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# Development and Validation of Scar Marker for Stem Rust Resistance Gene *Sr26* in Wheat (*Triticum aestivum* L.)

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## Abstract

The stem rust resistance gene *Sr26* imparts resistance to all the virulent pathotypes of stem rust (*Puccinia graminis* f. sp. *tritici*) including a new race of pathogen named TTKSK (syn.Ug99). Wheat genotypes including non-carrier Kalyan Sona (-*Sr26*) and carrier Kite (+*Sr26*) of the *Sr26* gene were analysed using polymerase chain reactions and a RAPD marker OPAE07620 was found to link with the desired trait. Linkage between the marker OPAE07620 and rust resistance phenotype was confirmed by analyzing F2 population obtained from a cross between a resistant and susceptible genotype. The Random Amplified Polymorphic DNA (RAPD) marker was converted into sequence characterized amplified region (SCAR) marker SCOPAE07620. The marker was validated in another segregating population and 45 wheat genotypes including carrier and non-carriers of *Sr26* gene. The marker developed in this study can be used for pyramiding of the *Sr26* gene with other major resistance genes that are effective against the TTKSK lineage (Ug99) which will help in attaining durable resistance against a disease that poses a serious threat to global wheat production.

**Keywords:** *Triticum aestivum* L.; Stem rust resistance; *Sr26*; SCAR marker; MAS

## Introduction

Wheat is the most widely grown cereal crop globally after maize and rice which provides 21% of food calories and 20% of the protein for more than 4.5 billion people in 94 developing countries [1]. The rust diseases of wheat, i.e., leaf rust, stripe rust, and stem rust are major biotic constraints in the world [2]. Stem rust, caused by *Puccinia graminis* f. sp. *tritici*, is one of the most destructive diseases of wheat. Stem rust epidemics have resulted in as much as 50% yield losses in recent years [3], A new race of the pathogen named TTKSK (syn. Ug99) detected in Uganda in 1999 [4] and its variants are virulent to many designated and undesignated stem rust resistance genes, losses due to Ug99 can be as high as 90% [3]. An effective method to combat emergence and spread of Ug99 races would be to stack several broadly effective resistance genes into new adapted variety using marker assisted breeding (MAB). Success of gene pyramiding depends on the availability of molecular markers tightly linked to resistance genes *Sr22*, *Sr26*, *Sr25* and *Sr35* that confer resistance to Ug99 and other virulent races [5] and breeding efforts to pyramid these genes are already underway.

*Sr26* is one the few known major resistance genes effective against the *Sr31*-virulent race Ug99 (TTKSK). *Sr26* was transferred from Knott's *Agropyron elongatum* Thatcher stock [6] into Australian cultivars such as Eagle, Kite and Jabiru. Translocation lines with shorter *Agropyron elongatum* segments were developed that have been observed not to exhibit the reported 9% yield penalty associated with the *Sr26* segment. The effectiveness of this gene against the TTKS family of races, its low frequency among Indian cultivars and the availability of donor lines with shortened alien segment makes *Sr26* ideal for use in MAB. Several DNA markers linked to various stem rust resistance genes in wheat have been identified and developed. The genes include *Sr2* [7,8], *Sr1RAmigo* [9], *Sr6* [10], *Sr9a* [11], *Sr24* [9,12], *Sr25* [13], *Sr26* [12,13], *Sr31*[14], *Sr35* [15], *Sr36* [16], *Sr38* [17], *Sr39* [18], *Sr40* [19]. These molecular markers help in deployment of several resistance genes in single cultivar which is otherwise a time-consuming method if done using rust reaction in field condition. Molecular markers might simulate selection gain [20] because they can be assessed in high-throughput techniques at a very early growth stage with high heritability and they are relatively cheap [21]. They can be used to characterize parental material better, thereby improving the efficiency and effectiveness of parental selection for crossing and to track genes in segregating progenies through the selection process. The marker reported in this study is based on co-segregation study for stem rust resistance conferred by *Sr26* and hence the results and procedures may be applied for use in MAS. It will assist in the stacking of resistance gene with other broadly effective resistance genes in order to develop wheat lines with potentially stable and durable stem rust resistance.

