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Review Article

Breeding wheat for leaf rust resistance: past, present and future

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Abstract

Leaf rust of wheat caused by (*Puccinia triticina* Eriks) proliferate under optimum weather conditions and causes severe damage. Diseases appeared in form of epidemics pose a real threat to food security rising the cost of food production. Breeding for development of resistant varieties against disease has advantages for ecological and monetary reasons, predominantly for peasants in the developing world. Sufficient research work has been conducted regarding pathogen host interaction mechanism. Two mechanisms of resistance are acquainted very well. Complete resistance function from seedling to adult growth stages whereas partial resistance becomes effective at the pre-booting stage and is more durable. Eighty leaf rust-resistant genes have been documented. Among these leaf rust-resistant genes *Lr12*, *Lr13*, *Lr22a*, *Lr34*, *Lr35*, *Lr37*, *Lr46*, *Lr48*, *Lr49*, *Lr67*, *Lr68*, *Lr74*, *Lr75*, *Lr77*, and *Lr78* are adult plant resistant (APR) genes. Fear of genetic erosion is also well known. It means cultivars grown on a wide range with narrow genetic backgrounds and this situation is undesired as it may invite an epidemic. It has been experienced repeatedly in past decades. Wide genetic diversity in parents can promise to achieve maximum output from the breeding programmes. Sources of resistance other than Triticum aestivum are rich in diversity and consequently have been addressed adequately. Usage of relatives of wheat plant as a source of novel genes belonging to genera *Triticum*, *Aegilops*, *Thinopyrum* and *Secale* has generated more desired output. Molecular markers are being applied to explore diversity in pathogen as well as in host effectively although conventional approaches are being used as well. Status of research work carried in Pakistan has also been discussed in abridged form. This review has been conducted with an objective to summarize research work academic as well as applied, carried to develop strategies to incorporate genetic resistance in wheat against leaf rust.

Keywords: Leaf rust, Brown rust, *Puccinia triticina* Eriks. (Pt), Disease resistance, Molecular markers, Genetic erosion

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Introduction

Bread wheat (*Triticum aestivum* L. em. Thell.) is one of the chief staple foods along with rice, and maize, fulfill more than 50% the calories demand of the world population (Afzal et al., 2020). Wheat occupies status of king crop in terms of acreage under cultivation, tonnage produced and quantity traded (Enghiad et al., 2017). Production of wheat crop has increased vastly worldwide and facilitated in curtailing malnutrition with the deployment of semi dwarf varieties advanced since 1960's (Khan et al., 2013). There is about 123% increase in population from 1960 to 2009 in spite of a substantial increase in global population, per capita agricultural production has still overtaken population growth and there is 29% more food is available that was in 1960 (The Royal Society, 2009). Despite the brilliant achievements we have to escalate wheat production to nourish the continually-increasing population.

The target can be accomplished by evolving high yielding and rust resistant varieties. The inadequate genetic diversity might cause susceptibility to several biotic and abiotic stresses. Instinctive genetic diversity for related breeding characters including resistance for diseases is an important source that assist breeders to determine and incorporate useful variability that may be valuable for combating against these problems (Sansaloni et al., 2020). Continuous emergence of highly virulent pathogens causing rusts, bunts, smuts, blight, spot blotch, anthracnose, leaf blight, tan spot of wheat, and powdery mildew making worse pressure on food security (Afzal et al., 2021). Change in climate led to development of novel plant pathogenic strains which might lead to serious epidemics in near future (Santini and Ghelardini, 2015).

Three wheat rust diseases (Leaf rust, Stem Rust & Stripe Rust) are major biotic constrictions to sustain production of wheat worldwide continue to threaten food security for thirty hundred years (Afzal et al., 2018; Khan et al., 2013). Rusts in wheat are successful plant pathogens being highly fertile and having capacity to travel long distances (Afzal et al., 2021). Wheat cultivation is mostly hit by rusts and substantial damage occurs in short period of time than any other disease. Severe yield losses have been recorded in numerous regions caused by cereal rusts (Chen et al., 2014; Wellings, 2011; Kolmer et al., 2009). Various species of *Puccinia* are characterized in terms of different life cycles. Rust fungi have a specific characteristic of creating novel strains frequently. Dissimilar response of cultivars to rust as recorded in previous year indicates incidence of novel strain. Wheat production is constrained significantly almost everyplace wheat is grown, by one or more of the rusts in wheat (Hovmøller et al., 2010). Wheat experts are challenged by the development of novel strains of rust pathogens throughout the world (Figlan et al., 2020). Human population is increasing geometrically, whereas, food production is increasing arithmetically, there-by the gap between demand and production is widening. Across the world, yields gain in major staple crops are not stagnated. The choices are: increase area under crop cultivation, increasing the productivity or mitigate the losses, even though the first two are also of its prime importance later possibility is sustainable and achievable, the task of provision of nourishment a growing population appraised to around nine hundred million by mid of current century to meet the projected demands 100% increase in wheat production (Röös et al., 2017).

