Mapping QTL Associated with Yield and Yield Components and Ascochyta Blight in Chickpea

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Keywords: Chickpea. *Cicer arietinum*. Recombinant Inbred Lines. Ascochyta blight. Quantitative Trait Loci. Photoperiod.

Abstract

Chickpea (*Cicer arietinum* L.) is the third important legume crop in production among the world pulse crops. A better understanding of the genetic basis of yield and disease traits and their association with flowering time could contribute to their use in the breeding strategies of the crop in the Canadian Prairies. The objective of this study was to evaluate a set of Recombinant Inbred Lines (RILs) of chickpea for yield and disease traits and to locate Quantitative Trait Loci (QTL) associated with these traits. This study used a RIL population derived from across between ICCV 96029 (a desi market class, an extra early maturing, highly susceptible to ascochyta blight and CDC Frontier (a kabuli market class, late maturing, moderately resistant to ascochyta blight. A population consisting of 92 RILs together with the 2 parents were grown in a field at Elrose, Saskatchewan in 2011 in a micro plot with 2 replications. Measurements on agronomic traits were made on an individual plant basis and the means of five plants were used for analysis. Plants were air-dried at 35°C for 48 h before measuring the above-ground biomass. Traits measured were grain yield (in gm /plant), above ground biomass (in g/ plant), number of grains /plant, number of pods/ plant, and 1000 seed weight. Harvest index (HI) = grain weight/total above ground dry weight. The result indicated that, there was significant difference in plant height (in cm), number of seeds /plant, number of seeds/pod, 1000 seeds weight (in gm/plant) and Harvest Index (%). The same lines were evaluated in the greenhouse for Ascochyta blight reaction and in the growth chambers for their flowering responses to different photoperiod. Mapping of QTL will be performed on the line mean data for single years of the field observation and for different photoperiod treatments in the growth chamber.

Introduction:

Chickpea (*Cicer Arietinum* L.) is a self pollinated diploid annual grain legume. Chickpea (*Cicer arietinum* L.) is a self pollinated diploid (2n=2x=16) annual grain legume with a genome size of 740 Mbp (van der Maesen 1987). It is the third most important legume crop after dry bean and pea. The world chickpea production is close to 11.2 million ha. The crop is grown in over 40 countries and is use for human consumption because of high protein and calcium content of the crop compared to other pulse crops (Hulse 1991).

Chickpea is introduced to Canada recent and is grown on brown and dark brown soils zones. Canada ranks ninth in the global production. A total of 18.5 million ha cultivated land is available in Saskatchewan for cereals and oil crops. Incorporation of chickpea in the farming system in rotation with cereal is very essential because of the crops ability to fix nitrogen and contribution break disease and insects life cycles boosting the economic return of the growers.

Chickpea breeding was started in the Crop Development Center of the University of Saskatchewan in early 1990s. Since then a number of varieties with high yield, moderately tolerant for ascochyta blight were developed and released for production (Warkentin et al 2005.). In this environment the crop faces a unique challenge as flowering and maturation often occurs under unfavorable conditions. Growth time is limited by hot or cold temperatures, rainfall distribution and biotic stresses. The available wet condition favours a continued growth and formation of new flowers and flowers leading to delayed maturity as a result of which yield and quality of the crop is reduced. A better understanding of the genetic basis of yield and disease traits and their association with flowering time could contribute to their use in the breeding strategies of the crop in the Canadian Prairies.

Hypotheses

1. Flowering time in chickpea is under genetic control and modulated by environmental conditions
2. Earliness and ascochyta blight susceptibility are positively correlated in chickpea

Objectives

1. To determine the genetic basis of the association between flowering time and resistance to Ascochyta blight
2. To map the chromosome regions that control flowering time, days to maturity, photoperiod insensitivity and resistance to Ascochyta blight
3. To assess the number and chromosome position of loci associated with each of the above traits, estimating their effect, and identifying molecular makers closely linked to these QTLs.

Methodology

1. Field evaluation of Chickpea RILs for their response to flowering and maturity

A replicated field experiment was conducted at Elrose using 92 chickpea Recombinant Inbred Lines (RILs) derived from CDC Frontier and ICCV 96029 together with their parents. CDC Frontier is a Kabuli market class, moderately tolerant to Ascochyta blight and flowering and maturing late, developed and released for production by the Crop Development Center, University of Saskatchewan, Canada. ICCV 96029 is a desi market class highly susceptible to Ascochyta blight, flowering and maturing early, developed by International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) India.

