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Advanced Breeding Strategies to Mitigate the Threat of Black Stem Rust of Wheat

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A B S T R A C T

The spread of stem rust race Ug99 and variants are threat to worldwide wheat production and efforts are being made to identify and incorporate resistance. A primary source of concern at present is that Ug99 (TTKSK and its variants TTKST and TTTSK) has overcome major sources of stem rust resistance genes *e.g.* Sr31, Sr38 and other important gene complexes which confer resistance to stem rust. Deployment of cultivars with broad spectrum rust resistance is the only environmentally viable option to combat these diseases. Therefore, identification, mapping and deployment of effective resistance genes are critical components of global efforts to mitigate this threat. Identification and introgression of novel sources of resistance is a continuous process to combat the ever evolving pathogens. Few stem rust resistance (Sr) genes derived from the primary and secondary gene pool of wheat confer resistance to TTKSK and its variants. Breeding resistant cultivars is the most realistic approach to protect wheat from stem rust. Deployment of combinations of effective genes "stacked" or "pyramided" in combination with APR genes should improve the durability of resistance in commercial cultivars by reducing the probability of corresponding simultaneous mutation events in the pathogen. Gene pyramiding is facilitated by the ability to use molecular markers closely or completely linked to resistance genes. Though Ug99 type of races have posed a threat to the wheat cultivation worldwide, several developing countries of South and West Asia have taken proactive steps to meet this challenge.

Introduction

Stem rust or black rust of wheat is caused by the fungus *Puccinia graminis* Pers. f. sp. *tritici* Eriks. and E. Henn. Stem rust is known for causing severe devastations periodically in all wheat- growing countries of the world. The most effective and environmentally sound method to control these diseases is through the deployment of resistant cultivars. Although a number of rust resistance genes have been identified in wheat (McIntosh *et al.*, 2014), a major problem has been their short-lived effectiveness due to the fast emergence of virulent races of the pathogen that are capable of overcoming the resistance. For last several decades, epidemics of stem rust have been effectively controlled in most wheat growing regions because of the worldwide deployment of effective stem rust resistance genes in wheat varieties and removal of important alternate hosts, such as

Barberis vulgaris L. from the proximity of wheat fields (Singh *et al.,* 2006, 2008a, b; Jin *et al.,* 2006, 2009a).

However, stem rust has again become a major threat to the world wheat production with a new race of stem rust pathogen, Ug99, with virulence to a widely used resistance gene Sr31, was detected in Uganda in 1999 (Pretorius *et al.,* 2000), and was named TTKS based on the North American stem rust race nomenclature system (Wanyera *et al.,* 2006; Jin *et al.,* 2008a). Ug99 pathotypes defeat most of the race-specific resistance genes currently deployed worldwide and are considered to be the most virulent strain of stem rust to emerge in the last 50 years (Stokstad, 2007). Ug99 is virulent to Sr31 (derived from chromosome 1RS of rye, *Secale cereale* L.), a gene widely deployed in winter and spring wheat varieties in China, Europe, India and USA, and Sr38 (derived from 2NS of *Aegilops ventricosa* Tausch), a gene deployed in some European, American and Australian cultivars (Singh *et al.,* 2006, 2008a, b). Further concern has grown with the discovery of additional variants in the Ug99 lineage. Two new variants, TTKST and TTTSK, which were reported in 2006–2007 to be virulent to other widely deployed genes *Sr24* and *Sr36* (both were effective against race Ug99 or TTKSK) (Jin *et al.,* 2008b, 2009a).

In addition, Ug99 has migrated from East Africa to Sudan and Yemen in 2006 (Jin *et al.,* 2008a), and Iran in 2007 (Nazari *et al.,* 2009). Emergence and spread of these new races of stem rust pose an imminent threat to wheat production worldwide demand the rapid development of wheat cultivars with durable resistance to stem rust (Liu *et al.,* 2010). The proximity of Ug99 to highly vulnerable and vast wheat crops in the Indian subcontinent and China is concerning. Breeding of genetic resistance is considered to be the most effective approach to prevent or slow the spread of stem rust caused by Ug99 (Singh *et al.,* 2008a). At present, among the 58 catalogued resistant genes against stem rust, only less than half of them are effective to Ug99 (McIntosh *et al.,* 2014). There are a total of 26 stem rust resistant genes derived from common wheat, only three (*Sr28, Sr29* and *SrTmp*) are resistant to Ug99, and the effects of these genes are moderate under heavy disease pressure. Among the catalogued genes conferring some level of resistance against Ug99, 32 genes were introduced into wheat from its wild relatives. Because of limited resistance in the wheat gene pool, the discovery of novel resistance in wild relatives and its transfer to wheat by chromosome engineering is an effective strategy of disease control. New sources of Ug99 resistance in alien wheat species have been reported (Xu *et al.,* 2008, 2009; Jin *et al.,* 2009b, Liu *et al.,* 2013) and a resistance gene from *Aegilops speltoides* Tausch has been transferred into wheat (Faris *et al.,* 2008).

Stem rust at present provides a major challenge to the wheat breeders throughout the globe. The ever evolving new races of the pathogen (as ug99, TTKSK, etc) and their devastating nature make a think to the world breeding community to combat these threats, by means of use of efficient molecular techniques, use of alien novel genes and other breeding procedures to mitigate the potential threat.

This chapter provides a general outlook about the stem rust, pathogen type, breeding for disease resistance and travels throughout the span of development of various stem rust resistance genes. It also provides a general outlook of shuttle breeding and other marker assisted approaches devised for resistance breeding. At last this book chapter is prepared to make it very much clear that if the pathogen is not handled, it will create a disastrous situation in the world.

