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Morpho-molecular screening of chickpea (*Cicer arietinum* L.) genotypes for resistance to *Botrytis* grey mould disease

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The evaluation and identification of disease resistance to *Botrytis* grey mould (BGM) in chickpea (*Cicer arietinum* L.) is a critical step in the advancement of integrated disease control strategies. The current study was conducted to evaluate and identify chickpea genotypes for resistance to BGM disease in natural epiphytotic conditions. A set of 225 experimental chickpea genotypes were planted in 9 blocks during 2017-18, 135 genotypes were planted in 9 blocks during 2018-19, and 100 genotypes were planted in 4 blocks during 2018-19, including 5 checks *viz.,* PG 3 and PG 5 (Moderately resistant), H 208 and DCP 92-3 (Susceptible), and GL10006 (Resistant). The field experiment was conducted using an augmented block design-II. The results of field screening against BGM disease revealed that 17 chickpea genotypes were found with disease score 3 (resistant) out of 225 during *rabi*, 2017-18, 13 genotypes were found with disease score 3 (resistant) during *rabi*, 2018-19, and 11 genotypes were found with disease score 3 (resistant) during *rabi*, 2019-20. The 20 best genotypes were chosen from the screened genotypes based on disease score and seed yield per plant for molecular marker validation. The summary of molecular data revealed PG 17-9 (130 bp), PG 17-119 (130 bp), and PG 17-145 (130 bp) as resistant; PG 17-120 and PG 17-121 moderately resistant; and the remaining genotypes as susceptible. The field disease score data matched the genotype banding pattern by markers TR29 and TA144. TR29 and TA144 have polymorphism information content (PIC) values more than 0.5, indicating that they can be deployed for molecular tagging of *Botrytis* grey mould resistance genes in chickpea.

Keywords: *Botrytis* grey mould, BGM, Chickpea, Field screening, STMS

Chickpea (*Cicer arietinum* L.) is an important cool season grain legume crop, also known as Gram, Bengal gram, and Chana in Hindi[1,2]. It is cultivated in arid and semi-arid regions across the globe. Chickpea is a diploid (2n=2x=16) and self-pollinated crop[3,4]. It is categorized as one of the world's oldest and widely consumed legumes in the *Fabaceae* (*Leguminosae*) family and is an important food crop, especially in tropical and subtropical areas[5], with high nutritional value[6,7]. It is the third most important grain legume crop after soybean and pea, grown worldwide with an area of 17.8 million hectares and an annual production of 17.2 million tonnes[8]. It is grown over more than 50 countries[9]. The major chickpea producing countries are India, Australia, Pakistan, Turkey, Russia, Myanmar, Iran, Mexico, Canada, and USA. India leads the world in chickpea production, with around 68% of the total global production and 69% of planted acreage[9]. In India, chickpea is grown as a major rainfed crop on an area of 10.57 million ha, with a total production of 11.58 million tones and a productivity of 1056 kg/ha[10]. In India, Madhya Pradesh, Maharashtra, Uttar Pradesh, Rajasthan, Karnataka, Andhra Pradesh Chhattisgarh, Bihar, and Jharkhand are the major chickpea growing states and contribute more than 95% of the total chickpea production in the country. There are different causes of pulse production stagnation, such as a narrow genetic base, photoperiod sensitivity of released cultivars, and susceptibility to several biotic and abiotic stresses[11]. Traditional breeding relies on successful selection based on additive genetic effects[12-14]. Among the biotic stresses, *Botrytis* grey mould (BGM) and Ascochyta blight (AB) are major diseases that can cause a yield loss of 10 -100%[15,16]. BGM is an economically important disease of

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Suppl. Data available on respective page of NOPR

chickpea, particularly in areas where cold, cloudy, and humid weather persist. Several disease outbreaks of BGM have affected chickpea production in some major chickpea-producing countries in the recent past. The pathogen *B. cinerea* primarily lives on infected crop residues and seeds between seasons 17. Also, very little is known about the chickpea resistance mechanisms against *B. cinerea.* Effective screening techniques and multiple sources of resistance are the first step in the development of resistance plants. Different screening methods for *in vitro*, greenhouse and field conditions have been used to test the germplasm for BGM resistance[18]. Singh *et al.* developed a cut-twig technology that provides a non-destructive plant sample and is especially useful for large hybridization programmes[19]. Resistance to BGM identified in wild *Cicer spp.* might be transferred to cultivated types through wide hybridization. Tewari *et al.*, in their study identified a single dominant gene '*Bor1*' for BGM resistance in chickpea[20]. Rewal and Grewal identified two genes with dominant and recessive epistasis (13:3 ratio)[18] and Chaturvedi *et al.* Observed duplicate dominant form of epistasis was (15:1 ratio)[21]. Hence, there is a need to identify diverse genes for resistance from different sources so that these can be pyramided to obtain higher levels of resistance for better productivity. The markers closely linked with BGM resistance QTL identified in various earlier studies can facilitate identification of diverse genes and their pyramiding in a single genotype. Sources with higher levels of resistance, as compared to the cultivated species, are available in some wild *Cicer* species, including *C. judaicum* Boiss., *C. bijugum, C. echinospermum*, and *C. pinnatifidum*[22,23]. The identification of molecular markers linked to resistance genes would be invaluable tools for the development of resistant chickpea cultivars for most economically important diseases. Therefore, the present study was conducted to evaluate and identify chickpea genotypes having resistance to *Botrytis* grey mould disease.

Materials and Methods

The experiment was conducted at the N. E. Borlaug Crop Research Centre and the Pulse Breeding Laboratory of the G. B. Pant University of Agriculture and Technology in Pantnagar, Uttarakhand, during *rabi* 2017-18, 2018-19 and 2019-20 to evaluate and identify chickpea genotypes for resistance to *Botrytis* grey mould disease.

Plant material

For field screening, 225 chickpea genotypes were used in 2017-18, 135 in 2018-19, and 100 in 2019-20, with 5 checks: PG 3 and PG 5 (Moderately resistant), H 208 and DCP 92-3 (Susceptible), and GL10006 (Resistant). However, the number of checks remained constant (Suppl. Tables 1-3).

