

### **Exploitation of Novel Protocol for R-gene Transfer in Common Bread Wheat (*Triticum aestivum* L.)**

#### **Abstract**

Bread wheat (*Triticum aestivum* L.) is the second most important staple crop suffering from various fungal, bacterial and viral diseases leading to a decrease in yield and productivity of the crop. Breeders have found r gene responsible for developing resistance in the crop against these pathogens. This review attempts to describe the transfer of alien resistant gene (r gene) in wheat and also the techniques involved in it. R gene is covered in details with gene for gene hypothesis and the five classes of r gene. Different approaches both traditional ones, such backcross, pedigree, and recurrent selection have been discussed, as well as modern ones like mutation breeding, somaclonal variation, and genetic engineering, which address both genetic gain and genetic loss. The transfer of r gene for resistance to specifically powdery mildew (caused by *Erysiphe graminis*), karnal bunt (caused by *Tilletia indica*) and rust (Leaf rust, *Puccinia triticina*; Stem rust, *Puccinia graminis*; Strip rust, *Puccinia striiformis*) are outlined. Finally, we made an effort to discuss about possible future advances in R-gene transfer.

**Keywords:** R-gene, Diseases, Resistance, Novel protocol, *Triticum aestivum* L.

#### **Introduction**

Bread wheat (*Triticum aestivum* L.) have its place in tribe Triticeae of family Poaceae and it has been originated from Middle-East region of Asia. It has a genomic constitution of  $2n = 6x = 42$  hence it is an autogamous, allo-hexaploid species and it had three genomes involved in its evolution, named as A, B and D (AABBDD). It is the combination of the genomes of three diploid ancestrals, *Triticum urartu* ( $2n = 14$ , AA), *Aegilops* species ( $2n = 14$ , BB) and *Aegilops squarrosa* ( $2n = 14$ , DD) (Habtmu Seboka *et al.* 2009). It is one of the three main cereal crops and every year about 600 million tonnes of wheat is harvested (P. R. Shewry 2009). Wheat is rich in calories, proteins (glutens), vitamins, minerals and dietary fibers that helps in the prevention and treatment of some digestive disorders. The main constituents of wheat endosperm are starch (60-75%), proteins (6-20%), moisture (~10%), and lipids (1.5-2) (Surovy *et al.* 2020). It may contain a number of healthy antioxidants like phenolic acid (ferulic and vanillic acids), carotenoids, tocopherol, alkylresorcinols and lignans (Arshad *et al.* 2017). The phenolic acid helps in promoting anti-inflammation capacity of human beings (Kumar *et al.* 2019). Alkylresorcinols helps in inhibiting enzymatic activities, preventing bacterial and fungal infection, reducing cholesterol and promoting gut health and reducing the risk of colon cancer (Tian *et al.* 2020). On the other hand, lignan lowers the risk of heart disease, breast cancer, osteoporosis and menopausal symptoms (Rodríguez-García *et al.* 2019).

The production and productivity of wheat in India noted to be approximately 107.6 million tonnes and 3.4 tonnes per hectare and it occupies an area of about 31.5 million hectares. (Statista). The biggest grower of wheat in the nation is Uttar Pradesh. The area and production of wheat in this state is about 9.2 million hectare and 24.5 million tonnes,

respectively and productivity of 2.7 tonnes per hectare (Singh *et al.* 2020). Punjab is 3<sup>rd</sup> largest wheat producing state and the production of wheat in this state is 111.3 million tonne.

Wheat is affected by several biotic and abiotic stresses and physiological diseases are mostly the reason of the loss of yield and quality in the crop. It is affected by several biotrophic fungi, necrophytic species and nematodes, along with viruses and bacteria. Out of these, different fungal diseases like rusts, powdery mildew, Karnal bunt, loose smut are the most prominent and serves as a great challenge to wheat production (Simon *et al.* 2021). USDA had reported an average yield loss of 40 percent because of the fungal disease in wheat.

