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Studies on Inheritance of *Botrytis* Grey Mould Resistance in Chickpea (*Cicer arietinum* L.)

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ABSTRACT

Background: *Botrytis* grey mould (BGM) is a fungal disease of chickpea and can infect plants at any stage of development. In order to develop the resistant varieties, it is a necessary to understand the pattern of inheritance of its resistance and hence experiments were conducted during *rabi* 2014-17 at field area of GBPUAT, Pantnagar.

Methods: The six different generations of three crosses *i.e.*, DKG 876 × GNG1581, DKG 876 × H 208 and DKG 876 × DCP 92-3 were used as experimental material. All six generations were sown in compact family block design and data obtained from field disease screening were subjected to chi-square analysis.

Result: The results revealed that resistance to BGM was dominant over susceptibility. The inheritance of resistance against BGM showed monogenic dominant resistance in ratio of 3 (resistant):1 (susceptible). The results of present study showed the presence of a major gene in governing resistance to BGM in chickpea.

Key words: *Botrytis*, Chickpea, Inheritance, Resistance.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is an autogamous pulse crop belonging to family *Fabaceae*. India accounts for a substantial share of the world's chickpea area (70 per cent) and production (67%) (FAOSTAT, 2019). In India, more than 95 per cent production of chickpea is from Madhya Pradesh (M.P), Maharashtra, Rajasthan, Uttar Pradesh (U.P), Andhra Pradesh (A.P), Karnataka, Chhattisgarh, Bihar and Jharkhand (Gautam *et al*., 2021).

Chickpea grains are a rich source of protein (12 to 30%) along with Iron, zinc, selenium, calcium, magnesium, phosphorus, copper and potassium (Thavarajah, 2012; Jadhav *et al*., 2015). Chickpea is considered to be a healthy vegetarian food (Sravani, 2021). Chickpea crop is prone to various biotic stresses among which *Botrytis* grey mould (BGM) caused by *Botrytis cinerea* Pers. Ex. Fr is most devastating. BGM is emerging as an important disease of chickpea in the northern and eastern parts of the Indian Sub-continent (Sachdeva *et al.,* 2019). The BGM attacks all the aerial parts of the plant including growing tips and flowers results in formation of water-soaking lesions, which turn grey or dark brown and in most severe cases tiny black sclerotia may develop on the dead infected tissues (Davidson *et al*., 2004). BGM is a devastating disease and hence extensive studies on the mechanism of inheritance of resistance are urgently required. The limited reports available on genetics of BGM resistance suggests that the resistance may be controlled by a single major gene (Anuradha *et al.,* 2011) as well as few genes (Anuradha *et al.,* 2011; Sachdeva *et al.,* 2019; Nehra *et al.,* 2020; Kushwah *et al.,* 2021). Hence it is very important to understand the exact mechanism of resistance to BGM. The study of inheritance of resistance to BGM would be helpful for targeting resistance against BGM. Hence in the present study efforts are being made to find out the mechanism of inheritance of resistance against BGM.

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MATERIALS AND METHODS

Plant material and field trial

The present investigation was conducted during the *rabi* seasons of the year 2014-15, 2015-16 and 2016-17 at Norman. E. Borloug, Crop Research Centre of GBPUA and T, Pantnagar, Uttarakhand, India. Three different Crosses (DKG 876 × GNG 1581, DKG 876 × H 208 and DKG 876 × DCP 92-3) were attempted during *rabi* season of 2014-15 between a BGM resistant variety *i.e.*, DKG 876 and three BGM susceptible variety GNG1581, H 208 and DCP 92-3. The F_1 was grown in *rabi* season of year 2015-16 and backcosses were attempted with both the parents. The F_1's was also allowed to selfed to produce seeds for F_2 generation. Generation of new hybrids were also attempted in *rabi* season of year 2015-16. During *rabi* season of year

2016-17, all the generations *viz.,* P_1, P_2, F_1, F_2, BC_1 (F_1 × resistant parent) and BC_2 (F_1 × susceptible parent) were sown in compact family block design. 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.

Disease screening and scoring

The screening for 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. Five plants from each parent and all the plants of F_1, backcross and F_2 generations were screened in field. 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 as given in Table 1 (Kaur *et al.,* 2013).