Layout plan

Considering each genotype as one treatment, the experiment was conducted in augmented block design-II[24-26] during the entire study. The 225 experimental chickpea genotypes were planted in 9 blocks with each block consisted of 30 genotypes including 5 checks *viz.,* PG 5, PG 3, H 208, DCP 92-3 and GL10006 during 2017-18. While during 2018-19, 135 genotypes were planted in 9 blocks, each consisted of 20 genotypes along with 5 checks, while during the year 2019-20, 100 genotypes were planted in 4 blocks, each block consisted of 30 genotypes including 5 checks. Each genotype was planted in a single row of 4 m length with row to row distance of 30 cm and plant to plant distance of 10-15 cm. The standard package of practices for chickpea cultivation was followed to raise a normal and healthy crop.

Screening of chickpea genotypes against Botrytis grey mould disease in field condition

Plants were inoculated with a spore suspension (50,000 spores / ml) of a 10-day-old culture of *Botrytis cinerea* at the onset of flowering. These genotypes were screened for BGM for three consecutive years. The genotypes were chosen based on disease score as per the score given by Kaur *et al.*[27]and, seed yield per plant. Based on the disease score and seed yield per plant the genotypes were selected and planted in the next season. The observations were recorded when susceptible cultivars showed the maximum score for the disease. These genotypes were tested for BGM disease resistance and seed yield per plant.

Disease rating

The genotypes were selected for screening against BGM at the Pantnagar location. Total numbers of plants were counted in each line. At the reproductive stage of the disease was identified, and data was recorded on a nine-point (1-9) scale on the basis of per cent plant parts affected by BGM disease[27].

Statistical analysis

The test entries are planted once without replication, *i.e.*, each block has a different set of un-replicated entries,

Table 1 — Disease response of different genotypes to BGM in field (rabi, 2017-18)

S. No.	Genotype	Disease rating	Disease reaction	S. No.	Genotype	Disease rating	Disease reaction	S. No.	Genotype	Disease rating	Disease reaction	S. No.	Genotype	Disease rating	Disease reaction
1	PG 17-1	3	R	30	PG 17-35	6	MS	59	PG 17-66	8	S	88	PG 17-97	9	S
2	PG 17-2	8	S	31	PG 17-36	8	S	60	PG 17-68	6	MS	89	PG 17-99	8	S
3	PG 17-4	3	R	32	PG 17-37	9	S	61	PG 17-69	6	MS	90	PG 17-100	7	MS
4	PG 17-5	3	R	33	PG 17-38	9	S	62	PG 17-70	6	MS	91	PG 17-101	6	MS
5	PG 17-6	8	S	34	PG 17-39	8	S	63	PG 17-71	6	MS	92	PG 17-102	7	MS
6	PG 17-7	7	MS	35	PG 17-40	7	MS	64	PG 17-72	6	MS	93	PG 17-103	8	S
7	PG 17-8	7	MS	36	PG 17-41	8	S	65	PG 17-73	8	S	94	PG 17-104	8	S
8	PG 17-9	3	R	37	PG 17-44	7	MS	66	PG 17-74	5	MR	95	PG 17-105	8	S
9	PG 17-10	8	S	38	PG 17-45	8	S	67	PG 17-75	7	MS	96	PG 17-106	8	S
10	PG 17-13	3	R	39	PG 17-46	7	MS	68	PG 17-76	5	MR	97	PG 17-107	7	MS
11	PG 17-14	8	S	40	PG 17-47	8	S	69	PG 17-77	8	S	98	PG 17-108	7	MS
12	PG 17-15	8	S	41	PG 17-48	6	MS	70	PG 17-78	8	S	99	PG 17-109	8	S
13	PG 17-16	6	MS	42	PG 17-49	6	MS	71	PG 17-79	7	MS	100	PG 17-110	7	MS
14	PG 17-19	7	MS	43	PG 17-50	7	MS	72	PG 17-80	6	MS	101	PG 17-111	6	MS
15	PG 17-20	5	MR	44	PG 17-51	8	S	73	PG 17-81	8	S	102	PG 17-112	6	MS
16	PG 17-21	8	S	45	PG 17-52	7	MS	74	PG 17-82	6	MS	103	PG 17-113	6	MS
17	PG 17-22	8	S	46	PG 17-53	6	MS	75	PG 17-83	3	R	104	PG 17-114	7	MS
18	PG 17-23	9	S	47	PG 17-54	3	R	76	PG 17-84	5	MR	105	PG 17-115	8	S
19	PG 17-24	7	MS	48	PG 17-55	8	S	77	PG 17-86	8	S	106	PG 17-116	7	MS
20	PG 17-25	7	MS	49	PG 17-56	8	S	78	PG 17-87	8	S	107	PG 17-117	6	MS
21	PG 17-26	8	S	50	PG 17-57	8	S	79	PG 17-88	3	R	108	PG 17-118	5	MR
22	PG 17-27	6	MS	51	PG 17-58	5	MR	80	PG 17-89	3	R	109	PG 17-119	3	R
23	PG 17-28	9	S	52	PG 17-59	6	MS	81	PG 17-90	7	MS	110	PG 17-120	3	R
24	PG 17-29	9	S	53	PG 17-60	8	S	82	PG 17-91	8	S	111	PG 17-121	6	MS
25	PG 17-30	9	S	54	PG 17-61	7	MS	83	PG 17-92	8	S	112	PG 17-122	7	MS
26	PG 17-31	8	S	55	PG 17-62	8	S	84	PG 17-93	9	S	113	PG 17-123	6	MS
27	PG 17-32	6	MS	56	PG 17-63	6	MS	85	PG 17-94	8	S	114	PG 17-124	8	S
28	PG 17-33	7	MS	57	PG 17-64	8	S	86	PG 17-95	8	S	115	PG 17-125	6	MS
29	PG 17-34	9	S	58	PG 17-65	8	S	87	PG 17-96	9	S	116	PG 17-126	6	MS
117	PG 17-127	6	MS	146	PG 17-156	7	MS	175	PG 17-187	6	MS	204	PG 17-216	6	MS
118	PG 17-128	6	MS	147	PG 17-157	6	MS	176	PG 17-188	7	MS	205	PG 17-217	7	MS
119	PG 17-129	6	MS	148	PG 17-159	6	MS	177	PG 17-189	7	MS	206	PG 17-218	3	R
120	PG 17-130	7	MS	149	PG 17-160	6	MS	178	PG 17-190	7	MS	207	PG 17-219	8	S
121	PG 17-131	8	S	150	PG 17-161	7	MS	179	PG 17-191	6	MS	208	PG 17-220	6	MS
122	PG 17-132	6	MS	151	PG 17-162	8	S	180	PG 17-192	7	MS	209	PG 17-221	6	MS
123	PG 17-133	6	MS	152	PG 17-163	8	S	181	PG 17-193	6	MS	210	PG 17-222	8	S
124	PG 17-134	6	MS	153	PG 17-164	9	S	182	PG 17-194	8	S	211	PG 17-223	8	S
125	PG 17-135	6	MS	154	PG 17-165	7	MS	183	PG 17-195	8	S	212	PG 17-224	8	S
126	PG 17-136	8	S	155	PG 17-167	7	MS	184	PG 17-196	6	MS	213	PG 17-225	8	S
127	PG 17-137	6	MS	156	PG 17-168	6	MS	185	PG 17-197	7	MS	214	PG 17-226	7	MS
128	PG 17-138	7	MS	157	PG 17-169	8	S	186	PG 17-198	6	MS	215	PG 17-227	6	MS
129	PG 17-139	6	MS	158	PG 17-170	7	MS	187	PG 17-199	8	S	216	PG 17-228	6	MS
130	PG 17-140	6	MS	159	PG 17-171	7	MS	188	PG 17-200	8	S	217	PG 17-229	6	MS
131	PG 17-141	8	S	160	PG 17-172	5	MR	189	PG 17-201	6	MS	218	PG 17-230	7	MS
132	PG 17-142	7	MS	161	PG 17-173	5	MR	190	PG 17-202	6	MS	219	PG 17-231	7	MS
133	PG 17-143	7	MS	162	PG 17-174	8	S	191	PG 17-203	7	MS	220	PG 17-232	8	S
134	PG 17-144	6	MS	163	PG 17-175	5	MR	192	PG 17-204	7	MS	221	PG 17-233	5	MR
135	PG 17-145	3	R	164	PG 17-176	8	S	193	PG 17-205	3	R	222	PG 17-234	6	MS
136	PG 17-146	3	R	165	PG 17-177	7	MS	194	PG 17-206	3	R	223	PG 17-235	7	MS
137	PG 17-147	7	MS	166	PG 17-178	8	S	195	PG 17-207	7	MS	224	PG 17-236	7	MS
138	PG 17-148	6	MS	167	PG 17-179	5	MR	196	PG 17-208	6	MS	225	PG 17-237	8	S
139	PG 17-149	8	S	168	PG 17-180	5	MS	197	PG 17-209	6	MS	C1	PG3	5	MR
140	PG 17-150	6	MS	169	PG 17-181	6	MS	198	PG 17-210	6	MS	C2	PG5	5	MR
141	PG 17-151	6	MS	170	PG 17-182	5	MR	199	PG 17-211	7	MS	C3	H 208	9	S
142	PG 17-152	6	MS	171	PG 17-183	5	MR	200	PG 17-212	3	R	C4	DCP 92-3	8	S
143	PG 17-153	6	MS	172	PG 17-184	6	MS	201	PG 17-213	6	MS	C5	GL 10006	3	R
144	PG 17-154	7	MS	173	PG 17-185	8	S	202	PG 17-214	8	S				
145	PG 17-155	6	MS	174	PG 17-186	7	MS	203	PG 17-215	8	S				

