

Research Paper

Molecular mapping of quantitative trait loci for ascochyta blight and botrytis grey mould resistance in an inter-specific cross in chickpea (*Cicer arietinum* L.) using genotyping by sequencing

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Ascochyta blight (AB) and botrytis grey mould (BGM) are the most devastating fungal diseases of chickpea worldwide. The wild relative of chickpea, *C. reticulatum* acc. ILWC 292 was found resistant to BGM whereas, GPF2 (*Cicer arietinum* L.) is resistant to AB. A total of 187 F₈ Recombinant Inbred Lines (RILs) developed from an inter-specific cross of GPF2 × *C. reticulatum* acc. ILWC 292 were used to identify quantitative trait loci (QTLs) responsible for resistance to AB and BGM. RILs along with parents were evaluated under artificial epiphytotic field/laboratory conditions for two years. Highly significant differences ($P < 0.001$) were observed for reaction to both pathogens in both years. Parents and RILs were genotyped-by-sequencing to identify genome wide single nucleotide polymorphism (SNPs). A total of 1365 filtered and parental polymorphic SNPs were used for linkage map construction, of which, 673 SNPs were arranged on eight linkage groups. Composite interval mapping revealed three QTLs for AB and four QTLs for BGM resistance. Out of which, two QTLs for AB and three QTLs for BGM were consistent in both years. These QTLs can be targeted for further fine mapping for deployment of resistance to AB and BGM in elite chickpea cultivars using marker-assisted-selection.

Key Words: chickpea, ascochyta blight, botrytis grey mould, ddRADseq, quantitative trait loci (QTL).

Introduction

Chickpea (*Cicer arietinum* L.) or Garbanzo beans is a self-pollinated diploid ($2n = 2x = 16$) crop with genome size of 738 Mb (Varshney *et al.* 2013). It is the second most consumed grain legume after dry bean grown worldwide and nutrient rich pulse crop that contains 17–31% protein, significant amount of essential amino acids, vitamins and minerals. Chickpea production and productivity is adversely affected by various biotic and abiotic stresses (Thudi *et al.* 2014). Among the biotic constraints, ascochyta blight (AB; caused by *Ascochyta rabiei* (Pass.) Lab.) and botrytis grey mould (BGM; caused by *Botrytis cinerea* Pers. ex. Fr.) are the most devastating fungal diseases of chickpea worldwide. AB can infect chickpea plants at any growth stage from plant emergence to seed maturity; however, the crop is more prone to disease at flowering and podding stages

which results in substantial yield loss and poor seed quality (Sharma *et al.* 2010). All the aerial parts of chickpea are susceptible to the BGM with growing tips and flowers being the most vulnerable (Bakr and Ahmed 1992). For effective control of both the diseases, fungicide applications are used, which resulted in insensitivity of pathogen isolates against several fungicides, besides causing environmental pollution (Chang *et al.* 2007, Wise *et al.* 2009). Therefore, development of chickpea cultivars resistant to AB and BGM is the most effective and sustainable approach. Resistance to both the pathogens has complex genetic nature and complete resistance against them have not been reported so far. Globally, several germplasm lines with moderate resistance to these pathogens have been identified and successfully used in chickpea breeding (Sharma and Ghosh 2016). Moreover, rapid evolution of pathogen and breakdown of resistance are major challenges.

In case of AB, the exact genetic and molecular mechanism of partial resistance against *A. rabiei* infection is still unknown. Depending on the isolates of the pathogen and the method of disease scoring, both qualitative and quantitative modes of inheritance for resistance against AB have

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been reported in chickpea. Initially, it was characterized as monogenic with additional modifier genes. Single dominant or recessive gene were found to impart AB resistance in both *desi* and *kabuli* types (Dey and Singh 1993, Tewari and Pandey 1986). However, recent studies using RILs demonstrated continuous distribution of disease response and suggested a polygenic inheritance (Deokar *et al.* 2019, Garg *et al.* 2018). For BGM, a few reports on genetics of resistance suggested that the resistance is controlled by few genes such as single dominant gene ‘*Bor1*’ for resistance (Tiwari *et al.* 1985); two genes with epistasis interaction (13:3 ratio) (Rewal and Grewal 1989); and two duplicate dominant genes with epistasis interaction (15:1 ratio) (Chaturvedi *et al.* 1995). However, complexity of disease and non-availability of high level of resistance suggests the polygenic inheritance of disease.

Several QTLs for resistance to AB contributing 12–50% of the total phenotypic variation have been detected in different mapping populations from inter- and intra-specific crosses (Cho *et al.* 2004, Collard *et al.* 2003, Flandez-Galvez *et al.* 2003, Sabbavarapu *et al.* 2013, Stephens *et al.* 2014, Tar’an *et al.* 2007, Udupa and Baum 2003). SSR markers linked to these QTLs have been used for marker-assisted backcrossing to introgress the AB resistance into adapted chickpea cultivars (Madrid *et al.* 2012, Tar’an *et al.* 2013). In case of BGM, very few reports are available for association of markers with QTL on different linkage groups (Anbessa *et al.* 2009, Anuradha *et al.* 2011, Kaur *et al.* 2013).

During last decade, chickpea research community has decoded the chickpea genome (Jain *et al.* 2013, Varshney *et al.* 2013) and developed several genomic (Agarwal *et al.* 2015, Nayak *et al.* 2010, Thudi *et al.* 2016) and transcriptomic resources (Hiremath *et al.* 2011, Kudapa *et al.* 2014) that has transformed chickpea from “orphan legume crop” to “genomics resource rich legume crop” (Varshney 2016). Besides, the past decade has witnessed the development of several high-throughput genotyping technologies which can mine thousands of SNPs across the genome. Double Digestion Restriction-site-Associated DNA sequencing (ddRAD-seq) technique is one such approach for high-throughput genotyping (Peterson *et al.* 2012), that uses two different restriction enzymes and size selection for recovering the appropriate number of regions arbitrarily distributed throughout the genome and maximizing the ability of multiplexing numerous samples in a single experiment.

