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Short Communication

Verification of an allele specific associated primer with wilt susceptibility in commonly used parental lines of chickpea

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Fusarium wilt caused by *Fusarium oxysporum* Schlechtend. Fr. f sp. *ciceris* (Padwick) is the most destructive and wide spread disease of chickpea (*Cicer arietinum* L.) causing significant yield losses in India. The screening of germplasm has led to identification of a large number of resistant genotypes/cultivars [1, 2]. Screening in wilt sick plots although efficient [2], maintenance of wilt sick plots in all the centers for selection of resistant genotypes is tedious. Therefore, efforts are being made to develop molecular markers. An allele specific associated primer (ASAP) for susceptibility was identified [3]. Although this primer typically amplifies a single fragment linked to the allele for susceptibility to fusarium wilt, a few recombinant lines that were resistant produced the band. It is essential to understand the association between this maker and wilt susceptibility in the commonly used parental genotypes of chickpea breeding programmes in the country.

Eight chickpea genotypes *viz.*, BG 256 (bold seeded with high seed yield), Karikadle (local variety, tolerant to pod borer), ICCV-10 (resistant to wilt), A-1 (national check), ICC-506 (tolerant to pod borer), WR-315 (wilt resistant check), JG-62 (wilt susceptible check) and K-850 (good combiner, nodulator and bold seeded) which are commonly used as donor parents in breeding programmes in India, were selected for this study. The reaction of selected genotypes against fusarium wilt race 1 was studied in pot culture according to the method described earlier [4]. A single spore isolate of wilt pathogen *Fusarium oxysporum* f. sp. *ciceris* race 1 was obtained from International Crops Research Institute for Semi Arid Tropics. (ICRISAT), Patancheru, India and maintained on fresh Potato Dextrose Agar (PDA). The fungus culture was multiplied in 100- 150 grams of sand maize meal in 250ml flasks incubated for 21 days at room temperature [5]. One hundred grams of the inoculum was mixed thoroughly with 3 kg of an autoclaved pot mixture in earthen pots of 30 cm diameter. The susceptible cultivar JG 62 was grown in the pots (5-10 seeds) and allowed to wilt. The seeds of the selected genotypes were sown in these sick pots for their wilt reaction. Five seeds /pot and two pots / genotype were maintained. The number of days from sowing to the appearance of initial wilt symptom and complete wilt were recorded for each genotype. Depending on the number of days taken for complete wilt, the genotypes were classified as early wilting (less than 25 days), late wilting (more than 30 days) and no wilting.

DNA was isolated from leaf tissue of the selected genotypes using the CTAB method [6]. DNA was used for polymerase chain reactions (PCR) following the protocol as described for, chickpea [3]. Allele specific associated primer CS-27_{700} conducted by Mayer *et al.* [3] was used in PCR amplification. The PCR products were separated on 2% (W/V) agarose gels, stained with ethidium bromide and scored for presence or absence of the band.

The study presents results of the reaction of selected genotypes of chickpea for resistance to the most prevailing race of the wilt pathogen *Fusarium oxysporum* race 1. Initial wilt symptoms were observed within 17 days in Karikadle and JG-62 and all the plants were completely wilted within 25 days in these two genotypes. The time of wilting differed taking more than 30 days up to 60 days in genotype A-1, ICC-506, BG-256, K-850 and ICCV-10 suggesting tolerance to wilt. The resistant genotype WR- 315 did not show wilting till maturity. Therefore, the genotypes have been grouped into three different groups *viz.*, early wilting, late wilting and no wilting. It has been reported that three independent loci govern resistance to race 1, of wilt pathogen [7]. Mayer *et al.* [3] developed an ASAP linked to h_1 locus and wilt susceptibility. In the present study, linkage between ASAP and Fusarium wilt susceptibility has been studied in commonly used parental genotypes in the breeding programmes throughout India. Of the eight genotypes, only two

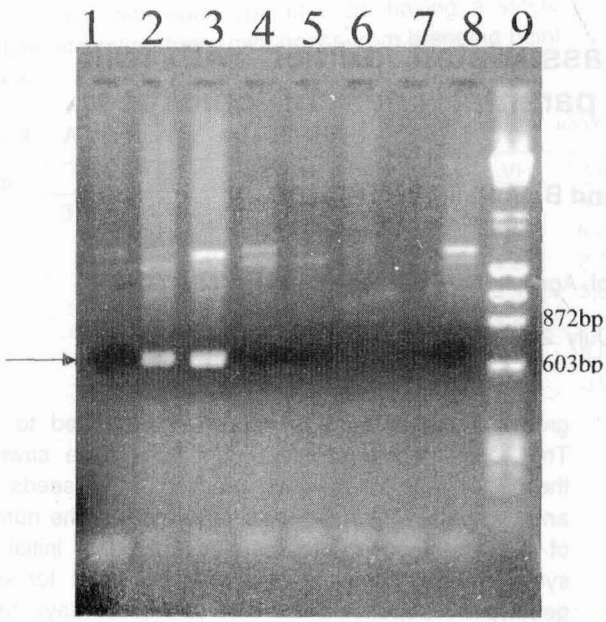

Fig. 1. ASAP amplification of parental genotypes of chickpea

genotypes *viz.*, JG-62 and Karikadle were positive for the amplified product with a specific band for susceptibility (Fig. 1). These two genotypes also showed early wilting. These results are in accordance with the results of Mayer *et al.* [3]. The remaining six genotypes showed either late wilting or no wilting and the spectfic band for susceptibility was absent. These results contradict Mayer *et al.* (3), who have observed amplification in moderately susceptible genotypes also. Our results clearly indicate that the ASAP used in the study amplifies a fragment linked to the allele for high susceptibility for *Fusarium oxysporum*, resulting in early wilting. The susceptibility in the selected genotypes is linked in coupling phase with $CS\text{-}27_{700}$. Among the early wilting genotypes, Karikadle a local collection is a known pod borer tolerant genotype and is extensively used in pod borer resistance breeding programmes of this region. Therefore, the molecular marker (ASAP) could be used for a quicker assessment of susceptibility to wilt in the segregating populations of crosses involving Karikadle. The pod borer tolerant selections, with the marker could be eliminated in the early generations itself to avoid wilt susceptibility. The ASAP used in the study also amplified a faint fragment of bigger size in three genotypes, Karikadle, ICCV-506 and WR-315, thus contradicting allele specific amplification in these genotypes. The persistent amplification of extra fragments could be due to poorly optimized marker or the presence of common classes of dinucleotides of particular fragment associated with susceptibility allele consistent across genotypes. The ASAP approach can expedite selection of only tolerant and/or resistant genotypes in the segregating generations by eliminating early wilting genotypes at the seedling stage. Further studies are needed to optimize the marker to avoid loss of specificity observed in this study.

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