Checks (C1, C2, C3, C4, C5)

Here, R= Resistant, MR= Moderately resistant, MS= Moderately susceptible, S=Susceptible

Table 2 — Classification of chickpea genotypes based on BGM disease rating (rabi, 2017-18)

Disease rating	Total genotypes	Name of the genotypes
3	17	PG 17-1, PG 17- 4, PG 17-5, PG 17-9, PG 17-13, PG 17-54, PG 17-83, PG 17-88, PG 17-89, PG 17-119, PG 17-120, PG 17-145, PG 17-146, PG 17-205, PG 17-206, PG 17-212, PG 17-218
5	14	PG 17-20, PG 17-58, PG 17-74, PG 17-76, PG 17-84, PG 17-188, PG 17-172, PG 17-173, PG 17-175, PG 17-179, PG 17-180, PG 17-182, PG 17-183, PG 17-233
6	65	PG 17-16, PG 17-27, PG 17-32, PG 17-35, PG 17-48, PG 17-49, PG 17-53, PG 17-59, PG 17-63, PG 17-68, PG 17-69, PG 17-101, PG 17-111, PG 17-112, PG 17-113, PG 17-117, PG 17-121, PG 17-123, PG 17-125, PG 17-126, PG 17-82, PG 17-80, PG 17-72, PG 17-71, PG 17-70, PG 17-127, PG 17-128, PG 17-129, PG 17-132, PG 17-133, PG 17-134, PG 17-135, PG 17-137, PG 17-139, PG 17-140, PG 17-144, PG 17-148, PG 17-150, PG 17-151, PG 17-152, PG 17-153, PG 17-155, PG 17-157, PG 17-159, PG 17-160, PG 17-168, PG 17-181, PG 17-184, PG 17-187, PG 17-191, PG 17-193, PG 17-196, PG 17-198, PG 17-201, PG 17-202, PG 17-208, PG 17-209, PG 17-213, PG 17-216, PG 17-220, PG 17-221, PG 17-227, PG 17-228, PG 17-229, PG 17-234
7	52	PG 17-7, PG 17-8, PG 17-19, PG 17-24, PG 17-25, PG 17-33, PG 17-40, PG 17-44, PG 17-46, PG 17-50, PG 17-52, PG 17-61,PG 17-75, PG 17-79, PG 17-90, PG 17-100, PG 17-102, PG 17-107, PG 17-108, PG 17-110, PG 17-114, PG 17-116, PG 17-122, PG 17-130, PG 17-138, PG 17- 142, PG 17-143, PG 17-147, PG 17-154, PG 17-156, PG 17-161, PG 17-165, PG 17-167, PG 17-170, PG 17-171, PG 17-177, PG 17-186, PG 17-188, PG 17-189, PG 17-190, PG 17-192, PG 17-197, PG 17-203, PG 17-204, PG 17-207, PG 17-211, PG 17-217, PG 17-226, PG 17-230, PG 17-231, PG 17-235, PG 17-236
8	66	PG 17-2, PG 17-6, PG 17-14, PG 17-15, PG 17-10,PG 17-26, PG 17-22, PG 17-31, PG 17-21, PG 17-65, PG 17-60, PG 17-45, PG 17-36, PG 17-47, PG 17-39, PG 17-62, PG 17-57, PG 17-41, PG 17-55, PG 17-64, PG 17-51, PG 17-56, PG 17-60, PG 17-66, PG 17-73, PG 17-77, PG 17-78, PG 17-81, PG 17-86, PG 17-87, PG 17-91, PG 17-92, PG 17-94, PG 17-95, PG 17-99, PG 17-103, PG 17-104, PG 17-105, PG 17-106, PG 17-109, PG 17-115, PG 17-124, PG 17-131, PG 17-136, PG 17-141, PG 17-149, PG 17-162, PG 17-163, PG 17-169, PG 17-174, PG 17-176, PG 17-178, PG 17-185, PG 17-194, PG 17-195, PG 17-199, PG 17-200, PG 17-214, PG 17-215, PG 17-219, PG 17-222, PG 17-223, PG 17-224, PG 17-225, PG 17-232, PG 17-237
9	11	PG 17-23, PG 17-28, PG 17-29, PG 17-30, PG 17-34, PG 17-38, PG 17-37, PG 17-96, PG 17-93, PG 17-97, PG 17-164

