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Recitation of R genes identified in common bean landrace KRC-5 and KRC-8 native to Himachal Pradesh against *Colletotrichum lindemuthianum* virulences

Shabnam Katoch, Abhishek Katoch, Shiwali Dhiman, Pratibha Sharma, S.K. Rana and P.N. Sharma*
Department of Plant Pathology
CSK Himachal Pradesh Krishi Vishvavidylaya, Palampur-176 062, India.
Corresponding author: pns1960@gmail.com

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Abstract

The predicted resistance genes present in three common bean landraces KRC-5, KRC-8 and Jawala were validated for their reaction against *Colletotrichum lindemuthianum* races prevalent in Himachal Pradesh. The landrace KRC-5 showed resistance to 17 races (3, 7, 87, 179, 211, 238, 259, 437, 503, 513, 529, 537, 737, 775, 935, 957 and 1395) while landrace KRC-8, possessed resistance against 7 races viz., 17, 437, 513, 529, 737, 957 and 1395. In the inheritance test, F_2/RIL populations obtained from Jawala X KRC-5 segregated into 3:1/1:1 ratio, further confirming the presence of single dominant resistance gene in land race KRC-5.

Key words: *Colletotrichum lindemuthianum,* virulences, KRC (Kinnaur Rajmash Collection), inheritance, resistance.

Common bean (*Phaseolus vulgaris*) locally known as "Rajmash" is one of the important pulse crops grown by the small and marginal farmers of North Western Himalayas including Himachal Pradesh (Sharma *et al.* 2007). It is cultivated around the world in an area of about 28 million hectares with an annual production of 20 million tonnes (FAO 2016); while in India-the area under common bean cultivation is 0.15 million hectares with an annual production of approximately 0.42 million tonnes (FAO 2016; Sharma 2017). This crop is vulnerable to the attack of many biotic stresses (of fungal, bacterial and viral origin) and causes significant yield losses (Pastor-Corrales 1988; Araya 1989; Fern´andez *et al.* 2000). Bean anthracnose caused by *Colletotrichum lindemuthianum* is a serious seed borne disease resulting in yield losses to the tune of 100 % in susceptible varieties during epidemic years (Shao and Teri 1985; Pastor-Corrales and Tu 1989). Moreover, the disease is of regular occurrence in crops raised through the farmers own saved seeds (Ferreira *et al.* 2013).

Although, the chemicals for anthracnose management has been recommended but are used rarely by the resource poor small & marginal farmers (Pastor-Corrales *et al.* 1995; Schwartz *et al.* 1982; Allen *et al.* 1998; Opio *et al.* 2001). In addition, chemical pesticides also have residue problems and negative impact on environment and human health. Therefore use of resistant variety is the only least expensive option which farmers can adopt easily (Schwartz *et al.* 1982; Allen *et al.* 1998, Opio *et al.* 2001). As it takes more time to develop a resistant variety against the specific pathogen, the better way of disease management is use of recommended certified disease free seeds or cultivars having strong resistance genes to protect the crop against prevalent races of the pathogen (Mohammed 2013; Gonçalves-Vidigal *et al.* 2013). After studying the prevalence of physiologic races and strength of R-genes present in local land races/ cultivars, the landraces having broad spectrum resistance could be included in different crop improvement projects (http://www.ipmcenters.org/cropprofiles/docs/ TNsnapbeans2012.pdf).

The highly variable nature of *C. lindemuthianum* (Sharma *et al.* 2007, 2018) makes the management of this disease more difficult. More than 200 pathogenic races have been described from different bean growing areas of the world (Sharma *et al.* 2018). Due to this, the improved varieties with time succumb to the disease and the highly variable nature of *C. lindemuthianum* serves as the major drawback in disease management (Mahuku and Riascos 2004; Mohammed 2013; Padder *et al.* 2017). The possible

solution to this resistance break down problem is to pyramid R-genes into a single line (Young and Kelly 1997). Hence its imperative to estimate the strength of R-gens present in a given source of resistance/ cv. before its exploitation either for deployment over time and space or transfer into susceptible recommended cultivars. In the present study, two common bean land races KRC-5 and KRC-8, resistant to anthracnose (Pathania *et al.* 2006), are being explored to know the novelty of the R genes present in these lines, so an attempt was made to know the gene strength and facilitate their introgression into recommended cultivars grown in the target areas.

