GENETICAL STUDIES OF ASCOCHYTA BLIGHT RESISTANCE IN CHCIKPEA

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ABSTRACT.

Ascochyta blight caused by Ascochyta rabiei (Pass.) Lab. is a devastating disease of chickpea (Cicer arietinum L.) worldwide. Available genetic variation for Ascochyta blight resistance in genus Cicer has prompted interest in the development and use of resistant cultivars that can be sown in autumn and, to increase seed yield in chickpea. Understanding the mode of inheritance of resistance to Ascochyta blight in chickpea would assist breeding efforts. The objective of this study were determining number of genes confer Ascochyta blight resistance and leaf size as well as action of them. Thus F1, F2 F3 progenies derived from a cross between Iranian local variety Bivanij (susceptible local variety) and ICC12004 along with their parents were sown in a RCB design at the International Center for Agricultural Research at Dry Area (ICARDA) under artificial infection conditions. Results showed that in F2 and F2 generations the ratio of susceptibility did not differ significantly from those of 9:7 and 5:3 theoretical ratios. There was a negative correlation between leaf size and blight score, which means that large leaf genotypes could be more susceptible to Ascochyta blight. Generation mean analysis for resistance to Ascochyta blight in this study revealed that additive effect has main role in Ascochyta blight resistant, however the leaf size besides of additive effect showed dominance effect as well. For theses traits we detected dominant x dominant interaction (l) in the opposite sign which reveals the evidence of a duplicate epistasis. These findings showed that the genotype of resistant parent could be as R1R1R2R2. According to these findings and available knowledge, it would be suggested an appropriate breeding program for gene pyramiding to produce multiple resistant genotypes in chickpea. For QTL analysis fifty-eight SSR markers and one morphological marker (flower color) were mapped on F2 individuals and F2:3 families derived from the cross ICC 12004 (resistant) × Bivanij (susceptible local variety) at the International Center for Agricultural Research at Dry Area (ICARDA). The linkage map comprised eight linkage groups, excluding flower color which didn't assign to any linkage group. Area under disease progress curve (AUDPC) was used to evaluate the F2 population and F3 families. Using composite interval mapping, three genomic regions were detected, which were in association with reaction to ascochyta blight. These QTLs on LG3, LG4 and LG6 accounted for 46.5% of the total estimated phenotypic variation for reaction to ascochyta blight. Fine mapping of the QTLs identified in this study would lead to the identification of markers that could be used for marker-assisted selection of chickpea genotypes with resistance to Ascochyta blight. These findings are particular pertinent considering that we used Ascochyta rabiei pathotype III and ICC 12004 (resistant to pathotype III) for the first time.

Keywords: ***Chickpea (Cicer arietinum L.), Ascochyta rabiei (Pass.) Lab., disease resistance, SSR, linkage map, QTL***