Chickpea is known to have narrow genetic base as compared to the most other legumes (Kushwah *et al.* 2020a, Stephens *et al.* 2014). Due to relatively low levels of polymorphism in cultivated chickpeas, inter-specific crosses between *C. arietinum* and *C. reticulatum* have been the primary focus for genetic studies (Singh *et al.* 2008). The amount of polymorphism in an inter-specific mapping population varies from 16% to 36% and 9.5% in intra-specific mapping population (Nayak *et al.* 2010). High-resolution genetic linkage maps can also be constructed by exploiting

the inter-specific polymorphisms between *C. arietinum* and *C. reticulatum* (Thudi *et al.* 2011). Thus, an inter-specific mapping population from a cross between *C. arietinum* and *C. reticulatum* has been used in the present study to identify the key genomic regions providing resistance against AB and BGM using ddRAD-seq based genotyping and phenotyping under artificial epiphytotic conditions.

Materials and Methods

Plant materials

A total of 187 interspecific RILs in F_8 generation were developed from a cross of GPF2 \times *C. reticulatum* acc. ILWC 292 using single seed descent method by Punjab Agricultural University, Ludhiana, Punjab, India in year 2017. The wild accession *C. reticulatum* acc. ILWC 292 is highly susceptible to AB but resistant to BGM, while the cultivated parent GPF 2 is resistant to AB (Basandrai *et al.* 2009, Islam *et al.* 2017) but highly susceptible to BGM. Randomly five plants were taken to record the observations on number of pods per plant, yield and biomass in each plot. The data of 100-seed weight were recorded on plot basis. HI was calculated as:

$$HI = (\text{seed yield/total shoot biomass}) \times 100$$

Field screening technique for AB

Field screening technique (Gurha *et al.* 2003) was used to screen the RILs and parents against AB at Ludhiana. High relative humidity (85%) was maintained at an ambient temperature of 25°C for a period of 48 h of incubation with the help of perfo-sprayer system for creating the epiphytotic conditions. The RILs along with parents were planted during two consecutive years 2017–18 and 2018–19 in alpha lattice design (17 \times 12) with three replications. Each RIL was planted in paired row of 2 m length at 30 cm \times 10 cm spacing. Two highly susceptible checks, L 550 and C 214, were planted as indicator-cum-infecter rows alternatively after every 8 test entries to spread and monitor the disease epidemic. At the onset of flowering, plants were inoculated with a spore suspension of *A. rabiei* isolate 8 of race 6 (3968) (4×10^4 spores ml^{-1}) in February (Singh 1990). The physiological race of this isolate along with nine other races of different isolates of pathogen were collected from North India and identified on the basis of pathogenicity on a set of 12 chickpea cultivars/lines which were used as differentials by Singh (1990). The disease symptoms started appearing 10 days after inoculation and observations were recorded 21 days after inoculation (Gurha *et al.* 2003).

Cut twig screening technique for BGM

For screening against BGM, cut twig method was used (Singh 1997). In this method, the tender shoots of the chickpea plants were cut and put in a tray containing water, immediately wrapped in wet cotton plug and placed into a test tube (15 \times 100 mm) containing fresh tap water. Three

twigs were tested from each RIL along with parents in replications for two consecutive years, i.e., 2017–18 and 2018–19. Two highly susceptible checks, JG 62 and H 208, for botrytis grey mould were used to spread and monitor the disease progress. Twigs were inoculated by spraying spore suspension of *B. cinerea* (10^4 spores ml⁻¹) and covered with moist polythene covers. These twigs were kept in moist chambers (polyethylene bags supported by iron cage) for 144 h (6 days) with 8 h dark and 16 h light periods provided through a fluorescent lamp (24" × 1.5", W 20, 32 lm/W.). Disease incidence were recorded (Gurha *et al.* 2003) after 6 days of inoculation.

Disease scoring for AB and BGM

The data for AB and BGM were recorded on 1–9 rating scale (Gurha *et al.* 2003) which was as follows: 1.0 = no infection; 1.1–2.0 = minute water-soaked lesions on leaves and stems; 2.1–3.0 = minute water-soaked lesions seen after careful examination; 3.1–4.0 = few small and few large lesions (>5 mm²); 4.1–5.0 = many small and large lesions; 5.1–6.0 = many small and large lesions, lesions coalescing (50–75% plant area infected); 6.1–7.0 = many small and large lesions, lesions coalescing, stem girdled (75–90% plant area infected); 7.1–8.0 = many small and large lesions, lesions coalescing, girdling stem breakage (>90% plant area infected); and 8.1–9.0 = 100% plants dead. The lines with disease score 3–5 were considered as resistant, 5–7 as moderately susceptible and 7–9 as highly susceptible.

Statistical analysis of phenotyping data

Analysis of variance was calculated to estimate the contribution made by each factor to the total variation using SAS-software version 9.3 (SAS Institute 2002, Cary NC). The contrast analysis used in SAS is based on t-test for comparison of means of two entries.

SNP genotyping by ddRAD-seq

Genomic DNA was extracted from parents and RILs using high throughput mini-DNA extraction method described in Mace *et al.* (2003). The RIL population and the parents were genotyped with ddRAD-seq (Peterson *et al.* 2012) using restriction enzymes *Pst*I and *Msp*I (Thermo Fisher Scientific, MA, United States). The ddRAD-seq of RILs was outsourced from SciGenom, India and data was received in the form of paired-end filtered and processed reads. The processed reads were aligned to the chickpea reference genome (https://www.ncbi.nlm.nih.gov/assembly/GCF_000331145.1) using BWA (Li and Durbin 2009) program with default parameters. The SNPs were called using freebayes (<https://github.com/freebayes/freebayes>) which is a bayesian variant detector that finds variants by haplotype based alignment. High-confidence biallelic SNP candidates were selected using VCFtools (Danecek *et al.* 2011) with the following criteria: (i) depth of coverage ≥ 5 for each data point, (ii) SNP quality score of ≥ 30 for each locus, and (iii) proportion of missing data of <20% for each locus.

