Effect of gamete selection on segregation of wilt susceptibility-linked DNA marker in chickpea

Gametophytic selection is emerging as an alternate to that based on sporophyte in crop improvement. The haplo–diploid gene expression[1,2] implies that selection exerted at the gametophytic level can increase the responsive allelic frequencies that can be detected also at the sporophytic level. The selection applied at gamete or pollen grain level has proven to be effective for a variety of selection pressures such as biotic stress[3,5]; abiotic stress[6–8] and herbicide tolerance[9–11]. However, most of the selection response studies conducted so far were based on complex traits due to the action of several minor genes leading to limited responses to selection[8,12,13]. Selection for simply inherited traits with established genes should permit to assess the potentiality of gametophyte selection. Even though gametophytic selection has been widely investigated, persistence of the response in succeeding generations has not been proven, except in a few cases[14,15]. Such evidences without confirmation in the later generation may lead to the question, whether the response to gametophytic selection could be due to transient epigenetic effects instead of selection of desired alleles. Therefore, the objective of the present investigation was to study the effect of gametic selection for resistance to wilt on the molecular marker associated with H_1 locus of susceptibility in chickpea.

Three independent loci govern resistance to race 1 of wilt pathogen in chickpea[16]. The results in our laboratory indicated dominance allele at both h_1 and h_2 loci result in early wilting and at any one produce late wilting and recessive at both the loci results in no wilting[17]. The primer pair CS-27F/CS-27R developed by Mayer *et al.*[18] termed as ASAP, amplifies a fragment of 700 bp linked to the allele (H_1) for susceptibility to race-1 of fusarium. The early-wilting susceptible genotype JG 62 with dominance at both H_1 and H_2 and resistant genotype WR 315 recessive at h_1 and h_2 were selected for this study. The genotype JG 62 is positive for susceptibility-linked ASAP marker, while WR-315 is negative. The genotype JG 62 is crossed to WR 315. The F_1 was raised under field condition. Two F_1 plants were sprayed with fusaric acid (Sigma cat. #F6513), one of the toxins produced by *Fusarium oxysporium* was at a concentration of 1500 µg/ml at flower-bud initiation stage for gamete selection. The remaining two F_1 plants were sprayed with water as control. The seeds from treated and control F_1s were harvested separately. The F_2 plants from both control and treated F_1s were grown in pots for isolation of DNA. The DNA was isolated from vegetative buds of F_2 plants. Fifty F_2 plants each for control and treatment were selected for DNA

Table 1. Segregation of allele-specific marker in F_2 populations obtained with and without gamete selection

Treatment	Total no. of plants	No. of plants		χ^2
		With DNA marker	Without DNA marker	
No gamete selection	50	34	16	0.96
Gamete selection	50	21	29	27.31