Materials and Methods

Breeding strategies for stem rust

Genes *Sr24* and *Sr26* are transferred from Agropyron elongatum. *Sr31* is located in the 1BL. 1RS translocation from "Pektus" rye. Undesignated gene on 1AL. 1RS translocation from "Insave" rye. *Sr36* from *Triticum timopheevi* and *Sr38* from *T. ventricosum* further reduced stem rust in 70's and 80's. Stem rust has been successfully brought under effective control through the use of host resistance in the past several decades until the occurrence of race TTKSK and its variants which have defeated most stem rust resistance (Sr) genes existing in commercial varieties. Most of the Sr genes have been characterized for their reactions to specific races of *P. graminis* f. sp. *tritici* including reactions at the seedling stage. Over the last century, these genes have been identified within common wheat and wild relatives (Olson, 2012). Pumphrey (2012) reported that about 30 major genes conferring resistance to Ug99-complex races, and one designated APR genes (*Sr2*) that contribute to stem rust resistance have been identified. Some of these, including *Sr22, Sr25, Sr27, Sr32, Sr33, Sr35, Sr37, Sr39, Sr40, Sr44, Sr45, Sr46* and a few genes with temporary designation are still resistant to Ug99 and its derivatives (Xu *et al.,* 2008). Although there are several genes showing considerable amount of resistance to Ug99 group of stem rust races yet, only *Sr22, Sr26, Sr35* and *Sr50* are known to be effective against all currently reported races of the group. *Sr25* is known to confer high level of resistance only in some specific genetic backgrounds, especially when present with adult plant resistance gene (Table 1). Dundas *et al.,* (2007) reported that most of these genes are derived from wild relatives of wheat and are located on chromosome translocations that include large donor segments that harbour genes possibly deleterious to agronomic and quality traits. Thus, they are virtually unusable in their current form. Translocations with small alien fragments have less likelihood of a linkage drag, which can depress essential agronomic and end-use quality traits (Liu *et al.,*, 2011b). The successful use of alien genes is mostly determined by the ability of the introduced alien chromosome segments to substitute for homoeologous chromosome segments of wheat. Translocations with small alien fragments have less likelihood of a linkage drag, which can depress essential agronomic and end-use quality traits. The development of wheat-alien compensating translocations with minimal alien chromatin by manipulating homoelogous recombination can enhance the commercial exploitation of wild relatives in wheat improvement (Sears, 1977; Friebe *et al.,* 1996; Qi *et al.,* 2007). To enhance the utility of genes in wheat breeding programme, currently there are ongoing research efforts to eliminate the deleterious linkage drag and to produce lines with smaller chromosome segments containing the resistance genes.

Genetic mapping for new stem rust resistance genes

Molecular mapping studies can identify chromosomal regions with important traits and tightly linked markers that can then be used as an effective tool in marker- assisted selection (Collard *et al.,*, 2005). Various molecular markers have been widely used to tag and map resistance genes in wheat; using high throughput simple sequence repeat (SSR), single nucleotide polymorphism (SNP) or Diversity Arrays Technology (DArT) markers gives the opportunity for genome-wide mapping (Singh *et al.,* 2013). However, simple sequence repeat (SSR) has emerged as the choice of marker in gene mapping studies. Rapid advance in DNA sequencing and molecular marker technologies has made

identification of new genes faster and more precise. Fine mapping of the resistance genes has also opened the possibility of cloning it and use in breeding programme avoiding linkage drag. Sr33 which is ortholog of barley Mla was cloned by Periyannan *et al.,* (2013). Recently a new APR gene Sr56 was identified by Bansal *et al.,* (2014). To date, 76 leaf rust, 72 stripe rust and 60 stem rust resistance genes has been designated (McIntosh *et al.,* 2014).

Marker diversity and their linkage to stem rust resistance genes

The new races of *Puccinia graminis* tritici have broken down the resistance of widely deployed stem rust resistance genes, especially *Sr31*. Development of resistant wheat varieties is one way of coping with this threat. (Ejaz *et al.,* 2012) conducted a study to determine the presence / absence of Sr genes in Pakistani adapted spring wheat so as to facilitate future Sr gene pyramiding. Stem rust resistance gene *Sr2* provides non hypersensitive response at adult plant stage (McIntosh *et al.,* 1995). Ejaz *et al.,* (2012) used six DNA markers to detect *Sr2* gene in Pakistani adapted spring wheat. Microsatellite marker Xgwm533 produced 120-bp fragment in 79% Pakistani wheat varieties, indicating the presence of *Sr2*. However, (Spielmeyer *et al.,* 2003) reported that some *Sr2* non carriers also produced 120-bp fragment. To reliably detect *Sr2* gene, the used of STS marker stm559tgag developed by (Hayden *et al.,* 2004) with the new forward primer referred to as stm559n (Pretorius *et al.,* 2012), which showed the same frequency as Xgwm533 for presence of *Sr2* gene with few exceptions. (McNeil *et al.,* 2008) found three BAC-derived markers, X3B042G11, X3B061C22, and X3B028F08, closer to *Sr2* gene than Xgwm533. These three markers produced polymorphic bands between positive and negative control in this study.

