Wheat rust research – Status, efforts and way ahead

Neha Gupta, Neha Batra¹ and Subhash Chander Bhardwaj*¹

ICAR-Indian Institute of Wheat and Barley Research, Regional Station, Flowerdale, Shimla, India
¹The IIS University, ICG Campus, Gurukul Marg, SFS, Mansarovar, Jaipur, India

Abstract

Wheat rusts caused by Puccinia species are the main biotic constraints in wheat production wherever wheat is grown. Their ability to spread aerially over the large distances, production of uredospores in enormous number and evolving new pathotypes, makes the management of wheat rusts a very daunting task. To overcome the threat of wheat rusts, efforts are going on worldwide for identification of pathotypes, anticipatory breeding, evaluation for rust resistance and deployment of rust resistant cultivars. Till now, more than 210 rust resistance genes and associated markers for many are available for the use of breeders. Some of the linked gene combinations like Lr34/Sr57/Pm38/Ltn1; Lr46/Yr29/Sr58/Pm39/Ltn2; Sr2/Yr30; Lr67/Yr46/Sr55/Ltn3 are known to confer durable resistance to different rusts. Efforts are on to introgress novel rust resistance from alien sources and revisiting rusts epidemiology. With the advent of genome sequences of wheat and wheat rusts, exploring the functional genomics and alternative approaches using next generation techniques are discussed in this review.

Keywords: Black rust, brown rust, yellow rust, resistance genes, epidemiology

1. Introduction

Wheat is the second important cereal following rice. It accounts for 20% of world’s food and calorie requirements of a major population in many developing countries (FAOSTAT, 2015). To meet the food requirements of ever increasing population, 60% increase in wheat production is projected by 2050 (Rosegrant and Agcaoili, 2010). India is one of the leading producers of wheat (Triticum aestivum L.) in the world. Wheat is synonym to the economic well being of India. During 2016-17, total wheat production of India was pegged at 97.44 mt from 30.72 mha (ICAR-IWBR, 2017). Indian wheat programme started around 1900 and progressed towards one of the most organized and successful systems in the world. Indian wheat production has shown not only self-sufficiency but is in a position to export 2-3 million tonnes during many years.

On the way to this achievement, many important steps like breeding for higher yields, resource utilization and protection against biotic constraints especially wheat rusts were taken (Tomar et al., 2014).

To increase and sustain wheat yield, the crop must be kept free from diseases (Bhardwaj et al., 2009). Rusts caused by three different species of fungus Puccinia are the main biotic impediments in our efforts to sustain and boost production of wheat. Their ability to spread aerially over the continents, production of infectious propagules geometrically and evolving new physiologic forms (pathotypes), make the management of wheat rusts a very challenging task. To counter the ever emerging threat of wheat rusts, efforts are going on worldwide. Identification of pathotypes, anticipatory breeding, evaluation for rust resistance and deployment of rust resistant cultivars are the important constituents of rust management. More than 210 rust resistance genes and associated markers for many are available for the use of breeders. However, the rust pathogens have always out done the efforts of the breeders and new virulent pathotypes have emerged which could overcome the immune and novel rust resistance genes.
2. History of wheat rusts

Wheat and rusts have co-existed since the times immemorial and rusts have been observed to be the earliest known diseases having Biblical records. The various rusts were recognized as serious pests by the ancients many centuries ago.

On 25th April each year the Romans had a festival the “Robigalia” during which a procession was marched out to the sacred trees where priest prayed Robigus to spare the crops. In addition to prayer, the wine was poured on the altar and sacrifice of sheep, reddish dogs, foxes and cows was done in an attempt to appease the Robigus in the belief that he would not send the rusts to destroy their crops. Most ancient reports, however, dealt with festivals and sacrifices to appease a God to keep away the dreaded rusts from their crops (Agrios, 2005). In 17th century, the French farmers had noticed that black stem rust was much worse in fields surrounded by hedges including barberry bushes, latterly, the Rouen city of France was the first to promulgate legislative measures in the year 1660 to control barberry bushes (Zadoks and Bouwman, 1985). Afterwards, in the late 1700s, other countries including Germany and the USA adopted the same law in hope to control stem rust on their cereals. Nobody knew at the time that barberries (Berberis vulgaris) were an alternate host in the stem rust life cycle (Puccinia graminis), it was only in 1865 that a famous mycologist, Heinrich Anton de Bary discovered the complete life cycle of the stem rust and demonstrated that P. graminis required two different hosts (Wheat and Barberry bush) during the different stages of its development and thus discovered the heteroecious nature of wheat stem rust pathogen. Subsequently, Johan Hubert Craigie (1927), a Canadian pathologist, successfully demonstrated that pycnia are the sexual structure of rust fungi. He designated two mating types (+) and (-) for haploid pycnia (spermatia and receptive hyphae) in wheat black rust (P. graminis f. sp. tritici).

