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Marker Assisted Selection for Mapping Population against Chickpea Wilt

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A B S T R A C T

Present investigation was carried out to select the resistant of amongst the mapping population lines through marker assisted selection. The investigation was carried out in the laboratory of Department of Plant Pathology and Biotechnology Centre, PGI, Dr. PDKV, Akola. The 30 chickpea genotypes were selected for screening from three mapping population of different crosses (JG 62(Susceptible) X ICCV 08113, PG 04305 (resistant) X JG 62 (susceptible) and PG 07101 (resistant) X JG 62 (susceptible). 10 genotypes from each population were selected. Marker assisted characterization of 30 chickpea genotypes differing for *Fusarium* wilt reaction was carried out using four molecular markers reported by earlier workers linked to disease resistant/susceptibility. In the present study, three different markers (namely, CS-27F/R 700bp, A07C 417bp and UBC-8251200bp) linked to susceptibility and three microsatellite based markers (TA-59 258bp) linked to resistance allele were validated. The molecular markers ASAP (CS27F/R 700bp), RAPD (A07C 417bp), ISSR (UBC-825 1200bp) and STMS (TA59 258bp) amplified against 30 genotypes of chickpea (F2 population). The results indicates that amongst 30 genotypes from the mapping populations 8 genotypes were resistant from cross I, 1 genotype from cross II and 7 genotypes from cross III shows amplification in CS27F/R 700bp. Other than theses genotypes bands are present in STMS primer, TA59 258bp which is linked to resistance to *Fusarium* wilt.

Introduction

Chickpea, a self-pollinating diploid (2n=16) species with a genome size of 740 Mbp, is the world's third most important food legume (Arumuganathan and Earle, 1991). It is a deep rooted crop belonging to the family Leguminosae. It is commonly known as *channa*, gram, *garbanzo bean* etc. It is originated in south-eastern Turkey around 7,500 from where it has spread to other countries of the world. India ranks first in the production and productivity of chickpea during 2015-16 covering 8.87 M. ha. And production 7.17 Mt) (Source: agricoop.nic.in) Fusarium wilt caused by a Deuteromycetes fungus *Fusarium oxysporum* Schlechtend. f. sp. *ciceris*, is the most destructive disease of chickpea and is wide-spread in chickpea growing areas of Asia, Africa, Southern Europe, Peru, Syria, Ethiopia, Mexico, Spain, Tunisia, Turkey, USA and the countries of the USSR (Westerlund *et al.*, 1974). Annual yield losses due to wilt have been estimated at 10-15% (Jalali and Chand 1992), but it is capable of causing 100% loss under favorable conditions (Halila and Strange, 1996). The fungus is a soil borne which lives in seeds and dead plant materials in the form of chalmydospore. The wilted seedlings shows a dull green color of the foliage; sudden drooping of the petioles, rachis and leaves. The plants, when uprooted, showed uneven shrinkage at the collar (Nene *et al.*, 1987). Nowadays, application of chemical pesticides is limited because of hazards environmental pollutions and health risks. So, using genetic resistance and cultivating resistant genotypes is the most suitable and practical method for management of Fusarium wilt of chickpea. The phenotypically selection of the resistant genotypes is the time consuming and complicated. So, using DNA based molecular marker is a major tool for the selection of the resistant cultivars; facilitating the process (Lindhout, 2002; Tanksley *et al.*, 1992). The selection and inheritance of the desirable traits is now become possible with the advancement of Marker Assisted Selection (MAS) which provides a beneficial source to exploit the potentiality of genes against agronomic traits (Choudhary, 2010; Allahverdipoor KH, 2011). Marker assisted selection (MAS) using DNA markers tightly linked to wilt resistance genes can be used to screen a large number of germplasm lines for the presence of these genes without actually subjecting them to the pathogen and to pyramid them into agronomical superior varieties. Chickpea wilt is one of the major limiting factor in chickpea production in India. This disease causes huge yield losses (10 to 90%) annually (Singh and Reddy, 1991). The use of wilt resistant chickpea cultivars, when they are available, is the most effective and ecofriendly method of managing the disease (Sharma *et al.*, 2005). Therefore, the present study was organized to select the resistant and susceptible lines in chickpea germplasm through ASAP STMS, ISSR and RAPD markers linkage to resistant genes to resistant gene pyramiding and to enhance resistant germplasm resources for increasing yield of chickpea.