Table 3 — Disease response of different genotypes to BGM in field (rabi, 2018-19)

S. No	Genotype	Disease rating	Disease reaction	S. No.	Genotype	Disease rating	Disease reaction	S. No.	Genotype	Disease rating	Disease reaction	S. No.	Genotype	Disease rating	Disease reaction	S. No.	Genotype	Disease rating	Disease reaction
1	PG 17-1	5	MR	30	PG 17-88	3	R	59	PG 17-134	6	MS	88	PG 17-172	6	MS	117	PG 17-209	6	MS
2	PG 17-4	5	MR	31	PG 17-89	3	R	60	PG 17-135	6	MS	89	PG 17-173	6	MS	118	PG 17-210	6	MS
3	PG 17-5	6	MS	32	PG 17-90	7	MS	61	PG 17-137	6	MS	90	PG 17-175	6	MS	119	PG 17-211	8	S
4	PG 17-8	6	MS	33	PG 17-100	7	MS	62	PG 17-138	9	S	91	PG 17-177	8	S	120	PG 17-212	3	R
5	PG 17-9	3	R	34	PG 17-101	7	MS	63	PG 17-139	6	MS	92	PG 17-179	6	MS	121	PG 17-213	6	MS
6	PG 17-13	6	MS	35	PG 17-102	7	MS	64	PG 17-140	6	MS	93	PG 17-180	6	MS	122	PG 17-216	6	MS
7	PG 17-16	6	MS	36	PG 17-107	7	MS	65	PG 17-142	9	S	94	PG 17-181	6	MS	123	PG 17-217	8	S
8	PG 17-20	6	MS	37	PG 17-108	7	MS	66	PG 17-143	6	MS	95	PG 17-182	6	MS	124	PG 17-218	3	R
9	PG 17-24	7	MS	38	PG 17-110	7	MS	67	PG 17-144	6	MS	96	PG 17-183	6	MS	125	PG 17-220	6	MS
10	PG 17-27	6	MS	39	PG 17-111	6	MS	68	PG 17-145	3	R	97	PG 17-184	6	MS	126	PG 17-221	6	MS
11	PG 17-33	8	S	40	PG 17-112	6	MS	69	PG 17-146	3	R	98	PG 17-186	8	S	127	PG 17-226	8	S
12	PG 17-35	6	MS	41	PG 17-113	6	MS	70	PG 17-147	7	MS	99	PG 17-187	6	MS	128	PG 17-227	6	MS
13	PG 17-48	6	MS	42	PG 17-114	7	MS	71	PG 17-148	6	MS	100	PG 17-188	8	S	129	PG 17-228	6	MS
14	PG 17-49	6	MS	43	PG 17-116	7	MS	72	PG 17-150	6	MS	101	PG 17-189	7	S	130	PG 17-229	6	MS
15	PG 17-53	6	MS	44	PG 17-117	6	MS	73	PG 17-151	6	MS	102	PG 17-190	8	S	131	PG 17-230	7	MS
16	PG 17-54	3	R	45	PG 17-118	6	MS	74	PG 17-152	6	MS	103	PG 17-191	6	MS	132	PG 17-231	8	S
17	PG 17-58	6	MS	46	PG 17-119	3	R	75	PG 17-153	9	S	104	PG 17-192	7	MS	133	PG 17-233	6	MS
18	PG 17-59	6	MS	47	PG 17-120	3	R	76	PG 17-154	9	S	105	PG 17-193	6	MS	134	PG 17-234	6	MS
19	PG 17-63	6	MS	48	PG 17-121	6	MS	77	PG 17-155	6	MS	106	PG 17-196	6	MS	135	PG 17-235	7	MS
20	PG 17-68	6	MS	49	PG 17-122	7	MS	78	PG 17-156	7	MS	107	PG 17-197	7	MS	C1	PG3	5	MR
21	PG 17-69	6	MS	50	PG 17-123	6	MS	79	PG 17-157	6	MS	108	PG 17-198	6	MS	C2	PG5	5	MR
22	PG 17-70	6	MS	51	PG 17-125	6	MS	80	PG 17-159	9	S	109	PG 17-201	6	MS	C3	H 208	9	S
23	PG 17-71	6	MS	52	PG 17-126	6	MS	81	PG 17-160	6	MS	110	PG 17-202	6	MS	C4	DCP 92-3	8	S
24	PG 17-72	6	MS	53	PG 17-127	6	MS	82	PG 17-161	7	MS	111	PG 17-203	9	S	C5	GL 10006	3	R
25	PG 17-74	5	MR	54	PG 17-128	6	MS	83	PG 17-165	7	MS	112	PG 17-204	9	S				
26	PG 17-76	5	MR	55	PG 17-129	6	MS	84	PG 17-167	9	S	113	PG 17-205	3	R				
27	PG 17-82	6	MS	56	PG 17-130	7	MS	85	PG 17-168	6	MS	114	PG 17-206	3	R				
28	PG 17-83	3	R	57	PG 17-132	6	MS	86	PG 17-170	7	MS	115	PG 17-207	7	MS				
29	PG 17-84	6	MS	58	PG 17-133	6	MS	87	PG 17-171	9	S	116	PG 17-208	6	MS				

Checks (C1, C2, C3, C4, C5)

Here, R= Resistant, MR= Moderately resistant, MS= Moderately susceptible, S=Susceptible

whereas a set of checks (c), numbering five, is replicated in each block to estimate error mean square and block effect. Randomization is done in such a way that all the checks (c) and a part of test genotypes fall only once in each block. Equal number of test genotypes was planted in each block to facilitate statistical analysis.

