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Inheritance and mapping of QTLs for *Ascochyta* blight resistance in chickpea (*Cicer arietinum*)

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ABSTRACT

There are very limited studies on the QTLs governing *Ascochyta* blight resistance which is a major problem in chickpea (*Cicer arietinum* L.) grown in North West Plain Zone of India in the cooler climates and world over. An interspecific mapping population consisting of 157 $F_{2:3}$ lines developed by crossing a resistant parent ILWC129 (*C. reticulatum*) with a susceptible variety GL769 (*C. arietinum* L.), was evaluated (2019–20) and genotyped (2021) for genetic studies and mapping of resistance to *Ascochyta rabiei* (syn. *Phoma rabiei*), isolate 8(3968). The inheritance pattern gave 1R:2H:1S goodness of fit ratio with χ^2 value of 0.4 indicating that resistance against isolate 8 of the pathogen governed by a single gene. Out of 831 SSR markers screened, 86 were found polymorphic with 10.3% polymorphism from which only 41 markers segregated in 1:2:1 ratio and could be mapped. Eight linkage groups with a cumulative distance of 1042.31 cM were formed with an average distance of 25.42 cM. This is probably the first report of inheritance studies and tagging of *A. rabei* race 8 (arr_8) resistance gene using molecular markers in an interspecific cross with *C. reticulatum*.

Keywords: *Ascochyta* blight, Chickpea, Linkage, Molecular mapping, QTLs

Chickpea (*Cicer arietinum* L.) is one of the most vulnerable crops in India facing huge losses in terms of productivity due to biotic and abiotic stresses. Of various biotic stresses of chickpea, Ascochyta blight caused by *Ascochyta rabiei* (syn. *Phoma rabiei*) is one of the most devastating diseases after *Fusarium* wilt. Under favourable environmental conditions, *Ascochyta* blight leads to complete yield loss (Sharma and Ghosh 2016). Not many efforts are being put forth towards the development of resistant varieties against the disease. We are still on the way to identify locus specific markers. Many researchers are working all over the world to enrich the molecular map of chickpea for Ascochyta blight resistance, the disease being tough to deal due to co-evolution of new pathotypes with its host (chickpea) depending on area and environmental conditions. Strengthening of breeding efforts as well as studies on inheritance of resistance will help to develop cultivars with durable resistance. However, there are very few accessions having resistance to Ascochyta blight in the cultivated gene pool, therefore in order to improve commercial cultivars for resistance, the cross compatibility of *C. arietinum* with its progenitor species *C. reticulatum* can be utilized to generate new resistant sources. Genetically it has been reported that resistance to Ascochyta blight is governed by both single recessive or dominant genes (Singh and Reddy 1990) but it breaks down easily and frequently by the appearance of new races of the pathogen. Therefore it necessitates to generate novel sources of Ascochyta blight resistance and map the QTLs associated with resistance so that they can be used in the breeding program for developing improved cultivars with greater level of resistance.

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MATERIALS AND METHODS

In the present study, an interspecific mapping population (157 $F_{2:3}$) was developed by crossing resistant parent ILWC129 (*C. reticulatum*) as male with agronomically good but susceptible variety GL769 (*C. arietinum*). Field screening was done using artificial epiphytotic field conditions (2019–20) at Department of Plant Breeding and Genetics (Latitude 30'.90"and Longitude 75'.85") and molecular work (2021) was conducted at laboratory facilities of School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana and partly at Division of Genetics, ICAR-IARI, New Delhi.

Spore culture and inoculum preparation for screening of $F_{2:3}$ population for Ascochyta blight: Virulent culture of *A. rabiei* isolate 8 (3968) was collected from infected chickpea

plants and used for single spore culture to prepare inoculums after multiplication on sterile seeds of chickpea. The process was followed by autoclaving chickpea seeds at 121°C for 25 min after being soaked overnight and inoculation on Capek Dox Agar with 1 cm disc of an actively growing culture of *A. rabiei* was done followed by incubation at 20°C for 8 days with 12 h photoperiod. Profusely sporulated seeds were stirred in sterile distilled water in order to facilitate the release of pycnidiospores into water. The suspension was filtered through muslin cloth and inoculum load was adjusted to 50,000 spores per ml following dilution and sprayed on the host plants.

