leading to huge losses in yield. Since wheat is grown in different agro climatic conditions in our country, the constraints to its production vary from one zone to other. Biotic stresses are the main constraints in wheat production worldwide. The black or stem rust (*Puccinia graminis* Pers. f. sp. *tritici* Erikss. & Henn) is important in warmer areas whereas the brown or leaf rust (*P. triticina* Eriks.) in the entire country and the yellow or stripe rust (*P. striiformis* Westend.) in cooler areas.

Tracking Rusts

As a national strategy under the overall umbrella of Indian Council of Agricultural Research, Indian Institute of Wheat and Barley Research (IIWBR) is coordinating All India Wheat and Barley Improvement Project through which rust pathotypes are being monitored, strategically resistance genes incorporated with major emphasis on adult plant resistance (APR) and slow rusting. The importance of crop health monitoring in Indian wheat programme was realized as early as 1920's and random surveys were conducted by Dr. K.C. Mehta and colleagues. From time to time there was integration of mobile surveys, trap nurseries, use of satellite for disease survey & evaluation of spore dissemination, infra-red image (s), analysis of rain samples for uredospores to monitor the disease situation. In addition mobile surveys are undertaken at various crop growth stages, along the specific routes. These roving or mobile surveys also help in keeping a vigil on the entry of a new pest or pathotype. A very good example is the detection of a new virulence (*Yr*9 virulence) of stripe rust in 1996 from the bordering areas of Punjab and identification of stripe rust resistant germplasm within two months of its confirmation. Subsequent surveys carried out through mobile units and trap nurseries generated considerable information on the appearance and spread of rusts in different parts of the country became an integral component of wheat improvement. During 1967-68 under the All India Co-ordinated Wheat Improvement Project (AICWIP) monitoring of diseases of wheat crop was systematically introduced in all wheat growing states to know the prevalence, spread of wheat diseases and performance of cultivated wheat varieties to rust. Co-operating scientists in different states monitor the wheat crop at 15 days interval starting from either December end or after the crop is more than 45 days of maturity.

Each state has different agro-climatic regions and the extensive and intensive monitoring is done in disease prone areas, however, other areas also monitored on alternate trips. A team of 2-3 scientists at fortnightly intervals move by car in different directions in a state and halt at a distance of 30 to 50 Kms to monitor 2-4 wheat fields on both sides of the road. Within wheat field randomly move around to look for diseases, pests, weeds and general crop health. For rusts prevalence, intensity and spread within and surrounding fields is monitored. In case there are 2-3 patches of the disease in a range of 100-200mts having traces to 40S it is considered to be of limited occurrence but if there are >5 such patches having infection varying from traces to 80S it is considered spreading and then the adjoining fields are monitored intensely more so if the field is grown with a susceptible variety. Sometimes the spread could be visualized just standing outside the fields such fields are considered to be severely infested where farmers are immediately apprised of the fact that if the crop is not sprayed losses could occur. The losses may vary from <1 to 100% depending upon the varietal susceptibility, stage at which infection occurs and the severity of infection on flag leaf up to grain formation stages. Many a times under severe situations of the rust infection the sporulation occurs on glumes and pericarp of the grain. Under any situation of rust prevalence from mild to severe form scientists of Agricultural Universities, KVK staff and officers of Department of Agriculture are contacted for awareness

campaigns to apprise the farmers of rust detection and spraying the crop with fungicide (s) and also making it available timely through Government agencies (Sharma *et al.,* 2013).

Besides, mobile surveys another strategy had been planting of Wheat Disease Trap Plot Nursery (TPN). To specify the exact purpose, the nursery was later on designated as Wheat Disease Monitoring Nursery (WDMN). The nursery has become an integral part of the Crop Protection programme and has assumed so much significance that it is regularly planted at various strategic locations and constant watch is kept on the appearance and spread of wheat diseases both in regular as well as off-season crop. Such nurseries usually contain varieties or entries with known genetic constitution so that the occurrence or appearance of a new pathotype or pest could be identified. Under the AICWIP, the Wheat Disease Monitoring Nursery (WDMN/TPN) is planted at multi-locations including those all along the western border. It contains a common set comprising of 15 lines having popular varieties, resistant varieties, a susceptible variety and some resistant lines. In addition, five predominantly cultivated zone specific varieties are also part of this 20 line nursery. It is serving as an important tool to know the wheat disease situation, progress and appearance of new variants or races on resistant materials. It also helps to keep a vigil on the occurrence of wheat diseases along the western border, especially for the stripe rust. Every year, the crop health is monitored and the field samples of the rusts are analyzed for their virulence (pathotype analysis). Distribution pattern of the rust virulences (pathotype) provides much-needed orientation for the wheat breeding programmes and executing the resistance-gene / varietal deployment.

On the same pattern as that of WDMN / TPN, a Regional Wheat Disease Monitoring Nursery, called as 'SAARC Nursery' is planted and conducted in Afghanistan, Bangladesh, India, Nepal and Pakistan in collaboration with CIMMYT, Kathmandu. This nursery was started in 1989-90 and first planted in 1990. It is composed of twenty varieties *viz.* Annapurna-1, WL 1562, HD 2204, PBW 343, HD 2687, HD 2189, HP 1633, RAJ 3765, PBW 373, Pak 81, Punjab 65, Chakwal 86, Faisalabad 85, Inquilab 91, Faisalabad 83, Rawal 87, Kohsar, Bakhtawar 94, Gourab and susceptible check drawn from the SAARC member countries. This nursery is also constituted at Regional Research Station, Shimla (ICAR-IIWBR - ICAR). The disease appearance in the nursery provides an evidence of comparative wheat disease situation and virulences prevalent in the SAARC countries. The nursery is monitored by the competent co-operators of the respective location. These observations help a breeder to re-cast the breeding strategy towards breeding for resistance to the new or likely to emerge virulences before these (pathotypes or virulences) become a real threat to wheat production in SAARC region.

Off Season Surveys

Frequent surveys are being taken during the off season in the high hills of Himachal Pradesh (Lahaul, Spiti and Kinnaur) and J & K (Ladakh). Major focus is on the occurrence of yellow rust and surveillance for the stem rust pathotype, Ug99. Survey is also being carried out in hills of Nepal having off season wheat, self-sown wheat and alternate/collateral hosts to understand the epidemiology of wheat rusts. Extensive surveys are conducted in the country to monitor the occurrence of stem rust pt. Ug99. Till today, there has not been any report of Ug 99 from anywhere in the country. Keeping in view the report of wheat blast disease in Bangladesh during 2016, extensive surveys are being carried out regularly by IIWBR, Karnal and co-operators in West Bengal, Bihar and other north eastern states of India for keeping strict vigil on wheat blast disease.

Post-Harvest Surveys

The post-harvest grain sampling and analysis for various seed borne diseases has been a regular activity. Analysis of grain samples was done for monitoring the status of Karnal bunt disease and to identify the disease free or low disease regions. Other seed-borne maladies like black point and discoloration (which may be confused by traders with KB), and ear cockle nematode were also monitored. Based on these, the 'low risk zone', 'high risk zone' and 'no risk zone' have been identified for Karnal bunt (KB). The states of Maharashtra, Karnataka, Gujarat and parts of M.P. have been declared as KB free. Parts of northern M.P. and Rajasthan have been in the low risk zone, while the NWPZ comprising of Punjab, Haryana, western Uttar Pradesh, foot hills of Uttarakhand and Himachal Pradesh, has been identified as endemic or 'high risk zone' for KB. There is scope to delimit the low KB areas even in the endemic zone itself. Some areas in Punjab and Haryana have been identified as low KB areas. These studies have great importance in view of the global trade.

Dissemination of Crop Health Information

Wheat Crop Health Newsletter was started during 1995-96 for faster dissemination of crop health information. This newsletter is being issued every month in every crop season regularly. Newsletter is also

made available on IIWBR web site (www. iiwbr.org). Wheat rusts information are also complied regularly in the form of Mehtaensis and issued twice in a year by IIWBR Regional Station Flowerdale, Shimla. Recently Vol 39, issue 2 was issued in July, 2019. For creating awareness among farmers for disease management, technical/research bulletins/cards were distributed which led to effective management of wheat diseases (Sharma *et al*., 2012a, b, c, d; 2013a, b; 2015).

Conclusion

In India, the concerted efforts of wheat surveillance has contributed significantly in keeping vigil on new pathogens and devising strategy for developing new resistant varieties ahead the pathogen can cause loss to the crop. In future, remote based technique either through remote sensing or intelligent robot can play important role in surveillance of wheat diseases. This will help in getting information in real time and necessary management options will be followed well in time.

Acknowledgment

All the co-operators of AICW&BIP for conducting regular surveys. Thanks to ICAR, CIMMYT and BGRI for strengthening the wheat rusts surveillance in SAARC countries through organizing training programmes.

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Genera of Rust fungi in India - Taxonomy and Preservation

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Phylum Basidiomycota comprises largely of rust, smuts, bunts and fleshy fungi which include mushrooms, toadstools, bracket fungi, fairy clubs, puff balls, stinkhorns, earthstars, bird's nest fungi and Jelly fungi. They live as saprophytes however some are serious agents of wood decay. Some toadstools are associated with trees form mycorrhiza, a symbiotic association while others are severe parasites *viz. Armillaria mellea* which destroys a wide range of woody and herbaceous plants.

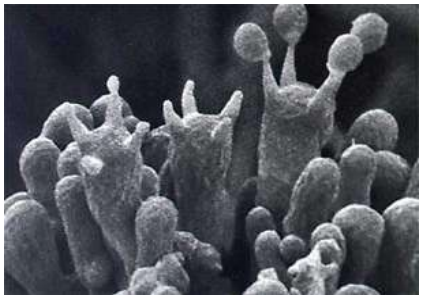
Rusts are the largest group of plant parasitic fungi in Basidiomycota that cause severe diseases of economically important crop plants like wheat, corn, cereals, legumes, beans and grasses. They are obligate in nature except a few and produce more than one spore forms in their life cycle. More than 160 genera of rusts have been recorded comprising 7,000 species world over, out of which 46 are monotypic. Geographically, rusts are distributed all over the world except Antarctica. The greatest numbers of species occur in temperate and near temperate regions. The most widely distributed species of economic significance are *Puccinia graministritici, P. recondita, Uromyces phaseoli* and *Tranzschelia discolor.* An important pathogen which is found around the globe is *Puccinia polysora*, popularly known as Southern corn rust. Most rust fungi occur on dicots than on monocot plants. Rust fungi parasitise plants that range from ferns to orchids and mints to composites. No rust fungus is however known to parasitise mosses or yet more primitive plants.

Important Characteristics of Phylum Basidiomycota

1. Members of this phylum are highly advanced fungi and have.
2. The name Basidiomycota is given because the fungi produce sexual spores on a special club shaped fruiting body called basidium.
3. A definite number of sexual spores called basidiospores (usually four in number) are produced on each basidium.
4. Fungi belonging to this group are referred as club fungi.
5. The group includes mushrooms, toadstools, shelf fungi, jelly fungi, puff balls, coral fungi, bracket fungi, birds nest fungi, stink horns, rusts and smuts.

General characteristics:

1. Produce sexual spores (basidiospores) on the outside of a specialized spore producing structure called basidium.
2. A typical basidium is a club shaped structure, bearing specially 4 basidiospores on pointed projections called sterigmata.
3. Basidiospores are haploid, uninucleate and are the result of plasmogamy, karyogamy and meiosis.
4. Dikaryotic phase dominates the life cycle.
5. Presence of clamp connections on the mycelium.
6. Presence of dolipore septum, except in rusts and smuts.
7. Absence of motile spores.

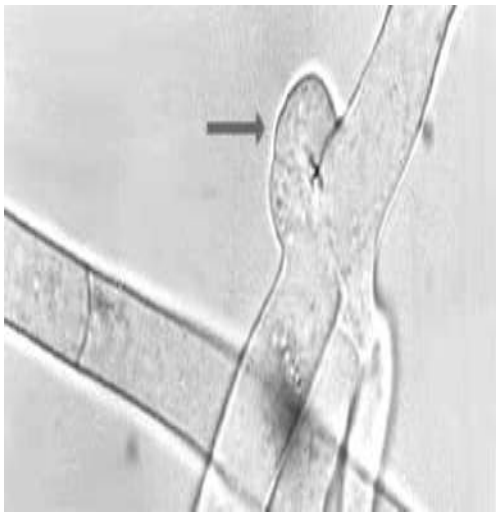


Somatic Structures

The mycelium consists of well developed septate mycelium. The mycelium passes through three distinct stages before the completion of life cycle. They are primary, secondary and tertiary mycelium.

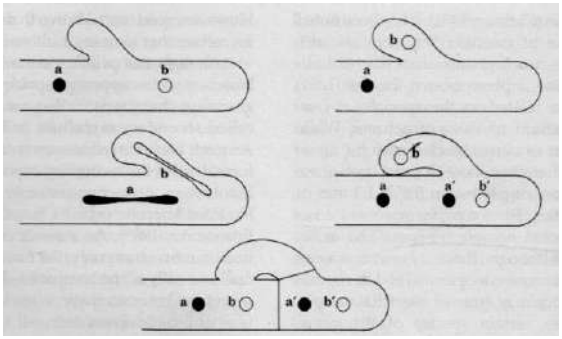
1. **Primary mycelium** (homokaryon or monokaryotic mycelium): It consists of hyphae with uninucleate cells. It usually develops from the germination of a basidiospore. It may be multinucleate at first when the nucleus of basidiospore divides many times as the germ tube emerges and grow. This multinucleate stage is short lived because septa are formed dividing the mycelium into uninucleate cells.

2. **Secondary mycelium** (dikaryon or dikaryotic mycelium): This originates from primary mycelium and its cells are dikaryotic (binucleate, n+ n nucleus) formed by somatogamy between compatible cells of monokaryotic mycelium or fusion of 2 basidiospores or spermatization. It exists during major part of the life cycle. Thus, this stage is an independent and extensive phase unlike the short dikaryotic phase of Ascomycotina. This is associated with special structures called clamp connections through which dikaryotization takes place (dikaryotization is a process by which monokaryotic primary mycelium is converted to dikaryotic secondary mycelium).

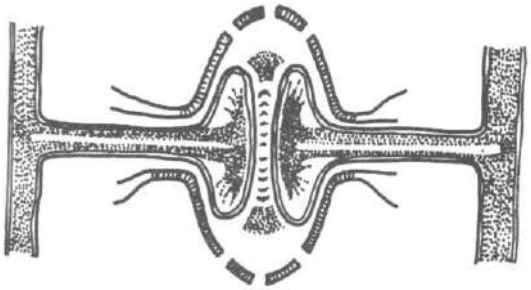


3. **Tertiary mycelium:** This is the binucleate mycelium which is organized into specialized tissues which form into fruiting bodies called sporophores (basidiocarps) in the members of higher Basidiomycotina.

Clamp connections: It is a hook like structure formed laterally in between the dividing nuclei in a dikaryotic hypha. It acts as a by-pass for the nuclei, as they cannot pass through septal pore ie. dolipore septum. It is meant for multiplication of dikaryotic cells.



Mode of development of clamp connection: When a binucleate cell is ready to divide, a small lateral branch called clamp connection arises from the cell betweenthe 2 nuclei (a and b) and begins to form a curved hook. Then the 2 nuclei divide simultaneously. One division orient obliquely so that one daughter nucleus "b" forms in the clamp connection and the other daughter nucleus "b1" forms in the dividing cell. The second division orients itself along the length of the dividing cellso that one daughter nucleus "a" forms near one end of the cell and the other "a1" approaches the nucleus "b1" of the first division near the other end of the cell. In the mean time, the clamp bents over and its free end fuses with the cell so that clamp forms a bridge through which one of the daughter nucleus "b" passes to the other end of the cell and approaches daughter nucleus "a ". A septum is formed to close the clamp at the point of origin and another septum vertically under the bridge to divide the parent cell into two daughter cells with "a" and "b" in one daughter cell and nuclei " a1 " and " b1 " in the other cell. The clamp remains permanently attached to hyphae. Its presence indicates that the hypha is dikaryotic.



Dolipore septum: Both primary and secondary mycelium consists of dolipore septum. The septum around the central pore swells at the centre forming a barrel shaped structure with open ends, thus forming a septal pore. The septal pore is surrounded by a cup like or dome shaped membrane called parenthosome or septal pore cap or nuclear pore cap. It is made up of a double membrane and its function is to shut the pore. The dolipore septum will not allow the movement of nuclei in hyphae but maintains continuity of cytoplasm.

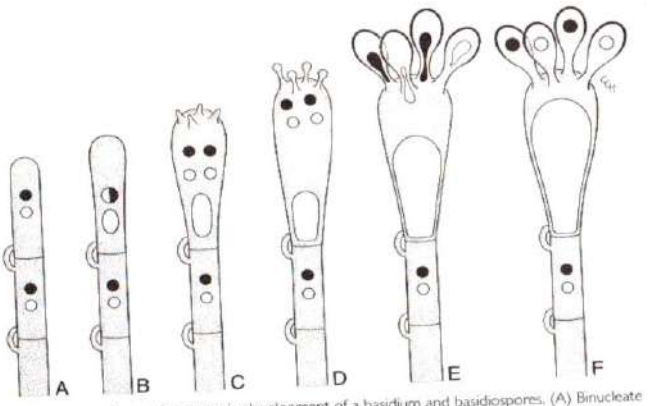
Asexual reproduction: Asexual reproduction takes place by means of budding (conidia), fragmentation of hyphae (arthrospores), uredospores. Conidial production is common in smuts while rusts produce uredospores (summer spores) that are conidial in origin and function.

Sexual reproduction: Sexual reproduction results in the production of basidium bearing haploid basidiospores. Basidiospores are formed as a result of karyogamy and meiosis taking place in basidium. In most of the members, sex organs (gametangia) are not produced and the somatic hyphae or detached somatic cells (arthrospores) undergo sexual process by somatogamy. In Puccinia sexual process is accomplished by spermatization through specialized organs called spermatia acting as male gametes and receptive hyphae as female organs. Thus, sexual cycle involves in typical cases, a monokaryotic phase and establishment of dikaryotic phase by somatogamy or spermatization of primary mycelium and then

karyogamy and meiosis in the basidium and return to monokaryotic phase by means of basidiospores. Thus, in the life cycle there is an alternation of monokaryotic and dikaryotic phases.

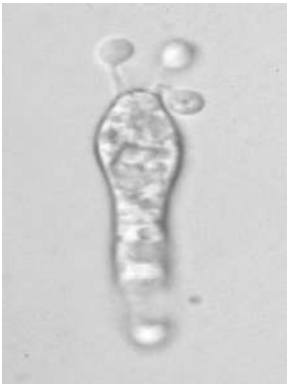
Basidium: Basidium is a club shaped, sexual, fruiting body bearing on its surface a definite number of (usually 4) basidiospores which are formed as a result of karyogamy and meiosis.

Development of basidium: A simple, club shaped basidium originates as a terminal cell of a binucleate hyphae and is separated from the rest of the hyphae by a septum over which a clamp connection is generally seen. At first, basidium is narrow and elongated and later it enlarges and becomes broader. Meanwhile, the 2 nuclei within the young basidium, fuse (karyogamy) and the zygote nucleus soon undergoes meiosis giving rise to 4 haploid nuclei. In the meantime, four small outgrowths termed as sterigmata push out at the top of the basidium and their tips enlarge eventually forming the basidiospore initials. During this time, a vacuole forms at the base of the basidium and as it increases in size, it pushes the contents of basidium out into basidiospore initials which finally become basidiospores.



Successive stages in development of a basidium and basidiospores. (A) Binucleate hyphal tip. (B) Uninucleate, diploid basidium following karyogamy. (C) Postmeiotic basidium with four haploid nuclei. Sterigmata have begun to develop. (D) Basidiospore initials on sterigmata and nuclei preparing to migrate into the spore initials. (E) Migration of nuclei into basidiospore initials. (F) Highly vacuolate, maturing basidium bearing four young uninucleate basidiospores.

Parts of basidium: Basidium is divided into 3 parts, Probasidium (portion where nuclear fusion takes place), metabasidium/promycelium (portion where meiosis occurs) and sterigmata (any portion between metabasidium and basidiospore). In smuts and rusts, fusion of 2 nuclei takes place in a specially formed thick-walled spore called chlamydospores and teleospores respectively. During the germination of chlamydospore/teleospore, fusion of 2 nuclei takes place in thespore, followed by meiosis. A germ tube called promycelium is formed which becomes transversely septate into 4 cells, each cell containing a haploid nucleus. The basidiospores are formed on the sterigmata on promycelium.



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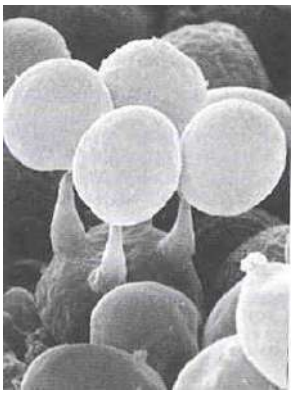

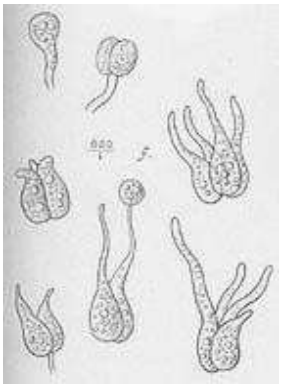

PHRAGOMOBASIDIA

Basidiospores: A basidiospore is typically a unicellular, uninucleate (exceptional2 nuclei) haploid structure. The basidiospores are formed exogenously on the basidium in contrary to the endogenous formation of ascospores. The basidiospores may be globose, oval, elongate or sausage shaped and may behyaline or coloured.

Dispersal of basidiospores: In majority cases, the spores are released violently and such spores are called ballistospores. Many possible mechanisms of spore discharge have been suggested. Buller was one of the first to examine critically the spore discharge. According to him, Basidiospores rest on the tip of sterigmata in an oblique fashion and a bubble or drop (called Bullers drop consist of liquid which forms at the hilar appendix ie, a minute projection of the spore near the point of attachment to the sterigmata) is responsible for basidiospore discharge .This drop keeps on increasing in size and its expansion results in explosive discharge of spore to a distance of about 0.1 mm. The spores are discharged insuccession at intervals of several seconds to minutes.

Life Cycle

The rusts have **polymorphic life cycle**. Production of many spore forms in the life cycle is called polymorphism. Generally five types of spores are seen during the life cycle viz., spermatia (uninucleate) in spermagonium, aeciospores (binucleate) in aecium, uredospores (binucleate) in uredium, teleospores (binucleate) in telium and basidiospores (uninucleate) on promycelium or metabasidium. The spermagonium represents gametic stage (male gamete spermatium and female sex organ receptive hypha), aecia represent the stage in which dikaryotisation occurs, uredia represent conidial or repeating asexual stage, telia represent sexual stage and act as encysted basidium in which karyogamy occurs and subsequently giving rise to basidiospores from promycelium or metabasidium.

6. **Autoceious rust:** If all the spore stages are produced on the same host then the fungus is called autoceious and the phenomenon is called **autoecism.** eg. *Melampsora lini* - linseed rust, *Uromyces appendiculatus* - bean rust.

7. **Heteroecious rust:** If spore stages are formed on two unrelated hosts ie. pycnia and aecia on one host and the uredia and telia on the other host, suchrusts are called heteroecious rusts and phenomenon is called **heteroecism.** eg. *Puccinia triticina* - black stem rust of wheat.

Primary host: The host in which heterocious rust produce the telial stage is called primary host (eg. wheat).

Secondary or alternate host: The host in which telial stage is not produced is called alternate or secondary host (eg. barberry).

8. Based on life cycle pattern, rusts are divided into macrocyclic, demicyclic and microcyclic rusts.

Macrocyclic rust (**long cycled rust):** Rusts in which all 5 spore forms are produced or produce at least one type of binucleate spore in addition to teliospores are called macrocyclic rusts. It may be autoceious macrocyclic rust (eg. *Puccinia helianthi*- sun flower rust) or heteroecious macrocyclic rust (eg. *Puccinia graminis tritici* - black stem rust of wheat).

Demicyclic rust: The rust in which uredial stage is absent. eg. *Gymnosporangium juniperi – virginianae*- cedar apple rust.

Microcyclic rust (short cycled rust): Rusts which produce no binucleate spore other than teliospore i.e., teliospore is the binucleate spore produced and both aecia and uredia are lacking, eg. *Puccinia malvacearum*- holly hock rust.

nclature stage "O" retained even today to avoid confusion.
Stage I: Aeciospores are the first binucleate spores formed in the life cycle.
Stage II: Uredospores are also called as repeating asexual spores as they function as conidia for the propagation of the rust fungus.
Stage III: Teleutospores represents the perfect stage because karyogamy and meiosis occur in them.
Stage IV: Basidiospores represent the sexual spores. Stages "O" and "I" occur on barbery while stages "II" and "III" occur on wheat. Basidiospores infect only barbery plant where as aeciospores can infect only wheat plant.

Rust Fungi - Genus *Puccinia*

Rusts are the most serious diseases of many crops in India. The causal agents of rusts are highly specialized plant pathogens with narrow host ranges. These are fungal organisms which belong to genus *Puccinia*, family Pucciniaceae, order uredinales and class basidiomycetes. Puccinia Pers. (1801) is an obligate plant pathogen genus and contains about 4,000 species. Of the rust diseases of wheat, the most common these days is called leaf or brown rust and is caused by *P. triticina* Eriks. develops rapidly at temperatures between 10° and 30°C. Stem rust, caused by *P. graminis* Pers. f. sp. *tritici* Eriks. & E. Henn., is also known as black rust is favoured by humid conditions and warmer temperatures of 15° to 35°C. Stripe or yellow rust, caused by *P. striiformis* West. f. sp. *tritici* Eriks. & E. Henn., is principally a disease of wheat grown in cooler climates (2° to 15°C), which are generally associated with higher elevations or cooler years. Wheat rust pathogens are generally obligate in nature. In principle, they produce five distinct fruiting forms (pycnium, aecium, uredium, telium and basidiospores) that appear in a definite sequence. Some of the spore stages parasitize one host, whereas the others may infect and parasitize a different alternate host. Rust fungi producing only teliospores and basidiospores are called microcyclic, and those additionally producing pycniospores, aeciospores and urediospores are called macrocyclic. Basidiospores, aeciospores and urediospores can infect host plants, and teliospores serve only as sexual, overwintering stage, which upon germination produces basidium. Aeciospores are formed after dikaryotization of

pycniospores which appear after infection of basidiospores. Aeciospores upon infection produce dikaryotic mycelia, which then bear urediospores. Some macrocyclic rusts complete their life-cycle on a single host and are called autoceious. Others, such as stem rust of wheat, require two different hosts (wheat and *Berberis*) are called heteroecious*.*

Maintenance of Herbarium Specimens

Preservation: A preservative is a substance used for killing or stopping the growth of an organism in or on the substrate on which it grows and the phenomenon is known as preservation. The reservation of diseased plant material or organism is done in two ways as mentioned below:

Preservation in dry form: For preserving a diseased specimen in dry form four major steps are necessary:

Collection: Diseased material is collected in the field and then it is properly pressed between the blotter sheets. Paper slips or bags are used to indicate locality, host, date and place of collection or other substrate on which it is present. For collecting damp material and fleshy fungi, the collection should be properly wrapped in moisture absorbing sheets to avoid their damage by moulds and insects, etc. Dry specimens (e.g., flowering plants) can be kept for very long period (even for hundred years or more), but preservation in natural condition is difficult. In short, an experienced collector during collection always pays attention to **Quality:** the representative material should be of good quality preferably should have reproductive parts, and **Quantity:** The material should be collected in sufficient quantities so that it can be made available to any one for investigations. It would be appropriate here to state that "Specimens in the herbarium need to be seen as working tools, like books and journals in a library. However, in a book only the description of the disease and a fungus can be seen, but in the specimen the disease and the fungus themselves are there to examine. Therefore, one good specimen is worth a thousand words".

Drying and accession: Drying is a process in which dehydration of the collected material occurs; this helps in long-term preservation. This is done in hot dry air or in the sun-light for a few days after carefully pressing the specimens between blotter sheets. Best drying is obtained by frequently changing the blotter sheets, particularly during the first few days. Sun-drying is economical and easy but for fleshy fungi, hot air or vacuum drying is the best. After drying, an entry is made for the specimen collected on the herbarium sheet and then it is accessioned in a large, strongly bound accession register (16 x 12"). This practice is followed in all important herbaria of the world and also in our national herbarium (Herbarium Cryptogamae Indiae Orientalis - H.C.I.O., IARI, New Delhi). The serial accession number is allocated to the specimen entered in a column on the extreme left in the register and the name of the fungus, host and country from which the specimen came along with the name of the collector are also entered. The right hand column is kept reserved for the remarks of the specialist who may examine the specimen later on.

Labeling and packeting: The accessioned material is kept in a stout, good, quality herbarium packet (6 x 4"). This is attached to a chart paper sheet (16.5 x 10.5"). The two sides of the packet are folded first, then the bottom flap and finally the top flap; this gives an appearance of an envelope. On the extreme lower left of the chart paper, entries regarding the name of the fungus, host (if applicable), locality date and identified by the scientist or specialist are mentioned on a printed lable (5 x 3") which is already pasted there. The entire sheet along with the folder is then placed inside a file cover (16.5 x 10.5"). On lower side of cover, accession number, name of the fungus, host and place of collection are given to locate it easily.

Storage and disinfection: These folders along with the specimens are then stored in steel or wooden almirahs. Before putting the folders inside the almirah, these are fumigated in order to protect them from climatic and environmental hazards, e.g., moulds, mites and insects. Fumigation is done by methyl bromide vapours in a fumigation chamber for 24-48 h. Naphthalene balls, para-dichlorobenzene, xylene, thymol or flit is sprayed/placed in a small container in each compartment of the shelf along with folders, whenever facility of methyl bromide fumigation is not available. The method effectively preserves the specimens in their original shape for years. For storing fleshy fungi, dehydration is carried out at 500C in an electric oven. Periodic fumigation or spraying of specimens in the folders is carried out to avoid any damage by mites and other insects (Miller and Richard, 1974).

Preservation by slide collection: Preservation of materials by slide collection can be obtained in 2 ways:

Microscopic slide preservation: Preservation of fungi sporulating in pure form in culture can be made by mounting the material in DPX mount or in 0.1% lacto phenol. Such slides can be stored for two to three years in a good shape. The cover-slip is ringed with the help of nail-polish or Canada balsam to avoid drying of the material.

Photographic slide preservation: For the preservation of dry spore masses of Agarics and Boletus, photographic slides of rare species of agarics showing spore prints are taken to study the structural features

of various species. These slides are then numbered and accessioned in the herbarium. A list is prepared for each genus and species. These can be used as visual aids in teaching as well as in identification.

Preservation in fluid: Different conventional methods of preserving fungi in original form are commonly employed by using various fixatives and preservatives depending upon the type of material. Fixatives are helpful in keeping the material indefinitely in its perfect state. The most commonly used fixatives and preservatives are mentioned below:

Fixatives: Formol-acetic alcohol (F.A.A.): Formalin - 13 ml; Acetic acid (glacial) - 5 ml; Ethyl alcohol (50%) - 200 ml. This fixative helps in restoring the natural shape of the material by checking its dehydration (Ainsworth, 1971). Flemming's week solution: Chromic acid (1%) - 25 ml; Acetic acid (1%) - 10 ml; Water - 60 ml; Osmic acid (2%) - 5 ml, is added before using this solution.

Preservatives: The general preservative used for museum specimens consists of 5 per cent formaldehyde (40%) in water. Formaldehyde-alcohol solution: Formaldehyde (40%) - 25 ml; Ethyl alcohol (95%) - 150 ml; Water - 1,000 ml. Alcohol helps in dissolving air and formaldehyde maintains the material in its natural form. Hesler's preservative: This is used for the preservation of coloured vegetables and fruits. It contains the following ingredients: Zinc chloride - 5g; Formaldehyde (40%) - 25ml; Glycerol - 25ml; Water - 1,000ml.

Preservative for diseased green plant parts: This is accomplished by boiling plant parts in a mixture of one part of glacial acetic acid saturated with copper acetate and four parts of water till the green colour reappears and then keeping it in a 5 per cent formalin. Another satisfactory method is to place the material in 5 per cent copper sulphate solution for at least six and not more than 25 hrs. The material is then washed for several hours in running tap water and preserved in sulphurous acid solution (5-6 per cent SO2) (15 ml) and distilled water (1000 ml) in sealed containers (Goncalves, 1931).

Maintenance of *Puccinia* Specimens at HCIO

The Herbarium Cryptogamae Indiae Orientalis (HCIO), a National herbarium established in 1905, not only serves as an educational resource for the National Agricultural Research System (NARS), but also conserves fungal biodiversity. Out of 1.5 million fungi, only 80,000 fungal species have been described so far, in which only some of the fungi can be stored in pure culture form, rests go unnoticed. Thus, it is the prime duty of the scientists to preserve them in disease specimen forms for the future generation before these elopes as never existed. In this context, role of HCIO becomes very important. At present, HCIO is enriched with about 51,000 specimens with Type Specimens (3800), New species recorded (570), New Indian Genera recorded (19), Indian Exsiccate sets (18) and Foreign Exsiccate sets (188) which are as old as from 1892, onwards. It has richest collection of ruts, smuts, powdery mildews and meliolales fungi. HCIO has a vast collection of Genus *Puccinia* with 718 species from a range of host plant families. For the researchers, students and others working in the field of Mycology and Plant Pathology, it is not always possible to visit HCIO and see the precious disease specimens. Therefore, 718 species of *Puccinia* is digitized deposited in HCIO by various workers with passport data.

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Molecular Phylogeny: Principles and Practice

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Bioinformatics, a new field of science includes biology, computer science, statistics and Information Technology. The sudden growth in the quantitative information in biology has resulted in realization of inherent bio-complexity issues which call for innovative tools to convert the information into knowledge. Bioinformatics, in one hand, involves computer specialists and statisticians for development of the tools and new algorithms for organizing and analyzing the data and in other hand helps biologists in understanding the structural and functional genomics, proteomics, protein engineering etc. using those tools (computational biology) in a biologically meaningful manner. In line with the theme of the "Central Dogma", bioinformatics utilizes the prediction approach to find out the sequence similarity in DNA that can lead to structural and functional similarity in protein and thus narrows down the search for understanding the functional role of a protein. The development of new rapid, inexpensive next generation high-throughput technologies sequencing over the last 10 years or so is changing the ways we think about the application of sequences to plant biology.

Different terminologies those are being routinely used bioinformatics analysis are described below:

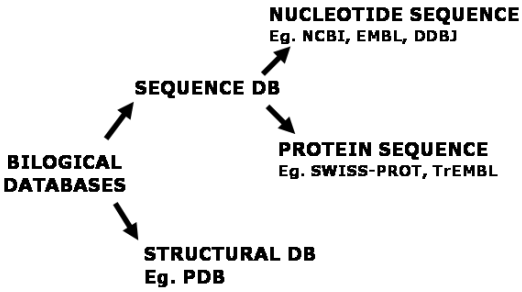
Biological Database:

Organized body of persistent data

Update

Query

Retrieve Components



Ways of submitting DNA sequence

- There are two principal ways of submitting DNA sequences to GenBank and EMBL.
- BankIt
- Sequin
- Webin-Align

Annotation

Refers to commentary or explanation of the information appended to DNA or protein sequences stored in databases.

Annotation can include:
known information about

- Source, Country, Organism
- protein(s) sequence
- predicted protein structure
- domain(s) of the protein.
- quaternary structure of the protein.
- protein function
- common post-translational modifications of the protein

Data Retrieval

Collection of data from databases

Data Mining

Generation of information from data in databases. E.g. – primer designing, gene finding, phylogenetic relationship study etc.

Gene Finding approaches

- Content based approach: The content based approach relies upon the differences in composition of nucleotide bases between the coding exons and noncoding introns. The periodicity of repeats and compositional complexity of codon triplets differentiate the exons from introns
- Site based approach: The gene has its own syntax. Start codon, stopcodon, donor and acceptor sequences, noncoding introns, ribosome binding sites, transcription factor binding sites, promoter sites, the poly adenylate sites etc are the specific signatures of genes
- Comparative method: The anonymous sequence is compared with cDNA sequence library.

Phylogenetic Relationship Study: Terminologies and Concepts

Homology:

This is a state of gene or morphological character that shares a common ancestry with a different gene or morphological character. For molecular sequence data, it is taken to mean that two sequences or even two characters within sequences are descended from a common ancestor.

This term is frequently misused as a synonym for 'similar', as in "two sequences were 70% homologous". This is totally incorrect! Sequences show a certain amount of similarity. From this similarity value, we can probably infer that the sequences are homologous or not.

Homology can not be measured only we can say whether homology is there or not but we can measure similarity. Homologous sequence must have similarity, but if there is similarity we can not say there is homology

Homologous Gene Super Family

A) Orthologous Gene

Same sequence and Same function but found in different taxa. E.g. - DNA Polymarase of Goat, DNA Polymarase of Human. Result of lineage Transfer.

B) Paralogous Gene

Found in same taxa. Same sequence but different function. E.g. - Hemoglobin, Myoglobin. Result of a gene duplication.

Alignment

An Alignment is an computational hypothesis which identify positional similarity or identity between bases/Amino Acids. Two ways: Local and Global Alignment.

Sequence Alignment Tools

- BLAST
- FASTA
- BLITZ
- BEAUTY, a modified BLAST

BLAST (Basic Local Alignment Search Tool)

- BLAST is the algorithm used by a family of five programs that will align your query sequence against sequences in a molecular database.
- Statistical methods are applied to judge the significance of matches.
- Alignments are reported in order of significance, as estimated by the applied statistics.
- BLASTN : Compares a nucleotide query sequence against a nucleotide sequence database.
- BLASTP : Compares an amino acid query sequence against a protein sequence database.
- BLASTX : Compares the six-frame conceptual translation products of a nucleotide query sequence (both strands) against a protein sequence database.
- TBLASTN : Compares a protein query sequence against a nucleotide sequence database dynamically translated in all six reading frames (both strands).

What We Know From BLAST

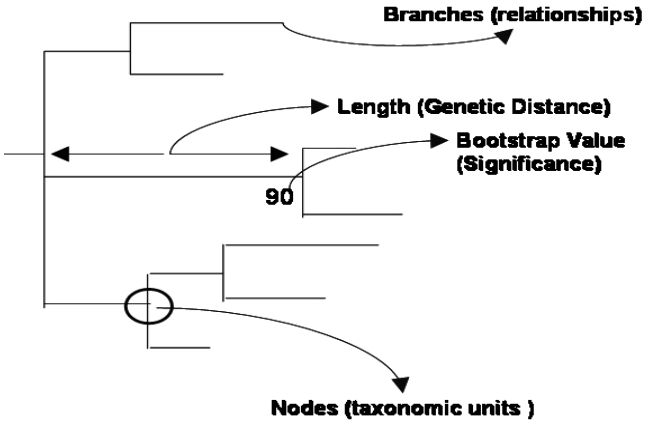
- Sequences that share similarity with query sequence
- Helps to retrieve those sequences

What We Do Not Know From BLAST

- Can not quantify the sequence similarity
- Can not tell us about the relationship between all those sequences

Multiple Alignment: Clustal W

Quick pairwise alignment: calculate distance matrix …> guide tree…> Progressive alignment following guide tree

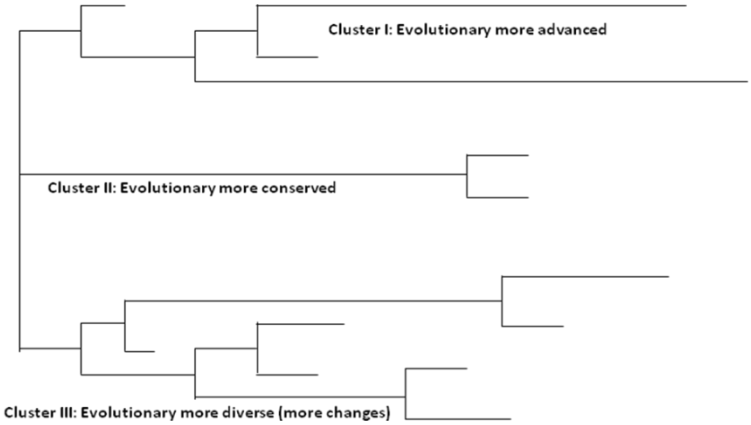


The branching pattern of a tree is called the TOPOLOGY
Representation of relationship through LINE : DENDROGRAM

Types of Dendrogram

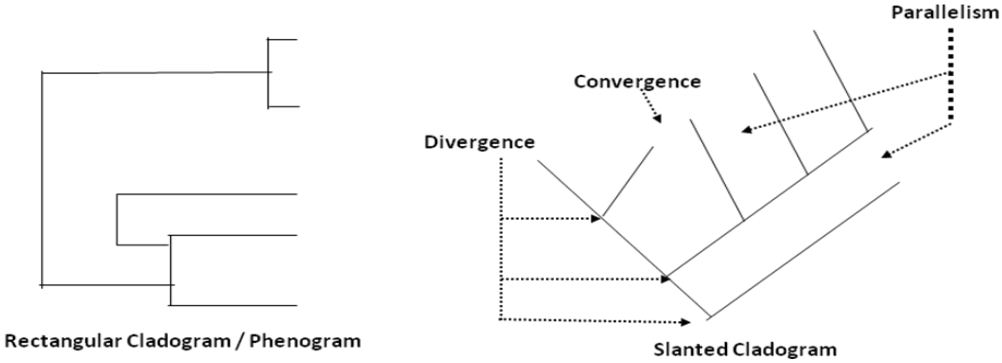
Phylogram

This is a phylogenetic tree that indicates the relationships between the taxa and also conveys a sense of time or rate of evolution. The temporal aspect of a phylogram is missing from a cladogram.



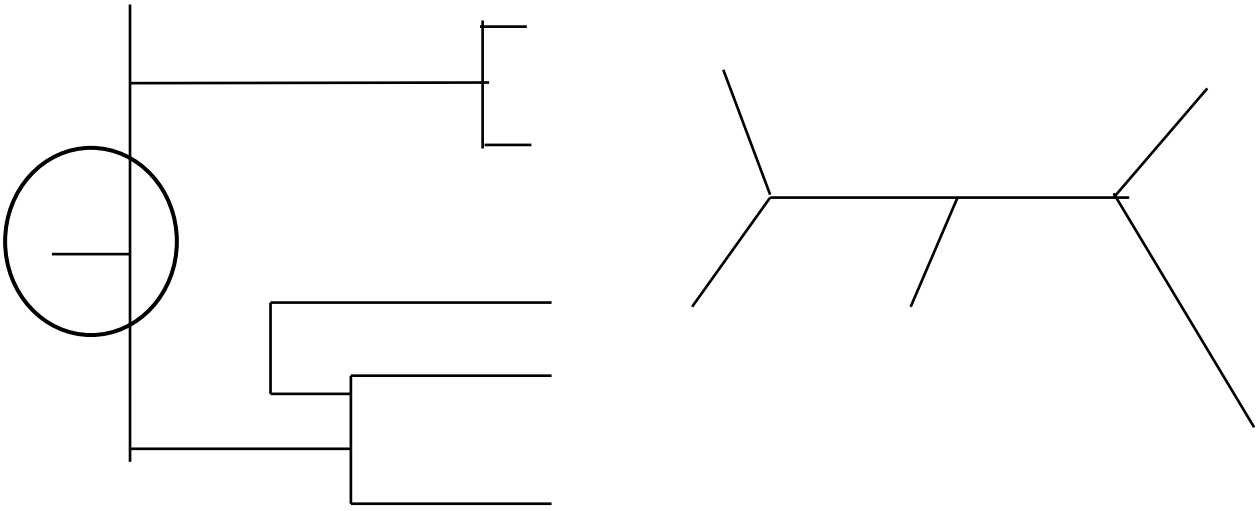
Cladogram

A dendrogram depicting the hypothesised branching order of a number of sequences. Cladograms do not give any indication of temporal change, but phylogram does.
Rectangular Cladogram / Phenogram – Suitable for grouping in taxonomic studies
Slanted Cladogram – Suitable for understanding convergence, divergence or parallelism in evolutionary studies



Presentation of a tree

Rooted tree : Assume that all taxa derived from a common ancestor
Unrooted tree: Assume that all taxa derived not from a common ancestor



Rooted dendrogram

Unrooted dendrogram

Methods for Constructing Phylogenetic Tree

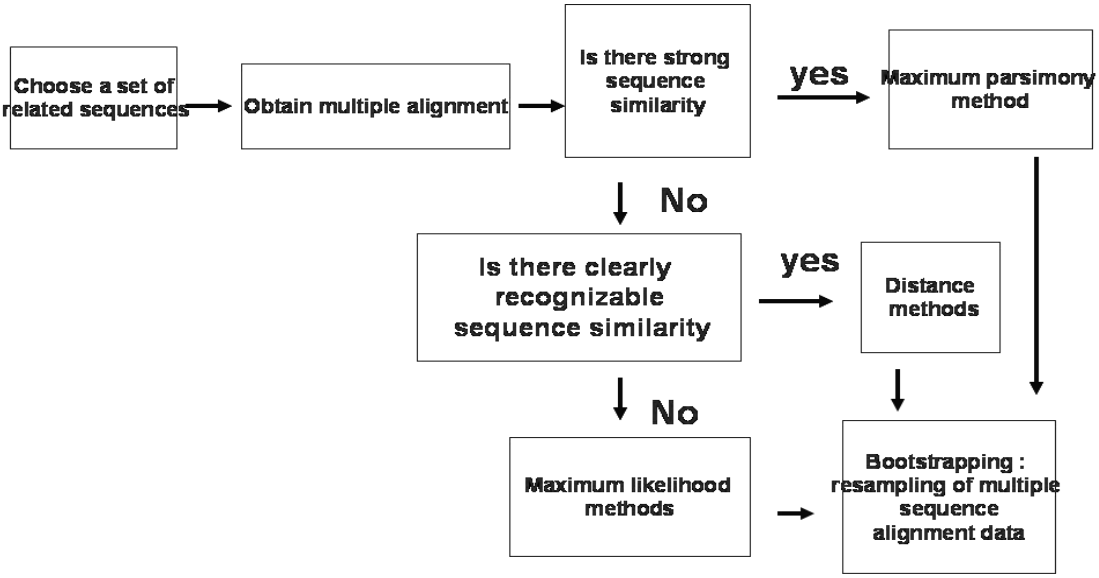
Character based and distance based method for tree development
Neibourhood joining tree - distance based method
Parsimony tree – character based tree. Use when sequences are quite similar, e.g. – strains of different viruses. Use small numbers of sequences for parsimony analysis.

Bootstraping:

- The bootstrap is a method for assessing the statistical significance the positions of branches in a phylogenetic tree.
- For each aligned pair, it samples scores from random positions in the alignment, adding the scores.
- When all the pairs have been sampled, it converts the scores to distances and computes a tree.
- This whole process is repeated many times and the frequency with which particular tree features are observed is taken as a measure of the probability that the feature is correct.

When to choose what type of tree:

Different bioinformatics analysis that are being routinely used for virus genomics studies are described below:



Survey, Sampling, Establishment, Multiplication, Inoculum Collection, Inoculation, Maintenance and Storage of Rust Pathogens

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Wheat is an annual grass that is grown under a wide range of environmental conditions in almost every parts of the world. Wheat crops suffers from many biotic & abiotic stresses in all stages of growth which interfere with normal functioning of physiological processes of plants and limit production. Rusts are among the earliest known diseases which co-existed with wheat crop since ancient period. Rusts occurs almost everywhere in the wheat growing areas and can cause huge losses. These are shifty pathogens and contain enormous variation to make promising wheat cultivars susceptible. Research on wheat & barley rusts is a continuous effort made by all the wheat & Barley growing countries. Survey & monitoring of occurrence & movement of rusts, trap nursery, sampling, pathotype identification & their maintenance, screening of germplasm and genetics of rust resistance are the areas of studies conducted on rusts and breeding rust resistant varieties.

Rust Survey

Survey and monitoring occurrence & movement of rusts and study of the pathogen's variation among and between the rust populations is a major objective of the rust surveys. Rust pathogens undergo continuous pathogenic changes for virulence factors. This phenomenon is resulting in the breakdown of effective resistance genes being deployed in resistant cultivars. Understanding the population structure and distribution of rust pathogens at national, regional & international levels would enable better understanding of the pathogenic variability and assist effective control measures and breeding programmes to plan for development of durable rust resistant cultivars.