There is an urgent need to take some action for the reduction of disease problem in wheat. Many conventional and non-conventional techniques are applied till now. The most common among all is R-gene transfer technology. Resistance genes (R-genes) are the genes present in plant genome that is responsible for plant disease resistance against pathogen by the production of R proteins. These R-genes encodes protein that helps in detecting the pathogen. These genes have been used in breeding from earlier times with varying degree of success. There are a number of mechanisms to convey resistance in plants including 1) Interaction of R protein directly with an Avr gene (Avirulence gene) which is the product of a pathogen according to Gene-for-Gene hypothesis. 2) Guard Hypothesis that mentions another protein that is protected by an Avr gene degrading R protein. 3) Detection of a Pathogen Associated Molecular Pattern or PAMP (also known as MAMP for microbe associated molecular pattern) by R protein and 4) Encoding of an enzyme by R protein that degrades the toxin produced by the pathogen. Here the information about R-gene, their transfer, and coding genes etc. are reviewed (Jatwa *et al.* 2017).

### **R genes and gene for gene hypothesis**

Harold Florin early 1940s with his work on flax rust fungus given the concept of plant pathogen interactions which is commonly known as gene for gene hypothesis. This particular concept is built on the fact that the plant disease resistance require two complementary gene: an avirulence (Avr) gene in the pathogen and a matching resistance gene in the host. At the time of pathogen infections of plant, in the lack of corresponding R proteins, Avr products show its virulent factor, destroying host cellular functions through interaction with plant encoded pathogenicity target. Against the infection, plant produce R proteins that sense Avr products specifically. This detection of Avr product lead to the activation of host defence which results in calcium fluxes, localized plant cell death, and generation of superoxide and nitric oxide (Erik *et al.* 1998). In twentieth century, plant breeders noticed that resistance in plant are regularly inherited as dominant trait. Many plant breeding programmes were developed for the identification of resistant sources in wild relatives of crop plants and then for finding the matching resistance R gene for agricultural benefits. Afterwards boom and bust cycle was observed. The boom and bust cycle isreferred as a widespread use of single resistance gene for the protection of multiple varieties of a grain from a disease. Many varieties become susceptible (bust) simultaneously when the disease overcomes this resistance gene (Rosewarne 2014).

### **Classes of “r” gene**

R genes are mainly divided into 5 classes on the basis of structural characteristics of their protein product. The first class have no leucine rich repeat (LRR) and is known to code for

serine or threonine kinase with the influence of Pto gene who is the only known member. The second class is known to code for receptor like protein with an extracellular LRR domain and a transmembrane domain. This class members involves cf gene products which is mainly found in solanaceous species and mainly codes for leaf mold rust resistance and for nematode resistance HS1 gene is involved. The third class mainly codes for receptor-like kinase along with an extracellular LRR, an intracellular protein kinase domain and a membrane-spanning region. The fourth class involves a majority of R genes that are known as the nucleotide binding-site leucine rich repeat (NBS-LRR) resistance genes. They are abundantly found in plants. Almost 200 NBS-LRR were found in Arabidopsis. NB domain generally binds with either ATP/ADP or GTP/GDP and the LRR domain often takes part in protein-protein interactions as well as binding of ligand. Depending on the structural features of the N-terminus, these NBS-LRR proteins can be further classified into TIR subclass which resembles intracellular signalling domains of drosophila and mammalian IL-1 receptors, and non-TIR subclass, having a coiled-coil (CC) domain mostly (Guet. *al.* 2014). The leucine-rich repeats (LRR) containing domain is present in many proteins associated with innate immunity in plants which serves as a first line of defence. This starts by sensing of pathogen-associated molecular patterns (PAMPs). Protein products of avirulence (AVR) gene is recognised by NBS (nucleotide-binding site)–LRR protein in plants. LRR domains mainly have a horsetail shape, with the concave face comprising of parallel  $\beta$ -strand and a convex face showing a more variable region of secondary structure including helices (Ng *etal.* 2011). In wheat NBS-LRR gene helped in cloning of functional resistance gene (Guet *al.* 2015). The NBS domain which is also called as NB domain are the proteins containing a block of sequence conserved in plant and animals. The last class of r gene have entirely different structure and thus cannot be fitted in any of the class. Powdery mildew resistance gene, RPW8, in Arabidopsis lies within this class. These are known to code for small proteins including only an amino-terminal transmembrane domain and a coiled coil domain. It unusually confers a wide spectrum of resistance. Classes 1 and 4 R genes are found to be partially or completely intracellular and recognises intracellular ligands. While class 2 and 3 R genes recognise due to the presence of transmembrane domain recognises extracellular domain. In the class 2,3 and 4 the LRR domain is mainly the reason for the recognition of ligands derived from the pathogen.