However, the *Sr2* gene-associated alleles of the first two markers were not similar to those reported by McNeil *et al.,* (2008). Therefore, these markers were not applied on all varieties. The results for marker X3B028F08 were consistent with McNeil *et al.,* (2008). Based on the results of this marker, 70% of Pakistani wheat varieties likely carry the *Sr2* gene. Ejaz *et al.,* (2012), suggested that this marker can be helpful in MAS for *Sr2*. The CAPS marker csSr2 is diagnostic to detect single nucleotide polymorphism for BspHI restriction site (Mago *et al.,*, 2011). The results of csSr2 marker were 87% and 82% similar to that of Xgwm533 and stm559tgag, respectively. However, after restriction with BspH1, only 9% of Pakistani varieties showed presence of the *Sr2* gene. This marker has been reported as more accurate for *Sr2* as compared to other markers reported previously. However, the results suggest that this marker probably underestimated the frequency of *Sr2* in Pakistani wheat germplasm (Ejaz *et al.,* 2012.,). Moreover, CAPS markers require an additional step of restriction digestion, which makes them costly and time-consuming compared to STS markers. It is, therefore, recommended to use both stm559tgag and BAC-derived marker X3B028F08 for screening of wheat germplasm in Pakistan. As *Sr2* is a race-nonspecific adult plant resistance gene, efforts should be made toward the development of a gene-specific marker to assist future incorporation of this gene into wheat varieties. (Ejaz *et al.,* 2012.,) used two closely linked (1.1 and 1.5 cM, respectively) microsatellite markers, Xwmc453 and Xcfd43, reported by Tsilo *et al.,* (2009) to detect the presence of Sr6. The marker Xwmc453 did not produce fragments associated with the presence/absence of Sr6, indicating that this marker is probably not diagnostic for Sr6 (Ejaz *et al.,*, 2012) On the contrary, marker Xcfd43 produced the expected fragments. Screening of Pakistani

varieties with this marker showed that 11% of varieties likely have Sr6. (Ejaz *et al.*,, 2012).

Stem rust resistance gene *Sr22* is effective against Ug99 and all other stem rust pathotypes, except races 316 and 317 from Israel (Periyannan *et al.*,, 2010). To date, this gene has only been incorporated in Australian commercial cultivar 'Schomburgk' (Singh, 1991; Khan *et al.*,, 2005). The limited use of this gene in cultivated wheat might be due to a yield penalty associated with this gene (Paull *et al.*,, 1994). The STS markers csIH81-BM and csIH81-AG are diagnostic to detect the presence/absence of *Sr22* (Periyannan *et al.*,, 2010). These markers showed absence of *Sr22* in Pakistani wheat varieties. It is, therefore, recommended to incorporate this gene into Pakistani wheat varieties to broaden their genetic base against Pgt races. Stem rust resistance gene *Sr24* confers resistance to stem rust race TTKS but not to its variants. Ejaz *et al.*,, 2012, results showed absence of this gene in Pakistani wheat varieties, so deployment of this gene in Pakistani cultivars should be encouraged. This will provide resistance to other prevalent Pgt races and may provide residual resistance to its variants as suggested by Knott (2008). Moreover, *Sr24* gene is also useful due to its linkage with Lr24. Klindworth *et al.*, (2011) reported the occurrence of this gene in U.S. winter wheat, which can be used as source for the introgression of *Sr24* (Ejaz *et al.*,, 2012).

Stem rust resistance genes *Sr25* and *Sr26* are effective against variants of Ug99, TTKST and TTTSK (Singh *et al.*,, 2006; Jin *et al.*,, 2007). Ejaz *et al.*, 2012., used STS marker Gb (Prins *et al.*,, 2001) to detect *Sr25* gene. The results showed absence of *Sr25* in Pakistani wheat varieties. This marker was also validated by Liu *et al.*, (2010) and Njau *et al.*, (2010). Liu *et al.*, (2010) also tested a more accurate codominant marker BF145935 for *Sr25*, which showed 198- and 180-bp fragments in *Sr25*-positive varieties, and 202- and 180-bp bands in *Sr25* non carriers. Ejaz *et al.*, 2012, preferred using Gb, as the 4-bp difference resulting from BF145935 was relatively difficult to resolve on agarose gel. This gene has been widely exploited in Australian and CIMMYT germplasm (Bariana *et al.*,, 2007). This gene needs to be incorporated into Pakistani wheat varieties so as to broaden their genetic base against the various Pgt races. The STS markers Sr26#43 (Mago *et al.*,, 2005) and BE518379 (Liu *et al.*,, 2010) were used in combination to serve as a co-dominant marker. These markers showed absence of the *Sr26* gene in Pakistani wheat varieties. Similar to *Sr25, Sr26* is also effective against Ug99 and *Sr24*-virulent races. Use of this gene has been limited to Australia where 'Eagle' was the first cultivar possessing *Sr26* (Martin, 1971). The limited use of this gene might be due to a 9% yield penalty associated with this gene (The *et al.*,, 1988). This problem was later solved with the development of new lines having reduced alien segment (Dundas *et al.*,, 2007). Thus, this gene can easily be transferred through Australian germplasm into Pakistani wheat varieties for broadening the genetic base of future wheat varieties against Pgt races. Before the emergence of Ug99, stem rust resistance was maintained mainly by Sr31 in most of the countries around the world except Australia (Singh *et al.*,, 2008). Ejaz *et al.*, 2012, used STS marker iag95 (Mago *et al.*,, 2002) and SCAR markers SCSS30.2576 and SCSS26.11100 (Das *et al.*,, 2006) to assay Pakistani wheat varieties for this gene; 35% of the varieties tested had the Sr31 gene.

Das *et al.*, (2006) reported that SCSS30.2576 and SCSS26.11100 were more reliable than previously developed STS markers. The results of the three markers were 98% similar, suggesting that these markers are equally reliable for detection of Sr31 gene. However, the two SCAR markers can be used as

codominant markers in segregating generations to distinguish homozygous dominant from heterozygous carriers of Sr31. Due to the large difference in the annealing temperatures of the two SCAR markers, these cannot be used in a multiplex PCR. Marker iag95 also has been successfully validated on South African germplasm (Pretorius *et al.*,, 2012).