Types of survey: There are two basic types of survey- *Roving / Mobile Survey* and *Fixed Plot Survey / Trap Plot Nursery (TPN)*. In the *Roving / Mobile Survey* observer travelling among large number of sites in short period and assessment of rust incidence/damage are made from randomly selected spots representing larger area. In the *Fixed Plot Survey* assessment of rust incidence/damage are made from a Fixed Plot selected or Trap Plot Nursery (TPN) grown in a field/location, and the data on rust incidence/damage are recorded periodic from sowing till harvest. Each survey type has its advantages & disadvantages and the choice of survey type must be made in order to minimize the disadvantages for a given set of objectives. It is usual to achieve this by adopting a combination of the two survey types.

Rust Sampling

Samples of rust infected wheat plants are collected to analysis and monitor the variability in rusts, pathotypes distribution and their spread, detection of new pathotypes and field response of cultivars. It is desirable to collect samples from wide range of host varieties and many localities in each district so that the collections are eventually distributed over entire wheat growing areas.

Sources of rust samples: Samples may be collected from different sources as below:

1. Farmer's field
2. Research farms
3. Trap plot nursery (TPN)
4. Off season crop / volunteer plants.

In the roving/mobile survey, random collections are made at every 20 Km or so depending upon geographical location, transportation facility and type of cultivar grown. The samples can be picked up from regular crop, summer crop, ratoon tillers and self-sown plants. At times the off-type plants in regular crop

also act as important source of samples. In addition to the commercial crops, samples from trap plot nurseries (TPN) also help greatly to generate information regarding the prevalence of pathotypes. Also TPN serves as a vital source for detection of new pathotypes, field response of a set of varieties and progress of a set of varieties and progress of the diseases. In addition to the *Roving/Mobile) Survey*, samples are collected from selective geographical areas. Samples of special interest are also sent by the wheat scientist positioned at different locations across the country.

Procedure for rust sample collection:

1. Samples should include a few well infected leaves (small bit of 2-3") or portions there of culms and portion of stem and even infected ear.
2. Samples should be dried for overnight in shade at room temperature in order to remove excess surface moisture. After drying, samples should be wrapped in newspaper or coarse paper envelopes and send as early as possible by ordinary mail.
3. Samples from resistant lines/genotypes may be interesting.
4. Following information may be given on each sample:

- Kind of rust (Yellow/Brown/Black) : ……..
- Infection type & percent severity : ……..
- Details of host (Wheat/Barley/Grass and Variety/Line) : ……
- Details of place of collection : ……
- Date of collection : ……..
- Cooperators name & address : ……..

Precautions to be taken during sampling:

1. Samples should be representative of a locality, variety and not repetitive.
2. Samples should not be taken from moist, dried and dead plant parts.
3. Blackish teliospores should not be collected, only uredial infections may be collected.
4. Glossy paper or polythene envelopes should not be used for collecting/mailing rust samples.
5. Samples should be dispatched immediately to ensure viability.
6. Samples should not be taken from the sites of artificial inoculations, otherwise should be indicated accordingly.

Establishment of Rust Samples

After receiving samples in the research laboratory, processing should start as quickly as possible to ensure spore viability. Rust samples are put on susceptible host immediately after making proper entries. For this purpose all the states and union territory of India have been arranged alphabetically and denoted by serial numbers accordingly. The serial numbers are also assigned to the neighbouring countries for keeping the records of the samples from those countries. The samples are entered serially in order of their receipt. Based on the serial number and state, samples are coded e.g. 110 number sample in the register is from Punjab, it would be coded as 110/21. Each rust is coded e.g. brown rust by Tr, black rust by Gr and yellow rust by St. Susceptible host cultivar for brown and black rust is Agra Local (except for pathotype 16 of brown rust for which Khapli is used) whereas for yellow rust now A-9-30-1 is being used in place of Kathia Local.

Modified techniques for establishment of stripe rust samples: Stripe rust (in other rusts when samples appear to be dead) samples are revived on 2% agar-agar. Simple 2% water agar is plated and samples of yellow rust are placed on it. Petri plates are placed in refrigerator for 24-48 hrs at 6°C. Stripe rust being partially systemic, a fresh crop of uredospores is produced (Fig. 1). Using this technique revival of stripe rust samples has increased considerably. Sometimes 80-90% of the sample are established. The samples from water agar or those received from field are inoculated onto susceptible host for multiplication of inoculum with the help of a lancet needle and kept in humid chamber for 48 hrs. The pots are then transferred on to the greenhouse benches. After 10-14 days, uredosporic dust can be used to inoculate set of differentials for pathotypes (races) analysis.

Fig. 1. Agar plate technique used for yellow rust sample

Raising Seedlings (Growing Plants) in Glasshouse

Seedlings are raised in glasshouse for inoculum multiplication, pathotypes identification, seedling resistance test & gene postulation, screening of advance generation lines/germplasm/genotypes and other important studies on rust pathogens.

Preparation of soil: A mixture of fine loam soil and farm yard manure in the ratio of 3:1 is ideal for growing seedlings. The mixture (soil) should be well sieved and steam sterilized at 1.054 kg/cm^2 pressure (121°C) for an hour in an autoclave to remove soil-borne fungi. The mixture (soil) can also be sterilized by treating with 5% Formalin and covering with polythene sheet for 48 hrs. The Formalin treated mixture (soil) is then spread over for evaporating Formaldehyde.

Glasshouse sowing: Four inch plastic pots sterilized with 2% Lysol solution are used for raising plants for inoculation, multiplication and other studies. Twelve to fifteen seeds of susceptible variety are placed (with endospermic end downwards) at half an inch depth in each pot (Fig. 2). Plastic pots are easy to handle, durable, light weight and efficient in retaining moisture.

Fig. 2. Raising wheat seedlings in four inch plastic pots

Fig. 3. Raising wheat seedlings in aluminium bread pans/trays

Rectangular pans or Aluminium bread pans/trays (11x4x3 inch size) which are able to accommodate 10 rows, are also used to raise plants for inoculum multiplication, identification of pathotypes screening of advance generation lines/germplasm and other important rusts studies (Fig. 3).

Three sets of differentials were previously used to be sown separately in three trays as Set-'0', Set-'A', Set-'B', each having capacity of 10 lines. Under the modified techniques, 3 sets of differentials are accommodated in one tray as three rows with each differential hill sown (Fig. 4). Five seeds are sown as one clump. Similarly, for seedling resistant test (SRT) 18 lines are hill planted in two rows with 7th line as susceptible check (Fig. 5).

Fig. 4. Three sets of differentials are accommodated in one tray

Fig. 5. Eighteen lines are hill planted in two rows for SRT in one tray

Specially designed wooden markers are used to facilitate sowing of differential sets (3 rows) or seedling resistance test material (2 rows) (Fig. 6a,b). After sowing, trays and pots are watered regularly to maintain the soil moisture.

The plants are raised in the spore proof seedling chambers with a provision of controlled temperature (25-30°C) and sufficient light. Generally, in about 6-7 days the seedlings are ready for inoculation. The pots/trays for inoculation are transferred to other glasshouse in wooden boxes having double layered muslin.

Fig. 6.a. Wooden marker used for sowing of differential sets (3 rows),
Fig. 6.b. Wooden marker used for sowing seedling resistance test material (2 rows)

Environmental Factors Associated with Rust Development

Temperature, humidity, free film of water and light are essential for the rust infection. Light and temperature are important for the expression of proper symptoms. Generally, uredospores germinate and produce infection hyphae only when a free film of water is available (Rowell *et al.,* 1958; Sharp *et al.,* 1958). Equally for rapid germination and appressorium formation by the uredospores, a congenial temperature and high relative humidity are essentially required. The optimum requirement for different rusts as given by Joshi *et al.* (1988) are given in Table 1.

Table 1. Optimum requirements for different rusts

Rusts	Free water	Temperature	Light and Humidity
Strip (yellow) rust			
Germination	Necessary	Minimum Just >0°C Optimum 9-13°C Maximum 23°C	Varies with temperature, favourable at temperature ≥15°C
Germ tube growth		Optimum approx. 10-15°C	
Penetration and formation of the sub-stomatal vesicle		Minimum 2°C Optimum 8-13°C Maximum 23°C	Light has little or no effect; high humidity necessary for at least 6 hrs at optimum temperature
Leaf (brown) rust			
Germination	Essential	Minimum >2°C Optimum 20°C (11-26°C) Maximum 32°C	Under some conditions light retards germination. High humidity necessary
Germ tube growth		Minimum 5°C Optimum 15-20°C Maximum 31°C	High humidity necessary
Appressorium formation Penetration and formation of the sub-stomatal vesicle		Optimum 20°C	High humidity necessary. No effect
Stem (Black) rust			
Germination	Necessary, dew for more than 2 hrs give high germination	Minimum 10°C Optimum 15-24°C Maximum 30°C	Intensities above $3.2x10^3$ lux hamper germination, which ceases at $11x10^3$ lux. Light inhibition at $4.3x10^3$ lux greater at 10°C than at 20°C
Germ tube growth, appressorium formation	Optimal with 10 hrs dew, 70% with 4 hrs dew	Optimum 20°C (16-27°C)	Slow drying in dark maintains viability of appressoria
Penetration and formation of the sub-stomatal vesicle	Dew necessary	Minimum 15°C Optimum 29°C Maximum 35°C	At least $5.5x10^3$ lux necessary

Multiplication of Rust Inoculum

Field collections of rust spores may not always be sufficient, either in volume or in the relative proportions of different biotypes for reliable nursery screening. Perhaps the best way of ensuring both the quantity and quality of collections is to supplement the inoculum gathered from the field with artificially multiplied spores. Various techniques have been developed to enable this to be carried out on a sufficiently large scale.

Seedling methods:

Clay pots and rectangular pans: Simple, traditional and known methods of multiplication of rust inoculum is to raise wheat seedlings in 4-inch clay pots (without any special treatment) in the greenhouse (Fig. 7). Between 12-15 seedlings are grown in each pot and only the first leaf is inoculated, and the inoculum is collected when sporulation reaches its maximum, usually after 10 to 14 days, under optimum conditions. This method requires a lot of glasshouse space and also doesn't produce sufficient inoculum.

Fig. 7. Raising brown rust inoculum on susceptible wheat seedlings in pots under glasshouse conditions

Perhaps a more efficient multiplication method involves growing seedlings in rectangular pans on aluminium bread pans/trays by which glasshouse space can be saved (Wilcoxson *et al.,* 1971). In a single pan/tray (11 x 4 x 3 inch size), one can grow 12 lines with six individual plants in each row (Fig. 8). The pan occupies about one third of the space required by the pots for growing the same number of plants and thus, nearly triples the available glasshouse space. In addition, sowing and inoculum collecting operations are considerably facilitated. However, this method is still useful only when small quantities of inoculum are required.

Fig. 8. Raising rust inoculum on susceptible wheat seedlings in rectangular pans under glasshouse

Using maleic hydrazide: Maleic hydrazide can be effectively used to enhance multiplication of *Puccinia graminis*, *P. recondita*, *P. striiformis* and *P. hordei* on seedlings (Joshi, 1965). This chemical is known to serve as growth inhibitor and also promotor of severity of many diseases like rusts and leaf spots. A concentration of 0.019 or 0.02% is applied to the soil when the primary leaf begins to emerge from the coleoptile. Pots so treated with this chemical are not watered 12 hrs before and 24 hrs after treatment. This treatment causes the suppression of second and third leaves and also leads to deeper pigmentation in the leaves. This methods of maintenance of cultures, apart from giving longer lease of life to the host and consequently to the rusts as well, removes the necessity of clipping off the uninoculated secondary leaves, which is necessary for proper maintenance of rust cultures in the glasshouse. Added to these advantages, there is manifold increase in the amount of inoculum. The amount of inoculum obtained from treated plants is 5-10 times more in comparison to the untreated control. This method has been found particularly useful for multiplication of nucleus inoculum or for the maintenance of single spore cultures of a race or biotype.

Detached leaf culture: This technique has also been used for maintenance and multiplication of rust culture in laboratory (Pillai and Wilcoxson, 1970). In this method, inoculated plants are kept in the glasshouse until flecks appears and then leaves are clipped off. The cut ends are immediately placed in a solution of 0.3 M sucrose and 40 ppm kinetin or 50 ppm benzimidazole, or about 2 parts sugar solution to 1 part kinetin or benzimidazole solution. The cultures are kept in laboratory at room temperature in winter months whereas during summer months they are kept in B.O.D. incubator with light arrangement at 23-25°C. In a few days, the leaves produce numerous uredospores (Fig. 9). Using this method, several spore crops can be taken from one set of leaves if cultures are carefully maintained. By detached leaf culture method, different races of stripe, leaf and stem rust can be cultured and maintained for a long time without any adverse effect. This techniques for culturing rust have many advantages, such as release of glasshouse space, easier maintenance of pure rust cultures and year-round rust studies can be planned.

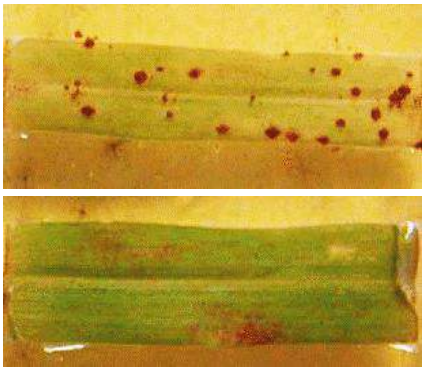

Fig. 9. Detached leaf culture of rust

Adult plant methods:

Raising inoculum on susceptible adult plants has been found to be considerably faster and most efficient method than the seedling methods. Normally 5-8 adult plants are grown in 10'' pots and are inoculated at either the tillering or the booting stage using uredospores suspension in water/soltrol oil or by dusting with a mixture of uredospores and talcum powder. The inoculated pots are kept in a greenhouse (Fig. 10) or plastic house / alkathene house and inoculum is collected periodically using a cyclone collector. It is estimated that, during one crop season, as much as 750-1000 grams of inoculum can be obtained from 700 pots. Moreover, these highly infected adult plants can be planted in between infector rows (discussed later), and it has been found that they form effective source of inoculum for creating epiphytotics.

Fig. 10. Raising rust inoculum on susceptible adult wheat plants under greenhouse conditions

Greenhouse space is frequently the limiting factor in the production of the large quantities of inoculum required for screening nurseries, due to high capital and maintenance costs involved. Studies have shown that considerably cheaper plastic house / alkathene house can be effectively be used in place of greenhouses if ventilation is adequate. Thus the multiplication of rust inoculum is possible even where physical facilities for research are somewhat lacking.

Collection of Rust Inoculum

There are a number of ways by which uredospores of stripe, leaf and stem rust may be collected for use in artificial inoculation work. Some of the effective methods of uredospores collection in use are given below:

Plant tapping collection:

Heavily rusted plants are tapped with a rod while they are held over either wax paper (Fig. 11), aluminium foil or even a funnel. This technique is successful where small amount of uredospores are to be collected. Same method is used in glasshouse for collecting uredomaterial from individual pots or from single spore cultures.

Fig. 11. Leaf rust inoculum collection on wax paper by rod tapping

Dried leaf collection:

Rusted leaves are removed from plants, placed in glassine envelopes, pressed and left to dry (Fig. 12). No more than six to eight leaves should be placed in each envelope so that drying is rapid and effective. Twenty-four hours will usually be sufficient for adequate drying at room temperature. This collection method is particularly useful in making field collections.

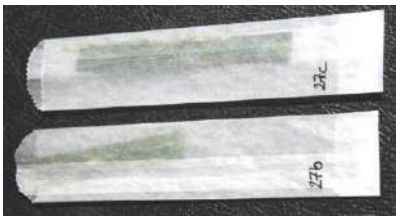

Fig. 12. Dried leaf collection in glassine envelopes

Cyclone collection:

The principal of cyclone spore collector was first described by Tarvet *et al.* (1951) and since then many models of the cyclone collectors are available, some for glasshouse and others for field collections. The detailed plans of these collections as described by Cherry and Peet (1966), Leath *et al.* (1966) and Browder (1968) are discussed here:

Cyclone collector for glasshouse work: These are small collectors for collecting uredospores into vials made from 5 mm borosilicate glass-tubing (Fig. 13). One can also collect spores in a gelatine capsule No. 100 fitted to the collector instead of glass vials. A collector designed by Leath *et al.* (1966) has been modified by Browder (1968) to facilitate rapid handling of cultures and to improve efficacy of spore collection. In

Fig. 13. Cyclone collector for rust inoculum in glasshouse

this collector, uredospores are impacted on the bottom of gelatine capsule or glass vials due to loss of velocity from downward cyclonic movement. The advantage of small cyclone collectors is that they immediately deposit the spores in the container, thus reducing handling time and risk of loss of material and also guard against possible contamination.

Cyclone collector for field operation: Mass collection of rust inoculum in the field can be done by using a cyclone spore collection. Details for this collector have been described by Cherry & Peet (1966). This collector has a pickup head which is placed over infected plants and the leaves are drawn into the collecting head (Fig. 14). Inside the head, leaves are agitated by air stream and most of the dry uredospores are removed from the rust pustules. Spores thus released are separated from the air as it passes through the cyclone and are quickly collected into the vials. A good tinsmith can make the collector locally from tin or brass.

Fig. 14. Cyclone collector for rust inoculum in field & spore collected

The collector requires a cadillac suction blower powered by either an electric or petrol generator. After collection, the inoculum is sieved in a 325 mesh sieve to remove dust particles and plant debris. Then the inoculum is dried in the laboratory at room temperature for few hours before storage.

Inoculation Techniques for Rusts

Greenhouse Inoculation Techniques:

Plants to be inoculated should be grown in good soil, under good light conditions, at suitable temperatures and in a rust-free environment. It is advisable to use both treated seed and soil to reduce the danger of root rots. Inoculation can be carried out when the seedlings have attained the appropriate stage or when the seedlings are between 5 and 10 cm tall.

All surface inoculation should be preceded by gentle rubbing of the leaf surfaces with a moistened finger or by spraying with a wetting agent (e.g. Tween 20). This helps to remove the outer waxy coating of the leaves and thereby increases the number of spores able to adhere to them. In addition, the leaves should be misted with distilled water both before and after inoculation and then placed in a moist atmosphere for 24 to 48 hours, in order to produce favourable moisture conditions for infection (Fig. 15.). The inoculum may be applied in one of the following ways:

Fig. 15. Fine water misting on inoculated plants

By spatula or lancet needle: this is one of the oldest methods and most convenient for inoculating single spore cultures or field samples. Before inoculation, seedlings are sprayed with water and gently rubbed to remove the thin layer of cuticular wax. In this manner the fine mist gets settled and surface run-off is minimum. By holding the infected leaves between the fingers and gently applying the needle from the lower end of the leaf upto the tip (Fig. 16). Generally black and brown rusts are inoculated on the dorsal side of the leaf whereas ventral side is preferred for yellow rust. This method is particularly suitable when the initial inoculum is scarce or when inoculations are to be made with spores from different pustule types in a mixed infection. During inoculation if greater pressure is exerted, the needle is likely to create leaf injury which may hinder the infection.

Fig. 16. Inoculation of rust with lancet needle

By toothpick: Another precise inoculation method (also suitable if inoculum is scarce) involves the use of a toothpick with a small piece of cotton at one end. The spores are picked up on the dry cotton, a small drop of water or light mineral oil is added and the cotton is rolled gently across the surface of the recipient seedling leaves (Fig. 17).

Fig. 17. Inoculation with toothpick

By finger rubbing: In this method, before inoculation seedlings are sprayed with water and gently rubbed to remove the thin layer of cuticular wax. In this way the fine mist gets settled and surface run-off is minimum. After cleaning the fingers, inoculum can be transfer gently by applying little pressure/rubbing from the lower end of the leaf upto the tip (Fig. 18).

Fig. 18. Inoculation by finger rubbing

Using a fine hair brush: Spores alone or mixed with talcum powder (if only small amounts of inoculum are available) are picked up on a fine camel hair brush and dusted over dry recipient seedlings or brushed directly onto the leaves (Fig. 19).

By pot brushing: This method is convenient for inoculating large numbers of plants when inoculum is abundant. A rusted plant is held close to a group of recipient plants and shaken gently to produce a uniform shower of spores (Fig. 20). Rusted plant is then brushed gently over the recipient plant surfaces several times to ensure adequate spore distribution.

Fig. 19. Inoculation using hair brush

Using a multiple inoculator: The multiple inoculator was developed by M.B. Moors at the University of Minnesota (Browder, 1972). It consists of a piece of sheet metal fastened to one finger tab of a large spring clamp (the type used in offices to hold thick stacks of papers) in which several "fingers", each tipped with a small piece of foam rubber, are held. Spore suspensions (in water or mineral oil) are placed on the sponges, which are then carefully pressed against the leaf surface. In this way it is possible simultaneously to inoculate single leaves with several different races or species of pathogen fairly rapidly.

Fig. 20. Inoculation by pot brushing

With a cyclone collector-duster: Small cyclone collector-dusters have proved valuable in making inoculations when inoculum is scarce. A glass cyclone (about 8 cm in length and 2 cm in diameter) is filled with 0.5 gram of talc. Spores are sucked from the source into the cyclone. An additional 0.5 gram of talc is added and the contents are thoroughly mixed. The mixture is then dusted onto the recipient plants by reversing the air flow through the cyclone.

Spray inoculation: When seedlings to be inoculated are more, it is cumbersome and time consuming to inoculate with lancet needle. In such situations spray inoculation is followed. For spray inoculation light weight non phytotoxic isoparaffinic oil (Soltrol or Pegasol) is used. The inoculum is suspended in a small quantity of oil and sprayed with the help of air blow pump (0.5 HP or so). With this method, to inoculate 20 pots, 2 ml inoculum suspension provides desirable infection. The suspension is automized with the help of a conical flask / hand atomizer (Fig. 21). When oil is not available, spore suspension in water with the help of little Tween 20 can be sprayed after removing the thin layer of cuticular wax. The inoculated pots/trays are sprayed with a fine mist of water and kept for 48 hrs in water saturated chambers. The inoculated materials are then transferred on to the glasshouse benches.

Fig. 21. Spray inoculation with hand automizer and pressure pack inoculation

Field Inoculation Techniques:

In the field-Inoculation methods on a field scale are rather different from those detailed above, as the objective is to create epidemic conditions in thousands of plants over a large area. Furthermore, there is no control over environmental conditions.

Fig. 22. Dusting spore-talc mixture in field

Dusting: One of the simplest and most effective ways of inoculating large numbers of plants in the field is with the use of a spore-talc mixture (Fig. 22). The ratio of spores to talc will depend upon factors such as effective cover, the quantity of inoculum available and the area to be inoculated. There are many different types and sizes of dusters available for applying inoculum in this way. Small hand dusters may be sufficient if it is only necessary to inoculate border rows; power-operated units are more suitable if the area to be inoculated is large. Dusting should always be carried out in the late evening, preferably just prior to dew formation, and when the air is still. This gives the best possible chance of achieving high levels of infection.

Fig. 23. Injecting rust inoculation into plant

Injection: A spore suspension in water with a little wetting agent (one drop of Tween 20 in 10 ml suspension) is used for injecting into the plant with the help of 2-5 ml hypodermic syringe (Fig. 23). The plants can be inoculated by injecting the portion just above the last existing node of the plant so that a drop of spore suspension appears at the top of the leaf whorl. Injecting 10-20% of the tillers in the border row produces desirable epiphytotic. While black and yellow rusts can be inoculated late at boot leaf stage, leaf rust should be inoculated before boot leaf emergence.

Fig. 24. Automatic B-D Cornwall syringe

Automatic syringe: The development of automatic syringes has done much to make the hypodermic syringe injection method more efficient. Automatic B-D Cornwall continuous pipetting syringes (Fig. 24) have been found quite effective in inoculating wheat plant at boot leaf stage or even in tillering stage with rust. In this type of syringe, a rubber tubing called "filling outfit" can be kept inside the container having bulk suspension of uredospores to give continuous supply of inoculum to the syringe at the time of inoculation. It has also an adjusting screw by which a measured quantity of spore suspension can be injected into the plant. This syringe is very easy to handle and saves lot of labour and time.

Fig. 25a. Spraying inoculum with hand sprayer, Fig. 25b. Battery operated ULV

Spray inoculation: Spore suspended in water with a little quantity of wetting agent can be sprayed on plants of any growth stage. If available, the use of non-phytotoxic iso-paraffinic oils is very effective. An automizer/ hand sprayer can be used for spraying the inoculum (Fig. 25a). Subsequently the plants are sprayed with a fine mist of water. Since these inoculations are done during the evening, the conditions remain favourable for infection. However, irrigating the field after inoculations helps in getting the desirable dew. At few places covering with moist cotton cloth or polythene sheet and intermittent spraying is also done to have good field epiphytotic of rusts. A battery powered ultra low-volume sprayer (ULVA) can also be used (Fig. 25b). As the spores disperse readily and uniformly in oil/wetting agent, a relatively small spore concentration can be used and a good cover still be obtained. This method has been found to be effective and its simplicity recommends it where large scale inoculations are necessary.

Susceptible infector row: In this method, the test plots are surrounded by susceptible border (infector) rows of a mixture of varieties known to be highly susceptible to one or two rusts such as Agra local, Kharchia, Kathia local, A-9-30-1, Lal Bahadur, NP 824 or any varieties which have become susceptible to new virulence in recent years. Usually these border rows are planted 2-3 weeks before the experimental material. Three sowings at a gap of 15-20 days are done. These border (infector) rows are inoculated to create a desirable infection (Fig. 26).

Fig. 26. Susceptible border (infector) rows of a mixture of varieties

Fig. 27. Rusted pot in between the border (infector) row

Spreader pots: Heavily rusted plants (grown in pots) are also transplanted in between the border (infector) rows (Fig. 27) and the field is irrigated after transplanting in order to ensure good establishment of the plants (Joshi, 1968). The transplanted infected plants, survive for 3-4 weeks and serve as a continuous source of inoculum to the experimental material.

Isolation of Single Spore/Pustule

This is usually done whenever a sample shows infection types different to those exhibited by already known pathotypes, it is designated as new pathotype. The plant showing characteristics differentiating infection type (resistance or susceptible) on the differentiating tester is selected and rest of the plants are clipped off. Since a distinct pustule is theoretically the result of single spore infection, it is considered to be the purest. Therefore, only one pustule of desirable reaction types is kept. This pustule is sprayed with fine mist of water for three consecutive days. It helps in washing down/germination of spores lying on the surface. On fourth day using a hand lens a single pustule (microscope for single spore), is transferred with a fine glass needle from a slide to a leaf of a susceptible wheat cultivar (Fig. 28). The inoculated seedlings are placed on dew chamber at 10°C for 24 hrs and then isolation booth within a growth chamber to multiply well. When sufficient inoculum is available, it can be used for races analysis or other purposes.

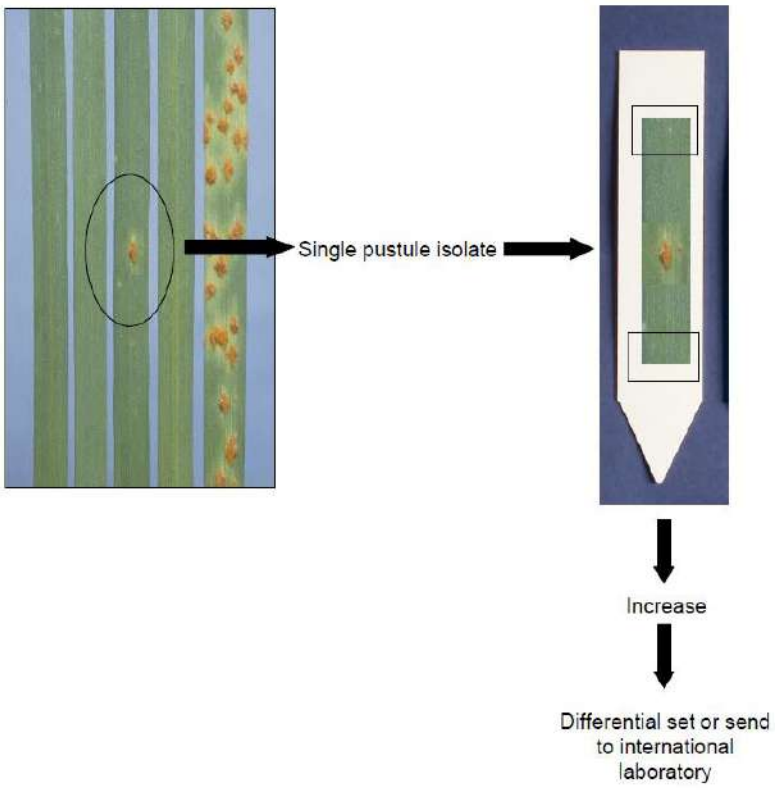

Fig. 28. Procedure for isolation of single pustule

Single spore isolation is done to maintain the purity of rust pathotypes. Single spore culture of many different pathotypes of all the three rusts are being maintained *in vivo* at ICAR-Indian Institute of Wheat & Barley Research, Regional Station, Flowerdale, Shimla.

Maintenance of Rust Cultures in Glasshouse

To prevent the movement of rust spores into/out of glasshouse, a double layered muslin is fixed as false ceiling in every glasshouse. Also, provision for a chimney at the top (for the exit of hot air), cooling and heating is kept. All the exhausts or inlets of fans are also covered with a double-layer of muslin. The entrance of every glasshouse is provided with trap doors to avoid the gush of air at the time of opening/closing of glasshouse door. Compartmentation of glasshouse is also done to facilitate working. The table/bench for inoculation with wash basin are also provided at the entrance. The cultures on the glasshouse benches are separated from each other with the help of a wooden and muslin screen.

For the control of powdery mildew in the glasshouse, a fine dust of ground elemental sulphur is sprinkled on the plants in pots/trays. Sulphur dust does not interfere in the expression of rust symptoms which is observed at the times with Karathane application. Also, old plants should be discarded so that these do not act as a source of powdery mildew. In adult plant local application of sulphur is found effective to control powdery mildew. Aphids can be very easily controlled either by killing manually or by spraying Nuvan (Dichlorvos 76% EC) @ 0.05% on the plants having infestation. Every possible care is always taken to avoid the spread of rust inoculum/infected planting materials from glasshouse. All the infected plants parts are cut and dipped in Lysol (4%) solution for 48 hrs for killing the rust inoculum inside the glasshouse.

Storage of Rust Inoculum

Rust inoculum is stored by different methods so that it could be effectively used when required. This inoculum is used for different purpose *viz.,* genetic analysis, genome sequencing, field and glasshouse screening of wheat & barley germplasm for rust resistance etc. The temperature, moisture and light are the main factors affecting the viability of rust spores. Drying of uredospores and storing at low temperature are the best means for spore preservation. When atmospheric humidity is more *i.e.* during rainy and cloudy days, the spores can be dried in Calcium Chloride or Silica Gel for 24-48 hrs or these can be dried at room temperature on less humid days.

Fig. 29. Storage of rust inoculum in Aluminium pouches and sealed

Simple way to store spores is at 2-4°C in a refrigerator. The inoculum thus stored remains viable for 1-12 months depending on drying and types of rusts. Yellow rust is more sensitive to temperature as compared to brown and black rusts. The inoculum can be stored using Aluminium foil pouches / Tin foil pouches which are kept at ultra-low temperature (-80°C). The dried inoculum in a small piece of butter paper is put in Tin Foil pouch and sealed (Fig. 29). These sealed Tin Foil pouches are kept freezers. The inoculum thus stored can be reactivated by giving heat shock in hot water bath at 40°C for 10 minutes. The inoculum so stored was found viable even after 5 years. It is also known that inoculum stored at this low temperature remains viable for >15 years. Stored inoculum after treatment can be used immediately or kept in refrigerator (2-4°C) for 1-3 moths for subsequent use.

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Pathotype (Race) Analysis of Wheat Rust Pathogens

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Rust diseases represent the most economically significant fungal diseases in wheat and other cereal crops worldwide. These are shifty pathogens and contain enormous variation to make promising wheat cultivars susceptible. The three rust diseases affecting wheat are stripe or yellow rust, leaf or brown rust and stem or black rust. Information concerning occurrence of where rusts is important, but the evidence on which pathotypes are present and at what frequency is vital for breeding rust resistance varieties and cultivar recommendation. Hence, identification of new pathotypes of wheat rust pathogens in initial stages is of utmost importance. The only way to determine virulence combinations and which specific pathotype(s) are present is to undertake seedling assays (race analysis) under controlled conditions, using an established composition of sets of differential. A brief overview of the general procedure used for race analysis is given below:

Procedures currently being used for Identification of Wheat Rusts Pathotypes in India

- The rust infected samples collected for field are dried for overnight in shade at room temperature in order to remove excess surface moisture. After drying, samples are wrapped in newspaper or kept coarse paper or glassine envelopes envelopes.
- Rust spores are transferred from infected tissue onto healthy leaves of a seedling of susceptible wheat cultivar. The inoculated seedlings are placed on dew chamber at 10°C for 24 hrs and then isolation booth within a growth chamber to multiply well.
- When sufficient inoculum is available, the fresh inoculum is then use to inoculate the differential sets (such rust differential sets are specific for each rust, see Table 1). For this bulk inocula is taken from the leaves. If it happens to be mixture of more than one pathotype then further isolations (single pustules isolations) are taken and it is purified. Use of single pustule isolates increases the chance of working with a single race. For single pustule isolations, spores are sub-cultured and multiplied again on the susceptible host. After multiplication, a differential set is used for inoculation.
- After development of rust pustules, seedlings reaction on the differential set are recorded using a standard 0-4 evaluation scale (Fig. 2 and Table 2). Low infection types (0-2) are considered as Resistant (R) and high infection types (3-4) as Susceptible (S). Races are identified based on the combination of virulence/avirulence patterns exhibited. These reactions have been further coded to the following binary notation:

Pustule	Reaction	Binary number
0;	R	0
;-	R	0
;	R	0
1	R	0
2	R	0
3	S	1
33+	S	1
3+/4	S	1
X	S	1

- Using binary number and decanary value the nomenclature of a virulence is given. The decanary procedure follows raising a number to the base 2, and total of no two numbers can be identical. Hence, a set which consists of 9 entries would have a decanary from 2^0-2^8, and decoded value from 1-256, as illustrated below*.

- When an isolate of leaf rust is tested on the *Lr* lines (genes), the Resistant reaction (R) is given the notation as '0' and Susceptible reaction (S) is denoted by notation as '1' following the binary notation or sequential value. Based on the virulence value ('1' for Susceptibility and '0' for Resistance) on Set-'A' and Set-'B', a pathotype is designated on the basis of binomial system of Habgood (1970). When distinguishing feature is available on the line in Set-'0' then it is suffixed to the value e.g. leaf rust pathotypes 77-5(121R63-1) gets value 121 on the basis of susceptible differentials in Set-'A' (exemplified below*) whereas 63 is the value on Set-'B'. The value -1 means that line at serial number 1 in Set-'0' is susceptible. The value of Set-'A' and Set-'B' is separated by capital alphabet G for black rust which denotes that pathotype is of *graminis* e.g. 62G29. Similarly in case of brown rust alphabet R is used to denote that pathotype is of *recondita* (old name for *triticina*) and for yellow rust alphabet S is used for *striiformis*.

*__Procedure for coding and decoding of rust pathotypes [e.g. Leaf rust pt. 77-5(121R63-1)]__

Details	***Lr* genes**								
	*Lr*14a	*Lr*24	*Lr*18	*Lr*13	*Lr*17	*Lr*15	*Lr*10	*Lr*19	*Lr*28
Decanary value	2^0	2^1	2^2	2^3	2^4	2^5	2^6	2^7	2^8
Decoded decanary value	1	2	4	8	16	32	64	128	256
Reaction of pt. 77-5	S	R	R	S	S	S	S	R	R
Binary value	1	0	1	1	1	1	1	0	0
Decanary value of pt. on set A	1	0	0	8	16	32	64	0	0
Summary of decanary value=1+8+16+32+64=121									

Table 1: The revised composition of sets of differentials used for the identification of wheat rust pathotypes in India

Set-0		**Set-A**	**Set-B**
Leaf or Brown rust (*Puccinia triticina*)			
IWP 94 (*Lr*23+)		*Lr*14a	Loros (*Lr*2c)
Kharchia Mutant		*Lr*24	Webster (*Lr*2a)
Raj 3765		*Lr*18	Democrat (Lr3)
PBW 343		*Lr*13	Thew (*Lr*20)
UP 2338		*Lr*17	Malakoff (*Lr*1)
K 8804		*Lr*15	Malakoff (*Lr*1)
Raj 1555		*Lr*10	Benno (*Lr*26)
HD 2189		*Lr*19	HP1633 (*Lr*9+)
Agra Local		*Lr*19	
Stem or Black rust (*Puccinia graminis tritici*)			
*Sr*24		*Sr*13	Marquis (*Sr*7b+)
NI 5439		*Sr*9b	Einkorn (*Sr*21+)
*Sr*25		*Sr*11	Kota (*Sr*28+)
DWR 195		*Sr*28	Reliance (*Sr*5+)
HD 2189		*Sr*8b	Charter (*Sr*11+)
Lok 1		*Sr*9e	Khapli (*Sr*7a+)
HI 1077		*Sr*30	Tc*6/*Lr26* (*Yr*9)
Barley Local		*Sr*37	
Agra Local			
Stripe or Yellow rust (*Puccinia striiformis tritici*)			
WHEAT	BARLEY		
WH 147	TDT	Chinese 166 (*Yr*1)	Hybrid 46 (*Yr*4)
Barley local	Barley local	Lee (*Yr*7)	Heines VII (*Yr*2+)
WH 416	WH147	Heines Kolben (*Yr*6)	Compair (*Yr*8)
PDW 215	Heils Franken	Vilmorin 23(*Yr3*)	*T. spelta album* (*Yr*5)
HD 2329	Fong Tien	Moro (*Yr*10)	Tc*6/*Lr26* (*Yr*9)
HD 2667	Himani	Strubes Dickkopf	Sonalika (*Yr2+*)
PBW 343	BHS 16	Suwon92 X Omar	Kalyansona *Yr2*(KS)
HS 240	Alfa 93	Riebesel47/51(*Yr*9+)	
Anza	Dolma		

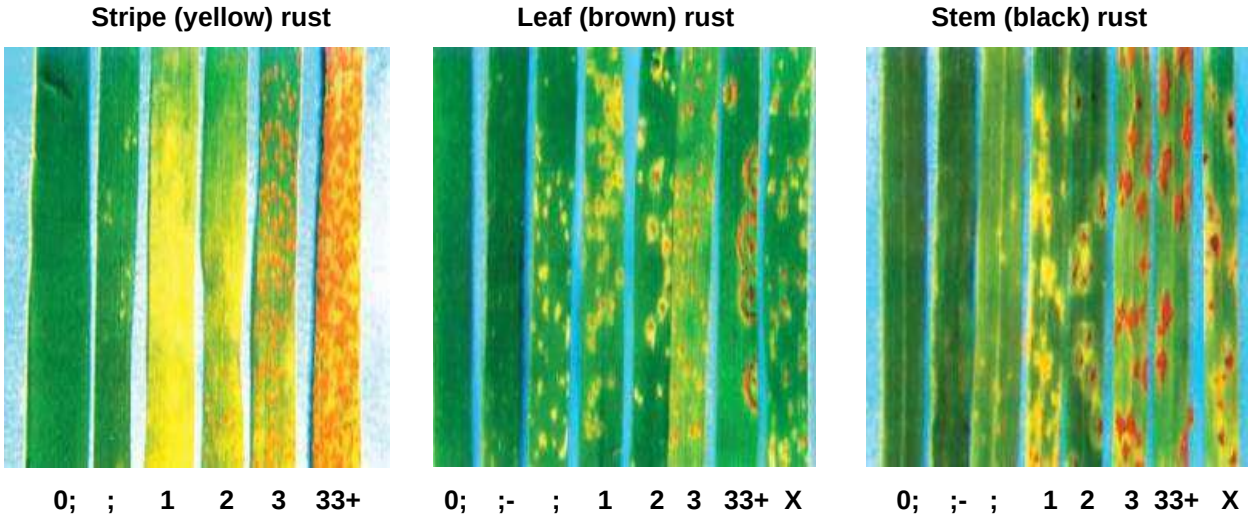

Fig. 2. Different Infection Types (ITs) produced by three Rusts of Wheat

Table 2: Description of different Infection Types (ITs) classes for rusts

Reaction Type/Grade	Category	Visible Symptoms
0; (naught fleck)	Immune	No visible infection
;- (fleck minus)	Nearly Immune	Slight necrosis / micro flecking visible
; (fleck)	Very Resistant	No uredia but hypersensitive flecks present
1 (one)	Very Resistant	Uredia minute, surrounded by distinct necrotic areas
2 (two)	Moderately Resistant	Uredia small to medium, surrounded by chlorotic (in case of black rust) or necrotic border
3 (three)	Moderately Susceptible	Uredia small to medium size, chlorotic areas may be present. In case of black rust coalescence infrequent
33+ (three three plus)	Susceptible	In black rust uredia medium to large coalescing, no necrosis but chlorosis may be present. In brown rust no chlorosis or necrosis, uredia profusely sporulating. In yellow rust uredia profusely sporulating
3+/4 (three plus/four)	Highly Susceptible	In black rust uredia large, coalescing, no necrosis but chlorosis may be present. In brown rust no chlorosis or necrosis, uredia profusely sporulating, rings may be formed. In yellow rust uredia profusely sporulating and form stripes in adult plants
X	Heterogeneous	Variable types of uredia
Y	Heterogeneous	Susceptible types of uredia at the tip and resistant towards the base of leaf
Z	Heterogeneous	Resistant types of uredia at the tip and susceptible types towards the leaf base

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Concepts of Rust Resistance in Wheat

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Introduction

Rusts remain the most important and economically significant fungal diseases of wheat & other cereal crops worldwide because of their wide distribution, their capacity to form new races that can attack previously resistant cultivars, their ability to move long distances and their potential to develop rapidly under optimal environmental conditions that result in serious crop yield losses. The three rust diseases affecting wheat crop are leaf (brown) rust, stem (black) rust, and stripe (yellow) rust.

Among the various strategies to combat with this disease, host resistance is the only efficient, economic and environment-friendly approach to eliminate the use of fungicides and minimize the losses in wheat from attack by rust pathogens (Singh *et al.,* 2004; Spielmeyer *et al.,* 2005). With the discovery of the genetic basis of resistance by Biffen (1905), physiological specialization in rust pathogens by Stakman and Levine (1962) and gene-for-gene interaction by Flor (1956), the utilization of the hypersensitive (race-specific) type of resistance has dominated in wheat improvement. This approach appeared to be very attractive from the crop cleanliness point of view and because it is simple to incorporate into improved germplasm. The phenomenon of the erosion of such genes, or their combinations, led scientists to look for alternative approaches to resistance management.

The multilineal approach promoted by Jensen (1952) and Borlaug (1953) emerged out of the frustrations associated with the frequent failures of race-specific genes. Van der Plank (1963) was the first epidemiologist to clearly define the theoretical basis of concepts of resistance. In the late 1960s and 1970s, there was a revival of the concept of general (race-nonspecific) resistance and its application in crop improvement (Caldwell, 1968). This approach was widely used for breeding stem rust resistance in wheat by Borlaug (1972), leaf rust resistance by Caldwell (1968) and yellow rust resistance by Johnson (1988).

Gene for Gene Interaction

Flor (1942) was first to study both the inheritance of pathogenicity and host response. Working with flax rust (*Melampsora lini* Desm.) and its host, flax (*Linum usitatissimum*). In his initial studies he dealt with a gene pair in the pathogen corresponding to a gene pair in host- the corresponding gene pairs (Flor, 1946,1947). Based on these studies he developed gene-for-gene hypothesis (Flor,1971). The hypothesis was further conceptualized by Person *et al*. (1962). They mentioned that " A gene for gene relationship exists when the presence of a gene in one population is contingent on the continued presence of a gene in another population and where the interaction between the two genes leads to a single phenotypic expression by which the presence or absence of the relevant gene in either organism may be recognized". Gene-for-gene systems have evolved through a series of steps in evolution of each. Therefore, if a host is resistant to pathogen, a virulent mutant would have an advantage over avirulent. Likewise, if a host is susceptible to a pathogen, a resistant mutant in host would be at advantage. So, the process continues and during the course of evolution resistant host and virulent pathogen continue to strive for the race of supremacy. Genetic analyses have indicated that resistance to rusts is controlled generally by dominant genes and virulence in pathogen is due to recessive genes (or avirulence is controlled by dominant genes). Resistance and avirulence are dominant and they result from active gene product from avirulent pathogen.

Incompatibility (resistance) is a recognition process which results from a resistant host and avirulent pathogen. Infection type produced as a result of pathogen attacking a host is a product of two genetic systems. For a single pair of genes (assuming that homozygotes occur). The interaction can be illustrated as given in Table 1.

Table 1: Gene for gene relationship

Pathogen	Host	
	RR	Rr
PP	Resistant	Susceptible
Pp	Susceptible	Susceptible

Only one set, RR/PP, results in resistant (low infection type). The host carries a gene for resistance for which the pathogen does not carry the corresponding gene for virulence. There occurs specificity in combinations involving the genes that are normally dominant, R and P. Any one of the three other combinations, RR/pp, rr/PP, rr/pp result in susceptibility or compatible reaction.

Types of Resistance in Wheat

Two distinct classes of genetic resistance differing in mechanism of operation and their epidemiological consequences are described by Vanderplank (1963). In addition, related nonhost species are gradually being utilised to identify new sources of resistance referred as 'Non-host resistance' (Bettgenhaeuser *et al.,* 2014; Kawashima *et al.,* 2016).

Race-specific resistance genes are effective against some but not all races of a rust pathogen and generally conform to the classical gene-for-gene model, where resistance depends on a specific genetic interaction between host-resistance (*R*) genes and pathogen avirulence (*Avr*) genes. Many race-specific rust resistant genes have been defined genetically and catalogued by McIntosh *et al*. (1995). Most of these genes can be detected in seedling evaluations using specific pathotypes. However, detection of a few others requires testing at post-seedling growth stages. Race-specific resistance is last very rapidly due to evolution of new virulence by the pathogens especially, when a single *R*-gene is deployed over large area (McIntosh *et al.,* 1998; Wan and Chen, 2012).

Race non-specific resistance is mainly polygenic, effective at the adult plant stage and operate against all pathotypes or races of a pathogen. The genetic nature of this type of rust resistance is usually complex and based on the additive interaction of a few or several genes having minor to intermediate effects. This type of resistance has often been described as slow rusting or partial resistance or adult plant resistance and is known to be long lasting and more durable (Singh *et al.,* 2004; Herrera-Foessel *et al.,* 2011). Slow rusting and partial resistances are almost synonymous terms. As defined by Caldwell (1968), slow rusting is a type of resistance where disease progresses at a retarded rate, resulting in intermediate to low disease levels against all pathotypes of a pathogen. Partial resistance, as defined by Parlevliet (1975) referring to leaf rust resistance in barley, is a form of incomplete resistance characterized by a reduced rate of epidemic development despite a high or susceptible infection type. The components that cause slow rusting of a cultivar are longer latent period, low receptivity or infection frequency, as well as smaller uredial size and reduced duration and quantity of spore production. All these components can affect disease progress in the field. Durable resistance, as defined by Johnson (1988), is that which has remained effective in a cultivar during its widespread cultivation for a long sequence of generations or period of time in an environment favourable to a disease or pest.

Non-Host Resistance is the most common and most effective form defence, which renders most of microbes incapable of attacking plants. They are termed as 'non-pathogen' and the plant as 'non-host'. The plant is totally exempt from infection and phenomenon is called 'immunity'. Rust fungi usually have narrow host ranges and poor ability to infect related nonhost species. Nonhost resistance (NHR), exhibited by plant species that do not support full infection by a non-adopted pathogen, offers promise as a source of new genes for crop protection (Bettgenhaeuser *et al.,* 2014). NHR can result from basic incompatibility when the non-adapted pathogen fails to recognize plant physical and chemical signals necessary for infection. For instance, the wheat stripe rust fungus *P. striiformis* f. sp. *tritici* shows a reduced ability to locate stomata in broad bean (*Vicia faba*) (Cheng *et al.,* 2012) and the flax rust fungus *M. lini* rarely penetrates rice stomata (Ayliffe *et al.,* 2011). In other cases, NHR occurs as a post penetration event. For instance, *Hemileia vastatrix*, the causal agent of coffee leaf rust, can successfully invade *Arabidopsis thaliana* leaves via stomata but fails to develop haustoria (Azinheira *et al.,* 2010), while rice exhibits post haustorial resistance when inoculated with various cereal rust pathogens (*Puccinia* sp.) (Bettgenhaeuser *et al.,* 2014; Ayliffe *et al.,* 2011).

