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Screening of *Lilium* germplasm against botrytis blight (*Botrytis cinerea* Pers. Fr.)

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Abstract
Botrytis blight of *Lilium* is one of the most important destructive diseases caused by the fungus *Botrytis cinerea* Pers. Fr. A total of 21 *Lilium* germplasm lines were screened under natural epiphytotic conditions followed by screening of moderately resistant to resistant cultivars under artificially inoculated controlled conditions against single virulent isolate of *B. cinerea* Pers. Fr. All the germplasm lines were found susceptible to the disease but to significantly varying extents. Among the tested germplasm lines, seven were categorized as resistant (Revena-1, Revena-3, A-4 pink palace, Rialto, Cobra, Tiber, Conca–D), five as moderately resistant (Navasin, Navona, Nello, Blackout, Tresor), four as moderately susceptible (Courier, Archachon, Indian summer set, Nashvillae), four as susceptible (Litouwen-6, V-4 Litouwen, Yellow diamond, Pavia) and remaining one as highly susceptible (Malesco-5). Of these twelve moderately resistant to resistant test germplasm lines, three showed resistant response (A-4 pink palace, Cobra, Tiber), five moderately resistant (Revena-1, Reven-3, Rialto, Conca-D, Blackout), three moderately susceptible (Navasin, Navona, Tresor) and remaining one susceptible (Nello) when tested under artificially inoculated controlled conditions. On the basis of per cent disease intensity (PDI) oriental lines were categorized as resistant, Asiatic lines as moderately resistant or moderately susceptible and LA's (Longiflorum X Asiatic) are susceptible in reaction. Among the test germplasm lines Malesco-5 showed highest disease severity of 50.84% whereas, germplasm line Tiber showed lowest disease severity of 1.97%.

Keywords: *Botrytis cinerea*, *Lillium*, Kashmir, gray mold, screening

Introduction
Lily (*Lilium* spp.) is one of the most valuable commercial market flower bulbs in the world, mainly owing to its various ornamental functions as a cut flower or a potted plant. It is a member of sub-class monocotyledonae and family liliaceae comprising approximately 100 species native to North America, Europe and Asia (Mathews, 2007) [9]. It is one of the most popular groups of ornamental, bulbous, out-crossing, perennial herbs worldwide due to their incomparable beauty, commercial importance and ornamental uses (Dhyani *et al*., 2010; Sahin *et al*., 2012) [3, 12]. Lilies are unmatched in the diversity of plant architecture, shape, colour, size and fragrance of flowers that can equally well be used as cut flowers, landscape plants, potted plants etc. with large prominent flowers in the world (Kamenetsky and Okubo, 2012) [6]. Lilies are propagated through seeds or vegetatively predominantly through bulbs, bulb scales, bulblets, and bulbils (McRae, 1998) [10].

A new disease was observed on *Lilium* plants in the temperate region of Kashmir valley during 2018 and 2019. This disease cause considerable degradation of lily flower quality and severely affects plant growth and development, resulting in reduced total production worldwide. The symptoms of the botrytis leaf blight of lily are mainly observed on leaves and flowers. Both the leaves and petals of the flower buds showed circular necrotic spots that enlarged until all the leaves and flowers became blighted and covered with a grey mold layer (Dhayani *et al*., 2012) [4]. The disease appeared during mid May on leaves and in the month of June on petals of flower buds, after the onset of the monsoon. Botrytis grey mold has occurred in 50 kinds of flowers such as Lily (Zhang *et al*., 2009) [14], Begonia, Peony, Rose, Rurse, *Primula obconica*, Impatiens, Gerbera and Phalaenopsis (Liu *et al*., 2004) [8]. Grey mold is especially a devastating disease for lily and medicinal and edible *Lilium*, both of which occur widely throughout the world (Kim *et al*., 2007) [7]. *Botrytis* infections are favored by a cool rainy spring season. Gray mold can be particularly damaging when rainy occurs over several days. Since 2018 and 2019, botrytis gray mold disease of Lily caused by *B. cinerea* has been found at different locations in Kashmir valley.

In the present study, different germplasm lines are screened against the Botrytis blight under field conditions for the identification of resistant sources.

Materials and Methods

Screening of germplasm against Botrytis blight of *Lilium* under natural epiphytotic conditions

Lilium cultivars set of 21 germplasm lines available in the experimental field of Division of Floriculture, SKUAST-Kashmir, were maintained unsprayed throughout the year 2018 and 2019. Plots of 2m × 2m size were prepared and bulbs of were sown at recommended spacing of 40cm × 15cm.There are about150 plants in each germplasm planted in three rows and were assessed for the occurrence and extent of Botrytis blight disease under natural conditions. The data on disease intensity was recorded after four months of planting which was the peak period of the disease. The plants from each germplasm were earmarked and assessed for the disease incidence and intensity. Following germplasm was screened:

S. No.	Germplasm name
LA's (Longiflorum X Asiatic)	
1.	Litouwen-6
2.	V-4 Litouwen
3.	Yellow diamond
4.	Malesco-5
5.	Pavia
6.	Courier
Asiatic	
7.	Archachon
8.	Indian summer set
9.	Navasin
10.	Nashvillae
11.	Navona
12.	Nello
13.	Blackout
14.	Tresor
Oriental	
15.	Revena-1
16.	Revena-3
17.	A-4 Pink palace
18.	Rialto
19.	Cobra
20.	Tiber
21.	Conca-D

Screening of germplasm under artificially inoculated controlled conditions

Germplasm found resistant to moderately resistant under uninoculated field conditions were further screened under artificially inoculated controlled conditions. *Lilium* bulbs were grown in the pots containing sterilized soil. After 15-20 days bulbs start sprouting. The 30 days old potted plants were then inoculated by spraying conidial suspension of *B. cinerea*. Inoculated plants were covered with clear polyethylene for 72 hours immediately after inoculation to maintain humid conditions and allow infection.

Per cent disease incidence was calculated by using the formulae:

$$\text{Per cent disease incidence} = \frac{\text{Number of infected leaves}}{\text{Total Number of leaves examined}} \times 100$$

The observation on percent disease intensity was recorded to assess the level of resistance and susceptibility of each test germplasm line by using a 0-5 scale of Sehajpal & Singh (2014) [13] with slight modification as under:

Category	Grade	Extent of infection
I.	0	No infection/ disease free
II.	01	1.0-10.0 percent leaf area affected
III.	02	10.1-20.0 percent leaf area affected
I V.	03	20.1-30.0 percent leaf area affected
V.	04	30.1-40.0 percent leaf area affected
VI.	05	>40 percent leaf area affected

Per cent disease intensity (PDI) was calculated as per the following formula:

$$\text{Per cent disease intensity} = \frac{\sum (n \times v)}{N \times G} \times 100$$

Where, $\sum$ = Summation
n = Number of diseased leaves in each category
v = Numerical value of the category
N = Total number of leaves examined, and
G = Highest grade value

The test cultivars were arbitrarily categorized into six different reaction groups on the basis of per cent disease intensity by using a slightly modified 0-9 scale of Prasad and Kumaraswamy, (2017) [11]

Disease grade	Intensity of infection%	Reaction
0	No infection	Immune
1	1-10	Resistant
3	10.1-20	Moderately resistant
5	20.1-30	Moderately susceptible
7	30.1-50	Susceptible
9	>50	Highly susceptible

Results and Discussion

Screening of germplasm under field conditions

The use of varietal resistance is one of the most effective and reliable strategy for management of Botrytis blight disease. Excessive use of chemicals and environment concern compelled the researcher to divert his attention towards environmentally safe practices including use of resistant varieties. Natural resistance within the germplasm exists as source that can be exploited every time when situation arises. Therefore, screening of existing germplasm for their resistance is primary requirement for evolving resistant varieties against the disease. Attempts were made to identify the *Lilium* cultivars showing resistance to *Botrytis cinerea* Pers. Fr. under natural conditions. Twenty one germplasm accessions were screened for confirmation of resistance against Botrytis blight disease under natural epiphytotic conditions in field. All the test cultivars were found susceptible to the disease but to significantly varying extents. The disease intensity at peak period of disease among the germplasm lines ranged between 1.97-50.84 per cent respectively. Maximum disease intensity was recorded in germplasm lines of LA's (Longiflorum x Asiatic) Malesco-5 followed by Pavia and V-4 Litouwen with average disease intensity of 50.84, 41.22 and 36.29 per cent, respectively. The least disease intensity of 7.75% and 1.97% was recorded in germplasm lines of Oriental *viz*., Tiber followed by Cobra (3.14%) and Revena-1 (5.76%) (Table 1). On the basis of per cent disease intensity (PDI), among test cultivars, oriental

lines *viz*., Revena-1, Revena-3, A-4 pink palace, Rialto, Cobra, Tiber and Conca –D were rated as resistant lines (1-10%). These results are in agreement with the findings of Beers *et al*., 2005 [2] who evaluate various *Lilium* cultivars and among the test cultivars, resistance to Botrytis is present in the Oriental lilies. The five asiatic cultivars *viz*., Navasin, Navona, Nello, Blackout and Tresor were rated as moderately resistant (10.1-20%), whereas, Courier, Archachon, Indian summer set and Nashvillae were moderate in disease susceptibility (20.1-30%). The germplasm line Malesco-5 were found to be highly susceptible (>50%). Remaining LA's (Longiflorum x Asiatic) germplasm lines were susceptible in reaction (Table 2). These results are in agreement with the findings of Antra and Belica (2004) [1] who evaluated 75 *Lilium* cultivars under natural conditions and found some cultivars as moderately resistant and some moderately susceptible. The cultivars with moderately resistant or resistant reaction can be used in hybridization program to evolve cultivars possessing desirable traits, besides resistance to Botrytis blight pathogen.