Materials and Methods

Plant material

The samples were collected from the field of Pulses Research unit, Dr. PDKV, Akola. The mapping population of F_4 generation from crosses of JG 62 (susceptible) X ICCV 08113(resistant), PG 04305(resistant) X JG 62((susceptible), PG 07101(resistant) X JG 62(susceptible) 10 genotypes from each population were selected. Parents used for crossing were ICCV 08113, PG 04305, PG 07101 and JG-62. All genotypes were sown in plastic pots and leaf samples were taken.

DNA isolation

Total genomic DNA was isolated from the young leaves following the CTAB method. The DNA obtained after extraction was confirmed by running it on 0.8% agarose gel (containing ethidium bromide @ 0.5 mg/ml) in a horizontal gel electrophoresis system.

PCR amplification and electrophoresis

The PCR was carried out in small reaction tubes, containing a reaction volume typically of 20µl that was inserted into a thermal cycle (Eppendorf) that heated and cooled the reaction tubes within it to the precise temperature required for each step of the reaction. PCRs were run on the programmable thermal cycle 94^{0}C Denaturation, 50^{0}C Annealing, 72^{0}C Extension. Annealing temperature varied from primer to primer. Electrophoresis of one RAPD, one STMS, ISSR and one ASAP primer PCR amplified analysis on1.5% agarose gel was carried out in 1xTBE buffer in horizontal gel electrophoresis

Results and Discussion

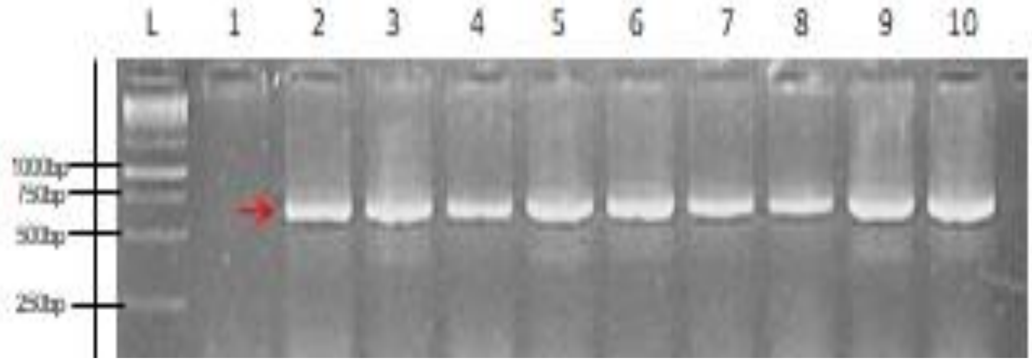
An investigation was undertaken to study the resistance and susceptibility banding pattern in different genotypes from mapping population of F_2 generation by using different four (STMS, ASAP, RAPD and ISSR) primers (Table 1).

The ASAP marker CS-27F/R gave amplified product of 700 bp in susceptible genotypes only. In cross I (JG 62 X ICCV 08113), amplification was observed in 3rd and 4^{th} genotypes, whereas, absent in other eight genotypes. In cross II (PG 04305 X JG 62), amplification were observed in nine genotypes (2, 3,4,5,6,7,8,9 and 10), whereas, absent in only one genotype. In cross III (PG 07101 X JG 62), amplification were observed in three genotypes (1, 2 and 3), whereas, absent in other seven genotypes. Mayer *et al.*, (1997) was observed that ASAP marker CS-27F/R linked to disease resistance gave amplification of 700 bp susceptible genotypes only. The STMS marker TA59 gave amplified product of 258 bp in resistant genotypes only. In cross I (JG 62 X ICCV 08113), amplification was observed in eight genotypes (1, 2, 5, 6, 7, 8, 9 and 10) genotypes, whereas absent in other two genotypes. In cross II (PG 04305 X JG 62), amplification were observed in only one genotype (1) whereas absent in 0ther nine genotypes. In cross III (PG 07101 X JG 62), amplification were observed in seven genotypes (3, 4, 5, 7, 8, 9 and 10), whereas absent in other three genotypes. Winter *et al.*, (1999) characterized and mapped 120 STMS on the chickpea genome map. The primer TA-59, TA-96 and TR-19 were mapped on same linkage group on which gene for disease resistance was present (Winter *et al.*, 2000). The ISSR marker UBC-825 produced amplification of 1200 bp in susceptible genotype and intermediate genotype ISSR marker, (UBC-825) screened on the 30 genotypes of crosses (JG 62 (susceptible) X ICCV 08113 (resistant), PG 04305 (resistant) X JG 62 (susceptible) and PG 07101 (resistant) X JG 62 (susceptible)),the banding pattern were shown in plate4 (a, b and c). Ratnaparkhe *et al.,* (1998) identified new ISSR primer (UBC-825_{1200}) by changing the anchored region of the ISSR primers previously reported to linked with disease resistance gene.

