

# Multiple genes confer anthracnose resistance in French bean accessions of Garhwal Himalayas

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## Research Article

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## Abstract

The Indian Himalayan region is very rich in the genetic variability of French bean and therefore considered as the secondary centre of origin of French bean. Though, a good diversity of French bean is present in Uttarakhand hills of Western Himalaya but it is almost unexplored yet. Unfortunately, French bean is attacked by some of the phytopathogens those cause heavy crop losses. *Colletotrichum lindemuthianu* (Sacc. & Magnus) Scrib is one of the sever pathogens that causes anthracnose disease in French bean worldwide. Identification or development of a resistant variety/cultivar is an environmentally safe approach. Diversity analysis of anthracnose resistant loci in common bean is important to identify new sources of resistance gene(s). So the study was designed with the objectives to collect and screen the local accessions of French bean from Garhwal hills for anthracnose resistance. A total of 100 accessions were collected from 6 different districts of Garhwal region of Uttarakhand and all were screened for anthracnose resistance. For this, 13 SCAR (Sequence cleaved amplified region) primers specific to anthracnose resistance were used in this study.

Results revealed a high level of genetic diversity within the population for anthracnose resistance loci. Gene diversity ranged from 0.354 to 0.499 with a mean of 0.457. Pair wise genetic distance ranged from 0 to 2.236. The accessions were also screened for anthracnose resistance under field and polyhouse conditions. There was a moderate correlation ( $R = 0.56$ ) between field trial and trial under controlled condition. Thirteen of these accessions possessing two genes (*Co-10* and *Co-42*) showed complete resistance for anthracnose disease under field and polyhouse conditions. The anthracnose resistant accessions may further be used in future breeding programmes to develop new and more resistant varieties of French bean against anthracnose disease.

## Introduction

Anthracnose diseases in French bean (*Phaseolus vulgaris* L.) caused by *Colletotrichum lindemuthianum* (Sacc. & Magnus) Scrib is considered as one of the most threatening disease. The disease causes massive loss of crop yield worldwide, preferably in the regions with prevailing high humidity and moderate temperatures (13 to 27°C) (Vieira and Paula Junior 2004; Mendez-Vigo et al. 2005). In Uttarakhand the cultivation of French bean is mainly done by small to medium scale farmers and the enterprise creates on farm employment opportunities for the rural community. However, the disease alone accounts for around 50 % losses in yield and also reduces quality of the produce (Sharma and Sugha 1995). Fungus requires cool and humid conditions to grow and the environmental conditions of Uttarakhand hills favor the development of this disease. Because of its seed borne nature and pathogenic variability disease can cause rigorous yield loss. The pathogen's reproduction mechanism is responsible for continuous resistance development, which is mainly observed in commercial cultivars, since most of them have vertical resistance that is easily overcome by emerging races of this fungus (Rodriguez-Guerra et al. 2003). However, among the various strategies adopted to control anthracnose disease, use of disease resistant varieties is considered as the most efficient and economical method (Mahuku et al. 2002; Pastor-Corrales et al. 1995; Schwartz et al. 1982).

Currently, twenty-one anthracnose resistance loci have been characterized and are denoted by the symbol *Co* followed by a numerical designation (Kelly and Vallejo 2004). In addition to these, four allelic series have also been identified to contribute is resistance in French bean against *C. lindemuthianum*. However, different varieties/ accessions of French bean possess variable resistance loci to confer disease resistance (Ferreira et al. 2013). Though, a large number of literature on research on anthracnose resistance in French bean is available, but, still bean breeders are putting great efforts to assess the specific gene(s), which should be deployed in resistance breeding programmes. In addition to identification of resistance by conventional methods, the use of molecular markers has contributed significantly to characterize resistant genes for anthracnose disease. Use of molecular markers for screening of germplasm reduces

the time and costs involved in the whole process. Since DNA markers are closely linked to genes and are not influenced by environmental factors, they show epistatic or minimum/none pleiotropic effects (Agrawal et al. 2008).

Among the available molecular markers, the SCAR (Sequence characterized amplified regions) markers have been playing great importance on common bean analyses for anthracnose resistance. These markers have been optimized in breeding programs dedicated to search anthracnose resistant cultivars by implanting assisted backcrosses (Miklas and Kelly 2002), during characterization of accessions in the beginning of the selection process or to obtain superior lineages (Beraldo et al. 2009). Until now, there are 14 SCAR markers have been linked to anthracnose resistance genes.

Wide but unexplored genetic diversity of French bean is found in the Uttarakhand hills that has noticeable variation particularly in regards to the edible parts and growing habits. Unfortunately, much effort has not been made to characterize and explore this diverse gene pool of French bean. There is a need for more inclusive analysis of genetic diversity and resistance for different diseases of germplasm available in this region. Therefore, the present work was aimed to identify anthracnose resistant accessions of French bean through the evaluation of germplasm collected from Garhwal Himalayas. Efforts are also done to identify the presence of resistance loci and their contribution in asserting the resistance against anthracnose disease using SCAR markers.

