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Evaluation of French bean germplasm from Garhwal Himalayas for resistance to angular leaf spot

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Research Article

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Abstract

Angular leaf spot (ALS) caused by *Pseudocercospora griseola* is a major disease of french bean (*Phaseolus vulgaris* L.) worldwide. A good diversity of French bean is present in the Garhwal Himalayas of Uttarakhand, India, which is unexplored. The purpose of this study was to identify ALS-resistant accessions among local landraces of French bean in this region. One hundred seventy-six local accessions were collected from different villages of Garhwal, Uttarakhand. All the accessions were screened by four SCAR primers SN02 (*Phg*-2), SAA19, SM02, SBA16 (*Phg*-3), one STS primer TGA1.1 (*Phg-1*) and one SSR primer Pv-at006 (*Phg*-5). All the accessions were also screened for ALS resistance under field condition in the years 2019 and 2020. The disease-resistant score was recorded on 1–9 scale. After field screening, 48 accessions (19 resistant, 24 moderately resistant and five susceptible) were selected for *in-vitro* screening under screen house condition. These 46 accessions were artificially inoculated by two different isolates of *P. griseola* P5 and P9, which are the most virulent pathotype characterized by microbiology lab, College of Forestry, Tehri, Uttarakhand. After *in-vitro* screening, seven accessions (GFB-25, GFB-26, GFB-30, GFB-32, GFB-93, GFB-97 and GFB-136) were found resistant to both the isolates P5 and P9. The *P. griseola*-resistant accessions may further be used in future breeding programmes to develop new and more resistant varieties of French bean against ALS.

Introduction

French bean (*Phaseolus vulgaris* L) is a vital source of protein in the human diet and consumed worldwide (Broughton *et al.*, 2003). The French bean is the most widely cultivated species of the genus *Phaseolus* and accounts for approximately 95% of the world's *Phaseolus* bean production (Gonçalves-Vidigal *et al.*, 2013). It is diploid ($2n = 2x = 22$) in nature and predominantly self-pollinated, with a 3–5% average out-crossing rate (Ramalho and Abreu, 2006) although occasionally higher values are also obtained (Ibarra-Perez *et al.*, 1997). The State of Uttarakhand has a huge diversity of French bean which is uninvestigated yet (Prabha *et al.*, 2021). According to baseline data on horticultural crops in Uttarakhand (2018), 5776 hectares area of Uttarakhand is under French bean with a production of 38,112 MT. In Uttarakhand, a higher genetic diversity can be seen but the main drawback of the crop is occurrence of different diseases. Angular leaf spot (ALS) is one of them which is caused by the fungus *Pseudocercospora griseola* (Sacc.). ALS alone can result in 80% losses globally in the production of French bean (Busogoro *et al.*, 2002); losses can be dependent on the environmental conditions, pathogenicity of the isolates, level of susceptibility of the cultivar and the stage of plant growth (Paula and Zambolim, 1998; Tryphone *et al.*, 2015). Use of fungicides is an option to control ALS disease, but in tropical countries French bean is commonly grown by small farmers, who cannot afford the expenses of these chemicals (Nay *et al.*, 2019). Use of fungicides is also harmful to the environment as well as to the human health. The most effective and eco-friendly way to control the disease is the use of resistant cultivars. However, development of French bean cultivars with durable ALS resistance is difficult due to the broad and changing virulence diversity of the ALS pathogen that renders varieties that are resistant in one year or location and susceptible in another (Pastor-Corrales *et al.*, 1998; Mahuku *et al.*, 2002; Nay *et al.*, 2019). Therefore, it is necessary to screen available bean germplasm for resistance to ALS.