## Materials and Methods

### Plant materials

A total of 45 wheat genotypes (seeds were procured from IARI, New Delhi; DWR, Karnal and NBPGR, IIWBR Regional Station (previously DWR Regional Station), Flowerdale, Shimla) were used for validation of marker in this study (Table 1) of which 12 were carriers of *Sr26* and remaining 33 were non-carriers of *Sr26*. A segregating F2 population was developed from cross between Kalyan Sona (susceptible) and Kite (resistant; +*Sr26*). Another cross was made between Kalyan Sona and Takari (+*Sr26*) for validation of the marker.

| S. No. | Varieties/ Genotypes | *Sr26* status | SCAR status | S. No. | Varieties/ Genotypes | *Sr26* status | SCAR status |
|---|---|---|---|---|---|---|---|
| 1 | Takari | + | + | 24 | HW-2001 | - | - |
| 2 | Flinder | + | + | 25 | HW-2003 | - | - |
| 3 | Jabiru | + | + | 26 | HW-2004 | - | - |
| 4 | Harrier | + | + | 27 | FLW-2 | - | - |
| 5 | Apollo | + | + | 28 | A-9-30-1 | - | - |
| 6 | HW2002 | + | + | 29 | NW-1012 | - | - |
| 7 | HW2023 | + | + | 30 | PBW-373 | - | - |
| 8 | HW2027 | + | + | 31 | UP-2338 | - | - |
| 9 | HW2090 | + | + | 32 | HW-1085 | - | - |
| 10 | HW2094 | + | + | 33 | HD-2687 | - | - |
| 11 | HW2096 | + | + | 34 | HD-2189 | - | - |
| 12 | HW2099 | + | + | 35 | HS-240 | - | - |
| 13 | NIAW-301 | - | - | 36 | Marquis | - | - |
| 14 | PBW-435 | - | - | 37 | Reliance | - | - |
| 15 | Lok-45 | - | - | 38 | Kanchan | - | - |
| 16 | PBN-51 | - | - | 39 | Vidisha | - | - |
| 17 | Hindi-62 | - | - | 40 | Vaishali | - | - |
| 18 | NP-846 | - | - | 41 | Kota | - | - |
| 19 | Sonora-64 | - | - | 42 | Kavkaz | - | - |
| 20 | HI-385 | - | - | 43 | W3531 | - | - |
| 21 | Kharchia-65 | - | - | 44 | RL-6087 | - | - |
| 22 | A-206 | - | - | 45 | PBW-343 | - | - |
| 23 | Bijaga Yellow | - | - | | | | |

**Table 1:** List of wheat genotypes used for validation of marker.

Stem rust inoculation and scoring: Seedlings in field plots, about one month old were inoculated with the pathotype 122 (7G-11, obtained from IIWBR, Regional Station, Flowerdale, Shimla) and disease reactions on the leaves of each plant were recorded at 14 days after inoculation using disease rating scale [22]. To ensure heavy inoculums build-up of stem rust, Cv. Agra Local rows were planted and artificially inoculated with stem rust collected from experimental plots in the previous year. F3 generation rows corresponding to F2 generation plants were also scored for rust resistant phenotype and were characterizing as (RR, rr, Rr) homozygous for resistance or susceptibility, or segregating for resistance to stem rust and also deduce the genotype of respective F2 individuals.

DNA isolation, RAPD and Gel electrophoresis: DNA was extracted from the non-inoculated, fully expanded, 21 days old leaves according to Eswaran et al. [23]. RAPD analysis was carried out using 100 random decamer primers procured from Operon Technologies Inc., Alamaenda, CA, USA (kits A, B, C, D, E). The PCR reactions were performed in 25 µl volume, containing 10 mm Tris-HCl (pH 9.0), 2.0 mm $MgCl_2$, 50 mm KCl, 200 µm of each dNTP, 25 pmol of primer, 1.0 Unit of Taq DNA polymerase (Bangalore Genei Pvt. India Ltd.) and 50 ng of template DNA. The PCR was carried out in Mastercycler gradient PCR machine (Eppendorf) using the following thermal profile: 94°C – 5 min, 42°C – 5 min, 72°C – 5 min (one cycle); 94°C – 1 min, 42°C – 1 min, 72°C – 1 min (45 cycles); final extension at 72°C – 10 min.