Table 1: Screening of germplasm against Botrytis blight caused by *Botrytis cinerea* Pers. Fr. under natural epiphytotic conditions

S.no.	Germplasm name	Disease intensity (%)		Pooled	Disease reaction
		2018	2019		
1.	Litouwen-6	30.35	33.15	31.75	S
2.	V-4 Litouwen	35.44	37.15	36.29	S
3.	Yellow diamond	29.66	31.34	30.50	S
4.	Malesco-5	49.33	52.35	50.84	HS
5.	Pavia	39.78	42.66	41.22	S
6.	Courier	25.64	28.39	27.01	MS
7.	Archachon	27.36	30.24	28.80	MS
8.	Indian summer set	28.56	31.45	30.00	MS
9.	Navasin	15.75	17.34	16.54	MR
10.	Nashvillae	24.53	26.45	25.49	MS
11.	Navona	13.45	14.59	14.02	MR
12.	Nello	18.75	20.55	19.65	MR
13.	Blackout	12.33	13.55	12.94	MR
14.	Tresor	16.33	18.15	17.24	MR
15.	Revena-1	4.97	6.55	5.76	R
16.	Revena-3	5.83	7.00	6.41	R
17.	A-4 Pink palace	6.50	7.15	6.82	R
18.	Rialto	7.59	8.50	8.04	R
19.	Cobra	2.55	3.73	3.14	R
20.	Tiber	1.44	2.50	1.97	R
21.	Conca-D	7.00	8.50	7.75	R

Table 2: Grouping of various germplasm into different reaction categories for botrytis leaf blight disease caused by *Botrytis cinerea* Pers. Fr. on percent disease intensity by using slightly modified 0-9 scale.

Disease grade	Disease intensity	Germplasm name
Immune	No infection	--
Resistant	1-10	Revena-1, Revena-3, A-4 pink palace, Rialto, Cobra, Tiber, Conca –D
Moderately resistant	10.1-20	Navasin, Navona, Nello, Blackout, Tresor
Moderately susceptible	20.1-30	Courier, Archachon, Indian summer set, Nashvillae
Susceptible	30.1-50	Litouwen-6, V-4 Litouwen, Yellow diamond, Pavia
Highly susceptible	>50	Malesco-5

Screening of germplasm under artificially inoculated controlled conditions

In order to validate the resistance, twelve cultivars found moderately resistant to resistant under uninoculated field conditions were further screened against *B. cinerea* under artificially inoculated controlled conditions (Table 4). Three cultivars (A-4 pink palace, Cobra, Tiber) were found resistant with average disease intensity ranging from 5.33 to 9.63 per cent, five cultivars (Revena-1, Reven-3, Rialto, Conca-D, Blackout) exhibited moderately resistant response with average disease intensity ranging from 10.50 to 16.94 per cent, three cultivars (Navasin, Navona, Tresor) were found moderately susceptible with average disease intensity ranging from 20.66 to 23.44 per cent and remaining one cultivar (Nello) exhibited susceptible response with disease intensity of 30.65 per cent (Table-3). None of the cultivars exhibited highly susceptible response. On the basis of per cet disease intensity (PDI), among tested germplasm three lines (A-4 pink palace, Cobra, Tiber) were categorized as resistant, five lines (Revena-1, Reven-3, Rialto, Conca-D, Blackout) as moderately resistant, three lines (Navasin, Navona, Tresor) moderately susceptible and remaining one (Nello) as susceptible (Table-4). The above observations are supported by Doss *et al*. 1986 [5] who evaluate 20 ornamental lily clones derived from *Lilium longiflorum* Thunb. (Easter Lilies) for resistance to Botrytis blight and through resistance evaluations *Longiflorum* hybrids showed less variability in resistance and were grouped as susceptible lines

Table 3: Screening of *Lilium* germplasm against Botrytis blight (*Botrytis cinerea* Pers. Fr.) under artificialy innoculated controlled conditions

S.No.	Germplasm name	Disease intensity (%)	Disease reaction
1.	Navasin	21.54	MS
2.	Navona	20.66	MS
3.	Nello	30.65	S
4.	Blackout	16.94	MR
5.	Tresor	23.44	MS
6.	Revena-1	14.76	MR
7.	Revena-3	12.41	MR
8.	A-4 Pink palace	9.63	R
9.	Rialto	10.50	MR
10.	Cobra	7.14	R
11.	Tiber	5.33	R
12.	Conca-D	11.75	MR

Table 4: Grouping of *Lilium* cultivars into different reaction categories based on their response to Botrytis leaf blight (*Botrytis cinerea* Pers. Fr.) under artificially inoculated controlled conditions

Disease grade	Disease intensity (%)	Germplasm name
Immune	No infection	-
Resistant	1-10	A-4 pink palace, Cobra, Tiber
Moderately resistant	10.1-20	Revena-1, Reven-3, Rialto, Conca-D, Blackout
Moderately susceptible	20.1-30	Navasin, Navona, Tresor
Susceptible	30.1-50	Nello
Highly susceptible	>50	-

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