Five ALS resistance loci (*Phg-1*, *Phg-2*, *Phg-3*, *Phg4* and *Phg-5*) have been approved by the Bean Improvement Cooperative Genetics Committee (http://arsftfbean.uprm.edu/bic/wpcontent/uploads/2018/04/bean_Genes_List_2017.pdf) (Gonçalves-Vidigal *et al.*, 2011, 2013; Oblessuc *et al.*, 2012, 2013; Keller *et al.*, 2015) although several authors have detected

quantitative control for the disease, and numerous QTLs have already been identified, showing the complex inheritance of ALS resistance (Lopez *et al.*, 2003; Caixeta *et al.*, 2005; Oblessuc *et al.*, 2012; Keller *et al.*, 2015; Perseguini *et al.*, 2016; Bassi *et al.*, 2017; Pereira *et al.*, 2019; Librelon *et al.*, 2020). Three independent and dominant *Phg* loci (*Phg*-1, *Phg-2* and *Phg-3*) and two major QTLs (*Phg-4* and *Phg*-5) are included (Carvalho *et al.*, 1998; Sartorato *et al.*, 1999*a*; Correa *et al.*, 2001).

During co-evolution process between pathogen and host, *P. griseola* can be divided into Andean and Mesoamerican races, and it is observed that Mesoamerican races infect both Mesoamerican and Andean bean genotypes, while Andean races preferentially infect Andean genotypes (Guzman *et al.*, 1995; Pastor-Corrales and Jara, 1995; Crous *et al.*, 2006). Thus, genetic breeding strategies may use this knowledge to pyramid both Andean and Mesoamerican resistance genes to durable ALS resistance. Furthermore, Andean beans can be used as a source of resistance for introgression of genes to Mesoamerican genotypes, as in the case of the carioca variety (Nay *et al.*, 2019). Among five resistant genes, *Phg-1*, *Phg-4* and *Phg-5* loci are from French bean accessions of Andean gene pool, whereas *Phg-2* and *Phg-3* are from beans of Mesoamerican gene pool (Sartorato *et al.*, 1999*a*). All these genes alone or in combination can provide high resistance to different races of *P. griseola* all over the world (Nay *et al.*, 2019). Several authors reported molecular markers linked to ALS resistance genes in their studies (Pastor-Corrales *et al.*, 1998; Mahuku *et al.*, 2002). SCARs (sequence cleaved amplified regions), STS (sequence tagged site) and SSR (Simple sequence repeat) primers show many advantages in studies of germplasm screening, as they are co-dominant, characterize single loci and can perceive high level of polymorphism and reproducibility. Molecular markers reveal the number of resistance loci in the accessions which can help in selection of breeding material for future breeding programmes.

Breeders have developed many bean cultivars, which are resistant to some *P. griseola* races, but due to changing virulence diversity, the genotypes no longer show resistance to different pathogenic races (Almeida *et al.*, 2021). Therefore, new sources of multiple resistances to *P. griseola* need to be identified. Pyramiding several resistance genes in one variety is a breeding tool to develop wide and durable resistance into French bean varieties (Ddamulira *et al.*, 2015). Merging both Andean and Mesoamerican resistance genes into the single accession or variety will possibly result in substantial resistance to many ALS pathotypes (Gil *et al.*, 2019). Therefore, screening of the available French bean germplasm is necessary against different *P. griseola* races. As the Garhwal region of Uttarakhand has abundant diversity of French bean, this study was designed with the aim to screen the French bean germplasm from Garhwal, Uttarakhand to identify potential sources of resistance to ALS.

Materials and methods

Plant material

One hundred seventy-six accessions of French bean (online Supplementary Table S1) collected from five districts of Garhwal, Uttarakhand, India. An ALS-resistant line Cornell 49-242 was obtained from Dr P. N. Sharma, Head, Department of Plant Pathology, CSK Himachal Pradesh Krishi Vishwavidyalaya, Palampur, Himachal Pradesh, India. In addition to its application in breeding, Cornell 49-242 is a popular line used in various countries because of its resistance to several strains of *P. griseola*. Further, each accession was planted at farmer's field at New Tehri, Tehri Garhwal, Uttarakhand for multiplication and to check disease severity in the field condition. These accessions were screened for ALS resistance under field and *in-vitro* condition.