Leaf Rust

Leaf or brown rust caused by *Puccinia triticina* Eriks.(*Pt*) (Anikster et al., 1997), is the most geographically dispersed rust of wheat (Huerta-Espino et al., 2011). Characteristic symptoms of leaf rust are dusty, reddish-orange to reddish-brown fruiting bodies that seem on the surface of leaf. Numerous spores are produced in these lesions, which can cover nearly the complete upper portion of leaf surface. Colour and shape of pustules distinguish leaf rust and Stripe rust (Figure 1). Importance of leaf rust has augmented extremely during the preceding decade, because of the incidence of more lethal strains of pathogen *P. triticina* (Pt) and the collapse of the resistant genes that had been deployed broadly created a situation hazardous to worldwide wheat production. The pathogen prevails dominantly in regions with mild temperatures and humid circumstances (Huerta-Espino et al., 2011). Rust surveillance reports demonstrate that leaf rust incidence is more common in practically all zones under wheat cultivation than stripe rust or stem rusts of wheat (Bolton et al., 2008; Huerta- Espino et al., 2011). Worldwide leaf rust is the most important problem in Asia (central, south and southeast), North and south America, Europe, New Zealand, Australia, and North Africa (Carver, 2009). Based upon the spread of uredospores by wind currents during each cropping season, Huerta-Espino clustered wheat region of the globe in to diverse

epidemiological areas including: Australia–New Zealand, South America, Western Europe, the Far East, Southeast Asia, Northern Africa, Southern Africa, Eastern Europe, Egypt, West Asia, South Asia, the USA, Canada and Mexico (Huerta-Espino et al., 2011). This disease is anticipated to impair crop production hereafter even more, because of forthcoming alteration in environment (Jiang et al., 2018).

Figure-1: Infection of leaf and stripe rust on same leaf. a- Leaf rust has orange brown pustules randomly distributed on the leaf. b-Yellow streaks followed by long yellow elongated uredial pustules arranged in rows are of stripe rust.

Alteration in nomenclature of casual organism of leaf rust

Nomenclature of the leaf rust experienced many changes. Several taxonomist contributed their efforts to describe the true position in taxonomic order of the causal agent of the leaf rust in various eras (de Candolle, 1815; Winter,1884; Eriksson, 1899; Cummins and Caldwell, 1956; Wilson and Henderson, 1966; Anikster et al., 1997). It was proposed as *P. recondita* (Cummins and Caldwell, 1956) and was accepted internationally by scientific community. Savile (1984) and Anikster et al. (1997) recommended *P. triticina* in view of the results of current genetic and morphological research. Anikster et al. (1997) placed leaf rusts in different species based on their different alternate hosts *Triticum speciosissimum* and *Anchusa* spp. This categorization of fungi causing leaf rusts is based on sexual incompatibility (Anikster et al., 1997). The causal organism of wheat leaf rust is recognized a dissimilar species from leaf rusts infecting rye and other families of wheat based on erotic incompatibility. Reports of phylogenetic ribosomal DNA sequence investigating (Zambino and Szabo, 1993), size, shape, and structure of the spore (Savile, 1984) and morphology of infection structure (Swertz, 1994) also support that microorganism causing leaf rust in wheat is unique from leaf rusts infecting other species. Leaf rust of *T. aestivum* L. i.e., bread wheat or *T. turgidum* L. var. *durum* or pasta wheat is now nominated as *Puccinia triticina* Eriks.

Economic significance of leaf rust

Among three wheat rusts leaf rust prevails the utmost threatening food security worldwide (Hovmøller et al., 2010). This is typically less damaging than those from other two rusts (Stem & Stripe rust), but hurts the crop more severely due to its frequent recurrence (Thabet and Khadiga Najeeb, 2017). *P. triticina*, mostly infects the leaf blades, though it attacks glume and the leaf sheath too. Therefore, disturbs the photosynthesis directly which leads to reduction of photosynthates needed for healthy growth, development, and functioning.

Appearance of disease at earlier stages may damage production more severely (Singh et al., 2002). A study conducted recently revealed 60–70% disease severity on the flag leaf (leaf below the inflorescence) at heading stage may reduce yield above 30%, however the equivalent infection at the milky stage may damage crop produce slight as a 7% (Hunger and Edwards, 2019). Several epidemics of the disease have been documented in history causing severe damage. Leaf rust prevails in the Yellow-Huai-Hai River regions in China (Kang et al., 2010). In Pakistan, the crop suffered an assessed general injury of 86 million US $ during the year 1978 (Hussain et al.,1980). A research work explored that USA suffered $\geq$50% cereal yield decline during the rust epidemic years from 1918 to 1976 (Roelfs, 1978). According to Mikhailova et al., 2009 in different wheat growing regions up to six leaf rust epidemics occur in every decade in Russia. In South Africa, 21-million-hectare under wheat farming is disposed to leaf rust related losses to approximately 30% each year. Over 90% of the wheat zone in Central Asia is disposed to leaf rust (Singh et al., 2004). Huerta-Espino et al., 2011 reported leaf rust has potential to damage crop produce valued up to 197 million Australian dollars annually in Australia, the deployment of resistant varieties curtailed damage to about 12 million Australian dollars. In Egypt Abdel-Hak et al., 1980 reported leaf rust depressed yield up to 50%. Depression in yield caused by rusts in wheat were assessed by conventional means up to 2% or more than one million ton annually in North America during 1960s (Wiese, 1977).

