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Research Article

Introgression of stem rust resistance gene *Sr36* into durum wheat back ground using marker assisted backcross breeding

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Abstract

India is one of the major producers of durum wheat in the world. Rusts are the important diseases which are considered to be most important constraints in the global wheat production. Stem rust caused by *Puccinia graminis* sf. sp. *tritici* has been particularly severe on durum wheat. Even though the durum wheat offers resistance against certain aggressive and more virulent pathotypes of 40 group like 40A (62G29) and 40-1 (62G29-1), it is highly susceptible to certain other pathotypes belonging to 117 group. Wheat stem rust resistance gene *Sr36* (syn. *SrTt-1*), derived from *Triticum timopheevii* shows effectiveness to many stem rust pathotypes including 117-6 (37G19) which is highly virulent to durum wheat. Presently, *Sr36* gene is present in bread wheat background, whereas, none of the durum wheat genotypes found to have *Sr36* gene. An effort is being made to transfer *Sr36* gene into most popular durum variety HI 8498 by utilizing molecular marker assisted backcross breeding.

Keywords: Durum wheat, stem rust, *Sr36*, marker assisted backcross breeding

1. Introduction

India is one of the major durum wheat (*Triticum turgidum var durum*) producers with around 2.5 million tons production per year. Stem rust (*Puccinia graminis sf.* sp. *tritici)* has historically been one of the major constraints in realizing stabilized durum wheat yields in central India. Durum wheat provides better nutrition as it is rich in protein, β-carotene and essential micronutrients like iron and zinc. The durum wheat is 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.

Most of the Indian durum genotypes showed resistance to stem rust pathotypes 40A (62G29) and 40-1 (62G29-1) which are highly virulent and aggressive on bread wheat varieties. However, they showed susceptibility to several pathotypes of stem rust race 117 group, 117-6 (37G19) being most virulent, followed by 117A (38G2), 117-1 (166G2), 117-3 (167G3) and 117A-1 (38G18) (Mishra *et al.,* 2009).

Many major and minor rust resistance genes have been utilized in fighting against rust diseases. However, majority of these resistance genes have been utilized in the improvement of bread wheat with little or none attention to durum wheat. 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(37G19) which is highly virulent on durum wheat (Mishra *et al.,* 2009).Bread wheat stocks CItr 12632 (= W1656) and CItr 12633, had served as the original sources of *Sr36* in wheat breeding programs worldwide (McIntosh *et al.,* 1995). *Sr36* is located on chromosome 2BS, and has been deployed in many Australian wheat cultivars (Bariana *et al.,* 2001) and some soft winter wheat cultivars in the USA (Jin and Singh, 2006). Presence of the gene *Sr36* is not documented in any of the known durum genotypes.

HI 8498 (Malavshakti) is currently the most popular durum wheat cultivar in Central India due to its high yield with earliness, disease resistance, and excellent grain quality and 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 (62G29) and 40-1(62G29-1); however it is susceptible to 117 group.

Breeding for rust resistance is a continuous process due to high evolutionary nature of rust pathogens. So an effort is being made to transfer this important resistance gene from bread wheat background to durum wheat.

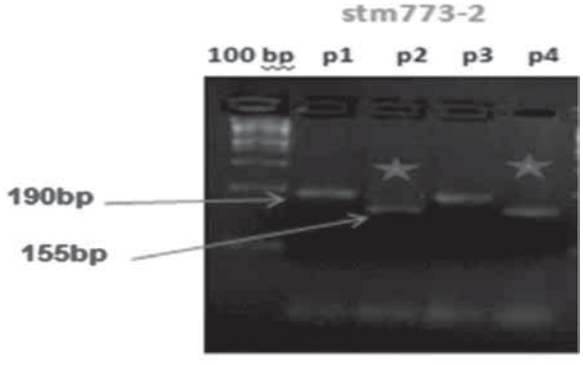
2. Materials and methods

As the presence of the *Sr36* gene is not documented in any of the known durum genotypes, initial survey was taken up to look for the gene. Hence, 74 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 were found to carry the gene (Sushil, *et al*., 2013). Australian bread wheat cultivar 'Songlen' which was documented to carry *Sr36* used as donor for this resistance gene. The presence of the gene was also confirmed with the closely linked molecular marker. Stem rust pathotype117-6(37G19) was used for tracking resistance derived from the donors. Cross was performed between Songlen / HI 8498(recurrent parent).