Field screening of adult plants: The technique for the development of disease and evaluation for disease reaction was done as suggested by Gurha *et al*. (2003). The $F_{2:3}$ progenies were sown in a row length of 2 m with 40 cm spacing between the rows. A susceptible check, L550 was planted after every 8 test rows as indicator cum infector rows. Recommended package of practices were used to raise a good crop. Conditions in the field were made epiphytotic by using perforated sprayer system as inoculation takes place at around 25ºC temperature and beyond 85% humidity. Knap-sack sprayer system was used to wet the plants before inoculation during the evening hours. The spore suspension containing 4×10^4 spores per ml was prepared at the Department of Plant Breeding and Genetics, PAU, Ludhiana. Artificial inoculation of isolate 8 (3968) of *A. rabiei* was done and sprinklers were run in the experimental area from 10:00-16:00 h for 21 days at an interval of one hour. After 10–15 days, symptoms of disease appeared, and the 1-9 disease rating scale was used to record disease score during the last week of March (Singh *et al.* 1998, Garg *et al.* 2018). Based on the disease reaction, the $F_{2:3}$ population was categorized into resistant and susceptible. Different genetic ratios were used to evaluate the data in order to identify the best fit ratio.

Genotyping of F_2 mapping population (GL769 × ILWC129) using SSR markers: Young leaves were harvested from 157 F_2 plants along with both the parents (GL769 and ILWC129). DNA was isolated using CTAB extraction procedure.

SSR primers used in study: Parental polymorphism was carried out through screening a total of 831 SSR markers selected from the published maps of Winter *et al.* (1999), Lichtenzveig *et al.* (2005) (H-series), Nayak *et al.* (2010) (ICCM series), Bharadwaj *et al.* (2011a), Thudi *et al.* (2011) (CaM series) and Subodh *et al*. (2015) (NC series). The polymorphic markers were used for 157 $F_{2:3}$ families. ABI thermal cycler was used for the marker amplifications using a touchdown amplification profile.

Molecular mapping of Ascochyta blight resistance: For each segregating marker, a χ^2 goodness of fit analysis was performed using MAPDISTO (1.7.6.5) in order to test deviation from 1:2:1 expected segregation ratio and 2.5 logarithms of odds (LOD) was used for grouping them with 0.3 recombination distance. Table 2 illustrates map distances acquired from recombination frequencies using Kosambi (1944) mapping function and MAPCHART.

Table 1 Segregation of *Ascochyta* blight resistance at adult plant stage in field conditions at PAU, Ludhiana (Phenotypic) and the SSR marker TA 42

Phenotypic segregation ration at Adult Plant stage for Ascochyta blight (R, Homozygous resistant; H, Heterozygous; S, Homozygous susceptible)					
R	H	S	Total	Genetic ratio	$\chi 2$
37	76	43	157	1R:2H:1S	0.6
Segregation of SSR marker (TA42) linked with Ascochyta blight resistance gene					
SSR marker	GL769 (Allele A)	Heterozygote (Allele H)	ILWC129 (Allele B)	$\chi 2$ (1:2:1)	
TA42	41	80	35	0.57	

Table 2 Characteristics of linkage groups generated in an interspecific F_2 mapping population in chickpea

Linkage group	No. of markers	Average distance between the markers (cM)	Total length (cM)
1	11	48.7	487.8
2	4	37.9	113.7
3	6	25.2	126.3
4	3	33.3	66.7
5	2	30.2	30.2
6	6	25.76	128.8
7	4	17.47	52.4
8	5	9.1	36.2

RESULTS AND DISCUSSION

Inheritance studies: As a result of field screening which was carried out at adult plant stage on $F_{2:3}$ lines, susceptible parent GL769 was identified with an average score of 8.0 and the resistant parent ILWC129, with average score of 3.0. The contrasting reaction of parents suggested the development of epiphytotic condition in the field suitable for evaluation of experimental materials. Out of 157 $F_{2:3}$ lines tested, 76 were categorized as heterozygous (H), 43 as homozygous susceptible (S) and 39 as homozygous resistant (R) (Table 1). This segregation pattern for AB isolate 8 gave a perfect fit into 1R:2H:1S ratio with χ^2 value of 0.4 as expected for single gene. The results of the study clearly shows that the resistance at adult plant stage for isolate 8 of the pathogen is governed by a single gene.