Materials and Methods

The performance of genes identified in two common bean landraces KRC-5, KRC-8 along with a popular recommended cv Jawala (susceptible to many *C. lindemuthianum* virulences) was recited under greenhouse conditions. The fungal cultures of 24 races of *C. lindemuthianum* maintained in the Molecular Plant Pathology Laboratory, CSK HPKV Palampur were used for inoculation of the test genotypes.

Multiplication of *C. lindemuthianum* culture and race confirmation

The fungal cultures of different races were multiplied on Mathur's medium and maintained at 22±2°C as per the descriptions of Sharma *et al.* (2018). The identity of the test isolates was reconfirmed on a set of international differential bean cultivars viz., Michelite, MDRK, Perry marrow, Cornell 49.242, Widusa, Kaboon, Mexico 222, PI 207262, TO, TU, AB 136, G2333 (CIAT 1988). The freshly sporulating culture of each isolate was inoculated individually on each differential cultivar and the inoculated plants were maintained at 22±2°C till disease appears (maximum for 15-18 days). To avoid the loss of virulence, each race (0, 3, 7, 16, 17, 87, 99, 145, 158, 179, 211, 238, 259, 437, 503, 513, 529, 537, 737, 775, 935, 957, 1015 and 1395) culture was transfered to susceptible cv. Jawala after every 3rd subculture.

Inoculum preparation

Conidial suspension of *C. lindemuthianum* races was prepared separately in sterilized distilled water by harvesting acervuli from freshly sporulating cultures (15 days old). The spore concentration in suspension was determined using a haemocytometer and spore density of 1.2 x 10^{6} conidia per ml (Balardin and Kelly 1998; Bigirimana and Hofte 2001) was used for plant inoculations. The prepared inoculum suspended with few drops of Tween-20 (0.1% v/v) was used for carrying out the phenotypic studies.

Method of inoculation and evaluation of disease reaction

The germinated seed dip method (Champion *et al*. 1973) was used to study the reaction type of test isolates on differential cultivars and local landraces. Seeds were germinated by using rolled towel method (Kommendahl and Lang 1971). Surface sterilized seeds were placed in double layers of moistened germination paper and kept at 25 ± 1°C in seed germinator with 12-hour photoperiod. 3 days old germinating seeds without seed coat were dipped in standard spore suspension for 5 minutes and thereafter, sown in 3.0 cm deep plastic trays (30 X 15cm) containing sterilized river sand. Three seeds of each individual were used for inoculation per race and further the inoculated plants were kept in growth chamber (Saveer Biotech) at 22 ± 1°C with more than 90 per cent relative humidity and 12-hour photoperiod for 6 days. The disease reaction of different races on Jawala, KRC-5 and KRC-8 was recorded on 7th day by following 0-5 point (Drijfhout and Davis, 1989) scale where 0: No disease symptoms; 1: Pin point lesions on leaves; 2: Small lesions (3 mm) but not sunken, no sporulation on leaves; 3: Large sunken lesions (> 3 mm), no sporulating lesions; 4: Large deep lesions upto stem centre and sporulating lesions and 5: Seedling killed by the pathogen. Plants showing reaction type 0, 1 and 2 or either of these were graded as resistant (-) while those showing reaction type 3, 4 and 5 or either of these were susceptible (+).

Response of KRC-5 x Jawala populations to selected races of *C. lindemuthianum*

In order to check the inheritance of resistance pattern in land race KRC-5 against some races of the pathogen, an experiment was conducted using F_2 population derived from Jawala x KRC-5 cross. The F_2/ RIL (F_{2-8}) plants were inoculated separately with four virulent races *viz.,* race 3 (105 seeds), 211 (100 seeds), 537 (89 seeds) and 935 (96 seeds) of *C. lindemuthianum* as per the procedure described above. The pattern of inheritance was estimated by counting the numbers of resistant and susceptible plants and the obtained data was subjected to Chi-square analysis to test the goodness of fit to Mendelian ratios.