Leaf rust management through breeding

Growing rust resistant cultivars in farmer field is the most practical method of disease management (Figlan et al., 2020). Application of fungicides at proper stage

is recommended in many regions to control rusts in wheat (Afzal et al., 2020; Rees and Platz, 1975). Combination of resistance against rusts have been a major goal in wheat breeding as growing rust resistant cultivars in farmer field has been considered the profitable technique of disease management. The conservation and allocation of leaf rust resistance genes into locally adapted varieties has been a foremost objective of programs of wheat improvement through breeding worldwide. Genetic basis of resistance has been addressed sufficiently; data generated is applied effectively in plant disease management through breeding.

Rowland Harry Biffen pronounced the hereditary base of resistance to stripe rust in the beginning of 1900s. Developing disease resistant genotypes is a complicated process. Cultivation of disease resistant varieties not only enhances crop production but ensures stabilized productivity. Growing disease resistant plants assures sustainable agriculture (Biffen, 1905). Subsequently, the detection of genomic diversity for rust resistance in wheat has been continuing element of breeding programs but requires substantial financial investment. To characterize genetic diversity, plants are screened at seedling and then near maturity phases. Test material is generally inoculated artificially and spreader rows (highly susceptible genotypes) are cultivated among test entries. Test entries which show resistant response, can be exploited as parents in the breeding for crop improvement. Frequently several circles of selection are essential to recover biotic and abiotic characters beforehand an upgraded variety is released for general cultivation in the field. Nearly one century before, two wheat cultivars Malakof and Webster investigated to observe their response against leaf rust (McIntosh et al., 1995). These two genotypes with genes nominated Lr1 and Lr2 having resistance against the leaf rust (Ausemus et al., 1946).

Wheat breeding for rust resistance is not only advantageous for wheat growers, but have fiscal reimbursements to wheat improvement programs. One hundred eighty-seven rust resistance genes (58 Stem rust, 80 Leaf rust, 49 Yellow rust) have been catalogued (McIntosh et al., 2017). Among these some genes were found either repeated or terminated, thus were detached enlisted as *Lr4*, *Lr5*, *Lr6*, *Lr7*, *Lr8*, and *Lr40*, *Lr41*, *Lr43* (McIntosh et al., 2013).

Genetic Erosion

The scientific community highlighted a fear concerning "Genetic Erosion" approximately half a century ago. This term was used first by Harlan (1972) to define the possibly alarming narrowing of the germplasm base of the developed food crops. Langridge et al., 2001 attributed domestication, frequent practice of improved germplasm, lack of extensive genetic recombination as the factors responsible for shriveled genetic diversity of main crops together with bread wheat. This situation is unwanted agronomically as well as from plant pathological point of view. In Pakistan approximately 70% of the area was cultivated with variety "Inqilab-91" and "PBW343" was mainly cultivated in India. Those both carried the similar genetic resistance gene (*Yr27*) that resulted in epidemic in recent past. The risk of the immensely infectious novel race of stem rust *Ug99* to genetically similar genotype derived from IB. IR translocation cultivated on extensive zone is a serious threat (Afzal et al., 2015; 2021). The Veery derivatives derived from1B.1R translocation were grown widely in various regions because of their preferred agronomic characteristic and resistance against disease. This gene bank exhibited substantial advantage in terms of grain yield and widespread adaptation with more disease resistance traits. The higher yielding ability of these genotypes is attributed to post anthesis stress tolerance of this germplasm resulting in high grain weight (Moreno-Sevilla et al., 1995). At one phase the incidence of 1B.1R translocation reached around 70% in spring wheat germplasm evolved in CIMMYT (Singh et al., 2006). This translocation, carries genes *Yr9*, *Sr31*, *Lr26*, & *Pm8* conferred resistance against three rusts and powdery mildew (Purnhauser et al., 2010). The 1B.1R translocation became widespread in wheat cultivars released in Indo-Pakistan, China, USA, and several other countries. Due to the high frequency of 1B.1R translocation wheat lines in the international cultivation sphere, Lr26 based cultivars dominated within our germplasm during the mid-1980s and later (Khan et al., 2002). The wide spread global popularity of the germplasm with 1B-1R translocation created monoculture situation and when used initially it provided resistance to stem rust, leaf rust and yellow rust but with the development of new virulent races, these genes are ineffective now (Singh et al., 2006). Several important resistant genes, lost their effectiveness with the emergence of novel races. Genetic erosion in host plays key role to obligate pathogen to evolve

novel strains. Consequently, genetic diversity occupies the status of pivot in plant breeding. An effective disease management strategy is defined as based on broad genetic support (Rahmatov, 2016).

