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Secreening of common bean (*Phaseolus vulgaris* L.) germplasm for resistance to angular leaf spot disease (*Phaeoisariopsis griseola* (Sacc.) Ferraris) under cold arid conditions of Ladakh

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Abstract
The Himalayan mountain range has significant bearing on the climate of India, as its towering height created a vast rain shadow zone in the north. Among the cold arid parts of India, Ladakh in Jammu & Kashmir is one of the highest (2900m to 5900m asl) and coldest. Leh and Kargil are two districts, which jointly form Ladakh region, the principal cold desert of India. Leh with an area of 45,110 sq km is the largest district in the country located in an altitudinal range from 2900 to 5900 m above mean sea level. Being cold arid region, the temperature ranges between -35°C in winter to +35°C in summer. In general, area has short mild summer to long cold winter. An experiment was conducted to identify promising accessions suited for cultivation under cold arid conditions of Leh, Ladakh. Twenty five accessions of French bean were screened for resistance to angular leaf spot disease during the spring seasons of 2018 in the Experimental field of High Mountain Arid Agriculture Research Institute (HMAARI) Leh, Ladakh, Sheri Kashmir University of Agricultural Sciences and Technology of Kashmir. The study revealed that under natural epiphytotic conditions none of the accessions was resistant in reaction. Seven accessions viz., Acc-252, WB-185, Acc-811, Acc-1643, Acc-4564, WB-22 and Acc-1692 were moderately susceptible. The other eight accessions viz., WB-1247, Local (Ladakh), Acc-966, Acc-1129, Acc-662, Acc-719, WB-216 and SKUAWB-5001 were susceptible in reaction. All other accessions viz., Acc-1492, WB-956, Acc-1690, Anupama, WB-275, Acc-1144, Acc-335, Acc-21529, WB-6 and SKUAWB-5000 exhibited highly susceptible reaction.

Keywords: accessions, angular leaf spot, screening, French bean, common bean

Introduction
Ladakh constitutes the easternmost trans- Himalayan part of Jammu & Kashmir state of India, bordering Pakistan and China constituting of two districts viz. Leh and Kargil. Leh district is situated between 32°N to 36°N latitude and 75°E to 80°E longitude at an altitude ranging from 2900-5900 m amsl. It is separated from the Indian subcontinent by the Great Himalayan Range and edged by the Karakoram Range to the North (Dame and Nusser, 2011) [6]. The region is characterised by extreme temperature variations, low precipitation mostly in the form of snow, high wind velocity, sparse plant density, thin atmosphere with high UV-radiation and fragile ecosystem. The temperature drops down to -35 ºC in winter. Long harsh winters reduce the cropping season to just five to six months in a year. Single-cropping is dominant, as double-cropping is possible only in a limited area falling below an altitude of approximately 3000 m. Agriculture production is entirely based on irrigation. The region remains cut-off for over six months in a year due to heavy snowfall. Availability of locally grown fresh vegetables is restricted to summer months and therefore, there are seasonal differences in dietary intake of food. The availability of fresh vegetable decreases significantly during the winter months, so here is great scope of pulses during harsh winter months. In winter when no fresh vegetables are available in Ladakh, common bean can be used in diet.

