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Pyramiding of resistance genes *Sr36 and Sr2* in durum wheat background (HI 8498) through marker assisted selection for resistance to stem rust race 117- group pathotypes

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Abstract. Stem rust (*Puccinia graminis* f. sp. *tritici)* has historically been one of the major constraints in realizing stabilized durum wheat yields in central India. Pyramiding of genes into a single genotype has been one of the preferred strategies in wheat rusts management. Currently, HI 8498 (Raj 6070/Raj 911) is the most popular durum wheat cultivar in central India. However, it is susceptible to a number of Indian pathotypes of stem rust race 117-group. Hence, a planned breeding programme was taken up to develop derivatives of HI 8498 with improved resistance by pyramiding stem rust resistance genes *Sr36* and *Sr2* through marker assisted selection. These were targeted as besides being broad-spectrum resistance genes, they have had their origin in tetraploid wheats, *Sr36* in *Triticum timopheevii* and *Sr2* in *T. turgidum* var. *durum*. While, durum wheat genotype IWP 5070 was chosen as donor parental line for *Sr2* gene, based on the presence of 'pseudo-black chaff (*pbc*)', a tightly linked phenotypic marker; Australian bread wheat variety 'Songlen' was used as donor for *Sr36*, since none of the 75 durum wheat genotypes tested was found positive for this gene using tightly linked co-dominant microsatellite marker *Xstm773-2*. Moreover, Songlen is known to carry *Sr2* gene as well. The markers being used for foreground selection in the HI 8498 derivatives are '*pbc*' and CAPS marker *csSr2* for the gene *Sr2* and SSR marker *Xstm773-2* for the gene *Sr36.* The stem rust resistance in the 'HI 8498' derivatives (BC_3F_1) carrying *Sr2* and *Sr36* individually has improved significantly (terminal disease severity 0 to 5S), compared to the background cultivar HI 8498 (30S – 40S). Furthermore, a total of 770 representative SSR markers distributed throughout the wheat genome were screened for polymorphism between parental genotypes HI 8498, Songlen and IWP 5070. Of these, 165 markers showed polymorphism and are being used as effective markers in Marker Assisted Background Selection (MABS) to identify 99 % of recurrent parent genome (RPG) i.e., HI 8498 in BC_3F_1 generation of both the populations involving *Sr36* and *Sr2* genes to facilitate their pyramiding in common recipient parent background.

Keywords. Durum wheat – Stem rust resistance – MAS – Gene pyramiding.

Cumul des gènes de résistance SR36 et Sr2 chez le génotype de blé dur (HI 8498) par la sélection assistée par marqueurs pour la résistance à la rouille noire de la race du groupe pathotypes 117

Résumé. *La rouille noire (Puccinia graminisf. sp. tritici) a toujours été l'un des principaux obstacles à la réalisation de rendements stables pour le blé dur dans le centre de l'Inde. Le cumul de gènes dans un seul génotype a été l'une des stratégies privilégiées dans la gestion des rouilles du blé. Actuellement, HI 8498 (Raj 6070/Raj 911) est le cultivar de blé dur le plus populaire dans le centre de l'Inde. Cependant, il est sensible à un certain nombre de pathotypes indiens de la rouille noire de la race du groupe 117. Ainsi, un programme de sélection planifié a été proposé pour développer des dérivés de HI 8498 avec une meilleure résistance à la rouille noire par le cumul des gènes de résistance SR36 et Sr2 à travers la sélection assistée par marqueurs. Ces gènes ont été ciblés comme étant des gènes de résistance à large spectre, ils tirent leurs origines de blés tétraploïdes, T. timopheevii pour SR36 et T. turgidum var. durum pour Sr2. Alors que le génotype de blé dur IWP 5070 a été choisi comme lignée parentale donneuse pour le gène SR2, basée sur la présence de « pseudo-black chaff (pbc) », un marqueur phénotypique étroitement lié; la variété australienne de blé tendre Songlen a été utilisé comme donneur pour SR36, puisque aucun des 75 génotypes de blé dur testées a été trouvé positif pour ce gène en utilisant le marqueur microsatellite co-dominant Xstm773-2 étroitement lié. En outre, Songlen est connu pour porter aussi le gène Sr2. Les marqueurs utilisés pour la sélection de premier plan dans les dérivés de HI 8498 sont 'pbc' et le marqueur CAPS csSr2 pour le gène*

