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Short Communication

Molecular validation and screening of *Triticum dicoccoides* Korn. accessions for stripe rust résistance gene *Yr*15 with SSR marker

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Biotic Stresses in wheat are a major constraint that limits the production. Among several diseases, which attack wheat, the most common are those caused by fungal pathogens. Besides other two rusts, stripe rust or yellow rust, (*Puccinia striiformis* Westend f. sp. *tritici* Eriks.) of wheat is more destructive particularly in temperate, cooler and humid regions than the tropics. It is most devastating disease of wheat throughout the world. China is the largest epidemic region in the world [1]. In India, stripe rust is confined to northern hills, foot hills of Himalayas and north western region of the country. Stripe rust also appears in southern hill zone. Because of its early appearance it can cause severe damage resulting into poor tillering and shriveled grain. The most effective, efficient and economical mean for control of stripe rust is the use of genetic resistance. There are about 40 resistance genes (*Yr*1-*Yr*40), which have so far been identified and assigned to specific chromosomal location, while 23 are provisional [2]. Most of the cultivars grown in northern Indian plains are susceptible to stripe rust and only a few genes namely, *Yr*A, *Yr*2, *Yr*2Ks, *Yr*9, *Yr*18 and *Yr*27 have been postulated in the present day cultivars, which are not effective against one or more pathotypes. Gene *Yr*9 is most frequently occurring among the varieties released after 1995. Of the 40 *Yr* genes designated so far, only five have been transferred from wild relatives of wheat. One such resistance gene *Yr*15, located on 1BS has been transferred from wild emmer, *Triticum dicoccoides* (2n=4x=28, genome AABB) into common wheat [3] and it is effective against virulent Indian pathotypes of stripe rust [4] in seedling and adult stage. *Yr*15 has been introgressed into Sunstar*6/C80-1 and HD2329 a popular Indian cultivar. To confirm the introgressed gene, genetic analysis through test of allelism or comparison of the infection type with differentials is necessary. However, with the development of DNA markers, the confirmation of a target gene can be authenticated, provided molecular marker (s) closely linked with the gene are available. Wild emmer provides extensive and promising genetic resources for disease resistance in wheat. In addition to, *Yr*15, another gene conferring resistance to stripe rust *Yr*35 has also been transferred into wheat [5]. About 35 accessions of *T. dicoccoides* received from ICARDA have been evaluated for stripe rust resistance [4]. Twenty one randomly chosen amongst them were screened with the *Yr*15 linked molecular marker to study DNA polymorphism. The results on validation of *Yr*15 linked molecular marker and screening of *T. dicoccoides* accessions are reported here.

The material comprised the *Yr*15 introgressed lines Sunstar*6/C80-1//V763-2312 (Selection Pusa T3479) and HD2329*5/C80-1//V763-2312 a donor V763-2312 and Negative controls, namely, HD2687, WH147 and HD2329 for validation of molecular marker. Twenty one diverse accessions of *T. dicoccoides* obtained from ICARDA, namely, GSP-3, IG46148, IG46522, GSP-6, GSP-13, GSP-1, GSP-14, 00-01/21, IG46391, IG46386, GSP-9, IG46353, IG46434, GSP-25, IG46506, GSP-2, IG46304, *T. dicoccoides*-4, IG46431, IG119405, *T. dicoccoides* (WTC IARI) were also screened for the presence of *Yr*15.

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DNA extraction

DNA Samples were isolated by CTAB method [6] from the fresh leaves of seedlings grown for 10-15 days. These were then purified by RNase treatment. Quantification was then done in 0.8% Agarose gel by using ë uncut (50 ng/µl) as marker.

PCR conditions

Identification of molecular marker for *Yr*15 was done using SSR primer Xgwm273 [7]. 10µl PCR mixture was prepared for PCR analysis comprising: 50ng template DNA, 12ng of each of forward and reverse primers, 0.1mM dNTP's, 1X PCR buffer (10mMTris, pH 8.0, 50mM KCl and 50mM Ammonium sulphate), 1.8mM $MgCl_2$ and 0.25 unit Taq polymerase (MBI, Fermentas). The volume was made up to 10ml by autoclaved MQ H_2O. PCR was run for 40 cycles at 95°C for 4 min, 95°C for 1 min, 60°C for 1min, 72°C for 2 min and final extension step of 72°C for 5 min and final storage step at 4°C. Equal amount (10ml) of 1X gel loading dye was mixed with the PCR products. For gel analysis, 3% Metaphor gel was prepared and 7ml of PCR product + dye was run for 3 hour at 70 Volt. Gel was visualized under UV light using Gel Documentation Equipment for scoring the bands.