## **Method of forming resistant variety**

### **A. Conventional Methods**

**Backcrossing:** Backcrossing is a scheme where transfer of a characteristics or trait, a gene, chromosome segment or an anonymous gene from donor parent to genomic background of recurrent parent is done. The progeny in the successive generation are selected for the gene of interest and backcrossed to the recurrent parent to ensure the share of genome from the donor parent to be zero as generation accumulate excluding the part hosting for the gene of interest (Hospital, F. 2005). Backcrossing is a very beneficial tool for the transfer of disease resistance from resistant crop to a high yielding crop.

**Pedigree Breeding:** The method of breeding for genetic improvement of self-pollinated species in which genotypes which are superior are selected from a segregating generation and in each generation a proper record of the ancestry is also maintained. (Begna, T. (2021))

**Recurrent Selection:** The method was given by Comstock *et al.* 1949. It stands for successive cycle of selection and recombination to get elite line from the improved population. Recurrent selection was mainly developed for outcrossing crop species but it is applied to self-pollinated crops for some years (Goldringer *et al.* 1998).

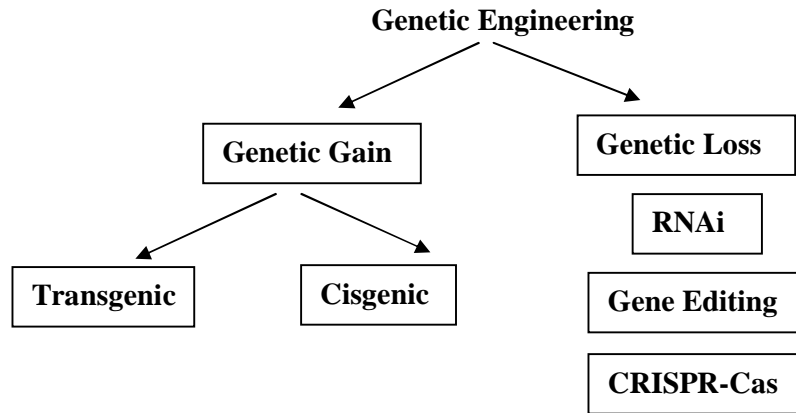
## B. Modern methods

**Mutation Breeding:** Mutation can be defined as a sudden heritable change in the genomic constituent of an organism. They may be spontaneous that is naturally occurring or induced, caused by physical or chemical mutagens. Mutation breeding is the use of mutation for crop improvement. This breeding method unlike other methods like selection and hybridization improve defect in an elite cultivar without sacrificing its agronomic and quality characters. This method also plays a significant role in disease resistance. Mutagenic crop improved for disease resistance involves rice, wheat, bean, green pea etc. Recently in 2017, development of Niab Kinnow mutant variety of *Citrus reticulata* from budwoods of local kinnow irradiated at 20 Gy of gamma rays was reported. Also in 2004, *Oryza sativa* variety Wonchu mutant has been developed from irradiated seeds of 250 Gy of gamma rays (Danish and Raina 2018).

**Somaclonal Variation:** Somaclonal variations are the genetic and epigenetic changes induced in vitro between clonal regenerants and their respective donor plants. Somaclones are tissue culture derived plants. These variations are mainly induced by point mutation, alternation in number and structure of chromosome, methylation of DNA sequences etc. These variations are found to be useful for crop improvement. There are two main approaches towards isolation of somaclonal variations (i) Screening that involves only observation of a large number of cells for the detection of variant individuals, (ii) Cell selection which involves application of suitable selection pressure that permits survival of variant only. These selection pressures include exposure to phytopathotoxin, pathogen-wall material or secreted elicitors which help in obtaining disease resistant somatic variant for crop plants. By gradually increasing concentration of culture filtrate or phytotoxin the culture is selected in such selection method. (Pérez-Clemente *et al.* 2012)