The systematic investigations on cereal rusts in India were initiated by Rai Bahadur Professor Karam Chand Mehta of Agra College in 1922-23. In 1930, with the assistance of Imperial Council of Agricultural Research (ICAR), he strengthened rust research program at Agra and three other locations (Shimla, Almora and Murree i.e. now in Pakistan) were selected for research work. Ultimately Shimla (Flowerdale) was found most suitable site to grow wheat and maintain the cultures of obligate rusts pathogens around the year (Nayar et al., 1994). The required natural ambience for wheat rust research could be found at Shimla with least efforts. His outstanding contributions were the discovery of the life cycle of stem rust of wheat in India and epidemiology of wheat rusts. Through experimentation and circumstantial evidences Prof. Mehta proved beyond doubt that barberry, an alternate host of wheat stem rust pathogen, does not play any functional role in the perpetuation of the rust fungus in India (Mehta, 1940). He laid a very firm foundation and set up a systematic school of wheat rust research in India. Subsequently, along with Dr. BP Pal, breeding for wheat rust resistance was also started in India during 1934 (Tomar et al., 2014). Wheat rust pathogens are the obligate parasites which need living host. However, with the axenic culturing of Gymnosporangium juniper-virginianae by Hotson and Cutter in 1951, there was a question on the fidelity of rust fungi. Subsequently, the wheat rust pathogen (P. graminis f. sp. tritici race 126-ANZ 6,7) was also grown on artificial media successfully (Williams et al., 1966). During culturing mycelium grows but spore production doesn’t occur.

3. Wheat rust losses

Both black and yellow rusts of wheat can cause 100% losses whereas brown rust can cause 50% yield loss (Anonymous, 1992). Historic account of wheat rust epidemics in India has been given by Nagarajan and Joshi (1975). Epidemic had occurred at Jabalpur as early as 1786 and subsequently in 1805, 1827, 1828-29, 1831-32. In the main wheat belt of India rust epidemics have been observed around 1843 at Delhi and during 1884 and 1895 at Allahabab, Banaras and Jhansi. Later on in 1905 rust epidemic was reported to have occurred in Punjab and sub mountainous regions of Gorakhpur. Another report of epidemic in Indo Gangetic plains is of 1910-11. Both brown and yellow rusts caused losses in Western Uttar Pradesh between 1971-73. Another epidemic of brown rust occurred in 1993 in about 4 million hectares of north western India (Nayar et al., 1997).

4. Pathogen: Taxonomy, Symptoms

Wheat rust is macrocyclic in nature. De Bary in 1864-65 after extensive researches finally resolved the heteroecious nature of P. graminis. Eriksson and Henning in their publication “Die Getreideroste” in 1896 laid the foundation for work on rust specialization (Chester, 1946). There are following five stages in the life cycle (Fig. 1) of wheat rusts.
Fig.1 Life cycle of *Puccinia graminis f.sp. tritici*.

In this figure, Pycnial stage (0); Aecial stage (I); Uredial stage (II); Telial stage (III); Basidial stage (IV). Pycnial stage is called stage -0 because before 1927 the role of pycnial stage in the life cycle of rusts was not understood. Craigie (1927) in Canada determined its function in the life cycle and in the variation of rust fungi. The pycnial stage (stage -0) and aecial stage (stage -I) occur on alternate hosts for completion of life cycle of the pathogen. The genus *Puccinia*, was named in honour of a Florentine physician and teacher, by P.A. Micheli. However, genus *Puccinia* in relation to wheat was established by Persoon (1794). It belongs to the family Pucciniaeae, order *Uredinales*, class *Basidioymycetes* (*Teliosymyctes*) of *Basidioymycotina*. More than 8000 species of *Pucciniomycotina* have been described including putative saprotrophs and parasites of plants, animals and fungi. The overwhelming majority of these (~90%) belong to a single order of obligate plant pathogens, the Pucciniales (ertwhile *Uredinales*) or rust fungi (Aime et al.,2006).

Christiaan Hendrik Persoon (1797) named *P. graminis* as the causal organism of wheat stem rust. Although in earlier records, wheat stem rust was not distinguished from wheat leaf rust (Chester, 1946). It was de Candolle (1815) who showed that the incitant of wheat leaf rust is a different fungus and described it as *Uredo rubigovera*. Cummins and Caldwell (1956) suggested *P. recondita* as a causal organism of wheat leaf rust.

