Inheritance of Resistance to *Ascochyta rabiei* in Chickpea and Development of Marker Assisted Selection for Disease Resistance

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1. INTRODUCTION

Chickpea (Cicer *arietinum* L.) is the third most important pulse crop in the world after dry bean (Phaseolus vulgaris) L.) and pea *(Pisum sativurn* L.). Chickpea is a self pollinated crop, has a chromosome number of 2n=l6, and is classified in the tribe *Cicereae* under the *Fubuceae* family. It originated in the Fertile Cresent (region adjoining southeastern Turkey and Syria (Ladizinsky and Addler, 1976 a, b).

Chickpea is classified into two types: desi, which has purple flowers and a small angular seed with a thick, colored seed coat; and kabuli, which has white flowers, a large seed which resembles a chick's head and a thin, white seed coat. Chickpea is consumed by people in the form of garbanzos (whole seed), falafel, hummus, dhal (decorticated and split chickpea) and besan (flour) which is used in infant foods, biscuits and breads. Kabuli chickpea seed >10 mm in diameter is demanded by the garbanzo canning industry and the salad bar trade.

On a global basis about 8.4 million MT of chickpea is produced with an average yield of 0.74 t/ha; India and Pakistan account for 74 % of the production, all of which is consumed locally (FAO, 1997). Roughly 0.5 million MT of chickpea (mostly desi) is traded worldwide; most of the trading is done by the top two exporters Turkey and Australia. The major importers of chickpea are Spain, Lebanon, and the United States, and they prefer premium large-sized kabuli seed. Smaller Western European markets of approximately 50,000 tonnes exist for 10 mm size kabuli and are currently being filled by 8-9 mm kabuli chickpea from Turkey.

Starting in the late 1980s lines of chickpea were evaluated in Saskatchewan to assess their potential in the Brown and Dark Brown soil zone, but they were susceptible to ascochyta blight which destroyed the crop. Ascochyta blight is caused by the polycyclic fungus Ascochyta *rubiei* (anamorph) or *Didynzellu rubiei* (teleomporph) which decimates fields of susceptible chickpea plants within three weeks of rainy weather in the presence of adequate inoculum. Warm moist weather, which is conducive to chickpea growth, is also favorable for the development of A. *rubiei*. Generally, hot dry weather and low humidity inhibit development of the disease.

Commercial chickpea production in Saskatchewan started with in 1994 with 120 hectares, rose to 3250 hectares in 1996 and about 10,000 hectares in 1997. Production was based on the ascochyta susceptible cultivars Tyson (Cheston, ILC 235) desi, and UC 27 kabuli in 1994, and starting in 1995 the ascochyta resistant cultivars Myles desi and Sanford, Dwelley, and B90 kabuli. Approximately 80 % of the production was kabuli type in 1997. Approximately 20,000 hectares (40,000 tonnes of product) will be produced in 1998. Resistance to ascochyta is required to minimize losses and consistently provide a high quality product for the evolving export and domestic markets.

Sources of resistance are available and can be incorporated into lines of chickpea that are adapted to the Brown and Dark Brown soil zones. Several chickpea lines with resistance to ascochyta blight have been identified (Haware et. al., 1995; Singh et. al., 1993a; Gurdip et. al., 1991; Singh et. al., 1992; Singh et. al., 1993b). These are being used as resistant parents in breeding programs. Development of cultivars with durable resistance requires the incorporation of two or more genes for resistance into the new cultivar. However, when two or more genes for resistance to ascochyta blight are segregating in an F_2 population, it is difficult to determine whether the resistance is due to the first gene, the second gene or both genes. Development of molecular markers, e.g. random amplified polymorphic DNA (RAPD), closely linked to each of the genes for resistance to ascochyta blight will enable researchers to select those plants carrying both genes for resistance and develop cultivars with durable resistance.

Accordingly, the objectives of this study were:

a) determine the mode of inheritance of resistance to ascochyta blight in chickpea, and
b) develop a RAPD marker closely linked to a gene for resistance to ascochyta blight in chickpea.

2. MATERIALS AND METHODS:

In 1995,26 chickpea lines, most of them reportedly resistant to ascochyta blight, were rated for ascochyta resistance to the Preston Aveneue *Ascochyta rabiei* population. Crosses were attempted between resistant lines and susceptible lines and three crosses were selected for further study.
A susceptible kabuli line ICCV6-3, and three resistant desi lines (GLG 83 119-4, P1279-2-1, and PBGl-4) were crossed with and the F,, F_2, and $F_{2:3}$, populations were grown. The kabuli parent with recessive white flowers was used as the female and purple flower color of the F, plants confirmed that they were true hybrids. The F_2 plants and $F_{2:3}$ were rated for ascochyta reaction.

