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

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### Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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**Review Article** 

### 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 as 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.

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# **1. INTRODUCTION**

Bread wheat (Triticum aestivum L.) has 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 = 42hence it is an autogamous, allohexaploid 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) (Habtamu 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 [1]. The phenolic acid helps in promoting anti-inflammation capacity of human beings [2]. Alkylresorcinols helps in inhibiting enzymatic activities, preventing bacterial and fungal infection, reducing cholesterol and promoting gut health and reducing the risk of colon cancer [3]. On the other hand, lignan lowers the risk of heart disease, breast cancer, osteoporosis and menopausal symptoms [4].

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 tonnes.

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 biotic and abiotic stresses and physiological diseases. Out of these, fungal diseases such as rusts, powdery mildew, karnal bunt, and loose smut pose a significant challenge to wheat production, making them the most prevalent and concerning issues in this regard (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 Genefor-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 [5].

### 1.1 R Genes and Gene for Gene Hypothesis

In the early 1940s, Harold Florin introduced the concept of plant pathogen interactions through his research on flax rust fungus which is widely recognized as the gene-for-gene hypothesis. This particular concept is built on the fact that the plant disease resistance requires 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 led 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. programmes plant breedina Manv 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 is referred 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 aene (Rosewarne 2014).

### 1.2 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 involves cf gene products which is mainly found in solanaceous species and mainly codes for leaf mould 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 bindingsite 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 [6]. The leucinerich 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 β-

strand and a convex face showing a more variable region of secondary structure including helices [7]. In wheat NBS-LRR gene helped in cloning of functional resistance gene [6]. 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 aminoterminal 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.

# 2. METHOD OF FORMING RESISTANT VARIETY

### 2.1 Conventional Methods

**Backcrossing:** Backcrossing is a method used to transfer specific traits, such as genes, chromosome segments, or anonymous genes, from a donor parent to the genomic background of a recurrent parent. 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 [8]. 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 [9].

**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 [10].

### 2.2 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 Gv of gamma ravs was reported. Also in 2004, Oryza sativa variety Wonchu mutant has been developed from irradiated seeds of 250 Gy of gamma rays [11].

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 have been identified as valuable tools for crop improvement. There are two main steps towards isolaton of somaclonal variationts (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 pressure include exposure to phytopathotoxin, pathogen-wall material or secreated 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 [12].

**Genetic Engineering:** Genetic engineering can be defined as the use of biotechnology for altering an organism genetic material. It is better than conventional methods as it cause lesser number of undesirable changes and hence we get a crop with desired agronomic traits in fewer generation. Also, it allows interspecific gene transfer so than 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.

**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.

**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: Chart 1.

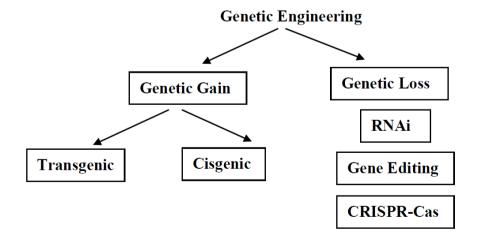


Fig. 1. Division of genetic engineering

### Designing the gene construct

Introduction of transgene into the plant using gene transfer techniques

# Growing transgenic plantlets

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Confirming the plant is transgenic

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Observing effect of transferring gene into the plant

### Chart 1. Process of forming transgenic plant

**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 [13].

**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.

**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 is 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.

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 approaches 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 acts 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 [14].

**Gene Pyramiding:** The term was introduced first by Watson and Singh in 1953. It is a method of staking 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.

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 [15]. 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 varietv by repetitive process of backcrossing.

# 3. 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 vield of wheat. Therefore, the introduction of durable disease resistance was a significant challenge for breeders [16-20]. 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. that in domestic species and their [6]. 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.

# 3.1 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 [21-24]. Mature infection appears as grev 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 [25] 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 relatives 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 exhibited resistance to 10 isolates of Bgt, while CS was susceptible to all of those isolates. Further the powdery mildew resistance in TA3465 was mapped to the short arm of 4S and was named as Pm66. Shi et al. [26] observed a new gene named as Pm25 for powdery mildew resistance wheat in NC96BGTA5 (germplasm line). Linked with the gene was three amplified polymorphic DNA OPX061050, OPAG04950, marker. 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  $f_2$  and BC<sub>1</sub>F<sub>1</sub> population.

# 3.2 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 gets unusually spread in Northwest India and between 1974-75, it got distributed in whole Northern India from West Bengal to Western Borders [27]. 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. [28] observed that according to the mean KB score, SH wheat has 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. [29] 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 aestivumc vs. WL711 and Sonalika. The process was done by screening eighty six accessions of 21 Aegilops species under greenhouse condition. Mujeeb-Kazi et al. [30] 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.