Screening of P. griseola under field condition

First the 176 French bean accessions were screened for ALS under field condition. The experiment was conducted at New Tehri Town in two consecutive years, 2019 and 2020 in the months of May to November. New Tehri is located at coordinates 30.3739′N and longitude is 78.435379′E with an altitude of 1750 m asl. One hundred and seventy-six accessions along with resistant line Cornell 49-242 were planted in the field in randomized block design (RBD) in the years 2019 and 2020 and categorized into three classes viz., resistant (1.0–3.0), moderately resistant (3.1–6.0) and susceptible (6.1–9.0) (Balardin *et al.*, 1997). This screening was done to check the disease severity in natural environment. After screening of all the accessions under field condition, accessions which were found resistant in field were selected for *in-vitro* screening for ALS resistance along with some moderately resistant and susceptible accessions (Gulsum *et al.*, 2021).

Screening of selected accessions under in-vitro condition

From both the field trials, 48 accessions were selected to screen for ALS resistance under polyhouse conditions along with resistant line (Cornell 49-242). The experiment was conducted in completely randomized design (CRD). All the accessions were grown in plastic pots (9 inches). The pots were prepared by mixing soil with sand and decomposed manure (1:1:1). Seeds of each accession were disinfected with 1% NaOCl for 2–3 min and then washed with distilled water for 2 min. Two *P. griseola* isolates P5 and P9 were obtained from the well-characterized repository of Microbiology Lab, Department of Basic Sciences, College of Forestry, Ranichauri, Uttarakhand, India. Both the isolates (P5 and P9) were grown on *Streptopenicillin* amended PDA plates. The culture plates were then incubated at 25 ± 2°C for 7 days. Conidia were scraped from incubated plates in to 10–20 ml of sterilized distilled water, and the final volume was made up to 50 ml with sterile distilled water. Spore suspension was filtered through the sterile muslin cloth, and spore concentration was adjusted to 50×10^5/ml. Three to four drops of Tween-20 (0.01%) were added to it just before spraying. After 21 days, the plants were sprayed with freshly prepared spore suspensions of *P. griseola* isolates P5 and P9. The disease reaction of each accession was assessed after 7 days of inoculation. The plants were categorized on a 1–9 scale as resistant (1.0–3.0), moderately resistant (3.1–6.0) and susceptible (6.1–9.0) (Balardin *et al.*, 1997).

Evaluation of disease symptoms in field and in-vitro

At the onset of the disease, the lesions appear as brown spots with a tan or silvery centre on leaves which were initially confined to the leaf tissue between major veins, giving it an angular appearance. In highly susceptible accessions, many lesions were observed in stem and pods, while approximately 90% of the leaf area was affected by the lesions. In pods, lesions were oval or circular and initially superficial with margins that were almost black and reddish-brown centres, which were sharply defined. The disease

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caused by *P. griseola* was assessed by each inoculated plant using Centro International de Agricultura Tropical (CIAT) 1–9 scale adapted from Balardin *et al.* (1997). This scale was also used to select the accessions under field conditions.

Detection of P. griseola*-resistant loci in 176 accessions along with resistant line*

DNA extraction and PCR amplification of Phg *resistance gene*

The DNA was extracted of all the 176 accessions along with the resistant line (Cornell 49-242) from fresh and young leaves of plants using CTAB (cetyl trimethyl ammonium bromide) method by Devi *et al.* (2013) with few modifications. For molecular screening of 176 French bean accessions, a total of six primers were used, which were present on five loci (*Phg-1*, *Phg-2*, *Phg-3* and *Phg-5*) (Table 1). The PCR (polymerase chain reaction) reaction mixture was prepared in 10 µl volumes, containing 50 ng DNA, 2× PCR buffer, 1 µM primer, 100 µM of each dNTPs and 0.3 U Taq DNA polymerase. The PCR amplifications were done by 35 cycles of initial denaturation (at 95°C for 5 min), denaturation (at 94°C for 30 s), annealing (temperature varied according to primer specifications) for 1 min, synthesis (at 72°C for 1:30 min) and extension (at 72°C for 5 min) (Table 1).