Linkage map construction and QTL analysis

Linkage map was constructed using the OneMap (version 2.0.4) package in R (<https://www.r-project.org/>) via the “group” command with a minimum LOD (logarithm of the odds) of 3 and maximum recombination fraction of 0.45 (Margarido *et al.* 2007). The map distances were drawn using MapChart 2.2 software (Voorrips 2002). QTL analysis was performed with the composite interval mapping (CIM) executed in the Windows QTL Cartographer V2.5 software package (Wang *et al.* 2007) using genotypic and phenotypic data. The CIM analysis was performed using forward and backward stepwise regression. For each trait, experiment-wise significance thresholds ($p \leq 0.05$) were determined with 1000 permutations for QTL detection. The position of the QTLs was identified based on LOD peak location with 95% confidence interval. The percentage of phenotypic variance and additive effect described by QTLs was also estimated. The phenotypic contribution (R^2) was estimated as the percentage of variance explained by each QTL in proportion to the total phenotypic variance, while additive effect was estimated to find the positive or negative effect for the target trait.

Results

Phenotypic evaluation of RIL population along with parents

During the screening against AB, GPF2 found to be resistant, while *C. reticulatum* acc. ILWC 292 found to be highly susceptible during both the years (Fig. 1). Characteristic symptoms like concentric rings on pods (Fig. 1a) and brown lesions on stem and leaves (Fig. 1b) appeared during AB incidence. In case of BGM screening, *C. reticulatum* acc. ILWC 292 was found to be resistant, while GPF2 was found highly susceptible during both the years (Fig. 2). The contrast analysis of parents for screening of AB and BGM depicted that there were highly significant differences between parents in both years (Table 1).

Significant variation was also observed for both AB and BGM pathogens in the RILs in both years (Fig. 3). Out of the 187 RILs evaluated for AB disease, 24 RILs were resistant having AB score of 3.0–5.0, 95 RILs were moderately susceptible having AB score of 5.1–7.0, while 68 RILs were highly susceptible having AB score of 7.1–9.0 during 2017–18 (Fig. 3a). During 2018–19, 25 RILs were found to be resistant having AB score of 3.0–5.0, 101 RILs were moderately susceptible having AB score of 5.1–7.0, while 61 RILs were highly susceptible having AB score of 7.1–9.0 (Fig. 3b). A total of 23 common RILs were observed to be resistant having AB score of 3.0–5.0 in both years.

For BGM, out of the total 187 RILs evaluated, 22 RILs were resistant having BGM score of 3.0–5.0, 104 RILs were moderately susceptible with score of 5.1–7.0, while 61 RILs were highly susceptible with score of 7.1–9.0 during 2017–18 (Fig. 3c). During 2018–19, 22 RILs were found to be resistant having BGM score of 3.0–5.0, 99

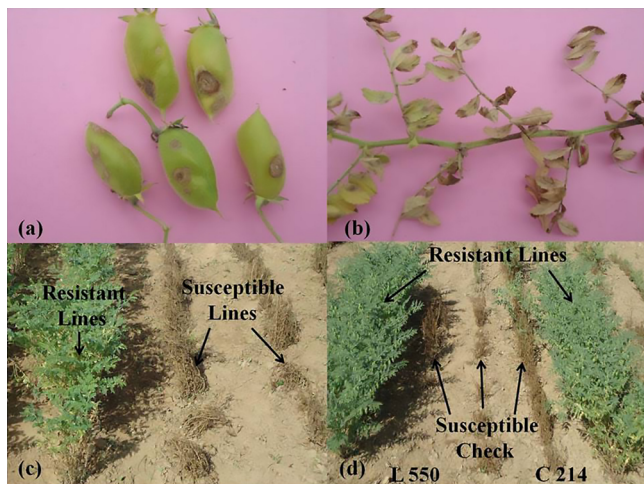


Fig. 1. Screening of recombinant inbred lines along with parents for ascochyta blight resistance. Symptoms of ascochyta blight (a) on pods and (b) on leaves. (c) and (d) Field screening technique of ascochyta blight showing resistant and susceptible lines and susceptible checks at Ludhiana, Punjab, India.



Fig. 2. Screening of recombinant inbred lines along with parents and susceptible checks for botrytis grey mould resistance. Cut twig screening technique of botrytis grey mould resistance (a) for parents and (b) for susceptible checks. (c) and (d) controlled environmental condition for botrytis grey mould screening using cut-twig screening technique.

RILs were moderately susceptible with score of 5.1–7.0, while 66 RILs were found to be highly susceptible having BGM score of 7.1–9.0 (**Fig. 3d**).

The ANOVA showed highly significant differences ($P < 0.001$) in RILs for genotypic variance for reaction to both AB and BGM pathogens in both the years (**Table 1**). Out of the 187 RILs, six lines showing resistance against AB and eight lines showing resistance against BGM during both the years were also promising for yield and yield related traits like number of pods per plant, biomass, 100-seed weight and harvest index (**Table 2**). These lines are being evaluated in multi-location trials. One line, RIL 41, having resistance to both AB and BGM during both the years, was also found promising for yield and yield related traits (**Table 2**).