Most Pakistani wheat varieties are highly susceptible to Ug99 but are resistant to local stem rust races (Mirza *et al.*,, 2010a). The results of (Ejaz *et al.,* 2012) showed the presence of Sr31 in these varieties, indicating that Sr31 probably is effective against Pakistani stem rust races. Moreover, susceptible genes can still provide resistance along with effective genes, a phenomenon known as ghost or residual resistance (Knott, 2008). So other stem rust resistance genes need to be incorporated into these varieties. Varieties 'Kiran-95', 'Tandojam-83', and 'Sarsabz-86' were found susceptible to a local stem rust race (Khanzada, 2008) named RRTTF (Mirza *et al.*,, 2010b) present in southern Pakistan. Among these cultivars, 'Tandojam-83' showed presence of Sr31, whereas the other two showed absence of Sr31. However, our results do not provide evidence that local race(s) carry virulence for Sr31, so the local races need to be tested against all stem rust resistance genes to know their virulence / avirulence pattern. Stem rust resistance gene Sr38 confers resistance against stem rust race TPPKC (Klindworth *et al.*,, 2011) and is linked with Yr17 and Lr37. This gene was found in very low frequency (9%) in the Pakistani wheat varieties tested. Due to its linkage with stripe and leaf rust resistance genes, this gene cluster should be incorporated in future Pakistani wheat varieties to increase its frequency and to confer multiple rust resistance. Gold *et al.,* (1999) developed SCAR markers to detect Sr39 gene in Canadian wheat. However, Ejaz *et al.,* failed to produce the amplicon diagnostic for Sr39 gene in Pakistani-adapted spring wheat. Instead, Ejaz *et al.,* 2012, observed three monomorphic bands ranging from 100 to 200 bp in size. Hence, there is need for further testing of this marker and for development of a more reliable marker for Sr39. This gene has not been exploited extensively and there is no report of quality deterioration associated with Sr39/Lr35 segment. Therefore, this gene should be introgressed into Pakistani wheats.

Genetics and molecular mapping of stem rust resistance

Genetic analysis of stem rust resistance revealed that two independent genes in WR95 were effective against different isolates of stem rust at seedling stage (Gireesh *et al.,* 2015). The resistance against isolates 40A and 21A-2 was found to be conferred by a recessive gene, whereas a dominant gene was observed for resistance against isolates 11 and 11A.The stem rust isolates 40A and 11A were used for mapping of the recessive and the dominant genes for stem rust resistance present in the line WR95, respectively. The recessive gene in WR95 conferring resistance to isolate 40A was mapped on long arm of 5D chromosome (Gireesh *et al.,* 2015).

The only other known gene located on chromosome arm 5DL is Sr30, which also behaves as a recessive gene (Knott and Mclntosh 1978). Bariana *et al.,* (2001) also mapped Sr30 to 5DL in cultivar Cranbrook, Hiebert *et al.,* (2010) mapped Sr30 in Webster to 5DL. The closest marker Xcfd12 linked to Sr30 was found monomorphic in the NI5439/WR95 population. BSA in NI5439/WR95 mapping population identified two flanking markers, Xcfd3 and Xwmc215 to 5DL which showed linkage to the recessive gene in WR95 at the distance of 8.6 and 12.8 cM, respectively. To validate the results,

markers on 5DL were used in BSA and genotyping of another F2 population derived from the cross Agra Local/ WR95. It was found that Xwmc215 and Xcfd7 were closest markers linked to the recessive gene in WR95 at a distance of 12.3 and 11.2 cM, respectively (Gireesh *et al.,* 2015). The order of markers and the map distances in the two populations were comparable, though Xcfd3 was monomorphic in the later population. The marker order in the map is in conformity with ITMI map (Song *et al.,* 2005).

To ascertain the identity of the recessive gene for stem rust resistance present in WR95, it was tested against isolates 11, 11A, 15-1 and 21A-2 which are virulent to Sr30. WR95 showed resistance to all these isolates whereas Webster carrying Sr30 was found susceptible. To determine whether resistance against isolates virulent on Sr30 is also conferred by the same gene, the F2 populations were subjected to genetic analysis against isolates 11,11A and 21A-2. The segregation pattern of F2 population derived from the cross Agra Local/WR95 suggested a single recessive gene against isolate 21A-2. The results suggest that recessive stem rust resistance gene (srWR) in WR95 is probably Sr30 but carries a different allele of it (Gireesh *et al.,* 2015).

The dominant gene in WR95 was mapped to telomeric region of 2BL chromosome in F2 (Agra Local/WR95) mapping population using isolate 11A. Three putative markers Xwmc317, Xcfa2278 and Xgwm388 identified in BSA were used for genotyping of F2 population. Linkage analysis mapped the dominant stem rust resistance gene in WR95 to 2BL telomeric region and Xwmc317 was found to be the closest marker at the distance of 8.2 cM (Gireesh *et al.,* 2015). However, 2BL also harbour *Sr9, Sr28* and *Sr16.* Hiebert *et al.,* (2010) mapped gene Srweb on 2BL with Xgwm47 as the closest marker at 1.4 cM. Sr9 was also mapped on 2BL and Xgwm47 was the closest marker at the distance of 0.9 cM (Tsilo *et al.,* 2007). Rouse *et al.,* (2012) mapped *Sr28* on 2BL in SD1691 and identified Xwmc332 as the closest marker at the distance of 5.8 cM. In order to ascertain the identity of dominant resistance gene in WR95, we genotyped the F2 population with marker Xgwm47, which is closely linked to Sr9 and Srweb. The map position of Sr9/ Srweb and the dominant gene (SrWR) in WR95 suggests two different loci for these genes, almost 20 cM apart. Since *Sr28* is not effective against stem rust isolates 11, 11A, 15-1, 21A-2 and 40A and the dominant gene in WR95 was mapped using isolate 11A, the possibility of *Sr28* in WR95 is explicitly ruled out. Virulent/avirulent isolates for Sr16 are not available with us, though Sr16 is considered to be not so effective gene against Indian stem rust isolates (Tomar and Menon 2001). Therefore, 2BL region of WR95 carries either Sr16 or a new gene. However, till the precise map position of Sr16 is known it is difficult to determine the exact identity of gene in WR95 (Gireesh *et al.,* 2015). Molecular markers linked to Sr16 are not available to differentiate the two loci based on map position.