PCR analysis: A SSR marker, *STM773-2* (*Xstm773-2*F 5'- AATCGT CCACATTG GCTTCT -3' and *Stm773-2*R 5'-CGCAACAAAATCATGCACTA -3') which is closely linked to *Sr36* (Tsilo*et al*., 2008) was used for foreground selection (*Sr36*). PCR was performed in a 96-well plate with 10 µL of final reaction mixture containing 3.5 µL ddH_2O, 1.5 µL 10X PCR buffer containing 15mM $Mgcl_2$, 0.8 µL of 10 m MdNTPs, 1 µL of each forward and reverse primer, 0.2 µL of 3U µL–1 Taq DNA polymerase (All reagents obtained from Genei), and 2 µL of genomic DNA. The reaction mixture was initially denatured at 94°C for 10 min, followed by 35 cycles of 94°C for 1 min, annealing temperature of 60°C for 1 min and 72°C for 2 min, with a final extension step of 72°C for 10 min and 4° C indefinitely. About 2 µL of gel loading dye was added to the PCR products and was subjected to gel electrophoresis in SFR gel (3%) (M/S Amresco) at constant 90 volts for 2 hours and visualized under UV gel documentation system for the presence of DNA bands and the amplified fragment co-segregating with the *Sr36* gene was used for foreground selection of *Sr36* gene. Parental polymorphism using SSR markers was done between recipient parent HI 8498 and donor parent Songlen using 730 markers covering all the chromosomes (Sourdille *et al.,* 2004).

3. Results and discussion

The different amplicons were noticed with *Xstm773-2* marker i.e., 155bp – Resistant and 190 bp – Susceptible. 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 bpi.e., susceptible (absence of *Sr36*).



P1-HI 8498; P2-Songlen; P3-IWP5070; P4-*Sr36* NIL

Fig 1. Band pattern of plants with *Sr36* with *stm773-2* marker

Parental polymorphism of parents: Around 730 SSR primers distributed in all the chromosomes of wheat were utilized to identify polymorphism between the parents, out of which 151 SSR primers were found to be polymorphic, and these were used for background selection of parent HI 8498 (Fig 2).

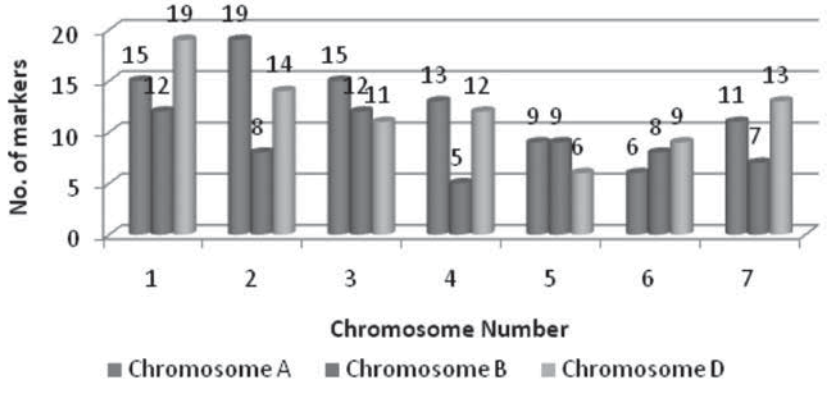
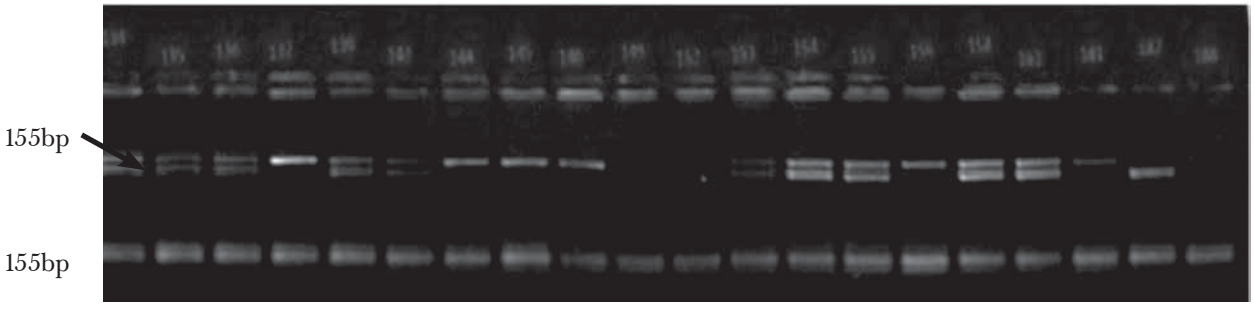


Fig 2. Chromosomal distribution of polymorphic SSR primers

Screening of backcross population for foreground selection of Sr36 gene: In BC_1 population, out of 56 plants, 21 plants showed the presence of the band 155bp i.e., resistant (presence of *Sr36*), whereas, other plants had band 190bpi.e, susceptible (absence of *Sr36*). Plants positive for *Sr36* showed good stem rust resistance in the field along with good expression and were used to develop the BC_2 population by crossing with the recurrent parent HI 8498. In BC_2 population, out of 60 plants, 65 *per cent* of the plants (39 plants) showed the presence of the band 155bp, with good stem rust resistance; whereas, other plants (35%) had band 190 bp i.e., susceptible (absence of *Sr36*). In BC_3 population, out of 92 plants, 27 *per cent* of the plants (24 plants) showed the presence of the band 155 bp i.e., resistant (presence of *Sr36*) (Fig 3) with good stem rust resistance and durum wheat 'HI 8498' plant type; whereas, the remaining plants (73 %) had band 190 bp i.e., susceptible (absence of *Sr36*). The stem rust resistance in the 'HI 8498' derivatives (backcross populations) carrying *Sr36* individually has improved significantly (terminal disease severity 0 to 5S), compared to the background cultivar HI 8498 (30S – 40S).