The recent morphological and genetic studies of the pathogen showed that *P. recondita* is not the incitant of wheat leaf rust rather *P. triticina* should be the preferred name as shown by Savile (1984) and Anikster et al. (1997). Although, stripe rust disease of wheat was first described by Gadd in 1777 but Schmidt (1827) named the stripe rust fungus as *Uredo glumarum*. Westendorp (1854) used *P. striiformis* for stripe rust collected from rye (Stubbs, 1985). Later on, Eriksson and Henning (1896) showed that stripe rust resulted from a separate pathogen, which they named *P. glumarum*. This name of stripe rust pathogen was in vogue until Hylander et al. (1953) revived the name *P. striiformis* Westend.

5. Epidemiology of wheat rusts in India

There is no evidence so far regarding the functional alternate hosts for wheat rusts in India. Wheat rusts are known to survive and carry over to the next generation with the help of uredospores which are called as repeating spores. Moreover, the circumstantial and experimental evidences suggest that alternate hosts may not at all play any role in the recurrence of wheat rusts in India. Our recent experimentation on the role of aeciospores from at least ten species of *Berberis* from different parts of India and Nepal in infecting wheat through the recent method of Yue Jin has failed to yield any infection. It is widely known that wheat rusts survive on off season, self sown
wheat plants in Himalayas and Southern India. However, our recent studies have indicated that some of the grasses growing in Yamuna nagar area of Haryana, Ropar district of Punjab, Una district of Himachal Pradesh and other catchment area near river basins or other water sources may also act as collateral host for brown rust of wheat (Bhardwaj, 2013) and may be for other rusts too.

6. Wheat rusts as international pathogens

Rusts are able to spread over long distances. Yellow rust of wheat was not known in Australia before 1979 (Wellings and McIntosh, 1981) when pathotype 104E137 identical to the one that was found only in Europe was noticed. Yellow rust was subsequently reported from New Zealand next year (Beresford, 1982). The introduction of yellow rust into Australia appears to be human aided, while introduction into New Zealand appears to be by way of dispersal of uredospores by wind (Wellings, 2011). Studies have revealed the long distance spread of *P. graminis tritici* uredospores from Australia to New Zealand across 2000 Km distance of ocean (Mc Ewan, 1969). Identical biochemical patterns and connective winds from Australia indicate long distance dispersal and deposition of viable uredospores across 5000 Km of ocean from southern parts of Africa to that of Australia.

In 1990, *Yr*9 virulence was identified in Syria and in April 1994, the virulence attacking cultivars such as Pak81, Pirjabak85, Seri 82 possessing *Yr*9 became susceptible in Pakistan (Nagarajan and Saari, 1995). In 1996, a virulent pathotype on *Yr*9 and another virulent to *Yr*9 and *Yr*27 in 2002 were identified from the bordering areas of Punjab (Prashar et al., 2007) and subsequently have been identified from Nepal and Bhutan also.

7. Wheat Rust Resistance

Resistance is broadly classified into three main categories (Agrios, 2005) non host resistance, true resistance (vertical and horizontal) and apparent resistance (disease escape, tolerance, acquired resistance etc.)

Non-host resistance occurs across the species and is not much harnessed. Vertical (race specific) resistance is generally controlled by few or more major genes with larger effect while horizontal (non race specific) resistance is controlled by many genes having minor effect, thereby the name polygenic or mutligenic. Horizontal resistance is affected by environment more than vertical resistance. Vertical resistance is more prone to breakdown than horizontal resistance. In horizontal resistance though there is visible rust infection, however, the yield losses are not significant statistically. For having a perfect management package, a blend of diverse seedling (all time), slow rusting (horizontal resistance) and adult plant rust resistance (both non race specific and race specific) is desirable. Wheat rust resistance genes of both R and APR classes are designed as *Lr*, *Sr* and *Yr* for leaf, stem and stripe rust, respectively (Ellis et al., 2014).

Host resistance is the most efficient, cheap and environmentally most secure means of rust management. When adequate genetic resistance is achieved in a cultivar, no other prophylactic measures are necessary. A few historic cultivars, such as Thatcher and Hope (Hare and McIntosh, 1979) for black rust, Americano 25, Americano 44d, Surpreza, Frontana and Fronteira (Perez and Roelfs, 1989) for brown rust, and Wilhelmina, Capelle-Desprez, Manella, Juliana and Carstens VI (Stubbs, 1985) for yellow rust, have maintained some resistance for many years.

In most, if not all the cases, the failures have been due to the rush of releasing new varieties even when these do not conform to the disease yardsticks or due the inadequate knowledge of the virulences present in the pathogen population. Generally, there have been haphazard efforts to breed for rust resistance. The key points for the management of wheat rusts has always been to avoid large scale planting of single genotype/similar resistance and deploy varieties with diverse resistance, if possible then resistance based on more than one effective gene. It will not only delay the epidemics of wheat rusts but also increase the self life of wheat varieties and discourage the evolution in pathogens.