Common beans (*Phaseolus vulgaris* L.) is one of the most important leguminous vegetable crop, grown throughout the world for its green pods as well as dry beans (Rajmah) having its origin in South Mexico and Central America. Common beans also called as Rajmash or Rajma (Hindi) or haricot bean or kidney bean or common bean or snap bean or french bean, occupies premier place among grain legumes in number of countries including India. Among grain crops, pulses (Food legumes) rank third after cereals and oilseeds in terms of total world production. Pulses are rich in proteins and represent an important source of dietary protein for humans and animals. The proteins are generally composed of high amount of lysine, while the amount of methionine and cysteine is less. However, consumption of legumes and cereals results in a balanced diet of energy and protein. Common bean is an important legume crop in the daily diet of more than 300 million people of the world's population (Hadi *et al.*, 2006; Meziadi *et al.*, 2016) [9, 19]. In particular, in developing countries, the importance of common bean is beyond limit as a source of cash and full food nutrients (Popelka *et al.*, 2004; Hadi *et al.*, 2006; Akhavan *et al.*, 2013; Meziadi *et al.*, 2016) [27, 9, 2, 19]. Legumes are also an important source of some essential minerals (Grusak, 2002) [8]. The legumes have been observed to reduce blood cholesterol levels (Andersen et. al., 1984) [3]. It is consumed in different forms including the leafy vegetable, pods, green grains and as dry beans (Katungi *et al*., 2009) [12]. It is a source of Vitamin – B, calcium, iron, phosphorus and zinc which are essential for human growth, health and development. According to Wortmann (Rusuku *et al*., 1997) [29], in the developing world, this leguminous crop is produced subsistantly by women farmers' who market approximately 40% of their produce estimated at US $452 million, while the rest of the crop is used for home consumption. It is largely grown in Himachal Pradesh, Jammu and Kashmir, Uttar Pradesh, North Eastern Hills, Darjeeling, South plateau Hills (Nilgiri and Palni hills) Mahabaleshwar, Ratnagiri (Maharastra) and Chickmanglore (Karnataka) having mild climate with humid environmental conditions. Although there has been increase in bean production due to expansion into marginal agricultural lands, productivity has not shown any encouraging improvements. Typical bean yields obtained on farmers fields are only 20% to 30% of the genetic potential of improved varieties (Wortman *et al*.,1998) [33]. One of the reasons for low productivity is lack of effective disease management practices including lack of disease resistant cultivars. The development of cultivars with improved resistance to biotic and abiotic stresses has long been a primary goal for many bean breeding programs (Miklas *et al*., 2006) [20]. Among the diseases Angular leaf spot (ALS) of common bean caused by the imperfect fungus *Phaeoisariopsis griseola* (Sacc.) Ferraris, is one of the most damaging and widely distributed disease. This disease mainly infects leaves and pods, inducing premature leaf dropping and consequently reduction in grain quality (Mahuku *et al*., 2009) [15]. Losses in grain yield caused by ALS can reach 80% (Singh and Schwartz, 2010) [31]. Use of resistant varieties combined with other disease management practices is regarded as the most practical approach to disease control at the farm level. The greatest setback to this strategy is the high pathogenic variability occurring on *Phaeoisariopsis griseola* (Sacc.) that renders bean varieties that are resistant in one location or year susceptible in another and also its incidence and severity has increased in the areas of common bean production (Stenglein *et al*., 2003) [32]. It is considered that the use of resistant cultivars is an efficient, safe and in expensive technique accessible for bean growers (Ferreira *et al*., 2000) [7]. In fact, this strategy is the most effective and sustainable method for controlling bean diseases (Oliveira *et al*., 2008) [23]. An important step towards achieving this goal involves testing of the available germplasm to identify potential doners for resistance. Although currently there are no well adapted parents available for breeding, hence to draw genes from both local and conserved material, this study was undertaken to identify potential sources of resistance to angular leaf spot from the accessions maintained and collected from major bean growing regions of the state. Common bean has a great scope in Ladakh as the road remains closed for almost six months during winters and no fresh vegetable is available during that season.

Materials and Methods

Twenty five common bean accessions (Table 2) were screened against the angular leaf spot disease under natural conditions at High Mountain Arid Agriculture Research Institute Leh, Ladakh Shere Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-K) during kharif 2018. The experiment was laid out in a complete randomized block design with three replications. The plot size was 3.0 × 2.0 m^2 with spacing of 30 × 10 cm. Recommended agronomic practices were followed to raise a good crop. From each representative collection, 10 plants were selected randomly, kept unsprayed throughout the season and were tagged for the assessment of the disease. All the leaves of the ten plants were counted and then grouped as healthy and diseased. The disease incidence and intensity was assessed in the month of September. Per cent disease incidence was worked out as per the following formula given by (James, 1974) [11]:

$$\text{Percent disease incidence} = \frac{\text{No. of diseased leaves}}{\text{Total no. of leaves examined}} \times 100$$

For assessment of disease intensity, Twenty five accessions of common bean were screened at flowering stage of crop. Observation on angular leaf spot was recorded from flowering till maturity of the crop using Centro International de Agricultura Tropical (CIAT) 1-9 scale adapted from (Inglis *et al*., 1988) [10] and (Razvi et. al., 2017) [28] which is given in Table 1.