Data analysis

Disease scores of the accessions were subjected to analysis of variance using RBD/CRD to calculate the significance by magnitude of the F value ($P = 0.05$). The K-means algorithm was used to make clusters based on disease reaction under *in-vitro* conditions. The objective of using a non-hierarchical cluster function is to minimize the sum of the squared distances of accessions from their cluster.

$$J = \sum_{n=1}^{N} \sum_{k=1}^{K} r_{nk} \|x_n - m_n\|^2 .$$

Results

In our study, we evaluated *Phg*-resistant loci in French bean accession from Uttarakhand Himalayas by using molecular markers which will contribute in breeding strategies for ALS disease. Therefore, a total of 176 accessions with a control line were screened with five SCAR primers, one STS and one SSR primer (online Supplementary Table S1) linked to *Phg-1*, *Phg-2*, *Phg-3* and *Phg-5* genes, which confer combined or independent resistance to ALS.

All the primers (SAA19, SBA16, SM02, SN02, Pv-at006 and TGA1.1) amplified the specific DNA fragments. The primer SAA19 (associated with gene *Phg-3*) produced a specific amplicon of 650 bp and out of 176 accessions, 89 accessions showed specific bands. SBA16 (*Phg-3*) produced amplicon of 560 bp and 28 accessions showed specific bands (online Supplementary Table S1, Fig. 1). The plant DNA of 96 accessions showed specific binding with the primer SM02 (*Phg-3*) and produced amplicon of 460 bp and the plant DNA of 62 accessions amplified with the primer SN02 (*Phg-2*) and produced amplicon of 890 bp. The STS ($TGA1.1^{570}$) and SSR (pv-at006, 132 bp) primers both produced specific amplicon in five and four accessions, respectively (online Supplementary Table S1, Fig. 1). Primers $TGA1.1^{570}$ and pv-at006 amplified with the DNA of French bean accessions with large seed size (GFB-25, GFB-26, GFB-35, GFB-102, GFB-157 and GFB-163).

All the 176 accessions of French bean were sown in field in the two consecutive years 2019 and 2020 and the disease incidence was recorded on 1–9 scale for both years. Out of the 176 accessions, 19 accessions were found resistant, 96 were moderately resistant and 61 accessions were susceptible under field condition (online Supplementary Table S1). Accessions GFB-93 and GFB-97 were found highly resistant under field condition, their resistant score was 0.5 in both the field trials (2019 and 2020) (online Supplementary Table S1). Disease score of accessions GFB-32, GFB-35 and GFB-58 ranged from 1 to 1.5 in both field trials, showing good resistance under field condition. The accessions which were found resistant (19) in both the field trials were again screened under *in-vitro* screening with some moderately resistant (26) and susceptible accessions (3). Moderately resistant and susceptible accessions were selected on the basis of disease-resistant loci identified by molecular markers and their performance in field trials. The accessions with different combinations of loci were selected for *in-vitro* screening for, e.g. loci

Table 1. Details of primers used in the study for screening of angular leaf spot resistance genes in French bean accessions collected from Garhwal Himalayas

S. No.	Primer	Chromosome No.	Sequence	Annealing temp. (°C)	References
1.	SAA19	Ouro Negro dominant gene	F: TGAGGCGTGTCAATGGATATAA	56	Queiroz *et al.* (2004)
			R: GAGGCGTGTTGATAATTC TGG		
2.	SBA16	Ouro Negro dominant gene	F: TTCCACGTCTATTTTGCATCA	58	Queiroz *et al.* (2004)
			R: CACGCATCACGCAGAACT		
3.	SM02	Ouro Negro dominant gene	F: CAACGCCTCATTAAATTGGA	58	Queiroz *et al.* (2004)
			R: CGCCTCTAAACGGGAGAAAC		
4.	SN02	Phg-2	F: ACCAGGGGCATTATGAACAG	59	Nietsche *et al.* (2000), Miklas *et al.* (2002)
			R: ACCAGGGGCAACATACTATG		
5.	$TGA1.1^{570}$	Phg-1	F: CAGAGGATGCTTCTCACGGT	50	Gonçalves-Vidigal *et al.* (2011)
			R: AAGCCATGGATCCCATTTG		
6.	Pv-at006	Phg-5	F: TTCAACACCAAAGACA	68	Keller *et al.* (2015)
			R:GGTGTTCCTCATTTT		

Yield of primers were ranging from 62.7 nmol (SAA-19) to 29.1 nmol (SH13), which was further diluted to prepare stock solution of 10 µM.

