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Short Communication

Phenotypic and marker aided identification of donors for spot blotch resistance in Wheat

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Abstract

In this study, multi-locational phenotypic data for spot blotch disease and other agronomic traits of two mapping populations (set-I: Sonalika / BH1146, set-II: Kanchan / Chirya1) generated over two consecutive years (2011-2013) was utilized for identifying potential lines possessing spot blotch resistance. Some promising lines (seven lines in set-I and six lines in set-II mapping populations) based on the performance for resistance and other agronomic traits (days to heading, plant height, spike length and thousand grains weight) were shortlisted and further validated using molecular markers. Diagnostic markers for spot blotch (*Xgwm*371, *Xgwm*425, *Xgwm*445) and SSR markers (*Xbarc59*, *Xbarc*232) associated with percent disease severity identified during preliminary RILs screening were also used to validate these lines for spot blotch resistant. These lines have potential and thus could be utilized for direct testing or as improved donor to incorporate spot blotch resistance in wheat.

Keywords: Spot blotch, linked SSR markers, resistant lines

Spot blotch or *Helminthosporium* Leaf Blight (HLB) caused by *Bipolaris sorokiniana*, is one of the major biotic constraint to wheat production in Africa, South America, warm wheat growing regions of South Asia and particularly to Indian subcontinent, affecting the livelihood of small scale farmers, who depend on the wheat cultivation (Duveiller *et al.,* 2005). Besides spot blotch, this fungus is also the causal agent of other diseases like common root rot, foot rot, seedling blight and seed rot of wheat (Chowdhury *et al.,* 2013, Rajita *et al.,* 2014).With recent changes in cropping pattern leading to delayed sowing and climate change particularly sudden rise in temperature in the month of February with rainfall, the disease is appearing in more severe form. The delayed sowing experiences a warm and humid weather in February which is favorable to spot blotch. The most economical way to manage the disease is following resistance breeding approach. Information on the extent of the genetic variability, heritability and other genetic parameters of spot blotch resistance with other agro-morphology attributes, is pre-requisite for genetic improvement (Singh *et al*., 2007).Therefore, it has now become more important to understand the molecular genetics of spot blotch and to develop varieties/breeding material resistant to spot blotch through introduction of new alleles. In this direction, we developed recombination inbred lines in different back grounds and identified few resistant lines for spot blotch disease along with validation of molecular markers responsible for tolerance to this disease.

Experiment details: The two populations involving contrasting diverse parents (Sonalika/BH1146 and Kanchan/Chirya1) for spot blotch resistance were screened in their $F_{9\text{-}10}$ generation under high disease pressure at two hot spot locations (Coochbehar and Kalyani) in eastern India and also at DWR, Karnal under field and epiphytotic conditions in polyhouse. These recombinant inbred lines were screened in individual row plots for two consecutive crop years 2011-13 for spot blotch resistance along with other morphological traits. Observations were recorded on spot blotch score (final recording around late dough stage), days to heading, plant height (cm), spike length (cm) and 1000-grains weight (g) at appropriate stage of crop. Genomic DNA was isolated from 15 days old seedlings of the parents and RILs (F_8). Diagnostic markers reported earlier *Xgwm*371, *Xgwm*425, *Xgwm*445(Kumar *et al.,* 2010)

and SSRs-*Xbarc59*, *Xbarc*232were used to validate the promising lines for spot blotch resistant. The PCR product was resolved on 3% (w/v) agarose.

*Phenotypic evaluation:*The phenotypic data for spot blotch and other agronomic traits of importance taking mean performance based on three replications during two consecutive years was taken and analyzed. Screening for spot blotch was done on double digit scale (00-99). Total RILs (set-I=220, set-II=215) were categorized into HR, R, MR, MS, S, HS representing highly resistant (00-03), resistant (13-25), moderately resistant (35-46), moderately susceptible (57-68), susceptible (79-89) and highly susceptible (99) respectively (Fig.1).

The infection response (disease score) and then percent disease severity were used as criteria to identify potential donors. Some promising lines were identified from the set-I combination (Sonalika x BH1146) included 07 lines (LBRIL 3, LBRIL 8, LBRIL 18, LBRIL 46, LBRIL 61, LBRIL 102 and LBRIL 142).