Data Collected

Five random plants from each plot were harvested and air dried at 35 ^{0}C for 48 hours and the following traits were measured: Main stem length (cm), Biomass (in g/plant), Number of seeds/plants, Number of pods/plants, 1000 seed weight (in g), Harvest Index (HI) = Grain weight /total above ground dry weight.

Table 1. Analysis of variance for some agronomic components of the chickpea RILs evaluated at Elrose in 2011.

Source of Variation	Sum of Squares	Mean Square	F Value	Pr > F
Plant Height (cm)	2911.41	1.99	3.03	0.0001 ***
No of seeds /plant	485512.19	5220.56	1.41	0.0510*
No of seeds/pod	5.26	0.06	1.98	0.0010***
1000 seeds weight (in g)	258486.89	2779.44	3.35	0.0001***
Harvest Index (%)	9091.69	97.76	1.51	0.0249*

*, **, *** indicates significant at p= 0.5, 0.01 and 0.001 respectively

Table 2. Pearson correlation coefficients among yield and yield components of the chickpea RILs evaluated at Elrose in 2011.

	Stem length	*Inter-node*	*Node*	*Branches*	*Biomass*	*Pods*	*Seeds weight*	*No of seeds*
Inter- node	*0.42****							
Node	*0.55****	*-0.29**						
Branches	*0.09*	*0.36****	*-0.02*					
Biomass	*0.35****	*0.15*	*-0.14*	*0.28**				
Pods	*0.26**	*-0.003*	*0.36****	*0.38***	*0.54****			
Seeds weight	*0.08*	*0.01*	*-0.41****	*0.08*	*0.83****			
No of seeds	*0.09*	*-0.08*	*0.21**	*0.42****	*0.54****	*0.88****	*0.24**	
HI	*-0.41****	*-0.23*	*-0.14*	*0.16*	*-0.04*	*0.10*	*0.15*	*0.36***

*, **, *** Significant at P < 0.05, 0.01 and 0.001 respectively.

The same set of RILs and their parents were evaluated at Pasqua in a micro plot, single replication in 2011. The following data were collected: Ascochyta blight score1 and the second ascochyta blight score two weeks after the first score is done. Days to flowering and maturity were also scored.

Table 2. Pearson correlation coefficients among Ascochyta blight scores and days to flowering and maturity of the RILs evaluated at Pasqua in 2011.

	Ascochyta Blight 1	Ascochyta Blight 2	Days to Flowering
Ascochyta Blight 2	0.66***		
Days to Flowering	- 0.14	-0.24*	
Days to maturity	- 0.23 *	-0.24*	- 0.05

*, ***, Significant at $P < 0.05$ and 0.001 respectively.

2.1. Indoor Evaluation of chickpea RILs for their response to flowering under long days

Indoor evaluation of the 92 RILs and their parents was conducted for their response to flowering under long days. The chambers were adjusted to 16/8 hr and 22/16 ^{0}C day and night respectively and a light intensity of 370 µmol $m^{-2}s^{-1}$. Each line was planted in 3 pots/line with 2 plants/pot in a 1 gallon pots, Sunshine mix # 4. Soluble fertilizer every 2 weeks and watering was done as per the requirement of each pot. Days to flowering was scored as a number of days from seedling emergence to appearance of the first floral opening on a plant.

Table 3. Anova for response to flowering under long days in chickpea RILs and the two parents evaluated in the growth chamber.

Variables	Sum of Squares	Mean Square	F Value	Pr > F
Days to flowering run 1	3010.98	33.09	9.32	0.0001***
Days to flowering run 2	6528.33	75.91	17.23	0.0001 ***
Days to flowering run 1&2 Combined	8863.66	96.34	17.98	0.0001***

*** Significant at P < 0.001.

2.1. Indoor valuation of chickpea RILs for their response to ascochyta blight

Evaluation of RILs and their parents for their reaction to Ascochyta blight was conducted in greenhouse in summer 2011. The experiment was conducted in four rounds using the 92 lines together with the two parents ICCV 96029 (a susceptible parent) and CDC Frontier (a resistant parent) as checks. The susceptible check was planted with every test line. An isolate Ar- 170 at rate of $2x10^5$ was used 2 ml/plant 3 weeks after planting. The test lines were kept in a humidifying chamber for 48 hours and then transferred to the mist chamber for 2 weeks. Ascochyta blight score 1 was rated after 2 weeks of inoculation (1- 9 scale) and the Ascochyta blight score 2 was rated after 3 weeks of inoculation (1- 9 scale) (fig 2.)

