Marker assisted approach for incorporating durable rust resistance in popular Indian wheat cultivars

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ABSTRACT

Rust diseases are common foliar fungal diseases of wheat in India. A major challenge is to avoid rust epidemics as cultivars remain under constant threat of new virulent races. The most promising long-term control strategy against leaf, stripe and stem rusts, is to breed and deploy cultivars carrying durable resistance. To address this issue, molecular marker approach is being utilized for enhancing rust resistance mainly through identification of durable rust resistance gene and pyramiding of different seedling and adult plant resistance genes. Recent studies have indicated that leaf tip necrosis (LTN) is not a reliable marker for confirming presence of durable rust resistance gene *Lr34* in wheat genotypes. Considering this, wheat genotypes postulated to carry *Lr34* gene by virtue of having LTN were screened with 6 microsatellite markers including *gwm1220, gwm130, gwm295, SWM 8 , SWM 9, SWM10* and one STS marker (*csLV34*) reported to be linked with *Lr34* locus. *SWM10* and *csLV34* were found to be useful markers to know the presence of *Lr34* in breeding lines. Indian wheat genotypes confirmed to possess *Lr34* gene through this study, have been identified which are being recommended for utilization to enhance durable rust resistance in breeding programmes. Gene pyramiding using seedling and adult plant resistance genes through 'simultaneous and step wise transfer' approach was followed using molecular markers reported to be linked with different rust resistance genes such as *Lr24, Lr28, Lr35, Lr37* etc. Their subsequent utilization was made for screening BC_1F_1 and double cross F_1 populations where the chi-square analysis showed no segregation distortion in the marker allele(s). With the emergence of new virulent pathotype, possible menace of stem rust looms large and thus genotypes possessing atleast two stem rust resistant genes effective against *Ug99* were selected. Marker assisted pyramiding/introgression of *Sr24, Sr25* and *Sr26* along with durable adult plant resistance genes such as *Sr2* is underway. MAS approach, supported by host–pathogen interaction has been found quite useful for enhancing rust resistance in wheat genotypes.

INTRODUCTION

The leaf rust resistance gene *Lr34* carry the most durable forms of resistance. Leaf tip necrosis, thought to be associated reliable morphological marker, turned out not the most reliable one. Molecular markers reported by different Research labs for MAS of *Lr34* gene were scrutinized. The most informative markers were utilized to screen Indian wheat genotypes. There are certain wheat cultivars such as Lok1, HUW234, HD2733, HD2687, WH147, PBW 343 etc which are in cultivation in India since long time (occupying about 10 million hectares). These are quite popular with the farmers either because of some components of adaptability in them or because of market demands. But otherwise they are prone to leaf and / or stripe rusts. Any attack of prevalent pathotypic races may cause substantial yield losses in them. This called for strengthening of efforts to bring in genetic resistance against these rusts. Marker assisted selection was performed in early segregating generations to select the plants generated in the background of these cultivars with different effective leaf rust (*Lr24, Lr28, Lr34, Lr35, Lr37*) and stripe rust (*Yr10, Yr15*) resistance genes.

Stem rust disease is generally prevalent in warm wheat growing areas such as the peninsular and central India, hills of Nilgiris, summer wheat crop areas of Himachal Pradesh and Jammu and Kashmir. However, under favourable environment it may occur in other parts of the country also. During February 1999, high levels of stem rust infection was observed on entries in wheat (*Triticum aestivum*) grown in Uganda. A highly virulent stem rust pathotype, *Pgt*-Ug99, was identified. Pathogenicity of *Pgt*-Ug99 was studied in seedling tests of wheat varieties containing *Sr31*, as well as other stem rust differential lines (Park and Bariana, 2005). Virulence of this race to *Sr31* was confirmed. Therefore, an immediate requirement to face the challenge posed by it was to identify wheat lines resistant to this race.