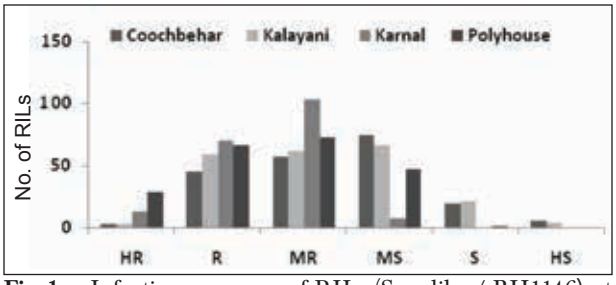

Fig 1. Infection response of RILs (Sonalika / BH1146) at three locations and in poly house.

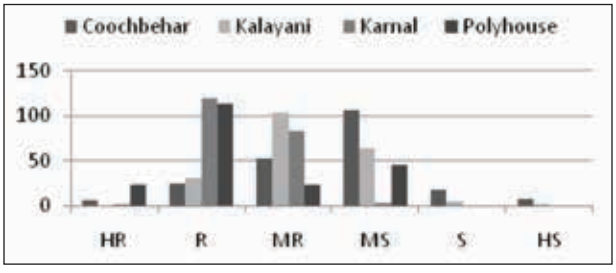

Fig 2. Infection response of RILs (Kanchan / Chirya 1) at three locations and in poly house.

P1(S)	P2(R)	LBRIL	8	46	18	61	102	03	142
79	13	Disease Score	02	13	24	02	24	02	03
77.78	3.7	Disease Severity	0	9.88	3.7	0	9.88	9.88	0

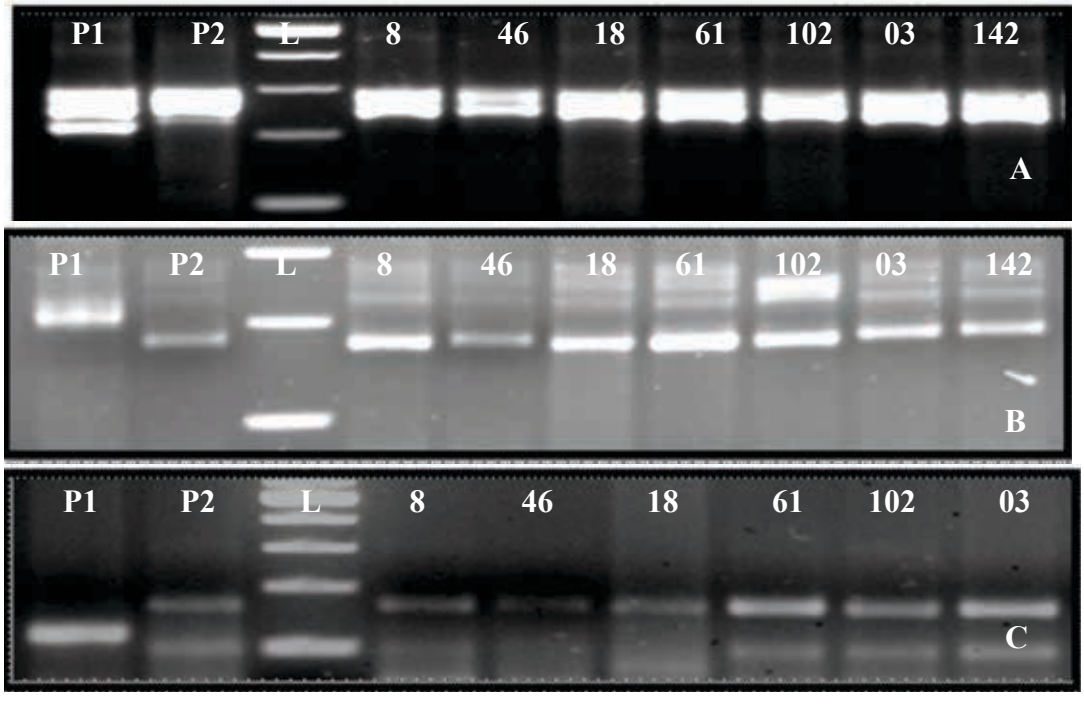

Fig 3. Molecular profile of promising lines of cross (Sonalika x BH1146) where P1- Sonalika (susceptible) P2- BH 1146 (resistant) and LBRIL-8, 46, 18, 61, 102, 03, 142 with molecular marker (A) *Xbarc59*(B)*Xbarc*232 (C)*Xgwm*445.