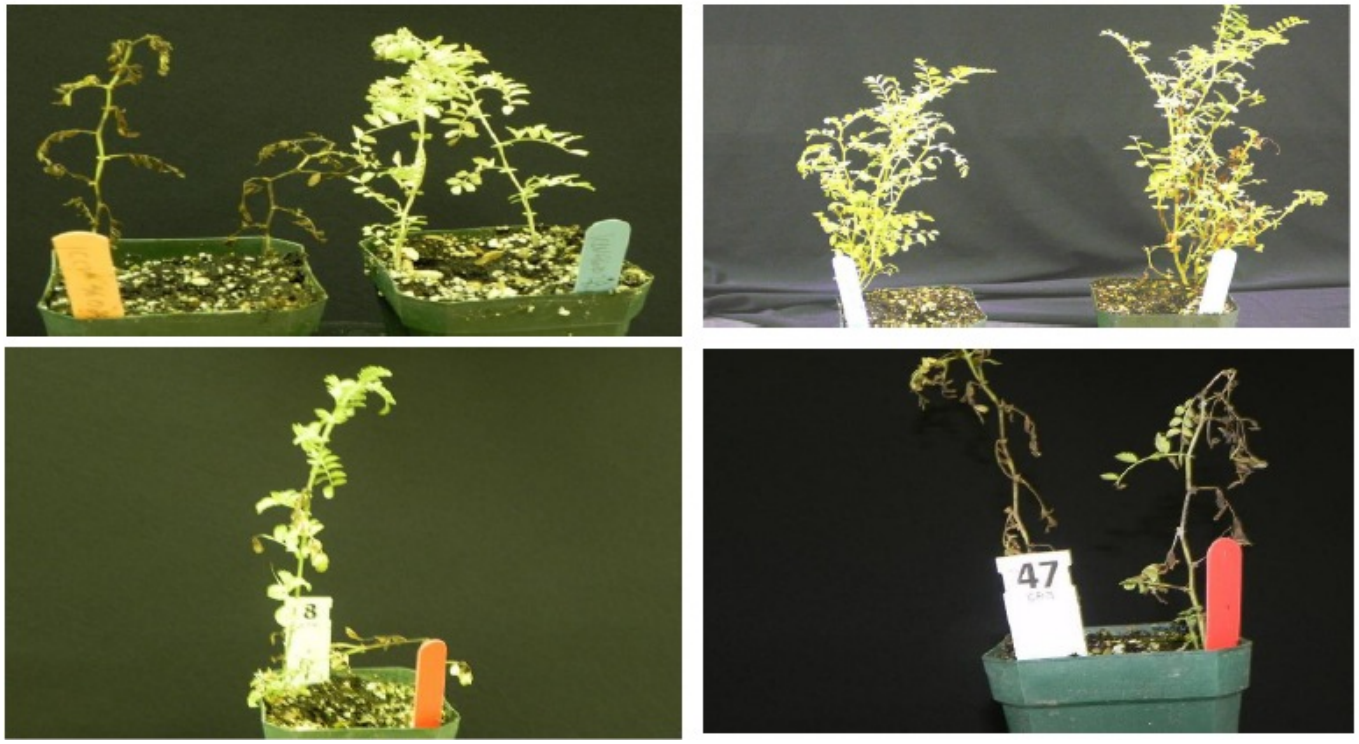

Fig 1. Reaction of the RILs to ascochyta blight in the greenhouse susceptible (A) and resistant (B) checks inoculated and water treated Resistant (C) and a highly susceptible (D) lines