Table 4 — Classification of chickpea genotypes based on BGM disease rating (rabi, 2018-19)

Disease rating	Total genotypes	Name of the genotypes
3	13	PG 17-9, PG 17-54, PG 17-83, PG 17-88, PG 17-89, PG 17-119, PG 17-120, PG 17-145, PG 17-146, PG 17-205, PG 17-206, PG 17-212, PG 17-218
5	4	PG 17-1, PG 17-4, PG 17-74, PG 17-76
6	77	PG 17- 5, PG 17-8, PG 17-13, PG 17-16, PG 17-20, PG 17-27, PG 17-35, PG 17-48, PG 17-49, PG 17-53, PG 17-58, PG 17-59, PG 17-63, PG 17-68, PG 17-69, PG 17-70, PG 17-71, PG 17-72, PG 17-82, PG 17-84, PG 17-111, PG 17-112, PG 17-113, PG 17-117, PG 17-118, PG 17-121, PG 17-123, PG 17-125, PG 17-126, PG 17-127, PG 17-128, PG 17-129, PG 17-132, PG 17-133, PG 17-134, PG 17-135, PG 17-137, PG 17-139, PG 17-140, PG 17-143, PG 17-144, PG 17-148, PG 17-150, PG 17-151, PG 17-152, PG 17-155, PG 17-157, PG 17-160, PG 17-168, PG 17-172, PG 17-173, PG 17-175, PG 17-179, PG 17-180, PG 17-181, PG 17-182, PG 17-183, PG 17-184, PG 17-187, PG 17-191, PG 17-193, PG 17-196, PG 17-198, PG 17-201, PG 17-202, PG 17-208, PG 17-209, PG 17-210, PG 17-213, PG 17-216, PG 17-220, PG 17-221, PG 17-227, PG 17-228, PG 17-229, PG 17-233, PG 17-234
7	23	PG 17-24, PG 17-90, PG 17-100, PG 17-101, PG 17-102, PG 17-107, PG 17-108, PG 17-110, PG 17-114, PG 17-116, PG 17-122, PG 17-130,PG 17-147, PG 17-156, PG 17-161, PG 17-165, PG 17-170, PG 17-189, PG 17-92, PG 17-197, PG 17-207, PG 17-230,,PG 17-235
8	9	PG 17-33, PG 17-177, PG 17-186, PG 17-188, PG 17-190,PG 17-211, PG 17-217, PG 17-226,PG 17-231
9	9	PG 17-138, PG 17-142, PG 17-153, PG 17-154, PG 17-139, PG 17-167, PG 17-171, PG 17-203, PG 17-204

Molecular marker validation of *Botrytis* grey mould resistant genotypes

Plant Material

The top 20 genotypes were chosen based on their performance in terms of seed yield per plant and disease score over three years. The validation of these genotypes by molecular markers included a total of 25 genotypes, including 5 checks, namely PG 5, PG 3, H 208, DCP 92-3, and GL 10006 (Suppl. Table 4).

DNA Extraction and PCR amplification

The genomic DNA was extracted from 30 days old seedlings following the CTAB (Cetyl tri-methyl ammonium bromide) procedure with minor modifications[28]. The quality of DNA was checked and resolved in a 0.8% agarose gel. PCR amplification was carried out in 10 µl reactions containing 10 ng/µl of DNA template, 1 µl TBE buffer (containing 200 mM Tris-HCl with pH 8.3, 500 mM KCl, 15 mM MgCl 2), 0.25 µl of 1 mM dNTP, 0.5 µl of 5 µM forward and reverse primers, and 0.25 µl of Taq DNA polymerase (4 U/µl) (Initial denaturation for 5 min at 94°C, followed by 35 cycles for 1 min denaturation at 94°C, 1 min annealing at 55°C, and 2 min extension at 72°C with a final extension for 5 min at 72°C). PCR products have been mixed with bromophenol blue gel loading dye and analyzed on 3 % agarose gel. The bands were visualized after 0.5 mg/ml EtBr gel staining and were documented using a Gel imager (UVP, UK).A standard 100 bp DNA ladder was used as marker to determine the band size.

Molecular marker analysis

For molecular screening of chickpea genotypes against BGM disease, a total of 25 STMS / microsatellite primers were used (Suppl. Table 5). In molecular marker analysis, tightly linked markers with a locus and amplification via polymerase chain reaction (PCR) at an appropriate annealing temperature were used. By comparing the amplified products to resistant and susceptible checks, the amplified products were scored separately for each site-specific primer. For the primers, only clear and seemingly unambiguous bands were scored. The data was then compared to field data, and conclusions were drawn for each primer-genotype combination.

Result

Field screening of chickpea genotypes against *Botrytis* grey mould disease

Pantnagar is a hot spot for *Botrytis* grey mold. Chickpea germplasm with BGM resistance is uncommon. Field screening of chickpea genotypes for BGM disease was done for three years in a row by spraying BGM inoculum over the genotypes (Figs 1 & 2). The disease symptoms appeared in about 10 days after the inoculations, and disease severity was recorded using 1-9 scale given as per Kaur *et al* [27]. During the year 2017-18, out of 225 chickpea genotypes that were screened against BGM, only 17 genotypes were identified as resistant (score 3), while 14 genotypes were identified as moderately resistant (disease score 5). The remaining genotypes, on the other hand, have disease susceptibility score (Tables 1 & 2). Disease score and seed yield per plant were used to select genotypes. In 2018-19, the selected 135 genotypes were planted in 9 blocks and screened for BGM. The disease rating for 13 of these

Table 5 — Disease response of different genotypes to BGM in field (rabi, 2019-20)

