Research Article

Genetics of Resistance of Chickpea (*Cicer arietinum* L.) Against Botrytis Grey Mould Disease

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Abstract

The present investigation was conducted in *rabi* seasons during 2015-16, 2016-17 and 2017-18 at G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand with the objectives to study the inheritance of resistance to botrytis grey mould (BGM) disease in chickpea. The experimental material comprised of resistant parents viz., GL10006, DKG876 and susceptible parents viz., DCP92-3 and H208. Screening for resistance to botrytis grey mould disease was done in field by spraying artificial inoculums of pathogen and scoring was done using 1-9 scale. Resistance was found dominant over susceptibility in F_1 generation of resistant x susceptible crosses (GL10006 X DCP92-3, DKG876 X H208 and GL10006 X H208). In F_2 generation, 3(resistant): 1(susceptible) was found for all crosses indicated that resistance is governing by single gene and resistance is dominant over susceptibility. Backcross with resistant parents gave all resistant parents. The backcross of the F_1 with susceptible parent gave segregants into 1(resistant): 1(susceptible) ratio. The backcrosses also confirmed the results obtained in F_2 generation.

Keywords: Chickpea, genetics, resistance and botrytis grey mould

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Introduction

Chickpea is a self-pollinated diploid (2n = 2x = 16) crop species with a genome size of 740 Mb and presently grown on global area of 12.65 million ha with 12.09 million tons production [1]. In India it was grown in 9.63 million ha area with production of 9.38 million tons having productivity of 974 Kg/ha in the year 2016-2017 accounting for 60-65% of global chickpea production [2]. The genus Cicer consists of 43 species with 9 annuals, 33 perennials and one unclassified [3]. Ladizinsky and Adler (1976) considered *C. reticulatum* as the wild progenitor and southeastern Turkey as the centre of origin for the cultivated chickpea [4]. It can be considered as a model legume crop having a smaller genome than other legume crops. Its substantial nutritive value makes it a valuable source for both food and feed [5].

Chickpea is susceptible to various biotic stresses. Botrytis grey mould (BGM) caused by *Botrytis cinerea* is the second most important foliar disease of chickpea after ascochyta blight caused by *Ascochyta rabiei*. The first occurrence of BGM on chickpea was reported from India by Shaw and Ajrekar 1915 [6, 7]. The disease reached epidemic proportions in India during the 1978–79 crop season, destroying about 20,000 ha of chickpeas [8]. BGM can attack the chickpea plant at any stage of development, but the disease usually appears around flowering time, when the canopy is fully developed and the weather is warm and humid (20-30^{o}C, 70-100% RH). The flowers are more easily infected than other parts of the plant and can subsequently abort. Botrytis can also infect pods and be carried into the next season through infected seed [9].

Despite extensive investigations on pathological, physiological and molecular characteristics of *B. cinerea* causing grey mould type diseases on chickpea and several other hosts, the nature of infection processes and genetic basis of pathogen variability have not been clearly established. The IDM of BGM has proved more effective than any of the individual disease management components on large scale. IDM includes package practices, chemical control and development of resistant cultivars. Among these the development of resistant cultivars is the safest, eco-friendly, economical and sustainable alternative to stabilize the chickpea production [10]. The preliminary step for the development of resistant cultivars needs efficient screening techniques and diverse sources of resistance. Several screening techniques suitable to botrytis grey mould resistance like cut twig method and screening under field and greenhouse conditions have been developed [11-13].

The limited reports available on genetics of BGM resistance suggests that the resistance is controlled by few genes. A single dominant gene 'Bor1' for resistance was identified by Tiwari *et al*. (1985) [14], while two genes with dominant and recessive epistasis (13:3 ratio) were reported by Rewal and Grewal (1989) [15] and duplicate dominant epistasis (15:1 ratio) by Chaturvedi *et al*.(1995) [16]. Some of the resistant chickpea lines such as ICC1069, P349-2 and NEC2451 have been widely used in breeding (Haware *et al.*, 1992) [17] but higher levels of host resistance still need to be identified. Furthermore, these resistances are unlikely to hold in the longer term as pathogen diversity indicates likely breakdown of host resistance.