Types of resistance

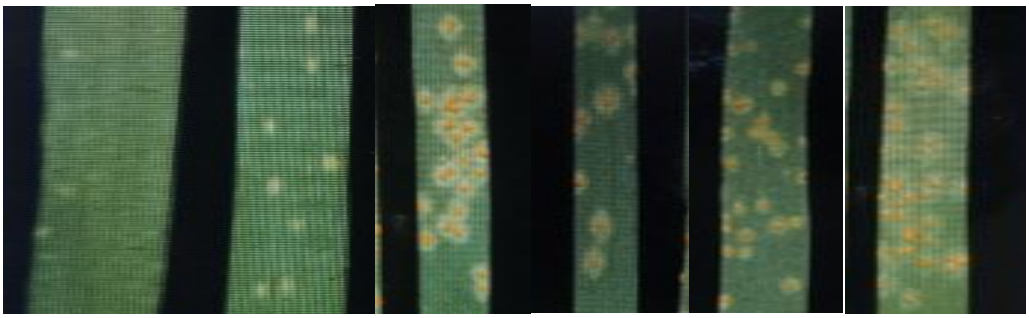
Two different mechanisms exist that control inheritance of disease resistance in plants (Afzal et al., 2021). Monogenic inheritance is determined by a single gene which is transferred from parents to descendants. In the same locus two alleles of this gene are located. This inheritance pattern characterizes sporadic variations in traits and is also termed as qualitative inheritance**.** Polygenic inheritance where a trait is determined by two or more genes. These two genes can be located in two or more loci. This pattern of inheritance is termed as quantitative inheritance and demonstrate a continuous variation of a specific trait. This pattern of inheritance does not follow Mendel Principles of inheritance, and hence is called as non-mendelian inheritance.

Resistance conferred by genes that are distinguished at the seedling growth stage and continue functioning throughout the plant life, thus known as 'all-stage resistance' also called as R resistance (Singh et al., 2016). R resistance is monogenic (Cloutier et al., 2007) and results in a hypersensitive response (HR) (Mondal et al., 2016), leading to generating novel strains virulent against the genes which were resistant before. That is why it is not unusual that a resistant genotype based on R type resistance causes the discarding of new variety after a very short time of their releases (Niks et al., 2015). Quantitative resistance is categorized by reduced speed of disease development (Slow rusting) by increasing latent period and does not follow genetic interaction with the pathogen as in case of complete resistance. Plant breeders depend on both types of resistance to develop disease resistant genotypes through breeding. Working with monogenic resistances is easy but often not durable. Thus, quantitative resistance is favored. Elongated latent period, low susceptibility, small size of uredia, lessen interval and number of spore production are the factors that cause reduced disease development under field conditions (Sareen et al., 2012; Wilcoxson, 1981; Navi et al., 1989). The phenomenon of slow rusting is to live and let live, a situation in pathogen and host that ensures long lasting resistance (Sareen et al., 2012).

Adult plant resistance also known as APR is effective typically in adult plants, and resistance conferred by such genes is fractional, contrary to most R genes. In general APR genes but not all confer resistance to all strains of a rust pathogen species and a subclass of these confer resistance to numerous pathogen species belonging to Mycota. Quite a lot of genes resistant against leaf rust are classified, and slow rusting APR genes have been postulated in high yielding varieties (Huerta-Espino et al., 2011). The genetics of APR to leaf rust in bread wheat line PI 250413 collected initially from Pakistan was inured b x a single recessive gene. This b x a recessive gene not known previous studies inherited independently of LrI2, Lr13 and Lr22, three known APR genes (Dyck and Samborski, 1979). Ellis et al., 2014 described three genes *Lr34/Yr18/Pm38*, *Lr46/Yr29/Pm39* and *Lr67/Yr46* categorized as slow rusters. Appearance of leaf tip necrosis (LTN) phenotype is linked with these three APR genes (Hiebert et al., 2010). These genes are dominant in CIMMYT release germplasm lines singly or in combination with other minor gene (Hiebert et al., 2010; Herrera-Foessel et al., 2011). Area under disease progress curve (Das, 1990) is used to identify slow ruster and fast ruster (Madden et al., 2007).

Sources of genes resistant against leaf rust

Gene symbols for wheat have been designated in the catalogue. Eighty leaf rust resistant genes (*Lr1* to *Lr80*) have been designated besides numerous undesignated genes and are used in wheat breeding. Most leaf rust resistance genes are race specific demonstrated by a hypersensitive response (HR) of sudden cell death that happens at the border between fungal haustoria and host cells in the epidermal and mesophyll coatings. Different genes treat typical phenotypes (Figure 2). For example, the response of wheat genotypes with *Lr3* is branded by clearly defined hypersensitive flecks while genotypes with *Lr2a* have only very light flecks. *Lr3ka*, *Lr3bg* and *Lr11* are displayed by small uredinia encircled by chlorosis, and genotypes with *Lr16* have trivial uredinia surrounded by necrosis.



1 2 3 4 5 6

Sr. No.	IT*	Host Response	Symptoms
1	0	Immune	No uredia or other macroscopic sign of infection
2	0;	Nearly immune	No uredia, but hypersensitive necrotic or chlorotic flecks
3	1	Very resistant	Small uredia surrounded by necrosis
4	2	Moderately resistant	Small to medium uredia surrounded by chlorosis or necrosis
5	3	Moderately susceptible	Medium-sized uredia that may be associated with chlorosis
6	4	Very susceptible	Large uredia without chlorosis or necrosis

*Infection type

Figure-2. Infection types of wheat leaf rust used in disease assessment at seedling stage adopted by Johnston and Browder (1966).