Linkage analysis of F_2 population: Parental genotypes were screened using 831 SSR markers. Out of 831 SSR markers, 86 were polymorphic showing an overall polymorphism of 10.3%. Software Mapchart, version 2.3 Voorrips (2002) was used to develop the linkage map. Only those markers that could be ordered at a LOD score of >2.5 were directly included into the linkage map framework, while rest of the markers were excluded from the analysis. Of the 86 polymorphic markers genotyped on

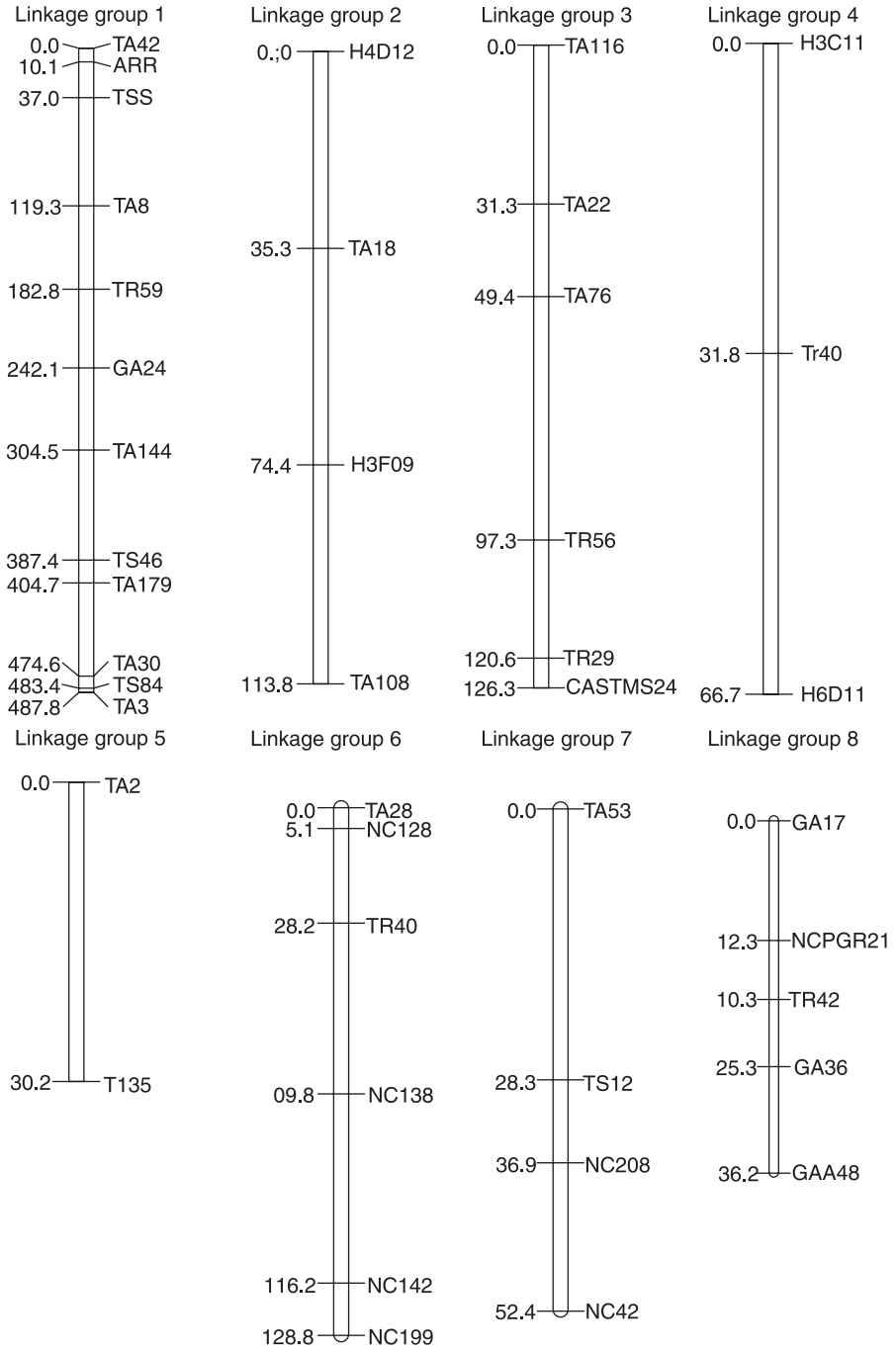

Fig 1 Linkage groups (1-8) of GL 769 × ILWC 129 F_2 population. The genetic distances of SSR markers in cM *right* and locus name *left* side of each linkage group. The cumulative map distance is 1042.2cM covered by 41 markers.