Materials and Methods

Experimental material

The parental materials used in present investigation were having different reaction for the Botrytis Grey Mould disease *viz.* resistant reaction (GL10006 and DKG876) and susceptible reaction (H208 and DCP92-3). The pedigree details and salient features of the parental lines used in genetic analysis are presented in **Table 1**.

Table 1 List of parental chickpea genotypes, their pedigree and characteristics

Genotypes	GL10006	DKG876	DCP 92-3	H208
Traits				
Pedigree	GG 1267 X GL 96010	ICCV 88500 X ICCV 96030	Selection from local germplasm line collected from adjoining areas (as L-412)	A widely adapted variety; from a cross (S 26 X G 24) X C 235
Maturity Duration	135 days	132 days	144 days	150 days
100 seed weight	23.00 g	18.00 g	23.00 g	20.00 g
Reaction to botrytis grey mould disease	Resistant	Resistant	Susceptible	Highly susceptible

Experimental method

Experimental lines were sown in 4 m long rows. The row-to-row distance was maintained at 30 cm and plant to plant at 10-15 cm. The standard package of practices for chickpea cultivation was followed. Crosses were attempted using hand emasculation followed by immediate pollination between resistant and susceptible parents for botrytis grey mould disease during *rabi* season of 2015-16. The F_1 seeds of desired crosses obtained in previous season were planted in *rabi* 2016-2017 in between their parental lines to observe botrytis grey mould in comparison to their parents. The backcrosses were attempt with both the parents. When, the F_1's were backcrossed with female parent (P_1), it was designated as BC_1P_1. Similarly, when it was backcrossed to the male parent (P_2), it was designated as BC_1P_2. The F_1's, F_2's, BC_1P_1 and BC_1P_2 along with their parents were sown during *rabi* season of 2017-18. The number of rows of each generation depended on the number of seeds available.

Disease screening

Screening for chickpea BGM was done under natural epiphytotic conditions. To create disease pressure in field, at the onset of flowering, plants were inoculated by spraying a spore suspension (50,000 spores / ml) of 10-days old culture of *Botrytis cinerea.* The observations were recorded when susceptible cultivars showed the maximum score of BGM. Thus plants of F_1's, backcrosses and F_2 generations were screened in field against *botrytis* grey mould (BGM).

Disease Rating

The genotypes were selected for screening against *botrytis* grey mould (BGM) caused by *Botrytis cinerea* at Pantnagar location. Total numbers of plants were counted in each cross. At reproductive stage disease was identified and data was recorded according to per cent plant parts affected by BGM. Disease data was scored for per cent plants affected on nine point (1-9) scale, where; 1 = free from disease and 9 = susceptible.

For assaying the overall disease reaction, individual plants with 1 to 5 score were considered as resistant/tolerant and 7 and 9 as susceptible.

Statistical Analysis

The data were subjected to chi-square analysis as per standard statistical procedures. Chi-square test was applied to test the goodness of fit for the appropriate genetic ratios in crosses.

Mean disease score

Mean disease score was calculated by using the following formula:

$$\text{Mean disease score} = \frac{\Sigma(\text{Infection score X frequency})}{\text{Total no.of plants}}$$

Segregation for detection of inheritance pattern:

The segregation for individual trait was analysed by χ^2 test to determine the goodness of fit of the observed segregation with the expected ratio. The χ^2 value was calculated as:

$$\chi^2_{cal} = \frac{\Sigma(O-E)^2}{E}$$

Where, O = the observed and E = the expected frequency of phenotypes in each class of segregation and Σ = summation over all the classes.

The χ^2 test for goodness of fit was applied to determine the F_2 and back crosses ratio's of different phenotypic classes under the expected segregation ratio.

Test of significance of deviation from expected ratio:

If the observed value of χ^2_{cal} due to deviation from expected ratio, is to be non-significant at 5 per cent probability level. The χ^2_{cal} value proves that the deviation from expected ratio is non- significant and all the families agree the expected segregation ratio.