Mechanisms (Components) of Slow Rusting / Adult Plant Resistance

Slow disease progress in the field due to:

- ✓ Reduced infection frequency
- ✓ Lower Rust Severity, CI, AUDPC & *r*
- ✓ Increased latent period
- ✓ Smaller uredia or smaller infection area
- ✓ Reduced spore production

Some Examples of Slow Rusting / Adult Plant Resistance Genes

- The four catalogued genes confer resistance to multiple pathogens

  *Yr*18/*Lr*34/*Sr*57/*Pm*38/*Bs* : 7DS

  *Yr*29/*Lr*46/*Sr*58/*Pm*39 : 1BL

  *Yr*30/*Sr*2/*Pm*? : 3BS

  *Yr*46/*Lr*67/*Sr*?/*Pm*? : 4DL
- *Lr*68*:* a new slow rusting gene in chromosome arm 7BL
- *Pseudo black-chaff* linked to *Sr*2 Complex (*Sr2*/*Yr*20/*Lr*27): a slow rusting gene in chromosome arm 7BL for Ug99
- Leaf tip necrosis (LTN) linked to slow rusting

*Sr*2 Gene Complex linked to Pseudo black-chaff

Sr2 transferred to wheat from 'Yaroslav' emmer in 1920s by McFadden and linked to Pseudo-black chaff phenotype (~ 90years)

PBC expression on visual observation using a 0-4 scale

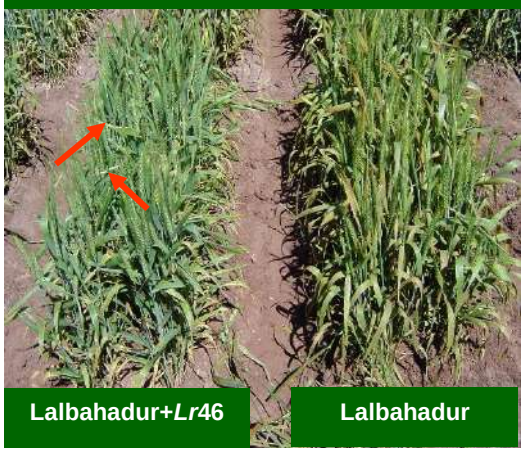
0 = no pigmentation

4 = high level of PBC

Leaf tip necrosis and Slow rusting resistance

- *Lr34/Yr18/Pm38*, *Lr46/Yr29/Pm39* and *Lr67/Yr46/Pm?* linked to some level of leaf tip necrosis expression
- Slow rusting resistance without leaf tip necrosis also known

Leaf tip necrosis associated with *Lr*46



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Screening & Phenotyping Seedling and Adult Plant Resistance to Rusts in Wheat

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The three rust diseases (stripe, leaf and stem) occur almost everywhere in the wheat growing areas and cause significant losses. Genetic resistance is the only efficient, economic and environment friendly approach to minimize the losses due to rusts in wheat. Screening wheat genotypes/germplasm for rust resistance is a continuous process to identify new effective sources and further their utilization in the rust resistance breeding programme for development of more durable resistance wheat varieties to combat with rust pathogens. Wheat genotypes/germplasm are screened/evaluated against rusts for assessment of their resistance at seedling stage under glasshouse as well as adult plant stages under field conditions. Field evaluation of disease screening nurseries is also undertaken at various hot-spot locations and under artificially inoculated conditions across the country.

Seedling Resistance Test (SRT) and Characterization of Resistance Genes

Screening of wheat germplasm at seedling stage is the fastest and reliable methods for selecting the resistant genotypes/germplasm against rust pathogens. A large number of pre-breeding/advanced lines/exotic and other materials of wheat are screened at seedling stage for their resistance against various pathotypes of all the thee rust pathogens.

Seedling Resistance Test (SRT):

The seedlings are raised in aluminium trays (11x4x3 inch) in uniformly fertilized soil as mentioned in previous chapter. Each tray contains 18 lines hill planted in a row with 7th line as susceptible check. Each testing material is accompanied by a set of differentials which acts as a check for the purity of pathotypes and response of common resistance genes under the same set of conditions. Optimum conditions required for rust mentioned in previous chapter are followed and maintained. Ten days old seedlings are used for inoculation with uredospore suspension of the pathotype prepared by mixing spore dust with a few drops of water and a pinch of tween-20 to break the surface tension. Adequate water is added to make the suspension of spore and filled in a glass atomizer and sprayed uniformly on the seedlings. Inoculated seedlings are sprayed with a thin mist of water and kept in moist chambers for 48 hrs for incubation at 16-18˚C temp, 100% relative humidity and 12 hrs daylight. The seedlings are then transferred to greenhouse benches and kept at 16±2˚C temp in relative humidity of 80-90%, and illuminated at about 15,000 lux for 12 hrs. Under optimum conditions clear visible host-pathogen interaction is expressed in 12-15 days after inoculation. After 12-15 days of inoculation, Infection types (ITs) on the test lines are recorded using a standard 0-4 evaluation scale (Fig. 1 and Table 1).

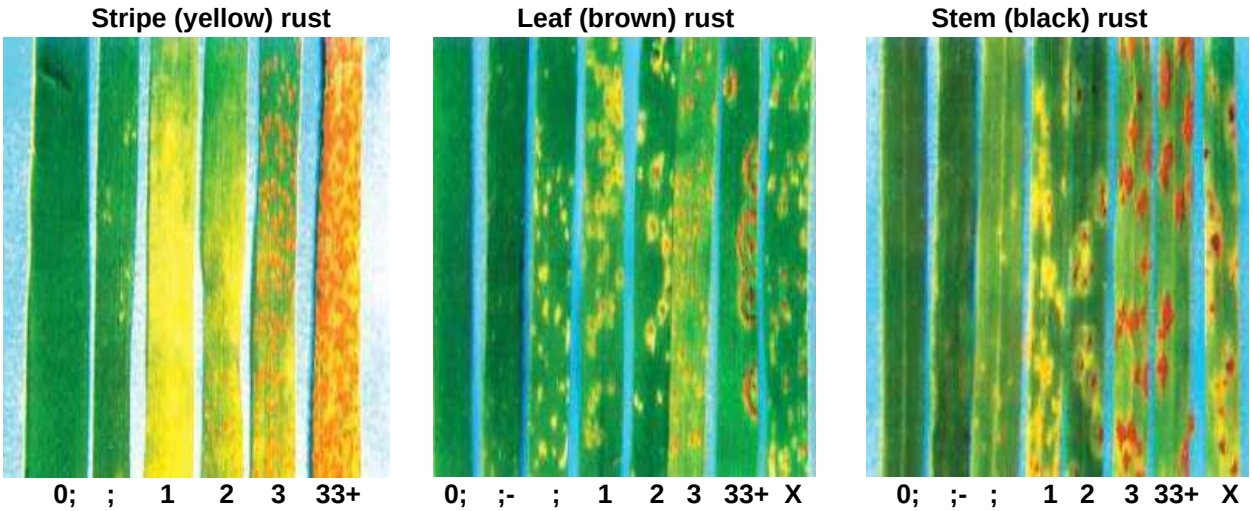

Fig. 1. Different Infection Types (ITs) produced by three Rusts of Wheat

Table 1: Description of different Infection Types (ITs) classes for rusts

Reaction Type/Grade	Category	Visible Symptoms
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; (fleck)	Very Resistant	No uredia but hypersensitive flecks present
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2 (two)	Moderately Resistant	Uredia small to medium, surrounded by chlorotic (in case of black rust) or necrotic border
3 (three)	Moderately Susceptible	Uredia small to medium size, chlorotic areas may be present. In case of black rust coalescence infrequent
33+ (three three plus)	Susceptible	In black rust uredia medium to large coalescing, no necrosis but chlorosis may be present. In brown rust no chlorosis or necrosis, uredia profusely sporulating. In yellow rust uredia profusely sporulating
3+/4 (three plus/four)	Highly Susceptible	In black rust uredia large, coalescing, no necrosis but chlorosis may be present. In brown rust no chlorosis or necrosis, uredia profusely sporulating, rings may be formed. In yellow rust uredia profusely sporulating and form stripes in adult plants
X	Heterogeneous	Variable types of uredia
Y	Heterogeneous	Susceptible types of uredia at the tip and resistant towards the base of leaf
Z	Heterogeneous	Resistant types of uredia at the tip and susceptible types towards the leaf base

Characterization of Seedling Rust Resistance Gene(s):

Reaction of wheat genotypes/lines to different rust pathotypes are considered for selecting the resistant lines for use in rust resistance breeding programme or evaluation for agronomic suitability. The presence of rust resistance genes (*R*-genes) can be assumed by comparing the reaction / Infection Type (IT) patterns of the pathotype array on test genotypes/lines with those of controls with known resistance genes (Browder 1973). A high IT on test line showed that the genotype did not possess a resistance gene(s) for which existing pathotype are avirulent. Additional information, such as the genetic linkage between the different resistance genes, characteristic infection types and phenotypic marker are also used to infer the presence of some of the resistance genes. An illustration of postulation of leaf rust resistant genes is given in Table 2.

Table 2: Illustration of postulation of *Lr*1, *Lr*13, *Lr*23 and *Lr*26

R-gene	12A 5R13	49R37 12-3	77 45R31	77-1 109R63	77-4 125R23-1	77-5 121R63-1	104-1 21R31-1	104-2 21R55	107-1 45R35
*Lr*1	0;	0;	3+	3+	3+	3+	3+	3+	;-
*Lr*13	X+3	33+	33+	3+	3+	3+	X	X	X+3+
*Lr*23	3+	X	;1	12+	3+	3+	3+	3+	;
*Lr*26	0;	3+	0;	3+	0;	3+	;-	3+	3+
*Lr*26+23 (K 8804)	0;	0;	0;	1	0;	33+	0;	33+	0;
*Lr*13+1 (HS 369)	0;	0;	3-	33+	3+	3+	X	X	0;
*Lr*26+1 (VL 759)	0;	0;	0;	3+	0;	3+	0;	3+	0;

Procedure for Postulation / Gene matching technique: Details of the procedure for postulation are given by Nayar *et al.* (1994). For application of gene matching technique following points must be considered:

- Differential host response is required.
- Reactions of the lines should be equal to or less than that of resistant gene(s). higher infection type (IT) rules out the possibility of the presence of that resistant gene.
- It is presumed that when more than one resistant genes are postulated, the effect is additive.
- It is assumed that largely background of hosts will not interfere with the expression of resistant gene.
- The linked traits can also be used as supplementary evidences.

This information can effectively be utilized in recommending cultivars with different resistant genes for deployment in different areas based on the pathotype distribution.

Adult Plant Resistance (APR) Evaluation

Many of the resistant factors express only at adult plant stage, therefore, it is also important to evaluate the wheat genotypes/germplasm at adult plant stage. The adult plant screening can be done under glasshouse, by multilocation testing and in the field.

Glasshouse Screening of Adult Plant Resistance:

Five seedlings of each genotypes/germplasm/variety/test lines are raised in plastic pots of 9 inch diameter separately. Optimum conditions for growth are provided as mentioned in previous chapter. When plants are about at Boot leaf stage, sowing of the same lines are done to obtain the seedlings for observing the comparative response on the seedlings as well as adult plants of the lines to be tested alongwith the suitable checks. Both seedlings as well as adult plants of the lines to be tested alongwith the suitable checks. Both seedlings as well as adult plants are spray inoculated with same inoculum suspension in light weight mineral oil (as exemplified in previous chapter) with the help of a pump. The inoculated pots are kept in humidity chambers especially designed for this purpose with the help of cotton cloth. Humidity is maintained with the help of mist mats. After 48 hrs of incubation plants are shifted on to glasshouse benches. The comparative host-pathogen interactions are recorded after a fortnight. Based on the comparative information of seedling response and adult plant response, adult plant resistance can be identified. Nayar *et al.* (1993) has given the detailed methodology for this type of studies.

Multilocation Screening of Adult plant resistance in different disease 'Hot-Spot':

Multilocation testing of wheat genotypes/breeding materials in different disease 'hot-spot' location is the only effective method to evaluate for adult plant resistance under field conditions. To avoid escapes and have precise information, relevant & predominant pathotypes are mixed to create artificial epiphytotics. With this aim in view of wheat germplasm/lines/entries are screened in "Plant Pathological Screening Nurseries (PPSN)" of AICRPW&B (ICAR-IIWBR, Karnal) in different locations across the country where a particular disease(s) appears under natural condition in epidemic form. These nurseries include entries from advance wheat trials. These help in identifying cultivars with multiple disease resistance and resistance to many of the prevalent pathotypes in the field. The resistant material from PPSN is selected to form elite PPSN which is planted at many locations so that wheat breeders may use the selected disease resistant lines in breeding programme. On similar pattern, "Preliminary Disease Screening Nursery (PDSN)" of ICAR-IARI wheat breeders are also screened for adult plant resistance against all the three rust different hot spot locations across the country.

Field Screening of Adult Plant Resistance (Slow Rusting Resistance):

Filed screening may comprise of natural infection or artificial inoculations. As rust infection is dependent on meteorological conditions, artificial epiphytotics are created with known pathotypes in order to ensure desirable rust infection. Various methods for creating artificial epiphytotic conditions are given below:

Injection method: Inoculum suspension in water with a little wetting agent (one drop of Tween 20 in 10 ml suspension) is used for injecting into the plant with the help of 2-5 ml hypodermic syringe. The plants can be inoculated by injecting the portion just above the last existing node of the plant so that a drop of spore suspension appears at the top of the leaf whorl. Injecting 10-20% of the tillers in the border row produces desirable epiphytotic. While black and yellow rusts can be inoculated late at boot leaf stage, leaf rust should be inoculated before emergence of boot leaf.

Spraying: Spore suspended in water with a little quantity of wetting agent can be sprayed on plants of any growth stage. If available, the use of non-phytotoxic iso-paraffinic oils is very effective. An automizer/ hand sprayer can be used for spraying the inoculum. Subsequently the plants are sprayed with a fine mist of water. Since these inoculations are done during the evening, the conditions remain favourable for infection. However, irrigating the field after inoculations helps in getting the desirable dew. At few places covering with moist cotton cloth or polythene sheet and intermittent spraying is also done to have good field epiphytotic of rusts. A battery powered ultra low-volume sprayer (ULVA) can also be used. As the spores disperse readily and uniformly in oil/wetting agent, a relatively small spore concentration can be used and a good cover still be obtained. This method has been found to be effective and its simplicity recommends it where large scale inoculations are necessary.

Susceptible infector row: In this method, the test plots are surrounded by susceptible border (infector) rows of a mixture of varieties known to be highly susceptible to one or two rusts such as Agra local, Kharchia, Kathia local, A-9-30-1, Lal Bahadur, NP 824 or any varieties which have become susceptible to new virulence in recent years. Usually these border rows are planted 2-3 weeks before the experimental

material. Three sowings at a gap of 15-20 days are done. These border (infector) rows are inoculated to create a desirable infection.

Spreader pots: Heavily rusted plants (grown in pots) are also transplanted in between the border (infector) rows and the field is irrigated after transplanting in order to ensure good establishment of the plants (Joshi, 1968). The transplanted infected plants, survive for 3-4 weeks and serve as a continuous source of inoculum to the experimental material under screening.

Field Assessment of Adult Plant Resistance (Slow Rusting Resistance):

Adult plant resistance (slow rusting resistance) are assessed through host response and epidemiological parameters estimates *i.e.,* rust severity & response, coefficient of infection (CI), area under rust progress curve (AURPC), relative area under rust progress curve (rAURPC), apparent infection rate (*r*) and latent period (LP).

Scales for Recording Rust Intensity:

Many scales for recording the rust reactions are available. The scales given by Peterson *et al.* (1948) provides a range of diagrams of visible lesions and the percentage of the leaf area covered by the lesions (Fig. 2). Where A- is Percentage of leaf area covered by lesions and B- is Visual percentage. Al CIMMYT, a uniform scale of 5, 10, 20, 40, 60 and 100 is followed for all the rusts.

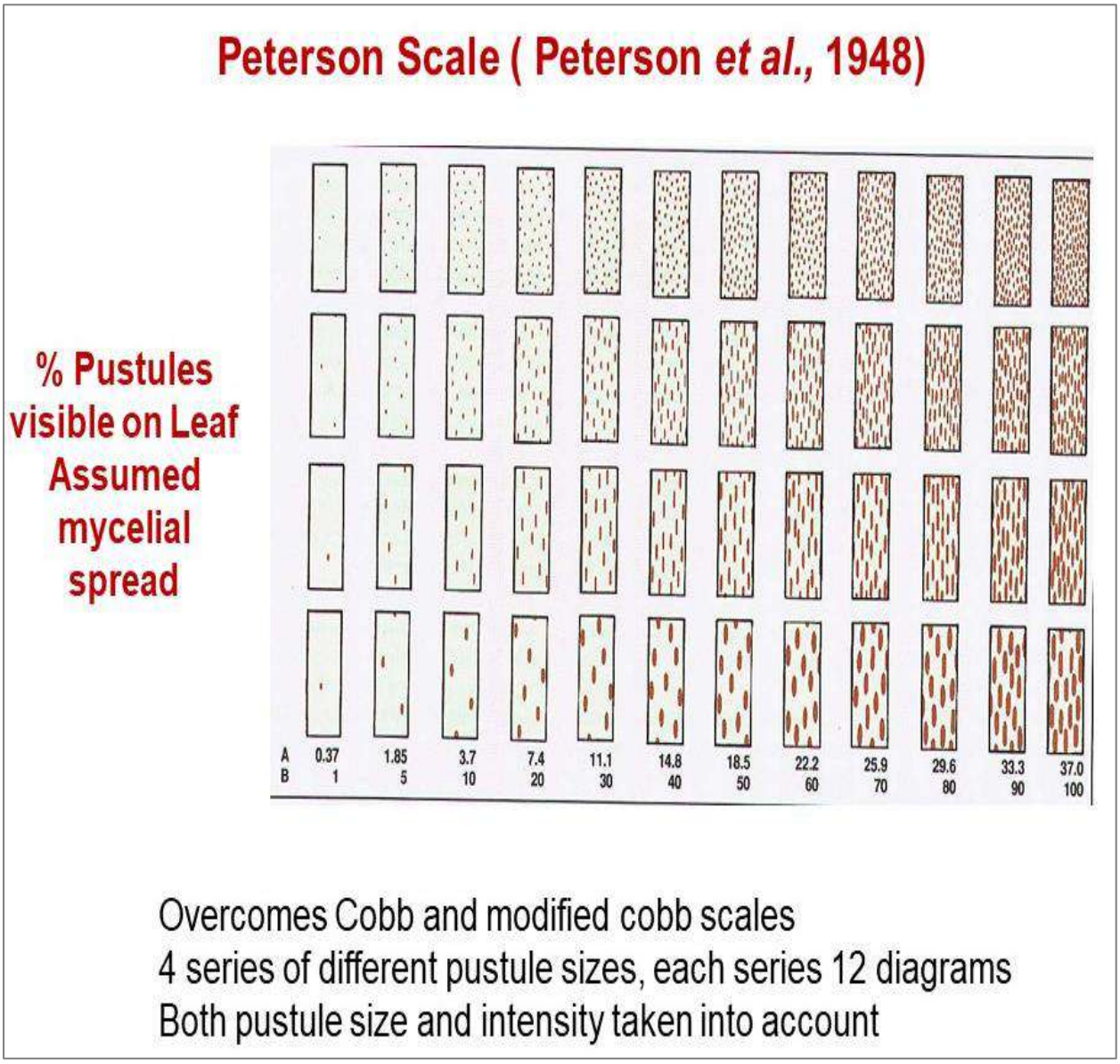

Fig. 2. Peterson scale for scoring rusts infection (Peterson et al., 1948)

Rust Severity and Response:

Loegering (1959) gave a detailed outline for recording black and brown rusts on the basis of severity and response. Severity is recorded as percent of infection based on the percentage scale. As severity is determined by visual observations, reading can not be absolutely precise. Below 5 percent severity, the intervals used are traces to 2 percent. Usually, 5 percent interval is used between 5 to 10 and 10 between 20 to 100 percent severity (Fig. 3).

The response of the variety has been referred to the adult plant infection type and is recoded based on Roelfs *et al.* (1992) as follows:

O : No infection (0.0)

R : Resistant – necrotic areas with or without minute uredia (0.2)

MR : Moderately Resistant – small uredia surrounded by necrotic areas (0.4)

X : Intermediate – variable size uredia, some with necrosis or chlorosis (0.6)

MS : Moderately Susceptible – medium uredia with no chlorosis (0.8)

S : Susceptible – large uredia, no necrosis or chlorosis (0.8)

Figures in bracket are response vales of the infection types. Reading of severity and response are recorded together with severity first e.g. TS: Trace severity of a susceptible infection.

20R : 20 percent severity of Resistant (R) type infection

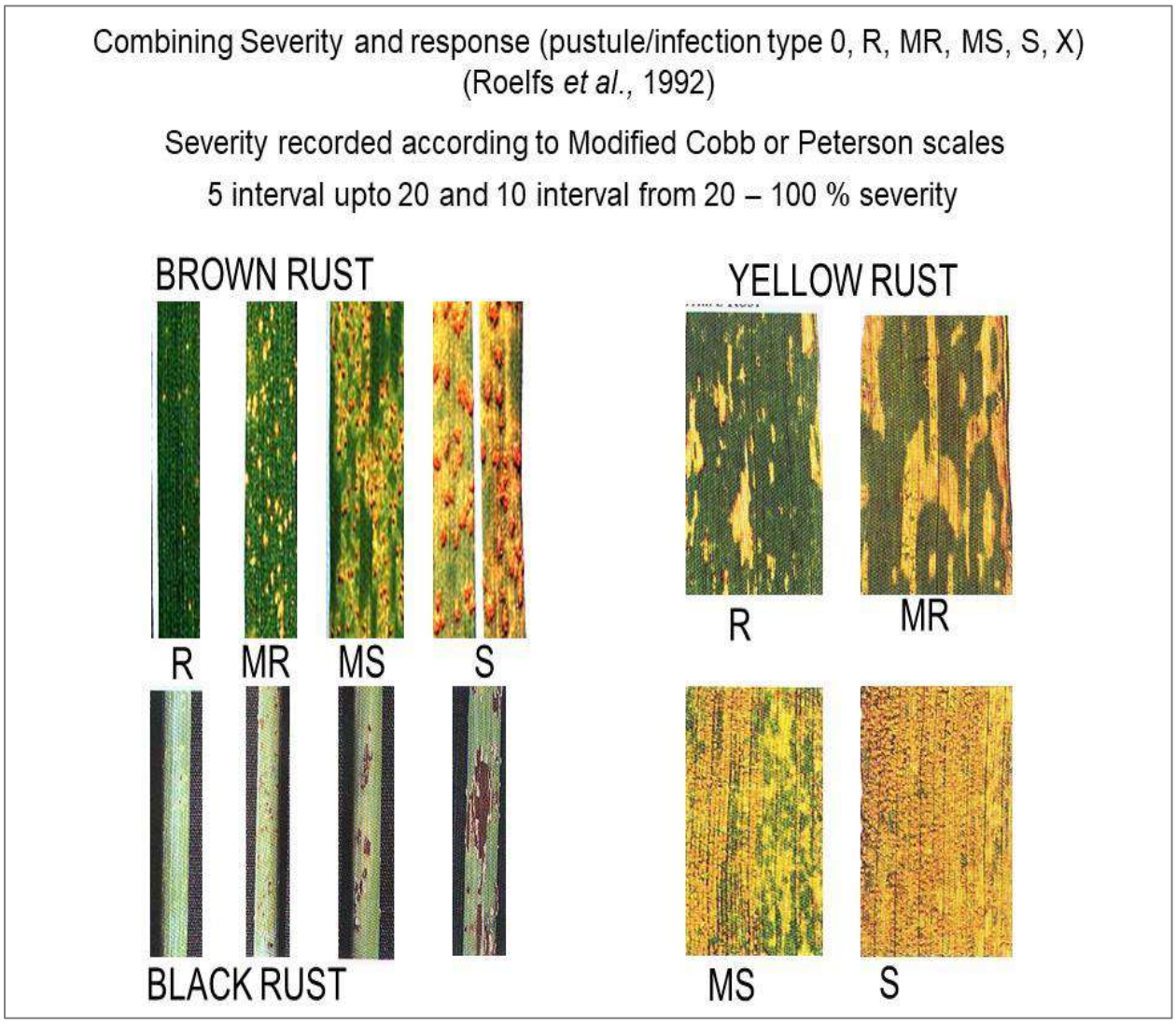

Fig. 3. Different Adult Plant Infection Types (ITs) produced by three Rusts of Wheat Roelfs et al. (1992)

Variability in Reactions: Under natural conditions variability in infection types is observed. This may occur due to the presence of different variants or heterogenous varieties. In such cases proper mention is made of:

1. Varietal mixture having classes (10R, 20S)
2. A range of reactions (5R-10S)
3. A range of reaction on each plant (Plant 1:5 MR-S; Plant 2:10S)

Coefficient of Infection (CI) Values:

The coefficient of infection (CI) which is calculated by multiplying the percentage of infection by response value of the concerned infection types (Stubbs *et al.,* 1986). The CI values are used for estimating AURPC, apparent infection rate (*r*) and other statistical analysis. The reaction/ infection type and their response value are given in Table 3.

Table 2: The reaction/ infection type and their response for calculating CI

Reaction Type	Response Value
R = Resistant	0.2
MR = Moderately Resistant	0.4
MS = Moderately Susceptible	0.8
S = Susceptible	1.0
X = Mesothetic	0.6

For example:

CI values for:

10S would be : 10X1 = 10
20MR : 20X0.4 = 8
5R-15S : 5 +15/2 = 10; 0.2 + 1.0/2 = 0.6 hence CI 10X0.6 = 6.0

Average coefficient of infection (ACI): average of multi-locations

Area Under Disease Progress Curve (AUDPC):

Periodic observations on disease severity, in any field or area, when plotted against time on a graph paper (both on arithmetic scale) give curved lines representing progress of disease over time. These cumulative disease curves are known as Disease progress Curve (DPCs). More frequent the observations the smoother will be DPC. DPCs are highly sensitive indicators, they reflect any fluctuation in factors affecting course of disease development. The formula given by Shaner and Finney (1977) for calculating AUDPC and rAUDPC for the cultivars is exemplified below:

$$\text{AUDPC} = \left[\frac{\left(\frac{D_1 + D_2}{2} \times T\right) + \left(\frac{D_2 + D_3}{2} \times T\right) + \left(\frac{D_3 + D_4}{2} \times T\right)}{n - 1} \right]$$

Where,

D = Disease severity at different dates (D_1, D_2, D_3 and so on)
T = Time interval (days) between two observations
n = Total number of observations

$$\text{rAUDPC} = \frac{\text{Line AUDPC}}{\text{Check AUDPC}} \times 100$$

For example:

Varieties	CI Values for Yellow rust					AUDPC	rAUDPC
	13th Feb.	20th Feb.	27th Feb.	06th Mar	13th Mar		
	D_1	D_2	D_3	D_4	D_5		
DBW 17	1	5	10	20	20	178.5	13.07
DBW 39	1	10	20	40	40	353.5	25.89
DBW 71	2	4	6	6	6	98	7.71
Check	30	50	80	100	100	1365	100

For wheat variety DBW 17:

$$\textbf{AUDPC} = \frac{(1+5/2 \times 7) + (5+10/2 \times 7) + (10+20/2 \times 7) + (20+20/2 \times 7)}{5-1}$$

AUDPC = 178.5

$$\textbf{rAUDPC} = \frac{\text{Line AUDPC}}{\text{Check AUDPC}}$$

$$\textbf{rAUDPC} = \frac{178.5}{1365}$$

rAUDPC = 13.07

Apparent Infection Rate (*r*):

Apparent infection rate (*r*) is the increase or decrease in disease per unit time. It is estimated in terms of disease severity recorded on cultivars at different times (Van der Plank, 1968).

$$r = \frac{2.3}{t_2 - t_1} \log_{10} \frac{x_2(1-x_1)}{x_1(1-x_2)}$$

Where,

x_1 = Disease index at time t_1
x_2 = Disease index at time t_2
t_1 = Time of disease rating(x1)
t_2 = Time of disease rating (x2)
r = Apparent infection rate

Categorization of Slow Rusting / Adult Plant Resistance level based on values of different parameters

Category / level of Slow Rusting / Adult Plant Resistance	FRS value (%)	CI Value	rAURPC value	*'r' value*
High	**1-20%**	**0-20**	**up to 30% of the check**	**less than 0.05**
Moderate	**21-40%**	**21-40**	**up to 40% of the check**	**0.06 to 0.09**
Low	**41-60%**	**41-60**	**up to 60% of the check**	**0.09 to 0.11**
Susceptible	**61-100%**	**61-100**	**100%**	**more than 0.37**

The cultivars having MS or MR infection type may carry slow rusting/adult plant resistance genes (Singh *et al.,* 2005; Singh *et al.,* 2015).

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Physiological Specialization in Wheat Rusts and Identification of Pathotypes

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Wheat rust pathogens are very dynamic and fast evolving. Any introgression of new resistance is easily matched by acquisition of virulence. Evolution of rust pathogens is a continuous process and many a times a new pathotype of wheat rust pathogens renders a resistant wheat variety susceptible. The development of new wheat varieties is a continuous process as the pathogen keeps on evolving continuously. During early eighties, *Lr*26 based resistance (1B.1R) was used in wheat to manage brown rust, however, pathotype 5R37 and 109R63 were identified in 1983 and 1985, respectively. Also there are instances, like emergence of pt. 62G29-1 virulent on *Sr*24, where pt. was detected much before the identification of a variety based on this type of resistance. Similar phenomenon was observed in recently identified pathotypes 253R31 and 377R60-1 with virulence to *Lr*19 and *Lr*28, respectively as till date there is no variety with these types of resistance in India. Thus as a part of management strategy, identification/ naming of new pathotypes or variation in rust pathogens is important not only to evaluate upcoming wheat material but also in varietal deployment. Therefore, pathotype identification/designation are the backbone of our national wheat programme which has provided valuable information for keeping wheat rusts in India under control. The information on virulence on different genes, postulation of vertical resistance genes and identification of resistant lines is mandatory for any successful wheat breeding programme.

Physiologic Specialization and Identification of Pathotypes of Wheat Rust Pathogens

The rust pathogens are highly variable. In each rust many pathotypes are known to occur. A rust infected field of susceptible variety may have many pathotypes. The evolution of new pathotypes occur in each rust generally rendering resistant variety susceptible.

The phenomenon of physiological specialization also termed as specialization of parasitism, was first noted by Schroeder (1879) in *Puccinia graminis*. Eriksson (1894) gave experimental evidence for this through crop inoculation experiments in *P. graminis*. He recognized five varieties in this rust viz. *tritici* on wheat, *avenae* on oat, *secalis* on rye, *agrostidis* on *Agrostis sp.* and *poae* on *Poa sp.*, and termed these as *formae specialis* (f.sp.). different varieties of P. graminis can be identified on the basis of their pathogenicity and also on morphology of spores.

Each *formae specialis* of rust species contains enormous variation. These variants called pathotypes, are differentiated on the basis of infection types on a set of hosts called differentials. In addition to some molecular tools, cytological studies may also be used for categorization of pathotypes. The host pathogenicity technique is quite reliable and accurate method of identification of pathotypes.

Specialization of parasitism in black rust was demonstrated by Stakman (1914) and existence of races in black rust of wheat was known in 1917 (Stakman and Piemeisel, 1917). Accordingly, a set of differential varieties for identification of pathotypes (races) was proposed for black rust by Stakman and Levine (1922), for brown rust by Johnston and Mains (1932) and for yellow rust by Gassner and Straib (1932) (Table 1). To separate minor differences within races, additional (supplementary) differentials were added later on in order to identify the new pathotypes (races) arising due to changed varietal pattern. Subsequently an International system for identification of races of brown rust was given by Johnston and Mains (1932) and for yellow rust by Gassner and Straib (1932).

With the discovery of gene for gene theory of Flor (1955), and it use later by Stakman *et al*. (1962), it was realized that the system of identification pathotypes (races) requires modification in order to use the virulence survey results in more meaningful purpose. Consequently, numerous methods using near-isogenic lines were suggested around the world (Watson and Luig,1963; Johnson *et al*.,1972; Roelfs and McVey,1974). Various research institutions developed their own systems of races analysis and race designation, but some joined in using a common system, in order to facilitate communication. Countries monitoring virulence frequencies are primarily interested in knowing which resistance genes are still effective in order to use them in breeding programs; other countries and regions are interested in virulence frequencies, virulence combinations, race frequencies, evolution, and diversity of *Puccinia* species. In USA and Canada, a series of 12 near-isogenic lines, in the group of 4 (McVey *et al*.,1999) which was modified

to 20 with the evolution of *Sr*31 virulences (Jin *et al.,* 2008). The constitution of these sets is given in Table 3. Likewise 12 differentials in sets of 4 in a Thatcher background were used as differentials for identifying pathotypes of *P. triticina* (Long and Kolmer, 1989; McVey *et al.,* 2004). Differentials are grouped in sets of four. When the four lines are classified for resistance or susceptibility, there are 16 possible combinations. These combinations are coded from B to T omitting the vowels. The pathogenicity of a race is coded using five letters, each indicating its pathogenicity on one set of four lines. For example, code BBBBB indicates that all 12 differentials are resistant; whereas TTTTT indicates all 12 differentials are susceptible. Provision is made for supplementary differential sets, as currently used in Mexico (Singh, 1991), USA (McVey *et al.,* 2004), on a global basis (Huerta-Espino, 1992), Kolmer *et al.* (2007, 2009), and other countries (Table 2).

Table1: Standard differentials for the identification of black, brown and yellow rust pathotypes

Stem or Black rust	**Leaf or Brown rust**	**Stripe or Yellow rust**
Little club Marquis Reliance Kota Aurnautka Mindum Spelmar Kubanka Acme Einkorn Vernal Khapli	Malakoff Carina Brevit Webster Loros Mediterranean Hussar Democrat	Michigan Amber Ble Rouge d'Ecosse Strubes Dickkopf Webster Holzapfels Fruh Vilmorin 23 Heines Kolben Carstens V Spalding Prolific Chinese 166 Rouge P. Barbu *Triticum dicoccum* var. *tricoccum* Fong Tien Heils Franken Pet kuser Roggen Lee Richersberg 42
Additional differentials		
Charter Yalta E535	Thew NI 5439 IWP 94	Kalyansona Sonalika

Table 2: North American stem rust nomenclature code sheet

Four gene differential sets				
Set 1	*Sr*5	*Sr*21	*Sr*9e	*Sr*7b
Set 2	*Sr*11	*Sr*6	*Sr*8a	*Sr*9g
Set 3	*Sr36*	*Sr9b*	*Sr30*	*Sr*17
Set 4	*Sr*9a	*Sr*9d	*Sr*10	*Sr*Tmp
Set 5	*Sr*24	*Sr*31	*Sr*38	*Sr*McN
Pgt letter B	L	L	L	L
C	L	L	L	H
D	L	L	H	L
F	L	L	H	H
G	L	H	L	L
H	L	H	L	H
J	L	H	H	L
K	L	H	H	H
L	H	L	L	L
M	H	L	L	H
N	H	L	H	L
P	H	L	H	H
Q	H	H	L	L
R	H	H	L	H
S	H	H	H	L
T	H	H	H	H

In Europe, many systems of reporting races have been used, including an octal notation system (Bartos *et al.,* 1996; Goyeau *et al.,* 2006; Hanzalova and Bartos, 2006). Likewise South Africa (Pretorius *et al.,* 1987) and Australia (Park, 2008) use their own systems.

The rust samples collected from the fields are established on susceptible wheat variety. The fresh uredospores are then inoculated on sets of differentials (such rust differentials are specific for each rust Table 4. For this bulk inocula is taken from the leaves. If it happens to be mixture of more than one pathotype then further isolations are taken and it is purified. Data are then compiled to determine the frequency of pathotypes and their distribution. It then helps us predict the responses of different varieties of the area. Such significant findings are then communicated to breeders and the community of wheat workers. The system which is currently being used for the identification of pathotypes is described below.

Indian System of Identification of Wheat Rust Pathotypes

Based on the experience on wheat rusts in India and systems proposed in other countries, to begin with, a system for the analysis of brown rust pathotypes in India was proposed by Nagarajan *et al*. (1983). The system has 3 sets of differentials. Set-'0' contained popular cultivars of bread wheat and durum, a susceptible cultivar and a resistant line. Set-'A' comprised 8 near-isogenic lines of different *Lr*-genes. Set-'B' had six selected lines from old international differentials. Keeping into account number of lines in three sets and near isogenic lines, the system permits to evaluate more than 14 resistant genes. Recasting of Set-'0' of differentials was done (Table 3) by Nayar *et al*. (1997) and Bhardwaj (2011). Subsequently on similar pattern pathotype analysis systems were developed for black rust (Bahadur *et al*.,1985) and yellow rust (Nagarajan *et al*.,1985). In yellow rust some modifications of the differentials were also made in Set-'A' and Set-'B' because of the appearance of pathotype with matching virulence for *Yr*9 in February, 1996. Riebesel 47/51 having *Yr*9 which is the fifth line in the Set-'B' was found to be resistant to this pathotype whereas some other lines known to possess *Yr*9 like Tc*6/*Lr*26 were susceptible. Therefore, Riebesel 47/51 is included in Set-'A' at serial number eight and at serial number five in Set-'B' will be Tc*6/*Lr*26. With this change, Set-'A' of the differentials will be exactly similar to world set of the yellow rust differentials. Also for yellow rust of barley, Set-'0' of differentials has been separately constituted.

Table 3: The revised composition of sets of differentials for the identification of rust pathotypes

Set-0		**Set-A**	**Set-B**
Leaf or Brown rust (*Puccinia triticina*)			
IWP 94 (*Lr*23+)		*Lr*14a	Loros (*Lr*2c)
Kharchia Mutant		*Lr*24	Webster (*Lr*2a)
Raj 3765		*Lr*18	Democrat (Lr3)
PBW 343		*Lr*13	Thew (*Lr*20)
UP 2338		*Lr*17	Malakoff (*Lr*1)
K 8804		*Lr*15	Malakoff (*Lr*1)
Raj 1555		*Lr*10	Benno (*Lr*26)
HD 2189		*Lr*19	HP1633 (*Lr*9+)
Agra Local		*Lr*19	
Stem or Black rust (*Puccinia graminis tritici*)			
*Sr*24		*Sr*13	Marquis (*Sr*7b+)
NI 5439		*Sr*9b	Einkorn (*Sr*21+)
*Sr*25		*Sr*11	Kota (*Sr*28+)
DWR 195		*Sr*28	Reliance (*Sr*5+)
HD 2189		*Sr*8b	Charter (*Sr*11+)
Lok 1		*Sr*9e	Khapli (*Sr*7a+)
HI 1077		*Sr*30	Tc*6/*Lr26* (*Yr*9)
Barley Local		*Sr*37	
Agra Local			
Stripe or Yellow rust (*Puccinia striiformis tritici*)			
WHEAT	BARLEY		
WH 147	TDT	Chinese 166 (*Yr*1)	Hybrid 46 (*Yr*4)
Barley local	Barley local	Lee (*Yr*7)	Heines VII (*Yr*2+)
WH 416	WH147	Heines Kolben (*Yr*6)	Compair (*Yr*8)
PDW 215	Heils Franken	Vilmorin 23(*Yr3*)	*T. spelta album* (*Yr*5)
HD 2329	Fong Tien	Moro (*Yr*10)	Tc*6/*Lr26* (*Yr*9)
HD 2667	Himani	Strubes Dickkopf	Sonalika (*Yr2+*)
PBW 343	BHS 16	Suwon92 X Omar	Kalyansona *Yr*2(KS)
HS 240	Alfa 93	Riebesel47/51(*Yr*9+)	
Anza	Dolma		

Based on the virulence value (1 for susceptibility and 0 for resistance) on Set-'A' and Set-'B', a pathotype is designated on the basis of binomial system of Habgood (1970). When distinguishing feature is available on the line in Set-'0' then it is suffixed to the value e.g. pt. 77-5(121R63-1) gets value 121 on the basis of susceptible differentials in Set-'A' (Table 4) whereas 63 is the value on Set-'B'. The value -1 means that line at serial number 1 in Set-'0' is susceptible. The value of Set-'A' and Set-'B' is separated by capital alphabet G for black rust which denotes that pathotype is of *graminis* e.g. 62G29. Similarly in case of brown rust alphabet R is used to denote that pathotype is of *recondita* (old name for *triticina*) and for yellow rust alphabet S is used for *striiformis*.

Table 4: Procedure for coding and decoding of rust pathotypes

Details	***Lr* genes**								
	*Lr*14a	*Lr*24	*Lr*18	*Lr*13	*Lr*17	*Lr*15	*Lr*10	*Lr*19	*Lr*28
Decanary value	2^0	2^1	2^2	2^3	2^4	2^5	2^6	2^7	2^8
Decoded decanary value	1	2	4	8	16	32	64	128	256
Reaction of pt. 77-5	S	R	R	S	S	S	S	R	R
Binary value	1	0	1	1	1	1	1	0	0
Decanary value of pt. on set A	1	0	0	8	16	32	64	0	0
Summary of decanary value=1+8+16+32+64=121									

The pathotype identification system based on near-isogenic lines helps in deducing the avirulence/virulence structure of pathotypes, monitor the variability in the rust population effectively and undertake anticipatory breeding before these assume alarming proportions. The information thus generated is compiled & published periodically (Bhardwaj *et al.,* 2010b). This host pathogenicity technique is quite reliable & accurate method of races identification & later of the host as well. As an example from the 2001 survey, pt. 78S84 was picked up from PBW 343. It was in very low frequency & remained so till 2005 & build up later.

Gene-for-Gene System

Flor (1942) was first to study both the inheritance of pathogenicity and host response. Working with flax rust (*Melampsora lini* Desm.) and its host, flax (*Linum usitatissimum*). In his initial studies he dealt with a gene pair in the pathogen corresponding to a gene pair in host- the corresponding gene pairs (Flor,1946,1947). Based on these studies he developed gene-for-gene hypothesis (Flor,1971). **T**he hypothesis was further conceptualized by Person *et al*. (1962). They mentioned that " A gene for gene relationship exists when the presence of a gene in one population is contingent on the continued presence of a gene in another population and where the interaction between the two genes leads to a single phenotypic expression by which the presence or absence of the relevant gene in either organism may be recognized". Gene-for-gene systems have evolved through a series of steps in evolution of each. Therefore, if a host is resistant to pathogen, a virulent mutant would have an advantage over avirulent. Likewise if a host is susceptible to a pathogen, a resistant mutant in host would be at advantage. So the process continues and during the course of evolution resistant host and virulent pathogen continue to strive for the race of supremacy. Genetic analyses have indicated that resistance to rusts is controlled generally by dominant genes and virulence in pathogen is due to recessive genes (or avirulence is controlled by dominant genes). Resistance and avirulence are dominant and they result from active gene product from avirulent pathogen. In genetic study on PBW 343, a predominant wheat variety of Northern India, it was revealed that resistance to black, brown, yellow rusts is being controlled by dominant genes (Datta *et al.,* 2010). Likewise resistance of HS 424 to rusts is also governed by dominant genes (Data *et al*. 2007). In cases where they are recessive, it may involve dosage effect and may require 2 or 3 dosages for a active gene product. Resistance in some cases is also controlled by recessive genes. Resistance of Agra Local to a pathotype of *Puccinia triticina* is controlled by 2 recessive resistance genes. One of these resistance genes was present in IWP94 also (Datta *et al*., 2008).

Incompatibility (resistance) is a recognition process which results from a resistant host and avirulent pathogen. Infection type produced as a result of pathogen attacking a host is a product of two genetic systems. For a single pair of genes (assuming that homozygotes occur). The interaction can be illustrated as given in Table 5.

Table 5: Gene for gene relationship

Pathogen	**Host**	
	RR	Rr
PP	Resistant	Susceptible
Pp	Susceptible	Susceptible

Only one set, RR/PP, results in resistant (low infection type). The host carries a gene for resistance for which the pathogen does not carry the corresponding gene for virulence. There occurs specificity in combinations involving the genes that are normally dominant, R and P. Any one of the three other combinations, RR/pp, rr/PP, rr/pp result in susceptibility or compatible reaction.

Characterization of Rust Resistance Genes and Identification of Resistant Lines

As the rust pathogens evolve to neutralise genes for resistance, we must continue to introduce further genes for resistance. To do this we must identify new sources of resistance and establish through genetic studies that they are determined by new genes (or new gene combinations) that can be successfully integrated into breeding programmes. Screening often must be done using key pathotypes identified in the pathogenicity survey. These tests lead to the postulation of different rust resistance genes which then can be promoted for release. Advance material trial entries of each year are subjected to multi-pathotype testing. This technique enables us to postulate the presence of vertical resistance genes in these elite lines. These lines are also tested at adult plant stage also. This would identify lines having race-specific or non-race specific type of resistance. Many such lines having adult plant resistance of race-specific nature have been identified. We also used molecular markers to establish gene identity and combine genes into single cultivars.

Genetics of Rust Resistance of Indian Wheat

Information on genetic of wheat rust resistance is widely available. Based on the available information it can be concluded that brown rust resistance of Indian wheat is based on *Lr*1, *Lr*3, *Lr*9, *Lr*10, *Lr*13, *Lr*14a, *Lr*17, *Lr*19, *Lr*22, *Lr*23, *Lr*24, *Lr*26 and *Lr*34. Among these *Lr*26, *Lr*13, *Lr*23 and *Lr*34 have been characterized in many wheat lines. Durum wheat has resistance based mainly on *Lr*23. Presently *Lr*24, *Lr*25, *Lr*29, *Lr*32, *Lr*39, *Lr*42, *Lr*45 are resistant to all the pathotypes of *P. triticina* in India. Virulences on *Lr*9, *Lr*19 and *Lr*28 were identified only recently. *Sr*2, *Sr*5, *Sr*6, *Sr*7a, *Sr*7b, *Sr*8a, *Sr*8b, *Sr* 9b, *Sr*9e, *Sr*11, *Sr*12, *Sr*13, *Sr*17, *Sr*21, *Sr* 24,*Sr*28, *Sr*30, *Sr31* have been characterized in Indian wheat material. Among these *Sr2, Sr*11 and *Sr*31 were very common in bread wheat whereas *Sr*7b, *Sr*9e and *Sr*11 conferred black rust resistance in many durum lines. *Sr*26, *Sr*27, *Sr31, Sr*32, *Sr*33, *Sr*35, *Sr*39, *Sr*40 and *Sr*43 confer resistance against Indian population of *P. graminis tritici*. Yellow rust resistance of wheat in India is based on *Yr*2, *Yr*A, *Yr*9, *Yr*18 and *Yr*27. The gene *Yr*5, *Yr*10, *Yr*11, *Yr*12, *Yr*13, *Yr*14, *Yr*15, *Yr*16 are resistant against *P. striiformis tritici* in India.

Further Reading

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Isolation of New Pathotypes of *Puccinia* species, Coding and Decoding of Pathotype Names

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Wheat rusts are very devastating pathogens. While stripe and stem rusts can cause 100% loss, leaf rust can inflict 50% yield reduction. Therefore, identification of new pathotypes of wheat rust pathogens in initial stages is of utmost importance. It is important not only in identifying sources of rust resistance but also for pre-emptive breeding also. Different countries have different systems of pathotype identification. Based on the experience on wheat rusts in India and systems proposed in other countries, a system for the analysis of brown rust pathotypes in India was proposed by Nagarajan *et al*. (1983). The system has 3 sets of differentials. Set-'0' contained popular cultivars of bread wheat and durum, a susceptible cultivar and a resistant line. Set-'A' comprised 8 near-isogenic lines (NILs) of different *Lr* genes. Set-'B' had six selected lines from old international differentials. Keeping into account number of lines in three sets and near isogenic lines, the system permits to evaluate more than 14 resistant genes. Recasting of set-0 of differentials was done (Table 1) by Nayar *et al*. (1997) and Bhardwaj (2011). Subsequently on similar pattern pathotype analysis systems were developed for black rust (Bahadur *et al.,* 1985) and yellow rust (Nagarajan *et al.,*1985).