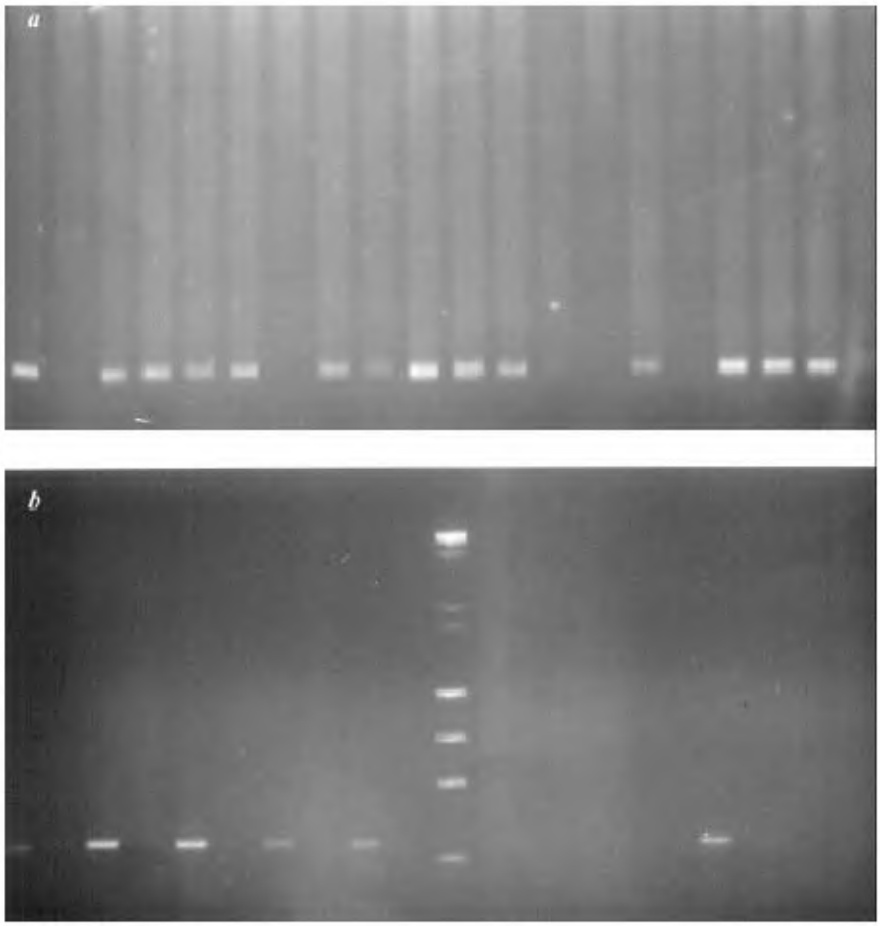

Figure 1. Allele-specific amplification in F_2 plants obtained (***a***) without and (***b***) with gamete selection.

isolation. DNA was used for polymerase chain reaction with ASAP for amplification[15]. The PCR products were separated on 1.1% (w/v) agarose gel stained with ethidium bromide and all the plants were scored for presence or absence of specific band. The segregation for a specific band was tested for single locus goodness-of-fit for the 3 : 1 ratio, applying Yates correction.

The genotype JG 62 with homozygous dominant H_1 gene is susceptible and WR 315 with homozygous recessive h_1 is resistant to fusarium wilt. The monohybrid ratio of 3 : 1 is expected for H_1 gene and amplification of ASAP marker in the F_2 generation of the cross JG 62 × WR 315. Among the 50 control F_2 plants, 34 showed amplification for the ASAP marker and the remaining 16 were negative for the marker (Table 1, Figure 1 *a*). The test for segregation of DNA marker closely fits the 3 : 1 ratio in control F_2, suggesting single locus goodness-of-fit as expected. The results are in accordance with earlier studies regarding the segregating behaviour of DNA marker[18]. Among the treated F_2, twenty-one plants were positive for the amplified product, while twenty-nine were negative. χ^2 test indicated significant deviation of treated F_2 from expected monohybrid ratio (3 : 1). The observed number of plants with ASAP amplification was significantly less than expected and the plants without ASAP amplification were more than that expected (Table 1; Figure 1 *b*). As the ASAP amplification is linked to the H_1 locus, susceptible plants were less than expected and resistant plants were more than expected. It is reported that the selection pressure can be applied at the whole plant level by exposing the plant to stress factor, and the progeny from stressed plants showed preferential segregation towards tolerant genome[6]. The deviations observed in the treated F_2 population against ASAP marker linked to susceptibility demonstrate directly that selection is acting on gametophyte to increase the frequency of desirable genes in the progeny. The F_1 is a heterozygote and produce gametes carrying dominant H_1 and recessive h_1 alleles in equal frequency, and both had the same competitive ability in the absence of selection pressure against dominant H_1 allele. Therefore, the segregation ratio for ASAP amplification was 3 : 1. However, in treated F_1, the selection was made at the diploid level in favour of recessive allele governing wilt resistance. Therefore, there is a deviation from the normal 3 : 1 ratio for DNA marker linked to susceptibility. The results indicate that gametophytic selection was able to favour those gametes containing alleles for resistance, thus emphasizing the potentiality of this method. It could permit rapid transfer of an allele conferring resistance to wilt into elite germplasm by subjecting the heterozygous plants to gamete selection. The effectiveness of gamete selection for simply inherited traits was also revealed in tobacco[19] and maize[11]. The feasibility of this approach seems to be promising for hastening the incorporation of desirable alleles in a short time. However, the results were based on only limited number of F_2 plants. It is essential to study a large number of F_2 plants and backcross progenies to unequivocally establish that the gamete selection is operative at the desirable allelic level. The study is in progress.

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Received 19 November 2003; accepted 24 November 2003

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