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### NEW SELECTION TECHNIQUES TO DETECT SOURCES OF RESISTANCE AGAINST STRIPE RUST IN WHEAT

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#### ABSTRACT

Stripe rust is the most important biotic constraint in wheat production. Deployment of resistant wheat cultivars is the most practical way to decrease yield losses attributed to stripe rust. Usage of wild germplasm of wheat is the best technique to detect new resistant genes for the evolution of new varieties and mainstream of these hold capacity for disease resistance. Traditional procedures of gene identification by using conventional screening through diversified races do not generate accurate data. In conventional breeding, transferring desired traits, for instance, stripe rust resistance, from a donor parent is offered into a genotype of interest, typically high yielding. Identification of the sources of resistance by conventional screening through diversified races and backcross breeding is time-consuming which could be avoided through the usage of DNA markers. Marker-assisted selection (MAS) is practiced for the improvement of a variety of traits in wheat around the world through introgression of disease resistance against diseases including stripe rust, which may in any case partially support in providing the anticipated result. MAS has been valuable for the development of quite a lot of significant traits such as resistance against diseases. Marker-assisted backcrossing, forward breeding, and MAS involving doubled haploid technology have been effectively used for this objective. Novel skills based on high throughput genotyping related with new marker systems (e.g., Diversity arrays technology DArT and as Single Nucleotide Polymorphism SNP), and new selection strategies such as (Advanced backcross QTL AB-QTL), mapping-as-you-go, marker-assisted recurrent selection, and genome-wide selection will have to be tried in future for upgrading of compound multigenic traits. The improvement made in all these features of marker-assisted wheat breeding, and the restrictions and future scenarios of this incipient technology have been reviewed in this article.

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#### INTRODUCTION

Food security is the main issue faced by most of the developing countries. Wheat (*Triticum aestivum* L.) is the

most important staple food consumed by 35% population of the world. Wheat is a leading field crop in terms of area under cultivation, tonnage produced,

consumption as staple food and the status in the marketing of food grain worldwide (Afzal et al., 2015). Common wheat is undoubtedly the utmost significant of all crops regarding the nourishment of mankind owing to its worth as a food source rich in energy (Gupta et al., 2010a). Wheat is grown in massive range of longitude and latitude under varied types of soil elevation or atmospheric circumstances. To feed population in 2050, with the demand of wheat set to increase 50%, the existing proportion of improvement will not be sufficient to accomplish the target (Senker, 2011). Due to constant increase in world population, there is an instant necessity to improve wheat yield significantly. World economies are focused on increasing wheat productivity (Afzal et al., 2021). Constant threat of change in climate (Asseng et al., 2015) and the appearance of very contagious pests and pathogens (Ahmed et al., 2013; Chakraborty and Newton, 2011) has made this outstanding challenge more complicated. Biotic and abiotic stresses are major issues that must be addressed as there exist no opportunity to increase area under wheat cultivation.

#### **Stripe rust of wheat**

Among many diseases of wheat, stripe rust is most aggressive (El-Orabey et al., 2020; Wellings, 2011). Disease is primarily prevalent in temperate regions, but since the beginning of 21<sup>st</sup> century, new destructive races have arisen, proliferated cosmopolitan, and caused severe epidemics in hot areas (Afzal et al., 2018). Stripe rust, appears as a re-emerging problem frequently and is expanding in new region where it did not prevail already. This has put as a disease that poses a threat to the world food security. Stripe rust damages 10-70% crop production in several regions under wheat cultivation (Chen, 2005). About 88% of the world's wheat production is prone to stripe rust (Schwessinger, 2017). Four major yellow rust epidemics were documented in 1978, 1997-98 and 2005 caused substantial losses to the economy of Pakistan (Bahri et al., 2011). Over 11 million hectare area under wheat cultivation in Pakistan and India hit by yellow rust epidemics, due to cultivation of three most important cultivars - MH 97, Inqilab 91, and PBW 343- possessing Yr27 (Selected Attila from germplasm of CIMMYT). In addition to this, nearly all the wheat cultivars, predominantly Anmol, Bahawalpur 97, Bhakhar, Chakwal 97, Faisalabad 83, Kiran 95, Kohistan, Mehran 89, Nowshera, Parwaz, Pasban 90, Sariab 92, Sarsabz, SH

2002, Soghat 90, T.J.-83, Takbeer, Tatara, Uqab 2000, Watan, Zardana, Zargoan 79, grown under rainfed and irrigated situations are no more resistant against this virulence (Khanzada et al., 2012; Tariq-Khan et al., 2020). Stripe rust hit wheat crop severely during the cropping season 2018-19 and 2019-20 in Northern Punjab and KPK (Observed by Author and Coauthor in a surveillance conducted funded by WPEP). Singh et al. (2004) reported that wheat cultivars, Attila, Bucbuc, Crow, Kauz, Nacozari, Opata, (derivatives or selections of germplasm oriented in CIMMYT, cultivated across the Central and West Asia and North Africa region abbreviated as CWANA), are under threat of yellow rust epidemic due to this new virulence.