1. Introduction

Chickpea (*Cicer arietinum* L.), a self-pollinating diploid annual, with 2x=2n=16 chromosomes. It is the third most important grain legume in the world after common bean (*Phaseolus vulgaris* L.) and pea (*Pisum sativum* L.)[19]. Primarily, chickpeas are grown in the Indian subcontinent, West Asia, North Africa, Ethiopia, Southern Europe, Mexico, Australia, North-Western United States and in the Brown and Dark Brown soil zones on the Canadian parties [8]. Average yield of chickpeas worldwide is about 700 kg/ha which is much below its potential [8], [16]. Yields are seen as low and unstable compared to other crops due to adverse effects of a number of biotic and abiotic stresses [8]. One of the greatest biotic stresses reducing potential yield in chickpea is ascochyta blight, caused by the fungus *Didymella rabiei* (Kovachevski) v. Arx. (anamorph: *Ascochyta rabiei* (Pass.) Labrousse) is the most devastating worldwide, causing up to 100 per cent yield losses in severely affected fields [7]. *Ascochyta rabiei* is heterothallic, thus when two compatible mating types are present genetic recombination can occur resulting in ascospore production [20], [21], [27]. Isolates of both mating types found in Iran indicating the occurrence of sexual recombination. Recombination could potentially lead to greater genetic and pathogenic variability in populations of *A. rabiei*. Pathogenic variability in *A. rabiei* populations has been reported in almost all chickpea growing regions in the world, including India, Iran, Pakistan, Turkey, Syria, the Palouse region of north-western United States and Canada [3], [6], [8], [12], [23]. Chongo et al. (2004) also confirmed the presence of genetic variability among *A. rabiei* isolates collected in the 1998 and 1999 growing seasons based on RAPD molecular markers [3]. Despite recognition of destructiveness of *A. rabiei* in chickpea production world-wide, very little head way on controlling the disease through resistance breeding has been made in the past century. Resistance in breeding lines of chickpea to ascochyta blight is not durable due to the high variability of *A. rabiei* populations wherever chickpeas are grown [8], [12], [14], [15], [23]. Resistance break down is possibly the greatest challenge in breeding for resistance to ascochyta blight in chickpea [13]. Cultivars available in ICARDA, lack complete resistance to *A. rabiei*. Partial resistance in cultivars adapted to the western Iran tends to break down after the onset of flowering. Partially resistant cultivars contribute to the development of new pathotypes of the disease by imposing selection pressure, possibly resulting in increased virulence or aggressiveness within the pathogen population [17]. With a genetically diverse population of *A. rabiei*, it is important not only to develop cultivars with durable forms of resistance, but also to monitor changes in the population structure to anticipate resistance breakdown in existing cultivars. Among current understanding of the genetics of ascochyta blight resistance (ABR) in chickpea strongly suggests polygenic inheritance of the trait. In an interspecific genetic background, Santra et al. (2000) mapped two QTLs which conditioned ABR over two years of field screening [9]. Likewise, preliminary QTL mapping in a wide-cross between *C. arietinum* and *Cicer echinospermum* (resistance source) revealed two to three QTLs for seedling resistance in controlled glasshouse bioassays [14]. Tar'an et al. (2007) identified one QTL on each of LG3, LG4 and LG6 accounted for 13%, 29% and 12% respectively, of the total estimated phenotypic variation for the reaction to ascochyta blight [18]. Although the genetic mechanism of ABR has been studied in identified resistant accessions of *C. arietinum*, the number and genomic locations of the genes or QTLs conditioning resistance has yet to be verified. The objective of this study were determining number of genes confer Ascochyta blight resistance and leaf size as well as action of them. Thus F_1, F_2, F_3 progenies derived from a cross between Iranian local variety Bivanij (susceptible local variety) and ICC12004 along with their parents were sown in a RCB design at the International Center for Agricultural Research at Dry Area (ICARDA) under artificial infection conditions.

2. Material and methods

F_1, F_2, F_3 progenies derived from a cross between Iranian local variety Bivanij (susceptible local variety) and an Indian accession ICC12004 along with their parents were sown in a Completely Randomized Block design (CRBD) at the International Center for Agricultural Research at Dry Area (ICARDA) under artificial infection conditions.

Bivanij is a high-yielding cultivar of *Kabuli* type with beige, relatively large seeds (400 mg), highly susceptible to *D. rabiei* and semi-erect growth habit. ICC12004 is resistant to the blight, with typical *Desi* small seeds (250 mg) and an erect growth habit. Isolate No. 13 of pathotype III (Udupa et al., 1998) was used for inoculation in both methods [1], [8], [25]. This isolate was cultured at room temperature under florescent light [2]. For every generation, the inoculation method was based on Buchwaldt et al., 2007, consisting in depositing a drop of spore suspension on detached leaves (10µL) [1]. Five plants of each generation were evaluated in the controlled environment. The parents, as well as the chickpea lines ILC1929 and ILC263 (susceptible), and ILC3279 (resistant to pathotypes I and II), were included as control genotypes. In this trial, the experimental design was a randomized complete block. Test plants were sown in a pair of seedling trays. Each pair of trays constituted one experimental block or replicate, and contained an individual plant of each of the $F_{2:3}$ families and control genotypes. Disease reactions were scored weekly after inoculation and AUDPC was calculated using the formula: $AUDPC=\sum [(x_i+x_{i+1})/2] (t_{i+1}- t_i)$. Isolate No.13 (PIII), was grown at room temperature under continuous fluorescent light. The suspension was filtered and adjusted to a final concentration of 2×10^5 conidia/mL using a hemacytometer. Isolate No.13 (PIII), was grown at room temperature under continuous fluorescent light. The suspension was filtered and adjusted to a final concentration of 2×10^5 conidia/mL using a hemacytometer. Genomic DNA of fresh leaves of young F_2 plants was extracted using CTAB protocol according to Weising et al., 1998 [26]. DNA of parental lines was screened for polymorphisms using 149 SSRs [11], [28]. The amplified DNA fragments were analyzed using ALFexpress DNA Sequencer [22] and DNA fragments were visualized via silver staining, using a silver staining kit [22]. The polymorphic primer pairs were further tested on population. Mapmaker/Exp version 3.0 (Lincoln et al. 1993) was used to create a linkage map when the LOD value obtained was >3 [5]. Using the linkage map (F_2) genotype data and family-mean AUDPC of the $F_{2:3}$ families, putative QTLs for resistance to ascochyta blight were identified by single-point analysis or one-way ANOVA at $P\leq0.05$ using the GLM procedure of SAS (SAS Institute Inc. 1996), and verified by composite interval mapping (CIM—Windows QTL Cartographer version 1.30; Wang et al. 2002) [10], [24].