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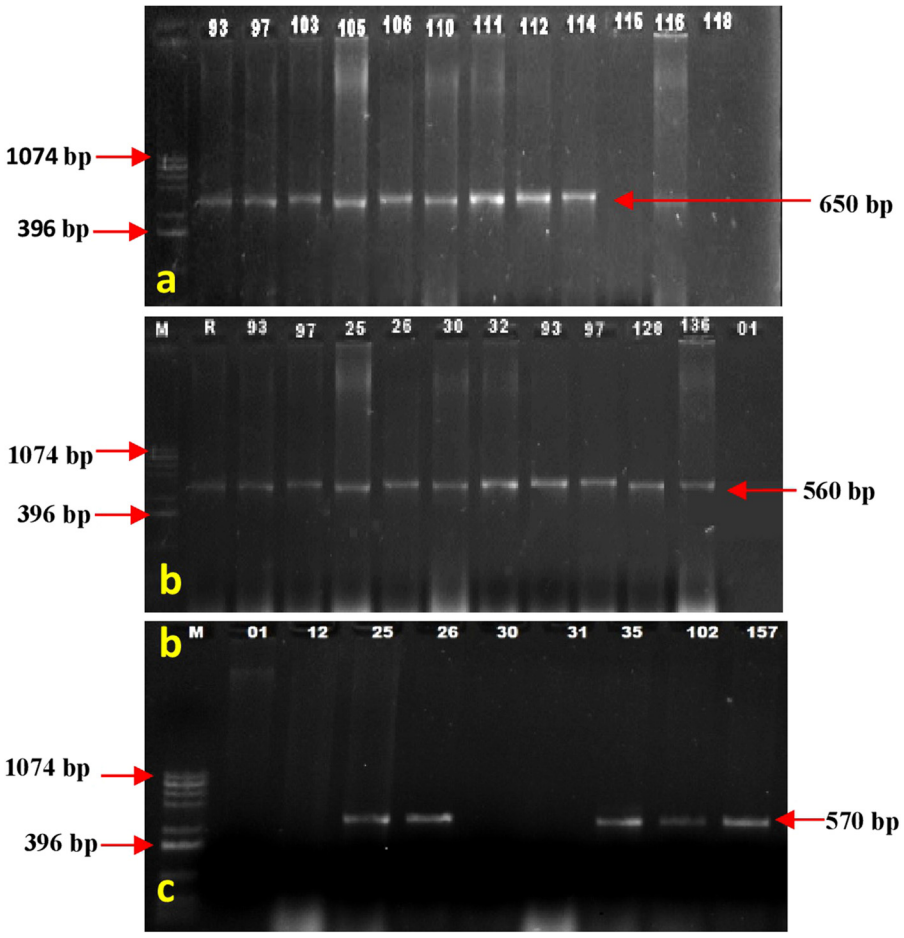

Fig. 1. Amplification of resistance gene in French bean accessions using disease-specific primers. (a) Amplification with primer SAA19 (650 bp). (b) Amplification with primer SBA 16 (890 bp). (c) Amplification with primer TGA1.1 (570 bp).

Phg-3 (GFB-9, GBF-8), *Phg-2* and *Phg-3* (GFB-77, GFB-81) and no loci (GFB-140) (online Supplementary Table S1). Finally, 48 accessions along with Cornell 49-242 were selected for artificial inoculation (Fig. 2).