Table 1: Evaluation scale for screening for angular leaf spot reaction

Scale	% leaflet area with lesions
1	1-10
3	11-25
5	26-50
7	>50
9	Defoliation

Where 1= plants with no symptoms,
3= plants with 5-10 % of the leaf area with lesions,
5 = plants with 20% leaf area infected and sporulation,
7 = plants with upto 60% of the leaf area with lesions and sporulation association with chlorosis and necrosis,
9 = 90 % of the leaf area with lesions frequently associated with early defoliation and plant death.
Plants with scores less than 3 were considered resistant.
Per cent disease intensity (PDI) was calculated as per the following formula given by FAO (Anonymous, 1967) [4]:

$$\text{PDI} = \frac{\Sigma\ (\text{NxV}) \times 100}{\text{N x S}}$$

Where,
Σ=summation;
N= no. of leaves in each category;
V= numerical value of leaves observed;
S= maximum numerical value/grade.

Results and Discussion

The evaluation study of 25 common bean accessions conducted during the year 2018 under natural epiphytotic conditions against angular leaf spot (*Phaeoisariopsis griseola* (SACC.) FERR.) indicated that disease occurred in variable proportion on all the tested cultivars (Tables 2). However, analysis of data showed a differential response among the accessions with regard to incidence as well as intensity.

Disease incidence

The disease incidence among the accessions ranged between 40.2 to 87.0 percent during kharif 2018. Maximum disease incidence (87.0 %) was recorded in the accession Acc-335 which was statistically at par with Acc-1144, Anupama, Acc-1690, SKUAWB-5000, WB-275 and Acc-21529 with average incidence of 86.3, 84.3, 80.7, 78.5, 78.4 and 77.9 respectively. The minimum disease incidence was recorded in accession Acc-1692 which was statistically at par with WB-22 and Acc-1643 with average incidence of 40.2, 40.3 and 42.5 per cent respectively. Rest of the accessions observed have significant differential response to the maximum and minimum disease incidence.