## **Material And Methods**

### **Seed Material**

One hundred accessions of French bean were collected from the different locations of six districts of Uttarakhand, India. However, a mixed germplasm of French bean was obtained from the farmers of most of the locations. The germplasm was therefore physically purified on the basis of seed colour, size, shape and texture etc. Accessions were then multiplied at the research block of Dept. of Seed Science and Technology, HNB Garhwal University to assess the presence of anthracnose resistance gene(s) by molecular and morphological markers. Two anthracnose resistant lines D line (Cornell-49242) and L line (G-2333) were procured from Dr. P. N. Sharma, Head, Dept of Plant Pathology, CSK Himachal Pradesh Krishi Vishwavidyalaya, Palampur, Himachal Pradesh and used as control in this study.

### **Molecular Screening**

Thirteen SCAR primers specific to anthracnose resistance and covering all the chromosomes of French bean were used for screening of germplasm (Table 1). Genomic DNA was isolated by cetyl trimethyl-ammonium bromide (CTAB) method as described by Doyle and Doyle (1990). Reaction mixture (10 $\mu$ l) for the amplification was prepared containing 50ng DNA, 2X PCR Buffer, 1 $\mu$ M primer, 100  $\mu$ M of each dNTP, 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 sec.), annealing (Temp. varied according to primer specifications) for 1 min., synthesis (at 72°C for 1:30 min.) and extension (at 72°C for 5min.).

Table 1

Details of SCAR primers used in present study for screening of anthracnose resistance in French bean accessions collected from Garhwal Himalayas.

S. No.	Primer	Chromosome No.	Sequence	Annealing Temp. (°C)	References
1.	SE <sub>ACT</sub> /M <sub>CCA</sub>	Co-1 <sup>2</sup>	F: ATTCACTTATAAAAAATAAAAT R :AACCATAACTGTTATCAGACC	52	Melloto et al. (2000)
2.	SH20/SCAreoli	Co-2	F: GAGACATCCATCAGACAACTCC R: GGGAGACATCTTCATTTGATATGC	65	Adam-Blodon et al. (1994)
3.	SF10	Co-10	F: GGAAGCTTGGTGAGCAAGGA R: GGAAGCTTGGCTATGATGGT	65	Alzate - Marin et al. (2003)
4.	SY20	Co-4	F: AGCCGTGGAAGGTTGTCAT R: CAGAGACCCTAGGCTTATCG	60	Kelly et al. (2003)
5.	SAS13	Co-4 <sup>2</sup>	F: CACGGACCGAATAAGCCACCAACA R: CACGGACCGAGGATACAGTGAAAG	72	Young et al. (1998)
6.	SH18	Co-4 <sup>2</sup>	F: CCAGAAGGAGCTGATAGTAGTCCACAAC R:GGTAGGCACACTGATGAATCTCATGTTGGG	60	Awale and Kelly (2001)
7.	SAB03	Co-5	F: TGGCGCACACATAAGTTCTCACGG R: TGGCGCACACCATCAAAAAGGTT	54	Queiroz et al. (2004)
8.	SAZ20	Co-6	F: ACCCCTCATGCAGGTTTTTA R: CATAATCCATTCATGCTCACC	60	Kelly et al. (2003)
9.	SC08	Co-4	F: AGAATGCCTTTAGCTGTTGG R:CAGAGAGGCTAGGCTTATCG	65	Queiroz et al. (2004)
10.	SBB14	Co-4 <sup>2</sup>	F: GTGGGACCTGTTCAAGAATAATAC R: GTGGGACCTGGGTAGGTAGAAAT	67	Awale and Kelly (2001)
11.	SZ04	Co-6	F: GGCTGTGCTGATTAATTCTGG R: TGCTCATTTTATAATGGAGAAAAA	45	Queiroz et al. (2004)

The primers received from the manufacturer were diluted to prepare working stock solution of 10µM concentration. However, for amplification of the DNA, 1µM of the specific primer(s) was used.

S. No.	Primer	Chromosome No.	Sequence	Annealing Temp. (°C)	References
12.	SW12	<i>Co-3/Co-9</i>	F: TGGGCAGAAGTTCTAGCATGTGGC R: TGGGCAGAAGCACAGTATGATTTG	70	Kelly and Vallejo (2004)
13.	SB12	<i>Co-9</i>	F: CCTTGACGCACCTCCATG R: TTGACGATGGGTTGGCC	68	Mendez - Vigo et al. (2002)

The primers received from the manufacturer were diluted to prepare working stock solution of 10µM concentration. However, for amplification of the DNA, 1µM of the specific primer(s) was used.

## Screening under field condition

Two consecutive field trials were conducted during April to September in 2018 and 2019 at farmer's field at Ranichauri to test the incidence of anthracnose disease on the accessions. The experimental sites are located at an elevation of about 1580–1630 m above mean sea level. Geographic position of experimental sites is between latitude 30° 15' N and longitude 78° 54' E under mid hill zone of Uttarakhand. Details of agrometeorological as observed at Agrometeorological observatory at Ranichauri are given in Supplemental Table 3. The accessions found moderately resistant and resistant under field conditions were further screened under polyhouse by artificial inoculation.