MATERIALS AND METHODS

A set of wheat genotypes postulated to possess *Lr34* gene based on published work of various researchers was selected for the present study. The *LTN* trait was recorded visually at stage 65-69 (Singh 1992; Zadoke *et al.* 1974). Genotypes were grouped LTN positive or LTN negative. For estimating the area under disease progress curve (AUDPC), the infection types (tR, R, tMS, MS, tS and S) were recorded following McNeal *et al.* (1971) Amplification was carried out with six simple sequence repeat (SSR) markers (*gwm1220, gwm130, gwm295, SWM8, SWM9, SWM10*) and one STS marker *csLV34*. Genetic linkage of molecular markers & genes responsible for leaf rust resistance was investigated. Permutations mean values of all markers were used for the study. Mean p value was taken by calculating the mean p value at field and at net house and permutation values for different leaf tip necrosis association was selected for the present study. Paired t-test was performed between the group of genotypes based on

presence / absence of *Lr34* and / or LTN. Marker assisted pyramiding of different leaf and stripe rust resistance genes was carried out by using 'simultaneous but stepwise transfer' method. This approach involved transfer of genes from different donor parents to HUW 234 (HUW12/SPRW//HUW12) as well as to LOK 1(S 308/S 331) parents in independent backcrossing programmes but simultaneously to combine them into one individual at the end. The markers utilized for MAS were from the research publications viz., *Lr24*(Prabhu *et al*. 2004); *Lr28*(Prabhu *et al*. 2003); *Lr35(*Gold *et al*. 1999); *Lr37(*Helguera *et al.* 2003); *Yr10(*Wang *et al*. 2002) and *Yr15(S*un *et al*. 1997). Efforts were put forth to incorporate various effective stem rust resistance genes *Sr24* and *Sr26*. A near isogenic line of Darf (Darf*6/3Ag3/Kite) developed using Kite, was the source genotype for *Sr24* and *Sr26*. Lines developed *viz.,* HW 2021, HW 2026 and HW 2027 were subjected to confirmation using MAS following the markers reported by Mago *et al.*, (2005).

RESULTS AND DISCUSSIONS

Marker assisted selection in various generations viz., BC_1F_1, BC_2F_1 and Double cross F_1 and F_2 was performed. Chi square value of expected and observed marker assisted screening data in different generations of various crosses showed 1:1 segregation (in backcross generations) and 3:1 segregation in F_2 generation. Also based on the observations recorded (disease reaction) on marker assisted selected plants in various generations *viz.,* BC_1F_1, BC_2F_1 and F_2, linkage distance between the trait and marker was estimated. Plants having gene combination *Lr24 + Lr37, Lr24 + Lr28, Lr35 + Lr28, Yr10+Yr15* etc were confirmed using markers. In general, plants selected having introgressed resistance genes showed enhance resistance without much deviation in the traits of adaptation. This would lead to early identification of potential cultivars / resistance sources for future breeding programmes.

Among 6 microsatellite markers used for screening a set of 40 genotypes, 7, 7,6,5,4 and 3 alleles were produced by *gwm1220, SWM8, gwm130, SWM9, SWM5* and *gwm295,* respectively. General Linear Model association showed maximum value of 0.997 for *XWM9* whereas; minimum value of 0.24 for *gwm295.* But even *gwm295* marker did not show any association with the *Lr34* gene. P value was maximum i.e. 0.919 for *Xgwm130* marker and was minimum for the marker *gwm295* (0.126). The two recently reported markers linked to *Lr34* gene namely *SWM10* (Bossolini *et al.,* 2006) and *csLV34* (Lagudah *et al.,* 2006) were used to screen all the 40 genotypes. Screening with microsatellite marker *SWM10* gave amplified fragment of 211bp, which was reported to co-segregate with the *Lr34* gene. Similarly, marker *csLV34* showed marker allele of 150bp size. The genotypes found out to be positive for *Lr34* gene (*SWM10* & *csLV34*) were also reported to be *Lr34* positive by various researchers on the basis of *LTN* and AUDPC data (Saini *et al*., 1988; Gupta and Saini, 1987; Singh and Gupta 1991; Bahadur 1998; Nayar *et al.* 1999). Hence both these markers proved to be good for screening out the genotypes possessing *Lr34* gene. As *csLV34* marker was STS based marker, it was found user friendly in comparison to *SWM10,* which gave resolution only on Polyacrylamide gel electrophoresis. In further studies, it was found that LTN may be associated with low disease severity, but is not a reliable marker for gene *Lr34*, as has been advocated by previous researchers. The results of amplification with both *Sr24* and *Sr26* markers in HW 2021, HW 2026 and HW 2027 were found in absolute conformity with the disease reaction of these lines under high inoculum load of *Pgt* which was 20R- MR, 20R -MR and 5R- MR, respectively.

ACKNOWLEDGEMENT

Authors gratefully acknowledge the financial support from Indian Council of Agricultural Research and Indo-Swiss Collaboration in Biotechnology in carrying out this work. Help rendered by Prof. Beat Keller, University of Zurich, is also acknowledged.

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