7.1 Source of wheat rust resistance: Due to the appearance of new virulent pathogen races like *Ug99*, breeding for rust resistance always requires a constant inflow of novel sources of resistance. While most of the rust resistance genes originate from hexaploid wheat, some have been introduced from related gramineae species. According to crossability with hexaploid wheat, other alien species are divided into three major gene pools: The primary gene pool, the secondary gene pool and the tertiary gene pool (Mujeeb-Kazi and Rajaram, 2002). Landmark beginning
was made by introgression of rye (*Secale cereale* L.) gene into bread wheat (1B/1R translocation or substitution) in 1973 (Mettin et al., 1973, Zeller, 1973) which carried *Lr26/Sr31/Yr9*. Sometimes yield reduction and genetic drag are associated with alien gene introgression in wheat cultivars. For instance, in *Sr26* introgression, 9% yield penalty was observed with the original 6AS.6AL-6Ae#1L segment, originally introgressed into the distal region of the long arm of hexaploid wheat chromosome 6A via an alien segment from *Agropyron elongatum* (syn. *Thinopyrum ponticum*) (Knott, 1961). *Sr26* is one of the few known major resistance genes effective against the *Sr31* virulent race *Ug99* (TTKS) and its *Sr24* virulent derivative (TTKST). A large number of rust resistance genes have been introgressed in to wheat from the alien sources (McIntosh et al., 1995).

7.2 Breeding strategies for effective incorporation of rust resistance: Wheat rusts can be effectively managed through a combination of strategies. Use of vertical resistance has proved to be short lived hence there is a need to first comprehend the horizontal resistance as suggested by Dr. Roy Johnson. It is possible to build this kind of resistance as has been shown by CIMMYT wheat Scientists and others. However, it needs to be seen, if this kind of resistance is long lasting. It is now becoming increasingly clear that pyramiding of 3-4 genes in a cultivar would provide durability to a cultivar. So wheat workers are now targeting this objective to build varieties with durable rust resistance. Traditional and molecular genetic research to further enhance the understanding of durable resistance based on minor, additive genes would receive high priority in future. To transfer resistance based on minor genes into a susceptible adapted cultivar or any selected genotype, a single backcross-selected bulk scheme is used where the cultivar/genotype is crossed with a group of 8-10 resistance donors, 20 spikes of the *F*$_1$ plants from each cross are then backcrossed to obtain 400-500 BC$_1$ seeds. Selection is practiced from the BC$_1$ generation onwards for resistance and other agronomic traits under high rust pressure. Because additive genes are partially dominant, BC$_1$ plants carrying most of the genes show intermediate resistance and can be selected visually. About 1600 plants per cross are space-grown in the *F*$_2$, whereas population sizes of about 1000 plants are maintained in the *F*$_3$-*F*$_5$ populations. Plants with desirable agronomic features and low to moderate terminal disease severity in early generations (BC$_4$, F$_4$ and F$_5$), and plants with low terminal rust severities in later generations (F$_4$ and F$_5$) are retained. The use of selected-bulk scheme where one spike from each selected plant is harvested and bulked until the *F*$_4$ generation is quite useful. Plants are harvested individually in the *F*$_5$. Bulking of selected plants poses no restriction on the number of plants that can be selected in each generation, as harvesting and threshing are quick and inexpensive, and the next generation is derived from a sample of the bulked seed. Because high resistance levels require the presence of 4 to 5 additive genes, the level of homozygosity from the *F*$_4$ generation onwards is usually sufficient to identify plants that combine adequate resistance with good agronomic features. Moreover, selecting plants with low terminal disease severities under high disease pressure means that more additive genes may be present in those plants. Subsequently, small plots of the *F*$_4$ lines are then evaluated for agronomic features and homozygosity of resistance, before conducting yield trials. Indian efforts for developing rust resistant wheat have been listed by Tomar et al. (2014).

7.3 Durable, slow rusting, adult plant resistance to rust: Durable resistance to a disease is resistance that remains effective during its prolonged and widespread in an environment favourable to the disease. The association of durable resistance with both major and minor genes, depending on different host-pathogen systems and the parasitic behaviour of pathogens and their degree of host specialization, has been discussed in detail (Parlevliet, 1993). Rather than displaying the immune phenotype, APR is generally activated at third leaf stage onwards, and tends to slow (rather than completely prevent) the progression of the pathogen (Singh et al., 2005). This ‘slow rusting’ is based on the low number of pustules, less uredial size, more latent period, incubation period and low uredospore production. Slow rusting lines have low area under disease progress curves (AUDPC). It is also a complex trait and is supposed to be conditioned by many genes (Singh et al., 1991) and may not follow the gene-for-gene hypothesis in true sense. Details have been given by Nayar et al. (2003). A simple formula: $7^*{(1+2/2)}+ 7^*{(2+3/2)}+7^*{(3+4/2)}$, where 1, 2, 3 are the disease co-efficients of 1$^*$, 2$^*$, 3$^*$, 4$^*$ rust observations, can be used to calculate the AUDPC.