Table 1: The revised composition of sets of differentials for the identification of rust pathotypes

Set-0		**Set-A**	**Set-B**
Leaf or Brown rust (*Puccinia triticina*)			
IWP 94 (*Lr*23+)		*Lr*14a	Loros (*Lr*2c)
Kharchia Mutant		*Lr*24	Webster (*Lr*2a)
Raj 3765		*Lr*18	Democrat (Lr3)
PBW 343		*Lr*13	Thew (*Lr*20)
UP 2338		*Lr*17	Malakoff (*Lr*1)
K 8804		*Lr*15	Malakoff (*Lr*1)
Raj 1555		*Lr*10	Benno (*Lr*26)
HD 2189		*Lr*19	HP1633 (*Lr*9+)
Agra Local		*Lr*19	
Stem or Black rust (*Puccinia graminis tritici*)			
*Sr*24		*Sr*13	Marquis (*Sr*7b+)
NI 5439		*Sr*9b	Einkorn (*Sr*21+)
*Sr*25		*Sr*11	Kota (*Sr*28+)
DWR 195		*Sr*28	Reliance (*Sr*5+)
HD 2189		*Sr*8b	Charter (*Sr*11+)
Lok 1		*Sr*9e	Khapli (*Sr*7a+)
HI 1077		*Sr*30	Tc*6/*Lr26* (*Yr*9)
Barley Local		*Sr*37	
Agra Local			
Stripe or Yellow rust (*Puccinia striiformis tritici*)			
WHEAT	BARLEY		
WH 147	TDT	Chinese 166 (*Yr*1)	Hybrid 46 (*Yr*4)
Barley local	Barley local	Lee (*Yr*7)	Heines VII (*Yr*2+)
WH 416	WH147	Heines Kolben (*Yr*6)	Compair (*Yr*8)
PDW 215	Heils Franken	Vilmorin 23(*Yr3*)	*T. spelta album* (*Yr*5)
HD 2329	Fong Tien	Moro (*Yr*10)	Tc*6/*Lr26* (*Yr*9)
HD 2667	Himani	Strubes Dickkopf	Sonalika (*Yr2+*)
PBW 343	BHS 16	Suwon92 X Omar	Kalyansona *Yr*2(KS)
HS 240	Alfa 93	Riebesel47/51(*Yr*9+)	
Anza	Dolma		

Coding and Decoding of Rust Pathotypes Names

Based on the virulence value (1 for susceptibility and 0 for resistance) on Set-'A' and Set-'B', a pathotype is designated on the basis of binomial system of Habgood (1970). When distinguishing feature is available on the line in Set-'0' then it is suffixed to the value e.g. pt. 77-5(121R63-1) gets value 121 on the basis of susceptible differentials in Set-'A' (Table 2) whereas 63 is the value on Set-'B'. The value -1 means that line at serial number 1 in Set-'0' is susceptible. The value of Set-'A' and Set-'B' is separated by capital alphabet G for black rust which denotes that pathotype is of graminis e.g. 62G29. Similarly in case of brown rust alphabet R is used to denote that pathotype is of *recondita* (old name for *triticina*) and for yellow rust alphabet S is used for striiformis.

Table 2: Procedure for coding and decoding of rust pathotypes

Details	***Lr* genes**								
	*Lr*14a	*Lr*24	*Lr*18	*Lr*13	*Lr*17	*Lr*15	*Lr*10	*Lr*19	*Lr*28
Decanary value	2^0	2^1	2^2	2^3	2^4	2^5	2^6	2^7	2^8
Decoded decanary value	1	2	4	8	16	32	64	128	256
Reaction of pt. 77-5	S	R	R	S	S	S	S	R	R
Binary value	1	0	1	1	1	1	1	0	0
Decanary value of pt. on set A	1	0	0	8	16	32	64	0	0
Summary of decanary value=1+8+16+32+64=121									

The pathotype identification system based on near-isogenic lines helps in deducing the avirulence/virulence structure of pathotypes, monitor the variability in the rust population effectively and undertake anticipatory breeding before these assume alarming proportions. The information thus generated is compiled and published periodically (Bhardwaj *et al.,* 2010b).

This host pathogenicity technique is quite reliable and accurate method of identification of pathotypes and later of the host as well. As an examples from the 2001 survey, pt. 78S84 was picked up from PBW343. It was in very low frequency and remained so till 2005 and build up later.

Isolation of New Pathotypes of *Puccinia* species on Wheat

During analyses of pathotypes on the sets of differentials different infection types are recorded. While recording pathotype data, some mixtures of races are easily detectable. Some of the samples are known to yield 3-4 pathotypes also. In USA, 80 pathotypes of stem rust were identified from a single wheat field. When the reaction matrix of a sample does not tally exactly with the known pathotypes, it is examined critically and looked for a new pathotype. Secondly, when a rust resistant line becomes susceptible, isolations of susceptible pustules are taken and again checked for difference. The test sample is purified further and compared with the closely related pathotypes. If it is distinctly different to the earlier described pathotypes, it is designated as a new pathotype. Subsequently, the new pathotype is used for identification of rust resistance sources and knowing its avirulence/virulence structure of the near isogenic lines (NILs). It is further included in anticipatory breeding.

New pathotype is added to the culture collection. At present there are more than 145 pathotypes of different rust pathogens in National Repository of India. A major part of these pathotypes forms a part of active collection whereas others have been put for long term storage in liquid nitrogen.

The designation of all the pathotypes of different rusts picked up since 1931 and maintained through these years at ICAR-Indian Institute of Wheat & Barley Research, Regional Station, Flowerdale, Shimla are given in Table 3-5.

Table 3: Pathotypes of *P. striiformis tritici* maintained at Flowerdale

Designation		**Detection details**		**Isolated from line/variety**	**Characteristic features**
13	67S8	1937	Palney	Local Wheat	Virulent on *Yr*5
14	66S0	1965	Kangra	-	-
14A	66S64	1970	Punjab	Kalyansona	Virulent on Kalyansona
19	70S0-2	1936	Rawalpindi	PB-8A	Virulent on Wheat & Barley
20	70S0	1937	Nilgiri Hills	Local Wheat	-
20A	70S64	1970	Punjab	-	Virulent on Kalyansona

24	0S0-1	1965	-	Barley Local	-
31	67S64	1936	Shimla	Local Wheat	-
38	66S0-1	1965	Himanchal Pradesh	-	-
38A	66S64-1	1970	Punjab	Kalyansona	Virulent on Kalyansona
57	0S0	1965	-	Barley Local	-
A	70S4	1937	Gurdaspur	Local Wheat	-
G	4S0	1965	-	Barley Local	-
G-1	4S0-3	1990	Nilgiri Hills	*Aegilops searsii*	-
I	38S102	1973	Nilgiri Hills	Sonalika	-
K	47S102	1982	Punjab	-	-
L	70S69	1988	Dalang Maidan	- -	Virulent on Hyb.46
M	1S0	1987	Shimla	-	Virulent on Chinese 166
N	46S102	1988	Dalang Maidan	-	-
P	46S103 46S119	1990 1996	Dalang Maidan Gurdaspur	- CPAN 3004	Virulent on Hyb.46 Virulent on *Yr*9
Q	5S0	1990	Bhowali	*A. kotschyei*	
T	47S103	1992	Nepal	-	-
U	102S100	1992	Nilgiri Hills	-	-
CI	-	1993	Leh	-	Virulent on Kalyansona
CII	-	1993	Leh	-	Virulent on Kalyansona
CIII	-	1993	Leh	-	Virulent on Kalyansona

Table 4: Pathotypes of *P. recondita tritici* maintained at Flowerdale

Designation		Detection details		Isolated from line/variety	Characteristic features
10	13R19	1931	Layallpur	Local Wheat	-
11	0R8	1947	Multai	A090	-
12	5R5	1966	Thordi	Local Wheat	Virulent on *Lr*3
12-1	5R37	1983	Gwalior	-	Virulent on *Lr*26
12-2	1R5	1979	Hansi	Local Wheat	Virulent on *Lr*23
12-3	49R37	1989	Dharwad	Bijaga yellow	Virulent on *Lr*26 & *Lr*15
12-4	69R13	1990	Pusa	Agra Local	Virulent on *Lr*20
12A	5R13	1974	Karnal	Local Wheat	Virulent on *Lr*20 & *Lr*23
17	61R24	1957	Nilgiris	Mal.4	-
20	5R27	1935	Hisar	UP Local	-
63	0R8-1	1931	Shimla	Local Wheat	-
77	45R31	1954	Pusa	Mal.4	Virulent on *Lr*3
77-1	109R63	1985	Nilgiris	Burgas-2	Virulent on *Lr*26
77-2	109R31-1	1984	Nilgiris	PAU Wheat	Virulent on *Lr*23
77-3	125R55	1989	Nilgiris	*Lr15*	Virulent on *Lr*26 and Avirulent on *Lr*20
77-4	125R23-1	1989	Nilgiris	Lal Bahadur	Virulent on *Lr*23 and Avirullent on *Lr*20
77-5	121R63-1	1992	Nilgiris	Crossing material	Virulent on *Lr*23 & *Lr*26
77A	109R31	1974	Dharwad	CC-62	Virulent on *Lr*10
77A-1	109R23	1976	Delhi	HD2009	Virulent on *Lr*10 and Avirullent on *Lr*20
104	17R23	1973	Janakpur (Nepal)	-	Virulent on *Lr*1 & *Lr*3
104-1	21R31-1	1985	Borkheda	Kalyansona	Virulent on *Lr*23 & *Lr*20
104-2	21R55	1991	Malan	Off type in Transec	Virulent on *Lr*23 & *Lr*26
104-3	21R63	1993	Nawal Parsi	Local Wheat &	Virulent on *Lr*23, *Lr*26

			Sunwal (Nepal)	*Lr20*	
104A	21R31	1975	Patna	HD1981	Virulent on *Lr*20
104B	29R23	1980	Hansi	C 306	Virulent on *Lr*23
106	0R9	1935	Haldwani	Local Wheat	-
107	45R3	1935	Khanewal	PB8A	-
107-1	45R35	1988	Dharwad	-	Virulent on *Lr*26
108	13R27	1935	Banaras	Pusa-4	-
108-1	57R27	1989	Deorighat	Self sown Sonalika	Virulent on *Lr*15
162	93R7	1957	Gwalior	NP720	Virulent on *Lr*3
162A	93R15	1961	Nilgiris	-	Virulent on *Lr*3 & *Lr*20

Table 5: Pathotypes of *P. graminis tritici* maintained at Flowerdale

Designation		**Detection details**		**Isolated from line/variety**	**Characteristic features**
11	79G31	1962	Maharashtra	-	Virulent on *Sr*11 and *Sr*30
11A	203G15	1974	Wellington	Durum	Virulent on *Sr*30 and Sr37
14	16G2	1959	Gwalior	Pissi local	-
17	73G7	1954	Jabalpur	RS6	-
21	9G5	1935	Layallpur	C518	Virulent on Kota
21-1	24G5	1985	Uttar Pradesh	UP2224	Virulent on *Sr*8
21A-2	75G5	1962	Indore	Meghdoot	Virulent on *Sr*30
24	18G3	1935	Himayat Sagar	Bansi	Virulent on *Sr*8 and Einkorn
24-A	5G19	1981	Powarkheda	MP195	-
34	26G13	1940	Pusa	Pusa-4	-
34-1	10G13	1991	Grotegaon	WH147	Avirulent on *Sr*8
40	104G13	1932	Poona	Pusa-4	Virulent on *Sr*9e and Sr30
40A	62G29	1974	Wellington	HD4515	Virulent on many *Sr* genes
40-1	62G29-1	1989	Wellington	HW2001	Virulent on *Sr*24
42	19G35	1932	Poona	Pusa-4	Khapli is susceptible
42B	7G35	1947	Mahableshwar	Khapli	*Sr*11 and Khapli susceptible
117	37G3	1945	Betul	Mixed varieties	*Sr*9e and *Sr*11 are susceptible
117A	36G2	1961	Karnataka	Local	-
117A-1	38G18	1977	Dharwad	NI5439	-
117-1	166G2	1987	Dharwad	EC183685	Virulent on *Sr*37
117-2	33G3	1987	Karnal	Tc6/Web. RL6016-2	Avirulent on *Sr*11
117-3	167G3	1987	Wellington	Local	Virulent on *Sr*13 and *Sr*37
117-4	166G3	1987	Dharwad	J-24	Virulent on *Sr*37
117-5	166G2-2	1987	Mahableshwar	Local Wheat	Virulent on *Sr*37 and NI5439
117-6	37G19	1990	Niphad	CPAN3031	-
122	7G11	1952	Bangal Kot	Local Wheat	-
184	53G1	1965	Karnal	Local-4	-
295	7G43	1962	Indore	E 938	Virulent on Khapli

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Pathogenomics of Major Fungal Pathogens of Wheat, Development of their Diagnostics and their Management

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Rapid and accurate diagnostics facilitate early intervention during plant disease outbreaks and enable disease management decisions that limit the spread of plant health threats. At present, conventional plant disease diagnostics rely on visible inspections of disease symptoms followed by basic laboratory tests through culturing and pathogenicity assays. Unfortunately, these conventional methods tend to be subjective, time-consuming, labour-intensive and reliant on specialised expertise and equipment, providing only limited phenotypic information. Recent alternative approaches have focused on serological and nucleic acid assays. Polyclonal and monoclonal antisera are frequently used to detect plant pathogens using techniques such as enzyme-linked immunosorbent assay (ELISA), immunostrip assays and immunoblotting. In addition, following a flurry of PCR-based diagnostic tests in the 1980s, the advent of the loop-mediated isothermal amplification (LAMP) assay at the turn of the twenty-first century provided the first rapid nucleic acid amplification method to accurately diagnose pathogens *in situ* in real time. Both serological and DNA-based methods typically require high initial financial investments and specialised expertise to develop new assays, are limited in sample capacity, frequently are not reliable at the asymptomatic stage, and provide limited information beyond the species level. The capacity to distinguish between individuals in a pathogen population with specific properties such as fungicide resistance, toxin production and virulence profiles is often essential to identify disease management approaches. In the past two decades, the genomics revolution has led to technologies that can rapidly generate genome-scale genetic information to define individual variants of a pathogen species. These emerging, data-driven, diagnostic tools have the potential to rapidly track shifting pathogen populations in near real-time, providing copious genetic information at the strain level that can be used in early warning systems and disease forecasting.

Genome Sequencing of Wheat Pathogens and Developing Diagnosis

Fungal pathogens are emerging or/ remerging in climate change scenario. In era of molecular biology, there is a need to understand pathogen evolution, biology, lifestyle, novel disease management using molecular approaches. The genomes of 1090 fungal species are available in the public domain. Plant pathogenic fungi comprised the largest category (35.5 %) in which plant pathogens are predominant. 191 genomes of pathogenic fungi are available in which 61.3 % cause diseases on food crops. The *T. indica* genome assembly size of 33.7 MB was generated using Illumina and Pac Bio platforms with GC content of 55.0 per cent. A total of 1,737 scaffolds were obtained with the N_{50} of 58,667 bp. The *ab initio* gene prediction was performed using *Ustilago maydis* as the reference species. Spot blotch of wheat caused by *Bipolaris sorokiniana* is an important disease of wheat, especially in slightly warm (25 ± 1 °C) and humid weather conditions. *Bipolaris sorokiniana* was sequenced using three platforms *viz*. Illumina HiSeq, Ion torrent and Nanopore. The generated whole genome size is 35.64 MB with G+C content of 50.2% having 235 scaffolds and N_{50} of 1654800. This whole-genome project has been deposited in DDBJ/ENA/GenBank under the accession number RCTM00000000. The BUSCO evaluation of completeness of the *B. sorokiniana* genome sequence predicted that it was 97.6% complete genome.

A quick and reliable PCR-based diagnostic assay has been developed to detect *B. sorokiniana* using a pathogen-specific marker derived from genomic DNA. The marker could detect the pathogen in soil and wheat leaves at pre-symptomatic stage. This sequence characterized amplified region (SCAR) marker designated as SCRABS (600) could clearly distinguish *B. sorokiniana* from other fungal plant pathogens, including other *Bipolaris* spp. The utilization of this diagnostic PCR assay in analysis of field soil and wheat leaves will play a key role in effective management of the disease (Aggarwal *et al*., 2011). Leaf rust of wheat caused by *Puccinia triticina* has significant impact on wheat production worldwide. Effective and quick detection methodologies are required to mitigate yield loss and time constraints associated with monitoring and management of leaf rust of wheat. Detection of *P. triticina* has been simplified by developing a rapid, reliable, efficient and visual colorimetric method *i.e*., loop mediated isothermal amplification of DNA (LAMP). Based on *in silico* analysis of *P. triticina* genome, PTS68, a simple sequence repeat was found

highly specific to leaf rust fungus. A marker ($PtRA_{68}$) was developed and its specificity was validated through PCR technique. A novel gene amplification method LAMP which enables visual detection of pathogen by naked eye was developed for leaf rust pathogen. The assay developed in the study shall be very much useful in the development of diagnostic kit for monitoring disease, creation of prediction model and efficient management of disease (Manjunatha *et al*., 2018). Similarly, genomic analysis also done to develop specific marker for *Puccinia striiformis tritici* causing stripe rust of wheat (Aggarwal *et al*., 2017).

Genomics for Disease Management

The evolutionary and epidemiological principles underlying the identification and deployment of resistance genes guide the discovery of new resistance genes, the development of resistance loci markers, and the deployment of gene cassette and other genome empowered strategies for durable disease management (Klosterman *et al.,* 2016). Draft sequencing of pathogen genomes can provide a rapid and cost effective means to understand life and disease cycle attributes and provide resources for tool development required for more detailed analyses. Xiang *et al.* (2016) showed how genomics can greatly accelerate and facilitate breeding of stripe rust-resistant wheat cultivars. Using molecular markers in wheat, a stripe rust resistance locus (*Yr* 76) in the wheat cultivar Tyee was mapped.

More than a decade ago, the genome of plant-pathogenic bacterium *Xylella fastidiosa*, was sequenced (Simpson *et al.,* 2000). Five years later, the rice blast fungus *Magnaporthe oryzae* was the first eukaryotic plant pathogen for which a genome sequence became available (Dean *et al.*, 2005), soon followed by the genome sequences of two oomycete plant pathogens, *Phytophthora sojae* and *P. ramorum* (Tyler *et al.,* 2006). Consequently, the number of available genome sequences has increased exponentially. Genome-wide studies aiming to identify effector candidates commonly first predict the secretome, *i.e.,* the repertoire of all secreted proteins, by querying for the presence of N-terminal signal peptides (Giralo and Valent, 2013; Lo Presti *et al.,* 2015). Comparative genomics-based effector discovery of closely related pathogens, or of different strains of the same pathogenic species, lead to identification of effectors and their core and lineage- specific effector repertoires, allowing to investigate effector dynamics and evolution. Gibriel *et al.* (2016) provided a fascinating review of some pathogen effectors identified in recent years through genomic approaches. Effectors can be used as tools in screening for disease resistance and for monitoring of effector diversity in pathogen populations. Knowledge of his diversity can lead to more focused and cost-effective management options which can be deployment of genes for host resistance or RNAi technologies. This more specifically can be applied in rapidly evolving plant pathogens such as *Puccinia* spp. causing wheat rusts. Earlier, genome comparison of multiple strains of the vascular wilt pathogen, a necrotroph *Verticillium dahliae* identified large lineage-specific genomic regions, that are only present in a subset of the *V. dahliae* population. These regions are enriched for secreted, *in planta* induced effector candidates that contribute to plant colonization (de Jonge *et al.,* 2013). Kiran *et al.* (2016) has decoded the genome of leaf rust of wheat races 77 and 106 which has helped in unraveling the molecular basis of its evolution and in identification of genes responsible for its various biological functions.

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Pathogenic and Molecular Variability among Pathotypes of *Puccinia* spp in India

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Wheat rusts are one of the major concerns for sustained wheat production globally. The rust pathogens, *Puccinia graminis* Pers. f. sp. *tritici* (*Pgt*) causing black (stem) rust, *P. triticina* Erikss & Henn (*Pt*) causing brown (leaf) rust and *P. striiformis* Westend f. sp. *tritici* (*Pst*) causing stripe (yellow) rust, are obligate biotrophic, macrocyclic and heteroecious fungi, and require graminaceous hosts for asexual or major and alternate hosts for sexual or minor phase. Geographically, in North part of India, brown and yellow rusts are capable of causing loss to wheat production, whereas in Central and Southern part, black and brown rusts are of more frequent occurrence. It is well documented that Yellow rust affects 10–12 m ha in North India, whereas black rust affects 7–8 m ha in Peninsular, Central India and other adjoining areas (Bhardwaj and Singh, 2019a; Bhardwaj *et al.,* 2019b). Leaf rust occurs throughout the wheat growing areas, but it appears late in the season so not a major problem in the main wheat belt of northern India. Historical account of wheat rust epidemics in India has been given by Nagarajan and Joshi (Nagarajan and Joshi 1975). Wheat rust epidemics were reported in Jabalpur as early as 1786 and later reported from Central India during 1879 and 1894-95. During 1946-47 epidemic of black rust occurred in central India and in 1948-49 in southern district of Bombay state. In the main food bowl belt of India, rust epidemics have observed around 1843 at Delhi and during 1884 and 1895 at Allahabad, Banaras and Jhansi. Significant yield losses dues to both brown and yellow rusts during 1971-73 in the western part of Uttar Pradesh have been reported. According to our estimates, meagre 5% yield losses due to rusts could result a loss of Rs. 39,200million (Bhardwaj *et al.,* 2016). As a consequence, rust attack on wheat becomes endless problem for successful cultivation. Considering the fast-evolving and long-distance dispersal nature of rust fungi, continuous efforts for tracking the evolutionary pattern and occurrence of new virulences of wheat rust pathogens together with development of broad spectrum rust resistant varieties is required. Over the years in India, significant research progress has been made in understanding the pathogenic and molecular variability among pathotypes of *Puccinia* species. In this domain, major contribution was played by ICAR-IIWBR, Regional station, Shimla for documenting the virulence pattern of different pathotypes of wheat rust pathogen since 1930s. At present, 150 pathotypes of wheat rusts have been maintained in a national repository with varying avirulence/virulence structure. At genomic level, employing next-generation DNA sequencing methodologies complete genome sequences of fifteen pathotypes of brown rust and three pathotypes of yellow rust have been generated, which provided inventory of more than 600 candidate effectors in the *Puccinia* spp. (9, 10). High quality draft genome sequences of fifteen *Pt* {77, 109R63 (77-1), 109R31-1 (77-2), 125R55 (77-3), 125R23-1 (77-4), 121R63-1 (77-5), 121R55-1 (77-6), 121R127 (77-7), 253R31 (77-8), 121R60-1 (77-9), 377R60-1 (77-10), 125R28 (77-11), 109R31 (77A),109R23 (77A-1) and 0R9 (106)} and three *Pst* {67S64 (31), 47S102 (K) and 46S119) pathotypes have been available in NCBI database. Besides this, significant efforts have been made to understand the molecular basis of *Lr24* mediated leaf rust resistance in wheat to *Pt* pathotype 121R63-1 (77-5), where the association of pathogenesis related proteins (PR proteins), systemic acquired resistance (SAR) and sugar transport-related proteins, *Pt* sRNAs has been established, which act as deciding the spectrum of virulence during wheat-*Pt* interaction (Dubey *et al.,* 2019; Savadi *et al.,* 2018). Moreover, novel sRNAs in *Pt* including two pt-mil-RNAs has been identified which contribute significantly to both growth and pathogenicity in *Pt*.

With the discovery of gene for gene hypothesis, numerous methodologies, using near isogenic lines (NILS) as differentials lines were preferred widely to study the physiological specialization in rust fungi. Globally, different types of systems of analysis and pathotype designation were devised, but few of them follow analogous system, which assisted ineffective communication and scientific knowledge translation. For instance, in Canada and USA and, a series of near isogenic lines (NILs) in a Thatcher (*T. aestivum*) background are employed as differentials to characterise the rust pathotypes (Long and Kolmer, 1989). This concept of using Thatcher NILs, where each containing a single *Lr* gene for resistance was first time described by Dr. Peter Dyck at the Cereal Research Centre, Agriculture and Agri-Food Canada in Winnipeg and later improvised as standard wheat leaf rust differentials throughout the globe. Similar scientists in Nepal, Bangladesh, Pakistan and other countries have their own systems, but pathotypes are reported using the North American code. In contrast, in India, two types of nomenclature systems (old and new) are adopted to name a particular rust pathotype. Initially, the variations in wheat rust species was assessed using international differentials and the pathotypes were named as per serial number based on the

virulence/avirulence of rust isolates on the international differential sets. With the adoption of Indian differential sets and binomial nomenclature system, each pathotype is being classified according to binary notation system (Nagarajan et al. 1983). Based on the experience on wheat rusts in India and systems proposed in other countries, a system for the analysis of brown rust pathotypes in India was proposed by Nagarajan *et al.* (1985). Indian system of wheat rust pathotype designation has 3 sets of differentials (Table 1). Set '0' contains popular cultivars of bread wheat and durum, a susceptible cultivar and a resistant line to act as a healthy check. Set 'A' has near isogenic lines (NILs) having rust resistance genes of respective wheat rusts, singly or in combinations, which are able to discriminate among the pathotypes; whereas Set 'B' has lines from the classic differentials or some other lines included to cater the present-day requirements of pathotype identification. Each pathotype is being classified according to binary notation system of pathotype nomenclature (New System). It is worthwhile to mention here that infection types on set 'A' and 'B' of differential lines form the basis of nomenclature, while set '0' gives the response of tested cultivars. In exceptional circumstances when characteristics distinction is not possible on the lines in Set 'A' and Set 'B', Set '0' is also considered for naming of pathotypes. The lines in Set 'A' and Set 'B' were arranged on the basis of their increased resistance to the available pathotypes except for *Lr24* in set 'A' of brown rust and Tc*6/*Lr26* in Set 'B' of yellow rust. The decanary procedure follows raising a number to the base 2, and the sum of the decanary value of any two pathotypes will not be identical.

Table 1: Set of differentials for the identification of wheat rust pathotypes in India

Set-0		**Set-A**	**Set-B**
Leaf or Brown rust (*Puccinia triticina*)			
IWP 94 (*Lr*23+)		*Lr*14a	Loros (*Lr*2c)
Kharchia Mutant (*Lr*9+)		*Lr*24	Webster (*Lr*2a)
Raj 3765 (*Lr*13+10+)		*Lr*18	Democrat (*Lr*3)
PBW 343 (*Lr*26+)		*Lr*13	Thew (*Lr*20)
UP 2338 (*Lr*26+34+)		*Lr*17	Malakoff (*Lr*1)
K 8804 (*Lr*26+23+)		*Lr*15	Malakoff (*Lr*1)
Raj 1555		*Lr*10	Benno (*Lr*26)
HD 2189 (*Lr*13+34+)		*Lr*19	HP1633 (*Lr*9+)
Agra Local		*Lr*19	
Stem or Black rust (*Puccinia graminis tritici*)			
*Sr*24 (3D/Ag Translocation)		*Sr*13 (Khapstein/10Marquis)	Marquis (*Sr*7b+)
NI 5439		*Sr*9b (CS/KF 2B)	Einkorn (*Sr*21+)
*Sr*25 (7D/ Ag Translocation)		*Sr*11 (ISr11Ra+W3015)	Kota (*Sr*28+)
DWR 195		*Sr*28 {Kota; + *Sr*28 (CH71)}	Reliance (*Sr*5+)
HD 2189		*Sr*8b (ISr8aRa + W3384)	Charter (*Sr*11+)
Lok 1		*Sr*9e (Vernstein)	Khapli (*Sr*7a+)
HI 1077		*Sr*30 (Festiguay)	Tc*6/*Lr26* (*Yr*9)
Barley Local		*Sr*37 (Line W)	
Agra Local			
Stripe or Yellow rust (*Puccinia striiformis tritici*)			
WHEAT	BARLEY		
WH 147	TDT	Chinese 166 (*Yr*1)	Hybrid 46 (*Yr*4)
Barley local	Barley local	Lee (*Yr*7, 22, 23))	Heines VII (*Yr*2+)
WH 416	WH147	Heines Kolben (*Yr*2, 6)	Compair (*Yr*8)
PDW 215	Heils Franken	Vilmorin 23(*Yr*3a, 4a)	*T. spelta album* (*Yr*5)
HD 2329	Fong Tien	Moro (*Yr*10)	Tc*6/*Lr26* (*Yr*9)
HD 2667	Himani	Strubes Dickkopf (*Yr*2, 3a, 4a)	Sonalika (*Yr*2+)
PBW 343 (*Yr*9, 27)	BHS 16	Suwon92 X Omar (*Yr*SU)	Kalyansona *Yr*2(KS)
HS 240 (*Yr*9)	Alfa 93	Riebesel47/51(*Yr*2, 9+)	
Anza (*Yr*A)	Dolma		

Understanding of rust pathogen population diversity, pathotype structure, and genetic lineages are extremely important for effective disease management through strategic deployment of rust resistance genes (varieties). Moreover, monitoring wheat rust pathotypes by a systematic surveillance programme is the most effective background tool to perform pre-emptive resistance breeding in wheat. Hence, a close disease monitoring system named as wheat disease monitoring nursery or Trap Plot Nursery was initiated

in India during 1967-68. It is an effective tool for monitoring the occurrence of wheat diseases especially rusts across different wheat growing zones of India. It also provides useful information on performance of newly evolved varieties and epidemiological aspects of wheat rusts. Moreover, regular extensive surveys for monitoring population diversity and pathotype structure of wheat rust pathogens are being conducted in India and neighbouring countries. These surveys also assisted in tracking the evolution of new and potentially damaging pathotypes and in determining the effectiveness of the resistance genes in wheat cultivars.

Population diversity of rust pathogens has been monitored effectually for decades using differential sets of host genotypes expressing known rust resistance gene(s). These investigations have brought out the understandings of the population dynamics of rust pathogens as well as their evolutionary and migratory routes. Nevertheless, differential sets have limited genetic resolution, and they are used regionally, making global tracking difficult. Such differential sets-based monitoring could be supplemented and limitations thereof be overcome by genomic DNA-based approaches, where even non-viable samples can be processed to get comparative global analysis and monitoring of rust pathogens. The study of population diversity, pathotype structure and genetic lineages among pathotypes causing yellow, brown and stem rust using molecular markers is an important area for inferring the impact of different forces that influence the pathogen evolution. It improves the understanding of pathogens' evolutionary patterns and allows prediction of pathogen evolution in agricultural ecosystems. Among several DNA-based molecular markers employed in the understanding of population dynamics and evolutionary pattern of plant pathogenic fungi, simple sequence repeat (SSR) or microsatellites are most widely accepted owing to their multiallelic, reproducible, and highly polymorphic nature. With the availability of genome sequence data, the use of gene-based functional markers (FMs) for studies on population genetics has augmented population diversity studies. Population diversity, pathotype structure and genetic lineages were determined for *Pgt* pathotypes from Indian-subcontinent using microsatellites markers (Table 2).

Table 2: Major molecular studies related to variability analysis of races of *Puccinia* spp in India

Title	Reference
Rust pathogen effectors: perspectives in resistance breeding	Prasad *et al.* (2019)
Discovery and profiling of small RNAs from *Puccinia triticina* by deep sequencing and identification of their potential targets in wheat.	Dubey *et al.* (2019)
Virulence and molecular analysis of atypical pathotypes of yellow rust pathogen in India	Gangwar *et al.* (2019b)
Characterization of three new *Yr9*-virulences and identification of sources of resistance among recently developed Indian bread wheat germplasm	Gangwar *et al.* (2019a)
Population distribution and differentiation of *Puccinia graminis tritici* detected in the Indian subcontinent during 2009-2015	Prasad *et al.* (2018)
Dissection of genomic features and variations of three pathotypes of *Puccinia striiformis* through whole genome sequencing.	Kiran K *et al.* (2017)
Population differentiation of wheat leaf rust fungus *Puccinia triticina* in South Asia	Prasad *et al.* (2017)
Draft genome of the wheat rust pathogen (*Puccinia triticina*) unravels genome-wide structural variations during evolution	Kiran *et al.* (2016)
Molecular characterization of Indian pathotypes of *Puccinia striiformis* f. sp. *tritici* and multigene phylogenetic analysis to establish inter- and intraspecific relationships	Aggarwal *et al.* (2018)

At whole genome level, comparative analysis revealed that the brown rust pathotype 45R31 (77) as a highly variable and adapted as compared to pathotype 0R9 (106) (Kiran et al. 2017). This is due to the fact that a higher percentage (5.52%) of recent segmental duplication was observed in the pathotype 45R31 (77) genome, compared with 3.86% in the pathotype 0R9 (106). Generally, segmental duplication is linked with genome organization and evolution and contributes to the genomic instability. Therefore, the relatively high number of segmental duplications in pathotype 45R31 (77) is likely to be a major factor in the evolution of large numbers of pathotypes in its lineage. Phylogenetic analysis of the 13 pathotypes of pathotype 45R31 (77) lineage on the basis of whole genome sequences, demonstrated that the relationships among these strains are associated with their virulence features on different *Lr* genes.

In case of brown rust pathotypes, whole genomes of three {47S102 (K) and 46S119 (Highly virulent) and 67S64 (31) (Least virulent)} pathotypes using next-generation sequencing technology revelled presence of more than 36% mobile repetitive elements in all the three genomes, where majority being contributed by

LTR retrotransposons (Kiran *et al.,* 2016). Similarly, a higher percentage of SDs (~6.47) was identified in the pathotype 46S119 genome, compared to other two pathotypes. SNP analysis revealed that pathotype 46S119 is potentially more variable than the much older races 47S102(K) & 67S64 (Bhardwaj *et al.,* 2019).

Research Challenges and Conclusions

Rust pathogens are highly dynamic in nature and undergo faster evolution even in the absence of sexual recombination phase in majority of wheat growing regions. Mutation, somatic hybridization, parasexual recombination and introduction are some of the guiding forces that helps rust pathogen evolution in the absence of sexual phase. Newly evolved aggressive and virulent isolates of these pathogen cause resistance breakdown of previously effective host resistance genes. Moreover, rust pathogens are believed to have ability to travel long distances, which helps them to move from one continent to the other through air current. Efforts to monitor changes in rust pathogens variability and to detect new virulences using host differentials are being done for years now, which have improved insights into the population dynamics of rust pathogens. Such understandings of pathogen populations including their evolutionary and migratory patterns have been utilized strategically to apprise rust resistance breeding programs. However, limited genetic differentiation associated with the use of differential sets along with their region-specific adoption makes these differentials less favorite for global tracking of rust pathotypes. Therefore, molecular marker assisted differentiation of rust pathotypes will be one of the ways to overcome the limitation of wheat differentials for rust pathogen tracking. Even with the molecular marker based genetic differentiation of rust pathogen population and their migration assessment, pure isolates of a pathogen are required to genotype them. Therefore, precise and meaningful genotyping of rust isolates to explore their evolutionary and migratory pattern, which decides rust resistance breeding approaches, remained one of the most challenging tasks of the plant pathologist.

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Multilocus Sequence Typing of Fungal Plant Pathogens

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Introduction

MLST tool using partial sequence analysis of seven to ten housekeeping genes has become the most popular typing approach for genetic diversity and epidemiological investigations of fungi and other microorganisms. In this approach, for each locus studied, different genetic sequences present within a species are assigned as distinct alleles. The combination of the identified alleles at each of the loci defines the allelic profile or sequence type for each isolate. The sequence generated can be used to determine whether the fungal populations are clonal or have undergone recombination patterns. This sequence-based approach produces in unambiguous, reproducible results and can be used to compare different isolates of a pathogen. MLST has been applied to many pathogenic microorganisms, and there is increasing interest in the variation among isolates and within microbial populations, especially in studies of microbial evolution, pathogenesis. Many studies have been used the MLST approach for detection disease outbreaks. Although MLST is simpler, faster, and less expensive than whole genome sequencing, it is more costly and time-consuming than less reliable genotyping methods.

Principle

MLST measures the DNA sequence variations in a set of housekeeping genes and characterizes strains by their unique allelic profiles. The principle of MLST is simple; the technique involves PCR amplification followed by DNA sequencing. Nucleotide differences between strains can be checked at a variable number of genes depending on the degree of discrimination desired.

Selection of MLST Fragment and Primer

The seven genes like Eukaryotic translation initiation factor 3 subunit A, Beta Tubulin, Actin-related protein 2, Phosphoglycerate kinase, Glyceraldeyde-3-phosphate dehydrogenase (Gapdh), Serine/threonine-protein kinase (STPK), Calcium-transporting ATPase were selected for genotyping. Primers were designed using IDT Oligo Analyzer. The designed primers were synthesized from GCC Biotech Pvt., India.

PCR Amplification and Sanger Sequencing

PCR was carried out for each gene. Briefly, in 25µl reaction volume consisting of 200 ng of genomic DNA, 200 µmol/L dNTP mix (dATP, dGTP, dCTP, dTTP), 0.1 µmol/L each primer, 3.5 mmol/L $MgCl_2$, 1.5 U *Taq* DNA polymerase, 9.5 µl water and 1X *Taq* buffer in a thermal cycler (Bio-Rad Laboratories India Pvt Ltd). The PCR conditions used were: 95°C for 5 min – initial denaturation; 95°C for 1 min – denaturation for 35 cycles at 55°-59°C for 30 sec –annealing (gradient); 72°C for 1 min – extension 72°C for 7 min – final extension. The amplified PCR products of each gene were sequenced. All the sequences need to be trimmed, edited, annotated and submitted to Genbank.

Phylogenetic Analysis

Sequences were trimmed accordingly and used for phylogeny. The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model. The bootstrap consensus tree inferred from 1000 replicates was taken to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pair wise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value or Mega 6.04 (Tamura *et al*., 2013).

Single Nucleotide Polymorphism Analysis

The FASTA files and PDF files were generated for sequenced genes. Single nucleotide polymorphism analysis was conducted with the following whole genome sequences 1. ENA|OAJ04384|OAJ04384.1 *Tilletia indica* actin-related protein 2 2. ENA|OAJ05357|OAJ05357.1 *Tilletia indica* tubulin beta chain 3.

A|OAJ00852|OAJ00852.1 *Tilletia indica* hypothetical protein 4. ENA|OAJ06275|OAJ06275.1 *Tilletia indica* Glyceraldehyde-3-phosphate dehydrogenase 5. ENA|OAJ01891|OAJ01891.1 *Tilletia indica* hypothetical protein 6. ENA|OAJ06449|OAJ06449.1 *Tilletia indica* phosphoglycerate kinase 7. ENA|OAJ03744|OAJ03744.1 *Tilletia indica* hypothetical protein. Single nucleotide polymorphisms (SNPs) were identified using Single Nucleotide Variant tool in CLC Genomics workbench 9.0.

Case Study in *Tilletia indica* (Karnal Bunt of Wheat)

MLST genotyping, seven multilocus sequence fragments were selected to differentiate and characterize these *T. indica* isolates. A phylogenetic tree was constructed based on pooled sequences of actin-related protein 2 (ARP2), β-tubulin (TUB), eukaryotic translation initiation factor 3 subunit A (EIF3A), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), histone 2B (H2B), phosphoglycerate kinase (PGK) and serine/threonine-protein kinase (STPK). Sequence based phylogeny indicated that the population of *T. indica* was highly diverse. The isolates of *T. indica* did not form clusters based on their region of origin (Fig. 1). Each gene and isolate-wise, mostly the frequency of single nucleotide polymorphisms (SNPs) was high. This genetic diversity information of *T. indica* will be helpful for epidemiological investigations and for developing management strategies of the Karnal bunt of wheat.

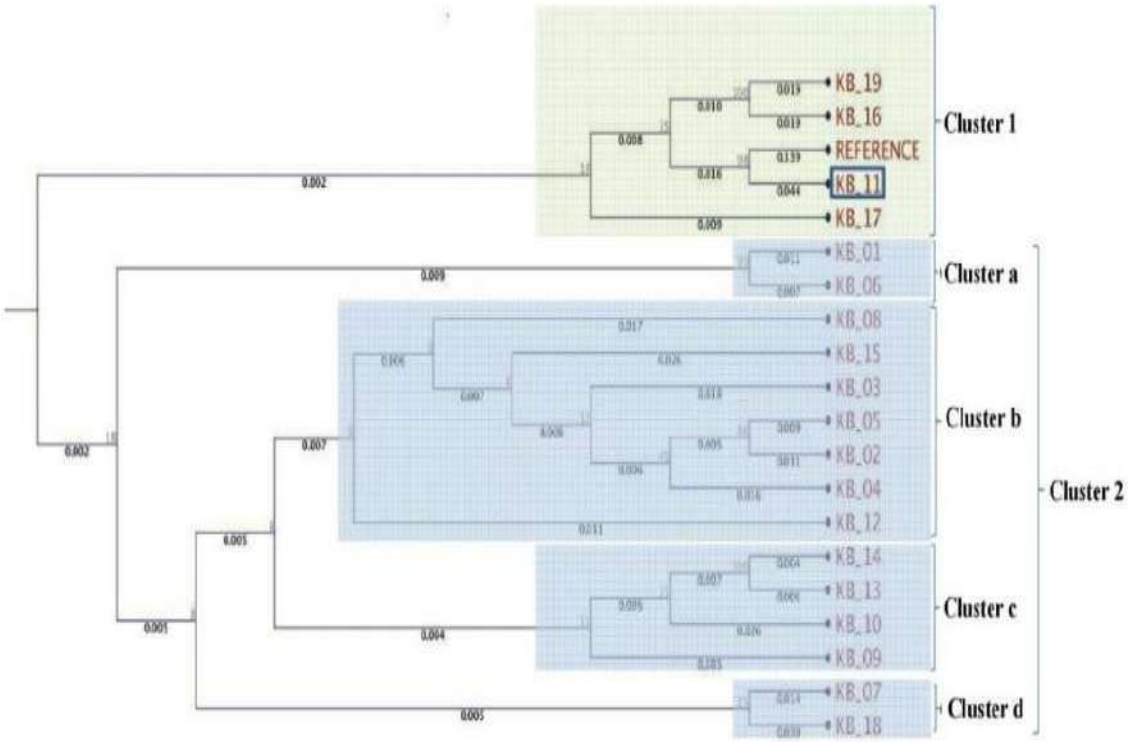

Fig. 1. Molecular phylogenetic analysis of T. indica isolates using seven gene sequences

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Development of PCR-based Assay for Detection of *Puccinia striiformis tritici* causing Stripe Rust in Wheat

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Introduction

Accurate and rapid identification of the organism(s) that cause plant diseases is of utmost importance for effective disease management. Conventional methods rely on identification of disease symptoms, isolation and culturing of organisms; and laboratory identification by morphology and biochemical tests. These techniques are used in describing the causal agents of diseases of various crops, however they are too variable, slow, and labour intensive to be of much use in most of the investigations. New, rapid screening methods such as DNA/RNA probe technology and polymerase chain reactions (PCR) technology are being increasingly used in all aspects of fungal diagnostics. The greatest efforts have been on the development of diagnostics based on antibodies and nucleic acid technologies. Nucleic acid-based methods (using probes and/or PCR) have increasingly been used in recent years to develop diagnostic assays for plant pathogens. These methods, particularly those based on PCR, are potentially very sensitive and specific.

Two general approaches are used to select target DNA sequences for use in diagnostics: one is to develop a method using known conserved genes, common to all fungi, but which have useful sequence variation within them that can be exploited; the other is to screen random parts of the fungal genome to find regions that show the required specificity. The main DNA region targeted for diagnostic development is ribosomal DNA. Ribosomal DNAs (rDNAs) have a number of useful features that make them well suited as targets for diagnostic methods. They are present in all organisms at high copy number and this allows very sensitive detection. Other genes are also becoming more widely used as targets for diagnostic development and pathogen characterization. The β-tubulin genes are perhaps the second most common targets for diagnostic development in fungi. Targets for development of a diagnostic method can also be sought by screening random regions of the genome to find sequences that are unique to a particular taxon. This was traditionally done by cloning fragments of the pathogen genome and testing individual clones (by DNA probing) to find the ones that are specific to the required target. These specific regions of DNA could then be sequenced to design primers for PCR-based diagnostics. PCR-based techniques such as RAPD (random amplified polymorphic DNA)/ URP (universal rice primers) analysis has been used to generate randomly amplified fragments from the genome. These are then separated by gel electrophoresis and the patterns of bands from related fungi are compared. Any potentially diagnostic bands are then sequenced and used to design specific SCAR (sequence characterised amplified region) primers.

Case Study

Stripe rust is considered as the current major rust disease affecting winter cereal production across the world. A quick, reliable PCR-based marker was developed here to detect, identify and rapidly monitor *Puccinia striiformis* f. sp. *tritici* (Pst) in wheat-growing areas (Table 1). Three respective sets of primers (Table 2), designed from β-tubulin, squalene monooxygenase and ketopantoate reductase genes selected from the full genome of *Puccinia striiformis* f. sp. *tritici*, amplified sequences of 239, 358 and 1518 bp, respectively, in Pst pathotypes. A fragment of 1518 bp unique to Pst pathotypes was amplified using primer set PstKeto F1_30/Pst KetoR1_1547 and distinguished the pathogen clearly from different *Puccinia* spp. and other fungal pathogens. The detection limit of the marker (KetoPstRA1500, accession no. KU240073) by conventional PCR assay was 10 pg. This marker could detect the pathogen in the host before symptom expression. The sensitivity and utility of the marker were further enhanced in a qPCR-based assay that was developed with a newly designed primer set PstKeto F1_1246/Pst KetoR1_1547, which amplified a product of 302 bp and detected as little as 10 fg of DNA. This PCR/qPCR based marker is suitable. A rapid PCR based diagnostic marker has been identified to distinguish the fungus from other rust pathogens of wheat and other fungi and to quantify the inoculum load on resistant/susceptible cultivars.

Table 1: List of pathotypes of three wheat rusts and other pathogens taken in the study

Sl.No.	Pathogen	Pathotype/ isolate	ITS accession
1	*Puccinia striiformis* f.sp. *tritici*	38A	KT305926
2	*P. striiformis* f.sp. *tritici*	19	KX061103
3	*P. striiformis* f.sp. *tritici*	K (47S102)	KT320891
4	*P. striiformis* f.sp. *tritici*	78S84	JQ360860
5	*P. striiformis* f.sp. *tritici*	46S119	JQ360861
6	*P. striiformis* f.sp. *tritici*	A (70S4)	KT320892
7	*P. striiformis* f.sp. *tritici*	20A (70S64)	KT320893
8	*P. striiformis* f.sp. *tritici*	31 (67S64)	KT320894
9	*P. striiformis* f.sp. *tritici*	I (38S102)	KT320895
10	*P. striiformis* f.sp. *tritici*	14	KX061104
11	*P. graminis* f.sp. *tritici*	40A	JQ360859
12	*P. graminis* f.sp. *tritici*	40-1	JQ360858
13	*P. triticina*	77-5	JQ360857
14	*P. triticina*	12-2	JQ360857
15	*P. triticina*	104-2	JQ360862
16	*Aspergillus niger*	ITCC 6117	–
17	*Bipolaris sorokiniana*	Bs75	HM195259
18	*B. oryzae*	Bo1	KU499526
19	*Fusarium graminearum*	ITCC 3437	–
20	*Blumeria graminis* f. sp. *tritici*	–	–
21	*Tilletia indica*	KB-1	KX369242
22	*Rhizoctonia solani*	ITCC 4110	–

Table 2: Primer sets designed from gene-specific sequences available in NCBI database for conventional PCR and qPCR

Gene	Primers	Primer sequence	Amplicon size (bp)	Tm
β-Tubulin	Pst Bt_212	5′CAA TCA CCG TCC CAG AGT TGACA3′	239	57.8
	Pst Bt_450	5′TAA TCA TAA GCC GAC ATG AGA GAA AC3′		54.8
Squalene monooxygenase	Pst Mono F1_1439	5′CAA CGT AAA AAA TCT TCT AC3′	358	46.1
	Pst Mono R1_1518	5′TGG CCA GAT TTA AAGAA3′		45.1
Ketopantoate reductase (conventional PCR)	Pst Keto F1 _30	5′TCA TCA ACA ACA ATC TAC 3′	1518	43.9
	Pst Keto R1_1547	5′CTA CTT TAT GTC AGT GTC T3′		51.3
Ketopantoate reductase (qPCR)	Pst Keto F1_1246	5′TCA CGA AAT CCT CCA AAA ATC3′	302	53.4
	Pst Keto R1_1547	5′ CTA CTT TAT GTC AGT GTC T3′		55.0

The materials and protocol for development of SCAR marker are as follows:

Materials

Chemicals CTAB buffer, chloroform, isoamyl alcohol, alcohol, chloroform, isopropanol, TE buffer, primers, dNTPs, MgCl2, Taq Polymerase, DD water, PDA (Potato dextrose agar) and PDB (Potato dextrose broth).