## Pathogenic inoculum

A pre-identified virulent strain *C. lindemuthianum* DY-27 was obtained from well characterized repository of Microbiology Lab, Dept. of Basic Sciences, College of Forestry, Ranichauri and used to study the resistance potential of French bean accessions. The fungal strain was grown on potato dextrose agar (PDA) plates amended with streptopenicillin (Sarabhai Zydus Pvt. Ltd., India). The 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 final volume was made up to 50 ml with sterile distilled water. Spore suspension was filtered through sterile muslin cloth, and spore concentration was adjusted to 5×10<sup>5</sup> ml<sup>-1</sup>. Three to four drops of Tween-20 (0.01 %) were added to it just before spraying.

## Pathogen inoculation

Twenty-one-day old plants were used for the infection assay. Three seedlings per pot for each accession were maintained. The experiment was conducted in triplicate under polyhouse conditions. Seedlings were then sprayed uniformly with spore suspension of *C. lindemuthianum* DY-27. Humidity in the polyhouse was maintained by regular sprays of water with overhead sprinklers. Disease reactions were scored visually after 7 days of inoculation on a scale of 1–9 (Pastor-Corrales et al., 1995). Anthracnose disease reactions from 1.0 to 3.0 were considered as resistant, 3.1 to 6.0 as moderately resistant and 6.1 to 9.0 as susceptible.

## Data analysis

Dendrogram and Heat map based on euclidean distance was prepared by using Heatmapper software (Babicki et al. 2017). A dendrogram was prepared using weighted pair-group method, which divides each cluster in a hierarchical manner. For population structure analysis, the STRUCTURE v2.3.4 programme (Pritchard et al. 2000) was used. The programme was run with a burn-in period of 10,000 and 10,000 MCMC interactions, in a continuous series of groupings (K) ranging from 1 to 10 in 10 repetitions. The best K (DELTA K) was estimated by the method proposed by Evanno et al. (2005) through the STRUCUTURE HARVESTER program (Earl and Vonholdt 2012).

Genetic correlations between both the disease environments (field and controlled conditions) were estimated from predicted disease reaction values in both the conditions, using the Pearson correlation coefficients (Steel et al. 1997).

$$r = \frac{\sum_i (x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum_i (x_i - \bar{x})^2} \sqrt{\sum_i (y_i - \bar{y})^2}}$$

The K-means algorithm was used to make clusters on the basis of disease reaction under field and *in-vitro* conditions. The objective of using non-hierarchical cluster function is to minimize the sum of the squared distances of accessions from their cluster.

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

## Results

### Screening of French bean germplasm with SCAR primers

Out of thirteen, 9 SCAR primers [SF10 (*Co-10*), SAS13 (*Co-4<sup>2</sup>*), SH18 (*Co-4<sup>2</sup>*), SAZ 20 (*Co-6*), SH20 (*Co-2*), SC 08 (*Co-4*), SZ 04 (*Co-6*), SW 12 (*Co-3/Co-9*) and SBB-14 (*Co-4<sup>2</sup>*)] could amplify the specific DNA fragments. Among these, SH-20 showed nonspecific binding with plant DNA and could not produce the specific amplicon. As per the earlier reports, the amplicon size should be either 1000 or 1300 bp, however, in this study SH-20 amplified a fragment of 550 bp only. Therefore, we did not consider this primer to portray the results. For rest of the primers, the amplified bands ranged from 360 bp to 1076 bp (Fig. 1a,b,c). Maximum number of accessions (65) were amplified with the primer SZ04, followed by SF10 (49 accessions) and SAZ 20 (38 accessions).

Diversity indices showing that an average fraction of polymorphism was 0.45% per resistance loci with maximum 0.66% alleles in *Co-6* and minimum 0.23% in *Co-4<sup>2</sup>* locus. The gene diversity values ranged from 0.354 (*Co-4<sup>2</sup>*) to 0.499 (*Co-10*) with an average of 0.457. The mean Polymorphic Information Content (PIC) value was found to be 0.452 with minimum value of 0.349 (*Co-4<sup>2</sup>*) and maximum of 0.497 (*Co-10*). Moreover, average Fixation index per loci was 0.138 with minimum value of 0.100 to maximum value of 0.172 (Table 2). The *Co-6* gene was present in 62 accessions followed by *Co-10* (52) and *Co-3/Co-9* (37) (Table 2). Results also revealed the presence of *Co-10* gene in all resistant accessions, followed by *Co-4<sup>2</sup>* (92.31%) accessions and *Co-6* (84.62%) (Fig. 1d).

Table 2

Diversity indices of anthracnose resistance loci amplified by SCAR primers in different French bean accessions.