7.4 *Lr35*, *Lr46*, *Lr67* and other minor genes for durable resistance to brown rust: Frontana and several CIMMYT wheats,
possessing excellent slow rusting resistance to brown rust worldwide, have adult plant resistance based on the additive interaction of Lr34 and two or three additional slow rusting genes (Singh and Rajaram, 1992). When susceptible cultivars display 100% brown rust severity, cultivars with only Lr34 display approximately 40% severity; cultivars with Lr34 and one or two additional minor genes display 10-15% severity; and cultivars with Lr34 and two or three additional genes display 1-5% severity. The presence of Lr34 can be indicated by the molecular markers linked to it. Earlier leaf tip necrosis was a parameter linked to Lr34 but now two more genes Lr46 and Lr67 do exhibit leaf tip necrosis. At the cellular level this kind of resistance was seemingly operating effectively. Knott (1989) has shown that adequate levels of multigenic resistance to black rust can be achieved by accumulating approximately five minor genes. In his studies the genes were different from S/2.

Singh (1992b) and McIntosh (1992) indicated that the moderate level of durable adult plant resistance to yellow rust of the CIMMYT-derived US wheat cultivar Anza and winter wheats such as Bezostaja is controlled in part by the Yr18 gene. This gene is completely linked to the Lr34 and Sr57. The level of resistance, it confers, is usually not adequate when present alone. However, combinations of Yr18 and Lr34 display approximately five minor genes result in adequate resistance levels in most environments (Singh and Rajaram, 1994). Genes Lr34, Sr57 and Yr18 occur frequently in germplasm developed at CIMMYT and in various countries. The recently identified slow rusting gene Yr29 is completely linked to gene Lr46, which confers moderate resistance to brown rust (William et al., 2003). Likewise Lr67/Sr55/Yr46 is also known to provide slow rusting/adult plant type of resistance to brown rust under Indian conditions. There had been statistically insignificant yield penalty due to brown, black and yellow rusts in the varieties based on Lr67 resistance. Durability of such resistance can be expected if the cultivar’s low disease severity is due to the additive interaction of several (4 to 5) partially effective genes.

The most effective approach now cloned slow rusting leaf rust resistance gene Lr34 located on chromosome arm 7DS, has maintained its moderate effectiveness for over 60 years of use (Krattinger et al., 2009). Gene Lr46, located on chromosome 1BL (William et al., 2003), was first identified in the CIMMYT-derived Mexican variety Pavon 76. This gene is widely distributed in germplasm from CIMMYT and other countries. It also confers slow rusting to black rust, yellow rust and slow mildewing to powdery mildew and is designated as S58, Yr29 and Pm39, respectively. Herrera-Foessel et al. (2014) demonstrated that APR locus Lr67/Yr46 has pleiotropic effect on black rust and powdery mildew resistance and is associated with defoliation by black rust. Genes are designated as S55, Pm46 and Lrn3, respectively.

Practical assessments of breeding populations and parental lines have been frequently described partial, slow-rusting and temperature-sensitive yellow rust resistance that can be measured as quantitative characters and thus referred to as quantitative trait loci (QTLs). Bariana and co-workers (2010) identified QTLs controlling APR to yellow rust in Kukri/Janz-derived doubled haploid (DH) population through molecular mapping and identified genotypes combining resistance from both parents.

8. Molecular studies

8.1 Molecular markers for rust resistance genes in wheat: Till date 213 genes imparting resistance to rust fungi (http://www.shigen.nig.ac.jp/wheat/komugi/genes/symbol ClassList.jsp) have been catalogued. The first molecular STS marker was developed by Schachermayr et al. (1994) for the Lr9 gene derived from Aegilops umbellulata, likewise the results on identification of several markers for other rust resistance genes were published. The development of new DNA-based assays has led to their application for designing direct and tightly linked markers – restriction fragments length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), cleaved amplified polymorphic sequence (CAPS), sequence characterized amplified regions (SCAR), sequence tagged sites (STS) and simple sequence repeats (SSR) microsatellites to identify individual resistance genes in wheat accessions.

