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Short communication

Validation of molecular marker AVRDC-PP12 linked to male sterility gene *ms10* of chilli

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ABSTRACT

The newly identified SSR marker AVRDC-PP12 linked to the male sterility gene *ms10* distinguished the heterozygous (*Ms10ms10*) fertile from the homozygous (*Ms10Ms10*) fertile plants in chilli segregating populations. To assess efficiency of AVRDC- PP12 in marker-assisted backcrossing for transfer of *ms10* gene; the marker was screened in four backcross progenies. The marker co-segregated with *ms10* gene in three progenies derived from the crosses 'MS-12 × VR-16', 'MS-12 × S-217621' and 'MS-12 × Selection Dev' with recombination frequency of 3.22, 4.16 and 3.27%, respectively. The marker failed to differentiate the parents in the cross 'MS-12 × DCL- 524' and hence was not used for further genotyping of the population.

Key words: *Capsicum*, nuclear male sterility, *ms10*, SSR markers.

Hybrids in chilli (*Capsicum annuum* L.) are very popular due to their superior performance and the F_1 seed is produced manually as well as by utilizing male sterility. By using male sterility, hybrid seed cost is reduced by about 50% (Yang *et al.*, 11). Nuclear male sterility was first documented in *Capsicum* spp. by Martin and Crawford (7). Till-date, approx. 20 independently inherited NMS genes have been identified. Male sterility in all, except one, is controlled by a single recessive gene (Dhaliwal and Jindal, 3). Unlike the CMS, which is influenced by low temperature, the NMS system in chilli is stable and commercially used in India and in many other countries.

Pochard (8) identified a male sterility gene *mc509* from a mutant population of bell pepper. The gene was subsequently re-designated as *ms10* (Wang and Bosland, 10). Singh and Kaur (9) transferred *ms10* gene from bell pepper through the conventional backcross method to a multiple disease resistant chilli cv. Punjab Lal. The new NMS line designated as 'MS-12' was utilized to develop commercial hybrids 'CH-1', 'CH- 3' and 'CH-27'.

In recent years, molecular markers have been used to improve efficiency of crop breeding programmes. The linked markers can distinguish the heterozygous fertile from the homozygous fertile plants in segregating generations, thus, facilitating the development of new NMS lines through marker-assisted backcrossing. We used AVRDC-PP12 SSR marker to facilitate transfer of *ms10* gene from 'MS-12' into an array of elite chilli breeding lines through marker-assisted selection.

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The NMS line 'MS-12' was used as a donor parent for the male sterility gene *ms10*. Four elite inbred lines 'VR-16', 'S-217621', 'Selection Dev', and 'DCL-524' were used as the recurrent parents to receive *ms10* gene. Four BC_2F_2 populations were generated from four crosses involving male sterile × male fertile parents. Male sterile and male fertile plants were assessed visually by observing the anther colour of freshly opened flowers and pollen formation. The male sterile plants (*ms10ms10*) showed purple colour anthers without pollen grains sticking to the anthers. The male fertile plants, genetically either *Ms10Ms10* or *Ms10ms10* showed green colour anthers with pollen grains sticking to the anthers. Pollen presence was further confirmed by touching anthers of freshly opened flowers on thumbnail or piece of black paper. Presence of a whitish/ creamy powdery mass (pollen) indicated that the plant is male fertile, while its absence indicated that the plant is male sterile. The genomic DNA of the selected BC_2F_2 plants along with their respective parents were amplified by using the SSR primer pair AVRDC-PP12 (Table 1) linked to the male sterility gene *ms10* (Aulakh *et al.*, 1). Frequency of male sterile and male fertile plants obtained genotypically was compared with the frequency of the two classes obtained phenotypically, and the results were interpreted.

$$\text{The recombinant frequency (\%)} = \frac{\text{No. of recombinant events occurred}}{\text{Plant population screened}} \times 100$$

The NMS gene *ms10* has been utilized for hybrids development in chilli. Due to the recessive gene control, its transfer through the conventional backcross method to other genetic backgrounds

Table 1. Characteristics of SSR marker used for screening of segregating populations of chilli derived from the male sterile × male fertile crosses.