#### **Management of stripe rust**

Development of stripe rust resistant wheat varieties is reliable and ecofriendly method to curtail yield damage. Consequently, wheat improvement through breeding for rust resistance has been addressed extensively. Sufficient data have been generated as findings of research work conducted and are being applied successfully. Ali et al. (2014) reported 26 Barberis spp. in the Himalayan region indicating that this Barberis zone is the main source of sexual recombination for non-Barberis zones substituting the race pattern in Europe (Hovmøller et al., 2016). Due to this fact, scientific community has not achieved success in stripe rust management attributed to high genetic diversity prevailing in this region. Mujeeb-Kazi et al. (2013) is in opinion Primary gene pool of wheat comprising wild and early domesticated relatives of wheat, landraces, old cultivars and breeding lines is considered the best way to identify the new resistant sources and durable rust resistant genes for developing the new varieties. More than 2,500 members of the NBS-LRR gene family have been described in wheat, 570 in *Triticum urata*, 842 in *Aegilops tauschii*, 316 in *Brachypodium distachyon*, and 420 in *Hordeum vulgare* (Gu et al., 2015), and majority of these have potential for disease resistance (Liu et al., 2017). To recognize the resistant genes by conventional technique through diversified races take a substantial period and have to maintain every year. Conventional methods of gene postulation/identification by using different races do not generate precise information. Furthermore, preservation of pure races is time consuming. Many agronomic characters for example yield, value, resistance against abiotic as well as biotic stresses are hereditary. Phenotypical evaluation for said

traits is not consistent (Collard et al., 2005). In traditional backcross breeding, a character, for instance stripe rust resistance, from a donor parent is presented into a variety of interest, usually high yielding. Selected progenies with introduced trait are crossed back to the high yielding variety for many generations. The goal is to develop a cultivar identical to the high yielding cultivar with the exception of the introduced disease resistance trait. Backcross breeding takes several generations and for some species many years.

However during the recent past sufficient development has been observed in the discipline of Biotechnology. Consequently new horizons of technology have opened and these techniques are being used successfully that has accelerated process of breeding for crop improvement. Description is as under;

#### **Application of molecular markers**

Recent discoveries and developments in genetics, genomics and crop physiology at the closing of previous century have opened up new prospects to decrease the effect of these stresses which would have been problematic if not impracticable (Begum et al., 2014). Wheat presents distinct trials through the intricacies of working with three genomes and the key role of alien germplasm and chromosome pieces in development. However, these challenges also signify most important prospects for realizing substantial genetic increases (Gupta et al., 2010b). In the presence of molecular markers and genetic maps (Semagn et al., 2006), MAB is possible for both traits which is controlled by single gene and quantitative trait loci (QTLs) (Francia et al., 2005). The successive nature of gel-based marker systems involves small output and high expenses per test. Generally used marker systems for example SSR and SNP are sequence data dependent. These restrictions limit the volume of breeding programs significantly to attain satisfactory outturns on asset to rationalize the usage of marker-assisted breeding for many traits in routine and predominantly quantitative traits.

#### **Marker assisted back crossing**

Marker assisted backcrossing (MABC) is one of the most useful strategies is the usage of molecular markers to detect and chose resistant genes. Marker assisted backcrossing (MABC) is followed to transfer rust resistance gene into the genetic background of susceptible parent. Recurrent parent (susceptible) is used as female and crossed with different donors as male to produce First filial seeds in three isolated

programmes. It is backcrossed thrice (with susceptible) to improve successfully the genome of recurring genotype with instantaneous forefront and contextual selection. The forefront selection is ended up to BC3F3. Self-fertilization of the alleles received from the donor and simultaneous background selection is performed for homozygosity repeatedly (three cycles). MABC contribute to evolve varieties characterized by high-yielding and resistant by combining a gene of interest into a well-adapted variety. MABC is recently advanced effective instrument by which using vast population sizes (400 or more plants) for the backcross first filial generations, it is probable to recover the recurrent parent genotype using only two or three backcrosses. Until now, several genetically modified genotypes characterized by their high production, stresses tolerance, quality and taste in wheat varieties have been evolved in countries under wheat cultivation through MABC within the short timeframe. Currently, MABC is being practiced broadly in plant breeding to evolve new Marker assisted backcrossing (MABC) is an attractive tool for breeding immensely using in hundreds of research objective at concentrating on the structure, purpose, development, mapping, and editing of genomic regions of interest.