DNA marker systems can be grouped into three classes (Gupta et al., 1999):

(i) Hybridization –based markers: These are also called restriction fragment length polymorphism (RFLP) markers. These are based on hybridization of DNA sequence called probes (usually labeled with radioactive isotopes) to genomic DNA restricted with restriction enzymes. Variation in number and position of restriction sites among individuals defines polymorphism. Several rust resistance genes (Lr1, Lr24, Lr35, Lr57, S2, S22, Yr15 and Yr40) were mapped using RFLP markers. This marker system is not routinely used now adays.
(ii) PCR-based markers: Random amplification of polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP) and microsatellite or simple sequence repeat (SSR):

(a) Randomly amplified polymorphic DNA (RAPD): Polymerase chain reaction (PCR) is used to amplify DNA fragments. The RAPD marker system involves the use of single primer (short single stranded DNA sequence) to amplify random regions throughout the genome (Williams et al., 1990). Rust resistance genes Lr19, Lr24, Lr34, Sr22and Yr15were tagged using RAPD markers. This system is not currently used because of lack of reproducibility, low levels of polymorphisms, amplification of multiple bands and dominant inheritance (Semagn et al., 2006a).

(b) Amplified fragment length polymorphism (AFLP): These markers combine the properties of RFLP and RAPD marker systems. These involve selective PCR amplification of fragments from a pool of restricted genomic DNA and selective amplification primers comprise of sequences complementary to adapters and one to three random nucleotides attached at the 3’ end. Primers specifically bind to the fragments containing matching ends to selectively amplify different sized fragments (Vos et al., 1995). AFLP markers have been reported for rust resistance genes Lr3, Lr26, Sr30, Sr31, Sr39, Yr7, Yr9 and Yr29 (Bariana et al., 2001, Mago et al., 2005, Dieguez et al., 2006, Rosewarne et al., 2006, Mago et al., 2009).

(c) Sequence tagged site (STS): This category of primers was designed to amplify DNA sequence that are specific to a locus and not found elsewhere in the genome are called STS markers (Gupta et al. 1999; Semagn et al., 2006b). STS markers for rust resistance genes Lr9, Lr20/Pm1and Lr24 (RAPD-derived; Schachermayr et al., 1994, Schachermayr et al., 1995, Neu et al., 2002); Lr35 and S22 (RFLP-derived, Seyfarth et al., 1999, Periyanann et al., 2011); Lr19, Lr26, Lr28, Lr37, Lr24, S26, S31, S38, S39, Yr5 and Yr6 (AFLP-derived; Naik et al., 1998, Prins et al., 2001, Mago et al., 2002, Mago et al., 2005, Smith et al., 2007, Mago et al., 2009) and Lr34, Sr13, Sr25and S26 (EST-derived; Lagudah et al., 2006, Liu et al., 2010, Simons et al., 2011) are available.

(d) Microsatellites or simple sequence repeats (SSRs): Microsatellites or SSRs markers are tandem repeats of a few base pairs (1-6) occurring throughout the genomes.

The number and type of repeats determine polymorphism among different organisms. High levels of polymorphism, low cost and amenability for automation are the major advantages of these markers (Hayden et al., 2006). SSR markers linked with rust resistance genes Lr19, Lr22a, Lr24, Lr34/Yr18, Lr39, Lr42, S2, S6, S22, S36, S35, S40, S4Web, Y5, Yr10, Yr36, YrCH42 and YrZH84 are available for marker assisted selection (Schachermayr et al., 1995, Raupp et al., 2001, Bariana et al., 2002, Sun et al., 2002, Spielmeyer et al., 2003, Khan et al., 2005, Spielmeyer et al., 2005, Uauy et al., 2005, Bossolini et al., 2006, Gupta et al., 2006, Li et al., 2006, Hiebert et al., 2007, Tsilo et al., 2008, Tsilo et al., 2009, Wu et al., 2009, Sun et al., 2010, Hiebert et al., 2010, Olson et al., 2010, Zhang et al., 2010).

(e) Insertion site-based polymorphism (ISBP): Transposable elements have unique insertion sites that are highly conserved between different cultivars of plants. ISBP markers were used as PCR based markers by Flavell et al., (1998). ISBP markers were also developed from the BAC end sequences of chromosome 3B (Paux et al., 2006).

(iii) DNA chip and sequenced-derived markers: All the molecular markers described above are gel-based and are labour-intensive and time-consuming. DNA-chip based methods are highly throughput and highly efficient.

(a). DArT markers: Diversity arrays technology (DArT): This marker system provide a cost effective whole-genome fingerprinting tool and efficient for species which have complex genomes and lack prior DNA sequence information (Jaccoud et al., 2001, Wenzl et al., 2004). 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.