Genotyping by ddRAD-seq, data analysis and SNPs discovery

The RILs along with parents (GPF2 and *C. reticulatum* acc. ILWC 292) were genotyped by sequencing following ddRAD-seq approach. A total of 16.75 million reads for *C. reticulatum* acc. ILWC 292 and 3.74 million reads for GPF2 were generated. In addition, a total of 550.74 million reads were generated for 187 RILs with an average of 2.94 million reads per line. The number of reads generated varied from 0.42 million reads (RIL171) to 9.78 million reads (RIL119). The reads were aligned to the chickpea reference genome and overall, 83.74% of total reads mapped to the reference genome. SNP calling and filtering identified 8519 high quality SNPs. Further, based on criteria to identify homozygous polymorphic SNPs between parents, a total of 1365 informative SNPs were extracted, which were used in linkage map construction and QTL mapping. These informative SNPs can be located on coding as well as non-coding regions.

Linkage map construction

Out of 1365 informative SNPs, 673 SNPs could be arranged on eight chromosomal linkage groups (**Fig. 4**). Rest of the SNPs showed linkage in several smaller sized chromosomal linkage groups which were not used for construction of linkage maps. The average linkage map distance constructed from the RIL population was 4569.09 cM with an average of 6.79 cM between the markers (**Supplemental**

Table 1. Reaction of parents and recombinant inbred lines for ascochyta blight and botrytis grey mould disease along with analysis of variance of two years (2017–18 and 2018–19)

Variable	ILWC 292	GPF 2	Contrast analysis between parents	Mean (RILs)	St Dev	CV	Genotypic variance
AB 2017–18	8.33	3.67	32.67**	6.32	1.32	20.81	5.06**
AB 2018–19	9.00	3.33	48.17**	6.27	1.31	20.90	5.32**
BGM 2017–18	3.00	8.67	48.17**	6.36	1.28	20.11	1.94**
BGM 2018–19	4.00	8.33	28.17**	6.28	1.27	20.22	1.82**

** = significant at 1% probability level, St Dev = standard deviation, CV = coefficient of variation.

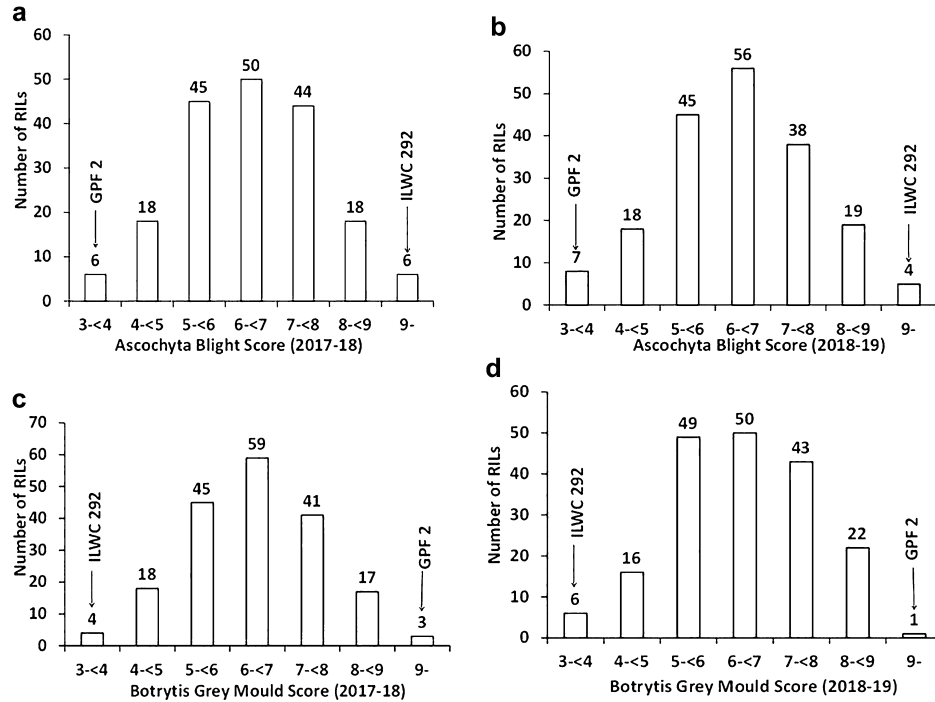


Fig. 3. Graphical representations of reaction of parents and recombinant inbred lines for ascochyta blight and botrytis grey mould screening for two consecutive seasons (2017–18 and 2018–19).

Table 2. List of promising recombinant inbred lines with number of pods/plant, biomass/plant, yield/plant, 100-seed weight and harvest index along with resistance against ascochyta blight and botrytis grey mould for two years (2017–18 and 2018–19)

RIL No.	AB score 2017–18	AB score 2018–19	BGM score 2017–18	BGM score 2018–19	Number of pods/plant	Biomass/ plant (g)	Yield/plant (g)	100-seed weight (g)	Harvest in- dex (%)
41	4.67	5.00	4.67	4.00	61.11	100.03	36.80	16.10	36.62
RILs having high yield as well as resistance against AB for both of the seasons									
44	3.00	4.33	5.00	5.67	52.55	87.57	34.77	15.97	39.72
48	4.00	4.33	6.33	6.00	55.89	89.13	36.70	15.27	41.07
56	4.00	4.67	5.67	5.33	55.55	96.83	42.93	16.30	44.25
58	4.00	3.33	5.00	5.00	60.67	98.07	47.70	18.17	49.22
61	4.67	3.67	5.00	5.33	49.55	87.20	35.67	14.90	41.02
127	4.33	4.00	6.33	6.00	66.89	98.33	46.63	17.30	47.45
RILs having high yield as well as resistance against BGM for both of the seasons									
1	8.33	9.00	3.00	3.33	63.11	97.30	38.73	17.53	39.86
2	6.33	5.00	3.33	3.67	76.44	105.80	45.17	17.40	42.76
7	7.67	6.33	3.33	3.00	62.45	87.03	40.10	13.90	45.79
12	5.33	6.67	3.33	3.00	50.33	92.43	34.70	14.93	37.53
14	8.67	8.33	4.67	4.33	53.33	101.60	42.37	16.43	41.72
42	6.33	7.33	4.67	4.00	40.22	81.07	37.30	15.70	46.17
46	5.33	5.00	4.67	4.67	53.22	83.27	35.03	16.37	41.97
51	9.00	8.33	4.00	3.67	66.44	102.03	40.10	15.23	39.37