The bread wheat genetic stock WR95 was thus found to carry two independent stem rust resistance genes located on chromosome 5DL and 2BL. WR95 showed recessive gene inheritance against stem rust isolates 40A and 21A-2. The recessive gene conferring resistance against 40A was mapped to 5DL chromosome which is flanked by markers Xcfd3 and Xwmc215. WR95 showed dominant gene inheritance against stem rust isolates 11 and 11A. The dominant gene SrWR was mapped towards telomeric region of 2BL chromosome and Xwmc317 was identified as the nearest marker (Gireesh *et al.,* 2015).

Marker Assisted Selection (MAS)

The development of DNA (or molecular) markers has irreversibly changed the disciplines of plant genetics and plant breeding. While there are several applications of DNA markers in breeding, the most promising for cultivar development is "marker assisted selection". MAS refers to the use of DNA markers that are tightly-linked to target loci as a substitute for or to assist phenotypic screening. By determining the allele of a DNA marker, plants that possess particular genes or quantitative trait loci (QTLs) may be identified based on their genotype rather than their phenotype (Ragimekula *et al.,* 2013). Five main considerations for the use of DNA markers in MAS (Mohler and Singrun, 2004) are;

Reliability

Molecular markers should co-segregate or tightly linked to traits of interest, preferably less than 5 cM genetic distance. The use of flanking markers or intragenic markers will greatly increase the reliability of the markers to predict phenotype.

DNA quantity and quality

Some marker techniques require large amounts and high quality DNA, which may sometimes be difficult to obtain in practice, and this adds to the cost of the procedures.

Technical procedure

Molecular markers should have high reproducibility across laboratories and transferability between researchers. The level of simplicity and time required for the technique are critical considerations. High-throughput simple and quick methods are highly desirable.

Level of polymorphism

Ideally, the marker should be highly polymorphic in breeding material and it should be co-dominant for differentiation of homozygous and heterozygous individuals in segregating progenies.

Cost

Molecular markers should be user-friendly, cheap and easy to use for efficient screening of large populations. The marker assay must be cost-effective in order for MAS to be feasible.

Mas Schemes in Plant Breeding

Early generation marker assisted selection: Molecular markers can be employed at any stage of a plant breeding programme. Hence, MAS has great advantage in early generation selections by eliminating undesirable gene combinations especially those that lack essential disease resistance genes. Subsequently, the breeders can focus on a lesser number of high priority lines of desirable allelic or gene combination (Ragimekula *et al.,* 2013). MAS-based early generation selection not only selects suitable gene combinations but also ensure a high probability of retaining superior breeding lines (Eathington *et al.,* 1997). An important prerequisite for successful early-generation selection with MAS are large populations and low heritability of the selected traits. The relative efficiency of MAS is greatest for characters with low heritability (Lande and Thompson 1990). This has important consequences in the later stages of the breeding program because the evaluation for other traits can be more efficiently and cheaply designed for fewer breeding lines (especially in terms of field space). However, in 2000 Barr *et al.,* stated that, "this is fantasy for public sector breeders, as MAS can only

be used in early generation screening for very important material", the main limitations being costs, availability of suitable markers, and staff resources for sample and data handling. Markers are also frequently used to select parents with desirable genes and gene combinations, and marker-assisted recurrent selection (MARS) schemes involve several successive generations of crossing individuals based on their genotypes. The achievable genetic gain through MARS is probably higher than that achievable through MABC (Ribaut and Ragot 2006).

Marker-assisted backcrossing (MABC)

Backcrossing is used in plant breeding to transfer favourable traits from a donor plant into an elite genotype (recurrent parent). In repeated crossings the original cross is backcrossed with the recurrent parent until most of the genes stemming from the donor are eliminated (Becker 1993). However, the donor segments attached to the target allele can remain relatively large, even after many backcrossing generations. In order to minimize this linkage drag, marker assays can be of advantage (Frisch *et al.*, 1999). There are three levels of selection in which markers may be applied in backcross breeding. Markers can be used in the context of MABC to either control the target gene (foreground selection) or to accelerate the reconstruction of the recurrent parent genotype (background selection) and to select backcross progeny having the target gene with tightly-linked flanking markers in order to minimize linkage drag (recombinant selection). According to Frisch *et al.*, (1999) in a computer simulation MAS can reconstruct a level of recurrent parent genome in BC3 which would only be reached in BC7 without the use of markers. However, the authors also state that large numbers of marker data points are required to achieve such results. MABC is especially efficient if a single allele is to be transferred into a different genetic background, for example, in order to improve an existing variety for a specific trait. To overcome the limitation of only being able to improve existing elite genotypes, other approaches like marker-assisted recurrent selection (MARS) have to be considered (Ragimekula *et al.*, 2013).

Marker-assisted recurrent selection (MARS)

The improvement of complex traits via phenotypic recurrent selection is generally possible, but the long selection cycles impose restrictions on the practicability of this breeding method. With the use of markers, recurrent selection can be accelerated considerably and several selection-cycles are possible within one year, accumulating favourable QTL alleles in the breeding population (Eathington *et al.*, 2007). Additionally, it is possible today to define an ideal genotype as a pattern of QTLs, all QTLs carrying favourable alleles from various parents. If individuals are crossed based on their molecular marker genotypes, it might be possible to get close to the ideal genotype after several successive generations of crossings. It is likely that through such a MARS breeding scheme higher genetic gain will be achieved than through MABC (Ribaut and Ragot 2006).

