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Newly Identified Anthracnose Resistant French Bean (*Phaseolus vulgaris*) Accessions from Garhwal Hills of Uttarakhand, India

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A B S T R A C T

Anthracnose is one of the major diseases of French bean, which alone causes up to 50 % loss in yield. This disease is very common in cold and humid areas. Use of resistant varieties instead of harmful pesticides is an effective and organic way to protect crop from diseases. Immense diversity of French bean is present in Uttarakhand hills and is required to be explored adequately. French bean germplasm was collected from Garhwal hills of Uttarakhand and screened for anthracnose resistance using SCAR markers. Accession GFB-3 and GFB-30 were found resistant for anthracnose under *in-vitro* and field conditions as well. Both the accessions showed the presence of multiple resistance genes for anthracnose in them.

Introduction

Anthracnose is considered as the most common disease of French bean (*Phaseolus vulgaris* L.), which is caused by C*olletotrichum lindemuthianum* (Sacc. & Magnus) Scrib. The disease causes massive loss in crop yield worldwide, preferably in the regions with prevailing high humidity and moderately low temperature (13 to 27°C). Wide but unexplored genetic diversity of French bean is available in Uttarakhand hills in western Himalaya (India). The immense genetic diversity of landraces of crops is the most useful and economically valuable part of biodiversity. Unfortunately, very little efforts have been done in this direction.

Considering this disease as the serious constraint to the French bean growth and yield, accessions of French bean were collected from six district of Garhwal regions. All the accessions were screened through *in-vitro* pathogenesis assay under controlled

conditions using *C. lindemuthianum* spore suspension (10^6 conidia/ml) Disease reactions were rated visually using a scale from 1 to 9. The plants scored from 1 to 3 were considered resistant, whereas the ones scored from as 3.1 to 6 were tolerant and 6.1 to 9 were susceptible.

Disease score of both the accessions was 1.5 (Pastor corrales *et al.*, 1995). Whereas, for amplification of resistance genes, DNA was isolated by using CTAB method following the method of Stewart and Lee (1993). PCR amplification of the genes was performed by using 10µl of reaction mixture containing DNA (50 ng), 2 µl 10X PCR Buffer, Primer (1µM), dNTP (1mM) and Taq DNA polymerase (0.3U) and specific temperature conditions as per manufacturers details.

Four field trials were conducted to test the anthracnose disease incidence at three different locations ranging in between 560 to 2300 m above mean sea level (Table 1). Data for natural disease development was collected routinely.

Results and Discussion

Disease reactions were rated visually using a scale from 1 to 9. The plants scored from 1 to 3 were considered resistant, whereas the ones scored from as 3.1 to 6 were tolerant and 6.1 to 9 were susceptible (Pastor Corrales *et al.*, 1995). Disease score of both the accessions was 1.5 and therefore considered as resistant to the disease. Very few leaf spots (1-3) with slight leaf yellowing was observed on inoculated plants (Fig. 1)

Out of thirteen SCAR primers 8 primers were amplified. Amplified primers were SF10 (*Co-10*), SAS13 (*Co-4*^{2}), SH18 (*Co-4*^{2}), SAZ 20 (*Co-6*), SH20 (*Co-2*), SC 08 (*Co-4*), SZ 04 (*Co-6*) and SW 12 (*Co-3*/*Co-9*). Accession GFB-30 was amplified with four primers SF-10, SAS-13, SH-18 and SZ-04 was found having three genes (*Co-10*, *Co-4*^{2}, *Co-6*) related to anthracnose resistance. On screening with SCAR markers GFB-3 accession amplified with three primers SF-10, SAS-13 and SZ-04 and was found having three genes (*Co-10*, *Co-4*^{2}, *Co-6*) related to anthracnose resistance.

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) *Co-4*^{2} allele is also considered as one of the best resistance source by breeders (Miklas and Kelly, 2002) The both the accessions having *Co-10* and *Co-4*^{2} gene together were found resistant for anthracnose.

Table.1 Screening of French bean accessions for anthracnose disease under field conditions

Year	Site/ Location	Period	Altitude (a msl)	Disease reaction
2016-2017	HNB Garhwal University, Chauras Campus	Oct-April	560m	No disease was reported in both the accessions
2017	Ranichauri, Tehri Garhwal	April- Sept	1700m	GFB-3- 1.0 GFB-30- 1.25
2018-2019	HNB Garhwal University, Chauras Campus	Oct-April	560m	No disease was reported in both the accessions
2019	Trjuginarayan, Rudraprayag	April- Sept	2300m	No disease was reported in both the accessions

Table.2 Qualitative and quantitative characters of the French bean accessions (GFB-3 and GFB-30)

Accession	Seed colour	Flower colour	Seed length (mm)	Seed diameter (mm)	Pod length (cm)	Number of seed per pod	Pod colour at physiological maturity	Days to 50 % maturity	100 seed weight (gm)	Yield (gm/ plant)
GFB-3	White with light green spot	White	10.1	3.6	11.72	6.4	Green	69	29.10	102.38
GFB-30	White with black spot	Purple	8.4	4.2	10.94	7.6	Yellowish green with red spots	62	25.34	26.56

Fig.1 Comparative performance of the line GFB-3 with resistant checks (D and L lines) under controlled conditions

Four field trials were conducted to test the anthracnose disease incidence. These accessions were screened with D and L lines (resistant to anthracnose) used as check under field condition. Almost no disease was recorded in field trials except the Ranichauri trial-2017. However, the disease incidence was very low in this trial too (Table- 1).

Out of the germplasm screened, these two accessions showed consistent performance in different trials for resistance against incidence of anthracnose. Accession GFB-3 and GFB-30 have multiple genes for anthracnose resistance with good qualitative and quantitative characters (Table-2).

A large number of *Co*-genes are substantiated in the anthracnose differential cultivars and accessions (Melotto *et al.*, 2000). Resistance provided by a single gene breaks up easily so it is needed to pyramid the genes for effective resistance for anthracnose disease.

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