S. No.	Genotype	Disease rating	Disease reaction	S. No.	Genotype	Disease rating	Disease reaction	S. No.	Genotype	Disease rating	Disease reaction	S. No.	Genotype	Disease rating	Disease reaction
1	PG 17-1	5	MR	28	PG 17-112	5	MR	55	PG 17-151	5	MR	82	PG 17-201	6	MS
2	PG 17-4	5	MR	29	PG 17-113	5	MR	56	PG 17-152	5	MR	83	PG 17-202	6	MS
3	PG 17-5	6	MR	30	PG 17-117	5	MR	57	PG 17-155	5	MR	84	PG 17-205	3	R
4	PG 17-9	3	R	31	PG 17-118	6	MS	58	PG 17-156	5	MR	85	PG 17-206	5	MR
5	PG 17-20	5	MR	32	PG 17-119	3	R	59	PG 17-157	6	MS	86	PG 17-207	7	MS
6	PG 17-27	5	MR	33	PG 17-120	3	R	60	PG 17-160	6	MS	87	PG 17-208	6	MS
7	PG 17-35	6	MR	34	PG 17-121	5	MR	61	PG 17-161	9	S	88	PG 17-209	6	MS
8	PG 17-48	5	MR	35	PG 17-123	5	MR	62	PG 17-165	9	S	89	PG 17-210	6	MS
9	PG 17-49	5	MR	36	PG 17-125	5	MR	63	PG 17-168	6	MS	90	PG 17-212	5	MR
10	PG 17-53	5	MR	37	PG 17-126	5	MR	64	PG 17-170	7	MS	91	PG 17-213	6	MS
11	PG 17-54	3	R	38	PG 17-127	5	MR	65	PG 17-172	6	MS	92	PG 17-216	6	MS
12	PG 17-58	5	MR	39	PG 17-128	5	MR	66	PG 17-173	6	MS	93	PG 17-218	3	R
13	PG 17-59	5	MR	40	PG 17-129	5	MR	67	PG 17-175	6	MS	94	PG 17-220	6	MS
14	PG 17-63	5	MR	41	PG 17-132	5	MR	68	PG 17-179	6	MS	95	PG 17-221	6	MS
15	PG 17-68	5	MR	42	PG 17-133	5	MR	69	PG 17-180	6	MS	96	PG 17-226	7	MS
16	PG 17-69	5	MR	43	PG 17-134	5	MR	70	PG 17-181	8	S	97	PG 17-230	7	MS
17	PG 17-71	5	MR	44	PG 17-135	5	MR	71	PG 17-182	6	MS	98	PG 17-233	6	MS
18	PG 17-72	5	MR	45	PG 17-137	5	MR	72	PG 17-183	6	MS	99	PG 17-234	6	MS
19	PG 17-74	5	MR	46	PG 17-139	5	MR	73	PG 17-184	6	MS	100	PG 17-235	7	MS
20	PG 17-76	5	MR	47	PG 17-140	5	MR	74	PG 17-187	6	MS	C1	PG3	5	MR
21	PG 17-82	5	MR	48	PG 17-143	6	MS	75	PG 17-189	9	S	C2	PG5	5	MR
22	PG 17-83	3	R	49	PG 17-144	5	MR	76	PG 17-190	8	S	C3	H 208	9	S
23	PG 17-84	5	MR	50	PG 17-145	3	R	77	PG 17-191	6	MS	C4	DCP 92-3	8	S
24	PG 17-88	3	R	51	PG 17-146	3	R	78	PG 17-193	6	MS	C5	GL 10006	3	R
25	PG 17-89	3	R	52	PG 17-147	8	S	79	PG 17-196	6	MS				
26	PG 17-90	7	MR	53	PG 17-148	8	S	80	PG 17-197	7	MS				
27	PG 17-100	7	MS	54	PG 17-150	8	S	81	PG 17-198	6	MS				

Checks (C1, C2, C3, C4, C5)

Here, R= Resistant, MR= Moderately resistant, MS= Moderately susceptible, S=Susceptible

Fig. 1 — (A) Disease inoculum, (B) Prepration of liquid inoculum of *botrytis* grey mould for spray, (C) Spraying of disease inoculum over chickpea genotypes

Fig. 2 — Screening of chickpea genotypes against *Botrytis* grey mould disease under field conditions

M: 100 bp DNA ladderLane 1: GL10006 (Resistant), 2:PG3 (Moderately resistant), 3:PG 5 (Moderately resistant), 4:DCP 92-3 (Susceptible), 5:H 208 (Susceptible), 6: PG 17-1, 7: PG 17-4, 8:PG 17-9, 9:PG 17-83, 10:PG 17-89, 11:PG 17-119,12:PG 17-120, 13:PG 17-121, 14:PG 17-123, 15:PG 17-145, 16:PG 17-146, 17:PG 17-151, 18:PG 17-152, 19:PG 17-160, 20:PG 17-183, 21:PG 17-205, 22:PG 17-206, 23:PG 17-212, 24:PG 17-218, 25:PG 17-233

Table 6 — Classification of chickpea genotypes based on BGM disease rating (rabi, 2019-20)

Disease rating	Total genotypes	Name of the genotypes
3	11	PG 17-9, PG 17-54, PG 17-83, PG 17-88, PG 17-89, PG 17-119, PG 17-120, PG 17-145, PG 17-146, PG 17-205, PG 17-218
5	42	PG 17-1, PG 17-4, PG 17-20, PG 17-27, PG 17-48, PG 17-49, PG 17-53, PG 17-58, PG 17-59, PG 17-63, PG 17-68, PG 17-69, PG 17-71, PG 17-72, PG 17-74, PG 17-76, PG 17-82, PG 17-84, PG 17-112, PG 17-113, PG 17-117, PG 17-121, PG 17-123, PG 17-125, PG 17-126, PG 17-127, PG 17-128, PG 17-129, PG 17-132, PG 17-133, PG 17-134, PG 71-135, PG 17-137, PG 17-139, PG 17-140, PG 17-144, PG 17-151, PG 17-152, PG 17-155, PG 17-156, PG 17-206, PG 17-212
6	31	PG 17-5, PG 17-35, PG 17-118, PG 17-143, PG 17-157, PG 17-160, PG 17-168, PG 17-172, PG 17-173, PG 17-175, PG 17-179, PG 17-180, PG 17-182, PG 17-183, PG 17-184, PG 17-187, PG 17-191, PG 17-193, PG 17-196, PG 17-198, PG 17-201, PG 17-202, PG 17-208, PG 17-209, PG 17-210, PG 17-213, PG 17-216, PG 17-220, PG 17-221, PG 17-233, PG 17-234
7	8	PG 17-90, PG 17-100, PG 17-170, PG 17-197, PG 17-207, PG 17-226, PG 17-230, PG 17-235
8	5	PG 17-147, PG 17-148, PG 17-150, PG 17-181, PG 17-190
9	3	PG 17-161, PG 17-165, PG 17-189