Marker	Primer sequence (5'-3')	Motif	Fragment size (bp)	PCR Tm	Source
AVRDC- PP12	(F)TCCTAACTCTTCCCACCACC (R)GGAGAAGTGTAGCTCCAGCC	(TC)10	132	55	AVRDC, Taiwan

is tedious and time consuming. In recent year, molecular markers have been used to improve efficiency of crop breeding programmes. Aulakh *et al.* (1) developed a SSR marker AVRDC-PP12 linked to the male sterility gene *ms10* in chilli. The marker distinguished the heterozygous (*Ms10ms10*) fertile from the homozyguous (*Ms10Ms10*) fertile plants in segregating generations, thus facilitating the development of new NMS lines through marker-assisted backcrossing. The marker has been screened in four breeding populations generated to transfer *ms10* gene into the elite breeding lines, *viz*. 'VR-16, 'S-217621', 'Selection Dev' and 'DCL-524'. Efficiency of the marker-assisted backcrossing was compared on basis of the phenotypic and the genotypic observations.

To test efficiency of *ms10*-linked marker AVRDC-PP12, genomic DNA of individual BC_2F_2 plants of four backcross populations along with their respective parental DNA was amplified with the marker and resolved in 6% polyacrylamide gel. The results showed that the marker AVRDC-PP12 co-segregated with *ms10* gene in three BC_2F_2 populations derived from the crosses 'MS-12 × VR-16', 'MS-12 × S-217621' and 'MS-12 × Selection Dev' into three genotypic classes as expected in one gene segregation, *i.e.* homozygous recessive (*ms10ms10*), homozygous dominant (*Ms10Ms10*) and heterozygote (*Ms10ms10*). The results showed that the marker can efficiently be used for marker-assisted selection (MAS) in progenies derived from the crosses 'MS-12 × VR-16', 'MS-12 × S-217621' and 'MS-12 × Selection Dev'. The marker failed to differentiate the parents in the cross 'MS-12 × DCL- 524' and hence was not used for further genotyping of the population.

In the backcross progenies, 62 plants of 'MS-12 × VR-16', 72 plants of 'MS-12 × S-217621', and 61 plants of 'MS-12 × Selection Dev' were analyzed for phenotypic and genotypic segregation. The frequency of recombinant events for the marker AVRDC-PP12 was recorded as 3.22, 4.16 and 3.27% in BC_2F_2 populations of crosses 'MS-12 × VR-16', 'MS-12 × S-217621' and 'MS-12 × Selection Dev', respectively (Table 2). Since the cross-over values are less than 5%, the marker can efficiently be used in MAS for the transfer of male sterility gene *ms10* into the three breeding lines. This would save the precious time and resources required to raise self or test cross progenies in the conventional backcross method.

Earlier, a SCAR marker named MS1-SCAR linked to *ms1* gene (Lee *et al*., 5), a CAPS marker GMS3-CAPS linked to *ms3* gene (Lee *et al*., 6), a GMSK-CAPS marker linked to *msk* gene (Lee *et al*., 4), two SCAR markers SCAR_P2 and SCAR_V17 linked to *ms8* gene (Bartoszewski *et al*., 2) and a high resolution melting marker (HRM) named GMSE linked to an undesignated gene were developed. The markers co-segregated with the linked genes, thus showing their potential use for MAS to transfer the linked genes and strengthening the heterosis breeding programme in peppers.

It is concluded that the markers AVRDC-PP12 distinguished homozygous dominant (*Ms10Ms10*) and heterozygous (*Ms10ms10*) genotypes in three out of four segregating populations incorporated with the *ms10* gene. Thus, the marker proved its applicability in MAS for the development of new NMS lines derived from the crosses between 'MS-12 × VR-16', 'MS-12 × S-217621' and 'MS-12 × Selection Dev'. The marker-assisted backcrossing would facilitate breeding for NMS lines incorporated with *ms10* gene and help to save the precious time and the resources.

Table 2. Segregation analysis of the marker AVRDC-PP12 for male fertility in the BC_2F_2 populations.

Cross	Total plants	Genotypic segregation			Recombinant frequency (%)
		Ms10Ms10	*Ms10ms10*	*ms10ms10*	
MS-12 × VR-16	62	18	34	10	3.22
MS-12 × S-217621	72	30	27	15	4.16
MS-12 × Selection Dev	61	25	31	5	3.27

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