Race-specific *Lr* genes are effective in seedling plants and remain effective in the adult plant stage (Bolton et al., 2008). Approximately 44% are derived from wild ancestor and non-progenitor species, whereas remaining are derived from *Triticum aestivum.* Most of genes among these wild relatives of wheat, particularly the tertiary gene pool, hold a huge diversity of disease resistance (R) genes (McIntosh et al., 2017; Qureshi et al., 2018). *Lr1, Lr3, Lr10 and Lr20* have been used in breeding often and distributed usually in wheat varieties globally (Dakouri et al., 2013). Genes with their resources other than common wheat are enlisted in the Table 1. New sources of resistant genes are indispensable to ensure sustainable wheat production against unceasingly and fast developing novel races of rust pathogens (Boshoff et al., 2018).

However, the resistance conditioned by some genes, is best expressed in adult plants. In wheat genotypes that have combinations of resistance genes, the gene with greatest resistant infection type is epistatic to genes with less resistant infection types. Another distinguishing feature of adult plant resistance genes is production of extended diversified proteins than proteins produced by R genes. *Lr12, Lr13, Lr22a, Lr34, Lr35, Lr37, Lr46, Lr48, Lr49, Lr67, Lr68, Lr74, Lr75, Lr77*, and *Lr78* are genes which become effective at adult stage (Singh et al., 1998; Suenaga et al., 2003; Hiebert et al., 2010; Herrera-Foessel et al., 2011; Herrera-Foessel et al., 2012; Singla et al., 2017; Kolmer et al., 2018a; Kolmer et al., 2018b). All APR genes are not durable eternally. A priority of breeding work is to improve the resistance to rust by describing more genes in wheat that confer resistance at adult stage and to comprehend how this works and how these interrelate when several R and APR genes are loaded into a single genotype (Ellis et al., 2014).

Conventional breeding

Conventional breeding programs depend on the screening of selected germplasm for making crosses with most required characters like disease resistance, terminal heat resistance, early maturity with high yield. Individual plants are screened as seedlings under glasshouse or in the field as adults to determine genetic variation for resistance against leaf rust. Especially, multilocational trials are conducted with an objective to assess the resistance at the adult stage to know durable resistance genes. The known sources of resistance are added in susceptible cultivars with desirable agronomic features. Frequently several cycles of crossing and screening are carried to improve

various abiotic and biotic characters before a genotype is released for commercial cultivation.

Borlaug began shuttle breeding (Borlaug, 1968). Procedure involves cultivating wheat germplasm at two dissimilar sites having different geographical and ecological presentation. Shuttle breeding approach accelerates the breeding program, as two consecutive generations are grown per year (Forster et al., 2014). This approach enabled selection for durable disease resistance, photoperiod insensitivity and wide adaptation result improvement in productivity of breeding (Ortiz-Ferrara et al., 2007). Phenotypic data in the field is recorded to detect the desired genotypes (Velu and Singh, 2013). Still, complications related with phenotyping under field conditions comprised of reliance on meteorological conditions, the prevalence of undesirable pathotypes, is time and labor intensive. Virulence in pathogen is developed in short duration so emphasizing speed breeding (Govindaraj et al., 2015). Despite, national wheat improvement programs are using this technique in many countries

Table-1. Sources of Leaf Rust Resistant Genes (Other than *Triticum aestivum*) (McIntosh et al., 2017; Chhuneja et al., 2015)

Sr. No.	Source	Genes
1	*Aegilops. Tauschii*	*Lr21, Lr22a, Lr32, Lr39/Lr41, Lr40, Lr42, Lr43*
2	*Ae. Speltoides*	*Lr28, Lr35, Lr36, Lr47, Lr51, Lr66*
3	*Triticum monococcum*	*Lr63*
4	*T. dicoccoides*	*Lr53, Lr64*
5	*T. timopheevi*	*Lr18, Lr50*
6	*Ae. Ventricose*	*Lr37*
7	*Ae. Umbellulate*	*Lr9, Lr76*
8	*Tinopyrum ponticum*	*Lr19, Lr24, Lr29*
9	*Secale cereale*	*Lr25, Lr26*
10	*T. intermedium*	*Lr38*
11	*Ae. Kotschyi*	*Lr54*
12	*Elymus trachycaulis*	*Lr55*
13	*Ae. Sharonensis*	*Lr56*
14	*Ae. Geniculate*	*Lr57*
15	*Ae. Triuncialis*	*Lr58*
16	*Ae. Peregrina*	*Lr59*
17	*Ae. Neglecta*	*Lr62*

Application of new techniques in wheat improvement:

Plant breeding with developed tools seems to play a principal role for the probable future. Application of molecular genetics in wheat is not easy. The hexaploidy besides the low level of polymorphism between elite varieties the crop provides substantial challenges for those trying to develop molecular markers and to usage in genomic studies. New studies are in progress to analyze the genetic base of various traits in wheat with the evolution of Amplified fragment length polymorphism (AFLP) and microsatellite marker systems. With intention of developing varieties having good degree of defense under disease pressure, numerous combinations of 'slow rusting" genes are prerequisite. Availability of molecular markers facilitates the process of gene pyramiding (Chukwu et al., 2019). Race specific genes originated from wild relatives are frequently allied to genes conferring unwanted characters. Breaking this link and implement R genes into breeding programs is not easy but requires expertise. Complications linked with genomic characterization of unfamiliar genes comprise little physical resolution of cytogenetic methods (Lukaszewski et al., 2005) and restricted potential of simple sequence repeats (SSR), short tandem repeats (STR), and simple sequence length polymorphisms (SSLP) shifting to the tertiary gene pool also known as gene pool three (GP3) (Mullan et al., 2005). These issues constrained the utilization of these valuable sources for development of wheat. An extensively debated genetic engineering technique, though not used widely, is host induced gene silencing (HIGS) of important genes in the pathogen. This line of work comprises articulating small interfering RNAs in the host that may be transferred to the pathogen and induce silencing of genes imperative for pathogen virulence (Nunes and Dean, 2012).

However, the progress in genomic tactics and the availability of numerous genome sequences has permitted the quick access to genes in wild species. This has qualified the advance of gene-specific molecular markers for fast gene characterization through MAS. Markers support in pyramiding of APR or R genes or in assortments to evolve wheat cultivars resistant against rust with greater durability (Singh et al., 2014).

Gene pyramiding: an approach to achieve durable resistance

Pyramiding of numerous resistance genes improves durability of resistance against wheat rusts including leaf rust. Durable resistance may be attained by incorporation of many genes encoding partial resistance pyramiding because classical breeding is not suitable as it involves concurrent trials of the wheat breeding materials with diverse races of pathogen before a selection is completed. Typically, it is impossible for a systematic program of breeding to

continue all required tests for rust races desired for this effort (Khan et al., 2013). Hence, MAS is a suitable alternate to facilitate rapid development of rust-resistant cultivars. STS or SCAR and CAPS are useful markers available for identifying leaf rust resistance genes such as *Lr1, Lr9, Lr10, Lr19, Lr21, Lr24, Lr25, Lr28, Lr29, Lr34, Lr35, Lr37, Lr39, Lr47* and *Lr51* (Chelkowski and Stepian, 2001). Genes *Lr1*, *Lr9*, *Lr19*, *Lr24*, *Lr26* and *Lr34* were used for MAS of wheat genotypes and *Lr1*(31.7%), *Lr19*(1.7%), *Lr26*(20%) and *Lr34*(10%) were found in genotypes under study. Genes Lr9 and Lr24 were not recorded in any of the tested genotypes (Ren et al., 2018). Microsatellite SSR and AFLP markers for Lr genes for instance Lr3bg, Lr18, Lr40, Lr46 and Lr50 have been advanced (Purnhauser et al., 2000). Though enzymatic marker (endopeptidase *Ep-D1c*) for *Lr19* has been commercialized (Chelkowski and Stepian, 2001) even then sufficient work is needed to exploit complete conveniences of biotechnology in crop breeding and genetics to crop improvement against stresses.

Gene cloning for developing leaf rust resistance in wheat

Nucleotide binding site (NBS)-leucine-rich repeat (LRR) proteins, confer resistance against diseases in plants. Lr1 (Cloutier et al., 2007), Lr10 (Feuillet et al., 2003) and Lr21 (Huang et al., 2003) are genes in wheat characterized by arrangements that translate these specific proteins for leaf rust resistance in wheat have been cloned. Molecular representation of these genes delivers an exceptional biotic scheme to explore the molecular mechanisms of host pathogen communication in resistant gene, development and assortment. This will permit more handling of disease resistant genes to recover the durability of resistance by genetic amendment of wheat.

Diversity in *Puccinia triticina*

Leaf rust is a challenging disease because the pathogen exhibits high diversity, there is a continuous advent of novel virulence profiles and the pathogen displays flexibility to a broad array of environments (McCallum et al., 2016). *Puccinia triticina* is common with varied population structure and experiences rapid evolution to generate novel races virulent against resistant cultivars (Prasad et al., 2020). As deliberated, the rapid change of the pathogen can overtake the improvement in resistant genes deployed in cultivars. That is why scientific community could not attain the goal of cultivation of wheat in rust free situation (Afzal et al., 2020). Several research works published on race analysis in *P. triticina* reveal pathogen is diverse highly all over the world.; (Ali et al., 2020; Hussain et al., 2015; Kolmer, 2019; Gultyaeva et al., 2016; Kolmer et al., 2012). Utmost presently vital strains (pathotypes) have changed through mutations in prevailing populations or travelled from other, frequently unfamiliar, zones (Huerta-Espino et al., 2011). Mains and Jackson (1921) were pioneer who demonstrated physiologic specialization in *P. triticina* produced on 11 differential wheat hosts (Mains and Jackson, 1926). Three of the differentials were not continued ultimately (Johnston and Mains, 1932), and the lasting eight became known international as typical differentials (Mains and Jackson, 1926). In Australia a selection of Mediterranean used has Lr2a in addition Lr3a (Singh and McIntosh, 1985). 0–4 scale is used to designate infection types (Stakman et al., 1962).