Table 1). The maximum inter-marker distance was observed on chromosome 6 with the value of 7.83, while the minimum inter-marker distance was observed on chromosome 8 with the value of 5.80. The maximum number of markers (200) was on chromosome 4, while the minimum number of markers (8) was on chromosome 8. On an average, the highest marker density was observed on chromo-

some 8 with 0.172 markers per cM, while the lowest marker density was observed on chromosome 6 with 0.128 markers per cM. Overall, the genetic linkage map had a density of 0.15 markers per cM on an average.

QTLs identified for AB resistance

Three QTLs were identified for resistance to AB on

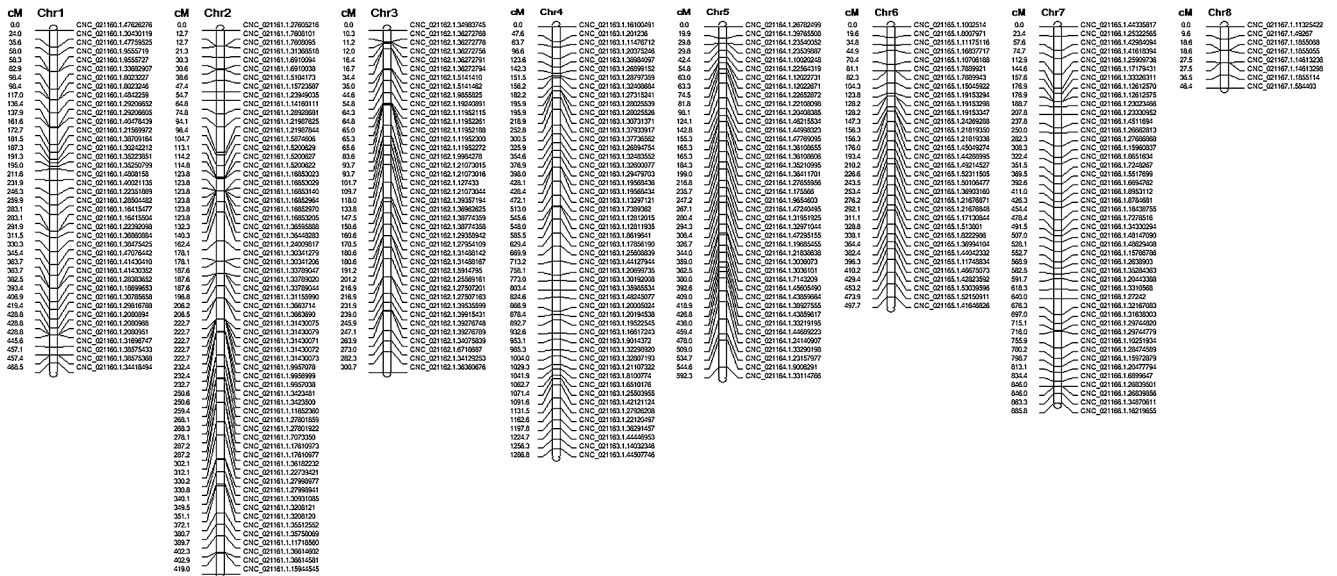


Fig. 4. Inter-specific genetic linkage map constructed using the RIL mapping population from a cross between GPF2 and *C. reticulatum* acc. ILWC 292.

chromosomes 4 (*qab-4.1*, *qab-4.2*) and 7 (*qab-7.1*) in both the years (Table 3, Fig. 5). The QTLs, *qab-4.2* and *qab-7.1* were identified in same genomic region in 2017–18 and 2018–19. The QTL *qab-4.1* was detected in a 22.3 cM interval on chromosomes 4. The QTL *qab-4.2* was mapped 8.1 cM distal to a group of right flanking SNP markers and 4.4 cM proximal to a group of left flanking SNP markers. The *qab-7.1* QTL was spanned by 8.3 cM between left and right flanking SNP markers that explained 6.91% and 8.26% of the phenotypic variation in 2017–18 and 2018–19, respectively. QTLs having positive or negative additive effect for a particular trait implied that the increase in the proportion of the phenotypic variation of that particular trait is contributed by the allele from GPF2 or *C. reticulatum* acc. ILWC 292, respectively. The QTL, *qab-4.2* explained 10.69% and 7.35% phenotypic variance in 2017–18 and 2018–19. Similarly, QTL, *qab-7.1* explained 6.91% and 7.41% phenotypic variance in 2017–18 and 2018–19.

QTLs identified for BGM resistance

Five QTLs (*qbgm-3.1*, *qbgm-4.1*, *qbgm-4.2*, *qbgm-5.1*, *qbgm-6.1*) for resistance to BGM were identified on chromosomes 3, 4, 5 and 6 in both the years. Of which, QTL, *qbgm-4.1*, *qbgm-4.2* and *qbgm-5.1*, were found to be consistent during both the years (Table 3, Fig. 6). The proportion of phenotypic variation explained by individual QTLs ranged from 7.24% (*qbgm-5.1*) to 10.89% (*qbgm-4.1*) during 2017–18 and from 6.20% (*qbgm-6.1*) to 17.19% (*qbgm-4.1*) during 2018–19. Out of these QTLs, three consensus QTLs namely, *qbgm-4.1*, *qbgm-4.2* and *qbgm-5.1*, have been identified in both the years (2017–18 and 2018–19).