Sr2 et le marqueur SSR Xstm773-2 pour le gène SR36. La résistance à la rouille noire chez les dérivés de HI 8498 (BC3F1) portant Sr2 et SR36 individuellement a amélioré de manière significative (sévérité terminale de la maladie de 0 à 5S), par rapport au cultivar HI 8498 (30S - 40S). En outre, un total de 770 marqueurs SSR représentatifs répartis sur tout le génome du blé ont été analysés pour le polymorphisme entre les génotypes parentaux HI 8498, Songlen et IWP 5070. Parmi ceux-ci, 165 marqueurs ont montré un polymorphisme et sont utilisés comme marqueurs efficaces pour la sélection du fond génétique assistée par marqueurs (MABS) pour identifier les 99% du génome du parent récurrent (RPG), à savoir, HI 8498 dans la génération BC3F1 des deux populations impliquant les gènes SR36 et Sr2 pour faciliter leur cumul dans le fond génétique du parent destinataire commun.

Mots-clés. *Blé dur – Résistance à la rouille noir – MAS – Cumul de gènes.*

I – Introduction

Durum wheat (*Triticum turgidum var. durum*) is the second most important wheat species globally as well as nationally, after bread wheat with a share of around 5% in the total wheat production of >90 million tons in India. Durum wheat cultivation offers many advantages like saving irrigation water due to its high water-use efficiency, field tolerance to loose smut and Karnal bunt diseases, employment generation through durum based fast food industry and provide better nutrition as it is rich in protein, β-carotene and essential micronutrients like iron and zinc. In fact, durum wheat was the predominant wheat species grown in central India, particularly in the Malwa plateau in Madhya Pradesh, parts of Gujarat, southern Rajasthan and Bundelkhand region of Uttar Pradesh. However, area under durum wheat cultivation continuously declined due to limited yield potential and susceptibility to rust diseases of old varieties, and by the seventies, durum wheat got almost out of cultivation. Hence, durum improvement programme was intensified at IARI – Regional Station, Indore.

A large number of rust resistance donors, particularly among the exotic collections, were identified and utilized in crop improvement. Three improved durum wheat varieties HI 8381 (Malav-shri), HI 8498 (Malavshakti) and HD 4672 (Malav Ratna) were released by the station, which immensely contributed to the revival or resurrection of durum wheat in Central India. While Malavshri and Malavshakti were released for irrigated timely sown conditions, and Malav Ratna was for rainfed and limited irrigation. HI 8498 (Malavshakti) is currently the most popular durum wheat cultivar in Central India under irrigated, timely sown (November sowing) conditions. Combining high yield with earliness, disease resistance, and excellent grain quality, it has proved to be a truly 'landmark' variety in the history of wheat crop improvement in central India. It showed resistance to stem rust pathotypes *40A* and *40-1* which exhibit high degree of virulence to bread wheat varieties. However, it showed susceptibility to several pathotypes of stem rust race *117* group like *117-6* (*37G19*), *117A* (*38G2*), *117-1* (*166G2*), and *117A-1* (*38G18*).

Breeding for durable resistance by pyramiding multiple resistance genes, both major and minor ones, is an effective strategy in managing plant diseases (Gupta *et al,* 1999, Singh *et al,* 2001). Therefore, a planned breeding programme was initiated to pyramid the genes *Sr2* and *Sr36* into HI 8498 background for improving resistance to the aforesaid pathotypes of stem rust race *117* group. Wheat stem rust resistance gene ***Sr36*** (syn. *SrTt-1*), derived from *Triticum timopheevii* (Allard and Shands, 1954), shows effectiveness to many stem rust pathotypes including *117-6* which is highly virulent to durum wheat (Mishra *et al* 2009). The gene ***Sr2*** was originally transferred from Yaroslav emmer wheat into hexaploid wheat (Mc-Fadden, 1930), which has been utilized in breeding for around 60 years as a source of durable and broad-spectrum adult-plant resistance. *Sr2* confers partial resistance only in the homozygous state because of its recessive inheritance due to which traditional breeding with *Sr2* is difficult to carry out. *Sr2* is closely linked to pseudo-black chaff ('pbc'), controlled by partially dominant gene which produces the characteristic stem and head melanism in wheat, but its levels of expression vary with genetic backgrounds and environments (Mc-Fadden, 1939, Kota *et al.,* 2006).