S. No.	Loci	No. of accessions amplified	Fp	Fa	GD	PIC	Fst	Ho
1	<i>Co-10</i>	35	0.52	0.48	0.499	0.497	0.733	0.172
2	<i>Co-4<sup>2</sup></i>	25	0.23	0.77	0.354	0.349	0.775	0.100
3	<i>Co-6</i>	62	0.66	0.34	0.448	0.446	0.679	0.192
4	<i>Co-3/Co9</i>	37	0.44	0.56	0.492	0.489	0.654	0.112
5	<i>Co-4</i>	52	0.40	0.60	0.480	0.479	0.726	0.114
<b>Mean</b>			0.45	0.55	0.457	0.452	0.713	0.138
<b>Fp:</b> Fraction of polymorphism, <b>Fa:</b> Frequency of alleles, <b>GD:</b> Gene diversity, <b>PIC:</b> Polymorphic information content, <b>Fst:</b> Statics per locus, <b>Ho:</b> Heterozygosity observed.								

The UPGMA analysis and heat map based on euclidean genetic distance matrix revealed considerable genetic variability of the set evaluated by the formation of five main groups at genetic distance of 1.4 (Fig. 2). Two major groups were formed at genetic distance 1.2. Accessions resistant to anthracnose were distributed throughout the groups. On Heat map analysis, monotonous colour depicted no average dissimilarity. Ten of the accessions (GFB-11, GFB-9, GFB-26, GFB-36, GFB-42, GFB-54, GFB-57, GFB-69, GFB-72 and GFB-85) grouped in monotonous cluster (blue colour). These accessions were not amplified with any of the primers and genetic distance for these accessions was recorded 0 for anthracnose resistance loci. The accessions GFB-50, GFB-5, GFB-81, GFB-93 and GFB-98 showed the presence of all amplified resistance loci and clustered in another monotonous blue matrix. The maximum genetic distance was 2.236 between the accessions (Fig. 2).

Assessment of genetic structure by Bayesian analysis (STRUCTURE) indicated division of the accessions into four groups (Fig. 3a). The groupings from STRUCTURE analysis with K = 4 is represented by Fig. 4b. The first group (red cluster) was composed of mostly elite accessions which were resistant for anthracnose disease. Second group (Blue cluster) consisted mainly of moderately resistant accessions, while the third and fourth groups (Blue and yellow cluster) were consisted of susceptible accessions.

## Screening for anthracnose resistance under field and controlled condition

The accessions were evaluated for disease resistance or susceptibility based on the amount of infection occurred (on a scale of 1–9). On the basis of disease scoring under field conditions 48 accessions were found susceptible, 21 moderately resistant and 31 resistant for anthracnose disease (Supplemental Table 1). The moderately resistant (21) and resistant accessions (31) were further screened under polyhouse conditions through artificial inoculation. Under polyhouse condition out of 52 accessions (resistant and moderately resistant), 13 accessions were found resistant, 32 were moderately resistant and 7 were susceptible for anthracnose disease (Supplemental Table 2). A significant correlation (R value 0.97) was observed between the disease reactions of all accessions under field trials in both the years (Table 3). For experiments under field conditions, coincidence percentage of accessions, that is accessions that showed no change in phenotype (resistant, moderately resistant or susceptible) was 82 %. However, 18 % of accessions showed change in their phenotype (resistant to moderately resistant or susceptible) when screened under polyhouse conditions by artificial inoculation.

Table 3

Estimation of Pearson correlation coefficient of common bean lines for anthracnose disease reaction for two different field trials and polyhouse (*in vitro*) trial.

	<b>Field Trial 1 (X value)</b>	<b>Field Trial 2 (Y value)</b>	<b>X and Y combined</b>
	742.87	764.50	737.97
<b>R value</b>	0.97		
<b>R<sup>2</sup> value</b>	0.96		
	<b>Average of Field Trial 1 and 2 (X value)</b>	<b>Polyhouse trial* (Y value)</b>	<b>X and Y combined</b>
	151.59	209.67	99.06
<b>R value</b>	0.56		
<b>R<sup>2</sup> value</b>	0.31		
*The accessions which were found resistant and moderately resistant (52 accessions) under field trial were selected for artificial inoculation. (Ai- Artificial inoculation).			
Results are significant at $p < .01$			

Screening of accessions under natural environment and under polyhouse conditions was moderately correlated, as reflected with 0.56 R value (Table 3). Accessions (GFB-10, GFB-19, GFB-27, GFB-45, GFB-48, GFB-49 and GFB-58) showed resistance towards anthracnose under field conditions but on artificial inoculation they showed vulnerability towards anthracnose disease (Supplemental Table 2). Thirteen accessions (GFB-1, GFB-3, GFB-5, GFB-30, GFB-39, GFB-50, GFB-74, GFB-75, GFB-76, GFB-81, GFB-83, GFB-93 and GFB-98) were found resistant for anthracnose (Table S1, S2). Details of accessions GFB-3 and GFB-30 have already been published (Prabha et al., 2020). Accessions GFB-1, GFB-3, GFB-30 and GFB-39 showed better resistance even than the reference lines (D and L). The K-mean cluster analysis that was done on the basis of disease reaction under field trial and polyhouse conditions, resulted in the formation of 9 clusters. Cluster 1, 3 and 4 were consisted of resistant accessions, while the clusters 5, 7, 8 and 9 were formed with the accessions those showed changes in their phenotype from resistant to moderately resistant or susceptible (Table 4).

Table 4

K-mean cluster analysis of French bean accessions for disease score of resistant and moderately resistant accessions under field trials and under *in vitro* condition.