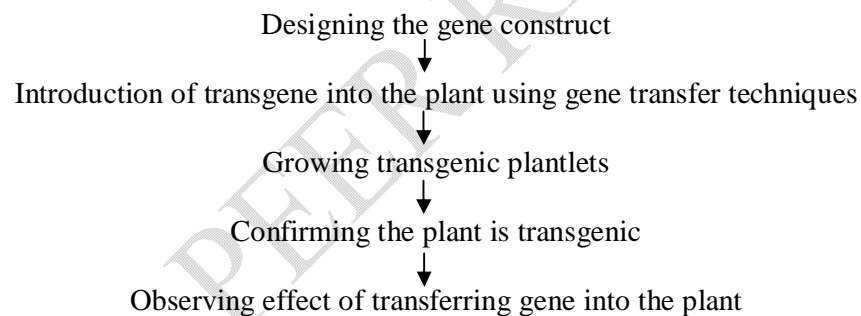
**Genetic Engineering:** Genetic engineering can be defined as the use of biotechnology for altering an organism's genetic material. It is better than conventional methods as it causes lesser amount of undesirable changes and hence we get a crop with desired agronomic traits in fewer generations. Also it allows interspecific gene transfer so that exploitation of genetic material would not be limited. Plant transformation leads to introduction of new genes in vegetatively propagated crops. All these features contribute in making genetic engineering a powerful tool for disease resistance.

Fig .1 Division of Genetic Engineering.



**Genetic Gain:** Genetic gain is the transfer of specific gene of interest from one species to other or within species which leads to gain of a foreign gene by the species to which it is transferred. It is widely used for creating resistant plant species. This technique includes transgenic and cisgenesis.

- a) **Transgenics-** These are the organisms whose genomic constitution has been altered through the introduction of one or more foreign DNA sequences from another species by using genetic engineering processes. Process of forming transgenic plant is as followed



- b) **Cisgenesis-** It is the process of alteration of the recipient genetic background with the help of a naturally derived gene from a cross compatible species in addition to introns and its relative promoters and terminator flanked in the normal sense orientation. The final cisgenic plant would be deprived of any kind of foreign DNA or selection marker and vector backbone sequence. It doesn't cause change in gene pool of the target plant but add any supplementary characters (Telem *et al.* 2013).

**Genetic Loss:** The process of creating disease resistance in a plant species by the loss gene causing susceptibility. Gene loss is a two steps process a) mutation that leads to a pseudogene and b) the deletion of the pseudogene.

- a) **RNAi-** RNA interference plays an important role in mounting defence against viral infection in plants. By the use RNAi pathway, the long double stranded RNA are transformed into small interfering RNAs (SiRNA) that precisely binds and cleaves the targeted viral mRNA in the cytosol that results to an effective protection of plant. RNAi can be activated by external application of dsRNAs in plants by the help of dsRNA injection or by development of transgenic plants which express dsRNAs targeting pathogens.

b) **Genome editing and CRISPR-Cas-** Genome editing is mainly group of technologies used to change an organism's DNA. This is used for addition, removal and alteration at particular location in a genome. Many genome editing techniques are developed so far and one of them is CRISPR-Cas. It is bacterially derived clustered regularly interspaced short palindromic repeats technology that is proven to be a one of the best approach for engineering resistance against plant viruses. It acts as antiviral defence machinery in many bacterial species. The process includes cleavage of a specific target site on the substrate viral DNA or RNA by an RNA guided nuclease (Cas protein) that leads to their degradation. Bacteria when infected by viruses, it captures small pieces of viral DNA in a specific pattern known as CRISPR array that help the bacteria in remembering that virus so that when the virus attack again, RNA segment will be produced by the bacteria that will attach to the specific site of the virus DNA and cut the DNA apart disabling the virus. A small piece of RNA with a short guide sequence in a cell's DNA that attaches to specific site same as the bacterial one was produced by the researchers. This guide DNA attaches to Cas9 and after introduction into the cell guide DNA recognises the specific sequence and Cas9 cut the DNA at that specific target site. Some examples are the RNA-guided endonuclease Cas9 from *Streptococcus pyogenes* (SpCas9), RNA-guided RNases Cas13a from *Leptotrichia shahii* (LshCas13a) etc. It act as a molecular scissors that creates breaks on sequence specific targets in the DNA or RNA of the substrate that make it useful tool for genetic engineering of antiviral defence. (Dong *et al.* 2019)