Both *Sr2* and *Sr36* are widely effective against the Indian stem rust populations including the race *117*-group pathotypes. Hence, transferring these genes into HI 8498 background could broaden and diversify its resistance base. However, pyramiding genes in a single line through classical breeding methods can be time consuming or even impossible, especially when more than one gene confers resistance against known races of *P. graminis* f.sp. *tritici*; as it becomes difficult to identify genotypes carrying combinations of more than one gene (Tsilo *et al.,* 2008). Hence, phenotypic and molecular marker assisted selection was resorted to for pyramiding the 'target' genes in common background.

II – Material and Methods

1. Material

Recipient parent HI 8498, a high yielding durum wheat variety with good adaptability was released for growing under timely sown conditions. Australian bread wheat cultivar 'Songlen' which was documented to carry *Sr36,* and IWP 5070, a durum wheat genetic stock carrying *Sr2* were used as donors for these resistance genes. Stem rust pathotype *117-6* was used for tracking resistance derived from the donors. Crosses between Songlen/HI 8498 and HI 8498/IWP 5070 were performed during *rabi* 2009-10. During the same time, parental polymorphism survey was done between recipient parent HI 8498 and the two donor parents Songlen and IWP5070 using 730 markers covering all the chromosomes (Sourdille *et al., 2004*). The F_1 seed obtained were sown in *rabi* 2010-11 at IARI –RS farm, Indore to produce BC_1F_1. All the plants in BC_1F_1 were screened for the presence of resistance genes *Sr36* and *Sr2* with the help of *Xstm773-2* and CAPS marker *csSr2* along with pseudo-black-chaff trait, respectively. The positive plants based on molecular analysis results and 'resistance phenotype' was utilized to produce BC_2F_1 populations. Markers which were polymorphic between HI 8498 and Songlen, and HI 8498 and IWP 5070 were used for background selection in backcross (BC_2F_1 and BC_3F_1) populations generated from the respective crosses to identify individuals with maximum genome of HI 8498. The foreground positive plants of both the populations with maximum recovery of recurrent parent HI 8498 were utilized for pyramiding of genes into the background of HI 8498. At all stages, the pathological screening in the field was done by syringe inoculating each plant with freshly collected uredospores of pathotype *117-6*.

2. TPCR analysis

For molecular analysis, DNA was extracted directly from two leaf stage of young plants with CTAB method to get pure form of genomic DNA for PCR analysis. Two pairs of primers were used for PCR analysis. Primers *Xstm773-2*F 5' AATCGTCCACATTGGCTTCT 3' and *Xstm773-2*R 5'-CGCAACAAAATCATGCACTA 3'were designed based on the published sequence (Tsilo *et al., 2008*), and the amplified fragment co-segregating with the *Sr36* gene was used for foreground selection of *Sr36* gene. The PCR conditions for *Xstm773-2* were *:* denaturing step: 95°C, 4 min, amplification step (40 cycles): 94°C, 30 sec; 60°C, 30 sec; 72°C, 30 sec, extension step: 72°C, 7 min (PCR amplified products were resolved on 3.5% metaphor gel for SSR marker). Similarly, Primers *csSr2*F 5'CAAGGGTTGCTAGGATTGGAAAAC 3' and *csSr2* R 5'AGATAACTCTTAT GATCTTACATTTTTCTG 3' were designed according to sequence information (McNeil *et al.,* 2008), and were used for detection of *Sr2* gene. The amplification products derived from the former primer pair are co-dominant, whereas those from the latter pair are dominant and require further digestion of amplified PCR product through *Bsp*HI restriction enzyme. The PCR conditions for *csSr-2* marker were : denaturing step : 95°C, 2 min, amplification step (30 cycles): 94°C, 30 sec; 60°C, 40 sec; 72°C, 50 sec, extension step: 72°C, 5 min. For CAPS analysis after completion of PCR, an additional 5 µl of mix consisting of 2.5 ml of 10x NEB buffer 4 and 0.5 µl of *Bsp*HI (10 U/ µl; NEB) was added and the tubes were incubated at 37°C for 1 h. After completion of restriction digestion the product was separated on a 2.5 % (w/v) agarose gel (R. Mago *et al.,* 2011).

III – Results and Discussion

Among the parents, amplification was noticed with *X**stm773-2*** marker only with different amplicon sizes i.e., 155 bp – Resistant and 190 bp – Susceptible utilizing *Sr36*-Near isogenic line (NIL) as positive control. It was observed that *Sr36*-NIL and Songlen had the band 155 bp i.e., resistant (presence of *Sr36*) (Fig. 1), while other varieties were having the band 190 bp i.e., susceptible (absence of *Sr36*).