Discussion

AB and BGM are the two most devastating fungal diseases of chickpea that cause substantial yield losses and poor seed quality worldwide (Pande *et al.* 2005, Udupa and Baum 2003). In chickpea, the wild relative can play an important role in bringing genetic diversity for resistance to complex diseases such as BGM (von Wettberg *et al.* 2018), where the limited resistance is available in cultivated gene pool. The wild relative of chickpea, *C. reticulatum* acc. ILWC 292 was found to be moderately resistant to BGM at PAU, India; however, it was susceptible to AB. On the other hand, GPF2, a cultivated chickpea, released by PAU is resistant to AB, but susceptible to BGM. The *C. reticulatum* acc. ILWC 292 can be directly crossed with cultivated chickpea and showed high level of fertility. The RIL population developed from the cross gave us opportunity to identify QTLs for both the important diseases. Additionally, PAU has well-established system of evaluation of AB and BGM under artificial epiphytotic conditions. Significant variation was observed for both AB and BGM screening in the RILs in both the years. The frequency distribution of RILs for AB and BGM screening in this study depicted normal distribution indicating that AB and BGM resistance is governed by polygene which is in accordance of several previous researchers (Anuradha *et al.* 2011, Stephens *et al.* 2014, Tar’an *et al.* 2013). RILs were also evaluated for yield component traits along with AB and BGM. One RIL (RIL 41) having resistance to both AB and BGM, showed comparable yield traits to elite cultivated parent GPF2 and avowed promise for further evaluation.

A large number of polymorphic markers are required for

Table 3. Summary of the QTLs identified for ascochyta blight and botrytis grey mould during two years (2017–18 and 2018–19)

Disease/Year	Ch	QTL name	LOD	Additive effect	R ² (%)	TR ²	Position (cM)	Contributing alleles	Left flanking marker position (cM)	Right flanking marker position (cM)	Left flanking marker	Right flanking marker
AB 2017–18	4	<i>qab-4.1</i>	3.53	0.58	15.47	0.378	260.9	GPF2	248.9	271.2	CNC_021163.1.32280291	CNC_021163.1.37933917
	4	<i>qab-4.2</i>	4.33	-0.55	10.69	0.312	979.0	<i>C. reticulatum</i> acc ILWC 292	970.6	983.1	CNC_021163.1.23799836	CNC_021163.1.24184658
	7	<i>qab-7.1</i>	3.57	0.49	6.91	0.282	491.5	GPF2	487.8	496.1	CNC_021166.1.34330294	CNC_021166.1.34330283
AB 2018–19	4	<i>qab-4.1</i>	–	–	–	–	–	–	–	–	–	–
	4	<i>qab-4.1</i>	3.13	-0.45	7.35	0.278	979.0	<i>C. reticulatum</i> acc ILWC 292	970.6	983.1	CNC_021163.1.23799836	CNC_021163.1.24184658
	7	<i>qab-7.1</i>	3.26	0.51	8.26	0.282	491.5	GPF2	487.8	496.1	CNC_021166.1.34330294	CNC_021166.1.34330283
BGM 2017–18	3	<i>qbgm-3.1</i>	3.88	-0.48	8.53	0.355	215.2	<i>C. reticulatum</i> acc ILWC 292	201.2	216.9	CNC_021162.1.25569161	CNC_021162.1.27507201
	4	<i>qbgm-4.1</i>	6.03	0.59	10.89	0.335	73.7	GPF2	63.7	75.6	CNC_021163.1.11476712	CNC_021163.1.7883450
	4	<i>qbgm-4.2</i>	3.50	-0.60	9.06	0.336	193.5	<i>C. reticulatum</i> acc ILWC 292	191.5	195.9	CNC_021163.1.38343874	CNC_021163.1.28025601
	5	<i>qbgm-5.1</i>	3.16	0.51	7.24	0.407	37.8	GPF2	29.8	39.3	CNC_021164.1.23539887	CNC_021164.1.19923058
BGM 2018–19	4	<i>qbgm-4.1</i>	7.08	0.70	17.19	0.358	73.7	GPF2	63.7	75.6	CNC_021163.1.11476712	CNC_021163.1.7883450
	4	<i>qbgm-4.3</i>	3.71	-0.59	7.75	0.317	193.5	<i>C. reticulatum</i> acc ILWC 292	191.5	195.9	CNC_021163.1.38343874	CNC_021163.1.28025601
	5	<i>qbgm-5.1</i>	3.83	0.64	11.77	0.378	37.8	GPF2	29.8	39.3	CNC_021164.1.23539887	CNC_021164.1.19923058
	6	<i>qbgm-6.1</i>	3.33	-0.46	6.20	0.321	0.0	<i>C. reticulatum</i> acc ILWC 292	0.0	18.7	CNC_021165.1.1002514	CNC_021165.1.8008006

Ch.- chromosome number, LOD- logarithm of odds, R² = proportion of the variance explained by genetic effect, TR² = proportion of the total variance explained by the model including covariates. Bold characters show QTLs which were common for both of the consecutive years (2017–18 and 2018–19).

linkage analysis and mapping quantitative traits in chickpea as it shows low levels of genetic polymorphism due to narrow genetic base (Kushwah *et al.* 2020b, Stephens *et al.* 2014). GBS overtook the conventional genotyping procedures involving the use of traditional markers such as RAPD, AFLP, SSR and many others in terms of time, labor and cost involved, with additional benefits of more polymorphism. The large length of current genetic map could be due to incorporation of SNP markers that showed segregation distortion in the genetic map. Segregation distortion has been observed in the intra-specific (*C. arietinum* × *C. arietinum*) as well as inter-specific (*C. arietinum* × *C. reticulatum*) crosses of chickpea by numerous authors (Abbo *et al.* 2005, Castro *et al.* 2011, Cobos *et al.* 2006, Flandez-Galvez *et al.* 2003, Kazan *et al.* 1993, Radhika *et al.* 2007, Tekeoglu *et al.* 2002, Winter *et al.* 2000). Various genetic or physiological aspects such as recessive lethal genes, gametic selection, zygotic selection can be the reason of segregation distortion (Castro *et al.* 2011, Li *et al.* 2007, Lu *et al.* 2002, Mano *et al.* 2005).