Cluster No	Accessions / cluster	Within SS
1	GFB-1, GFB-39, GFB-81	0.583
2	GFB-3, GFB-30	0.031
3	GFB-37, GFB-46, GFB-59, GFB-64, GFB-65, GFB-67, GFB-84, GFB-86, GFB-87, GFB-95, GFB-96, GFB-99	4.802
4	GFB-5, GFB-50, GFB-74, GFB-75, GFB-76, GFB-83, GFB-93, GFB-98	2.992
5	GFB-27, GFB-45, GFB-49	0.750
6	GFB-4, GFB-13, GFB-23, GFB-24, GFB-34, GFB-35, GFB-53, GFB-66, GFB-82, GFB-89	6.537
7	GFB-10, GFB-19, GFB-40, GFB-41, GFB-48, GFB-51, GFB-58	3.571
8	GFB-21, GFB-60, GFB-78, GFB-91	1.609
9	GFB-22, GFB-28, GFB-31	2.083
SS- Sum of square, 52 accessions were screened under <i>in-vitro</i> conditions.		

## Discussion

Eight of the selected primers showed polymorphism except primer SH20. However, these seven primers detected five different loci in our accessions. Number of amplified primers and the number of resistant loci were different because three primers SAS13, SH18 and SBB14 code for the same gene *Co-4*<sup>2</sup> (Young et al. 1998; Melotto and Kelly 2001) and SAZ20 and SZ04 code for gene *Co-6* (Queiroz et al. 2004; Kelly et al. 2003). Locus *Co-1* and *Co-5* were not detected in the accessions collected from Garhwal hills of Uttarakhand Himalaya. *Co-1* locus has been found in Andean gene pool of French bean while other loci (*Co-4*, *Co-5*, *Co-7* and *Co-9*) were found in gene pool of Mexican origin (Kelly and Vellejo 2004). Our study thus gives preliminary indication towards the origin of Garhwal Himalayan French bean as Mesoamerican. Since no work has been done so far to investigate the origin of the French bean germplasm in this region, more research efforts are needed to describe the origin of French bean germplasm available in Garhwal region.

In the present study, SCAR markers were successfully used to assess the genetic diversity of anthracnose resistant loci among 100 common bean genotypes collected from Garhwal hills. These SCAR primers were specifically selected to cover all the linkage groups. Our results were close to some earlier studies carried out on common beans by other researchers. Mean PIC, GD and frequency of allele were 0.452, 0.457 and 0.55 respectively (Table 2). The findings suggest that the bean loci identified by SCAR markers have moderate level of genetic diversity. The moderate level of heterozygosity observed in these accessions might be due to predominantly autogamous habit of common bean. There are several hypotheses which explain such intra-accession variation, such as, the mixed-mating reproductive system of bean, with up to 10% outcrossing (Ibarra-Perez et al. 1997), the small sample set tested and the self-pollinating system (Angioi et al. 2010) Perseguini et al. (2011) evaluated carioca bean cultivars by SSR markers and identified an average PIC of 0.47. Moreover, Delfini et al. (2017) analyzed a set of 39 Brazilian cultivars by using 17 SSR primers and reported a mean PIC of 0.33 with a mean number of alleles 3.4 per locus. The study therefore reflects that these markers are effective in accessing diversity for anthracnose resistant loci in French bean germplasm.

The results were in accordance with Darben et al. (2017), who found moderate level of diversity for anthracnose resistant loci for two anthracnose strains among Brazilian French bean accessions. Almeida et al. (2020) also found moderate diversity in common bean cultivars for resistance to *Pseudocercospora griseola*. The genetic distance and STRUCTURE analysis confirmed the moderate degree of diversity of the evaluated group, with the formation of 4 groups. Findings of the present study are in accordance with Perseguini et al. (2015) who analyzed the genetic diversity of 180 common bean accessions collected from Carioca. Resistant accessions were grouped in one group.

*Co-10* gene is explained in literature as the most potential gene for marker assisted breeding programme for Brazilian French bean germplasm (Alzate marin et al. 2003) but in our collection (Garhwal Himalayan germplasm) this gene alone was not found very effective in restoring resistance against anthracnose. For example, the accessions (GFB-2, GFB-7, GFB-15, GFB-38, GFB-56, GFB-88) possess *Co-10* gene but were found susceptible for anthracnose. However, *Co-10* gene in the presence of *Co-6*, *Co-3/Co-9* gene was able to induce moderate resistance in the accessions GFB-21, GFB-22, GFB-40, GFB-41, GFB-59, GFB-65, GFB-66 and GFB-67.