Marker-*stm773-2*; BC_3 population

Fig 3. Band pattern of plants in the BC_3 population for *Sr*36

3.1 Recurrent parent genome recovery (%) (RPG) through marker assisted background selection (MABS): Based on the information gained through parental polymorphism, polymorphic markers were utilized for MABS to select plants with high recurrent parent genome recovery. In $BC_3F_{1,}$ RPG ranged from 87.5 to 100 percent in the foreground positive plants, out of which 15 plants showed > 95% with homozygous bands as recipient parent, HI 8498 (Table 1). The rust reactions of these positive plants are in the range of 0 to 5S. Of these 15 plants, 3 plants showed 100 per cent RPG and similar plant type of HI 8498, which were selected for pyramiding of the gene *Sr36*.

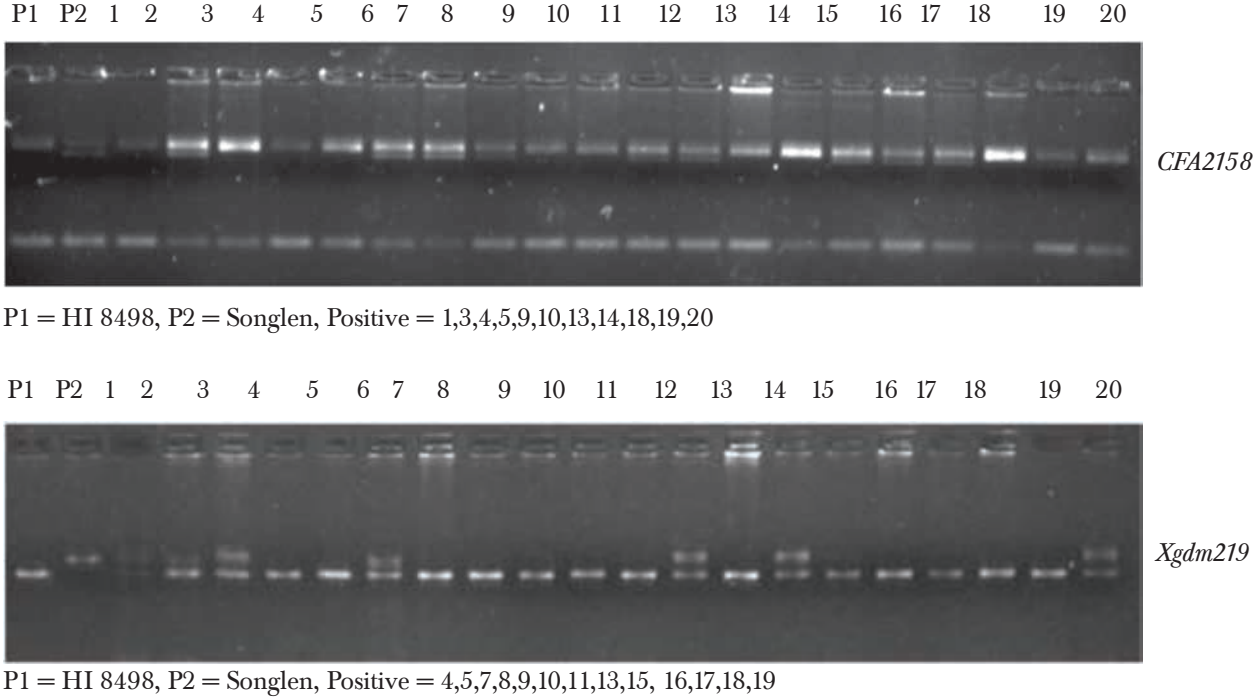


Fig 4. Band pattern of background selection of foreground positive plants for *Sr36*

Table 1. RPG recovery (%) of foreground positive plants for *Sr36* by MABS

Plant ID	Percentage recovery	Plant ID	Percentage recovery
WE 29	95.4	WE 130	95.4
WE 32	100.0	WE 131	97.2
WE 42	95.4	WE 143	97.2
WE53	100.0	WE 145	97.2
WE76	95.4	WE 151	95.4
WE 117	97.2	WE 161	95.4
WE127	100.0	WE 163	100.0

As many of major and minor genes have been knocked down by stem rust pathogen due to its many evolved variants it is more important to have more genes to fight against this pathogen. The stem rust resistance gene *Sr36* offers near immune reaction to many pathotypes belonging to the 117 group will be helpful in providing resistance to durum wheat on which these pathotypes are highly virulent. The variety HI 8498 once developed with the gene *Sr36* introgressed, will serve as a good source of resistance for developing stem rust resistant durum wheat varieties and genotypes in near future.

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