Table 7 — Amplification patterns of STMS primers (Number of alleles, range of amplified band size (bp), rare alleles and PIC Value)

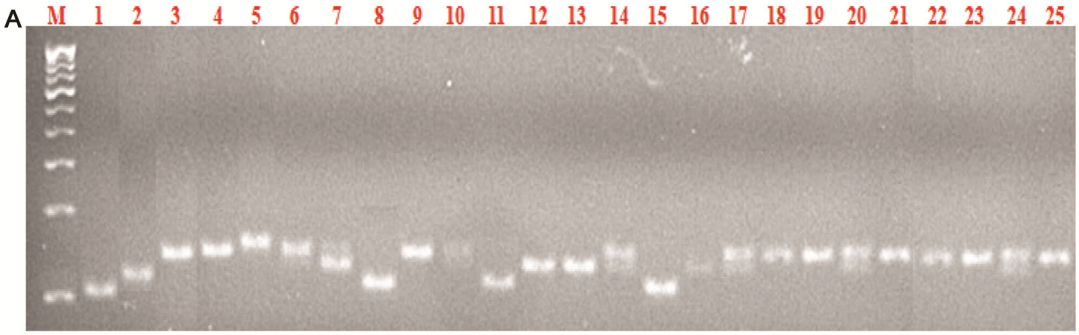
S.No.	Polymorphic markers	No of alleles	No. of polymorphic alleles	Amplified band size (bp)	Rare alleles	PIC Value
1	TR29	4	4	180	1	0.5728
2	TA 144	4	5	280	1	0.6496

genotypes was 3 (resistant), 4 (moderately resistant), and the remaining genotypes received a disease susceptibility score (Tables 3-6). Similarly, during 2019-20, these 100 genotypes were planted and screened. Only 11 genotypes received a disease rating of 3 (resistant), 42 genotypes received a disease rating of 5 (moderately resistant), and the remaining genotypes received a disease susceptibility score.

Molecular marker validation of *Botrytis* grey mould resistant chickpea genotypes

The chickpea genotypes were evaluated for three consecutive years, and then the best 20 genotypes were selected for molecular confirmation based on disease score and seed yield per plant. These 20 genotypes, along with the above 5 checks, were validated against BGM using 25 STMS markers (suppl. Table 5). Out of these 25 STMS markers, only two markers *viz.,* TR 29, TA 144 exhibited polymorphic bands. The polymorphic information (PIC) content ranged from 0.572-0.649 for these two polymorphic markers (Table 7). Monomorphic markers yielded zero PIC value and the same band sizes and, hence, were not considered further in the study. Higher PIC value was exhibited by marker TA 144 (0.649) followed by TR 29 (0.572). The marker TA 144 yielded five polymorphic alleles, whereas; maker TR29 exhibited four polymorphic alleles, and therefore, the amplification pattern of genotypes with these two primers was compared with the disease reaction.

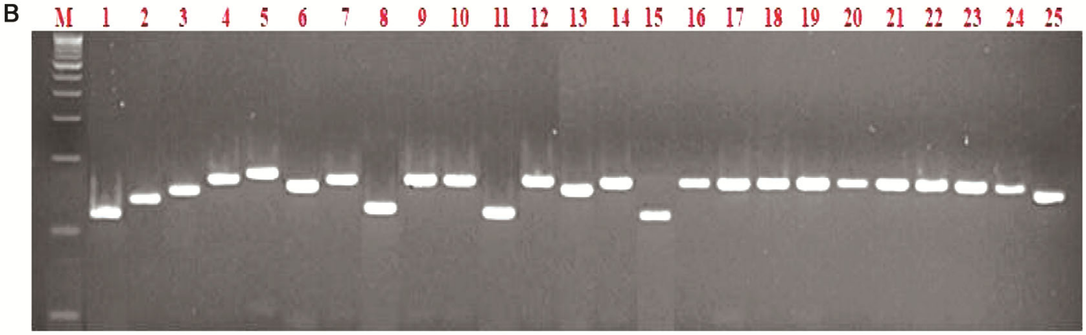
The genotypic screening of 20 genotypes along with five checks (Suppl. Table 4) showed amplification ranging between 130 -180 bp (Table 7) for the primer TR29 (Fig. 3A). Five known checks showed difference in band size from 130 to 180 bp. The genotype GL10006 (resistant check) showed band size of 130 bp, The genotype PG 3 showed band size of 140 bp, genotype PG 5 band size of 150 bp, DCP 92 -3 band size of 160 bp and the genotype H 208 showed band size of 180 bp. Out of 20 genotypes, three genotypes *viz*., PG 17-9 (130 bp), PG 17-119 (130 bp) and PG 17-145 (130 bp) exhibited band size similar to resistant check (Fig. 3A) and hence, these three genotypes were considered as resistant. Whereas, genotypes such as PG 17-120 and PG 17-121 showed band size similar to moderately resistant check, hence, these genotypes were treated as moderately resistant. The rest of the genotypes showed band sizes similar to susceptible genotypes and were therefore treated as susceptible genotypes. Similarly, genotypic screening of 20 genotypes along with five checks (Suppl. Table 4) showed amplification ranging between 230-280 bp (Table 7) for the primer TA144 (Fig. 3B). Five known checks showed a difference in band size from 230 to 280. The genotype GL10006 showed band size of 230 bp, PG 3 showed band size of 240 bp, PG 5 showed band size of 250 bp, DCP 92-3 showed band size of 260 bp and H 208 showed band size of 280 bp. Out of 20 genotypes, three *viz*., PG 17-9 (230bp), PG 17-119