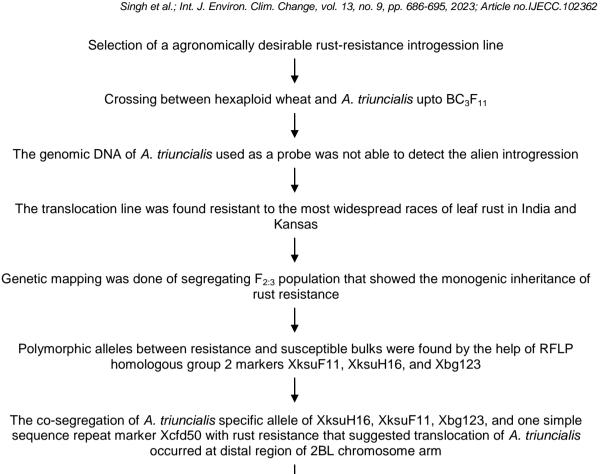
### 3.3 Resistance to Rust

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 condition [31-34]. 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 reddishbrown 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 number of spores are produced by lesions that are mostly dispersed by wind. This disease causes a great number of grain losses especially virulent African strain Ug99. According to Ellis et al. [35] 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. [36] 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, introgessed 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 strona 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. Kuraparthy et al. [37] used molecular marker to detect a cryptic introgression with leaf rust resistant gene by transferring gene into aestivum (common wheat) Triticum from Aegilops triuncialis L. Procedure they followed include.

Olson et al. [38] 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  $F_1$  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. [39] 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.



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

# Chart 2. Procedure followed by Kuraparthy et al. [37] for transferring gene into *Triticum aestivum* (common wheat) from *Aegilops triuncialis* L

### 4. 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 an 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 rational design-based varieties. molecular breeding is a promising approach and that will encourage future global food security.

### **5. CONCLUSION**

The study focuses on the exploitation different protocols for transferring R-genes in common bread wheat (*Triticum aestivum* L.) to enhance the crop's resistance against various pathogens, including fungal, bacterial, and viral diseases, which significantly impact yield and productivity. The protocol discussed in this study provides a valuable approach for introducing R-genes into bread wheat through different techniques, such as backcrossing, pedigree selection, and genetic engineering. By successfully transferring Rgenes, breeders and researchers can contribute to the development of disease-resistant wheat varieties, addressing the challenges faced by this important staple crop. Continued advancements in R-gene transfer protocols hold promising potential for improving the resistance of common bread wheat to pathogens and ensuring sustainable agricultural productivity in the future.

### **COMPETING INTERESTS**

Authors have declared that no competing interests exist.

# REFERENCES

- 1. Arshad MS, Joong-Ho K, Faqir MA, Muhammad S, Farhan S, Muhammad I, Zaid A, Muhammad N, Shahzad H. Wheat antioxidants, their role in bakery industry, and health perspective. Wheat Improvement, Management and Utilization; Wanyera, R., Ed.; IntechOpen: London, UK. 2017;24:365-81.
- Kumar N, Goel N. Phenolic acids: Natural versatile molecules with promising therapeutic applications. Biotechnology Reports. 2019;24:e00370.
- 3. Tian S, Sun Y, Chen Z, Zhao R. Bioavailability and bioactivity of alkylresorcinols from different cereal products. Journal of Food Quality. 2020; 2020:1-6.
- Rodríguez-García C, Sánchez-Quesada C, Toledo E, Delgado-Rodríguez M, Gaforio JJ. Naturally lignan-rich foods: A dietary tool for health promotion?. Molecules. 2019;24(5):917.
- Jatwa TK, Sharma M, Malav AK. R gene and its role in disease managements in plants. International Journal of Current Research in Biosciences and Plant Biology. 2017;4(5):61-4.
- Gu L, Si W, Zhao L, Yang S, Zhang X. Dynamic evolution of NBS–LRR genes in bread wheat and its progenitors. Molecular Genetics and Genomics. 2015;290:727-38.
- 7. Ng A, Xavier RJ. Leucine-rich repeat (LRR) proteins: integrators of pattern recognition and signaling in immunity. Autophagy. 2011;7(9):1082-4.
- 8. Hospital F. Selection in backcross programmes. Philosophical Transactions of the Royal Society B: Biological Sciences. 2005;360(1459):1503-11.