With the help of CAPS marker *csSr-2* (Fig. 2) with *Bsp*HI restriction enzyme, we had validated the presence of *Sr-2* gene linked to "pbc" phenotype in IWP 5070 viz., the presence of the band 172 bp, 112 bp and 53 bp i.e., resistant (presence of *Sr2 gene*) along with the pseudo-black chaff *(pbc*) phenotype in the field. Similar band pattern with CAPS marker *csSr-2* with *Bsp*HI restriction enzyme digestion was observed in IWP 5070 and Songlen as showed in Hope by R. Mago *et al.,* 2011, whereas, in HI8498, null type allele (lack of amplification) was observed as in Chinese Spring. Therefore, this CAPS marker behaved like a dominant marker.

Figure 1. Band pattern of plants with *Sr36* with *Xstm773-2* marker.

Figure 2. Band pattern of plants with *Sr2* with CAPS marker *csSr-2.*

1. Screening of durum wheat germplasm for presence of *Sr36* gene

Presence of the gene *Sr36* is not documented in any of the known durum genotypes. Hence, 75 durum wheat genotypes representing a cross section of Indian durum wheat germplasm were analyzed using closely linked molecular marker *Xstm773-2,* but none of the durum genotypes tested was found to carry the gene (Singh *et al., 2013*).

2. Parental polymorphism of parents

Marker assisted background selection is used for recurrent parent genome selection, which requires more number of polymorphic markers equally distributed in the genome of parents. So for this purpose, we have screened 730 SSR primers distributed in all the chromosomes of wheat, out of which 177 SSR primers were found to be polymorphic, and these will be used for background selection of parent HI 8498 (Fig. 3 and 4). 151 SSR primers were found polymorphic between Songlen and HI 8498, while, 77 primers between IWP 5070 and HI 8498. Due to differences at species and ploidy level, a large number of polymorphism was observed between Songlen and HI 8498, compared to IWP 5070 and HI 8498.

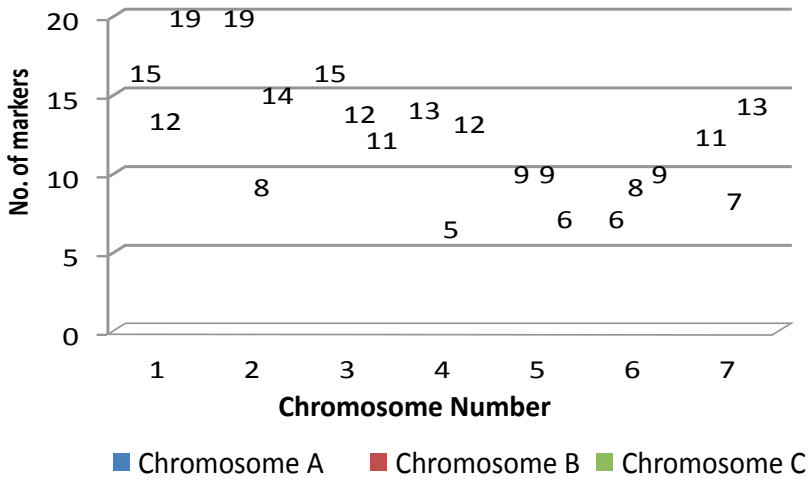


Figure 3. Chromosmal distribution of polymorphic SSR primers.

1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3

1= HI8498; 2 = Songlen; 3 = IWP5070 for 16 different markers

Figure 4. Parental polymorphism with different SSR markers.

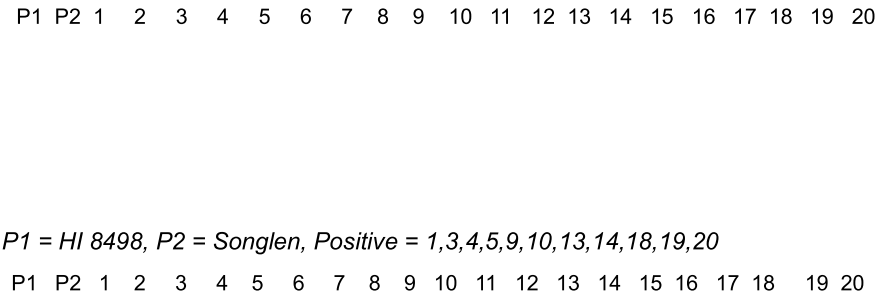


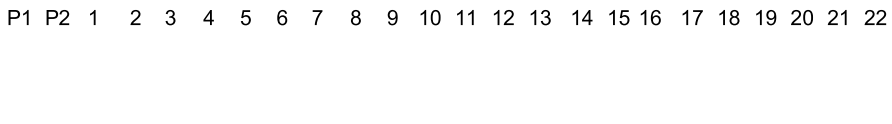
Figure 7. Background selection (MABS) of foreground positive plants for *Sr36.*