Glassware and plastic ware: Oakridge tube, 250 ml, borosil flasks, eppendorf tube, PCR tubes, micropipettes, tips, test tubes, petri plates.

Protocols

Multiplication of rust fungi and cultivation of other pathogens

Fifteen pathotypes of wheat rusts comprising three leaf rust pathotypes (77-5, 12-2 and 104-2), two stem rust (40A and 40-1) and 10 stripe rust pathotypes (38A, 19, K, A, 20A, 31, I, 14, 78S84 and 46S119) were collected. Monouredial cultures of these pathotypes were established and multiplied on susceptible genotype and inocula of all the pathotypes were stored in glass vials at −40 °C for further studies. Likewise, *Blumeria graminis* f. sp. *tritici*, was also maintained on the susceptible host. Other pathogens—*Aspergillus niger* (6117), *Bipolaris oryzae* (Bo1), *Fusarium graminearum* (3437), *Tilletia indica* (KB-1), *Bipolaris sorokiniana* (BS75), *Rhizoctonia solani* (4505)—were cultivated on potato dextrose broth (PDB) at 25 °C for a week. After incubation, mycelium was collected on sterilized filter paper and thoroughly washed with distilled water. This mycelium was frozen in liquid nitrogen and stored at −40 °C until DNA extraction.

Isolation of fungal genomic DNA: DNA was extracted from all isolates of *Puccinia striiformis* and other rust pathogens associated with wheat DNA by CTAB method.

PCR amplification

DNA from all isolates was amplified using primers. Steps given as under:

- Set up a 25 µl reaction in a 0.2 ml microfuge tube as follows

Template DNA (50 ng)	1.0 µl
10 X Taq polymerase buffer	2.5 µl
MgCl2 (25 mM)	1.5 µl
dNTPs (10 mM)	0.5 µl
Primer (10µM):	0.5µl
Taq Polymerase (5 U/µl)	0.5 µl
Autoclaved DD Water	18.5 µl

- Spin briefly in a microfuge to mix.

S.No.	PCR steps	**β-tubulin**	**6-squalene**	**Ketopentoreductase**
1	Initial denaturation	94ºC for 5min	94ºC for 5min	94ºC for 5min
2	Denaturation	94ºC for 40 sec	94ºC for 40 sec	94ºC for 40 sec
3	Annealing	56ºC for 40 sec	50ºC for 40 sec	48ºC for 40 sec
4	Extension	72 ºC for 1min	72 ºC for 1min	72 ºC for 1min
5	final extension	72ºC for 7min	72ºC for 7min	72ºC for 7min

- The mixture was then placed in PCR machine (i Cycler, BIO-RAD).
- Performed 35 cycles of PCR using the following temperature profile-
- Reactions were stopped at 4ºC

Gel electrophoresis: All PCR products were electrophoresed.

Selection of primers showing monomorphic bands in all pathotypes.

Gel elution: Selected monomorphic band was gel eluted using gel elution kit of Qiagen. Then this product is used for cloning purposes.

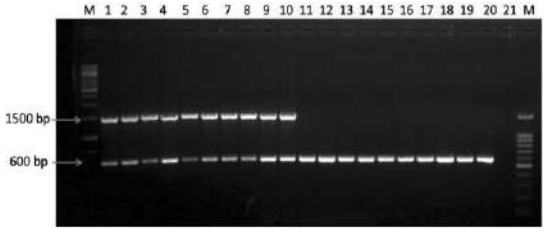

Fig. 1. Agarose gel showing PCR band amplified with *Puccinia striiformis* f. sp. *tritici*-specific marker (PstKetoF1_30 and Pst Keto R1_1547) and ITS universal primers (ITS 1 and ITS 4). *Lanes from left to right M* 1 kb molecular marker (Fermentas); *1–10* DNA templates of different *P. striiformis* f. sp. *tritici* pathotypes; *11 P. graminis* f. sp. *tritici* (40A); *12 P. triticina* (77-5); *13 Aspergillus niger* (6117); *14 Bipolaris sorokiniana* (Bs75); *15 Blumeria graminis* (=*Erysiphe graminis*); *16 Fusarium graminearum* (3437); *17 Tilletia indica* (KB-1); *18 Bipolaris oryzae* (Bo1); *19 Rhizoctonia solani* (4110); *20* wheat leaf; *21* sterile water; M: 100 bp molecular marker (Fermentas)

Cloning and Sequencing

Cloning of PCR product in pGEM-T Easy Cloning Vector

pGEM®-T Easy vector (3015 bp) from Promega is convenient system for the cloning of PCR products (Fig 2). This vector has single 3'-T overhangs at the insertion site which greatly improve the efficiency of ligation of a PCR product into the plasmids by preventing recircularization of the vector and providing a compatible overhang for PCR products generated by Taq polymerase which often add a single deoxyadenosine in a template independent fashion to the 3'end of the amplified fragments. Successful cloning of an insert in the pGEM®-T Easy vectors interrupts the coding sequence of β- galactosidase; recombinant clones are identified by colour screening on indicator plates containing Xgal and IPTG.

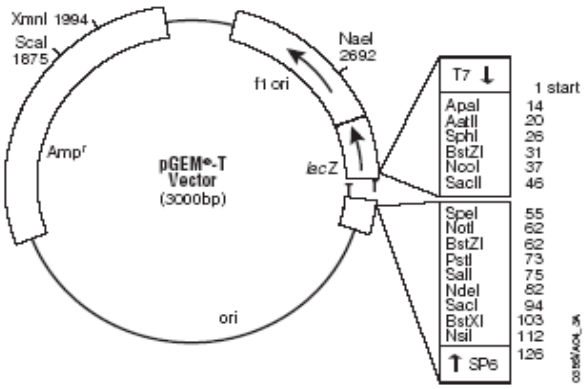

Fig. 2. pGEMT vector Map and Sequence Reference point

a. Optimization of insert: Vector molar ratios

1:3 ratio of the vector to DNA insert provided good result. The concentration of PCR product was estimated by comparing with DNA molecular weight markers on a agarose gel.

b. Ligation of PCR product to pGEM-T Easy cloning vector DNA

Ligation reactions were carried out between vector and PCR product. The reaction mix was prepared as follows:

pGEM-T Easy vector (50 ng/µl)	1 µl
PCR amplified product (50 ng/µl)	x µl
2X Ligation buffer	5 µl
T4 DNA ligase (3U/µl)	1 µl
Sterile distilled water	x µl
Total vol.	10 µl

Despite of standard reaction mix, positive and negative control mix were prepared by adding control insert and no DNA in ligation mix respectively. The ligation mixtures are incubated at 4ºC for 18hours.

c. Preparation of competent cells

The competent cells are prepared by modified CaCl2 method described by Mandel and Higa (1970).

- 50 ml Luria Broth (LB) was inoculated with overnight grown culture of XLblue strain of *Escherichia coli* and incubated at 37ºC for 1 h. and 15 min. with constant shaking at 200 rpm in a shaker incubator till the bacterial growth as measured by optical density reached 0.3 O.D. at 600 nm
- The culture was then aseptically transferred to 40 ml sterile screw capped tubes and kept on ice for 10 min.
- The cells were centrifuged at 4000 rpm for 10 min. at 4ºC in a Sigma 3K30 centrifuge to pelletize the cells.
- Decanted supernatant and re-suspend pellet gently in 10 ml ice cold buffer/100 ml culture aseptically.
- Kept on ice for 1 h.
- The cells were recovered by centrifuging at 3500 rpm for 10 min at 40C.
- Decanted supernatant, re-suspended pellets in 2 ml of chilled buffer and used for transformation after keeping on ice for 1 h.
- Buffer composition: CaCl2 (60mM), PIPES (Piperazine-N, N-bis (2- ethane sulphonic acid) 10 mM (pH 7.0), Glycerol (15 %).

d. Transformation of competent cells

- 200 µl competent cells were added to 20 µl of the ligation mixture in a sterile microfuge tube and was gently mixed and kept on ice for 1 h.
- Then it was given a heat shock at 42ºC for 90 sec. Incubated on ice for 5 min. 1 ml of LB medium was then added and the transformants were allowed to grow at 37ºC for 1 h in shaker incubator at 200 rpm.
- 200 µl of cell suspension were aseptically plated on Luria Agar (LA) plate containing ampicillin, X-gal and IPTG (50 µl of 50 µg/ml ampicillin; 100 µl of 2% X-gal and 10 µl of 0.1 M IPTG in 50 ml LA).
- The plates were incubated overnight at 37ºC.

e. Selection of transformants

The transformants were selected on the basis of blue/white colonies. The white colonies were selected and subsequently streaked on LA (Luria agar) Plates containing IPTG, X-gal and ampicillin.

f. Rapid screening of recombinant by colony PCR Method

Using same primer, white colonies were further screened by polymerase chain reaction. In this case a single colony was taken in each reaction mix in lieu of DNA sample.

Following was the colony PCR reaction master mix.

Set up a 25 µl reaction in a 0.2 ml microfuge tube

Template DNA (50 ng)	1.0 µl
10 X Taq polymerase buffer	2.5 µl
MgCl2 (25 mM)	1.5 µl
dNTPs (10 mM)	0.5 µl
Primer (10µM)	0.5µl
Taq Polymerase (5 U/µl)	0.5 µl
Autoclaved DD Water	18.5 µl

The reaction mixture was then placed in PCR machine. The temperature profile and cycle were same as used in amplification of DNA earlier.

g. Isolation of Recombinant Plasmid DNA by Miniprep Method

Isolation of recombinant DNA was done by modified alkaline lysis method (Brinboim and Doly, 1979) or plasmid isolation kit (Xcelris labs, India) as per instruction manual provided in the kit.

- Selected white colonies, positive in colony PCR reaction were individually inoculated in 5 ml of LB medium containing ampicillin (50 µg/ml) in sterile capped culture tubes.
- Tubes were then incubated overnight at 37ºC at 200 rpm in a shaker incubator.
- The overnight grown bacterial cells were then transferred to 1.5 ml sterile eppendorf tube and cells were harvested by centrifuging in a table top centrifuge for 1 min. Care was taken to remove the medium adhering to the cell pellet.
- The pellet was re-suspended in 100 µl of solution I {25mM Tris HCl (pH 8.0), 50mM Glucose, 10mM EDTA} and mixed vigorously by vortexing.
- The 200 µl of freshly prepared lysis solution i.e. solution II (0.2 N NaOH, 1 % SDS) was then added and mixed gently.
- 150 µl of ice cold solution III (3M Sodium acetate pH 4.8) was then added and mixed gently with lysed cell suspension and the mixture is kept on ice for 15 min.
- The chromosomal DNA and the bacterial cell debris were removed by centrifuging at 10,000 rpm for 20 min, at 4ºC in a table top centrifuge (Sigma 112).
- The supernatant was again centrifuged for another 20 min at 10,000 rpm at 4ºC to pelletize any unwanted bacterial debris.
- The supernatant was taken and equal volume of phenol: chloroform: isoamyl alcohol (25:24:1) was added. It was vortexed well, centrifuged in a table top centrifuge for 15 min. at room temperature.
- The clear aqueous phase was transferred to fresh eppendorf tube.
- The DNA in aqueous phase was precipitated by adding 0.8 volume of isopropanol and kept on ice for 10 min.
- The mixture was then centrifuged at 15000 rpm for 20 min at 4ºC.
- To the pellet 200 µl of 70% ethanol was added. The tube was rotated well so that the pellet from the wall gets suspended in 70% alcohol. This ensured removal of adhering salts by 70% alcohol. DNA was then pelletized by centrifuging at 15000 rpm for 5 min.
- The pellet was finally suspended in 30 µl sterile double distilled water.

Validation of the marker

Specificity and sensitivity of Primers: The universality of the marker was tested by detecting the presence of the pathogen on wheat leaves at different time intervals under artificially inoculated conditions in a greenhouse. Seedlings of wheat cultivar susceptible to pathotypes of *P. striiformis* f. sp. *tritici*, were grown in the greenhouse. Inoculation of stripe rust (pathotype 46S119) was carried out in a temperature-controlled greenhouse at 10–14 °C at the one-leaf stage (10-day-old seedlings). The trays with inoculated seedlings were kept in humid chambers for 48 h in diffuse light. Later, these were transferred to greenhouse benches to allow symptoms to develop. Leaf samples were collected for the PCR assay at 0 h, 8 h, 16 h, 24 h, 48 h, 72 h, 5 days and 10 days after inoculation and stored at −40 °C for further use. Water was sprayed on control (uninoculated) plants.

Reliability of primers: The effectiveness of specific primers was further confirmed using field samples. Symptomatic and symptomless wheat leaves were collected from the Indian Agricultural Research Institute farms. DNA was extracted and PCR amplification was performed with specific primers using the conditions described above.

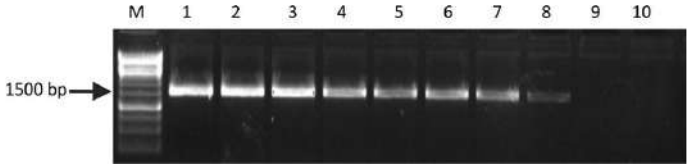

Fig 3. Sensitivity of PCR assays using PstKetoF1_30 and Pst Keto R1_1547 primers set. *Lanes M* 1 kb molecular marker (Fermentas); *1* 100 ng; *2* 75 ng; *3* 50 ng; *4* 25 ng; *5* 10 ng; *6* 1 ng; *7* 100 pg; *8* 10 pg; 9 1 pg as DNA template and *10* sterile water

Design and optimization of qPCR primers for real-time PCR assays

A primer set for PCR marker (KetoPstRA1500), specific for *P. striiformis* f. sp. *tritici*, was designed using the IDT oligoanalyser (https:// www.idtdna.com/Primerquest/Home/Index) and further utilized for qPCR detection assay. An *in silico* test for primer specificity was conducted by running the primer sequence against the nonredundant GenBank data (https://www.ncbi.nlm.nih.gov/blast/) with parameters set for the identification of short, nearly exact matches.

Generation of standard curve

DNA standard curve for qPCR assay was developed from 10-fold serial dilution of *P. striiformis tritici* (46S119) DNA (10 ng) in sterile ultrapure water (SUW). The standard curve was obtained by plotting the Ct values defined by the crossing cycle number versus the logarithm of the quantity of the serially diluted genomic DNA. Linear regression analyses of the logarithm base 10 of known concentrations of target DNA versus Ct values were performed for standard curve. A standard curve was also constructed from amplification of these samples containing both fungus and plant DNA. The amplification efficiency [E = 10(−1/ slope) − 1] was calculated for both the standard curves. The standard regression lines were used as the reference curve for transforming the experimental Ct values into amount of *P. striiformis* f. sp. *tritici* DNA (fg).

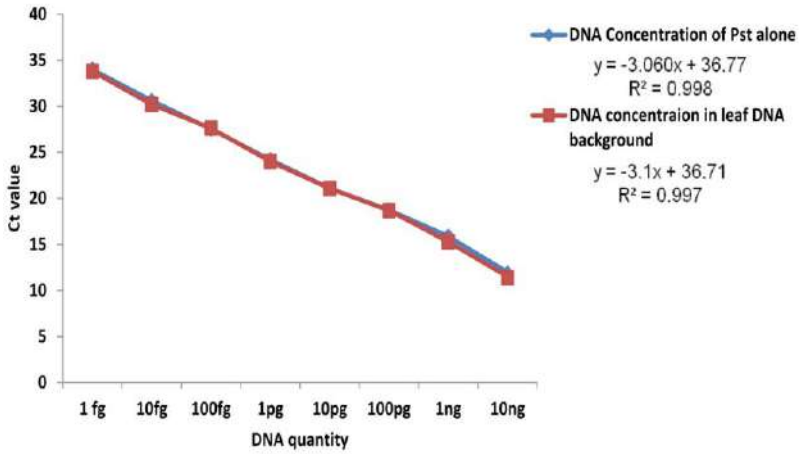

Fig. 4. Standard curve showing the log10 DNA amount (fg) plotted against the real-time PCR cycle threshold (Ct) for different dilutions of pure genomic DNA of Puccinia striiformis f. sp. tritici in sterile ultrapure water (SUW) as well as in fixed background of uninoculated leaf. R2 indicates a linear relationship between the DNA amount and Ct values

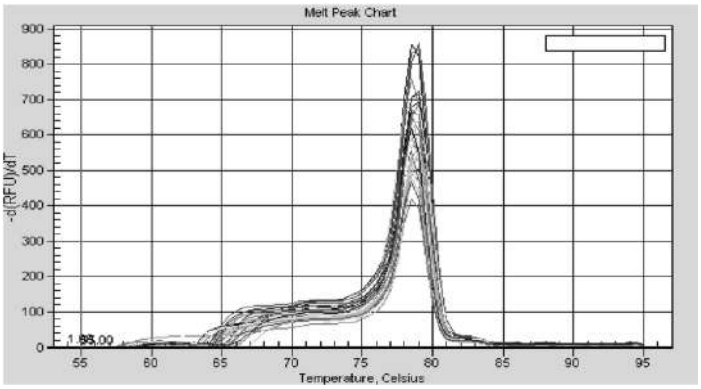

Fig. 5: Melting curve of qPCR products obtained using *Puccinia striiformis* f. sp. *tritici* specific primers. No peak was observed for the negative control. The single peak at 78 °C with *P. striiformis* f. sp. *tritici* (46S119) DNA as the template indicates the specificity of the Primers.

Expected outcome:

The present study led to the development of a species-specific PCR/qPCR marker, with high specificity, sensitivity and rapidity. The markers were useful to quantify spore mass in terms of fungal genomic DNA and evaluation of resistance. The assay would be especially invaluable for quantifying fungal colonization in wheat leaves that become infected but fail to display symptoms or suffer from damage of the disease. The developed assay, therefore, could provide a useful tool for rapid and reliable detection of *P. striiformis* f.sp. *tritici* in diseased plants as well as in field samples, thus reflecting the feasibility of monitoring of pathogens in the environment.

Reference

Aggarwal, R., Sharma, S., Gupta, S., Manjunatha, C., Singh, V.K. and Kulshreshtha, D. (2017). Gene-based analysis of Puccinia species and development of PCR-based marker to detect Puccinia striiformis f. sp. tritici causing yellow rust of wheat. *Journal of General Plant Pathology*, *83*(4): 205-215.

Molecular Characterization and Phylogenetic Relationship among Indian Pathotypes of *Puccinia striiformis tritici* causing Stripe Rust in Wheat

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Introduction

Wheat is one of the most important cereal crops in the world and serves as staple food for billions of people. Rusts are among the most widespread and economically important diseases of cereal crops. Stripe rust is the most damaging and important disease challenging wheat production worldwide including India. Stripe rust in wheat is caused by *Puccinia striiformis* f.sp. *tritici* (Pst) and it is present in most wheat-growing regions of the world. In some cases, if infection occurs at early stage and weather remains favorable up to adult stage, stripe rust can cause up to 100% loss. *P. striiformis* f. sp. *tritici* was commonly assumed to have a macrocyclic lifecycle but with missing pycnial and aecial stages until very recently when it was shown to be able to infect some *Berberis* species. More than 140 races of *Puccinia striiformis* f. sp. *tritici* have been identified in the US and a total of 28 pathotypes have been reported in India. Genetic resistance, i.e. growing resistant cultivars through deployment of stripe rust resistance genes (*Yr* gene), is the most economical, effective and environmentally friendly approach to control the disease. The existence of a large number of pathotypes/races shows the rapid evolution of the pathogen virulence and selections by host crop cultivars with various resistance genes. The genetic diversity of *Pst* has been investigated since 1990's using various molecular techniques. For *Pst* population in US, a high genetic diversity was found among 115 single-spore isolates using the random amplified polymorphic DNA (RAPD) technique. Later, isolates collected from the south central US since 2000 were found to be genetically distinct from older isolates (collected before 2000) using the amplified fragment length polymorphic (AFLP) technique. More recently, phenotypic and genotypic diversity studies using simple sequence repeat (SSR) markers in relatively small or large stripe rust epidemic regions in Northwest China suggested extensive genetic recombination in the Chinese population. In contrast, relatively low genetic diversities have been reported using AFLP markers for the Australian and European *Pst* populations as the pathogen appears to be Clonal.

Case Study

Stripe rust caused by *Puccinia striiformis* f. sp. *tritici* (Pst) is one of the most devastating diseases of wheat (*Triticum* spp.) worldwide. Indian isolates were characterised based on their phenotypic reaction on differential hosts carrying different *Yr* genes. Based on virulence/avirulence structure, isolates were characterised into ten different pathotypes viz. 70S0-2, 67S64, 70S4, 66S0, 70S64, 66S64-1, 38S102, 47S102, 46S119, and 78S84. These Indian pathotypes of *P. striiformis* f. sp. *tritici* and 38 pathotypes of other rust species (*P. graminis tritici* and *P. triticina*) were used in this study to analyze their molecular phylogenetic relationship. The nucleotides of rDNA-ITS, partial _-tubulin and ketopantoate reductase genes of all the pathotypes were sequenced directly after PCR. Based on sequence data of rDNA-ITS and β-tubulin, three phylogenetic groups corresponding to three different species of *Puccinia* were obtained. Asian isolates formed a distinct evolutionary lineage than from those derived from USA. The sequence similarity of Indian pathotypes with other Asian (China and Iran) isolates indicated the same origin of pathotypes. The results will allow rapid identification of Indian *P. striiformis* f.sp. *tritici* pathotypes causing stripe rust in wheat, assist in making predictions regarding potential rust pathotypes, and identifying sources of resistance to the disease in advance.

Materials

Chemicals CTAB buffer, chloroform, isoamyl alcohol, alcohol, chloroform, isopropanol, TE buffer, primers, dNTPs, MgCl2, Taq Polymerase, DD water, PDA (Potato dextrose agar) and PDB (Potato dextrose broth).

Glassware and plastic ware: Oakridge tube (250 ml), borosil flasks, eppendorf tube, PCR tubes, micropipettes, tips, test tubes, petri plates.

Protocols

Collection and maintenance of *P. striiformis tritici* (Pst)

A large number of wheat samples infected with *P. striiformis tritici* were collected from different wheat growing zones of the country. Single urediospores cultures were maintained on susceptible genotype 'Agra local' under glass house conditions. Urediospores were collected on sterilized butter paper and stored at -40°C for further use.

Virulence analysis of *Pst* isolates

The single uredinial isolates of different rust samples mentioned above were tested for avirulence/virulence on seedlings of differential hosts having different resistance genes. The differential hosts were grown in 10-cm diameter pots and inoculated at single leaf seedling stage, keeping three replicates per differential per isolate. The inoculation and disease recording was done as per standard procedure.

DNA extraction and template preparation

DNA was extracted from 100 mg of urediospores of each pathotype using ZR soil microbe DNA miniprep kit. The DNA obtained was stored at -20°C. One microliter of ribonuclease at10 mg/mL was added to the extracted nucleic acid and kept at 4°C overnight to completely digest the RNA. DNA was quantified by the Nanodrop spectrophotometer and DNA concentration was finally adjusted to 50ng/µLfor PCR amplification.

Primer design

To determine the phylogenetic relationship among 10 different pathotypes, the primers from the two genes (β-*tubulin* and *ketopantoate reductase*) along with ITS1- 5.8S-ITS2 region were designed using software Primer3. Primer quality was checked using IDT-oligoanalyser software to check the potential of secondary structures, self primer dimer and hetero primer dimer formation within and between the different primer sets.

PCR amplification

DNA from all isolates was amplified using primers. Steps given as under:

- Set up a 25 µl reaction in a 0.2 ml microfuge tube as follows

Template DNA (50 ng)	1.0 µl
10 X Taq polymerase buffer	2.5 µl
MgCl2 (25 mM)	1.5 µl
dNTPs (10 mM)	0.5 µl
Primer (10µM):	0.5µl
Taq Polymerase (5 U/µl)	0.5 µl
Autoclaved DD Water	18.5 µl

- Spin briefly in a microfuge to mix.
- The mixture was then placed in PCR machine (i Cycler, BIO-RAD).
- Performed 35 cycles of PCR using the following temperature profile-

S.No.	**PCR steps**	**Conditions**
1	Initial denaturation	94ºC (1 cycle) for 4min
2	Denaturation	94ºC for 1min
3	Annealing	55-66ºC for 1min
4	Extension	72 ºC for 2min
5	final extension	72ºC for 7min

- Reactions were stopped at 4ºC

Gel electrophoresis

The amplified products were resolved by electrophoresis in 1.2% agarose gels run at 80 V for 2 h in TAE buffer (1X) and stained with ethidium bromide at 0.5 µg/mL. The gels were visualized under UV light and

photographed with gel documentation unit. PCR fragments were excised from agarose gels and purified using QIAquick gel extraction kit (Qiagen). Amplified products were sequenced by ABI 3100 Genetic analyser. Sequences were BLAST analysed using NCBI database to check for their specificity.

Data analysis

For phylogenetic analysis, sequences were manually aligned using Bioedit ver. 7.0.9 to remove ambiguous base and primer sequences. Multiple alignments were performed in ClustalW. The sequences obtained for *ketopentaote reductase* gene, β-*tubulin* gene and the ITS region were trimmed, aligned, and the phylogenetic tree was prepared using the software Mega 6. For a comparison with results from earlier research, rDNA-ITS and β-*tubulin* sequence data of *P. striiformis tritici*, *P. graminis tritici*, and *P. triticina* from NCBI GenBank were used in a phylogenetic analysis. As no sequences were available for the *ketopentaote reductase* gene of Pgt and Pt, the Indian pathotypes of only Pst were characterized. The reference sequence of Pst was taken from the *ketopentaote reductase* gene sequences to analyse the phylogenetic evolution of the *P. striiformis* f.sp. *tritici* pathotypes in India. The evolutionary history was inferred using the NJ method. All positions containing gaps and missing data were eliminated from the dataset (complete deletion option). The evolutionary distances were computed using the maximum composite likelihood method. The Jaccard similarity matrices were used to perform cluster analyses using the neighbour joining (NJ) procedure. Support for the clusters was evaluated using boot strapping analyses with 1000 iterations. Nonparametric bootstrap (BS) was used to assess support for branching topologies. The final trimmed sequences were submitted in NCBI database for accession numbers (Table 1).

Table 1: Indian *Pst* pathotype sequences of ITS and β-*tubulin* and *ketopantoate reductase* submitted to NCBI for phylogenetic study

***Pst* Pathotypes**	**ITS**	**β-*tubulin***	***ketopantoate reductase***
19 (70S0-2)	KX061103	KX424983	KX249826
31 (67S64)	KT320894	KX424984	KX249827
A (70S4)	KT320892	KX424987	KX249833
14 (66S0)	KX061104	KX424982	KX249825
20A (70S64)	KT320893	KX424988	KX061102
38A (66S64-1)	KT305926	KT345695	KX249832
I (38S102)	KT320895	KX424981	KX249830
K (47S102)	KT320891	KT345694	KX249829
Yr9 pt. (46S119)	JQ360861	KX424986	KX249828
PBW343 pt. (78S84)	JQ360860	KX424985	KX249831

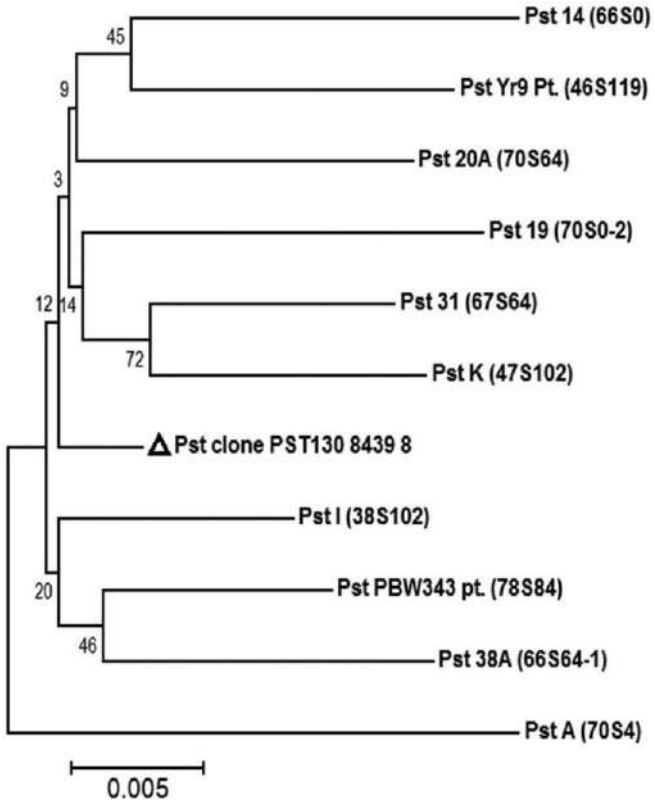

Fig. 1. Neighbor-joining phylogenetic tree based on nucleotide sequence of ketopantoate reductase gene showing phylogenetic relations among *P. striiformis* f.sp. *tritici* pathotypes

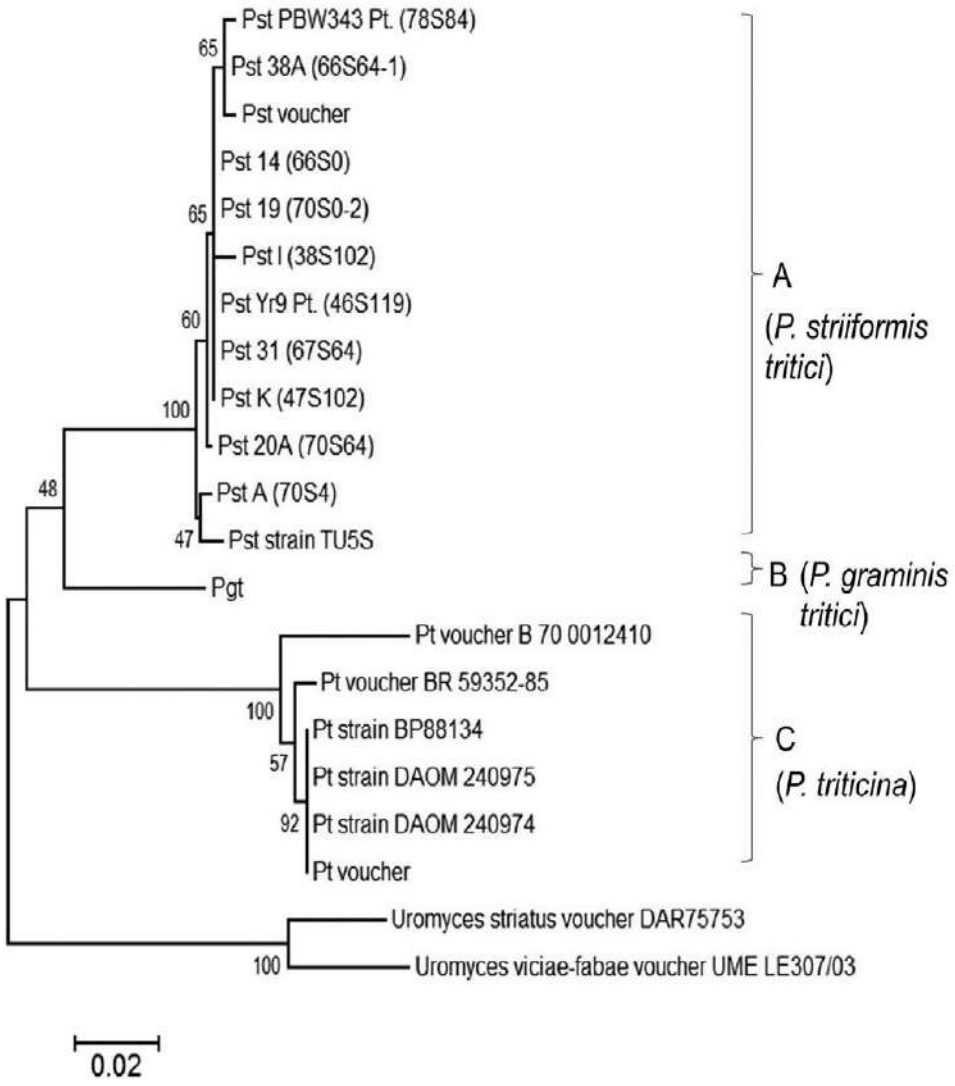

Fig. 2. Neighbor-joining tree based on nucleotide sequence of β-*tubulin* showing phylogenetic relations among pathotypes of *P. striiformis tritici*, *P. graminis tritici* and *P. triticina*

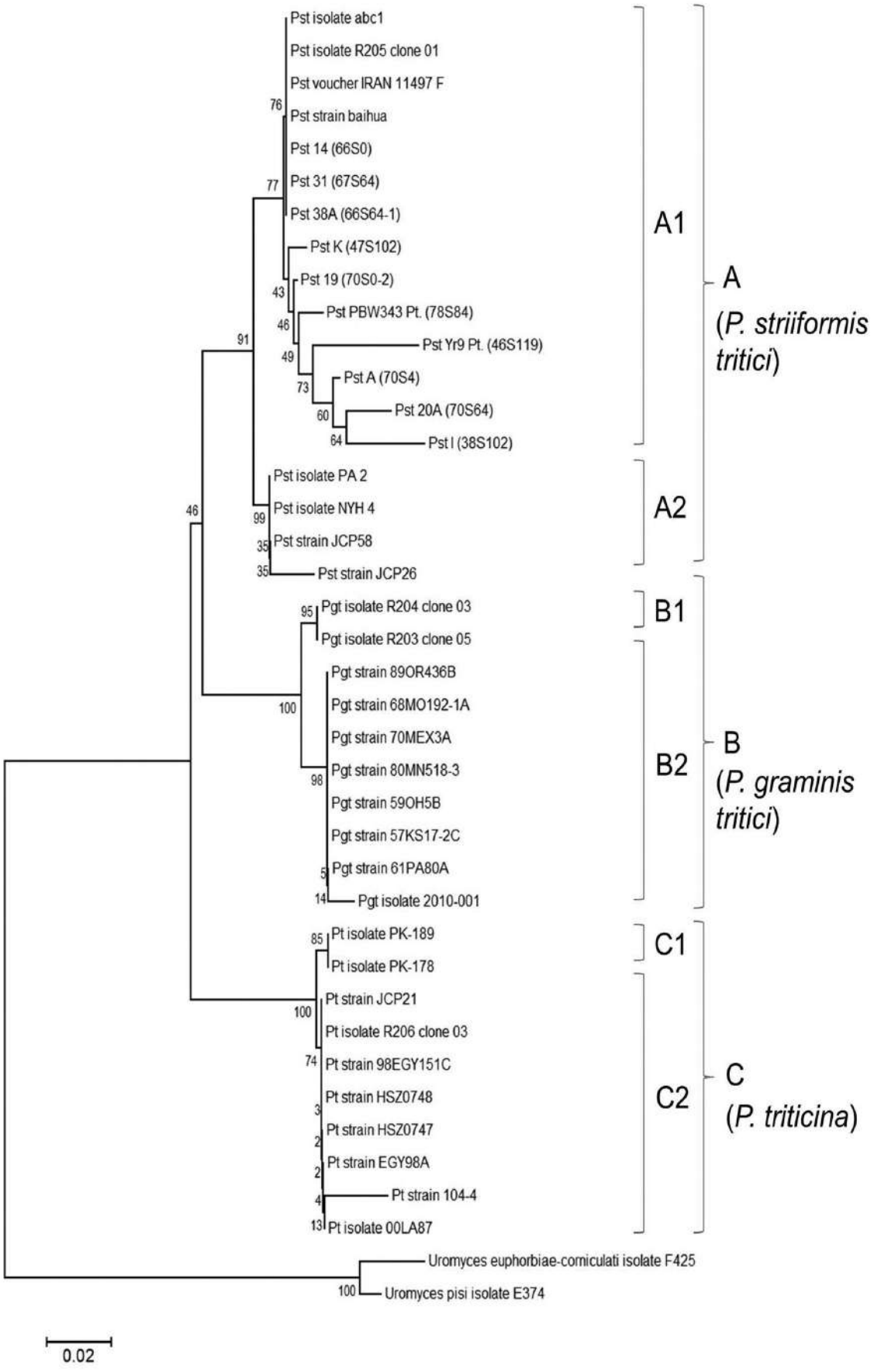

Fig. 3. Neighbor-joining phylogenetic tree based on nucleotide sequence of ITS showing phylogenetic relations among pathotypes of *P. striiformis tritici*, *P. graminis tritici*, and *P. triticina*.

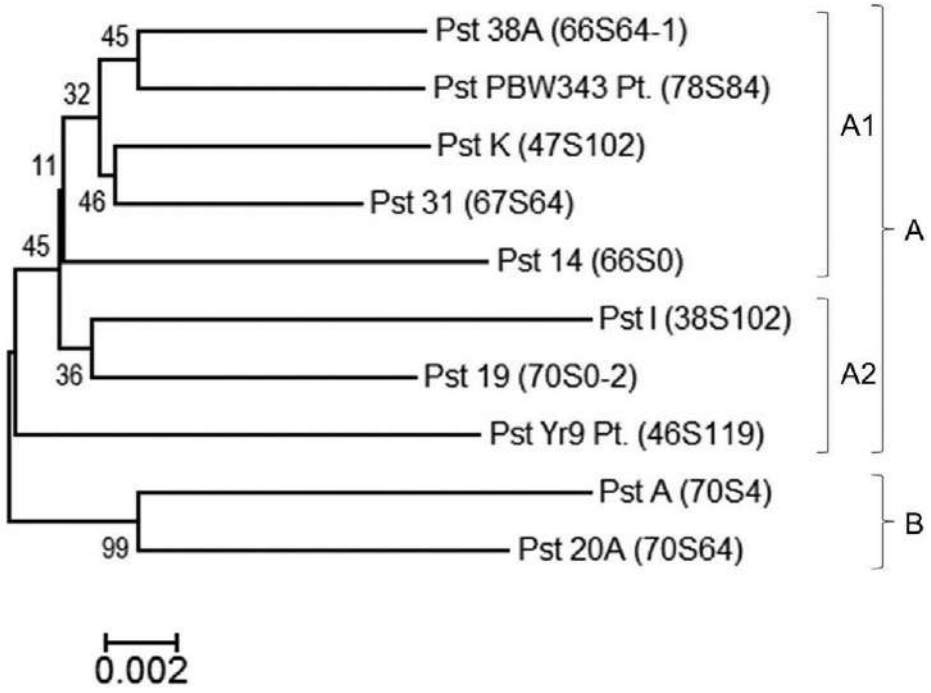

Fig. 4. Neighbor-joining tree based on combined nucleotide sequence of ITS, β-*tubulin*, and *ketopantoate reductase* showing phylogenetic relations among pathotypes of *P. striiformis tritici*

Expected Outcome

This study will allow rapid identification of Indian *Puccinia striiformis* f. sp. *tritici* pathotypes causing stripe rust in wheat and the resulting phylogeny will assist in making predictions regarding potential rust pathotypes of wheat and identifying sources of disease resistance in advance. These results indicate the need for more detailed phylogenetic analyses within the Pst clades, which will require the inclusion of additional taxa and loci.

Reference

Aggarwal, R., Kulshreshtha, D., Sharma, S., Singh, V.K., Manjunatha, C., Bhardwaj, S.C. and Saharan, M.S. (2018). Molecular characterization of Indian pathotypes of Puccinia striiformis f. sp. tritici and multigene phylogenetic analysis to establish inter-and intraspecific relationships. *Genetics and molecular biology*, 41(4): 834-842.

Genetics of Rust Resistance in Wheat

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Wheat is one of the world's most important crops. The three rust diseases: leaf or brown rust (*Puccinia triticina*), stem or black rust (*P. graminis tritici*) and stripe or yellow rust (*P. striiformis tritici*) are among the most serious constraints to realizing the potential wheat yields. Use of host resistance being effective, economic, and eco-friendly, has been the preferred method of managing wheat rusts. Rust resistance breeding in wheat gained momentum following Biffen's demonstration in 1905 of the Mendelian inheritance of stripe rust resistance. Since then, development and deployment of rust resistant wheat cultivars have considerably reduced the losses caused by the rust diseases worldwide. Furthermore, many insights have been gained into structural and functional aspects of the genetic architecture of rust resistance in wheat.

Genes for Rust Resistance in Wheat

In all, 60, 79, and 82 genes have so far been designated for resistance to stem rust, leaf rust, and stripe rust, respectively (Wheat Gene Catalogue 2013, 2013-14 Supplement, 2015-16 Supplement, 2017 Supplement; Chen *et al*., 2018; Feng *et al*., 2018; Nsabiyera *et al*., 2018; Qureshi N *et al*., 2018; Gessese *et al*., 2019; Pakeerathan *et al*., 2019). The genes for rust resistance in wheat are fairly well distributed with regard to their chromosomal location (Table 1). This scattered pattern of resistance loci in wheat in contrast to the clustered loci for rust resistance in corn and flax for instance, offers the breeder more advantage in terms of combining resistance with other desirable agronomic traits. Another advantageous feature from the breeder's viewpoint is that relatively only few loci (three *Sr*, five *Lr* and two *Yr* ones) are multiallelic, as listed below:

Sr7a, Sr7b; Sr8a, Sr8b; and Sr9a, Sr9b, Sr9c, Sr9d, Sr9e, Sr9f, Sr9g, Sr9h

Lr2a, Lr2b, Lr2c; Lr3a, Lr3b, Lr3c; Lr14a, Lr14b, Lr14ab; Lr17a, Lr17b; and Lr22a, Lr22b

Yr3a, *Yr3b*, *Yr3c*; and *Yr4a*, *Yr4b*

However, *Lr14a* and *Lr14b* are not true alleles but are closely linked loci, since both are present in a recombinant stock designated as *Lr14ab* (McIntosh *et al*., 1995). A number of rust resistance genes from other *Triticum* spp. and wild relatives have been transferred to common wheat (*T. aestivum*) as listed in Table 2.

Linkage between Rust Resistance Genes

A number of rust resistance genes show linkage, sometimes close or an almost complete one, which offers an added advantage toward multiple rust resistance breeding in wheat. The linked genes for rust resistance in wheat are listed below (McIntosh *et al*., 1995; Wheat Gene Catalogue 2013; 2013-14 Supplement; 2015-16 Supplement; and 2017 Supplement; Bariana *et al.,* 2016).

Sr2/*Lr27*/*Yr30*; *Sr15*/*Lr20*; *Sr23*/*Lr16*; *Sr24*/*Lr24*/*Yr71*; *Sr25*/*Lr19*; *Sr31*/*Lr26*/*Yr9*; *Sr38*/*Lr37*/*Yr17*; *Sr39*/*Lr35*; *Lr57*/*Yr40*; *Lr62*/*Yr42*; *Lr25*/*Lr48*; and *Yr51*/*Yr60*.

Mode of Inheritance

While *Sr2*, *Sr12*, and *Sr17* among the genes for stem rust resistance; *Lr14b*, *Lr30*, *Lr37*, and *Lr48* among the genes for leaf rust resistance; and the lone stripe rust resistance gene *Yr2* are recessively inherited, all other rust resistance genes in wheat studied showed dominant inheritance (McIntosh *et al*., 1995; Wheat Gene Catalogue 2013; 2013-14 Supplement; 2015-16 Supplement; and 2017 Supplement). In independent studies, the gene *Lr42* was reported as dominant (Czembor *et al.,* 2008) or recessive (Liu *et al*., 2013). The gene *Sr6* may behave as dominant or recessive depending on pathogen culture, temperature, and genetic background of the host (McIntosh *et al.,* 1995).

Expression of Resistance

Expression of resistance is influenced mainly by the temperature conditions, host growth stage, and the interaction between resistance/modifier genes. Effect of each of these factors on the expression of resistance is discussed in the following paragraphs.

Temperature is a very important factor regulating the expression of a number of genes for rust resistance. For example, leaf rust resistance genes *Lr11, Lr14a, Lr14b, Lr18, Lr34*, and *Lr37*; and stem rust resistance genes *Sr6, Sr12, Sr15, Sr17, Sr22, Sr34, Sr38*, and *Sr52* are generally more effective at temperatures below 20^{o}C. (McIntosh *et al.,* 1995; Qi *et al.,* 2011). In contrast, leaf rust resistance genes *Lr13, Lr16, Lr17, Lr23*; stem rust resistance genes *Sr13*, *Sr21*, and *Sr23*; and stripe rust resistance gene *Yr17* normally show more effectiveness at higher temperatures (McIntosh *et al.,* 1995; Chen *et al.,* 2018). Several soft white U.S. wheat cultivars show high-temperature adult plant (HTAP) stripe rust resistance. Adult plants are resistant at diurnal temperatures of 10 to 30^{o}C, but susceptible at 6 to 21^{o}C. Seedlings are susceptible at both temperature ranges (Qayoum and Line, 1985). The genes *Yr36*, *Yr39*, *Yr59*, and *Yr79* have been shown to condition HTAP stripe rust resistance in wheat. The genes *Lr34*, *Lr46*, *Lr67*, and *Lr68* were observed to be more effective in imparting adult plant leaf rust resistance under relatively cooler climatic conditions in India (Kumar *et al.,* 2019).

Host growth stage is another important factor affecting the expression of resistance to wheat rusts. Most of the designated genes for rust resistance can be detected in seedlings and remain effective throughout the plant life. Hence, these are described as seedling resistance or all-stage resistance (ASR) genes. They act in a 'gene-for gene' manner in which a resistance gene (R) in the host interacts with a corresponding avirulence (Avr) gene in the pathogen (Flor, 1956). It was hypothesized that this occurs through recognition of the Avr protein (elicitor) by the corresponding R protein (receptor) triggering the defense responses leading to resistance. In order to overcome the resistance, the Avr gene undergoes change due to mutation, somatic hybridization or sexual recombination resulting in the altered Avr protein which evades recognition by the R protein. Molecular evidence for this hypothesis has recently been provided by two studies. It was found that stem rust isolates evade recognition by the wheat immune receptor coded by *Sr35* through integration of a miniature inverted transposable element in *AvrSr35* (Salcedo *et al*., 2017 *Science* 358, 1604). Similarly, stem rust isolates escaped recognition by *Sr50*-receptor through sequence divergence and DNA insertion in *AvrSr50* (Chen *et al*., 2017 *Science* 358, 1607).

However, a number of resistance genes are expressed in post seedling stages only, and are called adult plant resistance (APR) genes. Stem rust resistance genes *Sr2, Sr55, Sr56, Sr57, and Sr58*; leaf rust resistance genes *Lr12, Lr13, Lr22a, Lr22b, Lr34, Lr35, Lr37, Lr46, Lr48, Lr49, Lr67, Lr68, Lr74, Lr75, Lr77* and *Lr78*; and stripe rust resistance genes *Yr11, Yr12, Yr13, Yr14, Yr16, Yr18*, *Yr29*, *Yr30*, *Yr33*, *Yr34*, *Yr36, Yr39, Yr46, Yr48, Yr49, Yr52, Yr54, Yr59, Yr62, Yr68, Yr71, Yr75, Yr77, Yr78*, *Yr79*, and *Yr80* are examples of APR genes. The APR is generally expressed from stem elongation to early head emergence with maximum expression occurring during the boot stage (Roelfs *et al.,* 1992). However, a study placed six APR genes for wheat leaf rust resistance in four different groups based on the earliest growth stage at which they could be detected under controlled glasshouse conditions. Thus, *Lr13* could be detected two weeks after sowing, *Lr37*- three weeks after sowing, *Lr12, Lr22a,* and *Lr35*- five weeks after sowing, whereas the gene *Lr22b* could be detected only nine weeks after sowing (Park and McIntosh, 1994). Some APR genes can be detected in seedling stage by manipulating the temperatures. For example, the genes *Lr13*, *Yr33*, and *Yr59* can be detected in seedling tests at elevated temperatures, whereas *Lr37* can be recognized in seedlings at low temperature (17˚C). In contrast, stem rust resistance genes *Sr25* and *Sr37* (Sawhney *et al.,* 1982), as well as *Sr36* (Roelfs, 1988) lose their effectiveness to some extent in the adult plants, particularly near maturity.