Marker Assisted Pyramiding (MAP)

Pyramiding is the process of simultaneously combining multiple genes/QTLs together into a single genotype. This is possible through conventional breeding but extremely difficult or impossible at early generations. Using conventional phenotypic selection, individual plants must be phenotypically screened for all traits tested. Therefore, it may be very difficult to assess plants from certain population types (e.g. F2) or for traits with

destructive bioassays. DNA markers may facilitate selection because DNA marker assays are non-destructive and markers for multiple specific genes/QTLs can be tested using a single DNA sample without phenotyping. The most widespread application for pyramiding has been for combining multiple disease resistance genes (Ragimekula *et al.,* 2013).

In order to pyramid disease resistance genes that have similar phenotypic effects, and for which the matching races are often not available, MAS might even be the only practical method, especially where one gene masks the presence of other genes (Sanchez *et al.,* 2000; Walker *et al.,* 2002). The Barley Yellow Mosaic Virus (BaYMV) complex as an example is a major threat to winter barley cultivation in Europe. As the disease is caused by various strains of BaYMV and Barley Mild Mosaic Virus (BaMMV), pyramiding resistance genes seems an intelligent strategy. Since, phenotypic selection cannot be carried out due to the lack of differentiating virus strains. Thus, MAS offers promising opportunities. Suitable strategies have been developed for pyramiding genes against the BaYMV complex. What has to be taken into account when applying such strategies in practical breeding is the fact that the pyramiding has to be repeated after each crossing, because the pyramided resistance genes are segregating in the progeny (Werner *et al.,* 2005).

Nisha *et al.,* (2015) developed wheat lines by virtue of possessing resistance to one or more type of rusts and powdery mildew has definite advantage over their susceptible recurrent parents. The combination of rust resistance genes *Sr2*, *Sr24* and *Sr36* in the genetic background of commercial wheat varieties 'Lok-1' and 'Sonalika' provides strong resistance against stem rust races in India, while its response against races prevalent in other geographical region has to be tested. Durability of resistance to multiple rusts and races can be strategically deployed in varieties with high yield potential. The pyramided lines may also serve as fairly good genetic background for the subsequent addition of genes conferring other desirable agronomic traits such as drought and salt tolerance etc.

Combined Marker-Assisted Selection

The strategic combination of MAS with phenotypic screening is known as 'combined MAS' (coined by Moreau *et al.,* 2004). It may have advantages over phenotypic screening or MAS alone in order to maximize genetic gain (Lande and Thompson, 1990). This approach could be adopted when additional QTLs controlling a trait remain unidentified or when a large number of QTLs need to be manipulated. In some situations a marker assay may not predict phenotype with 100% reliability. However, plant selection using such markers may still be useful for breeders in order to select a subset of plants using the markers to reduce the number of plants that need to be phenotypically evaluated. This may be particularly advantageous when the cost of marker genotyping is cheaper than phenotypic screening (Han *et al.,* 1997). This was referred to as 'tandem selection' by Han *et al.,* (1997) and 'stepwise selection' by Langridge and Chalmers (2005).

Simulation studies indicate that this approach is more efficient than phenotypic screening alone, especially when large population sizes are used and trait heritability is low (Hospital and Charcosset, 1997). Zhou *et al.,* (2003) observed in wheat that, MAS combined with phenotypic screening was more effective than phenotypic screening alone for a major QTL on chromosome 3BS for *Fusarium* head blight resistance (Table 2). In practice, all MAS schemes will be used in the context of

the overall breeding programme, and this will involve phenotypic selection at various stages to confirm the results of MAS as well as to select for traits or genes for which the map location is unknown.

Marker Assisted Breeding (MAB)

During the last 15 years, marker-assisted breeding (MAB) has gained importance among wheat breeders. The application of molecular markers has enabled breeders to select superior genotypes for traits that are difficult to select based solely on phenotype or to pyramid desirable combinations of genes into a single genetic background. MAB also offers the opportunity to improve response from selection because molecular markers can be applied earlier in the life cycle (for example gametic selection in the F1 seedling stage) (Randhawa *et al.,* 2013). MAB not only contributes improved precision for selection of specific traits but is also cost-effective compared with conventional plant breeding procedures. MAB also offers the opportunity to hasten transfer of desirable alleles from unadapted genetic backgrounds into a desirable germplasm through cross-breeding. To date, 30 different loci responsible for traits like resistance to various diseases, quality and agronomy (plant height, photoperiod response, grain weight, tolerance to abiotic stress, etc.) have been cloned, and 97 functional markers have been developed to categorize 93 alleles based on gene sequences (Liu *et al.,* 2012). Within traditional breeding systems, although MAB can be applied to all segregating generations, it is most commonly applied to early generations, including the F1 of complex crosses to enrich populations with favourable genes (Randhawa *et al.,* 2013).

The application of MAB in plant breeding programmes depends on several critical factors including the following:

The molecular marker and gene of interest should be very closely linked, the marker needs to be validated to show trait association in the desired genetic backgrounds grown under target environments (Sharp *et al.,* 2001) and the screening methodology should be cost-effective, time-saving and highly reproducible across laboratories (Randhawa *et al.,* 2013). In Canada, wheat breeders, agronomists, pathologists and physiologists have given special emphasis to improving adaptation to biotic and abiotic stresses (ability to produce stable grain yield over locally variable environmental conditions), earliness and end-use quality of wheat. Breeding for disease resistance, particularly against the rusts: leaf rust (Puccinia triticina), stem rust (*Puccinia graminis* f. sp. tritici) and stripe rust (Puccinia striiformis); Fusarium head blight (FHB); and insects including wheat midge (Sitodiplosis mosellana G ehin) and wheat stem sawfly (Cephus cinctus Nort.) has been practiced routinely in wheat breeding programmes (Randhawa *et al.,* 2013). The application of doubled haploid (DH) technology in wheat breeding programmes has increased the speed of cultivar development, particularly in winter wheat, where use of contra-season nurseries to achieve two breeding cycles per year is not possible. Wheat breeders screen parental plants for various alleles before DH production and haploid plants are subjected to marker assisted selection prior to chromosome doubling to ensure the retention of gene(s) of interest and to discard undesirable genotypes. (Randhawa *et al.,* 2013).