The pathogenicity test reveals a significant difference between both strains (P5 and P9). The mean disease severity of both the strains was 5.04 and 4.96, respectively. Out of 19 accessions which were found resistant in both the field trials, seven accessions were found resistant (GFB-25, GFB-26, GFB- 30, GFB-32, GFB-93, GFB-97 and GFB-136), one accession (GFB-128) was found resistant to strain P5 and moderately resistant to P9, two accessions (GFB-73 and GFB-74) were found moderately resistant to strain P5 and resistant to P9 while nine accessions (GFB-12, GFB-35, GFB-55, GFB-56, GFB-58, GFB-63, GFB-64, GFB-65 and GFB-102) were found moderately resistant after *in-vitro* screening by both the strains. Accessions GFB-08, GFB-18, GFB-104, GFB-50 and GFB-116 were found moderately resistant to strain P5, but they were susceptible to the strain P9. Similarly, GFB-44, GFB-45, GFB-46, GFB-69, GFB-107 and GFB-112 were found susceptible to strain P5 but to strain P9 they were moderately resistant. Seven accessions (GFB-07, GFB-71, GFB-81, GFB-112, GFB-116, GFB-130 and GFB-140) that were found moderately resistant in field trials were found susceptible after *in-vitro* screening by both the strains (online Supplementary Table S1, Table 2, Fig. 2). The coincidence per cent of disease reaction of accessions under screen house condition is 66.67%, which means 33.33% of accession changed their phenotypes from resistant to moderately resistant or susceptible, while 66.67% of accessions showed no change in disease reaction.

On the basis of screen house trial, the *K* mean cluster was prepared which distributed the accessions into 10 groups ($K = 10$). Groups 8 and 10 included all the resistant accessions while groups 1, 3, 4, 6 and 9 consist of moderately resistant accessions and groups 2, 5 and 7 consist of all susceptible accessions (Table 3).

Discussion

Six primers were used to screen the French bean accessions which identified four different ALS-resistant loci in accessions collected from Garhwal region of Uttarakhand. STS primer TGA1.1 detected locus *Phg-1*, SCAR primer SN02 detected locus *Phg-2*, three SCAR primers SAA19, SM02 and SBA16 detected locus *Phg-3* and SSR primer Pv-at006 detected locus *Phg-5*. According to Sartorato *et al.* (1999*a*), loci *Phg-1* and *Phg-5* are from Andean gene pool while *Phg-2* and *Phg-3* are from Mesoamerican gene pool of French bean. A preliminary indication of diversity of French bean accessions was observed in our study. We recorded that loci (*Phg-1*, *Phg-5*) were detected only in large seeded accessions (GFB-25, GFB-26, GFB-35, GFB-102, GFB-157 and GFB-163) of the Andean gene pool. Loci *Phg-2* and *Phg-3* were present in both Mesoamerican and Andean gene pools. However, more research is needed to describe the diversity of French bean accessions from Garhwal Himalayas.

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Fig. 2. Symptom of ALS disease on different French bean plants and leaves after *in-vitro* screening. (a) Resistant plant. (b) Moderately resistant plant. (c) Susceptible plant (growth of plant was hindered). (d) Healthy leaf, e.g. leaves showing different levels of disease severity.

The accessions GFB-30, GFB-32, GFB-97 and GFB-136 consist of only *Phg-3* loci and were found resistant against both ALS strains (P5 and P9) after artificial inoculation. This indicated that the *Phg-3* gene alone was effective in restoring resistance to ALS in some French bean accessions. Earlier it was reported that *Phg-3* gene present in Ouro Negro was very important for French bean breeding programmes in Brazil which confer resistance to at least seven *P. griseola* races, including highly virulent race 63–63 (Marin *et al.*, 2003, Souza *et al.*, 2011; Gonçalves-Vidigal *et al.*, 2013). On the other hand, 14 accessions which were having *Phg-3* gene were found moderately resistant and susceptible. The French bean accessions with single genes responsible for resistance to ALS will likely succumb to new virulent races of the ALS pathogen in the future because ALS has a virulent diversity. This has been reported several times that bean cultivars harbouring single genes for resistance to the rust and anthracnose pathogens were broken down (Kelly *et al.*, 1994; del Rio *et al.*, 2003; Pastor-Corrales *et al.*, 2010; Prabha *et al.*, 2021). Because of the intrinsic evolutionary changeability of *P. griseola*, gradually new strains of pathogen develop that overcome the resistance in bean varieties (Pedro *et al.*, 2006).