Gene Interactions

Epistasis is generally observed when two or more resistance genes are present together, that is, the gene conditioning the lowest infection type or the highest level of resistance is expressed. However, a number of cases of interactive effects of resistance genes have been documented. Enhancement of *Lr16* resistance by *Lr13* has been reported. Leaf rust isolates virulent to *Lr16* were avirulent to seedlings carrying both *Lr13* and *Lr16*, although *Lr13* was not expressed in the seedling stage to the pathotypes used in the study (Samborski and Dyck, 1982). In both seedling and adult plant tests, homozygous paired combinations of a

number of *Lr* genes either with *Lr13* (Kolmer, 1992) or with *Lr34* (German and Kolmer, 1992) had enhanced resistance relative to either parent. Leaf rust resistance in 'Gatcher' was reported to be due to complementary interaction of genes *Lr27* and *Lr31* (Singh and McIntosh, 1984). The gene *Lr34* appeared to interact with these two complementary genes to produce enhanced level of leaf rust resistance in some Chinese Spring substitution lines (Sawhney, 1992). The "*YrA*" resistance in Avocet R was found to be conditioned by two complementary stripe rust resistance genes, *Yr73* on chromosome 3DL and *Yr74* on 5BL (Dracatos *et al.,* 2015). The genes *Sr7a* and *Sr12* were observed to interact resulting in significantly higher levels of resistance than that conferred by either gene acting alone (Singh and McIntosh, 1986). The gene *Sr12*, or a gene closely linked to *Sr12*, was found to be responsible for 'Thatcher'-derived APR and this resistance was enhanced in the presence of *Lr34* (Hiebert *et al.,* 2016). Introgression of *Lr75* and a QTL on 7BL (believed to be *Lr14a*) from 'Forno' considerably increased the leaf rust resistance of the susceptible Swiss winter wheat cultivar Arina (Singla *et al.,* 2016). A QTL on chromosome 5BL enhanced the leaf rust resistance conditioned by the gene *Lr46* in an additive manner (Kolmer, 2015). Additive interaction for resistance to leaf rust and stripe rust was observed among *Lr46*/*Yr29* and three to four newly detected QTLs in RILs derived from the cross of Avocet x Kundan (DL 153-2) (Ren *et al.,* 2017). A combination of *Yr18* and a 6DL QTL derived from `Qing Shumai', a Chinese winter wheat landrace showing slow rusting resistance, elevated the stripe rust resistance consistently across both winter and spring wheat backgrounds, acting synergistically (Zhang *et al.,* 2017). The gene *Yr81* interacted with *Yr18* in an additive manner toward enhanced stripe rust resistance (Gessese *et al.,* 2019). The all-stage resistance gene *Yr82* interacted with *Yr29* in an additive manner to produce lower adult plant responses at the adult plant stage (Pakeerathan *et al.,* 2019). Additive effects on reducing the leaf rust severity were observed between the lesion mimic gene *lm* and *Lr34* in `Ning 7840', a Chinese wheat breeding line (Tao *et al.,* 2012). Combination of the genes *Lr68* and *Lr34* showed stronger resistance than either gene alone in all the nine tested environments suggesting an additive effect of these two genes (Lillemo *et al.,* 2011). In contrast, Silva *et al.* (2015) while studying the effect and interaction of *Lr68*, *Lr34* and *Sr2* genes in two wheat populations derived from 'Parula' at sites in Uruguay did not observe an additive effect of the combination of *Lr68* and *Lr34*. However, *Sr2* enhanced the effect of *Lr68*. Thus, the APR gene combinations are significantly influenced by the environmental conditions.

In contrast, some gene interactions may suppress resistance. For example, the resistance to Canadian leaf rust cultures conferred by the gene *Lr23* was suppressed by a gene in 'Thatcher', but this suppression was only partially effective under Australian conditions (McIntosh and Dyck, 1975). The suppressor of *Lr23*, named *SuLr23* was found to be orthologous and specific to the gene *Lr23* (Nelson *et al.,* 1997). Thatcher and the back-cross derivative Canthatch were susceptible to several stem rust races because of a suppressor on chromosome 7DL that inhibited the expression of relevant resistance genes (Kerber and Green, 1980). Later it was confirmed that Canthatch 7DL suppressor only suppressed stem rust resistance genes derived from either the A or B genome, and the pattern of the suppression is gene specific and independent of chromosomal location (Talajoor *et al.,* 2015). Suppressors on chromosomes 1D and 3D were reported to prevent the expression of stem rust resistance genes on chromosomes 1B, 2B, and 7B; and leaf rust resistance genes on 2B and 4B. The high frequency of suppressors in *Triticum aestivum* suggests that they might have a selective advantage (Bai and Knott, 1992). A *Yr18* suppressor was reported in four Chinese land races (Wu *et al.,* 2015). Fast rusting to stem rust was reported in lines carrying leaf rust resistance genes *Lr28* and *Lr32* (Tomar and Menon, 1999).

Mechanisms of Rust Resistance

In cereals, three different resistance mechanisms have been identified: (i) surface-localized receptors that recognize pathogen-associated molecular patterns (PAMPs) and activate PAMP-triggered immunity; (ii) race-specific resistance conferred by intracellular immune receptors that recognize pathogen effector proteins and activate effector-triggered immunity; and (iii) race non-specific broad-spectrum partial disease resistance via various molecular pathways.

In wheat, a number of rust resistance genes have been successfully cloned including stem rust resistance genes *Sr13*, *Sr21*, *Sr22*, *Sr33*, *Sr35*, *Sr45*, *Sr46*, *Sr50*, *Sr60*; leaf rust resistance genes *Lr1*, *Lr10*, *Lr21*; stripe rust resistance genes *Yr5*, *Yr7*, *Yr10*, *Yr15*, *Yr36*, *YrSP*, *YrAS2388R*; and multi-pathogen resistance genes *Yr18/Lr34/Sr57/Pm38*, *Yr46/Lr67/Sr55/Pm39*. Most of the cloned rust resistance genes in wheat encode intracellular nucleotide-binding leucine-rich-repeat receptors (NLRs), which recognize pathogen-

secreted effectors delivered into the host cytoplasm. Few others represent different protein families, which act through unique mechanisms and impart resistance to stripe rust (*Yr15* and *Yr36*), stem rust (*Sr60*) and to multiple pathogens including the three wheat rusts and powdery mildew (*Yr18/Lr34/Sr57/Pm38* and *Yr46/Lr67/Sr55/Pm39*). *Yr15* encodes a protein composed of putative kinase and pseudokinase domains in tandem, which was named WTK1 (wheat tandem kinase 1). Similarly, *Sr60* encodes a protein with two putative kinase domains. This gene was designated WTK2. Similar tandem kinase-pseudokinase (TKP) structures were discovered in a wide range of plant taxa which suggests that they belong to the same protein family, designated as TKP family. Interestingly, the presence of kinase-pseudokinase structure in both plant TKPs and the animal janus kinases indicates commonality in the molecular evolution of immune responses across these two kingdoms. *Yr36* is a high-temperature adult-plant resistance gene effective against a wide range of *Pst* pathotypes including the new aggressive races adapted to warmer temperatures. *Yr36* encodes a protein that has a combination of serine/threonine kinase and steroidogenic acute **r**egulatory protein-related lipid-**t**ransfer (START) domains and was therefore named *Wheat Kinase START 1* (*WKS1*). This protein phosphorylates PsbO, one of the subunits of the three extrinsic proteins (PsbO, PsbP and PsbQ) of the Photosystem II (PSII) super complex. The phosphorylation of PsbO works as a switch to perturb PSII function, inducing chlorosis and reduced *Pst* growth (Wang *et al*., 2019 *Mol Plant* 12:1639-1650). Further, WKS 1 phosphorylates a thylakoid-associated ascorbate peroxidase (tAPX) protein, inhibiting tAPX activity, which leads to the gradual accumulation of reactive oxygen species (ROS) and eventually, cell death several weeks later restricting pathogen growth and sporulation (Gou *et al.,* 2015). Changes in two critical amino acids in the resistant allele of *Yr46/Lr67* result in encoding a protein that has lost hexose transport function and could therefore disturb the balance of sugars between the extracellular and intracellular spaces of the leaf. This may reduce the availability of nutrients inside the host cell, hence the effectiveness of this gene against multiple biotrophic fungi (Moore *et al*., 2015. *Nat Genet*. 47:1494-1498). Alternatively, altering apoplastic sugar concentration may induce activation of defense responses (Dodds and Lagudah, 2016). The gene *Lr34*/*Yr18* has been shown to encode an ATP-binding Cassette (ABC) transporter (Krattinger *et al.,* 2009). However, the basis of resistance and the substrates of this ABC transporter are as yet unknown.

Pleiotropic adult plant resistance (PAPR) genes need a special mention as these impart resistance to multiple wheat pathogens. The locus *Lr34*/*Yr18*/*Sr57*/*Pm38*/*Sb1*/*Bdv1*/*Ltn1* on chromosome 7DS is the most important one among such genes, imparting resistance to leaf rust, stripe rust, stem rust, powdery mildew, spot blotch, and barley yellow dwarf virus. Brazilian wheat cultivar `Frontana' carrying this gene has been maintaining resistance since its release in 1943, and has been extensively used worldwide as a donor for durable leaf rust resistance. Interestingly, transgenic expression of the *Lr34* gene in other cereal species, such as durum wheat (Rinaldo *et al.,* 2017), barley (Risk *et al.,* 2013), rice (Krattinger *et al.,* 2016), maize (Sucher *et al.,* 2017), and sorghum (Schnippenkoetter *et al.,*) confers resistance to multiple adapted pathogens of these crops, suggesting that the role of this gene in infection is conserved across a wide taxonomic range. Thus, it has the potential to be used as a new source of basal / background resistance in other cereal crop species as well. The other two PAPR genes are *Lr67*/*Yr46*/*Sr55*/*Pm46*/*Ltn3* and *Lr46*/*Yr29*/*Sr58*/*Pm39*/*Ltn2* providing resistance to all three rusts and powdery mildew, and are located on chromosome 4DL & 1BL, respectively. All these three PAPR genes are associated with leaf tip necrosis (*Ltn*) which can serve as a phenotypic marker for keeping track of them in breeding populations. Although, PAPR genes are widely effective, the level of resistance imparted by them on their own is not adequate & needs to be complemented by other resistance genes. No significant additive effects on rust resistance were observed among different combinations of these genes in Indian conditions (Sivasamy *et al.,* 2014).

Future Thrusts

Despite all the progress made, recent spread of rust diseases in new areas, and appearance of new virulent strains of rust pathogens, perhaps due to the effects of climate-change and globalization, are posing new challenges to rust resistance breeding. Future thrusts toward realizing effective and long-lasing rust resistance in wheat include characterization of undesignated resistance genes through molecular mapping studies, cloning of the widely effective resistance genes and development of gene-specific markers, development of multiple resistance gene cassettes, field pathogenomics studies enabling rational deployment of resistance genes and pre-emptive breeding strategies, strengthening international collaboration for checkmating the rust pathogens, and harmonizing toward a universal system of nomenclature of wheat rust pathotypes.

Table 1. Chromosomal location of the designated genes for rust resistance in wheat

Chr[1]	*Sr* gene loci	*Lr* gene loci	*Yr* gene loci
1A		*Lr10*, *Lr59*	
2A	*Sr21*, *Sr32*, *Sr34*, *Sr38*, *Sr48*	*Lr11*, *Lr17*, *Lr37*, *Lr45*, *Lr65*	*Yr1*, *Yr17*, *Yr32*, *Yr56*, *Yr69*
3A	*Sr27*, *Sr35*, *Sr51*	*Lr63*, *Lr66*	*Yr76*
4A	*Sr7*	*Lr28*, *Lr30*	*Yr51*, *Yr60*
5A	*Sr60*		*Yr34*, *Yr48*
6A	*Sr8*, *Sr13*, *Sr26*, *Sr52*	*Lr56*, *Lr62*, *Lr64*	*Yr38*, *Yr42*, *Yr81*
7A	*Sr15*, *Sr7a*	*Lr20*, *Lr47*	*Yr61*, *Yr75*
1B	*Sr14*, *Sr31*, *Sr58*	*Lr26*, *Lr33*, *Lr44*, *Lr46*, *Lr51*, *Lr55*, *Lr71*, *Lr75*	*Yr3*, *Yr9*, *Yr10*, *Yr15*, *Yr21*, *Yr24*, *Yr29*, *Yr64*, *Yr65*
2B	*Sr9*, *Sr10*, *Sr16*, *Sr19*, *Sr20*, *Sr23*, *Sr28*, *Sr36*, *Sr39*, *Sr40*, *Sr47*	*Lr13*, *Lr16*, *Lr23*, *Lr35*, *Lr48*, *Lr50*, *Lr58*, *Lr73*	*Yr5*, *Yr7*, *Yr27*, *Yr31*, *Yr41*, *Yr43*, *Yr44*, *Yr53*, *Yr72*
3B	*Sr2*, *Sr12*	*Lr27*, *Lr74*, *Lr77*, *Lr79*	*Yr30*, *Yr57*, *Yr58*, *Yr80*, *Yr82*
4B	*Sr37*	*Lr12*, *Lr25*, *Lr31*, *Lr49*	*Yr50*, *Yr62*, *Yr68*
5B	*Sr49*, *Sr56*	*Lr18*, *Lr52*	*Yr19*, *Yr47*, *Yr74*
6B	*Sr11*	*Lr3*, *Lr9*, *Lr36*, *Lr53*, *Lr61*	*Yr4*, *Yr35*, *Yr36*, *Yr78*
7B	*Sr17b*	*Lr14*, *Lr68*, *Lr72*	*Yr2*, *Yr6*, *Yr39*, *Yr52*, *Yr59*, *Yr63*, *Yr67*, *Yr79*
1D	*Sr18*, *Sr33*, *Sr45*, *Sr50*	*Lr21*, *Lr42*, *Lr60*	*Yr25*
2D	*Sr6*, *Sr46*, *Sr54*, *Sr59*	*Lr2*, *Lr15*, *Lr22*, *Lr39*, *Lr54*	*Yr8*, *Yr16*, *Yr37*, *Yr54*, *Yr55*
3D	*Sr24*	*Lr24*, *Lr32*, *Lr69*	*Yr45*, *Yr49*, *Yr66*, *Yr71*, *Yr73*
4D	*Sr41*, *Sr55*	*Lr67*	*Yr22*, *Yr28*, *Yr46*
5D	*Sr30*, *Sr53*	*Lr1*, *Lr57*, *Lr70*, *Lr76*, *Lr78*	*Yr40*, *Yr70*
6D	*Sr5*, *Sr29*, *Sr42*	*Lr38*	*Yr20*, *Yr23*, *Yr77*
7D	*Sr25*, *Sr43*, *Sr44*, *Sr57*	*Lr19*, *Lr29*, *Lr34*	*Yr18*, *Yr33*

Chr[1] – Chromosome

Table 2. R-genes transferred to common wheat from other *Triticum* spp. and wild relatives

Source	Rust resistance gene/s
Triticum araraticum	**Sr40**
T. armeniacum	**Lr50**
T. dicoccoides	*Lr53, Lr64*; *Yr15*, *Yr35*, *Yr36*
T. monococcum	*Sr21, Sr22, Sr35, Sr60*; *Lr63*
T. spelta	*Lr44, Lr65, Lr71*; *Yr5*
T. timopheevii	*Sr36, Sr37*; *Lr18*
T. turgidum ssp. *dicoccon*	*Sr2, Sr9d, Sr9e, Sr13, Sr14, Sr17*; *Lr14a*; *Yr15*
T. turgidum ssp. *durum*	*Sr9g, Sr11, Sr12*; *Lr23, Lr61, Lr72*, *Lr79*; *Yr7, Yr24*, Yr53, *Yr56*
Aegilops comosa	*Sr34*; *Yr8*
Ae. geniculata	*Sr53*; *Lr57*; *Yr40*
Ae. kotschyi	*Lr54*; *Yr37*
Ae. neglecta	*Lr62*; *Yr42*
Ae. peregrina	*Lr59*
Ae. sharonensis	*Lr56*; *Yr38*
Ae. searsii	*Sr51*
Ae. speltoides	*Sr32, Sr39, Sr47*; *Lr28, Lr35, Lr36, Lr47, Lr51, Lr66*
Ae. squarrosa	*Sr33, Sr45*; *Lr22a, Lr40, Lr41, Lr42, Lr43*; *Yr28*
Ae. tauschii	*Sr33*; *Lr21*, *Lr32*, *Lr39, Lr42*
Ae. triuncialis	*Lr58*
Ae. umbellulata	*Lr9, Lr76*; *Yr70*
Ae. ventricosa	*Sr38*; *Lr37*; *Yr17*
Dasypyrum villosum	*Sr52*
Elymus trachycaulis	*Lr55*
Secale cereale	*Sr27, Sr31, Sr50, Sr59*; *Lr25*, *Lr26*, *Lr45*; *Yr9*
Thinopyrum elongatum	*Sr24*, *Sr25*, *Sr26*, *Sr43*; *Lr19, Lr24, Lr29*
Th. intermedium	**Sr44; Lr38; Yr50**
Th. ponticum	*Yr69*

Further Reading

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Role of Epigenetics in Wheat Rust Resistance

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The term epigenetics was coined by C. H. Waddington in 1942. Epigenetics can be defined as "stably heritable phenotype resulting from changes in a chromosome without alterations in the DNA sequence". The term also includes the biochemical changes in the chromatin. Epigenome is a collection of chromatin patterns in a cell including both heritable and transient changes. There are several mechanisms that contribute to epigenomic information in plants. This involves DNA methylation, histone modifications and RNA directed DNA methylation.

1. DNA methylation: DNA Methylation refers to covalent enzyme catalyzed transfer of methyl group to 5' position of cytosine thus converting cytosine to 5-methylcytosine. This modification is inherited on the parent strand through each round of DNA replication. Methylation of DNA occurs at three sequence contexts in plant genomes: CG, CHG and CHH where H=A, C or T.

a) CG methylation is maintained by maintenance methyltransferases MET1
b) CHG methylation is maintained by histone methyltransferases (HATs) and chromomethylase (CMT3)
c) CHH methylation depends upon activity of chromo methyltransferase (CMT2) or small RNA that guides de novo methyltransferase (DRM2) for methylation.

2. Histone modifications: This refers to modifications of histone proteins (proteins bound to DNA) which are catalyzed by a number of enzymatic processes like acetylation, methylation, sumoylation, ubiqutination and phosphorylation; the most well characterized modifications include acetylation and methylation of histones H3 and H4. These modifications alter the packaging structure which either activates the DNA for the transcription or makes the structure more condensed so that transcription machinery is unable to bind to it. Acetylation is related to activation of gene expression. Binding of an acetyl group reduces the positive charge of histone proteins and as a result interaction between DNA and bound histones is weakened. This loosens the compact DNA supercoils and hence DNA is more freely available for transcription. On the other hand, binding of methyl group may lead either activation or inactivation of the gene. Methyl groups alter the hydrophobicity of DNA resulting in altered DNA-histone interactions.

3. RNA directed DNA methylation (RdDM): Small regulatory RNA lead to secondary methylation of DNA. During RdDM, double-stranded RNAs (dsRNAs) are processed to 21-24 nucleotide small interfering RNAs (siRNAs) that guides *denovo* methyltransferase to target methylation of homologous DNA loci.

Plant breeding has traditionally relied on combining the genetic diversity present within a species to develop combinations of alleles that provide desired traits. Epigenetic diversity may provide additional sources of variation within a species that could be captured or created for crop improvement. There are numerous sources of variation, both natural and induced that could lead to the formation of epialleles. Natural sources of epigenetic variation include spontaneous changes, genetic changes in *cis*, such as transposon insertions, structural rearrangements and genetic changes in *trans* that could result in small interfering RNA (siRNA) signals including methylation, interactions among alleles in wide crosses and polyploids. Induced sources of epigenetic variation might include mutations in the epigenetic machinery, such as in the epigenetic recombinant inbred lines (epiRILs), chemical treatments with inhibitors of DNA methylation and other chromatin modifications, directed epigenome editing and treatments with tissue culture or other stresses (Springer and Schmitz, 2017).

Creation of epigenetic diversity, clonal selection, EpiRIL development and genome editing tools can be utilized for exploitation of epigenetics for stress management. These can lead to improvement in a elite variety without change in its original genomic content. A more reliable and demonstrated approach is through the disruption of genes encoding key epigenetic regulators. Indeed, numerous heritable epialleles have been produced in *arabidopsis* plants with mutations in either of two genes (met1 and ddm1) involved in the maintenance of DNA methylation. EpiRILs have been developed to assess the prevalence of stable epigenetic variation and its inheritance in experimental population and quantification of epigenetic variation on complex trait to identify EpiQTLs (Johannes *et al.,* 2009).

Biotic factors affect the epigenome of plants and can influence the plant phenotype. The first evidence in support of epigenome with different roles of epialleles, comes from *Arabidopsis thaliana* where epigenetic recombinant inbred lines (epiRILs) were developed from methylation deficient mutant and wild type strain (Johannes *et al.,* 2009). The epiRILs differed in their response to defense related hormones, salicylic acid and jasmonic acid (Latzel *et al.,* 2012) suggesting part of variation of plant defense responses in natural populations could happen due to underlying epigenetic phenomenon.

In the recent past, epimutations have been studied for abiotic and biotic stress tolerance in crop plants. Numerous studies deciphering the role of epigenetic phenomena playing a role in stress management are coming up (Surdonja *et al.,* 2017; Fortes & Gallusci, 2017; Akimoto *et al.,* 2007). Information on a past stress cue is retained and results in a modified response upon a recurring stress or a sustained response after the priming stress cue. Most of these stress-induced modifications are reset to the basal level once the stress is relieved, while some of the modifications may be stable, that is, may be carried forward as 'stress memory' and may be inherited across mitotic or even meiotic cell divisions. Epigenetic stress memory may help plants more effectively cope with subsequent stresses (Chinnusamy and Zhu, 2009). Stress memory can be of two types-Somatic stress memory & transgenerational stress memory. Thus, epigenetic changes are perceived by plant primary and secondary signals in response to stress which leads to change in histone variants or DNA methylations resulting into heritable transgenerational or long- term resistance within generation memory.

In the past, role of epigenetic modifications in incompatible/compatible interactions had been reported in different host-pathogen systems. Hypermethylation in rice due to bacterial blight and hypomethylation in wheat due to powdery mildew and leaf rust resistance had been observed (Sharma et al 2018; Pan et al 2012; Sha et al 2005). Many genes active in susceptible genotype got silenced in resistant genotype due to hypermethylation or vice-versa. Gene encoding methyltransferase (OMT1) was found to be induced during wheat-rust pathosystem suggesting involvement of DNA methylation in plant-pathogen interactions (Sharma *et al.,* 2018; Cassasola *et al.,* 2015). In *Arabidopsis*, hyper/-/hypo-methylation was shown to be associated with compatible/incompatible interactions involving a number of pathogens including the following *Pseudomonas syringae*, *Plectsphaerell acucumerina*, *Alternaria brassicicola*, *Hyaloperono spora arabidopsidis* and *Fusraium oxysporium* (Deleris *et al.,* 2016; Pavet *et al.,* 2006).

Resistance against leaf rust due to *Lr* genes is partly controlled by epigenetic modifications including histone acetylation that is known to respond to biotic/abiotic stresses. Some recent studies suggested that histone acetylation/deacetylation is a crucial regulatory mechanism during onset of specific plant diseases (Jeon et al. 2014). *N-acetyltransferase* and *peroxidase12*, largely matched with changes in H3K4/H3K9 acetylation patterns of the two promoter regions in a pair of near-isogenic lines (NILs) for the gene *Lr28*. However, in plants infected with fungal pathogens, the importance of such modifications has only just begun to be appreciated, so that only a handful of studies are available, which examined the role of epigenetic modifications in the form of histone acetylation in bacterial/fungal pathogenesis (Gomez-Diaz *et al.,* 2012; Ayyappan *et al.,* 2015). One such example in rice includes association of monoubiquitinated H2A and H2B histones with the promoter regions targeted by gene *BRHIS1* (encoding SNF2 ATPase) leading to suppression of immunity against the fungal pathogen causing blast (Li *et al.,* 2015). Several non-coding RNA were also found to be differentially expressed during disease resistance. For instance, the pathogen *Magnaporthe oryzae* in rice and Chinese race of pathogen *Puccinia striiformis* induced small RNAs in response to disease. It is thus obvious that much remains to be learnt to fully understand the role of epigenetic modifications in imparting resistance against specific diseases in plants.

Epigenetic mechanism are wide spread and major mediators of genomic effects of environment. Epigenetic diversity may provide additional sources of variation that could be captured/created for crop improvement. It is necessary to study underlying epigenetic mechanism for stress tolerance by merging of plant development/adaptation and plant breeding. Studies on Genetic and epigenetic controls can provide significant impact in breeding for development of improved varieties.

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Breeding for Rust Resistance in Wheat - A Molecular Genetics and MAS Approach

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Rusts pose a serious threat to wheat production all over the world. There are three types of wheat rusts, leaf or brown rust caused by *Puccinia triticina* Eriks., stem or black rust caused by *Puccinia graminis* Pers. f.sp *tritici* Eriks. & Henn. and stripe or yellow rust caused by *Puccinia striiformis* Westend. A yield reduction of about 50% or more can be caused by leaf and stem rusts alone, while stripe rust can cause a yield reduction of about 70% under epidemic conditions. In India all the three types of rusts are prevalent. While stem rust is confined to warmer areas, stripe rust mostly occurs in cooler parts of the country. Leaf rust is prevalent in all the regions, wherever wheat is grown. Though rust disease can be controlled by application of fungicides, development of genetic resistance is economical and environment friendly approach (Pink, 2002). The use of resistance genes in cultivars eliminates or significantly reduces the use of fungicides to control the rust. However, rapid evolution of genetic variation for pathogenicity commonly observed in rust populations requires a constant effort to identify and integrate new resistance genes within breeding programs (German *et al.,* 2007). Till now 79 *Lr* genes (MacIntosh, 2017 and Qureshi N, 2018), 60*Sr* (MacIntosh, 2017; Chen, 2020) genes and 82 *Yr* genes (MacIntosh, 2017) have been catalogued for leaf rust, stem rust and stripe rust respectively. Out of all the designated genes for rust resistance, *Lr19*, *Lr24*, *Lr34* for leaf rust resistance, *Sr26* for stem rust resistance and *Yr5*, *Yr10* and *Yr15* for stripe rust resistance are most commonly used in our marker-assisted breeding programme as they are providing high degree of resistance to their respective rusts. All these are seedling resistance gene except *Lr34* which is an APR gene and provides moderate resistance. The linked and validated markers of these rust resistance genes, their sequence, product size etc. are given in Table 1.

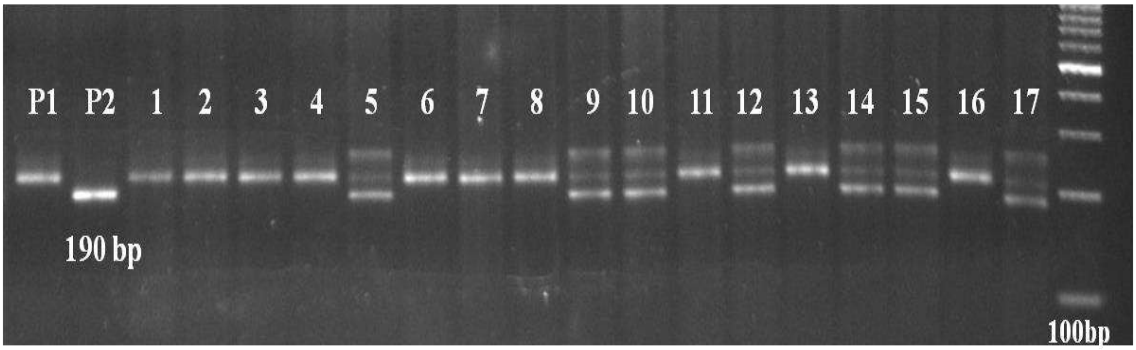
Marker assisted breeding provides an effective tool to transfer new rust resistance genes into popular wheat cultivars. In this approach, individual target genes are transferred first from donor to recipient varieties by limited backcrossing with background selection followed by selfing to develop Near isogenic lines (NILs). These NILs are having almost similar genetic background as that of the recurrent parent except for the gene of interest. These NILs with different rust resistance genes can be combined as per requirement to achieve resistance to more than one rusts. Marker Assisted Backcross Breeding (MABB) involves both marker assisted foreground and background selection. While in foreground selection we select the target gene using linked and validated molecular markers (Fig. 1 and Fig.2), background selection is done to identify plants with maximum recurrent parent genome (RPG). While foreground selection can identify the plants carrying target gene in each backcross generation without creating artificial rust epiphytotic condition, background selection can reduce the number of backcrosses to develop a NIL by identifying plants with maximum RPG. For calculating percent genome recovery of recurrent parent in each BC_1, BC_2 and BC_2F_2 generations following formula used.

Percent genome recovery = ((no of homozygous loci+1/2 (no of heterozygous loci)) / Total no of polymorphic primers (or) loci × 100

Table 1: Molecular markers linked to rust resistance genes

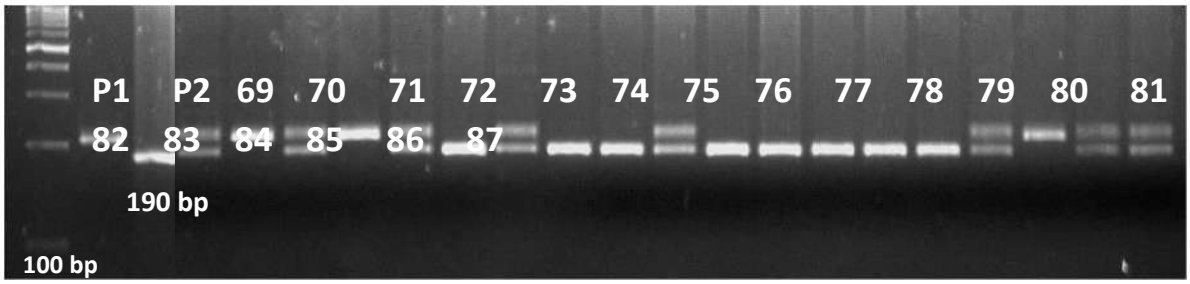
Rust resistance genes	Marker	Sequence of the Marker	Dominant/ Co-dominant	Base pairs	Ref.
Lr19	*Xwmc221*	F: 5'-ACGATAATGCAGCGGGGAAT-3', R: 5'-GCTGGGATCAAGGGATCAAT-3')	Codominant	190 and 210	(Gupta *et al*., 2006a)
Lr24	*Barc71*	F: 5'-GCGCTTGTTCCTCACCTGCTCATA-3', R: 5'-GCGTATATTCTCTCGTCTTCTTGTTGGTT-3'	Codominant	103 and 112	Mago R et al. (2005)
	SCAR (SCS1302$_{607}$)	F: 5'-CGC AGG TTC CAA TAC TTT TC -3' R: 5'-CGC AGG TTC TAC CTA ATG CAA-3'	Dominant	607bp	Gupta *et al*., 2006b

Lr34	*CSLV34*	F: 5'- GTT GGT TAA GAC TGG TGA TGG -3' R: 5'- TGC TTG CTA TTG CTG AAT AGT -3'	Codominant	150bp and 229bp	Lagudah et al., 2006
Sr26 (Coupling phase marker)	*Sr26#43*	F: 5'- AATCGTCCACATTGGCTTCT-3', R: 5'- CGCAACAAAATCATGCACTA-3'	Dominant	207	(Mago *et al.*, 2005)
Sr26 (Repulsion phase marker)	BE518379	F: 5'-AGCCGCGAAATCTACTTTGA-3', R: 5'-TTAAACGGACAGAGCACACG-3'	Dominant	303	(Liu *et al.*, 2010)
Yr5	*Xwmc175*	F: 5'GCTCAGTCAAACCGCTACTTCT-3', R: 5' CACTACTCCAATCTATCGCCGT-3'	Codominant	253	(Murphy et al., 2009a)
Yr10	*Xpsp3000*	F: 5' GCAGACCTGTGTCATTGGTC3', R: 5' GATATAGTGGCAGCAGGATACG3'	Codominant	286	(Wang et al., 2002)
Yr15	*Xgwm273*	5' ATTGGACGGACAGATGCTTT 3' 5' AGCAGTGAGGAAGGGGATC 3'	Codominant	156	(Roder et al., 1995)
	Barc8	5' GCGGGAATCATGCATAGGAAAACAGAA 3' 5' GCGGGGGCGAAACATACACATAAAAACA 3'	Codominant	300	(Murphy et al., 2009b)



P1: HD2932, P2: HD2867 + *Lr*19
Plant nos 5,9,10,12,14,15 and 17 heterozygous for *Lr*19/*Sr*25

Fig. 1. Representative gel picture of foreground selection in BC_2F_1 generation



Plant nos 74, 76, 77, 79, 80, 81, 82 and 83 are homozygous for *Lr*19 gene
P1: HD2932, P2: HD2867 + *Lr*19

Fig. 2. Representative gel pictures of foreground selection in BC_2F_2 generation

Using MAS approach several rust resistance genes, *Lr19*, *Lr24*, *LrTrk*, *Sr26*, *Yr5*, *Yr10* and *Yr15* have been transferred into the background of popular wheat varieties, HD2932, HD2733 and HD2967. Apart from seedling resistance genes, adult plat resistance (APR) genes for leaf rust resistance, *Lr34*, *Lr46*, *Lr67* and *Lr68* are also transferred into varieties HD2733 and HD3059 using MAS approach.

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Genetic Analysis and Molecular Mapping of Rust Resistance Genes

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The genetic analysis to decipher the nature and number of gene(s) controlling rust resistance is essential for effective utilization of the resistant lines as donor. This enables plant breeders to follow a breeding method to effectively exploit the resistance genes. Further, molecular mapping of resistance genes helps in rapid and precise incorporation in development new varieties through marker assisted selection (MAS) or transfer in leading varieties through marker assisted backcross breeding (MABB). The pyramiding of broad spectrum effective rust resistance genes is again very difficult through conventional method and can be achieved easily through closely linked molecular markers. Hence, genetic analysis and molecular mapping is one of the first steps towards effective and rapid utilisation of novel rust resistance genes.

Genetic Analysis

The first report of studies on the inheritance of resistance to a pathogen was given by Biffen (1905). The F_2 population derived from a cross between resistant and susceptible individuals to yellow rust segregated into a ratio of 3 susceptible to 1 resistant. The data clearly exhibited that the resistance to yellow rust was controlled by a single recessive gene. Subsequently, Mains *et al.,* in 1926 determined that the wheat cultivars Malakof and Webster had a gene that conditioned leaf rust resistance, later designated as *Lr1* and *Lr2*, respectively (Ausemus *et al.,* 1946). Identification of rust resistance genes and their genetic analysis led to designation of 79 Leaf rust (*Lr*), 78 Yellow rust (*Yr*) and 59 Stem rust (*Sr*) resistance genes (McIntosh *et al.*, 2017, Qureshi *et al.,* 2018). The genetic analysis of resistance genes is being done regularly through development of populations between resistant line and contrasting susceptible line. The phenotypic evaluation of parental lines, F_1, F_2 population, $F_{2:3}$ population and BC population is performed. The plants are classified to resistant and susceptible group and chi-square test is used to determine goodness-of-fit to compare the actual genetic ratio with those calculated for Mendelian segregation. The phenotype of F_1 indicates the dominant/recessive nature of resistance gene(s). Further, their segregation in F_2 population, $F_{2:3}$ population and BC populations determine the number of gene(s) and their interaction for resistance.

Molecular Mapping

Mapping of rust resistance genes through molecular markers is essential for rapid and precise transfer. Molecular markers offer numerous advantages over the phenotypic markers that were previously available to plant breeders. Molecular markers help in improving the efficiency of rust resistance breeding by carrying out selection based on linked markers without phenotyping. Besides, they are not environmentally regulated and are, therefore, unaffected by the conditions in which the plants are grown and are detectable in all stages of plant growth.

Molecular mapping of rust resistance gene can be performed in different type of mapping populations such as F_2, $F_{2:3}$, Doubled Haploid (DH) and Recombinant Inbred Lines (RIL) populations developed systematically through crossing of contrasting resistant and susceptible line. The populations are phenotyped precisely and can be genotyped through PCR based molecular markers (Gupta et al., 2006), SNP based arrays (Kolmer et al., 2018) or GBS (Wiersma et al., 2016). The linkage analysis is performed to identify the linked markers. The markers can be further validated in diverse populations. In addition, SNPs identified through SNP based array or GBS can be converted to PCR based markers for handy utilisation in MAS breeding program.

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Identification, Mapping and Fast-track Introgression of Rust Resistant Genes in Wheat (*Triticum aestivum* L.)

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Wheat is an important cereal of the world and offering one-fifth of total calorific and protein requirement of world's population. Wheat is cultivated on more than 80 million hectares annually around the globe and contributes 19% to the total cereal production. Human population is increasing at a fast pace and is expected to exceed 9 billion in 2050 and hence, to satisfy the global food demand, 60% increase in wheat production is required. Wheat diseases cause about 10-13% annual reduction in production. Our future generations will have to deal with changing climate and rapidly evolving pest and diseases and hence there is a need to generate systems, which enable us to produce more food in less space.

Wheat Genetics

Genus Triticum belongs to family Poaceae that is divided into three sub sections based on the ploidy level and genome, such as monococcon (diploid species), dicoccoidea (tetraploid species) and aestivum (hexaploid species). Diploid species (2n=2X=14) consists of only 'A' genome and tetraploid species (2n=4X=28) consists of 'A' and 'B' genome. Whereas, hexaploid species (2n=6X=42) consists of three different 'A', 'B' and 'D' genomes.

Wheat Rusts

Rust diseases are among the major biotic constraints to wheat cultivation. Rust fungi are widely spread plant pathogens causing diseases on angiosperm, gymnosperm, trees and cereals. They are bio- trophic in nature, widely adapted and have the capacity to evolve creating new virulent pathotypes. There are three kinds of rusts attack wheat, viz., stem rust or black rust (*P. graminis* f. sp. tritici Eriks.), stripe rust or yellow rust (*P. striiformis* West. f. sp. tritici Eriks. & E. Henn.) and leaf rust or brown rust (*P. triticina* Eriks.). Many epidemics of leaf rust, stripe rust and stem rust have been reported causing yield losses globally.

***Leaf Rust*:** Leaf rust is predominant among other wheat rusts and occurs more frequently than stripe rust and stem rust. Due to its wide adaptation, leaf rust fungi initiate infection and spreads very quickly under favorable conditions. The spores germinate at a large range of temperature regimes and light intensity (from nearly 2°C under low light intensity to 35°C in the presence of high light intensity). Leaf rust fungi can cause approximately 40-70 % yield losses.

***Stripe Rust*:** Stripe rust is also an important foliar disease and its early onset can cause tremendous yield loss in areas with cool weather. Infection starts under low temperature (9-13 °C) with low light intensity and free water on the host's leaf surface. Symptoms appear in 7-8 days after infection and spores will be visible after 13-14 days of inoculation. The stripe rust pathogen causes 10-70 % of yield reductions.

***Stem Rust*:** Stem rust of wheat is a serious fungal disease having capability to form new aggressive pathotypes with diversifying selection. This pathogen infects leaves, stems and glumes of wheat and arrest the water and nutrition translocation to kernels. Its infection may be high in hot days (25-30 °C) and mild nights (15-20 °C) with frequent dews.

High throughput Marker Technologies available in Wheat

SNP discovery:

SNPs present in an organism can be discovered through sequencing and comparison of genomic DNA sequenced data from two or more individuals of a species. Methods used for the sequencing of DNA can be broadly classified into first generation sequencing and next generation sequencing.

First-generation DNA sequencing

This method of DNA sequencing was also called Sanger–Coulson method. First-generation DNA sequencing is useful for sequencing 15-200 nucleotides. This method is more laborious and requires to prepare template DNA, restriction enzyme and gel(s) for electrophoresis.

Next generation sequencing (NGS) platforms

NGS opened a pathway for sequencing and genotyping of thousands to hundreds thousands samples through parallelised preparation library of genomic DNA without using restriction enzymes. Application of NGS had limitation for species with large complex genomes such as barley and wheat (16GB). To overcome these problems several sequencing techniques emerged using NGS as base platform by combining restriction enzymes as versatile tool such as reduced-representation libraries (RRLs) and genotyping by sequencing (GBS).

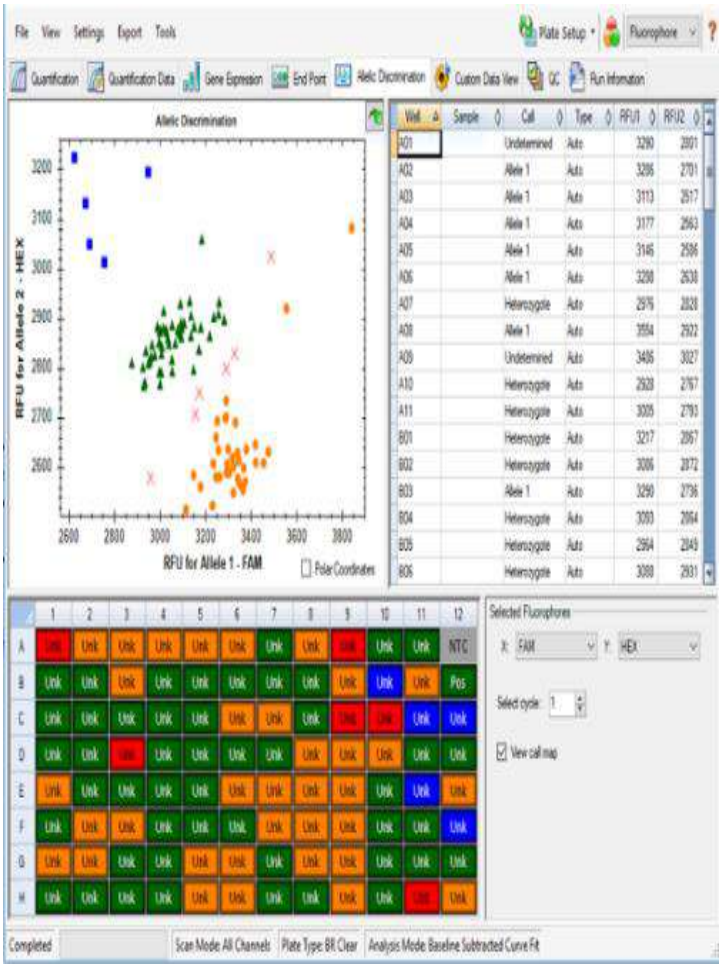
High throughput arrays in wheat

Advancements in next-generation sequencing technology have enabled whole genome re-sequencing in many species providing discovery and characterization of molecular polymorphisms. Based on this approach, scientists have developed a high-throughput array to interrogate 9,000 gene-associated SNPs in worldwide samples of 2,994 accessions of hexaploid wheat including landraces and modern cultivars in a chip format called it as "9K iSelect Beadchip Assay". Following observation of allelic ratio deviation between hexploid and diploid wheat SNP iSelect Array was developed comprising approximately 90,000 gene-associated SNPs. These 90K SNP iSelect Array provided dense coverage of the wheat genome for finding markers for the gene of interest.

Apart from 9K and 90K platforms, Diversity arrays technology (DArT) marker system was developed to provide a cost-effective whole-genome fingerprinting tool and efficient for species which have complex genomes and lack prior DNA sequence information. A single DArT assay is capable of typing of hundreds to thousands of single nucleotide polymorphism (SNPs) and insertion/deletion (indel) polymorphisms distributed throughout the genome. It involves assembly of a group of DNA samples representative of the target germplasm.

SNP genotyping and validation platforms

The modern chemistries and genotyping platforms used for SNP validation are Illumina's Bead Array technology-based Golden Gate (GG) and Infinium assays, Life Technologies' TaqMan assay coupled with Open Array platform (TaqMan Open Array Genotyping system, Product bulletin), and **KBiosciences' Competitive Allele Specific PCR (KASPar)** combined with the SNP line platform. Among these KASPar is the widely used assay with ease procedures.



Wheat genetic maps

After discovery of PCR-based SSR markers, first consensus map was build up by joining of independent four genetic maps of bread wheat. To highly saturate the existing wheat consensus map, various researchers developed variety of new markers (SSR, STS and ESTS) using advanced techniques and constructed new maps. Thereafter, recent advancement of NGS and discovery of abundant SNP markers, highly saturated maps with 90K SNPs have been constructed. Apart from above mentioned maps, DArT GBS map, 9K SNP map and chromosome-based maps are available.

Identification / Discovery of Resistant Genes

Landraces and wild relatives of wheat are considered as valuable sources of genetic variation for economic traits including rust resistance. Identification and characterization of genetically valuable diverse sources for rust resistance and its incorporation into the commercial cultivars determines the success of rust resistance breeding in wheat.

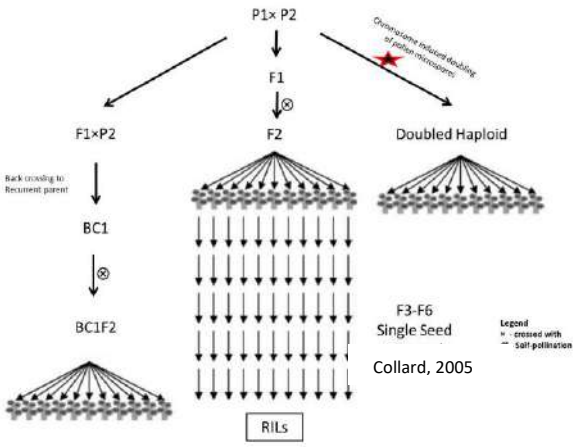
There are few steps in identification/discovery of resistant genes in wheat.

Gene postulation

Gene postulation is a classical method for detecting the presence of a particular gene(s) in a cultivar through phenotypic comparison with the differential line(s) carrying known gene. The principle behind the gene postulation is the gene-for-gene interaction between wheat genotypes and the rust pathogen to determine the probability of presence of the resistance gene. Characterised collection of wheat rust pathotypes is used to postulate the resistance genes in wheat on the basis of phenotypic expression as infection types. Knowledge of rust resistance gene conferring resistance against distinctive pathotypes played an important role in the wheat resistance breeding as well as in gene pyramiding.

Population development

Populations are developed by crossing of two contrasting parents showing polymorphism for the trait of interest. The number of lines used for will vary from 50 to 250 depending on number of targeted traits segregating in the population. There are three different types of population which are commonly used for genetic analysis and mapping, Doubled haploid (DH), recombinant inbred lines (RIL) and BC_1F_2. Genetics analysis of a gene in F_3 population can also be practiced. DH population is developed by chromosome doubling. RIL population is advanced from individual F_2 plants until F_6-F_8 generation by selfing. Single seed descend method is used to advance the generation from $F_{2:3}$ to get RILs. BC_1F_2 are derived by backcrossing F_1 with recurrent parent and selfing of the resulting BC_1F_1 plants.



Genetic analysis

Genetic analysis is commonly practiced method to determine the number of gene(s) present in a cultivar or genotype. The number of segregating resistance genes can be determined by phenotyping the F_3, BCF_2, DH or RIL population with specific rust races at the seedling stage or at the adult plant stage in the field against a mixture of rust pathotypes.

If a population is segregating for more than one gene, then isolation and characterisation of single gene F_3 families is the most important step. The major advantage of using BCF_2 populations compared to F_3 families is a smaller population is required to isolate single gene segregating families (1:1) or genetics analysis. An additional advantage of the backcross method is that the segregating resistances can be evaluated in a background with 75% of the susceptible recurrent parent.

Chromosomal location of gene

In last century chromosome, location was determined by studying the progenies developed by crossing test cultivars with monosomic series. But with the advances in marker technology, the chromosomal location of unknown genes can be detected by bulk segeregant analysis (BSA) or selective genotyping. In both methods, contrasting phenotypes carrying bulks or lines are screened using advanced automated 90K or 9K or DArT platforms.

Case Study:
1. Identification of stripe rust gene in a Watkinson's collection.
2. Identification of novel recessive leaf rust gene

Mapping rust resistant genes

Recent advances in automated next generation sequencing (NGS) platforms revolutionised wheat genomics and this technology coupled with advances in bioinformatics tools has resulted in faster and precise detection of marker-trait associations for genomic selection and genomic prediction. The availability of bread wheat reference sequence (IWGSC RefSeq v1.0) (http://www.wheatgenome.org/) is also highly

useful for development of closely linked markers. By using these molecular technologies, many disease resistances controlling chromosomal regions have been identified & formally named within the last decade.