Results and Discussion

Advantages of MAS over conventional methods

In addition to the cost and time savings, for a number of breeding scenarios, MAS methods

are likely to offer significant advantages compared with conventional selection methods.

Gene stacking for a single trait

MAS allows breeders to identify the presence of multiple genes/alleles related to a single trait, when the alleles do not exert individually detectable effects on the expression of the trait, *e.g.* when one gene confers resistance to a specific disease, breeders would be unable to use traditional phenotypic screening to add another gene to the same cultivar in order to increase the durability of resistance.

In such cases, MAS would be the only feasible option, provided markers are available for such genes.

Early detection

MAS allows alleles for desirable traits to be detected early *i.e.* in the seedling stage itself well before the trait is expressed phenotypically. This benefit can be particularly important in slow growing and long duration crops.

Recessive genes

MAS allows breeders to identify heterozygous plants that carry a recessive allele of interest whose presence cannot be detected phenotypically. In traditional breeding approaches, an extra step of selfing is required to detect phenotypes associated with recessive genes.

Heritability of traits

MAS is mainly useful in selection for traits with low heritability up to a point, gains from MAS increase with decreasing heritability.

Seasonal considerations

MAS offers potential savings compared with conventional selection when it is necessary to screen for traits whose expression depends on seasonal parameters.

Using molecular markers, at any time of the year, breeders can screen for the presence of an allele (or alleles) associated with traits that are expressed only during certain growing seasons. For example, CIMMYT's wheat breeding station in northern Mexico is usually used for screening segregating germplasm for leaf rust resistance. However, expression of leaf rust is not uniform in all growing seasons. When there are seasons with low expression of leaf rust, markers, if available, can be a valuable alternative as a tool for screening.

Geographical considerations

MAS is necessary to screen for traits whose expression depends on geographical considerations. Using molecular markers, breeders in one location can screen for the presence of an allele (or alleles) associated with traits expressed only in other locations.

Multiple genes, multiple traits

MAS offers potential savings when there is a need to select for multiple traits simultaneously. With conventional methods, it is often necessary to conduct separate trials to screen for individual traits.

Biological security considerations

MAS provides a potential advantages over selection based on the use of potentially harmful biological agents (e.g. artificial viral infections or artificial infestations with pathogens), which may require specific security measures.

Recent developments in DNA marker technology together with the concept of marker-assisted selection provide new solutions for selecting and maintaining desirable genotypes. Marker assisted selection can be performed in early segregating populations and at early stages of plant development for pyramiding the resistance genes, with the ultimate goal of producing varieties with durable or multiple disease resistance. Thus, with MAS it is now possible for the breeder to conduct many rounds of selection in a year. Molecular marker technology is now integrated into existing plant breeding programmes all over the world in order to allow researchers to access, transfer and combine genes at a faster rate and with a precision not previously possible (Ragimekula *et al.*, 2013).

However, potential limitations that might restrict the wide application of MAS in breeding were high costs and non-availability of suitable markers but, not as MAS is less efficient compared to phenotypic selection. On the contrary, especially in breeding of bi- or perennial crops markers were expected to lead to a high efficiency gain. Regarding the impact of MAS on breeding in near future an increase in relevance and application is unanimously expected. New technological developments such as automation, allele-specific diagnostics and diversity array technology will make MAS based gene pyramiding more powerful and effective. Especially the increased application of SNPs and improved technologies for sequencing will contribute to an increasing impact of MAS.

Table.1 Origin and usefulness of designated Sr-genes in conferring adult plant resistance to Ug99 race

Origin of Sr genes	**Stem rust resistance (Sr) genes**	
	Ineffective	**Effective**
Triticum aestivum	5, 6, 7a, 7b, 8a, 8b, 9a, 9b, 9f,10, 15, 16, 18, 19, 20, 23,30, 41, 42, Wld-1	28[a], 29[b], Tmp[a]
Triticum turgidum	9d, 9e, 9g, 11, 12, 17	2[b], 13[a,b], 14[a]
Triticum monococcum	21	22, 35
Triticum timopheevi		36[a], 37
Triticum speltoides		32, 39
Triticum tauschii		33[b], 45
Triticum comosum	34	
Triticum ventricosum	38	
Triticum araraticum		40
Thinopyrum elongatum		24[a],25, 26, 43
Thinopyrum intermedium		44
Secale cereale	31	27, 1A. 1R[a], R
Triticum aestivum	5, 6, 7a, 7b, 8a, 8b, 9a, 9b, 9f,10, 15, 16, 18, 19, 20, 23,30, 41, 42, Wld-1	28[a], 29[b], Tmp[a]

[a]Virulence for the gene is known to occur in other races.
[b]Level of resistance conferred in the field usually not enough.

(Ravi *et al.*, 2008.)

Table.2 Published markers for important genes in wheat

Trait	Locus	Source	Marker	Chromosome	Reference
Stem Rust	Sr2	*T. turgidum*	RFLP/STS	3BS	Johnston *et a.,* 1998
	Sr5	*T. aestivum*	RFLP	6DS	Parker *et al.,* 1998
	Sr9e	*T. aestivum*	RFLP	2BL	Parker *et al.,* 1998
	Sr22	*T. monococcum*	RFLP	7AL	Paull *et al.,* 1995
	Sr36	*T. timopheevii*	RFLP	2BS	Parker *et al.,* 1998

The MABC strategies will gain importance and more emphasis is needed on combined selection systems, rather than viewing MAS as a replacement for phenotypic or field selection. It is also critical that future endeavours in MAS are based upon lessons that have been learnt from past successes and especially failures in using MAS (Ragimekula *et al.,* 2013).