Details of this technology are provided on their website (http://www.diversityarrays.com/). DArT marker system has now evolved further and it is now referred to as DArTseq. DArT marker system involves sequencing of the genomic representations on the Next Generation Sequencing (NGS) platforms. Many wheat populations have been mapped using DArT and DArTseq system. DArT markers linked with Lr34/Yr18/Pm38, Lr46/Yr29/Pm39, S2, S6, S25and Y51are available for marker assisted selection (Lillemo et al., 2008, Tsilo et al., 2009, Yu et al., 2010, Randhawa et al., 2014).
9. DNA Polymorphism and genome sequencing in wheat rusts

Over the years, advances in the field of molecular biology have lead to virtually discover unlimited number of DNA markers for their use in plant pathology (Singh and Hughes 2006). The most popular markers employed in the variability studies of Puccinia sp. include RFLP, RAPD, AFLP, SSR, Internal Transcribed Sequence (ITS) and Single Nucleotide Polymorphism (SNP) (Cooke and Lees, 2004, McCartney et al., 2003). Whole genome sequencing is another aspect of molecular biology which has opened up research areas from where we can uncover a number of unsolved mysteries. The genome of P. graminis f. sp. tritici (Duplessis et al., 2011), P. striiformis (Cantu et al., 2011) and P. triticina have been sequenced at Broad Institute, USA. Recently 100-106Mb genome of pathotypes 77 and 106 of P. triticina was sequenced by ICAR-NRCPB, New Delhi (Kiran et al., 2016).

De novo sequencing of whole genomes of pathogens would be useful in understanding the evolution of new virulences, race identification, virulence pattern and population genetics of the rust pathogens. Comparative genome analyses would help in knowing the evolution of virulent races and use of information in disease management. Next generation sequencing (NGS) is a powerful tool that provides dramatic improvement in sequencing speed and depth together with a steep decline in associated costs compared to previous sequencing technologies.

10. Genome editing-mediated disease resistance

Genome editing with engineered nucleases (GEEN) is a genetic engineering tool which is used to insert, replace or remove the part of DNA in the genome of an organism using artificially engineered site-specific nucleases (SSNs) or molecular scissors such as zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs) and clustered regularly interspaced short palindromic repeat (CRISPR)/Cas. GE/GEEN has facilitated targeted in vivo gene editing in many organisms (Gaj et al., 2013). These SSNs cut DNA at desired locations in the genome. Some of the successful examples of application of these genome editing methods in plants include development of fragrant rice by knocking out OsBADH2 gene in rice (Shan et al., 2015) and Celiac-safe wheat by elimination of gluten by ectopic expression of glutenase genes in wheat (Wen, 2014).

Of the SSNs, TALENs have been utilized for in vivo modifications of plant R genes to modify their expression level and resistance range against different pathogens (Wang et al., 2014). TALEN technology was used to introduce site-specific mutations in the three homeoalleles of MLO gene encoding Mildew-Resistance Locus (MLO) proteins in wheat. In recent years, the availability of wheat genome sequences has further increased the genomic resources in wheat.

11. Genetics of rust resistance of Indian wheat

Nagarajan et al., (1987) documented rust resistance genes in wheat material, subsequently updates were also published (Nayar et al., 2001, Bhardwaj et al., 2010a). Inbetween diverse information on genetics of wheat rust resistance has been added (Nayar 1989, Sawhney 1994, Tomar and Menon 2001, Nayar et al., 2001, Walia and Kumar 2008, Bhardwaj 2011). Based on the available information, it can be concluded that brown rust resistance of Indian wheat is based on Lr1, Lr3, Lr9, Lr10, Lr13, Lr14a, Lr17, Lr18, Lr19, Lr22, Lr23, Lr24, Lr26, Lr28, Lr34, Lr46 and Lr49. Among these Lr26, Lr13, Lr23 and Lr34 have been characterized in many wheat lines. Presently Lr24, Lr25, Lr29, Lr32, Lr39, Lr45, Lr47 are resistant to all the pathotypes of P. triticina in India (Bhardwaj et al., 2010b). S22, S25, S26, S27, S28, S29, S30 and S31 have been characterized in Indian wheat material. Among these S22, S21 and S31 were very common in bread wheat whereas S27, S29 and S11 conferred black rust resistance in many durum lines. S26, S27, S31, S32, S33, S35, S39, S40, S43 and S44 (Jain et al., 2013) confer resistance against Indian population of P. graminis tritici. Yellow rust resistance of wheat in India is based on...
Yr2, Yr9 and Yr18. Yr5, Yr10, Yr11, Yr12, Yr13, Yr14, Yr15, Yr16, Ysp, Ynk (Prashar et al., 2015) are resistant against P. striiformis in India.