Mapping of a major gene

Linked markers identified through BSA or selective genotyping are converted into PCR based markers and are genotyped in the mapping population. Then the phenotypic data and marker data are analysed using software of choice e.g. MapManager QTX, MapDisto to calculate the recombination fraction using Kosambi mapping function.

Mapping a QTL

Detection of QTL was impossible by conventional phenotypic evaluation but new marker technologies made it possible. RIL or DH population is genotyped using high throughput marker platforms. QTL analysis means examining the association between the genotype (markers) and the phenotype. Therefore, accurate phenotypic evaluation is very important. The principle behind the QTL analysis is that during the chromosome crossover, targeted trait and the closely linked marker(s) are co-segregating into the progeny, thus allowing analysis in the progeny.

Case Study:
1. Development of co-dominant KASP markers co-segregating with Ug99 effective stem rust resistance gene Sr26 in wheat

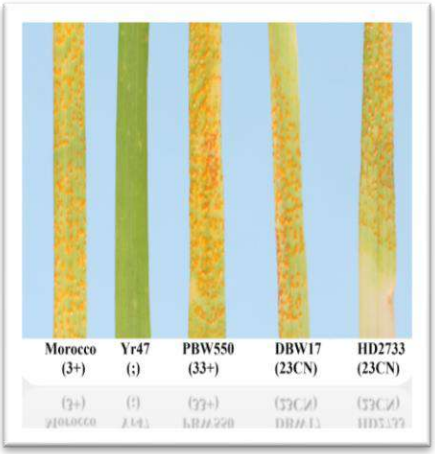
QTL may be called as 'minor' (R^2 < 10 %) or 'major' (R^2 > 10 %) based on the proportion of phenotypic variation explained as 'R^2' value and major QTL may be called as QTL if the phenotypic variation is constant over the different environment. In other way, QTL can be classified in to 'suggestive', 'significant' and 'highly significant' to ensure the true hints of linkage through the elimination of false positive claims.

For the QTL analysis, mapping populations are genotyped by previously published markers or GBS method or 90K SNP platform and linked polymorphic markers are used to construct genetic linkage map. Composite interval mapping is popular because it allows the analysis of linked QTL as well as additional markers in the linear statistical system. Statistical packages QTL Cartographer, PLABQTL, MapMaker, R/QTLBIM, R/QTL, QTL Express, Flex QTL, MCQTL, ICIM, etc. are publically available to perform the analysis. To draw the QTL and map figures MapChart software version 2.3 is commonly used.

Fast Track Introgression of Resistant Genes

- Testing effectiveness of target genes
- Screening for background genes
- Development of backcross derivatives or Doubled haploids (Speed breeding intervention)
- High throughput phenotyping
- Developing triple rust resistant lines
- Large scale multilocation testing and evaluation
- Entries to varietal release pipeline

Case Study:
Systematic introgression of triple rust resistant genes in to three Indian backgrounds trough MAS and Doubled haploid production



Conclusion

Major future area of interest is off-season phenotyping under controlled environment, developing known gene profile of identified entries and wheat- maize based doubled haploid technique for rapid fixation of genes. A successful breeding effort for rust resistance in wheat relies on understanding of pathogenic variation, identification of genetically diverse sources and deployment of combinations of resistance genes in new cultivar. The pathotypic evolution and wider adaptation of new pathotypes of rust pathogens resulted in demise of single gene carrying cultivars and elite germplasm; and many studies recommended the deployment of major (all stage resistance; ASR) and minor (adult plant resistance; APR) genes combinations in new wheat cultivars which can be achieved through marker assisted selection. The emerging trend towards high-throughput phenotyping, exploitation of modern genomic resources and faster breeding approach to deliver triple rust resistance and fortified versions of wheat cultivars is of outmost importance. The generated wheat elite materials will assist to achieve durable rust control and advance the level of global food security.

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Molecular Characterization of Rust Resistance Genes in Wheat

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Postulation of rust resistance genes through single race testing is time consuming and tedious. Therefore, use of molecular markers linked to or based on rust resistance genes can help in easy identification and utilization in breeding program. The molecular analysis for identification of rust resistance genes comprises of following steps:

1. Isolation of genomic DNA
2. Purification of DNA
3. Quantification and quality check
4. PCR amplification
5. Electrophoresis for resolving amplicon and Imaging

Isolation of Genomic DNA: For isolation of genomic DNA, fresh and tender leaves should be collected from seedlings at 3-4 leaf stage. The collected leaf samples should be flash frozen in liquid nitrogen and should be further stored in deep freezer at -80^{0}C. High quality DNA can be isolated from stored or fresh leaf samples following CTAB method (Murray and Thompson, 1980). Stock solution of 1M Tris-Cl (pH 8.0), 0.5M EDTA (pH 8.0), 4M NaCl and 10% CTAB are used for preparation of DNA extraction buffer. The stock solutions should be autoclaved and stored at room temperature. Five hundred ml working stock of CTAB buffer is prepared by mixing 50ml of 1M Tris-Cl (pH 8.0), 20ml 0f 0.5M EDTA (pH 8.0), 175ml of 4M NaCl, 100ml of 10 percent CTAB and 155ml of double distilled water.

Tender wheat leaves to be ground using a pestle and mortar in liquid nitrogen. The powdered leaf samples are transferred to a 2ml microcentrifuge tubes having 1ml of pre-heated (65^{0}C) CTAB extraction buffer and 0.2 % of β-mercaptoethanol. The powdered samples along with pre-heated extraction buffer are incubated at 65^{0}C in water bath or heating block for 1hr. The samples are mixed by gentle swirling at 10-15 minutes interval during incubation. After incubation and mixing, the samples in buffer are brought to room temperature. The samples along with buffer in tubes are mixed with an equal volume (1 ml) of chloroform: isoamyl alcohol (24:1). The tubes are again mixed for 5 min by gentle inversion. The samples are centrifuged at 10,000 rpm for 10 min at 4^{0}C. Proper care should be taken to balance the rotor of centrifuge. The supernatant from centrifuged tubes are taken out with a wide-bore pipette tip and kept in new micro centrifuge tubes followed by addition of 2/3rd volume of chilled iso-propanol. The sample is mixed again by quick and mild inversion for few times and incubated in freezer at -20^{0}C for 30 min. The coagulated DNA is centrifuged at 10,000 rpm for 5 min at room temperature and the supernatant is decanted. DNA pellets are washed by ethanol (70% v/v) through brief centrifugation. The washed DNA pellets are air dried and dissolved in TE (100 mMTris and 50 mM EDTA, pH 8.0) buffer according to amount of DNA. The dissolved DNA samples are stored at 4^{0}C for further processing and use.

Purification of DNA: Major impurities of crude DNA preparation are RNA, protein and polysaccharides. Inclusion of CTAB in DNA extraction buffer helps in elimination of polysaccharides to a large extent. The RNA is removed by treating the samples with DNase free RNase (10 mg/ml) denatured at 70^{0}C. Depending upon the amount of DNA, 5-10 ul of RNase A is added and incubated at 37^{o}C for 30 minutes to 1 hour. Proteins including RNase are removed by treatment with phenol: chloroform: isoamyl alcohol (25:24:1). The DNA sample is precipitated again as discussed earlier.

Quantification and quality check: Image of DNA on 0.8 % agarose indicates quality and contamination of DNA. During DNA isolation, steps like vortexing or rapid mixing of samples can result in shearing of DNA. The shearing of DNA can be confirmed by broad DNA band. Contamination of RNA can also be confirmed by presence of distinct band at bottom. The quantity of DNA can also be confirmed on agarose gel by comparing DNA band with known concentration (100ng, 200 ng) of λ uncut DNA. The DNA can also be quantified on spectrophotometer and purity can be confirmed by ratio of absorbance at 260/280 nm, which around 1.8 for a pure DNA sample. The pure genomic DNA is diluted to 20-25ng/µl working stock for further use in PCR.

PCR amplification: The diluted working stock of DNA is used to carry out PCR. Specific SSR primers diluted to an initial concentration of 15 µM and further diluted to a working concentration of 5 ppm. The marker analyses can be performed in 10-25 µl reaction volume. The Polymerase Chain Reaction (PCR) can carried out in the PCR plates/tubes depending upon number of samples on a thermal cycler with the following thermal profile: initial denaturation step of 94^0C for 4 min, followed by 35-45 cycles of 94^0C for 1 min (denaturation), at specific annealing temperature for 1 min (annealing temperature depends on primers) and 72^0C for 1 min (primer extension), with a final extension of 72^0C for 10 min and final cooling at 4^0C.
Electrophoresis for resolving amplicon and Imaging: The amplified PCR products are subsequently resolved on 3.5% metaphore gel in 1X TBE buffer. Loading dye is added in each PCR product and loaded in the wells of the solidified gel. DNA ladder of different size is used as reference for confirmation of PCR product. The PCR product is resolved on electrophoresis unit for 2-3 h at 120 volts. Finally, the gel is stained in ethidium bromide solution (10 mg/ml) and visualized under UV trans-illuminator in a gel documentation system. (Ethidium bromide added to the boiled and cooled metaphor during gel preparation)

Primers for molecular analysis: Primers designed for amplification of markers linked to many seedlings as well as adult plant resistant gene has been developed for all 3 wheat rust. The different primers for each of the mapped genes and their PCR condition are provided at (http://maswheat.ucdavis.edu). The diagnostic or gene based markers can verify the presence of specific genes. In addition, linked molecular markers can be used to guess the presence of specific genes.

Equipments required: High speed centrifuge, Micropipettes (2-20µl, 20-200µl and 200-1000µl), Waterbath/Heating block, Deep freezer (-20^0C) and Refrigerator (4^0C)

Preparation of stock solutions of CTAB buffer

1. 0.5 M EDTA (pH 8.0): 186.1 g of sodium salt of EDTA was dissolved in 800 ml of MQ/DD water; pH was adjusted to 8.0 with NaOH pellets. The final volume was adjusted to one litre with MQ water and sterilized by autoclaving.
2. 4 M NaCl: 233.8 g of NaCl was dissolved in 800 ml of MQ/DD water. The final volume was adjusted to one litre with MQ water and sterilized by autoclaving.
3. 1M Tris-Cl: 121.1 g of Tris-Cl salt was dissolved in 800 ml of sterile MQ water. pH was adjusted to 8.0 with concentrated 1N HCl. The final volume was adjusted to one litre with MQ/DD water and sterilized by autoclaving.
4. 10% CTAB: 100 gm of CTAB powder was dissolved in sterile MQ water and the volume was adjusted to one litre.

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Unravelling the Genomes of Bread Wheat and its Leaf Rust Pathogen for Accelerated Wheat Improvement

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Wheat (*Triticum aestivum* L.) is arguably the most widely cultivated crop in the world that contributes about a fifth of the total food calories for humans. Therefore, wheat productivity and production are key factors in the global economy. Wheat crop failure can lead to major food scarcity and social unrest. Wheat breeders have continuously strived to develop improved varieties by dissecting the genetics of complex agronomic traits including yield, end-use quality and resistance to a range of biotic and abiotic stresses. However, breeding efforts are limited by insufficient knowledge and understanding of wheat biology and the molecular basis of the key agronomic traits. To meet the demands of human population, there is an urgent need for wheat research and breeding to accelerate genetic gain as well as to enhance the wheat yield while maintain its quality. Access to a fully annotated reference genome sequence, with complete information on its gene space including regulatory sequences and genetic diversity has promoted the development of systematic approaches for understanding and selection of important traits. Wheat genome sequencing has lagged behind, primarily because of the challenges of assembling a genome that is more than five times as large as the human genome, hexaploid and highly redundant containing more than 85% repetitive DNA. International Wheat Genome Sequencing Consortium was formed in 2005 to provide a foundation for wheat molecular breeding and set out to deliver a high-quality annotated reference genome of bread wheat.

An annotated reference sequence representing all the 21 chromosomes of hexaploid bread wheat genome has been completed giving access to 107,891 high-confidence genes, including their genomic coordinates and regulatory sequences. This genome assembly has enabled discovery of tissue- and developmentally regulated gene expression networks using a transcriptome data representing different stages of wheat growth and development. The dynamics of change in complex gene families involved in climate adaptation and product quality were revealed at sub-genome level and mapped to known single genes or quantitative trait loci (QTLs). Aspects of the future value of the annotated assembly for molecular breeding and research have been illustrated by resolving the genetic basis of a quantitative trait locus conferring resistance to abiotic stress and insect damage as well as by serving as the basis for genome editing of the flowering-time trait.

The annotated reference genome of the bread wheat in 21 chromosome pseudomolecules have been analyzed to identify the distribution and genomic context of protein-coding and noncoding elements across the A, B, and D sub-genomes. With an estimated coverage of 94% of the genome and containing 107,891 high-confidence gene models, the assembly has enabled discovery of genome wide gene regulation patterns. Dynamics of complex gene families involved in environmental adaptation to various stresses and end-use quality have been revealed at sub-genome resolution and contextualized to known agronomic single-gene or quantitative trait loci. This community resource establishes the foundation for accelerating wheat research and application through improved understanding of wheat biology and genomics-assisted breeding. Analyses of the components of the genome sequence revealed the distribution of key elements and enabled detailed comparisons of the homologous A, B, and D sub-genomes. There are 3,968,974 copies of TEs belonging to 505 families, accounting for 85% of the genome with relatively equal distribution across the three sub-genomes. Total 112,744 full-length long terminal repeat (LTR)–retro transposons were identified that have been difficult to define from short-read sequence assemblies. These have been extensively rearranged through rounds of deletions and amplifications since the divergence of the A, B, and D sub-genomes about 5 million years ago. Still the TE families that shaped the Triticeae genomes have been maintained in similar proportions: 76% of the 165 TE families present in a cumulative length greater than 1 Mb contributed similar proportions (less than a twofold difference between sub-genomes), and only 11 families, accounting for 2% of the TEs, showed a higher than threefold difference between two sub-genomes. The TE abundance largely accounts for the size differences between the sub-genomes, e.g, 64% of the 1.2-Gb size difference between the B and D sub-genomes can be attributed to lower gypsy retro transposon content. No evidence was found for any major burst of transposition after polyploidization of the bread wheat genome.

Gene models were predicted with two independent pipelines previously utilized for wheat genome annotation and then consolidated to produce the RefSeq Annotation v1.0. Subsequently, a set of manually curated gene models was integrated to build RefSeq Annotation v1.1. In total, 107,891 high-confidence (HC) protein-coding loci were identified, with relatively equal distribution across the A, B, and D sub-genomes (35,345, 35,643, and 34,212, respectively. In addition, 161,537 other protein-coding loci were classified as low-confidence (LC) genes, representing partially supported gene models and gene fragments, and orphans. *In silico* predicted functions were assigned to 82.1% (90,919) of HC genes in the RefSeq Annotation v1.0, and transcription support was found for 85% (94,114) of the HC genes versus 49% of the LC genes. Within the pseudogene category, 25,419 (8%) of 303,818 candidates matched LC gene models. The D genome contained significantly fewer pseudogenes than the A and B genomes (81,905 versus 99,754 and 109,097, respectively.

The completeness of the RefSeq Annotation v1.1 gene set was benchmarked against BUSCO v3, representing 1440 Embryophyta near-universal single-copy orthologs and published annotated wheat gene sets. Of the BUSCO v3 genes, 99% (1436) were represented in at least one complete copy in RefSeq Annotation v1.1 and 90% (1292) in three complete copies. To further characterize the gene space, a phylogenomic approach was applied to identify gene homeologs and paralogs between and within the wheat sub-genomes and orthologs in other plant genomes. Analysis of a subset of 181,036 genes comprising 103,757 HC and 77,279 LC genes identified 39,238 homeologous groups—that is, clades of A, B, and D sub-genome orthologs deduced from gene trees - containing a total of 113,653 genes (63% of the filtered set). Gene losses and gene gains were determined for all homeologous loci of IWGSC RefSeq v1.0, assuming the presence of a single gene copy at the diploid level.

The selection of desirable genetic variation underlying agronomic traits in breeding programs is complicated if phenotypic selection depends on the expression of multiple loci with quantitative effects which are strongly influenced by the environment. This problem can be overcome by finding DNA markers tightly linked with the phenotype through forward genetic approaches or through genome editing. The potential of IWGSC RefSeq v1.0, together with the detailed genome annotation, to accelerate the identification of candidate genes underlying important agronomic traits was exemplified for two targets. A forward genetics approach was used to fully resolve a QTL for stem solidness (SSt1) conferring resistance to drought stress and insect damage. A diagnostic SNP marker was developed that was physically linked to the copy number variation that has been deployed to select for stem solidness in wheat breeding programs.

Knowledge from model species can also be used to annotate genes and provide a route to trait enhancement through reverse genetics. This approach here targeted flowering time, which is important for crop adaptation to diverse environments and is well studied in model plants. Six wheat homologs of the FLOWERING LOCUS C (FLC) gene were been identified as having a role in the vernalization response, a critical process regulating flowering time. IWGSC RefSeq v1.0 was used to refine the annotation of these six sequences to identify four HC genes and then to design guide RNAs to specifically target, using CRISPR-Cas9 based gene editing of one of these genes, TaAGL33, on all the sub-genomes [TraesCS3A01G435000 (A), TraesCS3B01G470000 (B), and TraesCS3D01G428000 (D)]. Editing was obtained at the targeted gene and led to truncated proteins after the MADS box through small deletions and insertions. Expression of all homeologs was high before vernalization, dropped during vernalization, and remained low post-vernalization, implying a role for this gene in flowering control. The expression pattern was not strongly affected by the genome edits. Plants with edited events in D sub-genome flowered 2-3 days earlier than controls. Further refinement could help to fully elucidate the importance of TaAGL33 gene for vernalization in monocots. These results exemplify how IWGSC RefSeq v1.0 could accelerate the development of diagnostic markers & design of targets for genome editing for traits relevant to breeding.

IWGSC RefSeq v1.0 is a resource with potential for disruptive innovation in wheat breeding. Breeders work with the genome at the whole chromosome level as each new cross involves modification of genome-wide gene networks that control the expression of complex agronomic traits such as yield. With the annotated and ordered reference genome sequence available, researchers can now easily access sequence-level information to define changes in the genomes of varieties in their breeding programs. Although hundreds of QTLs have been published in wheat, only a small number of genes have been cloned and functionally validated. IWGSC RefSeq v1.0 underpins immediate application by providing access to regulatory regions and will serve as the backbone to anchor all known QTLs to one common annotated reference genome. Combining this knowledge with the distribution of meiotic recombination frequency and genomic diversity will enable breeders to more efficiently tackle the challenge of parallel selection for adaptation to biotic and abiotic stress, end-use quality, and yield improvement. Strategies can now be defined more precisely to bring desirable alleles into coupling phase, especially in low recombination regions of the wheat genome. Here the full potential of the newly available genome may be realized through the implementation of DNA-marker platforms and targeted breeding technologies including genome editing.

Wheat production is severely affected by three types of rust diseases, leaf or brown rust (*Puccinia triticina* Eriks); stem or black rust (*Puccinia graminis* Pers. f.sp. tritici); and stripe or yellow rust, (*Puccinia striiformis* Westend f. sp. tritici). In India, leaf rust is the most important and prevalent of these three diseases. Stem and stripe rusts are restricted to certain parts of India, whereas leaf rust is prevalent all over the country and appears in the months of February–March, when the wheat is in anthesis, or grain filling stage. Leaf rust is comparatively more frequent worldwide and results in more yield losses, due to reduced kernel weight and decreased numbers of kernels per head, while economic losses due to stem and stripe rusts are relatively low. Losses in kernel weight among wheat varieties due to leaf rust infection can be up to 40% depending on the resistance level of wheat varieties. Leaf rust disease can be effectively managed using resistance (R) genes; however, the most challenging task for breeding programs is to incorporate durable leaf rust resistance genes into high yielding cultivars. The frequent appearance of new variants of *P. triticina* and shifts in virulence patterns are major problems for leaf rust management.

Decoding the genome of leaf rust pathogen will help in unraveling the molecular basis of its evolution and in the identification of genes responsible for its various biological functions. We generated a high quality draft genome sequences (approximately 100- 106 Mb) of two races of *P. triticina*; the variable and virulent Race77 and the old, avirulent Race106. The genomes of races 77 and 106 had 33X and 27X coverage, respectively. We predicted 27678 and 26384 genes and found that the genomes consisted of 37.49% and 39.99% repetitive sequences, in race 77 and 106, respectively. Genome wide comparison revealed Race77 differing substantially from Race106 with regard to segmental duplication (SD), repeat element, and SNP/InDel characteristics. Comparative analyses showed that Race 77 is a recent, highly variable and adapted race as compared with Race106. Sequence analyses of additional 13 pathotypes of Race77 clearly differentiated the recent, active and virulent, from the older pathotypes. Secretome analysis demonstrated that Race77 has more virulence factors than Race 106, which may be responsible for its greater adaptation. We also found that genes under greater selection pressure were conserved in the genomes of both races, and may affect functions crucial for the higher levels of virulence factors in Race77. The study provides insights into genome structure, genome organization, molecular basis of variation, and pathogenicity of *P. triticina*. The genome sequence data generated in this study have been submitted to public domain databases and will be an important resource for comparative genomics studies of the more than 4000 existing *Puccinia* species.

The annotated genomes of wheat and its leaf rust pathogen are genomic resources that can drive rapid innovation in wheat improvement as this a community resource establishes a foundation for accelerating wheat research and its application in genomics-assisted breeding. The database developed will facilitate a better understanding of the evolution of wheat genome by making use of the chromosome level high-quality reference assembly. Breeders work with the whole genome level because each new cross involves rearrangement of the genome-wide gene networks that control the expression of complex agronomic traits such as yield and quality. With the annotated and ordered reference genome in place, wheat scientists can now easily access chromosome-level information to precisely define and create necessary changes in the genomes for wheat breeding programs. This will be realized through the implementation of new DNA marker platforms and targeted breeding, particularly for developing wheat cultivars with immunity to rust diseases.

Strategies for Genome Sequencing of Fungal Plant Pathogens

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Introduction

A genome is an organism's complete set of DNA, including all of its genes. All living things have a unique genome. The technique that allows researchers to read and decipher the genetic information found in the DNA of any living organism is called genomic sequencing. The genomes of more than 1000 fungal species are already publically available and it's growing steadily. The fungal kingdom comprises some of the most devastating plant pathogens. Fungal genomics has enabled to rapidly develop tools to study pathogen biology and genetics. In a field where delayed action has profound consequences for livelihoods and food, genome sequences provide us with essential tools to prepare for the emergence of new plant pathogens and future disease outbreaks. Some salient information about genome features are given in Table 1.

Table 1: Salient features of fungal genome

Fungal Species	Genome size (MB)	GC content (%)	No. of genes	No. of Protein
Puccinia graminis tritici	81.6	43.3	16,309	15,979
Puccinia triticina	135.34	46.7	15,69	15,685
Magnaporthe oryzae	40.95	51.6	13,184	12,989
Bipolaris oryzae	31.33	50.5	12,002	12,002
Fusarium oxysporum f. sp. *lycopersici*	59.93	48.4	21,354	53,650

Brief Methodology and Techniques Involved in Genome Sequencing

Isolation, Library Preparation and Sequencing

Initially, the isolated fungus is characterized for internal transcriber region using ITS-4 and ITS-5 markers to confirm the fungus genus, species and isolation purity. Once the fungus is confirmed, gDNA is isolated from the pure fungus and used for library preparation. Generally, each NGS sample processing called library preparation starts with the shearing/fragmentation/tagmentation of genomic DNA into desired fragments and followed to end repair. After the end repair, each sample subjected for the multiplexing through adapter and barcode/index ligation reaction, called sequencing libraries. The prepared library subjected for the quality and quantity check to make sure prepared libraries are suitable for sequencing. As samples are barcoded, we can pool the various samples together through normalization and then equimolar pooling. Next, pooled library placed for clonal amplification through emulsion PCR (emPCR, in 454 GS FLX and Ion Torrent) and bridge amplification (cluster generation, in Illumina). In 454 GS FLX and Ion Torrent, after emPCR, sample processed for recovery and enrichment (as illustrated in Fig. 1). The finally enriched sample loaded in chip and then placed in machine for sequencing. Whereas in Illumina, sequencing followed immediately after cluster generation. Each machine generated sequenced sample stored in the form nucleotides fastq files, which is a standard output format.

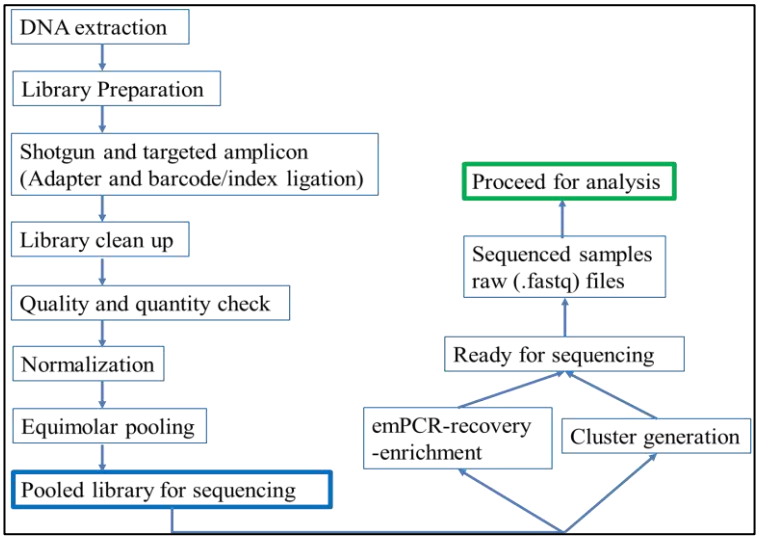

Fig. 1 Simple work flow of next generation sequencing library preparation and sequencing protocol

Brief Summary of Sequencing by Reversible Termination

The sequencing by reversible termination technology was applied in Illumina Genome Analyzer (SOLEXA) promoted in the year 2006. In this technology, the subjected study material is prepared through random fragmentation, which followed by the ligation of oligonucleotide adaptors and indexes, called prepared libraries which will be subjected for sequencing in the machine. The prepared libraries were amplified through bridge amplification technology (Adessi *et al.,* 2000; Fedurco *et al.,* 2006). In this process, the PCR forward and reverse primers complementary with adapters are hybridized on glass surface, prepared libraries are amplified using modified DNA polymerase in a procedure called cluster generation. In this sequencing technology, a modified DNA polymerase and different fluorophore-labelled nucleotides at 3' position are used. In each cycle of sequencing, integration of single nucleotide followed to cleavage of fluorescent reporter which is matching to the incorporated base and recorded by camera (Ju et al. 2006). The developments in this technology permitted the 300∗2 paired-end sequencing with a total average read length of 600 nucleotides (Goodwin *et al.,* 2016). The limitation of this technology is high error rate of transition (Ts) to transversion (Tv) SNPs and Ts/Tv ratio. This technology offers the following two distinct kind of library preparation, while both kind of libraries are sequenced on compatible Illumina sequencing machine in a default sequencing chemistry.

Paired End library preparation

- The paired-end sequencing library is prepared using Illumina Library Preparation Kit. The insert size of the PE sequencing libraries is ~300bp -550 bp.
- Each Sequencing library will be individually indexed/barcoded for sequencing.
- Most frequently used sequencing library for genomes and metagenomes.

Mate Pair library preparation

- Preparation of Mate Pair library with jumping distance of 3KB & 8KB average insert size.
- Each sequencing library will be individually indexed/barcoded for sequencing.
- Usually used for genome gap finish and polishing.

PacBio Sequel: Single Molecule Real Time sequencing

The third-generation sequencer comprises through DNA sequencing without applying the PCR extension step, as amplification introduces a bias in read content and existence of high GC content affects depth and coverage. The key advantage of this technology is the longer reads with an average length of 5–10 Kb. With this sequencing technology, the first commercially launched was single-molecule real-time (SMRT) by Pacific Biosciences (Eid *et al.,* 2009). In this sequencing chemistry, the sample library preparation consists of DNA molecule to be circularized by ligating the adapter to both the ends of template. The generated circular library is positioned into SMRT cell comprising 150,000 zeptoliter wells. Each well of SMRT cell comprises single immobilized modified DNA polymerase at the bottom. DNA polymerase binds with adapter sequence and then starts the template replication. The incorporation of complementary four different fluorescently labelled nucleotides into reaction well, the labelled nucleotide gets incorporated enzymatically, a light signal is produced and recognized as the matching nucleotide (Eid *et al.,* 2009). The data output of PacBio RS II machine is 0.5–1 billion bases per SMRT cell with very high error rate (typically 10–15%).

Nanopore Sequencing Technology

Another third-generation sequencer is MinIon instrument commercialized by Oxford Nanopore Technology in 2014. In this sequencing technology, sample is subjected through a nanopore through electrophoresis, using electrolytic solutions with fixed electric field. As the template passes through nanopore, change in current occurs, and the resultant magnitude is recorded. MinIon library generation involves DNA fragmentation and end repaire, and then poly A tail is added to 3' OH end. In these two dissimilar adapters, a hair pin adapter and Y adapter (shape based) are used. With the assistance of motor protein, sequencing templated dsDNA is unzipped at Y adapter and passes the ssDNA through nanopore. It is followed through base calling of ssDNA and hundred to thousand base pair read length is obtained, with an accuracy of 88% (Laszlo *et al.,* 2014). This technology provides long reads, low cost, and small size with real-time nature of sequencing and offers attention in genomics study.

10x Genomics Chromium Technology

This microfluidics based-library chemistry is a prime-trade product of 10x Genomics company founded by Saxonov, Hindson and Ness. Primarily, it is helpful to the scientists who utilizes heterogeneous tissues/ subpopulations for single-cell RNA based sequencing also known as scRNA-seq. During the run, individual cells are encapsulated into nanoliter droplets/emulsion which contain DNA-barcoded reads for reverse transcription. For cDNA synthesis, recovery droplets are broken up, and cDNA formed is subjected to library

preparation. This method enables the profiling of more than five hundred cells simultaneously, however, it requires custom-designed special equipment initially which need to be purchased separately than reagents.

In this technology, the studied samples are mixed with 10X barcoded gel beads and oil. It is then followed to collection of gel bound cells called single cell GEM (Gel bead in EMulsion). The collected GEMs are reverse transcribed in thermal cycler to get the 10X barcoded cDNA. Finally oil was removed to get the purified 10X barcoded cDNA. This prepared library checked for quality and quantity, and then used for sequencing. This technology successfully works with as little as 1 ng of genomic DNA. This technology prepared sequencing libraries are compatible with Illumina HiSeq sequencer such as HiSeq 4000 sequencer.

Efficient in the detection of structural variants (SVs) such as insertions, deletions, translocations and inversions. Haplotype phasing for SNVs, indels across >10 Mb haplotype region. Also suitable for single cell genome study. Important perquisite for these technology are the live cells (their quality), total cell count per experiment and FACS sorting. The convenient platform systems for droplet-based single-cell analysis are ddSeq single-cell isolator (BioRad), InDrop (1CellBio), single-cell controller (10x Genomics) and Fluidigm C1 platform (Fluidigm).

NGS Data Processing and Genome Assembly

Quality Filtration

Initially, the raw NGS files are processed for the filtering criteria is that any read with base quality score Q<20 is filtered, then following to read trimming from 5' end and 3' end, if required. The machine generated raw reads are filtered to obtain high quality clean reads using Quality Trimming tools to remove adapter sequences, ambiguous reads (reads with unknown nucleotides "N" larger than 5%), and low-quality sequences (reads with more than 10% quality threshold (QV) < 20 phred score (for phred scale phred33)). Few quality filtration tools are *Trimmomatic,* Cutadapt, *T*rim Galore, PRINSEQ, etc.

de novo Genome Assembly

The quality passed reads are utilized for genome assembly, which describes the process of taking a large number of short DNA sequences and putting them back together to create a representation of the original chromosomes from which the DNA originated. The word *'de novo'* means starting from the beginning. Assemblies can be produced which have less gaps, less or no mis-assemblies, less errors by tweaking the input parameters. The most frequently used tools for genome assembly, majority of them are command line interface (CLI) and available only for Ubuntu (free and open source) operating system. Among that CLC-Bio, SOAP denovo2, Velvet, IDBA-UD, SPAdes are the widely used. These tools details algorithm, input data type and dependencies are given in Table 2. This step is performed to optimize the generated assemblies by combining overlapping contigs and introducing appropriate gaps. A scaffold can be defined as a portion of the genome sequence reconstructed from end-sequenced whole-genome shotgun clones. Some of the scaffolding tools are SSPACE, PBJelly.

Table 2: List of some tools for genome assembly

Assembler	Algorithm	Assembly Method	Standard Input	Read length	Output format	Availability
CLC-Bio	de Bruijn graph	Denovo and Reference	fasta, fastq	Arbitrary	fasta, sam, bam	Licence
SeqManNgen	Patented	Denovo and Reference	fasta, fastq	Arbitrary	fasta, sam, bam	Licence
SOAP denovo2	de Bruijn graphs	Denovo	fastq	Arbitrary	fasta	Open source
MaSuRCA	Hybrid de (Bruijn graph +overlap-based)	Denovo	fastq	Arbitrary	fasta	Open source
Velvet	de Bruijn graphs	Denovo	fastq	Arbitrary	fasta	Open source
IDBA-UD	de Bruijn graph	Denovo	fastq	Arbitrary	fasta	Open source
CAP3	Overlap Layout Consensus	Denovo	fasta	Arbitrary	fasta	Open source
SPAdes	De Bruijn Graphs	Denovo	fastq	Arbitrary	fasta	Open source

Genome Annotation Work Flow

The standard genome annotation pipeline illustrated in Fig. 2, which consist of repeat masking to functional metabolic pathway and protein family identification. Sequenced organism transcript utilized for the identified genes regiments and accuracy during gene prediction. The predicted genes subjected for determination of metabolic pathways, protein family, conserved domains etc.

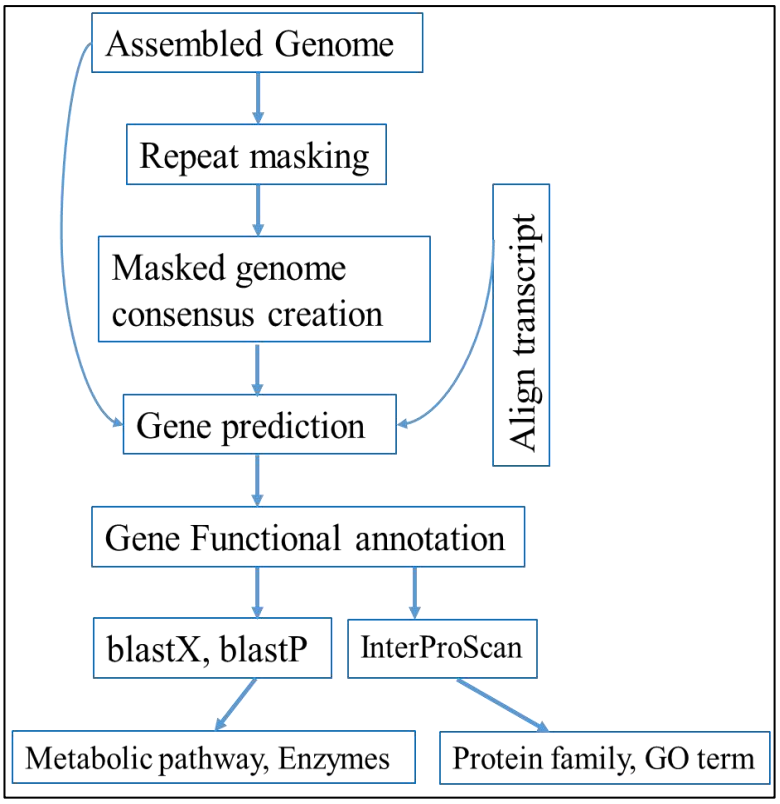

Fig. 2 The schematic work flow of genome annotation.

Generally, the NGS machines from shotgun metagenome generates the read length from 50 to 600 base. Among that majority were ranged from 300 to 600 bases, depending on the sequencing platform and chemistry. These short reads are assembled into longer sequences called contigs in a process call assembly. The assembly of short sequences become more important when the objective is to find the functional gene and metabolic pathways. The microbial genome sequencing is now become feasible for various laboratories in allied research, diagnostic, and clinical microbiology. In current time, numerous tools are available for genomics data analysis. These tools mainly varies from algorithms and code language. Other variations include hardware requirement, user interface, installations and their user-interface.

The advancement in genome sequencing has led the development of various bioinformatic tools for de novo genome assembly and annotation. Further as bioinformatic knowledgebase advanced, it offered to the development of various automated whole genome data based microbial genome binning and functional annotation, while requiring good computation resources. The ab-initio gene prediction based tools such as GeneMarkS, GLIMMER, AUGUSTUS were highly used. More details of each tool presented in Table 3.

Table 3: Tools used for *Ab-initio* gene prediction in genomes.

Tools	Input	Output format	Availability
GLIMMER	fasta	.txt	Open source
AUGUSTUS	fasta	.txt, .gff	Open source
GeneMark	fasta	.txt, .gff	Open source
ORF Finder	fasta	.txt	Open source
Prodigal	fasta	.txt, .gbk	Open source

Prediction of genes: The assembled scaffolds are then searched for their coding potential to get the protein coding gene sequences. The widely used tools for this purpose are Prodigal, Augustus, GeneMark and Maker-P. The predicted genes are annotated based on sequence homology to known proteins by performing a BlastX against protein databases such as non-redundant protein database at NCBI, UniprotKB, etc. The GO mapping provides ontology of defined terms representing gene product properties which are grouped into three main domains: Biological process, Molecular function and Cellular component.

Reference based genome analysis: The development of computational tools for reference based gene finder, the BLAST+, InterProScan, DIAMOND and Blast2GO were highly (Table 4). This method is executed, if there is a well assembled reference genome available for analysis. Further, from the reads gene identification, the high quality reads are first aligned to the reference genome. The genomes have to be indexed as per the aligner tool used for mapping the reads. The alignment tool needs to be specified if the reads are paired end or single end. Most popular aligning tools are BWA, Bowtie, TopHat.

Table 4: Tools used for reference based gene identification in genomes.

Tools	Input	Output format	Availability
BLAST+	fasta, fastq	.txt, sam, .xml	Open source
InterProScan	fasta	.txt, .xml	Open source
DIAMOND	fasta,fastq	.txt, .sam, .xml	Open source
Usearch	fasta,fastq	standard	Open source
RAPSearch	fasta,fastq	standard	Open source
PALADIN	fasta,fastq	standard	Open source
GhostX	fasta	.txt, html	Open source
Blast2GO	fasta,fastq	.txt, .xml	License

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Sequencing of Plant Genome: A Brief Overview of different Sequencing Chemistries

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The genome of higher eukaryotes are very complex and dynamic in nature as they possess giant amounts of DNA in their cells**.** These large amounts of DNA are densely packed in large no. of chromosomes that may be further complicated by increased ploidy levels. The nature of these sequences again vary between species, are also sometimes heterozygous in nature and it is now well known these are also regulated by epigenetic mechanism. These factors complicate the approaches and chemistries used for decoding the genomes of such organisms. Plant genomes are among the largest known among all the organisms on this planet. It varies between 125Mb for *Arabidopsis thaliana* to about 150Gb for a member of the legume family. Thus, massive amounts of sequence data has to be unravelled to assemble the genome in a chromosome specific manner.

First Generation Sequencing:

Sequencing approaches have evolved significantly with development of innovative and high throughput chemistries which have imparted a radical change in molecular and evolutionary biology. The science of DNA sequencing started with the landmark experiments of Maxam &Gilbert and F Sanger in the late 1970s. The manual sequencing operations gave way to automated and high throughput data generation by the beginning of the 21st century. The later part of the last decade saw a spurt of new generation chemistries that also catered to the sequencing of all types of RNAs, genotyping, methylome etc.

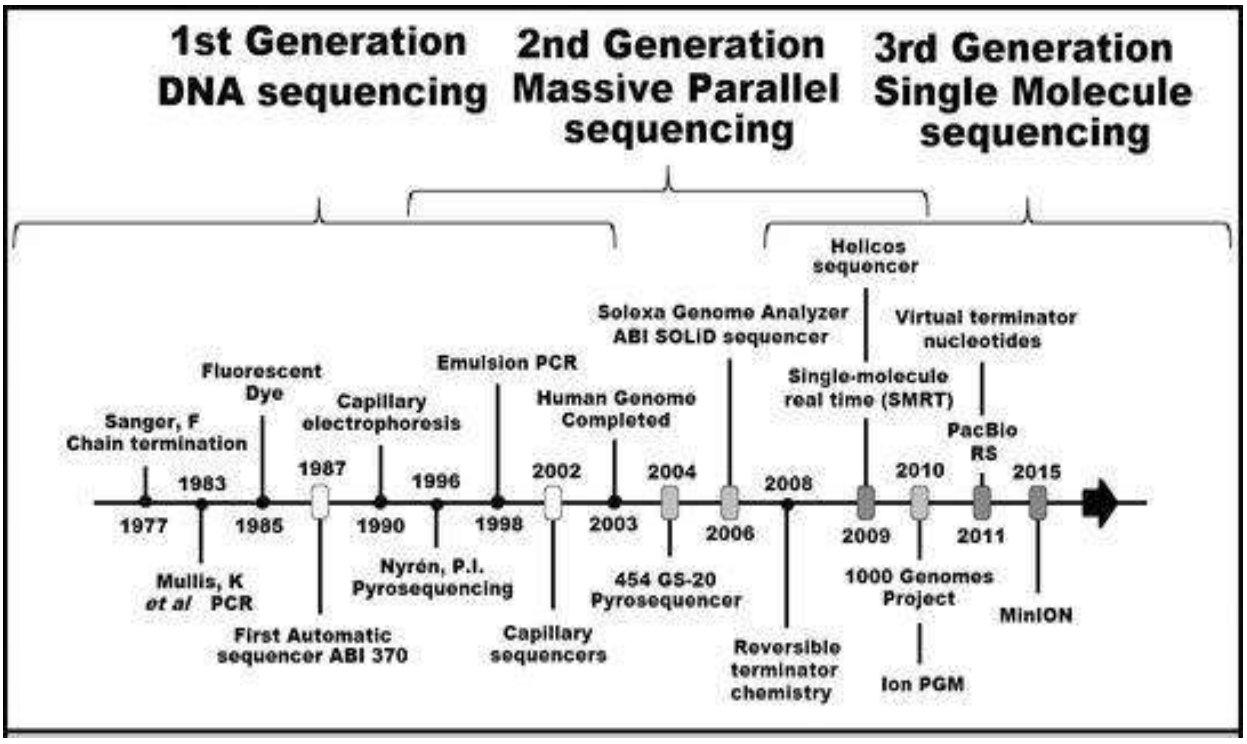


Fig 1. Chronology of DNA sequencing technology (Adopted from Pereira M.A et al., 2017)

Second Generation Sequencing:

It all started with the 454 technology also known as pyrosequencing, Illumina technology (Reversible terminator) followed by SOLID technology and ion torrent sequencing. There are two basic sequencing chemistries; one is sequencing by synthesis and another is sequencing by ligation and hybridization. Former chemistry is applicable in Roche 454, Illumina, and Ion torrent sequencing, while the latter is applied in SOLiD sequencing. In sequencing by synthesis strategy, incorporation of every nucleotide releases pyro

phosphate which is utilized by enzyme sulfurylase to convert it into ATP which is further involved in reaction catalyzed by luciferase. The principal of Ion torrent is similar to 454 where addition of every nucleotide in growing chain during DNA polymerization releases H+ resulting in change of pH, which is then detected by semiconductor chip. Detection system is direct, and no camera, light or scanning is required.

The major disadvantage of 454 and Ion torrent technology is that they are not suitable for homopolymer detection. If homopolymer repeats of the similar nucleotide (e.g. AAAAAA or GGGGG) are there on the template to be sequenced, complementary similar nucleotides will be incorporated thus release of more no. of hydrogen ions in a single cycle which leads to pH change obviously as a result voltage magnitude will also increase proportionally. By examining the magnitude, it is very difficult to determine the exact no. of nucleotide added during the DNA polymerization.

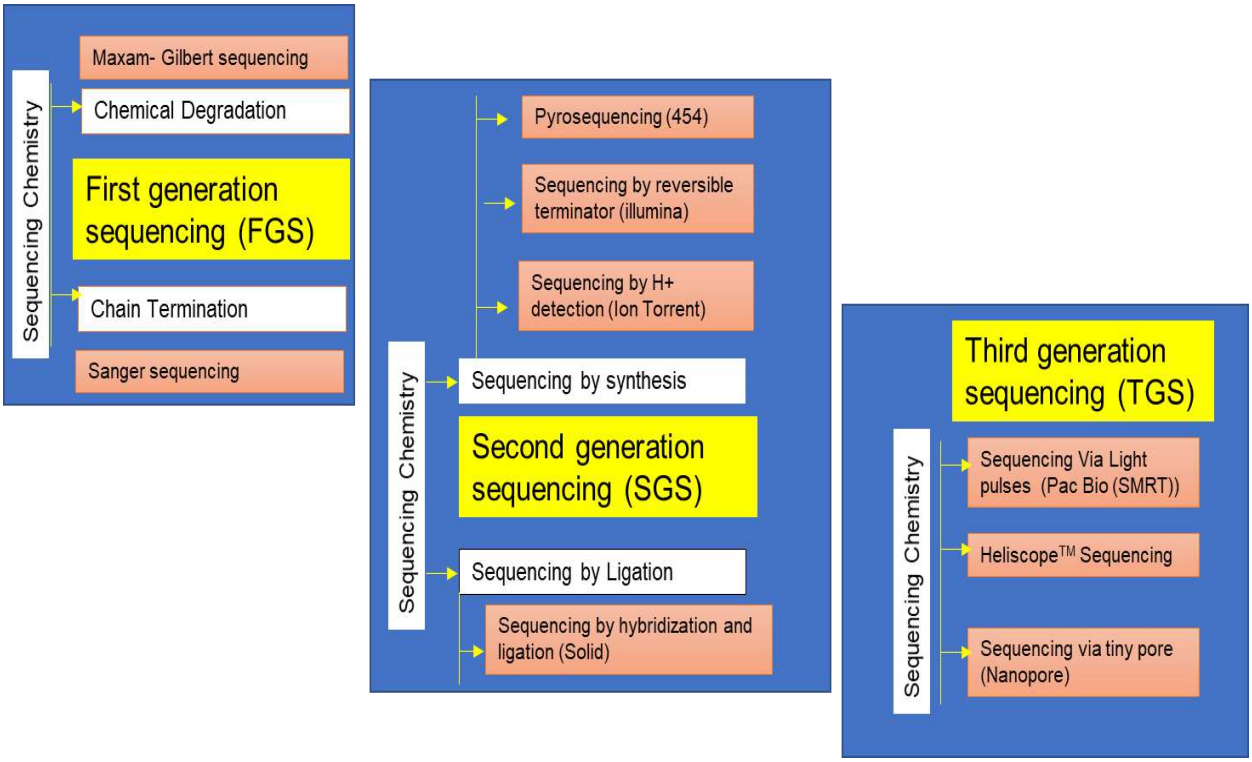


Fig 2. Various sequencing platforms with different sequencing chemistry

Third Generation Sequencing:

These PCR based chemistries have now evolved into single molecule sequencing approaches like those offered by PacBio and Nanopore. PacBio sequencing relies on sequence information during the polymerization process. In this case, the template is created by ligating hairpin adaptors to both ends of dsDNA in which leads to the formation of a closed single stranded circular DNA, also known as SMRTbell. Once this template is placed on to a chip known as a SMRT cell, it diffuses into a sequencing unit called a zero-mode waveguide (ZMW), where at the bottom of each ZMW a single polymerase is immobilized. This enzyme initiates the process of replication by binding to either hairpin adaptor of the SMRTbell. Nucleotides are labelled with four different fluorescent with distinct emission. As a base is held by the polymerase, a light pulse is produced that identifies the base. The replication processes are recorded by a ''movie" of light pulses, and the pulses equivalent to each ZMW can be read as a sequence of bases (called a continuous long read, CLR).