Further optimization of marker genotyping methods in terms of cost effectiveness and a greater level of integration between molecular and conventional breeding represent the critical aspects for the greater adoption of MAS in crop breeding in the near future. The increase in importance of MAS is not expected to be the same for all crops, for high value crops it may be of top priority. The new tools of molecular breeding will have a better opportunity for demonstrating their true values for crop improvement, when these techniques reach a higher degree of automation; it will be possible to use molecular markers leading to "gene revolution" in the world of agriculture (Ragimekula *et al.,* 2013).

Marker–Assisted Selection (MAS) for improving rust resistance

Pyramiding of several genes into one cultivar can be an effective strategy to use resistance genes to enhance durability of wheat resistance to leaf and stem rust (Leonard and Szabo, (2005). Durable resistance may be achieved by combination of several genes encoding partial resistance. Gene pyramiding through conventional methods is difficult and time-consuming because it requires simultaneous tests of the same wheat breeding materials with several different rust races before a selection is made. Usually, it is not feasible for a regular breeding program to maintain all necessary rust races needed for this type of work. Therefore, MAS is a powerful alternative to facilitate new gene deployment and gene pyramiding for quick release of rust-resistant cultivars. In the present time, the research of stem rust in wheat has focused on identifying more resistance genes to control Ug99. According to the Farm and Ranch Guide report, currently 50% of winter wheat and 70 to 80% of spring wheat used in the USA are susceptible to Ug99. Moreover, 75-80% of the breeding materials are susceptible to Ug99 and most stem rust resistance genes deployed in breeding programs have been overcome by this new fungus.

Microsatellite marker closely linked to resistance gene *Sr40* have been also obtained (Wu, 2003). To date three genes for leaf rust resistance in wheat Lr1, Lr10 and Lr 21 (Huang *et al.,* 2003) have been isolated, cloned and sequenced. They all have sequences that encode nucleotide binding site (NBS)-leucine-rich repeat (LRR) regions, which are characteristic of disease resistance genes in plants. Molecular description of these genes in wheat provides a unique biological system to study the molecular mechanisms of wheat-pathogen interaction and transduction as well as the resistance gene function, evolution and diversity. This will allow further manipulation of wheat resistance genes to increase the resistance durability by genetic transformation of wheat.

Borlaug Global Rust Initiative

Borlaug Global Rust Initiative (BGRI) (earlier

Global Rust Initiative) was implemented on September 9, 2005 at Nairobi, Kenya with the objectives: to monitor the spread of wheat stem rust race Ug99, to screen the released varieties and germplasm for resistance to Ug99, to distribute the sources of resistance worldwide, breeding to incorporate diverse resistance genes and adult plant resistance gene into high-yielding adapted varieties. Under the framework of BGRI, the evolution and migration of the Ug99 group of races are being monitored carefully so as to provide early warning to the farmers and wheat rust researchers in case of an epidemic. It will help the farmers as well as researchers in decision making. India, one of the strong partners of BGRI, is actively participating in the germplasm testing in Kenya and Ethiopia along with that from CIMMYT, ICARDA and various other countries. The success of BGRI lies in a timely replacement of stem rust susceptible cultivars with resistant ones having equal or better yield potential and other necessary characteristics (Bhardwaj *et al.*, 2014).

In conclusion, Major genes, when deployed singly, have the effect of generating directional selection toward virulence resulting in boom and bust cycles. The result of continuous boom and bust cycles are a diminished gene pool of effective stem rust resistance genes. Single genes deployed over large acreages have short life spans. Pyramiding several, major and minor, stem rust resistance genes into adapted varieties as opposed to breeding varieties with a single resistance gene is considered a more effective method to combat new races. Therefore, recent progress on molecular marker development and improved donor sources are accelerating the pyramiding and deployment of cultivars with more durable resistance to stem rust. To date, 60 genes have been designated for resistance to wheat stem rust. Over the last century, these genes have been identified within common wheat and wild relatives. Many Sr genes of common wheat origin have been deployed during major efforts to incorporate genetic resistance to stem rust in wheat cultivar development. After the detection of TTKST, a new variant of Ug99 in 2006 from Kenya the usefulness of the gene *Sr2* was reduced. However, it has been still reported effective against other races of Ug99 lineage. Although there are several genes showing considerable amount of resistance to Ug99 group of stem rust races yet, only *Sr22*, *Sr26*, *Sr35* and *Sr50* are known to be effective against all currently reported races of the group. Combining resistance genes to develop durable resistance is the prevailing strategy for gene deployment in wheat. Markers linked resistance with would be useful for marker-assisted pyramiding of this gene with other major and APR genes for which closely linked markers are available. Many genetics stock and varieties of wheat have been developed which are resistant to Ug99 and other important pathotypes of stem rust in Indian sub-continent.

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Index Keywords	
MAS	Marker Assisted Selection
MABC	Marker Assisted Backcrossing
MARC	Marker Assisted Recurrent Selection
MAP	Marker Assisted Pyramiding
Combined MAS	Combined Marker Assisted Selection
MAB	Marker Assisted Breeding
SSR	Simple Sequence Repeat
AFLP	Amplified Fragment Length Polymorphism
RAPD	Rapid Amplified Polymorphic DNA.
NB_LRR	Nucleotide Bind Leucine Rich Repeats
Dart	Diversity Array Technology
SNP	Single Nucleotide Polymorphism
QTL	Quantitative Trait Loci
BaYMV	Barley Yellow Mosaic Virus
Ug99	Uganda 99
BGRI	Borlaug Global Rust Initiative.
SrGH	Stem Rust Glossy Haguenot