1. **Gene Pyramiding-** The term was introduced first by Watson and Singh in 1953. It is a method of stacking more than one gene within a single genotype to get a desired combination of traits with the help of recombinant DNA technology or the conventional breeding. Conventional technique involves pedigree breeding, backcross breeding and recurrent selection.
2. **Marker assisted selection:** It is a process of incorporation of valuable traits into new cultivar with the help of molecular marker or DNA tags which remains attached to the gene of interest. Madsen, a soft winter wheat cultivar was one of the first wheat cultivar being developed by MAS which was released in the year 1986 (Malav *et al.* 2016). The process involves two steps – (1) pedigree, in which all target genotype is accumulated in one called root genotype, and (2) fixation step, that include getting an ideal genotype from one single genotype. There is an improved method called marker assisted backcrossing in which target allele is transferred to a popular cultivar from donor variety by repetitive process of backcrossing.

### **R gene in wheat**

The diseases in wheat are caused by fungal pathogens, few from the bacteria, virus and nematodes. This is major threat to productivity and yield of wheat. Therefore, the introduction of durable disease resistance was a significant challenge for breeders. However there were only 10 resistance gene recognised in bread wheat and its relatives. Most of them confers resistance to powdery mildew, stripe rust, and leaf rust. It was also found by Gu *et al.* 2015, that in domestic species and their progenitors, for analysing dynamic process of R gene, AABBDD- a freshly published genome sequences of bread wheat and its two ancestors, *Triticum urartu* (AA), and *Aegilops tauschii* (DD) are a decent resource.

### **Resistance to powdery mildew**

Powdery mildew is a most important fungal disease in wheat caused by the pathogen *Erysiphe graminis*. The disease is characterised by occurrence of fluffy, white powdery

growths of fungal spores upon leaf surface, awns, and glumes. Mature infection appears as grey or brown in colour along with black speckles. It causes a heavy yield loss of almost 40 percent and also hampers the growth and development. In the previous years, It has been observed that it can inflict as higher as five percent of loss in national yield per year in U.K. to overcome these losses, plant breeders have taken many steps that are as Reader & Miller (1991) observed that there is only a single gene Pm16 located at chromosome 4A which was resistant to powdery mildew in *Triticum dicoccoides*. It was observed that plants which were homozygous for Pm16 was free from symptoms while the plants which were heterozygous exhibited chlorosis in similar condition. Thus they concluded that the Pm16 gene was a most important source of resistance to wheat powdery mildew. Huanhuan *et al.* (2020) observed that *Aegilops longissimi* which is one of the wild relative of wheat is a good source of resistance to powdery mildew. Chromosome 4S from *A. Longissimi* was found to express a moderate resistance. They with the help of RNA sequencing, molecular marker, and in situ hybridization showed that among 16 Bgt isolates that were collected from different region of China, TA3465 was found resistant to 10. Whereas CS was found susceptible to all those Bgt isolates. Further the powdery mildew resistance in TA3465 was mapped to the short arm of 4S and was named as Pm66. Shi *et. al.* (1997) observed a new gene named as Pm25 for wheat powdery mildew resistance in NC96BGTA5 (germplasm line). Linked with the gene was three amplified polymorphic DNA marker, OPX061050, OPAG04950, and OPAI14600. They transferred this major gene for resistance to powdery mildew from wild einkorn wheat (*Triticum monococcum*) to hexaploid common wheat (*Triticum aestivum*). The new that was Pm25 was observed to be linked with Pm3a gene based on f<sub>2</sub> and BC<sub>1</sub>F<sub>1</sub> population.

### **Resistance to Karnal Bunt**

Karnal Bunt is a very harmful fungal disease in wheat caused by the smut fungus *Tilletia indica* and it was firstly discovered at the botanical Research station Karnal, Haryana, in Northwest India. For early times, it was a minor disease only found in Northwest India but during the year 1969-70, it get unusually spread in Northwest India and between 1974-75, it got distributed in whole Northern India from West Bengal to Western Borders (Joshi *et al.* 1983). The disease has been stated in various countries like Pakistan, Afghanistan, Nepal etc. It is a seed, soil, and air borne disease. The damage caused by the disease includes yield reduction, occurrence of fishy odour and taste to wheat flour and reduction in quality of the grains. Common symptoms are blackened area surrounding the base of the grain which is seen in grains are threshed and kernels are exposed.