3. Resaults and disccusion

Weighted analysis of variance showed that there is significantly difference for leaf size and reaction for Ascochyta blight disease (results not shown). Means along with their standard errors are tabulated in table 1. In F_2 and F_3 generations there were 109:90 and 115:81 resistant plants comparing to susceptible ones, respectively. These ratios were not significantly different from those of theoretical ones, say 9:7 and5:4, respectively. In this study according to Reddy and Singh (1993) chickpea varieties scaled as susceptible group (4/1-9/0) and resistant group (1/0-4/0), respectively.

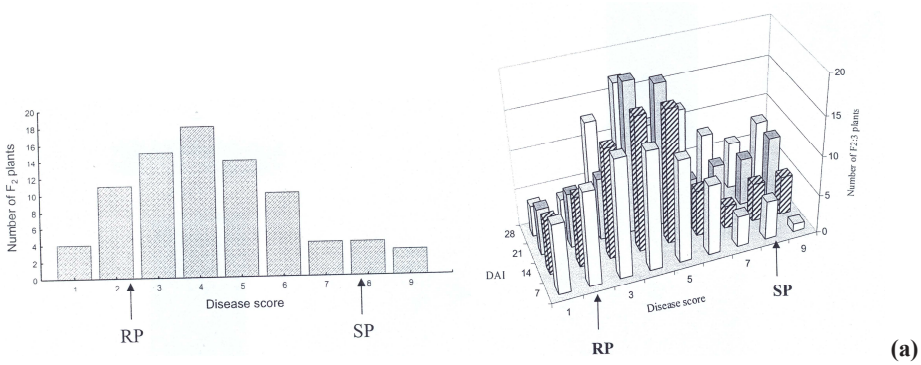


Figure1. The distributions of reaction to Ascochyta blight disease, for F_2 and F_3 generations (a, b, respectively). It is clear that both distributions show some skewness towards resistant parent, which is an indication of dominance for Ascochyta blight resistance controlling loci.

Table1. Mean value of response to Ascochyta blight disease and leaf size with plant number per generations derived from crossing between 2 Chickpea inbred lines (ICC12004×Bivanij)

Generation	Plant Number	Mean ± SD		Phenotypical correlation
		Response to A. blight	Leaf size(cm2)	
P_1	40	2.25±0.018	5.39±0.21	0.12
P_2	40	7.98±0.013	12.66±0.26	0.31*
F_1	32	4.28±0.008	10.21±0.41	0.28
F_2	199	4.34±0.016	10.59±0.17	0.11
F_3	195	4.69±0.015	10.13±0.12	0.15*
F_1	32	4.28±0.008	10.21±0.41	-
RF_1	34	4.51±0.009	10.03±0.28	-

P_1 = ICC12004 (R)
P_2 = Bivanij (S)

Table2. Estimates of gene effects for response to Ascochyta blight and leaf size in the cross between two chickpea inbred lines [ICC12004 (R) and Bivanij (S)]

Parameter	Response to A. blight	Leaf size
m	5.12±0.09	9.05±0.16
d	-2.86±0.09	-3.64±0.17
H	-2.18±0.52	5.26±0.85
I	-	-
L	1.34±0.49	4.14±0.95
X2	0.11	0.15
[h/d]	0.76	-1.45

m = mean
d = additive gene effect
h = dominance gene effect
I = additive × additive gene effect
L= dominance × dominance gene effect
[h/d] = degree of dominance

Table3. Components of variance of Basic generations and number of effective factors (EF_1,EF_2) for response to A. blight and Leaf size in crossing between two chickpea inbred lines [ICC12004(R) , Bivanij (S)] to Ascochyta blight disease .