- 9. Begna T. Conventional Breeding Methods Widely used to Improve Self-Pollinated Crops. 2021.
- Goldringer I, Paillard S, Enjalbert J, David JL, Brabant P. Divergent evolution of wheat populations conducted under recurrent selection and dynamic management. Agronomie. 1998;18(5-6): 413-25.
- 11. Raina A, Danish M. Mutagenesis in plant breeding for disease and pathogen resistance. Agric Res Technol. 2018; 13(1):1-2.
- 12. Pérez-Clemente RM, Gómez-Cadenas A. In vitro tissue culture, a tool for the study and breeding of plants subjected to abiotic stress conditions. Recent advances in plant *in vitro* culture. 2012;91-108.
- S Telem R, Wani H, B Singh N, Nandini R, Sadhukhan R, Bhattacharya S, Mandal N. Cisgenics-a sustainable approach for crop improvement. Current genomics. 2013; 14(7):468-76.
- Dong OX, Ronald PC. Genetic engineering for disease resistance in plants: Recent progress and future perspectives. Plant physiology. 2019;180(1):26-38.
- Malav AK, Chandrawat I, Chandrawat KS. Gene pyramiding: An overview. Int J Curr Res Biosci Plant Biol. 2016;3(7): 22-8.
- 16. Li J, Yang J, Li Y, Ma L. Current strategies and advances in wheat biology. The Crop Journal. 2020;8(6):879-91.
- McDowell JM, Woffenden BJ. Plant disease resistance genes: Recent insights and potential applications. TRENDS in Biotechnology. 2003;21(4):178-83.
- Multani D, Dhaliwal HS, Singh P, Gill KS. Synthetic Amphiploids of Wheet as a ource of Resistance to Karnal Bunt (*Neovossia Indica*). Plant Breeding. 1988;101(2):122-5.
- Sharma I, Bains NS, Nanda GS. Inheritance of Karnal bunt-free trait in bread wheat. Plant breeding. 2004;123(1): 96-7.
- 20. Van Der Biezen EA, Jones JD. Plant disease-resistance proteins and the genefor-gene concept. Trends in biochemical sciences. 1998;23(12):454-6.
- Bishnoi SK, He X, Phuke RM, Kashyap PL, Alakonya A, Chhokar V, Singh RP, Singh PK. Karnal bunt: A re-emerging old foe of wheat. Frontiers in Plant Science. 2020; 11:569057.

- Bonde MR, Peterson GL, Schaad NW, Smilanick JL. Karnal bunt of wheat. Plant Disease. 1997;81(12):1370-7.
- Dangl JL. Piece de resistance: Novel classes of plant disease resistance genes. Cell. 1995;80(3):363-6.
- 24. Demirer GS, Landry MP. Delivering genes to plants. Chemical engineering progress. 2017;113(4):40-5.
- 25. Reader SM, Miller TE. The introduction into bread wheat of a major gene for resistance to powdery mildew from wild emmer wheat. Euphytica. 1991;53(1):57-60.
- 26. Shi AN, Leath S, Murphy JP. A major gene for powdery mildew resistance transferred to common wheat from wild einkorn wheat. Phytopathology. 1998;88(2):144-7.
- Joshi LM, Singh DV, Srivastava KD, Wilcoxson RD. Karnal bunt: A minor disease that is now a threat to wheat. The Botanical Review. 1983;49:309-30.
- Villareal RL, Mujeeb-Kazi A, Fuentes-Davila G, Rajaram S, Del Toro E. Resistance to karnal bunt (*Tilletia indica* Mitra) in synthetic hexaploid wheats derived from *Triticum turgidum*× *T. tauschii.* Plant Breeding. 1994;112(1):63-9.
- 29. Warham EJ, Mujeeb-Kazi A, Rosas V. Karnal bunt (*Tilletia indica*) resistance screening of Aegilops species and their practical utilization for *Triticum aestivum* improvement. Canadian Journal of Plant Pathology. 1986;8(1):65-70.
- Mujeeb-Kazi A, Fuentes-Davilla G, Gul A, Mirza JI. Karnal bunt resistance in synthetic hexaploid wheats (SH) derived from durum wheatx *Aegilops tauschii* combinations and in some SHx bread wheat derivatives. Cereal research communications. 2006;34:1199-205.
- 31. Ellis J, Dodds P, Pryor T. The generation of plant disease resistance gene

specificities. Trends in plant science. 2000; 5(9):373-9.

- 32. Gullen A, Plungis J. Statista. The Charleston Advisor. 2013;15(2):43-7.
- Hammond-Kosack KE, Kanyuka K. Resistance genes (R genes) in plants. eLS; 2007.
- 34. Li H, Dong Z, Ma C, Xia Q, Tian X, Sehgal S, Koo DH, Friebe B, Ma P, Liu W. A spontaneous wheat-Aegilops longissima translocation carrying Pm66 confers resistance to powdery mildew. Theoretical and Applied Genetics. 2020;133:1149-59.
- 35. Ellis JG, Lagudah ES, Spielmeyer W, Dodds PN. The past, present and future of breeding rust resistant wheat. Frontiers in plant science. 2014;5:641.
- 36. Marais GF, Pretorius ZA, Wellings CR, McCallum B, Marais AS. Leaf rust and stripe rust resistance genes transferred to common wheat from *Triticum dicoccoides*. Euphytica. 2005;143.
- Kuraparthy V, Sood S, Chhuneja P, Dhaliwal HS, Kaur S, Bowden RL, Gill BS. A cryptic wheat–Aegilops triuncialis translocation with leaf rust resistance gene Lr58. Crop Science. 2007;47(5):1995-2003.
- Olson EL, Rouse MN, Pumphrey MO, 38. Bowden RL, Gill BS, Poland JA. Simultaneous transfer, introgression, and genomic localization of genes for resistance to stem rust race TTKSK (Ug99) Aegilops tauschii from to wheat. Theoretical and Applied Genetics. 2013; 126:1179-88.
- Sharma A, Srivastava P, Mavi GS, Kaur S, Kaur J, Bala R, Singh TP, Sohu VS, Chhuneja P, Bains NS, Singh GP. Resurrection of wheat cultivar PBW343 using marker-assisted gene pyramiding for rust resistance. Frontiers in Plant Science. 2021;12:570408.

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