Leaf samples were collected from individual F_2 plants and stored at -70°C for DNA extraction at a later date. Genotypes of the individual F_2 plants were confirmed by $F_{2:3}$ ascochyta rating. DNA samples from six homozygous resistant F_2 plants were bulked as were DNA samples from six homozygous recessive F_2 plants and used for bulked segregant analysis. These two DNA bulks were screened for polymorphisms using 550 decamer primers.

Linkage-l (Suiter et. al., 1983) was use to check for linkages between the resistance locus and RAPD markers in the F_2 population (56 plants) of ICCV6-3 x P1279-2-1.

3. RESULTS

Two crosses (ICCV6-3 x GLG 83 119-4 and ICCV6-3 x PBG1-4) gave a poor fit to a l resistant : 3 susceptible ratio in the F_2 and the 1:2: 1 ratio in the F,.
The cross ICCV6-3 x P1279-2-1 gave a good fit to a 1 resistant : 3 susceptible ratio in the F_2 and to a l homozygous resistant : 2 segregating :1 homozygous susceptible ratio for the F_3 families. Accordingly, this cross was selected for the RAPD marker study.

Six primers produced polymorphic bands, and contingency chi-squared tests indicated that RAPD marker UBC 293_{820} was linked with the recessive gene for resistance to ascochyta blight (9.1± 4.0 cM) and that RAPD marker UBC 636_{760} was linked with the recessive gene for resistance to ascochyta (22.0± 6.0 cM). Figures 4.6 to 4.9 illustrate typical results. MAPMAKER (Lander et al., 1987) indicated that RAPD markers UBC 290_{820}, and UBC 636_{760} flanked the recessive allele for resistance to ascochyta blight and were in coupling phase linkage. A third RAPD marker UBC 6 15 $_{1400}$ was also located distal to the RAPD marker UBC 293_{820}

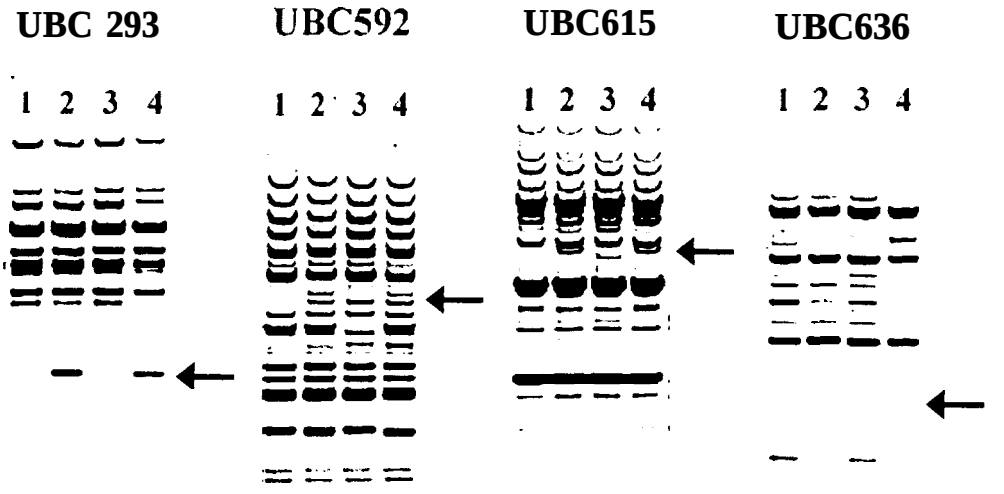

Figure 4.6 Detection of polymorphic RAPD bands from bulked segregant analysis in $F_{2:3}$ families of **P1279-2-1** x **ICCV6-3** chickpea, on agarose gel using UBC primers **293, 592, 615, 636.** Lane 1 = Resistant DNA bulk, lane 2 = susceptible DNA bulk, lane 3 = resistant parent P1279-2-1, lane 4=susceptible parent ICCV6-3. Arrows indicate the polymorphic bands for the respective UBC primers. These results indicate the successful use of bulked segregant analysis for the four RAPD markers.