Statistical analysis

Mean disease score was calculated by using the following formula:

$$\text{Mean disease score} = \frac{\sum(\text{Infection score} \times \text{frequency})}{\text{Total no of plants}}$$

The data obtained from field disease screening were subjected to chi-square analysis as per standard statistical procedures. The segregation for individual trait was analysed by χ^2 tests to determine the goodness of fit of the observed segregation with the expected ratio. The χ^2 value was calculated as:

$$\chi^2 = \frac{\sum (O-E)^2}{E}$$

Where,
O= Observed.
E= Expected frequency of phenotypes in each class of segregation.
∑= Summation over all the classes.

RESULTS AND DISCUSSION

Cross 1: DKG 876 × GNG1581

A critical analysis of results (Table 2) indicated that the mean disease score of parents DKG 876 was noted to be 4.4 and of parent GNG 1581 were 6. These scores indicated the resistant reaction of parent DKG 876 and susceptible reaction of parent GNG 1581. All the F_1 plants showed the disease score of 4.0 and hence showed resistant reaction. These results indicated that resistance was dominant over susceptibility. The disease reaction pattern in F_2 generation showed the disease score from 3 to 7 with a mean of 5.02, showing segregation for resistance. The segregation of F_2 population from the cross DKG 876 × GNG 1581 showed a wide range of response to BGM. Out of 135 F_2 plants, 95 plants were found as resistant and 40 were susceptible. The obtained data fit to the ratio of 3 (resistant): 1 (susceptible). The segregation pattern of 3:1 in F_2 generation showed that inheritance of resistance to BGM is controlled by single dominant gene. The backcross generations were also tested for the disease reaction. In the backcross of F_1 with resistant parent (BC_1), the mean disease score was 4.8 and all plants showed resistant response. On the other hand, cross between F_1 and susceptible parent (BC_2), total 13 plants segregate into resistant (6) and susceptible (7) with the mean disease score of 5.9. The segregation pattern in BC_2 showed a good fit in ratio 1:1. The results obtained from these backcrosses showed that resistance in this cross is controlled by single dominant gene and this also confirms the result obtained from F_2 generation.

Cross 2: DKG 876 × H 208

The data regarding the BGM disease response to different generations of cross DKG 876 × H 208 was given in Table 3. The mean disease score of parents DKG 876 was 4.4 (resistant) and of H 208 was 8.2 (susceptible). All the F_1 plants showed resistant reaction indicating that resistance was dominant over susceptibility. The segregation of F_2 population from the cross DKG 876 × H 208 showed a wide range of response to BGM. In case of F_2 generation, out of 130 F_2 plants 91 were noted as resistant and 39 were susceptible. The results of Chi square indicated that these numbers fit to the ratio of 3 (resistant): 1 (susceptible). The data of BC_1 generation indicated that all plants showed resistant response, while in case of BC_2 generation, out of 15 plants 7 was found as resistant and 8 was found as susceptible with the mean disease score of 6.2. The segregation pattern in BC_2 showed a good fit in ratio 1:1 and hence again indicated that resistance is controlled by single dominant gene.

Cross 3: DKG 876 × DCP92-3

In this cross the mean disease score of parents DKG 876 was 4.2 (resistant) and of DCP 92-3 was 7.9 (susceptible). All the F_1 plants showed the disease score of 4.3 and hence indicated that resistance was dominant over susceptibility (Table 4). In this cross out of 105 F_2 plants 71 were resistant and 34 were susceptible. The segregation pattern of 3:1 in 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 all plants showed resistant response with mean disease score of 5.1. On the other hand, cross between F_1 and susceptible parent, total 12 plants segregate into resistant (8) and susceptible (4) with the mean disease score of 5.5. The segregation pattern in BC_2 showed a good fit in ratio 1:1. The results obtained from these backcrosses showed that resistance in this cross is controlled by single dominant gene and this also confirms the result obtained from F_2 generation.

The inheritance studies conducted by using three different crosses *viz.,* DKG 876 × GNG1581, DKG 876 × H 208 and DKG 876 × DCP 92-3 indicated that resistance to

Table 1: Disease rating scale for *Botrytis* grey mould (BGM) in chickpea as suggested by Kaur *et al.,* (2013).