#### **Forward breeding**

Forward breeding is a sort of backcross breeding in which the original cultivar of interest is not used, but “best” cultivar of interest is used as the recurrent parent, allowing for the addition of current breeding developments into the backcross breeding program. Forward breeding takes benefit of improved cultivars and genetic data that may have been generated during the course of backcross breeding. Germplasm genotype is frequently a consideration in forward breeding. Marker assisted selection is mostly useful when selecting for multiple quantitative traits. One more amendment of backcross breeding involves “background selection” or “donor genome elimination”, where the backcross development is enhanced by coupling phenotypic selection with genomic selection for the genetic background of the recurrent parent.

#### **Doubled haploids**

Doubled haploids (DHs) are plants derived from a single pollen grain and doubled synthetically to develop homozygous diploids. A DH individual has two identical homologs, so that the quantity of recombination data is equal to a backcross. The benefit of exploitation a DH

population in molecular mapping is that all individuals are homozygous. Consequently, DHs may be transported between various labs and situations for evaluating the influence of the atmosphere on gene expression. DHs have had a substantial effect on reducing duration, employment, and cost in plant-breeding programs. This skill allows less technical programs with limited resources to achieve extraordinary results and is saving millions of dollars. The doubled haploid technique of procurement of homozygous lines of self-fertilized crops is becoming widespread, predominantly in wheat, paddy, and barley, and in cross-fertilized crops such as corn comes in farmer field two years earlier. Haploid plants can be screened with molecular marker linked to the genes of interest, or a genome wide selection approach can be used to only advance haploids that merit selection to be developed into DH lines. Furthermore, DHs can be evaluated in abiotic stress screening nursery, to make directed selections which DHs to forward into yield performance testing.

#### **Diversity arrays technology**

DArT markers, once these are transformed into specific PCR assays; but alteration wants to take account of marker redundancy, at the level of both segregation patterns and DNA sequences. Diversity Arrays Technology (DArT™) is a profitable hybridization-based marker skill that presents a high multiplexing level while being independent of sequence information. Marker conversion to PCR-based assays is generally involves many intermediate steps (McNeil et al., 2011; Xu et al., 2001). The presence of redundant markers could be described by the detail that DArT technology is based on a hybridization line. The arrangements of the probes may not be recognized, and the same probes are used with dissimilar names. On the other hand, probes having diverse DNA sequences may be allied together. Hence, the redundancy identified by us is typical of DArTs and may up to 40 %, as designated by other researchers (Raman et al., 2013; Schouten et al., 2012). The accessible indication is that converted DArTs typically continue the segregation pattern of the original DArT marker (Eckstein et al., 2008; Shahin et al., 2009).

#### **Single nucleotide polymorphisms**

Single nucleotide polymorphisms, frequently called SNPs (pronounced “snips”), are the most common type of genetic variation among population. Each SNP represents a difference in a single DNA building block, called a nucleotide. E.g., a SNP may replace the nucleotide cytosine

(C) with the nucleotide thymine (T) in a certain stretch of DNA. SNPs are the primary approach to realize evolutionary and genetic associations between/within species, elucidate of agronomic traits in crops, and explain susceptibility to diseases. Particularly in research of plant breeding and genetics, recognizing genetic loci that are responsible for trait diversity is important. With the advantages of stability, cost effective developments, and high throughput assays, SNP has become progressively significant in crop genetic studies. The advance of genotyping tools allows the discovery of the diversity in model and non-model crops, and it has been applied effectively in botany for several years. The shift to the high-throughput genotyping assays and progress of next generation sequencing technologies accelerated the detection of polymorphisms. But, the error-prone fashion of NGS analysis tools is still a large concern which may lead to erroneous-positive SNPs. There is a prerequisite for the progress of a tool for extracting bulk of data, support for the data analysis, and intellectual conclusion on the precision. To achieve this demand, industrialization has been carried rather than exploitation of binary structure of nucleotides. Integrated SNP Mining and Utilization (ISMU) Pipeline is one of the initial experiments to develop a machine learning method to SNP finding. The integrated approach alongside the recent inventions will permit an amplified information and application of SNPs in the future.

#### **Advanced backcross QTL analysis**

Advanced backcross QTL analysis is a technique of joining QTL analysis with variety development. It is tailored for the detection and transmission of useful QTL alleles from unadopted donor lines into recognized elite inbred lines. QTL analysis is deferred till the BC2 or BC3 generation and, during the progress of these populations, negative selection is exercised to decrease the frequency of harmful donor alleles. Simulations propose that advanced backcross QTL analysis will be effective in detecting additive, dominant, partially dominant, or over dominant QTLs. Epistatic QTLs or QTLs with gene actions ranging from recessive to additive will be detected with less power than in selfing generations. QTL-NILs can be derived from advanced backcross populations in one or two additional generations and utilized to verify QTL activity. These same QTL-NILs also represent commercial inbreeds improved (over the original recurrent inbred line) for one or more quantitative traits. The time lapse from

QTL discovery to construction and testing of improved QTL-NILs is minimal (1–2 years). If successfully employed, advanced backcross QTL analysis can open the door to exploiting unadopted and exotic germplasm for the quantitative trait improvement of a number of crop plants.