P1(S)	P2(R)	LBRIL	6	37	38	54	143	189
68	24	Disease Score	24	13	13	13	24	13
60	9.8	Disease severity	9.8	3.7	3.7	3.7	9.88	3.7

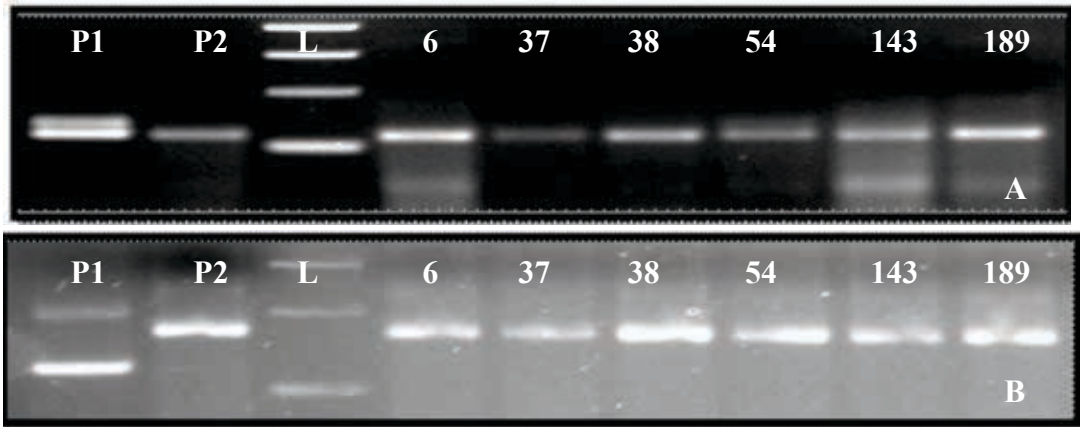

Fig 4. Molecular profile of promising lines of population (Kanchan x Chirya1), where P1- Kanchan (susceptible) P2- Chirya1 (resistant) and LBRIL-6, 37, 38, 54, 143, 189 with molecular marker (A) *Xgwm*425 (B) *Xgwm*371.

Similarly, the promising lines identified from set-II combination (Kanchan x Chirya 1) included 06 lines (LBRIL 6, LBRIL 37, LBRIL 38, LBRIL 54, LBRIL 143 and LBRIL 189) on the basis of high degree of resistance (00-13 score) against spot blotch disease across different locations with good agronomic traits (days to heading, plant height, spike length, grain weight) and resistance to other diseases also like rusts.

Genotypic analysis: In parental screening, out of 800 SSR markers surveyed covering all 21 chromosomes of wheat, around 150 markers were found polymorphic on Sonalika/ BH1146 and 140 were polymorphic on Kanchan/Chirya1. Initially, these polymorphic markers were used for screening selected 50 RILs (highly resistant and highly susceptible to spot blotch). From this preliminary study, some polymorphic markers showing an association with resistant and susceptible parent on the basis of single marker analysis and few previously identified closely linked markers for spot blotch resistance were used to screen some promising lines resistant to spot blotch (Fig 2). The closely linked markers -*Xgwm*111 (7DS5-0.36-0.61), *Xgwm*445 (C-2AL1-0.78), *Xgwm*425 (C-2AS5-0.78) and *Xgwm*148 (C-2BS1-0.53-0.75) were reported as potential diagnostic markers for spot blotch resistance (Kumar *et al.,* 2010). The detailed genotyping study on the entire set of RIL population, with all markers is being carried out for correlating the phenotyping data and considering the infection type at hot spots and ploy house conditions. Further efforts are in progress to identify/develop molecular markers linked with resistance, which would be highly beneficial to breeders to select and enhance the level of resistance in advanced mapping populations. It may be concluded here that since these identified promising lines possess high degree of resistance coupled with desired background might serve as potential donors for improvement of spot blotch resistance and other agronomic attributes in future wheat genotypes.

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