Single *Co-4<sup>2</sup>* genes was found in one accession (GFB-90) which was found susceptible to anthracnose under field trials. Various level of resistance was observed in different accessions for *Co-4<sup>2</sup>* allele with other genes. *Co-4<sup>2</sup>* allele with *Co-4*, *Co-6*, *Co-3/Co-9* gene provided tolerance (moderate resistance) to anthracnose as accessions GFB-28, GFB-46 and GFB-95 were moderately resistant for anthracnose. Though, *Co-4<sup>2</sup>* allele is also considered as one of the best resistance source by breeders (Miklas and Kelly 2002) but in our accessions this gene alone was not found very effective against anthracnose disease. *Co-3/Co-9*, *Co-4* and *Co-6* genes were not having enough potential in restoring resistance in Garhwal Himalayan French bean accessions as most of the accessions were found susceptible having these three genes singly or in combination. In Brazil and Mexico *Co-3/Co-9* gene was of little value because of frequent failure against anthracnose pathogen (Alzate-Marin et al. 2003). The original *Co-4* gene was found very weak as resistance of this gene was overcome by most of the anthracnose pathogens in Mexico (Balardin et al. 1997; Kelly 2000). *Co-6* gene was found effective in restoring resistance in Andean gene pool (Kelly et al. 2003) but was much less effective against Mesoamerican races (Falconi et al. 2003).

However, it was observed that the accessions having *Co-10* and *Co-4<sup>2</sup>* gene together were found resistant for anthracnose. All the 13 accessions those were found resistant under field and polyhouse conditions possess these two genes consistently with some other genes. However, GFB-4 was found as an exception to this as it contains both the genes eventhough it is moderately resistant for anthracnose (Supplemental Table 1, 2). While accessions GFB-75 and GFB-76 were having only these two genes and were found resistant for anthracnose (Supplemental Table 1). It clearly suggests that both the genes together confer resistance for anthracnose in Garhwal Himalayan region. Banoo et al. (2020) collected 188 common bean landraces from North-West region in India and screened against five important anthracnose races. They found that the presence of *Co-4* and *Co-2* genes in common bean landraces was encouraging for breeding durable anthracnose resistant cultivars for the region. French beans from Harshil and Chakarata are preferred by the consumers for their taste, cooking quality and digestibility. However, the accessions from Harshil were found tolerant while from Chakarata beans were susceptible to anthracnose disease (Supplemental Table 1). It is therefore necessary to identify or develop anthracnose resistant accessions for these regions so that the economic benefit of the farmers could increase.

Correlation coefficient is important in plant breeding because it measures the degree of association between two or more factors (biotic and abiotic) (Dewey and Lu 1959). It is clearly visible in Fig. 4 where trend line showing more resistant accessions when screened under field condition. Results of controlled condition inoculation and natural field infection were not highly correlated in this study (Table 4). Some accessions which were resistant under field conditions showed susceptibility for anthracnose under polyhouse trial (Supplemental Table 2). This greater experimental accuracy

is mainly due to sufficient amount of pathogen inoculums, controlled temperature and humidity in polyhouse, providing favorable environment for progress of disease. For disease screening of germplasm it is necessary to screen germplasm under *in-vitro* conditions. Our findings get support from Leite et al. (2016), who reported resistance in common bean progenies for white mold (*Sclerotinia sclerotiorum* Lib.) in field and greenhouse experiments. To determine the spectrum of resistance in the 14 bean genotypes, seedlings were inoculated with anthracnose inoculums under controlled condition (Gonc,alves-Vidigal et al. 2020). The advantage of *in-vitro* screening methods is that the most favourable conditions for disease progression can be provided. So, if the cultivar is identified as resistant to disease, the cultivar can further be considered for breeding programmes. In addition, these methods reduce the risk of the pathogen spreading to the surrounding environments.

A large number of *Co*-genes have been substantiated in the anthracnose differential cultivars and accessions (Melotto et al. 2000). These genes are presented as part of a multi-allelic series or in group with other *Co* genes. They are most likely subsisting resistance gene clusters and have been established at the molecular level for the B4 R-gene cluster (Ferrier-Cana et al. 2013). Multiallelic series and gene cluster bound the breeders' to choose the useful gene in breeding for anthracnose resistance. This is well known that the resistance provided by a single gene gets easily broken down. It is therefore needed to pyramid the genes for anthracnose resistance in case of French bean too. This is also suggested that while selecting for anthracnose resistance in a particular region, bean breeders should carefully choose a gene pair that, if deployed singly, would confer resistance to all known races in that region. New resistance source for anthracnose needs to be identified to expand the resistance spectrum of future bean cultivars.

Genes *Co-10* and *Co-4<sup>2</sup>* together were found able to provide maximum resistance against anthracnose disease in French bean accessions collected from Garhwal Himalayan regions. Information generated during this study may be helpful in getting information about the resistance level of accessions from different regions. The identification of agronomically superior and anthracnose resistant accessions will be useful in minimizing the linkage drag usually breeders come across while transferring disease resistance in already available high yielding but susceptible varieties. Further, subsequent analysis of diversity using more specific molecular markers is required to explicate more information on the overall genetic diversity, origin and particular gene(s) responsible for resistance in the Himalayan gene pool.

## Declarations

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### Conflict of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

### DATA availability

Data generated during this study are included in this published article and its supplemental files. Requests for additional information regarding the elite genetic materials in this study can be made to the authors and will be considered without undue reservation.