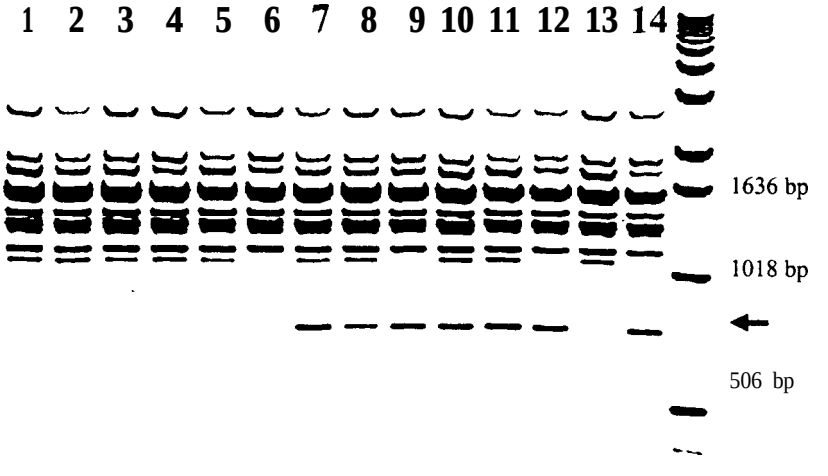

Figure 4.7 Screening primer $UBC293_{820}$ against the six individual plants in the resistant bulk of the $F_{2:3}$ families of **P1279-2-1** x **ICCV6-3** (lanes, **1-6),** the six individual plants in the susceptible bulk (lanes, **7-12), P1279-2-1** the resistant parent (lane **13), ICCV6-3** the susceptible parent **(lane14),** followed by a 1 Kb ladder. The arrow indicates the $UBC293_{820}$ banding pattern. These results indicate the successful use of bulked segregant analysis for this RAPD marker.

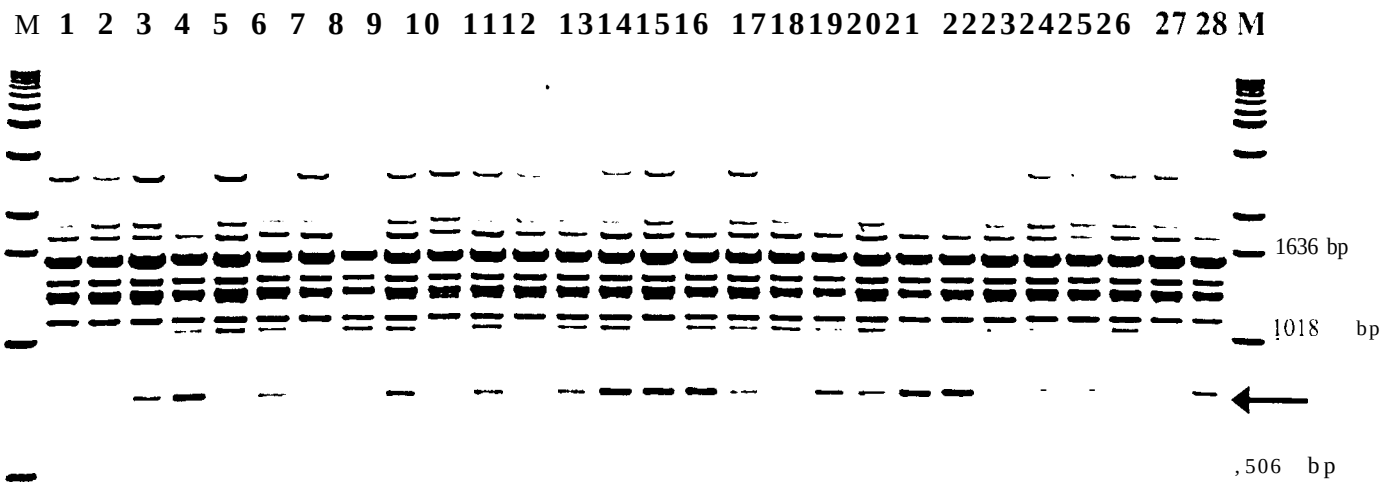

Figure 4.8 Agarose gel showing RAPD markers amplified for primer UBC293 in the F2 population of **P1279-2-1** x **ICCV6-3. F_2** plants from susceptible **$F_{2:3}$** families are marked by the presence of the band **$UBC293_{820}$** (lanes **2-4,6,9,11-22,24,25,28); F_2** plants from resistant **$F_{2:3}$** families indicate absence of the band **$UBC293_{820}$ (lanes 5,7,8,10,26,27);** recombinant between the linked marker **$UBC293_{820}$** and the resistance locus (lanes 1 and 23). The arrow indicates the linked marker **$UBC293_{820}$.** Lane M is the 1 Kb ladder.