273 is specific to stripe rust resistance gene *Yr*15. Concurrently 21 accessions of *T. dicoccoides* were also screened for the presence of stripe rust resistance using same primer, Xgwm273. These accessions have also earlier been screened against a mixture of stripe rust pathotypes under artificially inoculated conditions at Delhi. Among them, six accessions of *T. dicoccoides*, which showed resistance in adult stage, namely, IG46148, IG46522, IG46434, GSP-25, IG46506 and IG119405 amplified a band similar to that of *Yr*15 positive bread wheat cultivars and the remaining

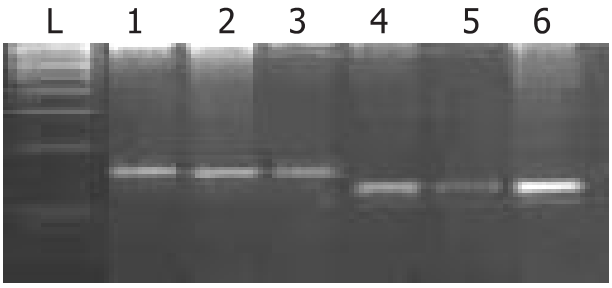

Fig. 1. Validation of *Yr*15 (M: Molecular weight marker, 1 = V763-2312, 2 = HD2329*5//V763-2312, 3 = Selection Pusa T3479, 4 = HD2687, 5 = WH147, 6 = HD2329)

Gene	Marker	Primer sequence	Product size	Distance (in cM)	Annealing Temp (in°C)
*Yr*15	Xgwm 273	5' ATTGGACGGACAGATGCTTT 3' 3'AGCAGTGAGGAAGGGGATC 5'	156bp	5.7	60

Most of the present day Indian cultivars are susceptible to stripe rust pathotypes. The effectiveness of seedling and adult plant resistance genes against Indian stripe rust pathotypes has been studied earlier [4, 8-9]. One such effective dominant gene *Yr*15 has been introgressed into popular cultivar HD2329 and an exotic line Sunstar*6/C80-1 utilizing a donor V763-2312 and the resistance present in them has also been assessed in seedling and adult stage [4].

The lines V763-2312, Sel. Pusa T3479 (Sunstar*6/C80-1//V763-2312), HD2329*5/C80-1//V763-2312 and negative control genotypes, HD2687, WH147 and HD2329, were used in gel-electrophoresis for validation of *Yr*15. As expected, genotypes, HD2329*5/C80-1//V763-2312, Sunstar*6/C80-1//V763-2312, and V763-2312 produced *Yr*15 band of 156 bp whereas, HD2687, WH147 and HD2329 did not (Fig. 1). Presence of a single band of 156 bp in all the tested bread wheat genotypes with *Yr*15 indicated that the primer Xgwm accessions showed polymorphic bands as they have slight variation in fragment size (Fig. 2). The presence of *Yr*15 band at a specific site in 6 accessions of *T. dicoccoides* indicate that these accessions might have received the gene through natural introgression during the coarse of evolution as distance between the gene *Yr*15 and SSR primer Xgwm273 is only 5.7 cM. All these six accessions have minor variation in their morphology and therefore, it is likely that the each accession got isolated in smaller pockets during the process of speciation and secondary origin. The results indicate that the identified marker is efficient and able to give correct resolution for this specific gene. However, the stripe rust resistance postulated in these accessions is similar to conferred by *Yr*15. The phenotyping with different yellow rust pathotypes is suggested to identify other accessions for genetic diversity for stripe rust resistance. Further, it is suggested that the effective gene such as *Yr*15 should be deployed in areas, where

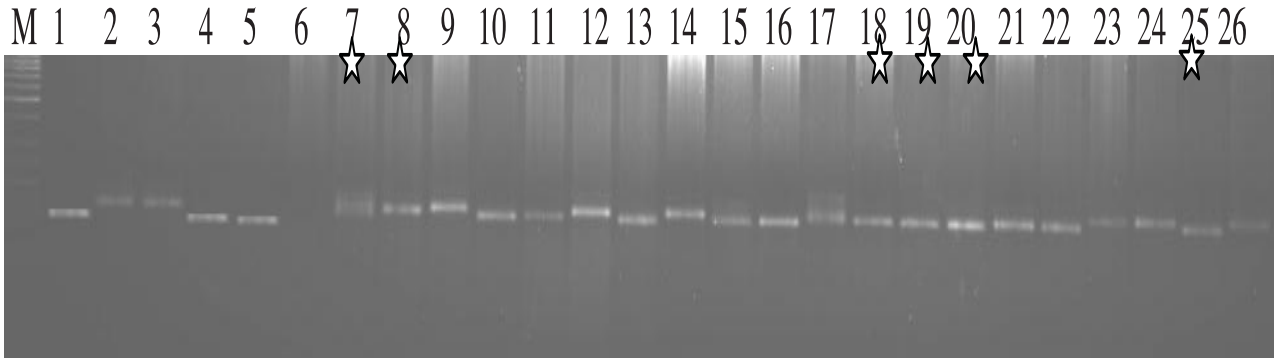

Fig. 2. Screening of *T. dicoccoides* accessions for *Yr*15 (M: Molecular weight marker, 1. HD2687, 2. V763-2312, 3. Selection Pusa T3479, 4. HD2329, 5. Agra Local, 6. GSP-3, 7. IG46148, 8. IG46522**, 9. GSP-6, 10. GSP-13, 11. GSP-1, 12. GSP-**

☆ indicating the band similar to Yr15 band position-156bp

yellow rust is likely to cause damage to wheat production. Selection Pusa T 3479 and HD 2329*5/C80-1//V 763-2312 are under field evaluation for their yield potential.

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