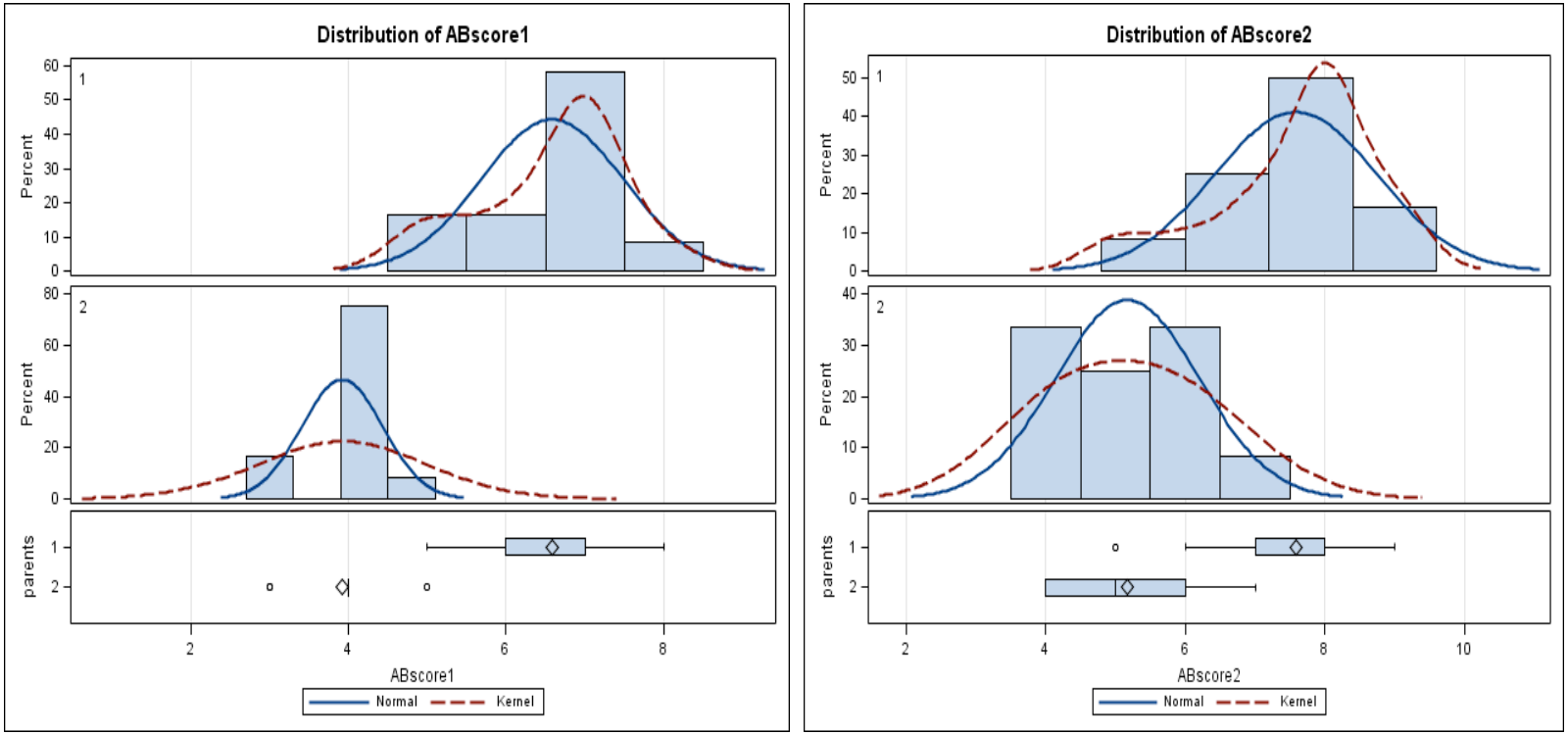

Fig. 2. Distribution of the Ascochyta blight Scores of the two parents (CDC Frontier and ICCV 96029) evaluated in 4 runs in the greenhouse, in summer 2011.