SCAR primer design and analysis: For developing SCAR markers the desired band was isolated, cloned and sequenced using primers SCOPAE07620F: 5' (AGTGGCAACTCGTCGGTGT) 3' and SCOPAE07620R: 5' (GTGTCAGTGAGAGTAAAGCGTAGGT) 3'). The PCR amplification was carried out in a 25 µl reaction mixture containing 8.0 pmol of each primer, 1.5 mm $MgCl_2$ and 50 ng of genomic DNA while the rest of the PCR components were the same. The thermal cycling conditions were as follows: 1 cycle of 95°C – 5 min, 60°C – 1 min, 72°C – 30 s; 35 cycles of 95°C – 1 min, 60°C – 1 min and 72°C – 30 s and a final extension at 72°C – 10 min.

## Results and Discussion

Identification of RAPD marker for the *Sr26* gene: A RAPD primer OPAE07 (5' GGAAAGCGTC 3') displayed an amplified DNA fragment of 620 bp designated as OPAE07620 specifically in resistant parent and resistant F2 individuals possibly carrying *Sr26* gene while absent in possibly non-carriers of the *Sr26* gene (Figure 1).

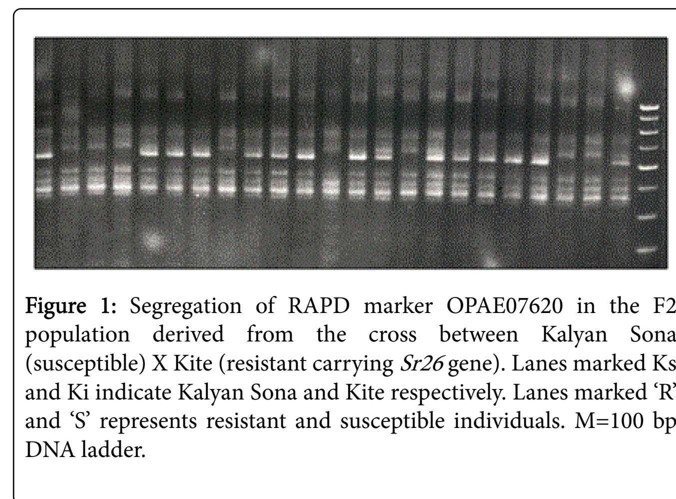

**Figure 1:** Segregation of RAPD marker OPAE07620 in the F2 population derived from the cross between Kalyan Sona (susceptible) X Kite (resistant carrying *Sr26* gene). Lanes marked Ks and Ki indicate Kalyan Sona and Kite respectively. Lanes marked 'R' and 'S' represents resistant and susceptible individuals. M=100 bp DNA ladder.

Linkage analysis: A F2 population of 120 plants (Kalyan Sona × Kite) was analysed for segregation of marker and rust resistance phenotypes. In the F2 population, phenotypic ratio of segregation as resistant to susceptible plants and presence of OPAE-07620 to its absence were observed to be 3:1 (Table 2) and showed a good fit to 3:1 ratio ($\chi^2$=0.711 and P=0.80-0.70). Thus, suggesting monogenic inheritance and dominant action of *Sr26* gene.

| F2 genotype | | | | | Calculated | | |
|---|---|---|---|---|---|---|---|
| Crosses | Resistant | Segregating | Susceptible | Total | $\chi^2$ value | Ratio | P value |
| Ks X Kite | RR | Rr | rr | | | | |
| Observed | 33 | 53 | 34 | 120 | 1.576 | 1:2:1 | 0.50-0.30 |
| Expected | 30 | 60 | 30 | 120 | 1.699 | 3:1 | 0.30-0.20 |
| $\chi^2$ value (table) =3.841 | | | | | | | |

**Table 2:** Phenotypic segregation ratio and $\chi^2$ test for rust reaction in F2 population derived from the cross Kalyan Sona X Kite.