the interspecific mapping population only 41 SSRs could be mapped (47.6% of polymorphic markers and 4.93% of total markers evaluated) covering a cumulative mapping distance of 1042.31 cM with an average density of 25.42 cM per marker (Table 2). There were eight linkage groups into which markers were mapped. The maximum number of markers (11) was mapped on linkage group (LG 1). Bharadwaj *et al*. (2011a) also reported such segregation distortions in *desi* x *kabuli* cross between BGD 112 and FLIP 90-166s. Legumes, in general and chickpea in particular, have low level of polymorphism for SSR's (Bharadwaj *et al*. 2011b) and ISSRs (Chaudhary *et al*. 2011). The genomic coverage of markers was restricted leading to low level of polymorphism between the parents resulting in large gaps on the map obtained. The diagrammatic presentation of these eight LGs is given in Fig 1. A summary of number of SSR markers distributed on each of eight linkage groups (LGs), their density and size of each linkage group is given in (Table 2). Of the eight LGs generated, LG 5 was the smallest with a size of 30.2 cM and LG1 was the largest having a size of 487.87 cM. Except linkage group 5, where only two markers could be mapped, all the remaining linkage groups could anchor more markers. LG 3 was mapped with six loci falling behind LG 1 which was mapped with a maximum of 11 loci. Eleven SSR markers were spanned at a distance of 487.87 cM linkage group 1.This linkage group depicted major gaps. Linkage group 2 included a total of four markers, viz. H4D12, TA18, H3F09 and TA108 spanning a total distance of 113.8 cM. A total of six SSR markers were included in linkage group 3. The markers TR29 and CASTMS24 were spanned by a distance of 5.5 cM, which was the closest distance mapped. Linkage group 4 included only three SSR markers. Two markers, TA2 and TS135 with 30.2 cM spanning distance were included in linkage group 5. Linkage group 6 had six markers, 7 had four markers and 8 had five markers. As the number of loci mapped on each LG was less, hence the linkage groups were small in size, and thus low map length was obtained. However, all the mapped loci were evenly mapped with no significant clustering on any chromosome. Development of this map implies that it can be saturated with more markers and is the best skeleton map if more markers could be associated for the anchorage of more loci in future investigations. Some skewedness towards clustering of markers was seen in linkage group 1. Such skewed segregation is expected in 3-4 clusters and is seen when wide crosses were used for mapping as in the present study.

Molecular mapping of Ascochyta blight resistance

gene: The framework linkage map constructed was used for the mapping of AB resistance gene. The $F_{2:3}$ population was found to be segregating in 1R:2H:1S ratio for single *Ascochyta* blight resistance gene (Table 1). Using present linkage map, AB resistance gene was mapped at a distance of 10.1 cM distal to SSR marker TA42 on linkage group 1 and has been temporarily designated as arr_8. TA42 was scored as co-dominant marker with segregation ratio of 35 (allele B): 80 (allele H): 41 (allele A) with χ^2 value of 0.57 (Table 1). More marker systems or additional SSRs need to be generated to find more closely associated markers with arr_8. The present study illustrates that this mapping information can be a starting point for the fine mapping of *Ascochyta rabei* resistance locus to isolate 8 (arr_8) locus.

It is concluded that inheritance of *Ascochyta* blight resistance to race 8 of the pathogen (studied using disease data of $F_{2:3}$ lines) was governed by a single gene in an interspecific population developed from a cross of *C. arietinum* with *C. reticulatum*. A marker, TA42 was identified as closely linked to the gene of interest. Eight linkage groups could be constructed covering a cumulative map distance of 1042.31 cM. In the present study, the STMS markers were very useful in mapping of AB QTL linked to isolate 8. Scope of increasing the number of markers on the map is always there for the construction of high density linkage map. The loci that this map had marked has a direct utility in marker assisted breeding as cultivated × wild cross throws insights into variability and diversity that can be utilized directly by the breeders in marker assisted breeding.

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REFERENCES

Bharadwaj C, Chauhan S K, Yadav S, Satyavathi C T, Singh R, Kumar J, Srivastava R and Rajguru G. 2011a. Molecular marker-based linkage map of chickpea (*cicer arietinum*) developed from *desi* × *kabuli* cross. *Indian Journal of Agricultural Sciences* **81**(2): 116–18.