Parameter	Estimate	
	Response to A. blight	Leaf size
σ^2_A	4.12	6.62
σ^2_D	2.06	5.09
σ^2_E	4.05	3.67
H	0.87	0.36
Gs*	1.78	1.01
EF_1	1.64	3.33
EF_2	1.56	3.16

* k= 1.16 for selection intensity of 30%

The frequency distribution of the disease reaction of the F_2 and $F_{2:3}$ mapping populations to ascochyta blight were approximately normal (Figure 1) consistent with the polygenic control of resistance. Leaf size and reaction to ascochyta blight were highly significantly affected by F_2 plants (Table 4).

Table4. One way ANOVA of the AUDPC of disease severity on F2 sibs derived from a cross between Bivanij and ICC12004 chick pea varieties.

S.O.V.	D. F.	Mean Square	
		Leaf size	Reaction to AB
F_2 sibs	82	31.73**	96.303**
Error	166	8.28	79.05

Coefficient of Variation= 17.34%

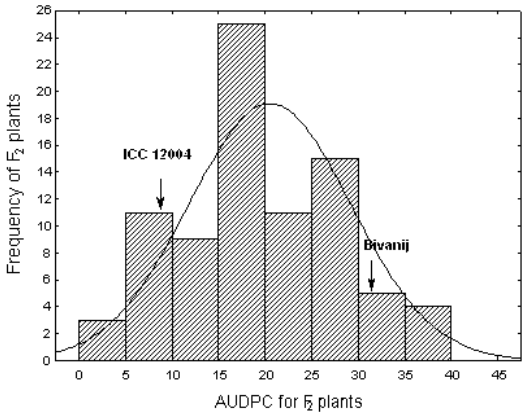
Significantly ($P < 0.01$) higher disease scores were recorded in F_3 families (Table 5). Figure 2 shows the relationship between AUDPC of ascochyta bight $F_{2:3}$ families and their variances in the populations derived from a cross between ICC12004 and Bivanij. There were not significant relationships between mean AUDPC for F_2 and F_3 generations due to Phenotyping based on mean scores of families for $F_{2:3}$ generation.

Table5. One way ANOVA of the AUDPC of disease severity between F3 families derived from a cross between Bivanij and ICC12004 chick pea varieties.

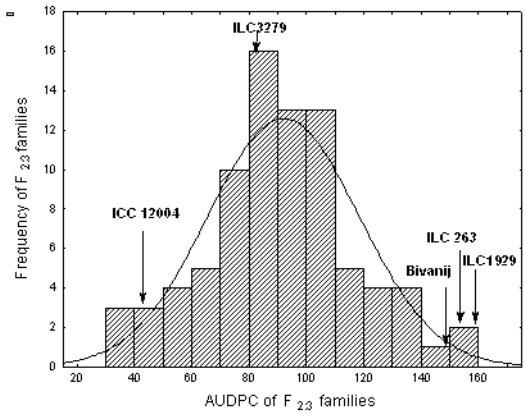
S.O.V.	D. F.	Mean Square	EMS
Between F_3 families	82	4865.7**	$\sigma^2\omega+7\sigma^2\beta$
Within F_3 families	498	2713.8	$\sigma^2\omega$

$\sigma^2\beta = 307.4$.