The Fertile crescent region of the middle east is the center of origin of *Puccinia triticina*, where alternative hosts exist; nevertheless, the population of *Puccinia triticina* is clonal in most parts of the world (Bolton et al., 2008; Kolmer, 2005). Wheat varieties under cultivation in Australia possess genes Lr1, Lr3a, Lr13, Lr13+, Lr14a, Lr17a, Lr17b, Lr20, Lr23, Lr24, Lr26, Lr27, Lr31, Lr34, Lr37, and Lr46 confer resistance against leaf rust frequently in combination (2 or 3 genes) and a lot of cultivars possess Lr34 (Wellings et al., 2012). The matching genes with virulence against pathogen were also perceived in the pathogen population for the successions of genotypes released (Park et al., 2002). In Australia the leading pathotype population of wheat leaf rust is 104-2, 3, (6), (7),11 detected first in1984 in Victoria, subsequently it underwent mutations repeatedly to generate its clonal ancestries, and the other pathotype prevailing less recurrently with clonal ancestries is 122-1, (2),3, (6), (7),11 (Park, 2012).

Status of leaf rust in Pakistan

Asian Countries including Pakistan, which are top producers of the wheat of the world could face up to 70% yield damage attributed to leaf rust (Singh et al., 2004), In Pakistan, the disease remains a serious hazard to wheat cultivation in Northern and Central Punjab, where the environment makes the circumstances promising for this disease. Now leaf rust becoming serious threat in northern areas and KPK due to change in climatic conditions. In a study the surveillance of three years showed Punjab wheat production effect more with disease than other areas

(Khan et al., 2020). Leaf rust prevails in Pakistan, (Yamin et al., 2021) most consistent in the central and southern areas of the country responsible for severe damage in produce (Channa et al., 2021) can cause damage yield up to 40% in susceptible cultivars (Khan et al., 2013). There were many rust epidemics observed in Pakistan with different level of losses (Duveiller et al., 2007). Four major wheat rusts epidemics were recorded in Pakistan during 1978, 1994-95, 1997-98 and 2005 (Bahri et al., 2011). During 1978 leaf rust epidemic in early stages hit the mega cultivar Mexipak and triggered a huge loss of 10.1% of amounting US$86 million (Hassan et al., 1979).

In Pakistan, alternative hosts for pathogen are not recognized; hence it is dependent on the clonal urediniospores phase from year to year. Pathogen subsists on wheat during the summer in western region highlands and then spreads to the wheat-producing parts of Indus basin in provinces of Punjab and Sindh (Nagarajan and Joshi, 1985).

Surveillance of rust exploiting seedling differentials is very enlightening in describing topographical spreading of virulence pattern of P. triticina, their virulence variation and how phenotypes modify in response to selection of host. Near isogenic lines are greatly effective while differentiating virulence/ avirulence structure of the leaf rust pathogen population. These lines are employed for specific rust resistance genes studies (Kolmer and Liu, 2000) to enable deciding comparative incidence of pathotypes and virulence phenotypes (McIntosh et al., 1995). To prevent yield losses, the continuing development of resistant cultivars requires data of the recognition of novel races and varying virulence patterns of rust fungus. For the identification of resistance genes, continuous modeling of prediction, recurrent monitoring is shaped in country. For development of wheat cultivars with resistance against leaf rust, the field inspections have been effective resource for biological investigation of pathogen (Channa et al., 2021). Isolates obtained from surveys of virulence can be exploited for assessing the genetic differentiation of *Puccinia triticina* genotype by means of procedure of molecular markers. Virulence investigation is beneficial to diagnose dominant virulence phenotypes in the leading wheat producing regions, and to detect the virulence deviation of the pathogen, to explore the concentration and dispersal of new phenotypes and designate if genotypes of wheat with key resistance genes of leaf rust have had a discriminating effect on the pathogen population. Keeping the above objectives, research is carried out regularly to recognize virulence variation of leaf rust isolates from several portions of Pakistan. Single uredinial isolates are investigated on 24 near isogenic (Thatcher wheat) lines which fluctuate for single Lr resistance genes to designate races. Work conducted by Channa et al., 2021 is expressed here as an example. Collections of disease samples were conducted from farmers' fields from diverse agroecological sites (Badin, Sanghar, Larkana, Tandojam, and Sakrand) of Sindh province, during two years (2015 and 2016) with an objective to detect the diversity in virulence. Results of the work showed spores from two sites (Tandojam and Sakrand) were not viable and could not be restored and only urediniospores of three locations (Sanghar, Larkana and Badin) were revived. None of the pathotypes had virulence to Thatcher wheat lines with leaf rust resistance genes Lr23 and Lr42. Though Lr24, LrB, Lr10, Lr14b and Lr20 genes showed susceptibility response with all tested pathotypes. Based on virulence, ten virulence phenotypes (MSPTDS, MJLTGS, MNPSDS, RTPTPS, MDPSDS, JDBQGJ, PNDQDS, RKTRGS, RTSTNS, and MSCTNS) were recognized among the ten isolates, nominated with six-letter code. Two phenotypes RTPTPS & RTSTNS showed broad spectrum, both were virulent to 19 resistance genes of leaf rust while pathotype JDBQGJ had narrow spectrum as compared to all other tested, with virulence to just 8 resistance genes of leaf rust. Among the sites virulence variability of leaf rust was recorded also. Most of recognized races were virulent to more than one of leaf rust resistance genes. Resistance genes (Lr42 and Lr23) recognized as effective can be utilized to attain leaf rust resistance in wheat. Further, the investigation offers virulence profile of the area may help to manage the leaf rust pathogen.