Phg-2 locus was present in many accessions but they did not show resistance in field as well as in screen house trials. This gene alone was not very effective for resistance against ALS in accessions from Garhwal, Uttarakhand. Our study was not in accordance with Sartorato *et al.* (1999*b*), who reported that, the *Phg-2* locus was effective in restoring resistance in Mesoamerican cultivar Mexico 54. Accessions GFB-25 and GFB-26 confirm the presence of *Phg-1*, *Phg-2*, *Phg-3* and *Phg-5* genes, which were effective for resistance against ALS in field as well as under *in-vitro* conditions. Caixeta *et al.* (2002) and Mahuku *et al.* (2004) also reported that the ALS-resistant genotype AND 227 has four ALS resistance genes (*Phg-1a*, *Phg-2*^{2}, *Phg-3*^{2} and *Phg-4*^{2}), Mexico 54 has three (*Phg-2*, *Phg-5* and *Phg-6*) and MAR-2 has two (*Phg-4*, *Phg-5*). The presence of a greater number of genes provides broad resistance in accessions.

Some accessions were moderately resistant to strain P5 but susceptible to strain P9 or *vice-versa*. Similar results were also reported by Sanglard *et al.* (2013), where they studied the resistant locus of Ouro Negro in relation to five other ALS-resistant sources ('AND 277', 'BAT 332', 'Cornell 49-242', 'MAR-2' and 'Mexico 54'). They reported that Cornell 49-242 and AND 277 were resistant to 62.23 race of ALS, while susceptible to 63.39 race.

Some accessions which were found resistant in the field showed less resistance or susceptibility for ALS under screen house condition. GFB-55, GFB-58, GFB-63, GFB-64, GFB-65, GFB-74 and GFB-102 were resistant under field conditions while they were moderately resistant in polyhouse condition by both the strains. Accession GFB-45 was resistant in field condition but found moderately resistant towards strain P9 and susceptible to strain P5. Oblessuc *et al.* (2012) recorded the mapping of seven ALS-resistant QTLs that had variable magnitudes of

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Table 2. Disease score of resistant and moderately resistant accessions under *in-vitro* conditions

S. No.	Accession No.	Resistance level under *in-vitro* condition with strain P5	Resistance level under *in-vitro* condition with strain P9	S. No.	Accession No.	Resistance level under *in-vitro* condition with strain P5	Resistance level under *in-vitro* condition with strain P9
1.	6	5.65 MR	5.4 MR	27.	71	5.88 MR	5.65 MR
2.	7	7.25 S	6.65 S	28.	73	4.40 MR	3.67 R
3.	8	5.40 MR	6.27 S	29.	74	4.38 MR	3.62 R
4.	12	4.63 MR	3.35 MR	30.	77	5.40 MR	4.5 MR
5.	15	7.53 S	7.4 S	31.	81	5.50 MR	6 MR
6.	18	5.63 MR	6.62S	32.	93	0.63 R	0.62 R
7.	20	8.63 S	7.5 S	33.	97	0.78 R	1.37 R
8.	25	2.50 R	2.87 R	34.	101	5.28 MR	4.4 MR
9.	26	2.88 R	2.65 R	35.	102	5.53 MR	4.37 MR
10.	30	2.40 R	3 R	36.	103	9.00 S	8.87 S
11.	32	2.38 R	2.87 R	37.	104	4.40 MR	6.4 S
12.	35	4.53 MR	3.37 MR	38.	105	4.40 MR	4.87 MR
13.	44	6.40 S	5.87 MR	39.	106	4.50 MR	6.5 S
14.	45	6.13 S	5.65 MR	40.	107	6.25 S	5.65 MR
15.	46	6.53 S	5.75 MR	41.	108	6.00 MR	5.25 MR
16.	47	5.13 MR	5.4 MR	42.	109	8.88 S	9 S
17.	50	5.40 MR	6.4 S	43.	112	5.63MR	5.4 MR
18.	53	5.50 MR	5.87 MR	44.	116	5.50 MR	7.4 S
19.	54	5.40 MR	4.65 MR	45.	128	2.65 R	4.40 MR
20.	55	4.13 MR	3.87 MR	46.	130	8.88 S	8.87 S
21.	56	4.25 MR	4.37 MR	47.	136	2.88 R	2.4 R
22.	58	4.40 MR	3.4 MR	48.	140	9.00 S	8.4 S
23.	63	3.88 MR	3.5 MR	49.	Control	0.55 R	0.4 R
24.	64	4.38 MR	3.62 MR	**C.D.**	**0.480**	**0.469**	
25.	65	3.88 MR	3.37 MR	**C.V.**	**4.720**	**4.681**	
26.	69	5.88 MR	5.5 MR				