SCAR marker analysis and validation: SCAR marker (SCOPAE07620) is a dominant marker and showed a tight linkage with the rust resistance phenotype yielded 620 bp band in Kite and resistant F2 plants which was absent in Kalyan Sona and susceptible F2 plants (Figure 2 and Table 2). Based on sequence data of the cloned RAPD marker and using software Primer 3.0, primers were designed and were synthesized by Numex Chemicals, Mumbai, India.

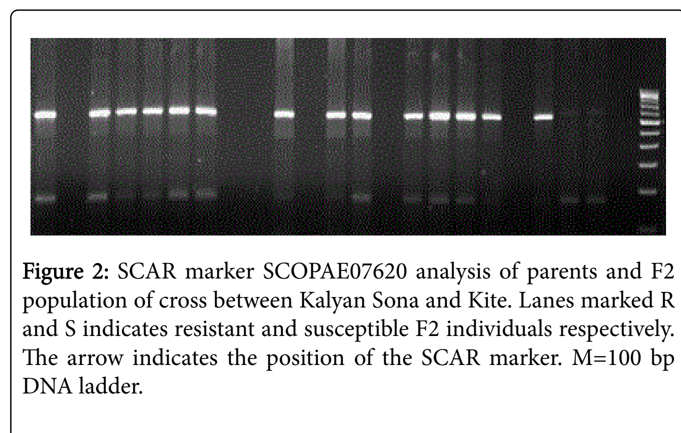

**Figure 2:** SCAR marker SCOPAE07620 analysis of parents and F2 population of cross between Kalyan Sona and Kite. Lanes marked R and S indicates resistant and susceptible F2 individuals respectively. The arrow indicates the position of the SCAR marker. M=100 bp DNA ladder.

Validation of SCAR marker: The SCAR marker SCOPAE07620 was validated using 45 wheat genotypes with known *Sr26* status. The SCAR marker was present in all 12 carriers of *Sr26* gene while absent in remaining 33 non-carriers of *Sr26* gene (Table 1). Validation was also done on other segregating population for *Sr26* gene obtained from cross between Kalyan Sona X Takari (resistant: carrying *Sr26* gene). The presence or absence of the marker co-segregated with the phenotypic status in the validating population.

## Conclusion

The reported SCAR marker SCOPAE07620 was developed using a cross between Indian cultivar with a carrier of *Sr26* gene of Australian origin. Hence this marker will be very valuable in Indian wheat breeding programme and its extensive use can be facilitated by transferring this gene in combination with other genes for enhancement of durability of rust resistance in the wheat lines. During the course of our research, Mago et al. [12] have reported AFLP marker for stem rust resistance gene *Sr26* using pair of NILs, however they did not include the co-segregation studies of marker with the phenotypic scoring in an appropriate segregating population. Also linkage analysis of the marker was not done and marker was only associated with the resistance gene. Liu et al. [13] also developed a diagnostic and co-dominant markers for *Sr26* using multiplex PCR. The marker reported in this study is a co-dominant SCAR marker tightly linked (5.7 cm) to the resistance gene which minimizes the risk of recombination separating the marker from the gene. Marker is polymorphic between gene donor as well as wide range of crop genotypes. Repeatability and reliability of marker was tested and validated on segregating population of appropriate size and hence it is useful for MAS. This study is based on association genetics of the obtained marker with the phenotypic scoring in a F2 segregating population of 120 individuals. The population size is large enough for obtaining linkage between marker and trait of interest. The marker obtained here is 5.7 cm away, thus it is co-inherited with the disease resistance gene *Sr26* which is dependent on the genetic proximity of this marker and the desired gene and can be effectively used in marker assisted selection. The newly identified marker was confirmed by validating it on another segregating population of cross between Kalyan Sona and Takari (carrying *Sr26*) and wheat genotypes with different *Sr26* status. This marker did not amplify in genotypes carrying other stem resistance genes confirming that it can be effectively used in marker assisted selection.

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