Bharadwaj C, Srivastava R, Chauhan S K, Satyavathi C T, Kumar J, Faruqui A, Yadav S, Rizvi A H and Kumar T. 2011b. Molecular diversity and phylogeny in geographical collection of chickpea (Cicer sp.) accessions. *Journal of Geneitcs* **90**: e94–e100.

Choudhary P, Suruchi M Khanna, Pradeep K Jain, Chellapilla Bharadwaj, Jitendra Kumar, Pramesh C. Lakhera and Ramamurthy Srinivasan. 2013. Molecular characterization of primary gene pool of chickpea based on ISSR markers. *Biochemical genetics* **51**(3-4): 306–22

Garg T, Mallikarjuna B P, Thudi M, Srinivasan S, Singh S, Sandhu J S, Kaur L, Singh I, Sirari A, Basandrai A, Basandrai D, Varshney R K and Gaur P M. 2018. Identification of QTLs for resistance to Fusarium wilt and Ascochyta blight in a recombinant inbred population of chickpea (Cicer arietinum L.). *Euphytica* **214**(3): 1–11.

Gurha S N, Singh G and Sharma Y R. 2003. *Diseases of Chickpea and their Management*, pp 195–227. Ali M, Kumar S and Singh N B (Eds). Chickpea Research in India. Indian Institute of Pulses Research, Kanpur

Kosambi D D. 1944. The estimation of map distances from recombination values. *Annals of Eugenics* **12**: 505–25.

Lichtenzveig J, Scheuring C, Dodge J, Abbo S and Zhang H B. 2005. Construction of BAC and BIRAC libraries and their applications for generation of SSR markers for genome analysis of chickpea, Cicer arietinum L. *Theoretical and Applied Genetics* **110**: 492–510.

Nayak S N, Zhu H, Varghese N, Datta S, Choi H K, Horres R, Jüngling R, Singh J, Kishor P B, Sivaramakrishnan S, Hoisington D A, Kahl G, Winter P, Cook D R and Varshney R K. 2010. Integration of novel SSR and gene-based SNP marker loci in the chickpea genetic map and establishment of new anchor points with Medicago truncatula genome. *Theoretical and Applied Genetics* **120**(7): 1415–41.

Sharma M and Ghosh R. 2016. An update on genetic resistance of chickpea to ascochyta blight. *Agronomy* **6**(1): 18.

Singh G, Sharma Y R and Bains T S. 1998. Status of Botrytis grey mold of chickpea research in Punjab, India. *Recent Advances in Research and Management of Botrytis Grey Mold of Chickpea,* pp. 7–14. S Pande, M A Bakr and C Johansen (Eds) (ICRISAT: Patancheru, AP, India).

Singh K B and Reddy M V. 1990. Patterns of resistance and susceptibility to races of Ascochyta rabiei among germplasms and breeding lines of chickpea. *Plant Disease* **74**: 127–29.

Subodh Verma, Gupta S, Bandhiwal N, Kumar T, Bharadwaj C and Bhatia S. 2015. High-density linkage map construction and mapping of seed trait QTLs in chickpea (Cicer arietinum L.) using Genotyping-by-Sequencing (GBS). *Scientific reports* **5**(1): 1–14

Thudi M, Bohra A, Nayak S N, Varghese N, Shah T M, Varma R P, Nepolean T, Gudipati S, Gaur P M, Kulwal P L, Upadhyaya H D, KaviKishor P B, Winter P, Kahl G, Town C D, Kilian A, Cook D R and Varshney R K. 2011. Novel SSR markers from BAC-End sequences, DArT arrays and a comprehensive genetic map with 1,291 marker loci for chickpea (Cicer arietinum L.). *PLoS One* **6**(11), p.e27275

Voorrips R E .2002. MapChart: software for the graphical presentation of linkage maps and QTLs. *Journal of Heredity* **93**: 77–78.

Winter P, Pfaff T, Udupa S M, Huttel B, Sharma P C, Sahim S, Arreguin-Espinoza R, Weigand F, Muehlbauer F J and Kahl G. 1999. Characterization and mapping of sequence tagged microsatellite sites in the chickpea (*Cicer arietinum* L.) genome. *Molecular and General Genetics* **262**: 90–101.