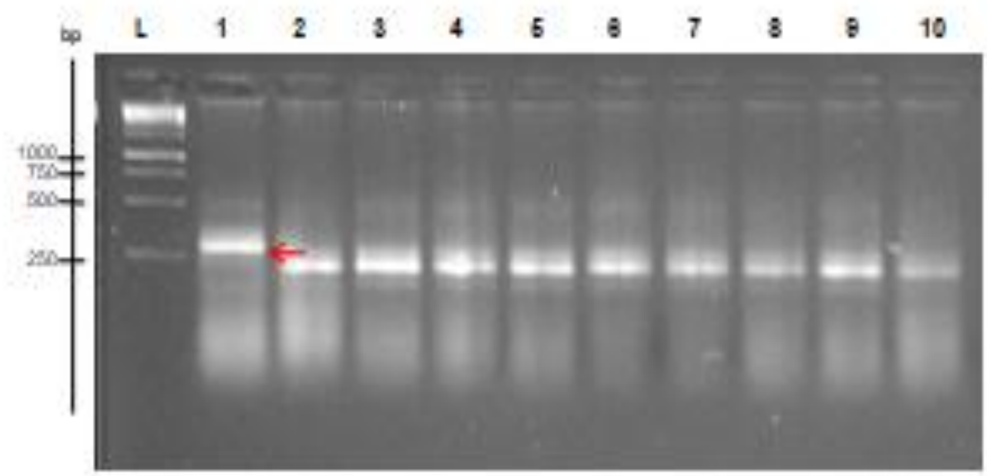
Table.1 List of primers used with their sequences

Sr. No.	Primer	Sequence (5'→3')	Annealing Temp. (ºC)
1	UBC- 825	ACACACACACACACT	45.1
2	TA- 59	F-ATCTAAAGAGAAATCAAAATTGTCGAA R-GCAAATGTGAAGCATGTATAGATAAAG	57.4
3	CS-27A	F-AGCTGGTCGCGGGTCAGAGGAAGA R-AGTGGTCGCGATGGGGCCATGGTG	62
4	A07C	GAAACGGGTGC	36

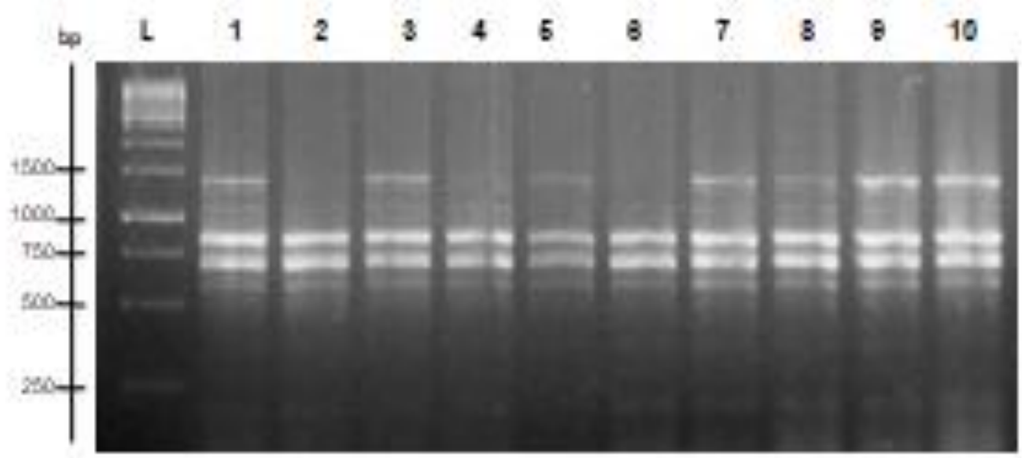
1) PCR amplification of DNA by using CS27 (ASAP) primer