### **Mapping As You Go**

The approach considered in this paper, referred to as the Mapping As You Go (MAYG) approach, repeatedly rescripts evaluations of QTL allele effects by remapping new elite germplasm generated over cycles of selection, thus ensuring that QTL estimates remain relevant to the current set of germplasm in the breeding program. Mapping As You Go is a mapping-MAS strategy that explicitly recognizes that alleles of QTL for complex traits can have different values as the current breeding material changes with time (Podlotch). Simulation was used to investigate the effectiveness of the MAYG approach applied to complex traits. The results indicated that greater levels of response were achieved and these responses were less variable when estimates were revised frequently compared with situations where estimates were revised infrequently or not at all.

### **Marker-assisted recurrent selection**

MARS is a scheme which permits selection of genotypes with performance and intercrossing among the selected individuals in the same crop season for one cycle of selection (Jiang et al., 2010). Therefore, MARS could enhance the efficiency of recurrent selection and accelerate the progress of the procedure particularly helping in integrating multiple favorable genes or QTLs from different sources through recurrent selection based on multi-parent populations (Asima et al., 2015). For complex traits such as grain yield, biotic and abiotic resistance, MARS has been proposed for “forward breeding” of native genes and pyramiding multiple QTLs (Eathington et al., 2007; Ribaut et al., 2010). In MARC, de novo QTL mapping is done for each breeding population. This helps in integrating the multiple QTL with small genetic effects governing the expression of complex traits. Once the major QTLs of interest are identified from MARS breeding population, subsequently the selected individuals are subjected to controlled pollination to develop lines with an optimum complement of QTLs from both parents. Marker-assisted Recurrent Selection (MARS) is a recurrent selection scheme using molecular markers for the identification and selection of multiple genomic regions involved in

the expression of complex traits to assemble the best-performing genotype within a single or across related populations (Ribaut et al., 2010). MARS is a scheme which allows high-performing genotypic selection and intercrossing among the selected individuals in the same crop season for one cycle of selection (Jiang et al., 2010). Therefore, MARS could enhance the efficiency of recurrent selection and accelerate the progress of the procedure particularly helping in integrating multiple favorable genes or QTLs from different sources through recurrent selection based on multi-parent populations (Asima et al., 2015). For complex traits such as grain yield, biotic and abiotic resistance, MARS has been proposed for “forward breeding” of native genes and pyramiding multiple QTLs (Ribaut et al., 2010). In MARC, de novo QTL mapping is done for each breeding population. This helps in integrating the multiple QTL with small genetic effects governing the expression of complex traits. Once the major QTLs of interest are identified from MARS breeding population, subsequently the selected individuals are subjected to controlled pollination to develop lines with an optimum complement of QTLs from both parents

### **CONCLUSION**

The common occurrence of *Pst* epidemics and universal dispersal emphasizes the necessity for an effective disease management strategy. The outline of development of *Pst*, population structures, migration courses, and reproduction styles are recognized. Concentration should be focused in the direction of recognizing of more *Pst* resistance genes from wild relatives. It is well recognized that to realize the goal of feeding growing population innovative combined tactics must be practiced with the traditional breeding programmes to quicken the breeding cycle by reducing net time and charges per unit production. Progress in molecular genetics has offered the chance to the investigator to integrate diverse important traits. It appears likely that novelties in breeding approaches will be important driver to the title role of Marker Assisted Selection in future and we are seeing even now innovative breeding systems that were not possible before application of molecular markers. Molecular markers are characterized by unaffected by atmosphere, exploited in all plant growth stages for selection of necessary characters along with target gene(s). To be convenient for selection purposes, molecular markers should be inexpensive and adaptable to large scale

analyses. Furthermore, they want to discriminate between different phenotypes with inevitability. Such situations could be accomplished by skills based on high output genotyping related with new marker systems, and new selection strategies such as AB-QTL, mapping-as-you-go, marker-assisted recurrent selection and genome-wide selection will have to be tried in future for upgrading of compound multigenic traits. In the molecular breeding programme MARS prove to be one of the most important approaches as this can be used for multiple QTLs controlling the expression of a complex trait which is a restriction in case of Marker Assisted Backcrossing.

#### AUTHORS' CONTRIBUTION

All the authors equally participated in collecting, organizing, writing and editing the manuscript.

#### CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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