The Wheat Productivity Enhancement Program aimed to boost the production of wheat in Pakistan by supporting research that led to the evolution of innovative disease-resistant ideal agronomical management of wheat varieties producing in abundance. The key objective of the project was to enable attempts of agricultural research institutes in Pakistan to restrain hostile effects of rusts in wheat—together with the immensely infectious Ug99 stem rust disease — through deployment of inherently resistant varieties. The administration of Pakistan's target to accomplish self-reliance in wheat production just became more realistic with the release of new varieties

of wheat (Subhani-2021, MH-2021, Dilkash-2021, Bhakkar-20 and MA-2020.). The varieties, were developed for different production environments in the Punjab province of Pakistan, drawn from germplasm from the International Maize and Wheat Improvement Center (CIMMYT). These new seeds could support the country's 8.8 million hectares of area under wheat cultivation turn out to be more productive, climate-resilient and disease-resistant — a welcome progress in an area where climate change circumstances impend continuous wheat production.

Many molecular studies have been conducted to identify resistant gene in cultivated and wild species to enhance the resistance of cultivated species against disease. The development of molecular markers for specific leaf rust genes permits the recognition of these genes autonomously of the phenotype. Molecular markers can be exploited in marker-assisted selection for an effective combination of genes in the pyramiding approach to produce a more durable resistance (Feuillet et al., 1995) For the purpose of genetic dissection scientist used molecular techniques like gene pyramiding and Marker assisted selection, markers are exploited to recognize sources of resistance (Naurin et al. 2021). Scientists succeeded in their efforts and identified many gene in wild and cultivated varieties of wheat (Inamullah et al., 2021; Ismail et al., 2021; Ali et al., 2016; Hussain et al., 2011). Use of these genes will be helpful in breeding programs for the development of resistant varieties and to make increase in production.

Conclusion

Wheat leaf rust, is one of the most damaging diseases of wheat have caused loss in yield during previous decades. Due to the fact that pathogen causing leaf rust in wheat is obligate in nature, this situation leads to evolution of novel race in response to deployment of cultivar with hypersensitive response. Awareness with virulence pattern of pathogen is crucial for each one engaged in wheat breeding for rust resistance. That is why crop is surveyed by experts regularly during the crop growth stage when there is opportunity of disease appearance, analysis of rust samples collected from diverse location is conducted under glasshouse conditions to monitor change in virulence pattern wherever research is conducted to develop varieties resistant against three rusts including leaf rust. Leaf rust virulence analyses is conducted in several labs all over the regions under wheat cultivation. Quite a lot of illustrations of the emergence of unusual races of unidentified source are attributed to frequent migration and mutation. Developing rust resistant genotypes need a continuous source of new and durable genetic resistance. Genetic diversity in host plays key role in crop improvement. Parents with diverse origin generate more desired output in crop improvement not only incorporating resistance against biotic stresses but against abiotic stresses as well. Enhancing, and stabilizing wheat yield through deployment of disease resistant cultivars is a multifaceted task and is not possible to address by a single technology or approach. Technology for instance speed breeding can aid to cultivate plants fast and accomplish several generations in short duration. A multidimensional technique is compulsory to assimilate the modern breeding skills to quickly detect new resistance factors lying concealed in collections of seed bank. This help widen the genetic base of contemporary wheat germplasm. Slow rusting varieties have been durable; however, this type of resistance is multigenic. Techniques in biotechnology are used to accelerate breeding wheat for durable resistance. Molecular markers are useful tools used in identification of diversity in host as well as pathogen and generate concise data. Gene pyramiding and cloning are techniques being used successfully to hasten the process of crop improvement against biotic stresses including leaf rust. Similarly, using of the modern genotyping platforms, could lead to the fast exposure of innovative genomic regions at the bottom of leaf rust resistance. Such tools sanction plant breeders to stay one step forward of the quickly developing pathogen.

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Contribution of Authors

Ijaz M: Reviewed and improved the article
Afzal A: Significantly promoted to the commencement and plan of the article and understanding the pertinent literature
Shabbir G: Reviewed and improved the article
Iqbal J: Drafted and remarked the work.
Rafique M: Supervised the project. Author and coauthors contributed fully developing manuscript in present arrangement.