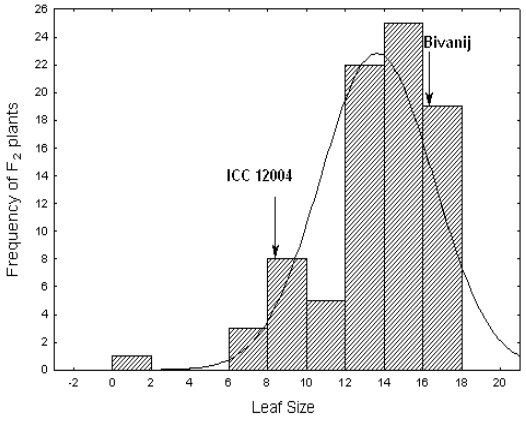
Mean blight scores of the resistant parent (AUDPC-F_2=32.2±6.2 and AUDPC-$F_{2:3}$ =148.4±0.2) were significantly different from that of the susceptible parent (AUDPC-F_2=8.1±3.14 and AUDPC-$F_{2:3}$= 44.1±0.32). To the best of our knowledge, this is the first study to identify and map QTLs confer resistance to payhotype III of ascochyta blight in an intraspecific population of chickpea. Out of 149 microsatellite markers tested, 58 markers revealed polymorphism between the parents ICC12004 and Bivanij, and 57 of them were mapped on the genome. The linkage map comprised eight linkage groups, excluding flower color which didn't assign to any linkage group. The SSRs that were common between the current map and previous maps (Winter et al., 2000; Udupa and Baum, 2003; Tar'an et al., 2007) were placed on the same linkage group but with slightly different orientation and distance [18], [22], [29]. Using composite interval mapping, significant association between SSR markers and putative QTLs for ascochyta blight reaction were found on three linkage groups. These QTLs on LG3, LG4 and LG6 determined 11, 17 and 19 percent, respectively and together these loci accounted for 47% of the total estimated phenotypic variation for reaction to ascochyta blight (Table 6).



(a)



(b)



(c)

Figure3 Frequency distribution for leaf area, AUDPC for F_2 and $F_{2:3}$ families, respectively.

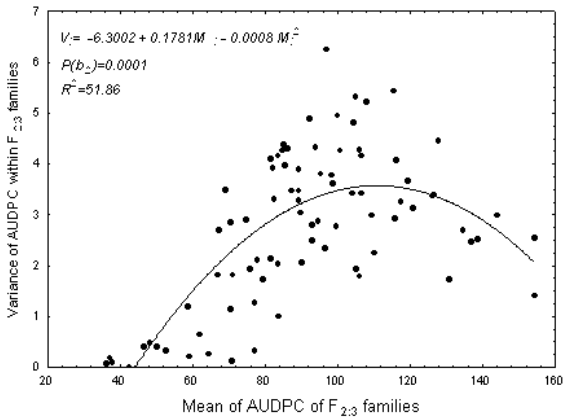


Figure4 Relationship between AUDPC of ascochyta bight $F_{2:3}$ families and their variances in the populations derived from a cross between ICC12004 and Bivanij.

Table6. Putative QTLs for ascochyta blight resistance in F_2 and $F_{2:3}$ generations by Composite Interval Mapping (CIM) method

Parameter	Linkage group	Interval (cM)[a]	Flanking markers	Position of QTL (cM)[b]	LOD[c]	Genetic effects[d]		Gene action[e]	R^2(%)[f]
						Additive	Dominance		
AUDPC-F_2	LG3	14.1	TA125-TA34	0.81	2.50	-4.82	1.96	PD	10.98
AUDPC-$F_{2:3}$	LG4	29.8	TA2-TA72	23.8	4.15	-6.42	-14.43	OD	16.96
	LG6	6.7	GA26-TA80	45.7	4.57	2.69	19.39	OD	18.61

[a] interval between two flanking markers(cM)
[b] QTL position from the left flanking marker(cM)
[c] Peak value of LOD test statistic observed for the QTL in question
[d] Additive and dominance gene effects
[e] A = additive gene action ($|d/a|<0.2$), PD = partial dominance ($0.2<|d/a|<0.8$), D = dominance ($0.8<|d/a|<1.2$), and OD = over dominance ($|d/a|>1.2$)
[f] proportion of phenotypic variance explained by the QTL.

The QTLs on LG3, LG4 and LG6 are flanked with TA125 and TA34, TA2 and TA72, and GA26 and TA80 respectively (Figures 5 and 6), on the current map and are co-localized with the QTLs reported by other investigators (Figures not shown) (Udupa and Baum, 2003; Tar'an et al., 2007) [22], [18]. None of the loci on LG2 was associated with resistance to ascochyta blight in our population. This result is in contrast to the findings of Chongo et al., (2004) and Udupa and Baum (2003), which suggested that a major gene located on LG 2 controlled quantitative resistance to *D. rabiei* [3], [22]. This was not surprising; since current population was evaluated for its quantitative reaction to one isolate belong to pathotype III of ascochyta blight. Fine mapping of the QTLs identified in this study would lead to the identification of markers that could be used for marker-assisted selection of chickpea genotypes with resistance to ascochyta blight.