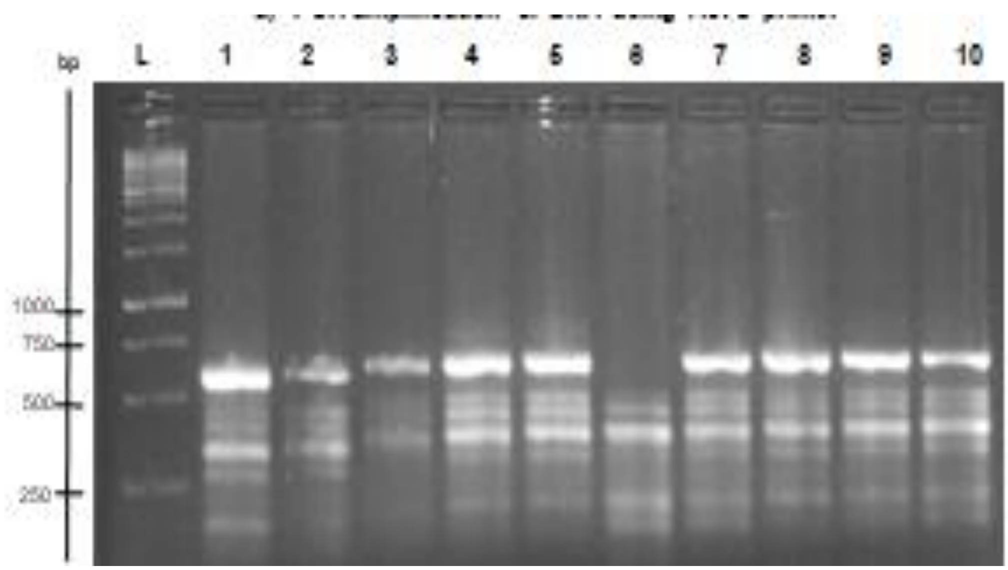
2) PCR amplification of DNA by using TA59 (STMS) primer



3) PCR amplification of DNA by using UBC825 (ISSR) primer



4) PCR amplification of DNA by using A07C (RAPD) primer



The RAPD marker, ($A07C_{417}$) linked to H2 locus was present in all genotypes and showed polymorphism in parental lines and resistant and susceptible bulks. RAPD (A07C) screened on the 30 genotypes of crosses (JG 62 (susceptible) X ICCV 08113 (resistant), PG 04305 (resistant) X JG 62 (susceptible) and PG 07101 (resistant) X JG 62 (susceptible)). A07C produce polymorphic banding pattern given in plate 5 (a, b and c). Williams *et al.,* (1990) work on the RAPD technique. After his pioneering work the RAPD technique has become one of the essential tools in molecular breeding. Soregaon *et al.,* (2007) an RAPD marker, A07C linked to H2 locus which amplifies 417bp DNA fragment linked to susceptible reaction.

In conclusion, molecular identification by RAPD, STMS, ISSR and ASAP marker are good tools in detection of resistant and susceptible genotypes. Screening of 30 genotypes by molecular markers showed variation in their susceptibility and resistance. 30 genotypes were selected from 3 crosses were analysed and observed that 16 genotypes were found resistant among 30 genotypes. The confirmation of resistance of chickpea genotypes to wilt exhibited wide range of response i.e. from resistant to highly susceptible against pathogen. Crossing of chickpea is good tool for developing of chickpea resistant variety. Marker Assisted Selection for checking resistant gene in F_2 population for further study.

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