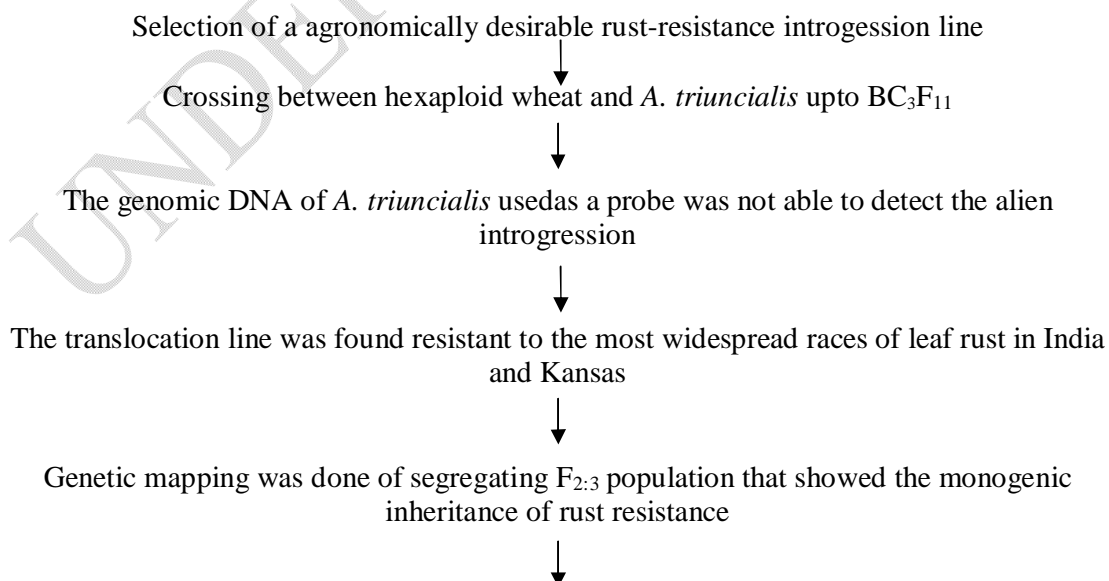
Villareal *et al.* (1944) observed that according to the mean KB score, SH wheat have shown high resistance to immune reaction to *Tilletia indica* due to resistance of their parents *T. turgidum* and *T. tauschii*. SH has shown 55.9% less mean KB infection than *T. aestivum* check cultivar “WL711”. They have taken four *Triticum turgidum* and nine *Triticum tauschii* parents to be evaluated for Karnal bunt resistance during 3 crop seasons. Ten tillers of each SH were taken arbitrary and injected with the suspension of sporidia in water. The spikes were individually threshed after maturity and checked for percent KB infection. According the mean KB score of all three seasons, 49% SH was immune to KB. Warham *et al.* (2009) observed 0-60% infection of karnal bunt infection *Aegilops* species over a severity scale from 0-5. These result show possible source of resistance in *Aegilops* when compared to 78% and 98% infection in the susceptible variety of *Triticum aestivum* vs. WL711 and Sonalika. The process was done by screening eighty six accessions of 21 *Aegilops* species under greenhouse condition. Mujeeb-Kazi *et al.* (2006) observed that only two of the ninety five elite synthetic hexaploid derived from the crossing of *T. turgidum* and *A. tauschii* had shown higher than 5.0% of infection over 3 years of testing and in 4<sup>th</sup> year testing, 53 synthetic hexaploids had

shown immunity that is 0% infection. They also observed a less than 3.0% infection in some advanced derivatives from the crossing of immune SH and susceptible bread wheat.

### Resistance to Rust

Rust disease is the utmost economically significant disease in wheat is fungal caused infection. The pathogen involved in of genus *Puccinia*. The disease has the capacity to cover long distances by windborne spores and mature easily within an optimum weather conditions. Rust is of three types in wheat- leaf, stem and stripe rust. 1. Leaf rust is caused by the pathogen *Puccinia triticina* and it cause symptoms like dusty, reddish-orange to reddish-brown fruiting bodies that is seen on the leaf surface. Several spores are produced by these lesions that are present on upper leaf surface. 2. Stem rust is caused by the pathogen *Puccinia graminis* which cause symptoms like raised spots (pustules) on stems and leaf sheath, sometimes on awns, glumes and seeds too. 3. Stripe rust is caused by *Puccinia striiformis* and cause symptoms like yellow or orange blister like pustules arranged in strips. Massive amount of spores are produced by lesions that are mostly dispersed by wind. This disease cause a great amount of grain losses specially virulent African strain Ug99. According to Ellis *et al.* (2014) there are two ways to control this rust disease- the first one is chemical control and the second one is genetic resistance. Genetic control has environmental and economic advantages so here are some of the works done on rust resistance:

Marasis *et al.* (2005) introduced *Triticum dicoccoides* by linking leaf rust and stripe rust resistant genes to protect common wheat seedling from a different pathogens of the mentioned pathotype. They had mapped the genes chromosomally through monosomic and telosomic analyses, RFLPs and C-banding. They have found an introgressed region at wheat chromosome arm 6BS. Due to reduced homology, introgressed region doesn't pair with Chinese spring 6BS arm at the time of meiosis but paired with 6BS of W84-17 and Avocet S. pollen transmission was strong in introgression region whereas varied with genetic background in egg cells. A normal phenotype was shown by homozygous resistant plants. The genes were named as Lr53 and Yr53. Kuraparthi *et al.* (2007) used molecular marker to detect a cryptic introgression with leaf rust resistant gene by transferring gene into *Triticum aestivum* (common wheat) from *Aegilops triuncialis* L. Procedure they followed include



Polymorphic alleles between resistance and susceptible bulks were found by the help of RFLP homologous group 2 markers XksuF11, XksuH16, and Xbg123



The co-segregation of *A. triuncialis* specific allele of XksuH16, XksuF11, Xbg123, and one simple sequence repeat marker Xcfd50 with rust resistance that suggested translocation of *A. triuncialis* occurred at distal region of 2BL chromosome arm



This suggested that this leaf rust resistant gene is new and was named as Lr58

Olson *et al.* (2013) had transferred stem rust resistant gene from *A. tauschii* to *T. aestivum*. They crossed TTKSK resistant *A. tauschii* (TA1662 and PI 603225) as male and stem rust susceptible *T. aestivum* (KS05HW14) as female. They got F<sub>1</sub> plant by the means of embryo rescue and then they performed backcross up to BC<sub>2</sub>F<sub>1</sub> genotype and then by the use of SSR and EST-STS markers found that on chromosome 1DS arm, stem rust resistance of both assents were located. By the help of allelism test, they found that stem rust resistance gene were transferred from PI603225 is Sr33. The gene transferred from TA1662 was found unique and designated as SrTA1662.

Sharma *et al.* (2021) performed gene pyramiding for the transfer of strip rust and leaf rust resistance in a wheat variety PBW343. At first they transferred leaf rust resistant genes Lr24 and Lr28 to PBW343. Then the marker assisted stripe rust resistant gene Yr5, Yr10, Yr15, Yr17, and Yr70 was introgressed into the variety having leaf rust resistant as the base for pyramiding. The genes from alien segment, *Aegilops ventricosa* (Lr37/Yr17/Sr38) and *Aegilops umbellulata* (Lr76/Yr70) was introgressed in PBW343. Finally by modified marker assisted backcross breeding, PBW723 was obtained and which was observed to have 81.57% of genetic background and it was released.

## Future Advances

Future success of the wheat breeding depends on the innovations and strategies based on classical and modern technology applications. Recent improvements in gene editing have enabled the production of targeted mutation in every copy of genome. Except some of the cases reported in this and other reviews, many more techniques are available and can be found. Some of these powerful techniques available are mutagenesis, improved transgenic technologies, high-quality genome sequencing, extended genetic mapping and advanced gene editing techniques. Mutagenesis and genome editing should be used in combination for the mutation of any of the wheat genome followed by the application of other approaches for revealing the biological function of mutated gene. For the advancement of wheat biology, strategies and techniques are on a accurate place. The entry of bioinformatics will significantly promote wheat functional genomics. Many more of agronomically valuable and beneficial genes which are good source for molecular breeding can be isolated followed by their characterisation by the application of new technologies and strategies for functional genomic analysis of model organisms. For the creation of elite wheat varieties, rational design-based molecular breeding is a promising approach and that will encourage future global food security.

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