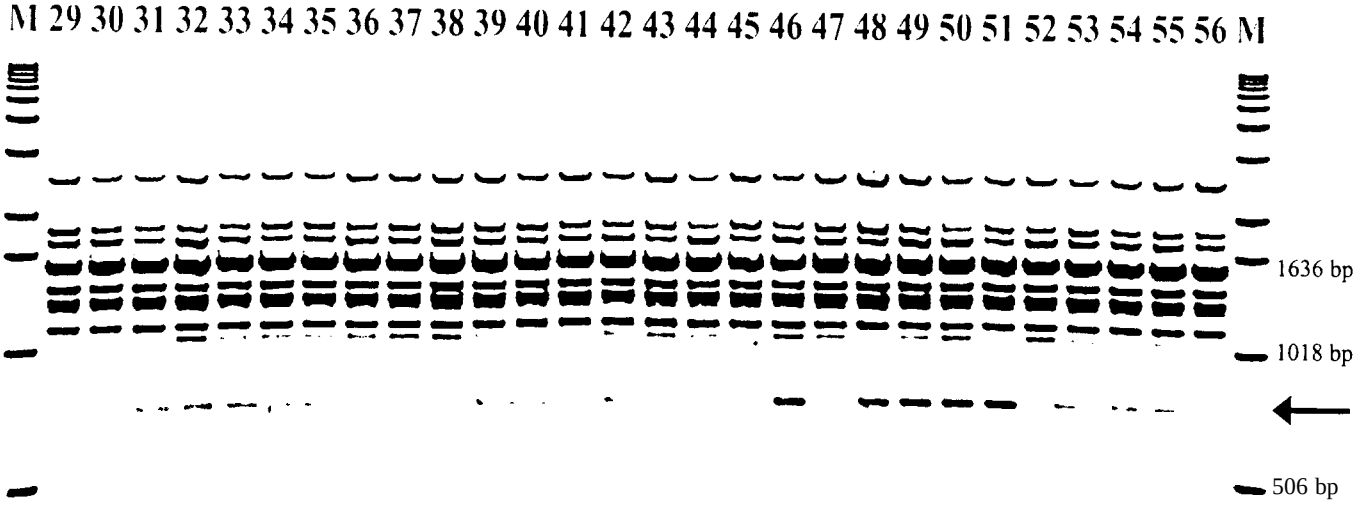

Figure 4.9 Agarose gel showing RAPD markers amplified for primer **$UBC293_{820}$** in the **F_2** population of **P1279-2-1** x **ICCV6-3. F_2** plants from susceptible **$F_{2:3}$** families (lanes **29-35,38-42,46,48-51,53,54,56); F_2** plants from resistant **$F_{2:3}$** families (lanes **36,37,43-45);** recombinant between the linked marker **$UBC293_{820}$** and the resistance locus (lanes **47,52,55).**