Nanopore sequencing relies on changes in ionic currents during translocation of DNA via a tiny channel with pore size in nm range. DNA to be sequenced is generally passed by means of a microscopic pore in lipid bilayer membrane. One protein unwinds the dsDNA helix into two strands whereas second protein makes a hole into the membrane. As the ion moves through the pore it generates a current. Bases are identified as they affect the flow of ions through the pores. Each base affects the ion flow to a different degree and the degree of electric current disruption by the corresponding base are measured. All the next generation sequencing platforms differ in term of sequencing chemistry, read length, data output, error rate, run time and cost per run. There are four common steps involved in all the high throughput platform viz, sample/library preparation, cluster generation, sequencing process and data analysis (Fig. 3).

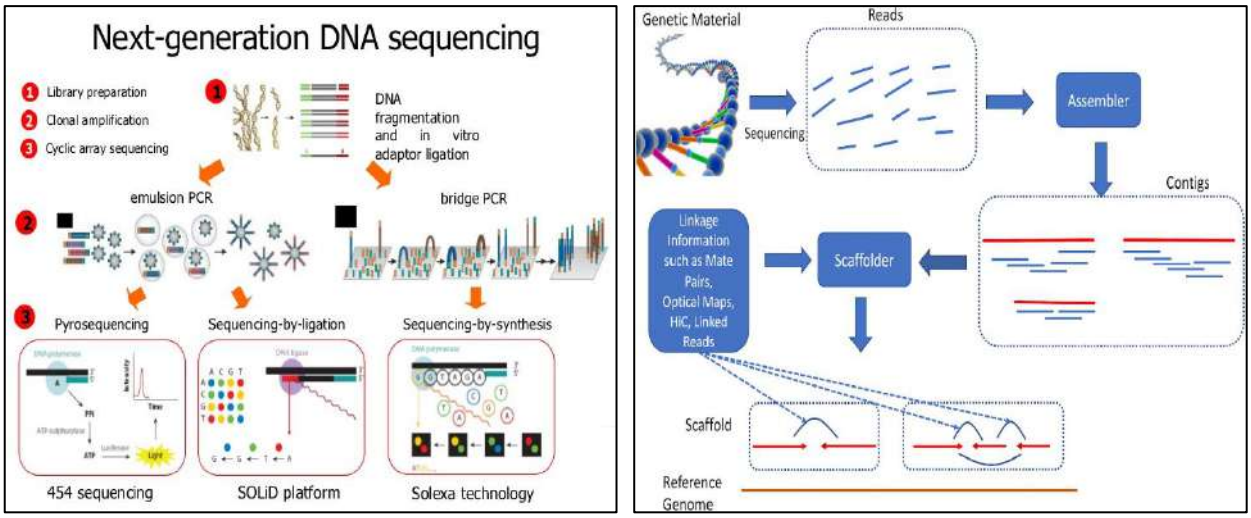
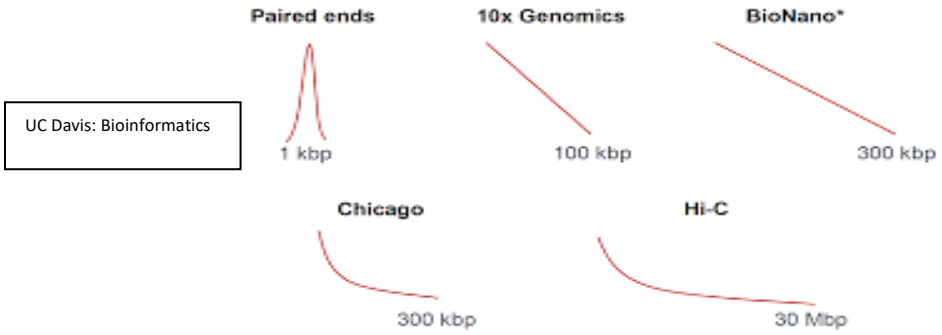


Fig 3. Introduction to Next generation sequencing workflow (http://ueb.ir.vhebron.net/NGS)

Technology like Illumina still dominates the quantum of data generation but newer platforms like those provided by PacBio and Oxford Nanopore have increased the speed by which the genomes can be assembled due to generation of larger sized reads. Illumina system offers a wide variety of application, like sequencing, genotyping etc. Its new system NovaSeq is capable of producing data in excess of 6TB per run. Its best suited for larger genomes and handling large plant populations for genotyping purpose. The current sequencing chemistries can generate huge amounts of data and each one of them have their own niche application.

Recently newer chemistries like the 10X genomics and the Dovetail genomics have added new dimension to sequencing approaches. Both these chemistries are now capable of constructing large insert DNA libraries, which are then sequenced on the Illumina HiSeq or NovaSeq platforms. This circumvents the need to generate mate pair library information. Additionally, the presence of Optical mapping technique like "BioNano" aids in better and chromosomal level assembly.



https://bioinformatics.ucdavis.edu/

None of them can single handedly decode the higher genomes due to differences in error rate, read lengths and output. Thus a appropriate mix (hybrid approach) of the short reads, long read chemistries and Optical mapping are required to sequence the entire genome for a true chromosome based assembly.

10x chromium, Hi-C and Optical Mapping

Availability of complete genome sequence of an organism can lead to paradigm shift in the research community. The genome sequencing provides an opportunity to study the comparative structural and functional genomics, evolutionary relational-ship among the organisms, and generates a strong interest towards translational research. Tremendous data on genome sequencing of various plants starting from Arabidopsis thaliana, followed by rice (Oryza sativa), and maize (Zea mays) and other major food, fiber, medicinal plants and energy crops are being generated and are available in public domain. With the advent of long-read single-molecule sequencing (Pac Bio), Hi-C sequencing, Optical mapping and advances in upgraded bioinformatics tools it has been possible to sequence and assemble complete genome of species with limited genetic resources.

Limitations of Short Read Sequencing

a) Because haplotype information is not retained for the sequenced genome or the reference, the reconstruction of long-range haplotypes is challenging using only short read data from a single genome.
b) Also, Short read fail to call large variations including inversions and translocations
c) Further, considering the presence of widespread repeats and paralogs, short reads makes scanning of entire genomic regions very difficult.

10x Chromium Sequencing

10x Linked-Reads amplify the power of short read sequencing and enable the analysis of a more complete genome. Linked-Reads are generated from short read sequences created with an in-line barcode (Fig 4). Because limiting DNA amounts are utilized, reads that share a barcode can be grouped as deriving from a single long input molecule. In this way, long range information can be assembled from short reads[1]

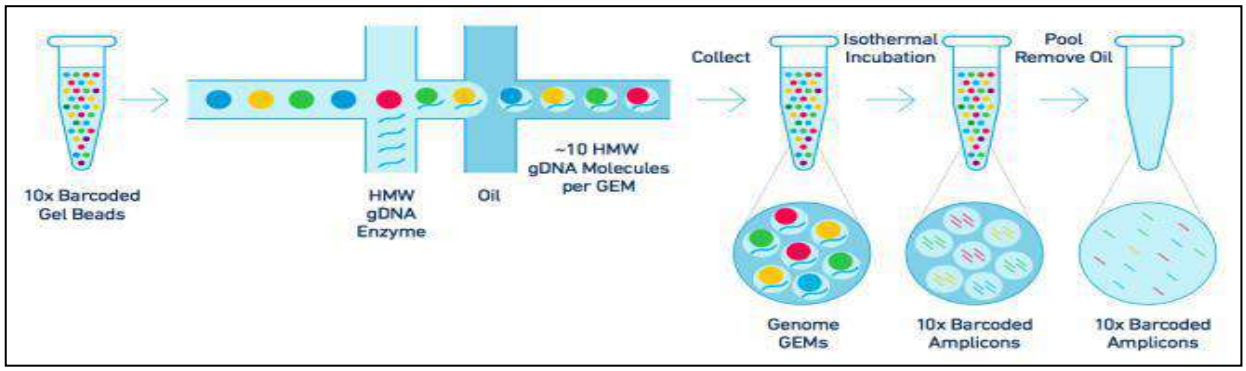


Fig 4: Chromium™ Technology mixes functionalized gel beads containing unique barcodes with enzymes and a limiting amount of genomic DNA to create >1,000,000 uniquely addressable partitions in minutes. Using a limiting dilution of molecules allows the correct mapping of reads to their corresponding molecules (Adopted from https://support.10xgenomics.com/de-novo-assembly/sample-prep).

Advantage: Allow large scale haplotype reconstruction, and improved genomic variant calling of wide range of variants, including SNVs, deletions, inversions & translocations by partitioning reads into distinct haplotypes.

HI-C Sequencing:

High degree of packaging and organization of chromatin and its dynamic structure drive to illuminate the various aspects of gene regulation process, chromosome morphogenesis, genetic instability, and gene inheritance and transmission. Spatial organization of chromatin can be studied using various approaches. which may further classify into microscopic and molecular assays. Among molecular assays techniques, Chromosome Conformation Capture (3C) is major assay method and many other methods (4C, 5C, Hi-C, Chip-loop, and ChiA-PET) have been developed based on 3C technique (Fig 5). In special variants of the 3C technology (ChIP-loop and ChIA-PET), immunoprecipitation is used to examine the role of the protein factor. Recently, new methods like 4C, 5C, and Hi–C have been established to exploit the Next Generation Sequencing (NGS) approach to examine the 3C ligation product library in a grand manner (Fig. 6 D and E)

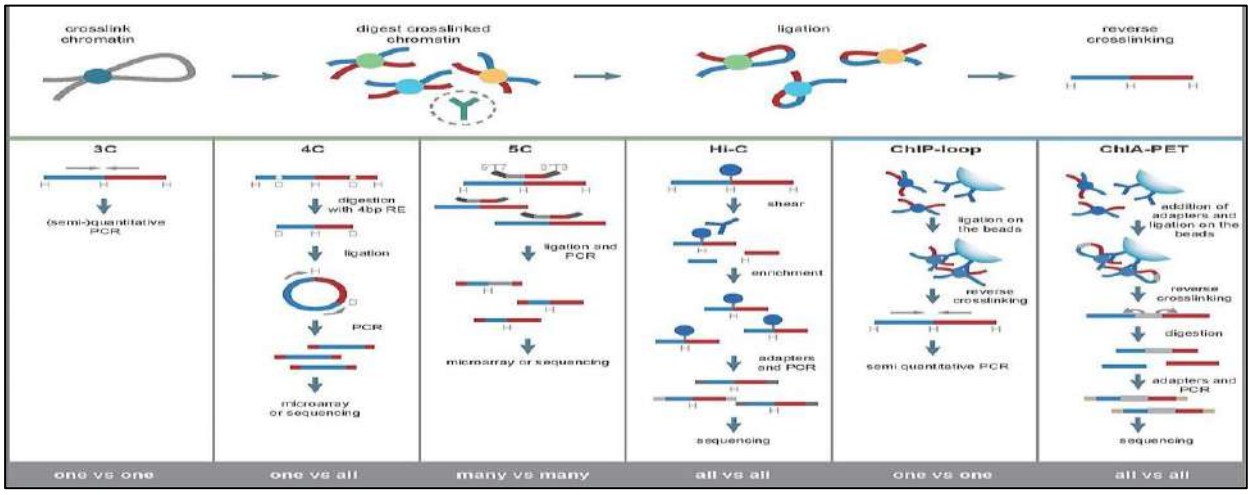


Fig. 5. Chromosome Conformation technologies (Source: Wikipedia)

The 3C method measure the average frequency of population in which two or more DNA fragments linked in three-dimensional space. This method actually quantifies the number of interactions between genomic loci present in 3D space (Fig. 6A). As soon as interacting loci are crosslinked with formaldehyde, chromatin is solubilized and get fragmented via restriction enzyme (Fig. 6B). Interacting fragments are then ligated together and purified to produce a genomic library of chimeric DNA molecules (Fig. 6C).

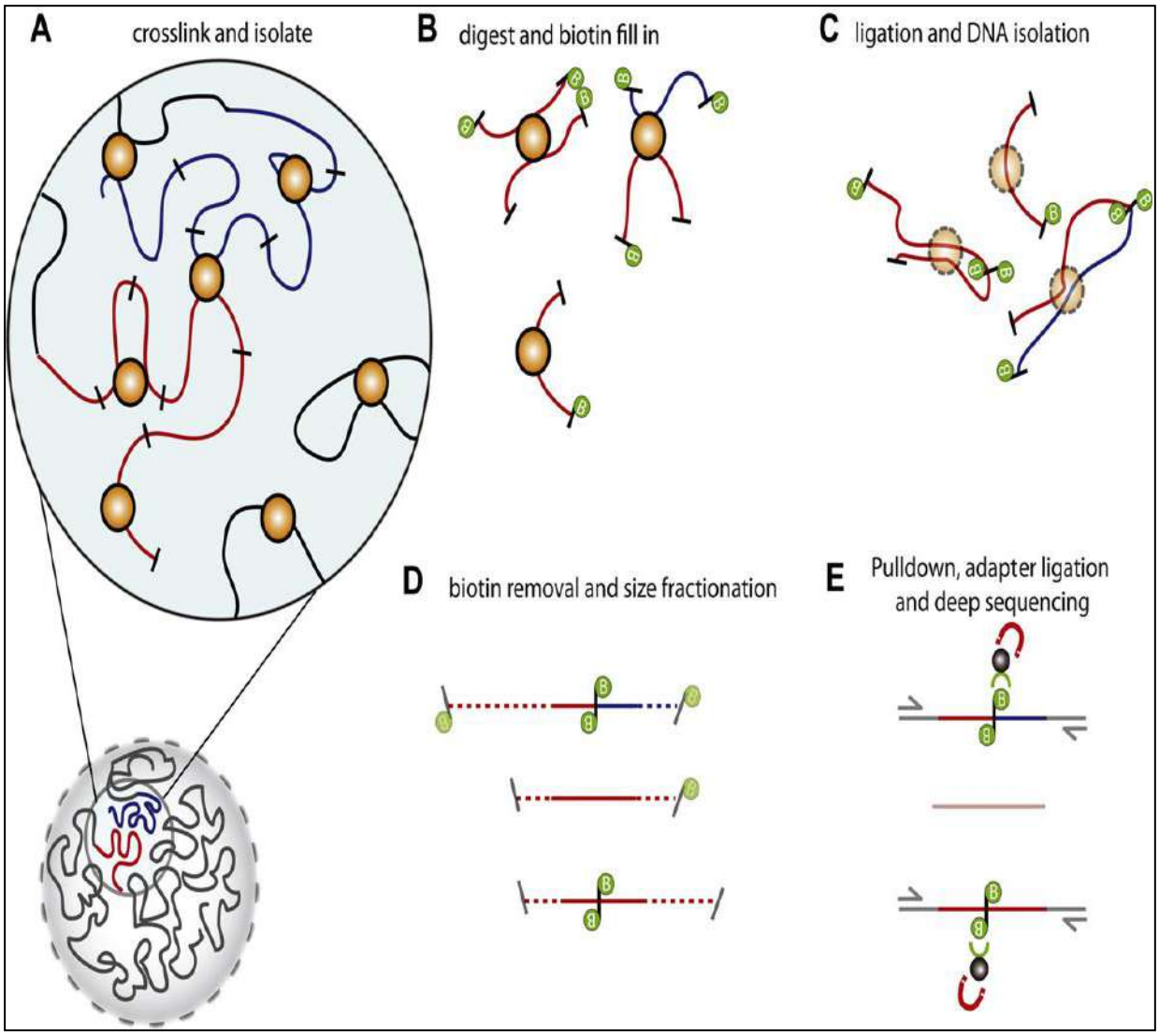


Fig 6. Overview of Hi–C technology. (A) Hi–C detects chromatin interactions, crosslinking protein/DNA complexes with formaldehyde. (B) Restriction digestion of chromatin and end biotinylation. (C) DNA ligation and formation of chimeric DNA molecules. (D) Removal of biotin and molecule fragmentation. (E) Molecules with internal biotin integration are removed with streptavidin coated magnetic beads which further adapted for sequencing. Quantification of chromatin interactions using high through put sequencing (Adopted from Belton et al., 2012).

Optical Mapping

Optical mapping is an approach to create ordered, genome wide restriction maps from single DNA molecules. Instead of solution digestion, large DNA molecules are subjected to restriction digestion on open glass slide surfaces and visualized under fluorescence microscope. DNA fragments that are stained with intercalating dye are visualized and sized using intensity of fluorescence. In this way Well-ordered restriction maps are produced from digital images of fully and partially digested molecules (Fig. 5). Clones are mapped redundantly and very accurately; the final map is an consensus of many individual maps. Optical Mapping is a high-throughput; single molecule system that generates ordered restriction maps (also called Rmaps) from high molecular weight genomic DNA molecules, ranging in size from 300 kilobases to a few megabases. The Rmaps are then used for the construction of genome-wide physical restriction maps using computational approaches, which provide insights into long range genome structure and genome variation.

Optical mapping Procedure: explained in Fig 7 & 8 (Adopted from Aston, C. *et al.,* 1999)

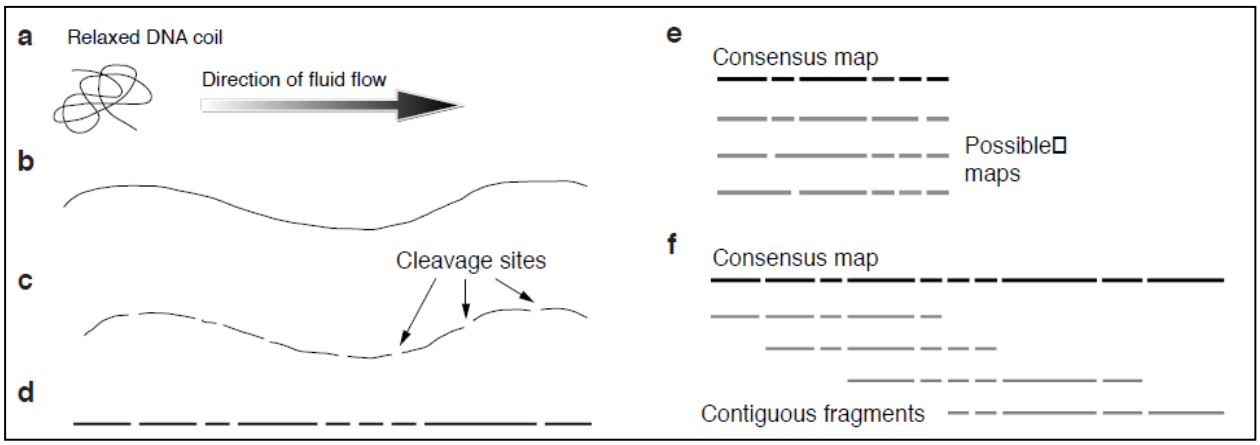


Fig 7: Steps in optical mapping: Large DNA molecules, placed on salinized glass surfaces (a). Fluid flows lengthen the DNA molecules (b) and charge interactions hold the stretched DNA molecules on the surface. The DNA, digested with a restriction enzyme; create cleavage site or gaps (c). The DNA is stained using intercalating dye and visualized by fluorescence microscopy. Digital images are recorded. The fragments are sized using integrated fluorescence intensity single molecule map are built (d). restriction fragments up to 800 bp can be sized. Consensus maps of cloned DNA are created using a probabilistic scheme (e) and maps of genomic DNA are aligned into contigs (f). Consensus maps are subsequently used as scaffolds for sequence assembly

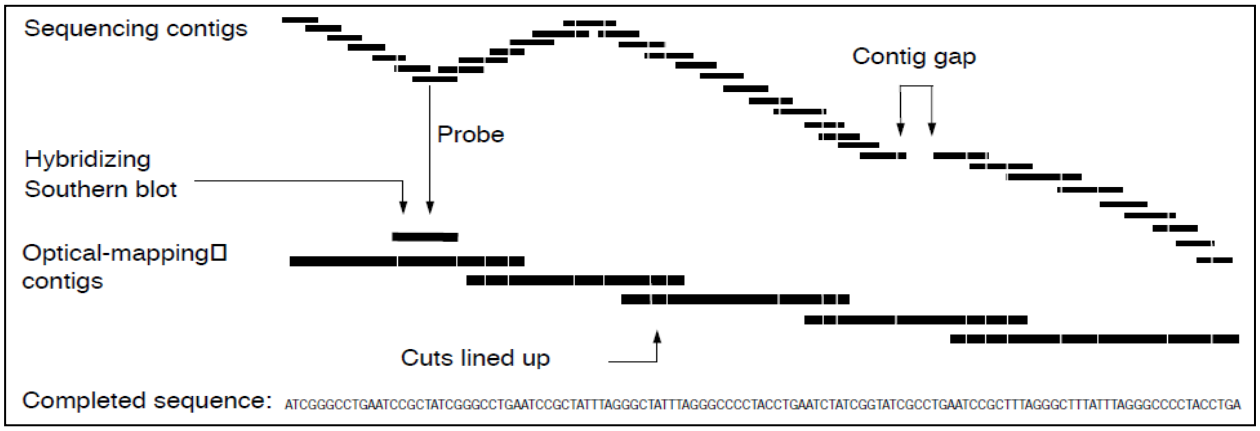


Fig 8: The analysis scheme represents sequence contigs aligned with a consensus optical map which provide a scaffold and generate alignment of overlapping restriction fragment followed by gap filing using gap-closure techniques such as long-range PCR.

Advantages: Use to Characterize the whole genome. High-resolution optical maps of DNA serve as a scaffold for the accurate alignment of contigs generated by shotgun sequencing.

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Bioinformatic Software and Tools for Next Generation Sequencing Data Analysis

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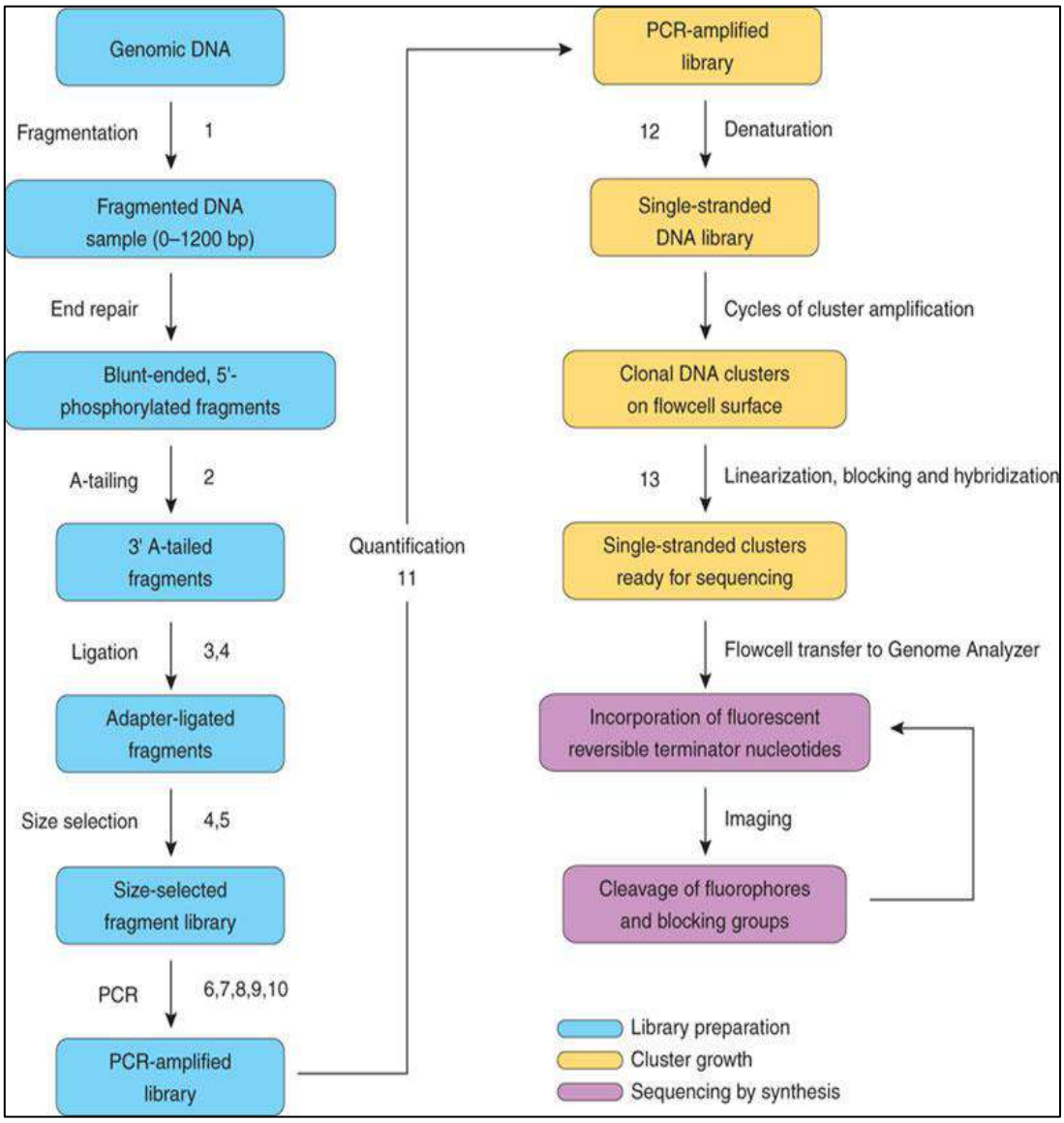
Next-generation sequencing (NGS) is a high-throughput method that allows fast sequencing of the DNA or RNA samples. NGS has immense of applications, including gene expression profiling, chromosome counting, detection of epigenetic changes, and molecular analysis, NGS is driving discovery and enabling the future of personalized medicine.

All the NGS platform include four common/ basic steps

1. Library preparation
2. Clonal amplification/ cluster generation
3. Sequencing
4. Data analysis



Basic Work Flow

Important Points to be Considered before Undertaking Genome Sequencing

Choice of sample: Generally, fresh tissues properly frozen are recommended for DNA extraction while tissue frozen at 20°C yield limited quantities and low quality of DNA.

Choice of DNA extraction method: DNA extraction methods are available for isolating abundant and good quality DNA. The available protocols can be modified according to one's need.

DNA quality requirements for sequencing: Bio**c**hemical purity and structural integrity of the DNA/ RNA are the two major key parameters that are of highest concern. Long read sequencing technologies require best quality high molecular weight (HMW) DNA. Lack of good starting material/ tissue will limit the choice of sequencing technology and will also affect the quality of obtained data. Besides, DNA extracts often contain carry-over contaminants like polysaccharides, proteoglycans, proteins, secondary metabolites, polyphenols, humic acids, pigments, etc., which can impair the efficacy of library preparation particularly in PCR-free library preparation protocol like PacBio and Oxford nanopore sequencing technology. Hence, it is very important to remove all the possible contaminants from the DNA sample before processing it for sequencing.

Choice of a suitable sequencing platform: While selecting a sequencing platform, one must consider the objectives of the investigation, genome size, and its complexity, as well as the information available in the public domain regarding quality and type of data. Quality read, yield, read length and cost heavily influence the selection of the sequencing method. Illumina sequencing provides the greatest output at the lowest reagent cost and offers a lot of flexibility e.g., different runs with varying read length, paired-end and single-end sequencing, and/ or multiplexing many samples. If longer read lengths are required, then one must consider Pacific Bioscience's RS II/ Sequel system or Oxford Nanopore Minion/Gridion.

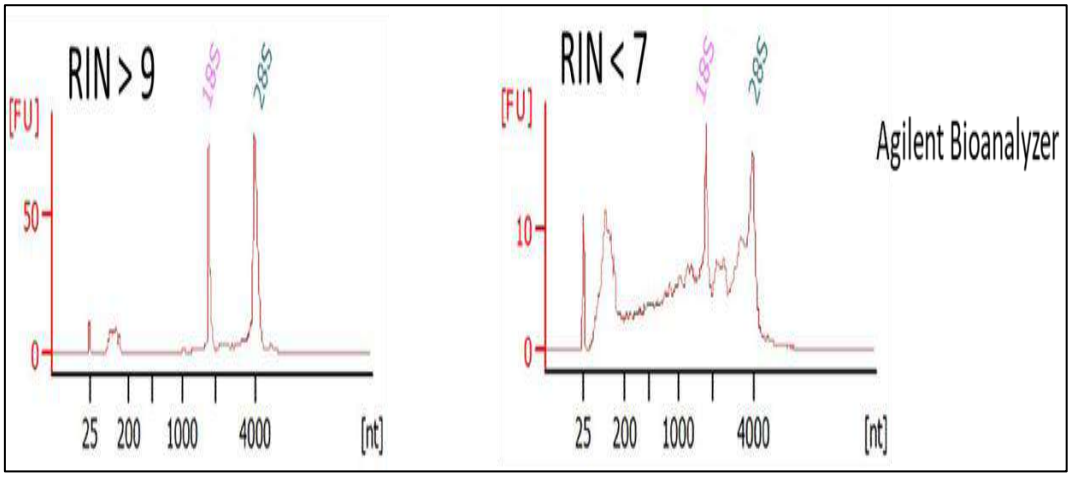
Library preparation: It depends on quality and accurate quantification of input DNA. Fluorometric-based methods are available for quantification, such as Qubit or PicoGreen. DNA library is validated quantitatively and qualitatively prior to sequencing to ensure that sufficient amount of good quality DNA is present in the prepared library. The quality of the sample library is assessed with the help of Agilent Technologies 2100 Bioanalyzer.

Quality Standards for DNA:

- Genomic DNA of high/ good quality should run as a high m.w. band on a 1-2% agarose gel, with the majority of DNA greater than 10Kb in size and with minimal lower molecular weight smearing. If the majority of the DNA is below 10Kb or smearing is visible, this suggests that the DNA is degraded or lower molecular smearing can be indicative of the presence of RNA contamination.
- Also, Absorbance measurements at 260 nm are commonly used to assess DNA quality. The ratio of absorbance at 260 nm to absorbance at 280 nm is used as an indication of sample purity. Values of1.8–2.0 indicate relatively pure DNA.

Quality Standards for RNA:

- ***Bioanalyzer:*** An RIN or RQS value greater than 8 is required, or greater than 7 for plant RNA and rRNA ratio (28S/18S or 23S/16S) of 1.5 to 2.5.
- ***Formaldehyde 1% Agarose:*** High quality RNA shows a 28S rRNA band at 4.5Kb that should be twice the intensity of the 18S rRNA band at 1.9Kb. m RNA will appear as a smear from 0.5-12Kb.
- The OD260/280 ratio should be 1.8-2.2

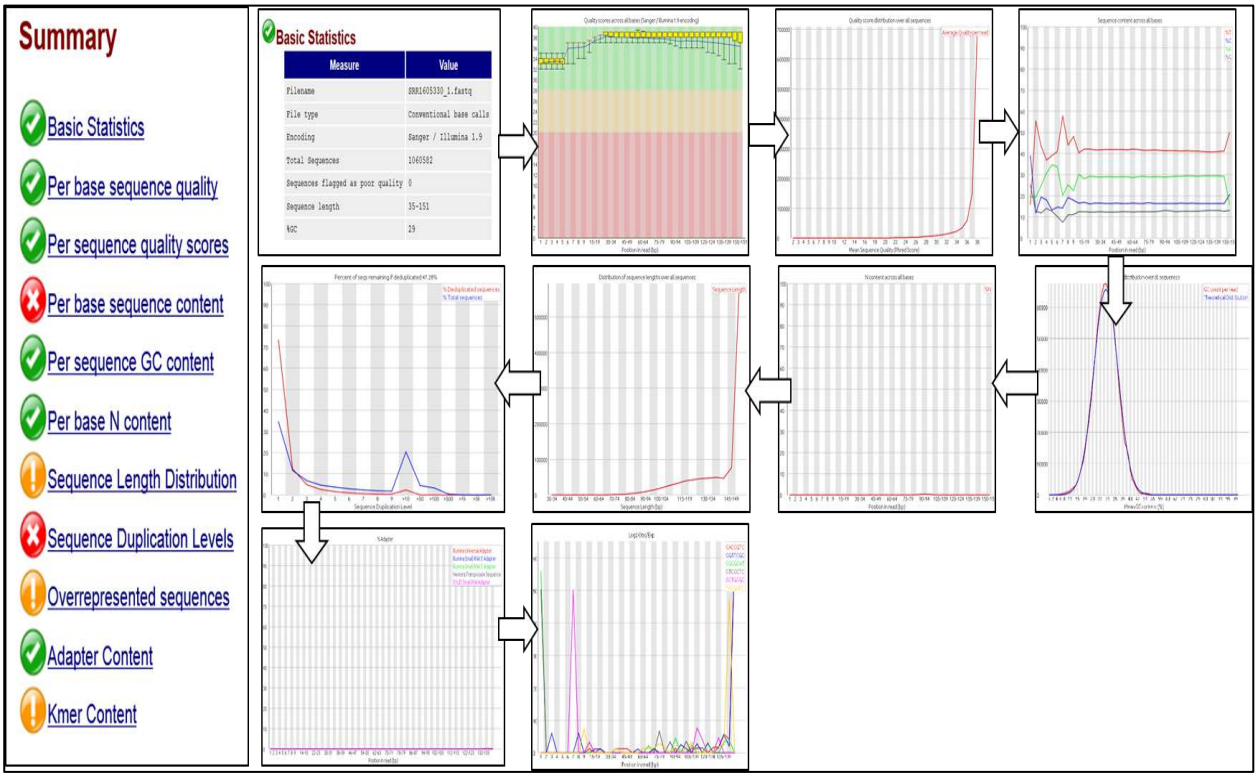


Fast QC:

Fast QC checks for high quality of the sequence data obtained (Figure 2). First it checks the raw data and verifies the quality of sequencing data. Figure 3 shows the quality control report for different data. Analysis of GC content is done using Perl script. Detection of repeat sequences is another important quality control in characterizing mitochondrial genomes.

The key role of FastQC are:

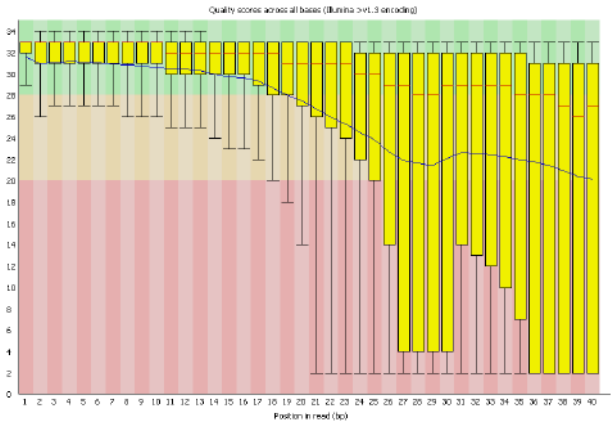
- Import of data from BAM, SAM or FastQ files (any variant)
- Providing a quick overview to look towards the problematic area
- Provide summary graphs and tables to quickly assess your data
- Export of results to an HTML based permanent report
- Offline operation system for generation of reports automatically



Basic Statistics: It includes the following

- Filename
- File type
- Encoding
- Total Sequences
- Filtered Sequences:
- Sequence Length
- The overall %GC

Per Base Sequence Quality: It depict an overview of the range of quality values across all bases at each position in the FastQ file.



For each position a BoxWhisker type plot and the elements of the plot are given below-

The central red line shows the median value while the yellow box characterizes the inter-quartile range (25-75%). The upper and lower whiskers denote the 10% and 90% points respectively whereas blue line signifies the mean quality and the quality scores is depicted on Y axis. Higher the quality score means better the base call. Very good quality calls are, reasonable quality and poor quality are represented by green orange and red respectively.

Per Sequence Quality Scores: This report provides information about the sequences having universally low-quality values.

Per Base Sequence Content: It plots out the proportion of each base position in a file for which each of the four normal DNA bases has been called.

Per Sequence GC Content: This module measures the GC content across the whole length of each sequence in a file and compares it to a modelled normal distribution of GC content.

Per Base N Content: If a sequencer is unable to make a base call with sufficient confidence then it will normally substitute an N rather than a conventional base call.

Sequence Length Distribution: This module generates a graph showing the distribution of fragment sizes in the file which was analysed.

Duplicate Sequences: This module counts the degree of duplication for every sequence in a library and creates a plot showing the relative number of sequences with different degrees of duplication.

Overrepresented Sequences: This module lists all of the sequences which make up morethan 0.1% of the total indicating that either it is highly biologically significant or the library is contaminated. For each overrepresented sequence, the program will look for matches in a database of common contaminants and will report the best hit it finds. Hits must be at least 20bp in length and have no more than 1 mismatch.

Kmer Content: The Kmer Content module performs a generic analysis of all of the Kmers in your library to find those which do not have even coverage through the length of your reads, thus helping in finding sources of bias including presence of read through adapter sequences building at the end of sequences.

Per Tile Sequence Quality: This graph allows you to look at the quality scores from each tile across all of your bases to see if there was a loss in quality associated with the flow-cell.

(FastQC content Adopted from https://www.bioinformatics.babraham.ac.uk/projects/fastqc/)

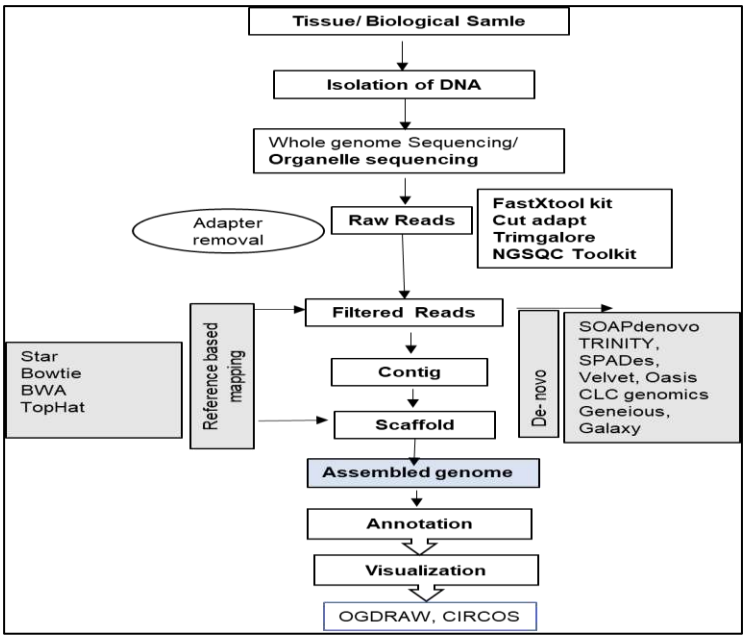


Fig: NGS Data Analysis Workflow

ALLPATHS-LG:
(ftp://ftp.broadinstitute.org/pub/crd/ALLPATHS/Release-LG/)
ALLPATHS-LG is a whole genome shotgun short reads (~100bp) assembler that can generate high quality genome assemblies. It is not designed to assemble Sanger or 454 FLX reads, or a mix of these with short reads. It requires high sequence coverage of the genome in order to compensate for the shortness of the reads. The appropriate coverage required depends on the length and quality of the paired reads of the order 100x or above. ALLPATHS-LG requires at least 2 paired-end libraries – one short and one long.

SPAdes (v3.13.0): Saint Petersburg Genome Assembler
(http://cab.spbu.ru/software/spades/)
Spades is a *de novo* assembler designed for prokaryotic and small eukaryotic genomes. It is multi-k and takes generally longer time and memory than other assemblers. It also supports assembly of IonTorrent and PacBio data for small genomes.

Canu (v1.8):
Canu is a tool which specializes in assembling the sequences generated from PacBio or Oxford Nanopore. Canu operates in three stages: (a.) correction (improve the accuracy of bases in reads) , (b.) trimming (trim reads to the portion that appears to be high-quality sequence) and (c). assembly (order the reads into contigs, generate consensus sequences and create graphs) [Koren et al., 2017].
Input sequences: FASTA or FASTQ format, uncompressed or compressed with gzip (.gz), bzip2 (.bz2) or xz (.xz). (NOTE: zip files (.zip) are not supported)

Trinity (v2.8.4):
(https://github.com/trinityrnaseq/trinityrnaseq/)
Trinity is a method which results in efficient and robust *de novo* reconstruction of transcriptomes from RNA-Seq data. It contains three independent software modules: *Inchworm, Chrysalis and Butterfly,* required to process large RNA-Seq datasets. *Inchworm* and *Chrysalis* steps can prove to be memory intensive.
Output: '*Trinity.fasta*' output file in the 'trinity_out_dir/' output directory (or your specified directory) will create when trinity assembly completes.
Input file: fastq/fasta files of illumina reads

SOAPdenovo2 (v2.04-r241):
(http://soap.genomics.org.cn/soapdenovo.html)
SOAPdenovo2 is a *de novo* assembler, which is designed particularly for short reads of plant and animal genomes, generated by illumina. It is an advanced version of SOAPdenovo with revised algorithm that reduces the consumption of memory, resolves more repeat regions, results in increased coverage, and optimizes the assembly for large genomes.

SOAPdenovo2 has two commands, **SOAPdenovo-63mer** and **SOAPdenovo-127mer**. The first one is suitable for assembly with k-mer values less than 63 bp, requires less memory and runs faster and the latter one works for k-mer values less than 127 bp (Luo *et al.,* 2012).
Input file: FASTA, Fastq and BAM

Commercial Softwares

CLC BIO:
(http://www.clcbio.com/)
CLC Main Workbench is a commercial bioinformatics software used to analyze the biological data. CLC bio's software permits the user to analyze, visualize, and compare multi omics data viz genomic, transcriptomic, and also epigenomic data obtained from all sequencing platforms such as Illumina, Roche 454, Pac Bio, Complete Genomics and other major high-throughput sequencing . CLC bio's software is platform independent and can be used for Mac OS X, Windows, and Linux. It possess various modules like Assembly; Cloning; Align and Phylogenetic Tree tutorial and Microarray modules (https://www.qiagenbioinformatics.com/).

Although, **CLC Genomics Workbench (v11.0.1)** is used for NGS data analysis as it comprises:

- Import of 454, Illumina Genome Analyzer, SOLiD and Helicos data
- *De novo* assembly/ Reference assembly of human-size genomes
- SNP/DIP detection
- Graphical display of large contigs
- Support for mixed-data assembly
- Paired data support
- RNA-Seq analysis and ChIP-Seq analysis
- Expression profiling by tags

Galaxy:
(https://galaxyproject.org/)
Galaxy is a freely available, open, web-based data analysis and workflow platform designed for biologist to analyze their own data with different toolsets already installed in it. It is written in Python and used for biological data analysis such as genomics, proteomics, epigenomics, transcriptomics and also gene expression analysis and genome assembly. (Giardine *et al.,* 2005).

Geneious:
(https://www.geneious.com/)
Geneious is easy, flexible integrated platform for molecular biology and NGS data analysis. It is graphical user interface and displays selected file(s) in a variety of different ways including sequence view (linear and circular), dot plot view, query-centric alignment view, protein domain view, 3D structure view, text view and notes etc. It is written in JAVA and applicable on any operating system eg. Linux, Apple OSX and MS Windows as a standalone download (Kearse *et al.,* 2012).

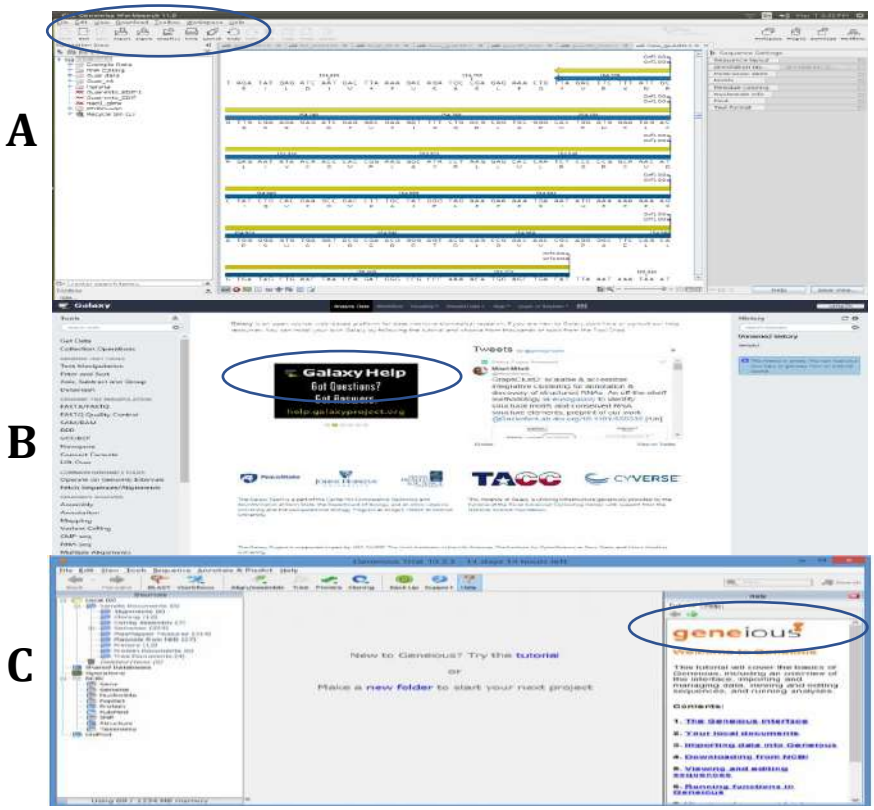
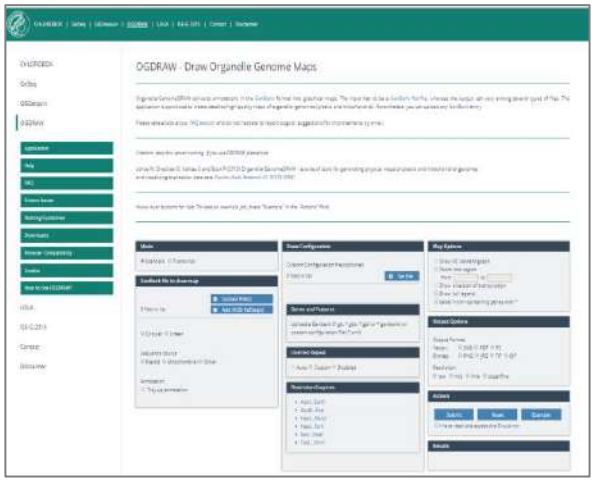


Fig: Web page of A) CLC B) Galaxy and C) Geneious

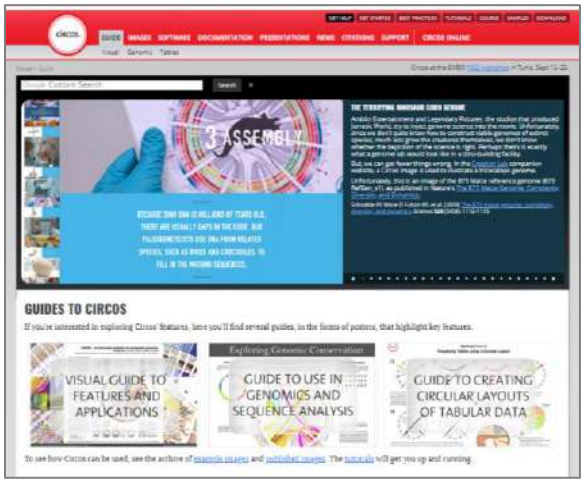
Bioinformatics Tools for Visualization

1. OrganellarGenomeDraw (OGDRAW)
2. Circos:
3. BRIG: BLAST Ring Image Generator
4. Anvi'o: An advanced analysis and visualization platform for 'omics data:

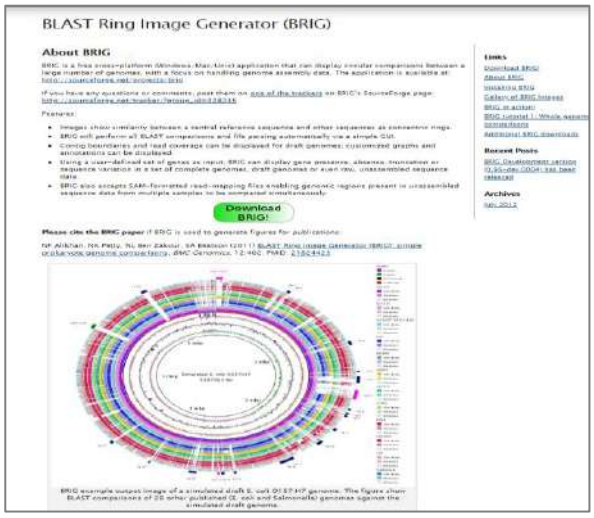
A



B



C



D

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Training Manual

"Pathophenotyping and Genome guided Characterization of Rust fungi infecting Wheat and other Cereals"

January 22 - February 01, 2020

Division of Plant Pathology & Division of Genetics, ICAR-IARI, Pusa Campus, New Delhi

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