S, susceptible; MR, moderately resistant; R, resistant
Bold values are significant an $p > 0.05$.

Table 3. *K*-mean cluster analysis of French bean accessions for disease score produced by both strains (P5 and P9) under *in-vitro* conditions

Cluster No.	No. of accessions	Within SS
1	GFB-54, GFB-56, GFB-77, GFB- 101, GFB-102, GFB-105	0.158
2	GFB-07, GFB-15	0.13
3	GFB-06,GFB-47, GFB-53, GFB-104, GFB-106	0.102
4	GFB-12, GFB-35, GFB-55, GFB-58, GFB-63, GFB-64, GFB-65, GFB-73, GFB-74	0.154
5	GFB-20	0
6	GFB-08, GFB-18, GFB-44, GFB-45, GFB-46,GFB-50, GFB-69, GFB-107, GFB-112, GFB-116	0.297
7	GFB-71, GFB-81, GFB-103, GFB-108, GFB-109, GFB-130, GFB-140	0.04
8	GFB-93, GFB-97, Cornell 49-242	0.345
9	GFB-128	0
10	GFB-25, GFB-26, GFB-30, GFB-32, GFB-136	0.011

SS, sum of square, 49 accessions were screened under *in-vitro* conditions.

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phenotypic effects under different environments (wet season, dry season and greenhouse condition). They observed that there is high correlation in ALS disease severity in the greenhouse condition then dry and wet season. This accuracy of disease development was due to the maintenance of accurate amount of inoculum, proper humidity and temperature for the progress of disease under screen house condition. Our findings indicated the close association with Gulsum *et al.* (2021) who found the resistant source and reaction of French bean to anthracnose by two isolates (k9 and T2) in 40 French beans by molecular markers and by producing pathogen inoculum artificially in M3 medium. They recorded that out of 40 cultivars, three cultivars were resistant to k9 strain, but susceptible to T2 strain and *vice versa*. This *in-vitro* screening method is very advantageous because the screen house provides all the favourable conditions for the disease to grow out.

This information will broaden the advantage of marker-assisted breeding, identification of new resistant sources and gene information will help breeders to choose the useful gene in breeding for ALS resistance. This gene information can be used in breeding programmes to target the introgression of chromosomal segment especially associated with resistance genes, rather than emphasizing on only introgression of single disease resistance genes. ALS is one of the most devastating diseases of French bean which badly affects its production (up to 80%). Because of high virulence diversity of *P. griseola* pathotypes, there is high possibility of overcoming resistance; therefore, it is important to combine various effective genes (gene pyramiding) for durable resistance.

Conclusion

In this study, seven French bean accessions (GFB-25, GFB-26, GFB-30, GFB-32, GFB-93, GFB-97 and GFB-136) were found resistant against ALS. Accessions GFB-25 and GFB-26 have four (*Phg-1*, *Phg2*, *Phg3* and *Phg-5*), GFB-30 and GFB-32 have two (*Phg-2* and *Phg-3*) and GFB-93, GFB-97 and GFB-136 have one (*Phg-3*) resistant gene to restore resistance against ALS. GFB 25 and 26 are of Andean origin while the rest five are of Mesoamerican origin. Combining the genes of both origins, different varieties with durable resistance to ALS can be developed. Information generated by this study is helpful in acquiring knowledge about the resistance level of accessions against ALS from Garhwal region. The identification of agronomically superior and ALS-resistant accessions will be useful in the relocation of disease resistance genes in previously available high-yielding but susceptible varieties.

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