Marker *TR 29*

M: 100 bp DNA ladder

Lane 1: GL10006 (Resistant), 2:PG3 (Moderately resistant), 3:PG 5 (Moderately resistant), 4:DCP 92-3 (Susceptible), 5:H 208 (Susceptible), 6: PG 17-1, 7: PG 17-4, 8:PG 17-9, 9:PG 17-83, 10:PG 17-89, 11:PG 17-119,12:PG 17-120, 13:PG 17-121, 14:PG 17-123, 15:PG 17-145, 16:PG 17-146, 17:PG 17-151, 18:PG 17-152, 19:PG 17-160, 20:PG 17-183, 21:PG 17-205, 22:PG 17-206, 23:PG 17-212, 24:PG 17-218, 25:PG 17-233



Marker *TA144*

M: 100 bp DNA ladder

Lane 1: GL10006 (Resistant), 2:PG3 (Moderately resistant), 3:PG 5 (Moderately resistant), 4:DCP 92-3 (Susceptible), 5:H 208 (Susceptible), 6: PG 17-1, 7: PG 17-4, 8:PG 17-9, 9:PG 17-83, 10:PG 17-89, 11:PG 17-119,12:PG 17-120, 13:PG 17-121, 14:PG 17-123, 15:PG 17-145, 16:PG 17-146, 17:PG 17-151, 18:PG 17-152, 19:PG 17-160, 20:PG 17-183, 21:PG 17-205, 22:PG 17-206, 23:PG 17-212, 24:PG 17-218, 25:PG 17-233

Fig. 3 — (A) Showing amplification pattern of 25 genotypes with marker *TR29*M: 100 bp DNA ladder Lane 1: GL10006 (Resistant), 2:PG3 (Moderately resistant), 3:PG 5 (Moderately resistant), 4:DCP 92-3 (Susceptible), 5:H 208 (Susceptible), 6: PG 17-1, 7: PG 17-4, 8:PG 17-9, 9:PG 17-83, 10:PG 17-89, 11:PG 17-119,12:PG 17-120, 13:PG 17-121, 14:PG 17-123, 15:PG 17-145, 16:PG 17-146, 17:PG 17-151, 18:PG 17-152, 19:PG 17-160, 20:PG 17-183, 21:PG 17-205, 22:PG 17-206, 23:PG 17-212, 24:PG 17-218, 25:PG 17-233; and (B) showing amplification pattern of 25 genotypes with marker *TA144*

(230bp) and PG 17-145 (230bp) produced band size similar to resistant check (Fig. 3B). Thus, these were considered as resistant. Comparison of field results with banding pattern of different genotypes revealed by markers TR29 and TA144 confirmed similar disease reactions in the three identified genotypes.

Discussion

The selection of high-yielding, disease-free chickpea lines is the most important criterion for future breeding programs. To select disease-free plants for the current study, chickpea genotypes were screened against BGM under natural epiphytotic conditions. A high level of resistance to BGM disease has been found in some chickpea genotypes. However, identifying and evaluating chickpea BGM resistant lines with the goal of linking the marker to the specific QTL is a novel approach in chickpea breeding. The present findings were consistent with previous findings on BGM. Sharma *et al.* evaluated 412 genotypes over a number of years at various locations, selecting genotypes based on disease score, seed yield and stability each year[29]. Individual analyses of field trials conducted at various locations revealed differences in the genotypes' responses to BGM. Variations in genotype frequency distributions demonstrated the need for multi-location and multi-year testing under natural epiphytotic conditions to ensure genotype stability. In the present study it was found that five genotypes, ICCV 96859, ICCV 96853, ICCV 05604, ICCV 96852, and ICCV 05605, had moderate BGM severity and stability. The ICCV 96859 genotype has the least severe BGM. Kaur *et al.* developed BGM resistant interspecific derivative lines of *C. arietinum* and *C. pinnatifidum*[27]. Similarly, Bhardwaj *et al.* conducted field screening under artificial inoculation conditions and found that chickpea line GL10006 was resistant to BGM while lines DCP 92-3 and GNG1581 were susceptible[1,30]. During field screening under natural conditions, Ranjana *et al.* identified reported that GL-10006, BG1003, HK-2, HK-4, KAK-2, GNG-1969 as highly resistant, GNG-1958, HC-1, BG-362, ICCL-10302, BG-1053, JGK-1 as resistant, BGM-547, BG-372, CSG-8962, BG-256 as moderately resistant, PBG-1 as moderately susceptible and DCP 92-3, GNG-469, GNG-1581 as susceptible[31]. The markers TA 144 and TR 29 were also validated by Ranjana *et al.* for *Botrytis* grey mould disease resistance and reported that, there is a correlation between the primers TA144, TR 29 and for BGM disease resistance[31]. Sachdeva *et al.* reported that marker TA144 was strongly correlated with resistance to BGM disease[32]. The comparison of field results in present study with banding patterns of different genotypes revealed by markers TR29 and TA144 confirmed that these genotypes had similar disease reactions. Inconsistencies in disease response with two primers, TR 29 and TA144, may be due to their location in different linkage groups. As a result, different QTLs are responsible for disease responses. Under natural field conditions, the disease reaction was found to be intermediate between the results of the two primers, which can be justified by the percentage contribution of each QTL to the overall disease reaction. The findings were consistent with the findings of Anuradha *et al*, who validated the markers TA 118 and TA144 using recombinant inbred lines (RILs) of a cross ICCV 2 x JG 62[33]. Thus, these validated microsatellite / STMS markers identified from this study will be useful in marker-assisted selection of desirable recombinants in resistance improvement programmes of chickpea.

Conclusion

The genotypes PG 17-9, PG 17-119, and PG 17-145 were found to be resistant, while PG 17-120 and PG 17-121 were found to be moderately resistant and the rest were susceptible. Markers TR29 and TA144 revealed a banding pattern of different genotypes, which corroborated field disease screening data. TR29 and TA144 have PIC values greater than 0.5, indicating that they can be used for molecular tagging of *Botrytis* grey mould resistance genes in chickpea. These validated microsatellite / STMS markers will aid in the selection of desirable recombinants for BGM disease resistance improvement programs in chickpea. The identification of BGM-resistant genotypes in this study offers valuable insights for chickpea breeding programs, providing potential donor lines to enhance disease resistance and yield stability in future varieties. The use of molecular markers for efficient selection, coupled with stable resistance across diverse environments, will streamline the breeding process. These advancements are expected to lead to more resilient chickpea varieties with improved resistance, yield stability, and sustainability, ultimately benefiting production, farmers, the agricultural sector, and food security.

Conflict of interest

All authors declare no conflict of interest.

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