Identification of QTLs is an important step for breeding of quantitative traits in plants. Resistance to AB and BGM are quantitative in nature as observed from near normal distribution in RIL population. The disease reaction was evaluated at flowering stage in present study, hence these QTLs can be used for imparting adult plant resistance in chickpea cultivars. Previous studies have identified several QTLs for AB resistance on chromosome 2 (Anbessa *et al.* 2009, Cobos *et al.* 2006, Iruela *et al.* 2007, Madrid *et al.* 2014), chromosome 3 (Anbessa *et al.* 2009, Kottapalli *et al.* 2009,

Tar'an *et al.* 2007), chromosome 4 (Anbessa *et al.* 2009, Cho *et al.* 2004, Garg *et al.* 2018, Iruela *et al.* 2007, Kottapalli *et al.* 2009, Lichtenzweig *et al.* 2006, Madrid *et al.* 2013, Tar'an *et al.* 2007), chromosome 6 (Anbessa *et al.* 2009, Tar'an *et al.* 2007) and chromosome 8 (Anbessa *et al.* 2009, Lichtenzweig *et al.* 2006) of chickpea employing different mapping populations. However, chromosome 4 has been consistently reported in several mapping studies spanning the QTLs (Deokar *et al.* 2019, Garg *et al.* 2018, Madrid *et al.* 2013, Sharma and Ghosh 2016) indicating its importance for imparting resistance to AB.

For resistance to BGM, five QTLs were identified on chromosomes 3, 4, 5 and 6 in both years, of which, QTL, *qbgm-4.1*, *qbgm-4.2* and *qbgm-5.1*, were found to be consistent. Till now, there is only one report on identification of QTLs for resistance to BGM. Anuradha *et al.* (2011) identified three QTLs responsible for BGM resistance, out of which, two QTLs were located on chromosome 3 while one QTL was located on chromosome 6. This is the first report on mapping of QTLs for BGM resistance in chickpea using high-throughput SNPs genotyping. Lack of chickpea genotypes having high levels of BGM resistance hampers the genetic studies and precise mapping of BGM. Thus, identification of several genomic regions from different sources of BGM resistance is an urgent requirement for gene pyramiding to obtain high level of resistance.

The present study has helped to identify promising chickpea RILs possessing resistance to AB and BGM that are being evaluated in multi-location trials. The identified QTLs for AB and BGM will help to provide opportunity