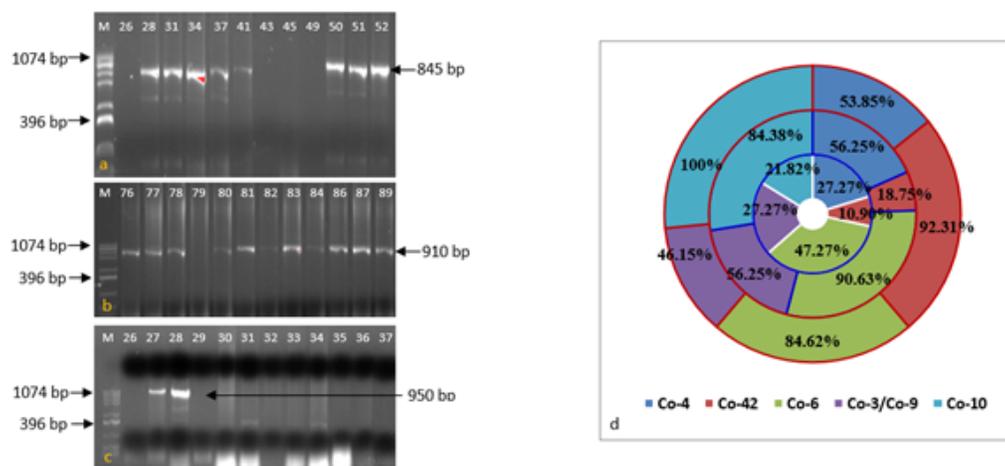
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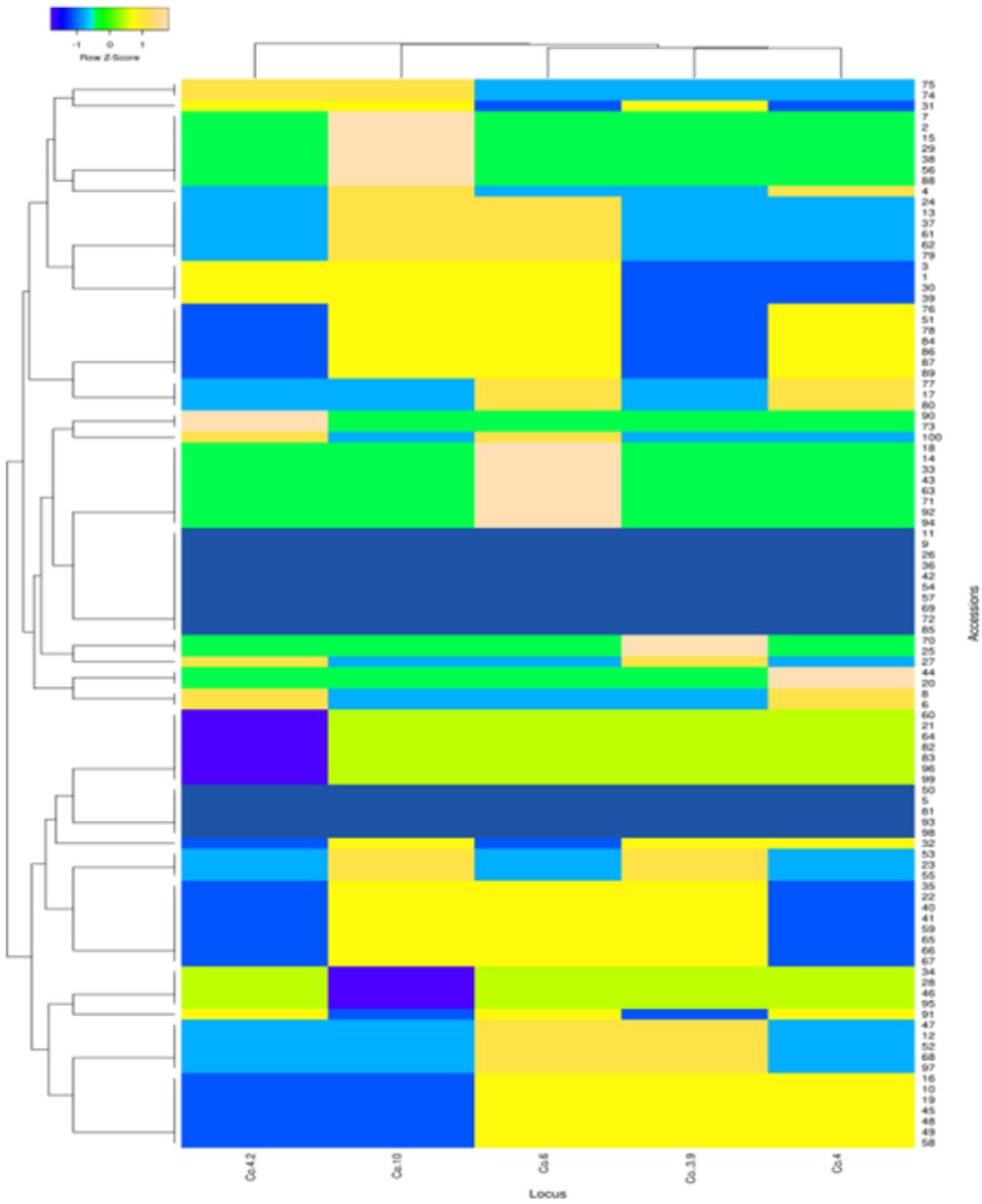
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## Figures