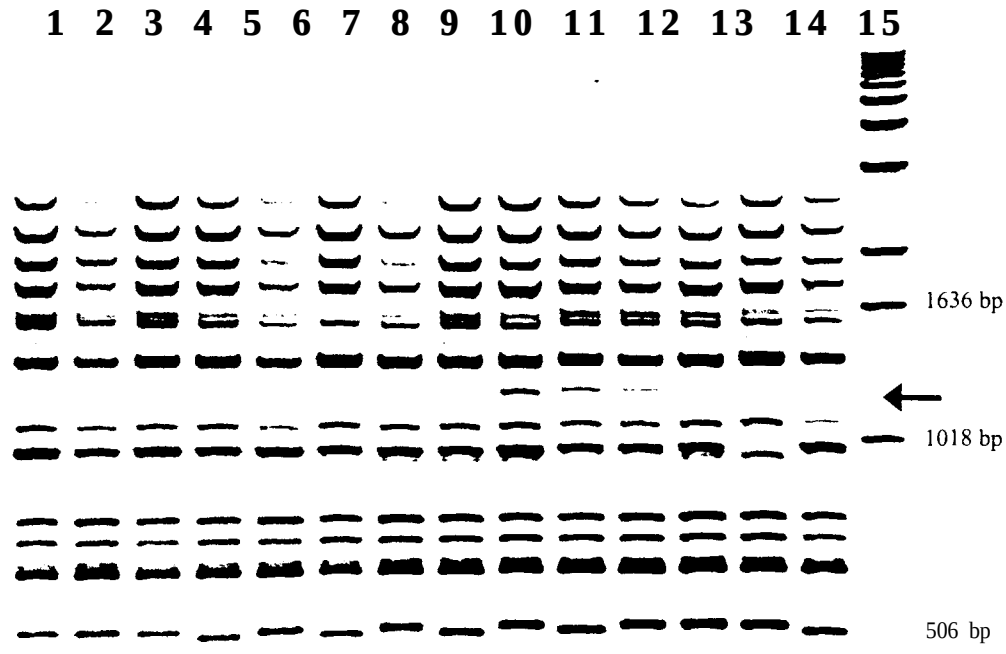

Figure 4.10 Screening primer UBC592 against the six individual plants in the resistant bulk (lanes **1-6),** the six individual plants in the susceptible bulk (lanes 7-12), P1279-2-1 the resistant parent (lane 13), ICCV6-3 the susceptible parent (lane 14) followed by a 1 Kb ladder. Arrow indicates the **$UBC592_{1300}$** banding pattern.

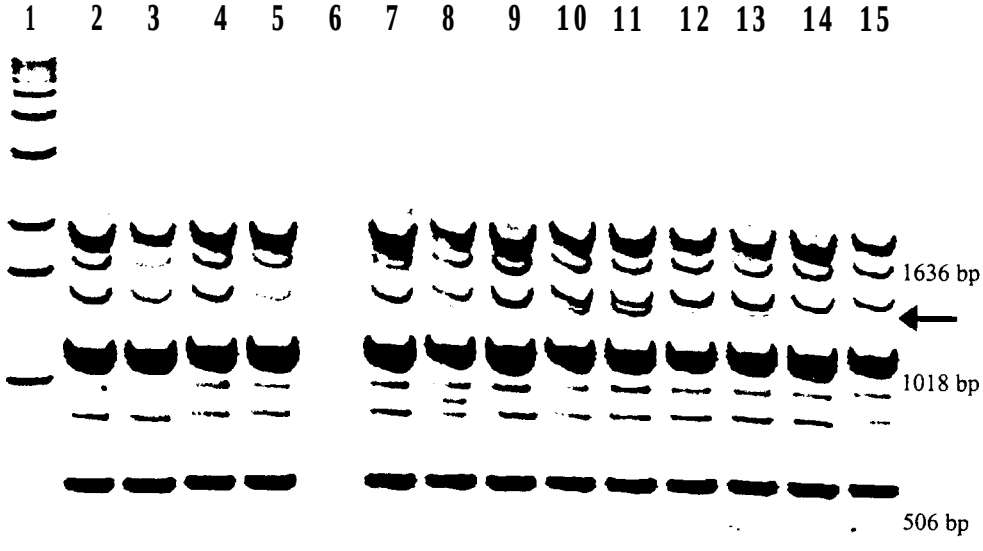

Figure 4.11 Screening primer UBC615 against the individuals in the resistant bulk (lanes **2-7),** the six individual plants in the susceptible bulk (lanes 8-13), P1279-2-1 the resistant parent (lane 14), ICCV6-3 the susceptible parent (lane 15) ; l=l Kb ladder. Arrow indicates the **$UBC615_{1400}$** banding pattern.

4. SUMMARY AND CONCLUSIONS

In the absence of an F_2 population which is greater than 800, recessive, monogenic best describes the inheritance of resistance in cross ICCV6-3 x P1279-2- 1. A single recessive gene for resistance to ascochyta blight in chickpea conditioned ascochyta resistance in P1279-2- 1. Six primers produced polymorphic bands in the F_2 of ICCV6-3 x P1279-2-1. RAPD marker UBC 293_{820} was linked in coupling phase with the recessive gene for resistance to ascochyta blight (9.± 4.0 cM). RAPD marker UBC 636_{760} was linked in coupling with the recessive gene for resistance to ascochyta blight (23.0±6.0 cM). These RAPD markers flanked the recessive gene for resistance to ascochyta blight. A third RAPD marker UBC 615_{1400} was also located distal to the RAPD marker UBC 293_{820}. Flanking markers can be used to select for resistance to ascochyta blight, but all selections must be double-checked by inoculation with ascochyta.

Amplified fragment length polymorphism (AFLP) could be used on new DNA bulks which would be reconstructed using the data form this RAPD study to identify new markers which are linked within 5 cM of the recessive resistance gene (r_{arl}).

5. REFERENCES

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