Results and Discussion

The present study was carried out with objective to screen the parents, F_1, F_2 and backcross generations of three crosses against BGM disease. The experiment included two resistant parents *i.e.* GL10006 and DKG 876 as well as two susceptible parents *i.e.* H208 and DCP92-3 to BGM disease. These crosses were generated to study the genetics of BGM resistance. For this purpose the F_1, F_2 and backcross generations were generated and artificially screened by spray of inoculums of BGM at field level. The crosswise results are given below:

GL10006 X DCP92-3

The number of plants with BGM disease response in different generations of this cross is given in **Table 2**. The mean disease score of resistant parent GL10006 was 4.2 and it was 7.8 for susceptible parent DCP92-3. All the plants in F_1, showed the disease score of 4.2, showing resistant reaction. This disease reaction in F_1 population indicated that resistance is dominant over susceptibility. In F_2, plants (score from 3 to 9) were showed segregation for resistance. These F_2 plants were classified into two groups on the basis of disease response. One group included resistant plants having 1, 3 and 5 disease score, while other group included susceptible plants having 7 and 9 disease score. Out of 181 plants in F_2 generation, 128 were resistant and 53 were susceptible. This distribution fits to the 3 (resistant): 1 (susceptible) ratio with chi-square value 1.769 (P-value 0.20-0.10). This segregation pattern of F_2 generation showed that inheritance of resistance to BGM is controlled by single dominant gene.

In the backcross of F_1 with resistant parent *i.e.* BC_1P_1 (GL10006 X DCP92-3) X GL10006, all the plants showed resistant response (3 and 5 disease score) with mean disease score 4.07. On the other hand, cross between F_1 and susceptible parent *i.e.* BC_1P_2 (GL10006 X DCP92-3) X DCP92-3, the observed frequency of resistant and susceptible plants was 7 and 6, respectively. This distribution fits to expected ratio of 1 (resistant): 1(susceptible) with chi- square value 0.076 (P-value between 0.80 and 0.70). The results obtained from these backcrosses confirmed that resistance in this cross is controlled by single dominant gene and this also confirms the result obtained from F_2 generation.

DKG876 X H208

The number of plants with BGM disease response in different generations of this cross is given in **Table 3**. The mean disease score of parent DKG876 and H208 was 4.2 and 8.2, respectively suggesting susceptible and resistant reaction. The plants in F_1, showed the disease score of 4.42, showing resistant reaction. This disease reaction in F_1 population indicated that resistance is dominant over susceptibility. In F_2 population of cross, having score from 3 to 9, were showed segregation for resistance. These F_2 plants were classified into two groups on the basis of disease response. One group included resistant plants having 1, 3 and 5 disease score, while other group included susceptible plants having 7 and 9 disease score. Out of 210 plants in F_2 generation, 149 were resistant and 61 were susceptible. This

showed a good fits to the ratio of 3 (resistant): 1 (susceptible) with chi-square value 1.834 (P-value 0.20-0.10). This segregation pattern of plants in F_2 generation showed that inheritance of resistance to BGM is controlled by single dominant gene.

Table 2: Botrytis Grey Mould score of parents, F_1, F_2 and backcross generations in cross GL10006 X DCP92-3

Parent/Cross	Gene ration	BGM score					Total no. of plants	Mean disease score	Observed frequency		Expected frequency		Expected Ratio	χ^2	P-value between
		1	3	5	7	9			R	S	R	S			
GL10006	P_1		2	3			5	4.2							
DCP92-3	P_2				3	2	5	7.8							
GL10006XDCP 92-3	F_1		4	6			10	4.2							
(GL10006 X DCP92-3) X GL10006	BC_1P_1		6	7			13	4.07	13	0	-	-	All resistant		
(GL10006XDCP92-3) X DCP92-3	BC_1P_2		4	3	2	4	13	5.92	7	6	6.5	6.5	1:1	0.076	0.80-0.70
GL10006XDCP 92-3	F_2		30	98	35	18	181	5.45	128	53	135.75	45.25	3:1	1.769	0.20-0.10

Table 3 Botrytis Grey Mould score of parents, F_1, F_2 and backcross generations in cross DKG876 X H208

Parent/ cross	Generation	BGM Score					Total no. of plants	Mean disease score	Observed frequency		Expected frequency		Expected ratio	χ^2	P-value between
		1	3	5	7	9			R	S	R	S			
DKG876	P_1		2	3			5	4.2							
H208	P_2				2	3	5	8.2							
DKG876X H208	F_1		4	10			14	4.42							
(DKG876 X H208) X DKG 876	BC_1P_1		5	10			15	4.33	15	0	-	-	All resistant		
(DKG876 X H208) X H208	BC_1P_2		5	3	3	4	15	5.8	8	7	7.5	7.5	1:1	0.066	0.80-0.70
DKG876X H208	F_2		46	103	38	23	210	5.36	149	61	157.5	52.5	3:1	1.834	0.20-0.10