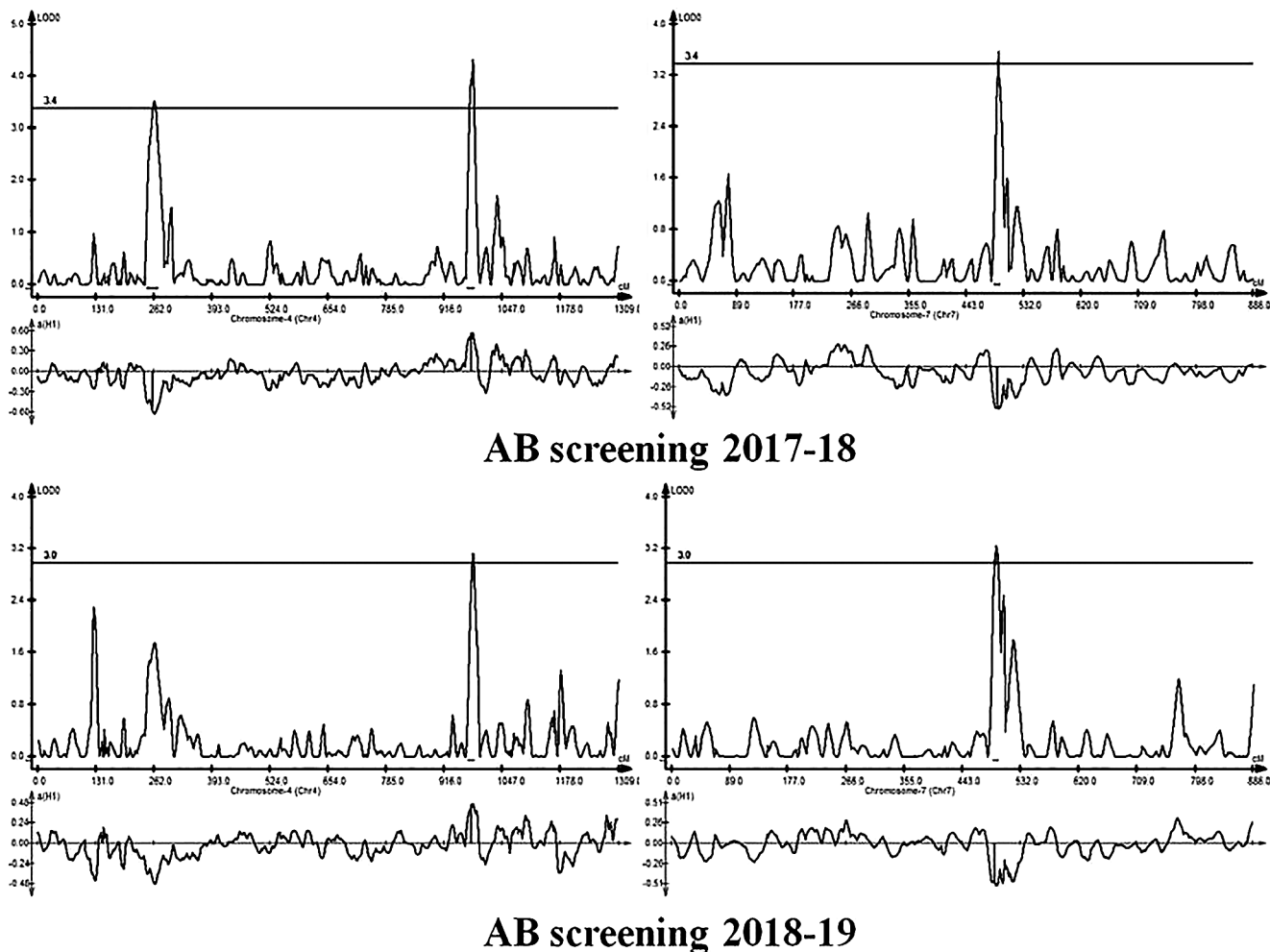


Fig. 5. Logarithm of odds ratio (LOD) curves obtained by composite interval mapping for quantitative trait loci (QTLs) mapped for ascochyta blight resistance in RIL population (GPF2 × *C. reticulatum* acc. ILWC 292) for two years, i.e., 2017–18 and 2018–19.

for fine mapping and cloning these QTLs, and identification of closely linked markers for marker-assisted transfer of resistance to AB and BGM in elite chickpea cultivars. These QTLs were specifically detected using artificial screening technique under epiphytotic field conditions. Thus, these QTLs can be responsible for providing adult plant resistance (APR) as the disease reaction was evaluated during flowering stage.

Author Contribution Statement

AK, SS and IS designed and conducted the experiments. AK and UR did screening for AB and BGM in experimental material. DB, AK, CB and ISY performed the data analysis. AK, DB, CB and SS prepared and edited the final manuscript.

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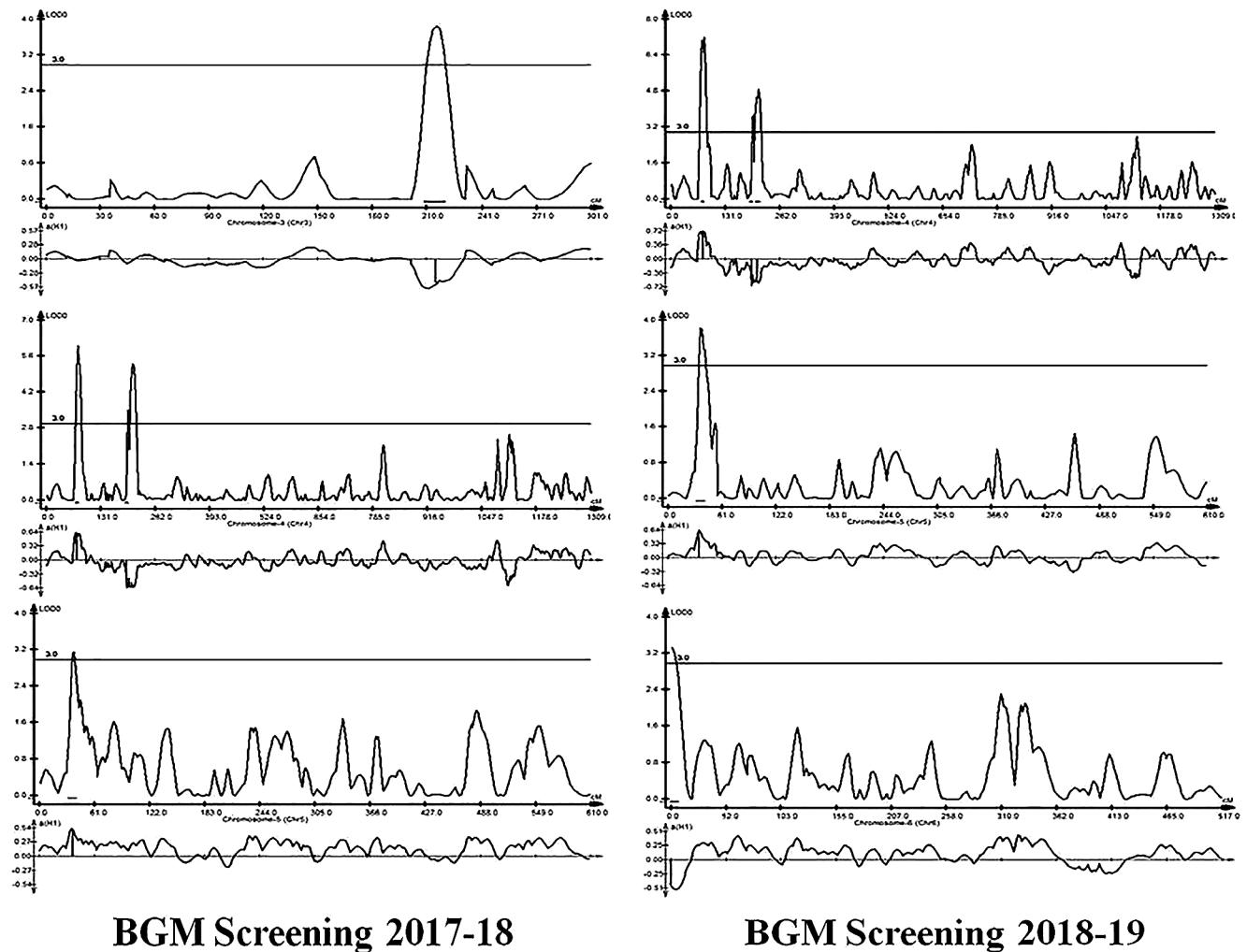


Fig. 6. Logarithm of odds ratio (LOD) curves obtained by composite interval mapping for quantitative trait loci (QTLs) mapped for botrytis grey mould resistance in RIL population (GPF2 \times *C. reticulatum* acc. ILWC 292) for two years, i.e., 2017–18 and 2018–19.

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