Scale	Disease response
1	Highly resistant (HR) (no infection on any part of the plant)
1.1-3	Resistant (R) (minute water-soaked lesions on 1-5% leaves)
3.1-5	Moderately resistant (MR) (lesions and soft rotting on 11-25% leaves and tender shoots
5.1-7	Moderately Susceptible (MS) (rotting and fungal growth on 41-55% of the leaves and shoots
7.1-9	Highly susceptible (HS) (extensive rotting and fungal growth on 71-100% of the leaves, shoots and stems

Table 2: Inheritance pattern of botrytis grey mould in chickpea using cross DKG 876×GNG 1581.

Generation	BGM score					Total no. of plants	Mean disease score	Observed frequency		Expected frequency		Expected ratio	χ^2 cal	χ^2 tab (0.05,1df)
	1	3	5	7	9			R	S	R	S			
		3	7			10	4.4							
P_2			5	5		10	6.0							
F_1		6	6			12	4.0							
BC_1P_1		3	10	2		15	4.8	13	2	15	0	1:0	0.267	3.841
BC_1P_2		1	5	7		13	5.9	6	7	6.5	6.5	1:1	0.077	3.841
F_2		36	59	40		135	5.02	95	40	101.25	33.75	3:1	1.543	3.841

Table 3: Inheritance pattern of botrytis grey mould in chickpea using cross DKG 876×H 208.

Generation	BGM score					Total no. of plants	Mean disease score	Observed frequency		Expected frequency		Expected ratio	χ^2 cal	χ^2 tab (0.05,1df)
	1	3	5	7	9			R	S	R	S			
P_1		6	4			10	4.4							
P_2				5	5	10	8.2							
F_1		4	6			10	4.2							
BC_1P_1		6	7			13	4.1	13	0	13	0	1:0	0.000	3.841
BC_1P_2		3	4	4	4	15	6.2	7	8	7.5	7.5	1:1	0.067	3.841
F_2		27	64	27	12	130	5.4	91	39	97.5	32.5	3:1	1.733	3.841

Table 4: Inheritance pattern of botrytis grey mould in chickpea using cross DKG 876×DCP 92-3.

Generation	BGM score					Total no. of plants	Mean disease score	Observed frequency		Expected frequency		Expected ratio	χ^2 cal	χ^2 tab (0.05,1df)
	1	3	5	7	9			R	S	R	S			
P_1		3	7			10	4.2							
P_2				6	4	10	7.9							
F_1		4	7			11	4.3							
BC_1P_1		2	9	3		14	5.1	11	3	14	0	1:0	0.643	3.841
BC_1P_2		2	6	3	1	12	5.5	8	4	6	6	1:1	1.333	3.841
F_2		26	45	13	21	105	5.7	71	34	78.75	26.25	3:1	3.051	3.841

BGM was dominant over susceptibility. Bhardwaj *et al.,* (2018) also reported the dominance of resistance to BGM over its susceptibility. Nehra *et al.,* (2020) also found the dominance of resistance over susceptibility in F_1 generation of crosses *viz*., GL10006 × DCP92-3, DKG876 × H208 and GL10006 × H208. In present study the results of chi square test indicated the presence of a single major gene. The presence of a single major gene in governing resistance to BGM in chickpea was also reported earlier by Tewari *et al.,* 1985; Bhardwaj *et al.,* (2018) and Nehra *et al.,* (2020) in their experimental material. The dominance nature and presence of a single gene for resistance against BGM is highly desirable as it facilitates easy incorporation and selection of desirable plants.

CONCLUSION

BGM resistant chickpea cultivars are urgently required for effective and sustainable control of BGM. To develop a suitable breeding programme for development of BGM resistant varieties the information on its mode of inheritance is needed. The present study indicated that the resistance is governed by a single major dominant gene. This single

dominant resistant gene can be targeted for deployment of resistance to BGM in elite chickpea cultivars.

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Author contributions

R.K. Panwar came up with the concept and designed the experiments. The manuscript was written by Rajneesh Bhardwaj. Rajneesh Bhardwaj and R.K. Panwar analysed the data and carried out the experiments. The data and manuscript were finalized by Rajneesh Bhardwaj, R.K. Panwar, S.K. Verma and A.K. Gaur. The final manuscript has been read and approved by all authors.

Conflicts of interest

The authors declare no conflict of interest.

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