Disease intensity

The disease intensity among the accessions ranged between 19.7 to 66.2 per cent during the year 2018 (Table 2). Maximum disease intensity was recorded in the accession Acc-335 which was statistically at par with Acc-1144, Anupama, Acc-1690, WB-275 and SKUAWB-5000 with average intensity of 65.2, 64.2, 60.3, 58.6 and 57.5 respectively. The least disease intensity was recorded in Acc-1692, which was statistically at par with Acc-1643, with average intensity of 19.7 and 20.2 per cent, respectively. Among 25 common bean accessions screened none of the accessions exhibited resistance reaction to the disease (rating between 0-10 % PDI), seven accessions *viz.*, Acc-252, WB-185, Acc-811, Acc-1643, Acc-4564, WB-22 and Acc-1692 were moderately susceptible (rating between 10.1-25 % PDI). The eight other accessions viz., Acc-1247, Acc-966, Local (Ladakh), Acc-1129, Acc-622, Acc-719, WB-216 and SKUAWB-5001 were susceptible. The other ten accessions viz., Acc-1492, WB-956, Acc-1690, Anupama, WB-275, Acc-1144, Acc-335, Acc-21529, WB-6 and SKUAWB-5000 were highly susceptible to angular leaf spot of common bean. The development of ALS resistance genotypes can be expected to increase profitability by reducing the amount of fungicides used to produce a crop. Plant species have a defense mechanism to avoid and resist pathogens and pests (Parlevlite, 2002) [24]. Identification and utilization of common bean resistant sources to *P. griseola* contributes greatly to management of the disease, since resistant varieties are the most practical and easily adapted strategy. However breeding for resistant varieties has been made difficult by the high pathogenic variability of *P. griseola.* An effective way of breeding for durable resistance to the highly variable pathogen is by use of minor genes. The advantage of the host resistance is that once the technology has been developed, it is packaged in seed which is easy to disseminate and does not require any additional handling by the farmers, other than the normal crop production practices. Several common bean lines with good levels of resistance to different isolates of *P. griseola* were identified (Pastor-Corrales *et al*., 1998; Mahuku *et al*., 2003) [25, 17]. Genotypes AND 277, MAR-2, Mexico 54, BAT 332 and Cornell 49249 were identified as potential sources of resistance having dominant genes that govern plant resistance to certain races of *P. griseola* (Nietsche *et al*., 2000; Sartorato *et al*., 2000; Aggarwal *et al*., 2004; Caixeta *et al*., 2005: Pereira *et al*., 2015) [7, 30, 1, 5, 26]. The common bean genotypes G10474 and G1090 were reported to have a single dominant gene conditioning resistance to two *P. griseola* pathotypes (Mahuku *et al*., 2004 and 2011) [14, 18]. Studies by (Mahuku and Iglesias, 2009) [16], revealed that common bean conditioning resistance to P griseola race 31-0. Resistance to ALS disease has been shown to be inherited quantitatively. (Oblessuc *et al*., 2012) [22] showed the existence of seven QTL's that had variable magnitudes of phenotypic effects under different environments. Kimno *et al*., (2016) [13] reported that only one out of the 34 entries studied showed field resistance to ALS and further revealed that there remains a need to identify further donors of resistance. Similar results were observed by Yayis and Shiferw (2019) [34], they reported that out of 300 common bean accessions evaluated only 14 common bean accessions were resistant to ALS. Hence the moderately susceptible accessions identified during the present investigation (Table 3) can be screened at different stages over locations and years to confirm their reaction to angular leaf spot so that promising accessions/resistant donors can be identified and used in future breeding programs for the development ALS resistant varieties.

Table 2: Field reaction of common bean accessions against angular leaf spot disease incidence (%) Disease intensity (%).

Varieties	Disease incidence	Disease intensity	Category
Acc -1492	74.5	55.3	HS
WB-956	70.9	50.2	HS
Acc -252	45.3	24.1	MS
WB-185	51.4	24.1	MS
WB-1247	60.6	30.9	S
Acc -1690	80.7	60.3	HS
Anupma	84.3	64.2	HS
WB-275	78.4	58.6	HS
Acc -811	45.1	22.3	MS
Acc -966	64.3	44.9	S
Local (Ladakh)	65.1	44.2	S
Acc -1643	42.5	20.2	MS
Acc -4564	44.7	23.7	MS
Acc -1129	70.3	49.2	S
WB-22	40.3	18.4	MS
Acc -1144	86.3	65.2	HS
Acc -335	87.0	66.2	HS
Acc -21529	77.9	56.4	HS
Acc -1692	40.2	19.7	MS
Acc -662	62.7	43.0	S
Acc -719	46.0	26.6	S

WB-216	57.8	40.0	S
WB-6	70.3	51.7	HS
SKUA WB -5000	78.5	57.5	HS
SKUA WB- 5001	70.4	49.4	S
Overall Mean	63.8	42.6	
CD	4.3	3.9	
CV	3.7	4.8	

*Mean of three replications

Table 3: Grouping of common bean accessions for angular leaf spot reaction based on per cent disease intensity

Resistant (R)	**0-10**	
Moderate susceptible (MS)	10.1-25	Acc-252, WB-285, Acc-811, Acc-1643, Acc-4564, WB-22 and Acc-1692
Susceptible (S)	25.1-50	WB-1247, Acc-966, Local (Ladakh), Acc-1129, Acc-662, Acc-719, WB-216, SKUAWB-5001
Highly susceptible (HS)	50.1 and Above	Acc -1492, WB-956, Acc-1690, Anupama, WB-275, Acc-1144, Acc-335, Acc-21529, WB-6, SKUAWB-5000

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