12. Chemical control

As an emergent tool for controlling wheat rusts, chemical method of wheat rust diseases management has been successfully used in Europe, permitting high yields (6 to 7 tonnes/ha) and where prices for wheat are supported (Buchenauer, 1982). Chemicals were also used to control a brown rust epidemic in 1977 in the irrigated Yaqui and Mayo Valleys of Mexico (Dubin and Torres, 1981). Elsewhere, chemicals have had limited use on high-yielding wheat in the Pacific Northwest of the United States for yellow and brown rust management. For controlling initial load of inoculums or under high yellow rust incidence in India, fungicides belonging to triazole group such as Propiconazole 25 % EC (Tilt), Tebuconazole 25 % EC (Folicur) and Triadimefon 25 % EC (Bayleton) have been used effectively at the rate of 0.1% for the management of wheat rusts.

13. Way ahead

With growing awareness of environmental and health hazards, only ecologically and bio-safe methods of disease management would be followed in the coming years. Rust resistance is going to be a major player in managing the wheat rusts in future. However, diversity by using diverse types of protection i.e. all time (seedling), adult plant and slow rusting resistance would always be desirable. Monitoring the new pathotypes, pathotype distribution, proactive breeding efforts for developing high yielding, climate resilient, resource responsive rust resistant wheat varieties would form the key factors to manage rusts. In addition, rust resistance gene pool has to be enriched regularly from primary, secondary and tertiary sources. More focused and extensive efforts would be initiated to study the perpetuation of wheat rusts and epidemiology in India. Genome sequences of wheat and wheat rusts are available. Exploring the functional genomics and alternative approaches using next generation techniques would be the tools for effective and intelligent management of wheat rusts. Use of genomic selection by overcoming the barriers of Marker assisted selection for using Single Nucleotide polymorphism, QTLs and other traits is going to be a strong tool for resistance breeding. Further efforts would go into developing of plant bodies (animal origin) based transgenics, use of Systemic Acquired Resistance, Induced Systemic Resistance by the application of effectors, transcription factors, Pathogenesis Related proteins, Ribonucleic Acid interference, Virus Induced Gene Silencing, trans-gene free genome editing, Genome wide selection, MAS, Gene cassettes and epigenetic tools are going to be other forefront technologies for managing the wheat diseases in future.

References


16. Chester KS. 1946. The nature and prevention of the cereal rusts as exemplified in the leaf rust of wheat. In Chronica botanica. Waltham, MA, USA. 269PP.


41. Jain SK, M Prashar, SC Bhardwaj, SB Singh and YP Sharma. 2009. Emergence of virulence to \( S_{25} \) of *Puccinia graminis tritici* on wheat in India. *Plant Disease* 93:840.


45. Kurarpathy V, P Chhuneja, HS Dhaiwal, S Kaur, RL Bowden and BS Gill. 2007. Characterization and mapping of cryptic alien introgression from Aegilops geniculata with novel leaf rust and stripe rust resistance genes \( Lr_{57} \) and \( Yr_{40} \) in wheat. *Theoretical and Applied Genetics* 114: 1379-1389.


47. Li ZF, TC Zheng, ZH He, GQ Li, SC Xu, XP Li, GY Yang, RP Singh and XC Xia. 2006. Molecular tagging of stripe rust resistance gene \( Yr_{ZH84} \) in Chinese wheat line Zhou 8425B. *Theoretical and Applied Genetics* 112: 1098-1103.


70. Nayar SK. 1989. ACIAR-ICAR collaborative Research Report "Genetics and Breeding for rust resistance in wheat". Pl. Breeding Inst., Univ. of Sydney, Australia. 50 PP.


85. Sawhney RN. 1994. for durable resistance to wheat rusts. Publications and Information Directorate Dr. K.S Krishnan MARG, New Delhi 110012. 52PP.

86. Sawhney RN. 1994. for durable resistance to wheat rusts. Publications and Information Directorate Dr. K.S Krishnan MARG, New Delhi 110012. 52PP.


95. Tozzetti GT. 1952. V. Alimurgia: True nature, causes and sad effects of the rusts, the bunts, the smuts, and other maladies of wheat and oats in the field. In L.R. Tehon, transl. Phytopathological Classics No. 9, p.139. St Paul, MN, USA, American Phytopathology Society. (Originally published 1767).

96. Tozzetti GT. 1952. V. Alimurgia: True nature, causes and sad effects of the rusts, the bunts, the smuts, and other maladies of wheat and oats in the field. In L.R. Tehon, transl. Phytopathological Classics No. 9, p.139. St Paul, MN, USA, American Phytopathology Society. (Originally published 1767).