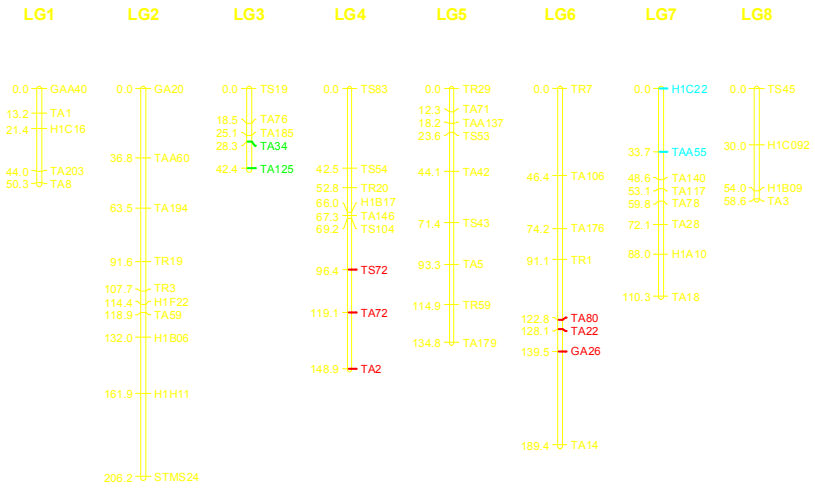


Figure 5 SSR linkage map of chickpea showing detected QTLs for leaf size and ascochyta blight resistance for F_2 and $F_{2:3}$ families in the populations derived from a cross between ICC12004 and Bivanij.

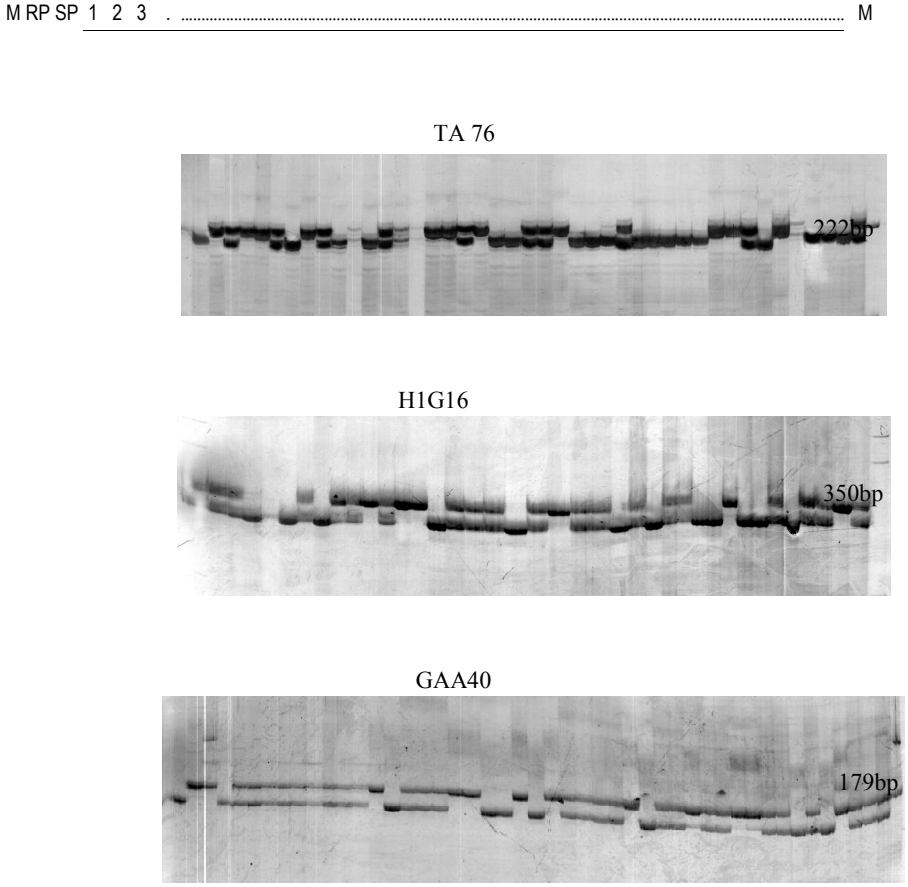


Figure6 Sequence Characterized 3 Microsatellite markers for few sibs of F_2 population; M: marker size, RP: resistant parent (ICC12004), SP: susceptible parent to ascochyta blight (Bivanij).

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