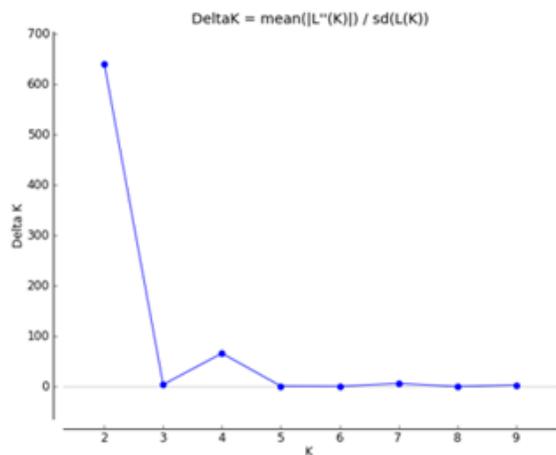
**Figure 1**

Amplification of resistance gene using SCAR primers. (a) Amplification with primer SAZ -20 (845 bp) (b) Amplification with primer SC-08 (910 bp) (c) Amplification with SAS-13 (950 bp). (d) Presence of different genes to conferring disease phenotype viz resistant (outer circle), moderately resistant (Middle circle) and susceptible (inner circle) in different accessions.

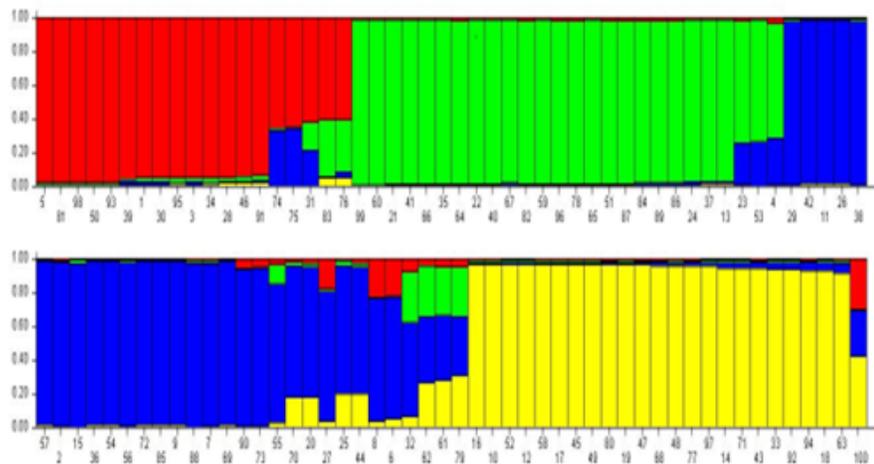


**Figure 2**

Heat map and dendrogram illustrating genetic diversity in French bean accessions for anthracnose resistant loci. Major five groups are formed. Monotonous colour means no diversity for anthracnose resistant loci.



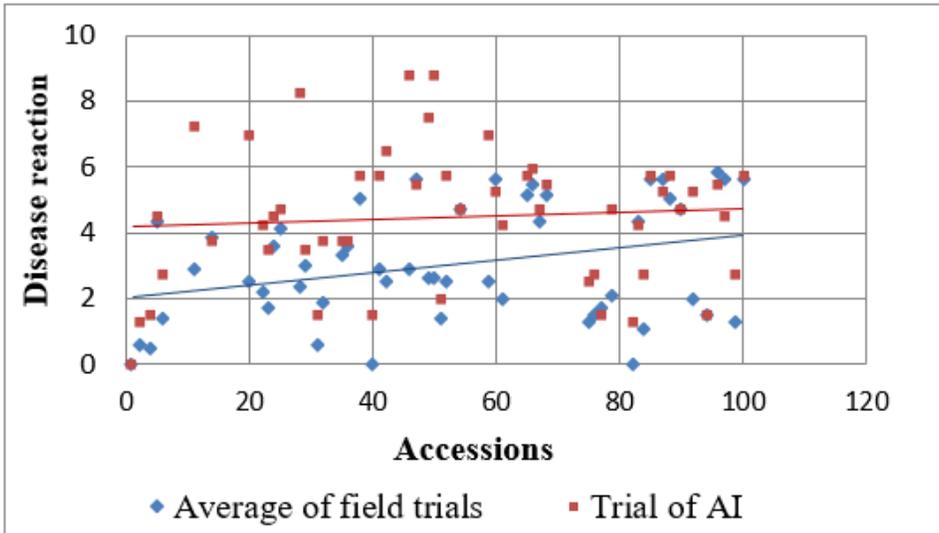
(a)



(b)

**Figure 3**

(a) Evanno analysis results for determination the best number of clusters (DELTA K). (b) Population structure analyzed for 100 accessions and grouped by k = 4. Each accession is represented by a vertical bar and the clusters are indicated by different colors.



**Figure 4**

Comparison of disease reaction of accessions under field trials and under artificial inoculation (AI). Accessions which were found resistant and moderately resistant under field conditions were selected for artificial inoculation.