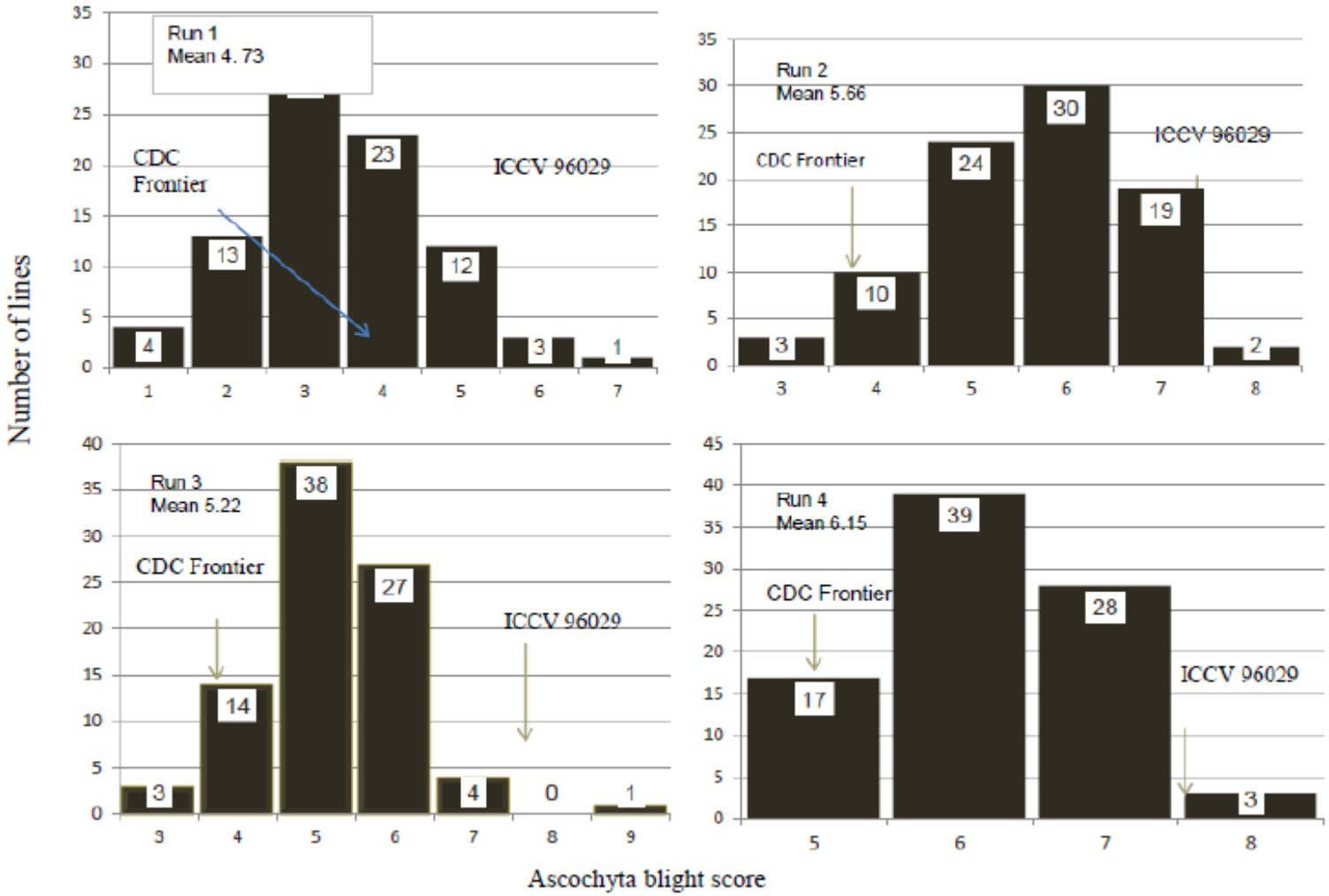

Fig. 3. Frequency distribution and mean of ascochyta blight disease ratings (1-9 scale) of the chickpea RILs evaluated in 4 runs in the greenhouse in summer 2011.

Table 4. Analysis of Variance for Ascochyta Blight in chickpea Recombinant Inbred Lines (RILs) together with the two parents (ICCV 96029 and CDC Frontier) evaluated in greenhouse in 2011.

Source of Variation	Sum of Squares	Mean Square	F Value	Pr > F
Ascochyta Blight score 1 Run 1	230.15	2.68	2.60	0.0001***
Ascochyta Blight score 2 Run 1	243.48	2.86	2.50	0.0001***
Ascochyta Blight score 1 Run 2	184.59	2.12	2.65	0.0001***
Ascochyta Blight score 2 Run 2	239.59	2.75	3.48	0.0001***
Ascochyta Blight score 1 Run 3	821.97	9.56	1.03	0.4226 ns
Ascochyta Blight score 2 Run 3	296.29	3.45	3.82	0.0001***
Ascochyta Blight score 1 Run 4	226.24	2.57	3.36	0.0001***
Ascochyta Blight score 2 Run 4	233.26	2.68	6.15	0.0001***
Ascochyta Blight score 1 All run combined	695.97	7.49	1.85	0.0001***
Ascochyta Blight score 2 All run combined	482.09	5.18	3.14	0.0001***

*** Significant at $P < 0.001$, ns non-significant.

Conclusions:

- Wide variability among the RILs for the traits measured
- The resistant parent flowered late while the susceptible parent flowered early in field and indoor evaluations
- Days to flowering and maturity were negatively and significantly correlated to ascochyta blight resistance

Future activities:

- A three replication micro-plot experiment will be repeated in the field at three locations in 2012.
- Evaluation of the RILs in the growth chambers under short days in 3 reps and 2 runs will be conducted.
- Genotyping using SNP markers followed by linkage mapping and QTL analysis**.**

References

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