In the backcross of F_1 with resistant parent *i.e.* BC_1P_1 (DKG876 X H208) X DKG876, all the plants showed resistant reaction (3 and 5 disease score) with mean disease score 4.33. On the other hand, in BC_1P_2, a cross between F_1 and susceptible parent (DKG876 X H208) X H208, the observed frequency of resistant and susceptible plants was 8 and 7, respectively. This gave a good fit to expect 1 (resistant): 1(susceptible) ratio with chi- square value 0.066 and P-value between 0.80 and 0.70. The results obtained from both backcrosses confirmed that resistance in this cross is controlled by single dominant gene and this also confirms the result obtained from F_2 generation.

GL10006 X H208

The number of plants with BGM disease response in different generations of this cross is given in **Table 4**. The mean disease score of parent GL10006 was 4.2 (resistant) and of H208 was 7.8 (susceptible). The plants in F_1 showed the disease score of 4.09, showing resistant reaction. This disease reaction in F_1 population indicated that resistance is dominant over susceptibility. In F_2 plants, having score from 3 to 9, showed segregation for resistance. These F_2 plants were classified into two groups on the basis of disease response. One group included resistant plants having 1, 3 and 5 disease score, while other group included susceptible plants having 7 and 9 disease score. Out of 138 plants in F_2 generation, 110 were resistant and 28 were susceptible. This distribution fits to the ratio of 3 (resistant): 1 (susceptible) with chi-square value 1.632 (P-value 0.30-0.20). This segregation pattern in F_2 generation showed that inheritance of resistance to BGM is controlled by single dominant gene.

Table 4 Botrytis Grey Mould score of parents, F_1, F_2 and backcross generations in cross GL10006 X H208

Parent/ cross	Generation	BGM score					Total no. of plants	Mean disease score	Observed frequency		Expected frequency		Expected ratio	χ^2	P-value between
		1	3	5	7	9			R	S	R	S			
GL10006	P_1		2	3			5	4.2							
H208	P_2				3	2	5	7.8							
GL10006 X H208	F_1		5	6			11	4.09							
(GL10006X H208) X GL10006	BC_1P_1		6	10			16	4.25	16	0	-	-	All resistant		
(GL10006 X H208) X H208	BC_1P_2		4	5	4	3	16	5.75	9	7	8	8	1:1	0.25	0.70-0.50
GL10006 X H208	F_2		24	86	21	7	138	5.15	110	28	103.5	34.5	3:1	1.632	0.30-0.20

The backcross populations for the cross GL10008 X H208 were also tested for disease reaction. In BC_1P_1 (GL10006 X H208) X GL10006, all the plants showed resistant reaction (3 and 5 disease score) with mean disease score 4.25. On the other hand, in BC_1P_2, a cross between F_1 and susceptible parent, the observed frequency of resistant and susceptible plants was 9 and 7, respectively. This gave a good fit to expect 1 (resistant): 1(susceptible) ratio with chi- square value 0.25 and P-value between 0.70 and 0.50. The results obtained from these backcrosses confirmed the conclusion drawn from the reaction of F_2 generation

The findings indicated that F_2 population derived from the crosses *viz.*, GL10006 X DCP92-3, DKG876 X H208 and GL10006 X H208 could be classified into two distinct groups *i.e*., resistant and susceptible groups. The F_2 populations from these crosses segregated in the ratio of 3 (resistant): 1(susceptible), demonstrating that the resistance to BGM in chickpea is governed by a single dominant gene. There are only limited reports available on genetics of BGM resistance suggests that the resistance is controlled by few genes. These findings are in agreement with the earlier reports of Rewal and Grewal (1989) and Chaturvedi *et al.* (1995) [15, 16]. They found that resistance for BGM is controlled by single dominant gene. The findings of Tewari *et al.* (1985) were also in similar direction to the present research findings that resistance was dominant over susceptibility and monogenically controlled [14]. Therefore, resistance to BGM can be incorporated successfully into elite lines of chickpea from identified donars through hybridization followed by pedigree.

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