Table 1. RPG of foreground positive plants of HI 8498 along with *Sr36.*

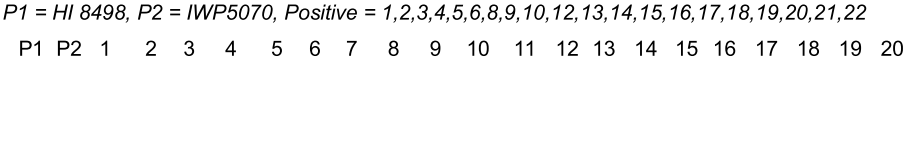
S. No.	Plant ID No.	Percentage recovery
1	WF1	95.8
2	WF3	95.8
3	WF4	95.8
4	WF6	95.8
5	WF16	97.5
6	WF17	100.0
7	WF18	100.0
8	WF33	97.5
9	WF37	97.5
10	WF42	100.0
11	WF46	97.8
12	WF50	98.9
13	WF58	95.8
14	WF60	97.5
15	WF61	95.8

Sr2: Based on the information gained through parental polymorphism, polymorphic markers were utilized for background selection to select plants with high recurrent parent genome recovery (RPG) *i.e*, band pattern for markers CFD238 and CFD54 is shown as example in Fig 8 and RPG recovery in BC_3F_1 population through GGT2 program was shown in Fig 9. In BC_3F_1, RPG ranged from 82 to 100 percent in the foreground positive plants, out of which 14 plants showed > 95% with homozygous bands as recipient parent, HI 8498 (Table 2). The rust reactions of these positive plants are in the range of 5R to 10S. Of these 14 plants, 4 plants showed 100% RPG and similar plant type of HI 8498, which were selected for pyramiding of the gene *Sr2*.

Figure 8. Graphical representation of RPG of HI 8498 along with *Sr36.*



P1 = HI 8498, P2 = IWP5070, Positive = 1,2,3,4,5,6,8,9,10,12,13,14,15,16,17,18,19,20,21,22



P1 = HI 8498, P2 = Songlen, Positive = 1,2,3,5,7,8,9,10,11,12,13,16,17,19,20

Figure 9. Background selection (MABS) of foreground positive plants for *Sr2.*

Table 2. RPG of foreground positive plants of HI 8498 along with *Sr2*.

S. No.	Plant ID No.	Percentage recovery
1	WE 29	95.4
2	WE 32	100.0
3	WE 42	95.4
4	WE53	100.0
5	WE76	95.4
6	WE 117	97.2
7	WE127	100.0
8	WE 130	95.4
9	WE131	97.2
10	WE 143	97.2
11	WE 145	97.2
12	WE 151	95.4
13	WE 161	95.4
14	WE 163	100.0

Figure 10. Graphical representation of RPG of HI 8498 along with *Sr2*.

5. Pyramiding of *Sr36* and *Sr2* in HI 8498 background

Foreground positive plants with maximum recurrent parent genome were selected in BC_3F_1 population derived from Songlen and IWP 5070 with HI 8498 background. Crosses were made among high RPG plants with resistance genes *Sr36* and *Sr2*, looking mostly like HI 8498 in field with good resistance to stem rust race *117-6*. DNA markers *Xstm773-2* and *csSr2* were utilized for individual selection of both the genes in a single plant which really helped us to discriminate the plants for the presence of both the genes in a single cultivar, which would have been impossible in the phenotypic screening because *Sr36*-resistance could mask the *Sr2* resistance which is expressed in adult plant stage. Positive homozygous plants for both the genes will be selected for multiplication and further utilization. Efforts are in progress to select homozygous plants in F_2 of the pyramided population with resistance genes *Sr36* and *Sr2*, looking mostly like HI 8498 in field with good resistance to stem rust race *117-6* using both PCR analysis and phenotyping. It was observed that the positive plants selected through MAS functioned normally as recipient parent HI 8498. Positive homozygous plants of this population involving *Sr36* and *Sr2* genes will help to facilitate their pyramiding in common recipient parent HI 8498 background, its multiplication and further utilization.

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