Results and Discussion

In the present study, the strength of R-genes present in KRC-5 and KRC-8 was validated using 24 *C. lindemuthianum* races present in various bean growing areas of Himachal Pradesh. The land race KRC-5 exhibited resistance to 17 races **(**3, 7, 87, 179, 211, 238, 259, 437, 503, 513, 529, 537, 737, 775, 935, 957 and 1395**)** whereas 7 races viz., 0, 16, 17, 99, 145, 158

Table 1. Reaction of common bean land races to different races of *Colletotrichum lindemuthianum* under green house conditions

Sr. No.	Race	Isolate no.	Origin	Jawala	KRC-5	KRC-8
1.	0	CL257	Sangla (Kinnaur)	S	S	S
2.	3	CL238	Polling (Barot)	S	R	S
3.	7	CL 247	Kalpa (Kinnaur)	S	R	S
4.	16	CL 254	Sangla (Kinnaur)	R	S	S
5.	17	CL260	Kullu	S	S	R
6.	87	CL228	Lambathach (Mandi)	S	R	S
7.	99	CL234	Nair chowk (Mandi)	S	S	S
8.	145	CL261	Shimla	S	S	S
9.	158	CL 297, CL 303	Shimla NBPGR Sangla farm	S	S	S
10.	179	CL 229	Thunag, Mandi	S	R	-
11.	211	CL 231	Chail chowk, Mandi	S	R	S
12.	238	CL 286	Kadon	S	R	S
13.	259	CL 237	Magrugalla, Mandi	S	R	S
14.	437	CL 271	Lohardi, Kangra	R	R	R
15.	503	CL232	Janjheli, Mandi)	S	R	S
16.	513	CL101	Bhulah, Mandi	R	R	R
17.	529	CL 137	Deol, Chamba	R	R	R
18.	537	CL 74	kullu	S	R	S
19.	737	CL 296	Shimla NBPGR	S	R	R
20.	775	CL 48	Karon (Lag valley) kullu	S	R	S
21.	935	CL 46b	Ghiyagi (Sainj) Kullu	S	R	S
22.	957	CL 268	Sarla Mandi	S	R	R
23.	1015	CL 272	Barot	S	S	S
24.	1395	CL233	Rajgarh, Mandi	S	R	R

R: Resistant, S: Susceptible

Table 2. Segregation of resistance in F_2 and RIL progenies of the cross between common bean resistant landrace KRC5 and susceptible genotype Jawala against *Colletotrichum lindemuthianum*

Parents/ Pathogen	Generation	Number of plants		Expected ratio (R:S)	(χ 2)	P-value
		R	S			
Race 3						
KRC5	P_2	10	-			
Jawala	P_1	-	10			
	F_2	77	19	3:1	1.12	0.25 < P < 0.5
Race 537						
KRC5	P_2	10	-			
Jawala	P_1		10			
	F_2	67	22	3:1	1.99	0.1 < P < 0.5

and 1015 were virulent on this accession. While landrace KRC-8, showed resistance against 7 races (race 17, 437, 513, 529, 737, 957 and 1395) and was susceptible to 17 races (Table 1). The cultivar Jawala, a highly susceptible to almost all races in our previous studies was found resistant to race 16, 437, 513 and 529, respectively. In 1997, Kumar et al. reported resistance in KRC-5 to 5 races of *C. lindemuthianum* both under field and laboratory conditions while recommended cv Jawala showed highly susceptible reaction. In conformity with our results, Pathania *et al*. (2006) also reported resistance in KRC-5 to six races viz., 3, 513, 529, 537, 775 and 935; and against three races (115, 513 and 529) in landrace KRC-8.

Response of KRC-5 x *Jawala* populations to some selected races of *C. lindemuthianum*

In addition to phenotypic screening, an attempt was also made to check the inheritance pattern of the resistance gene present in KRC-5 using four physiologic races (race-3, race-211, race-537 and race-935) on F_2 population (Table 2). Based on the observed segregation of resistance in F_2 population against four races of *C. lindemuthianum*, a null hypothesis was formulated that, the resistance in F_2 progenies (Table 2) segregated in monohybrid ratio of 3:1 and deviation of the observed data is not real, while in the F2-8 RIL population it was 1:1. The segregation data of F_2/RILs showed a good fit to 3R:1S/ 1R:1S ratio, thereby indicating that the resistance in KRC-5 against four races is controlled by a single dominant gene. The inheritance of resistance in KRC-5 and KRC-8 landrace using race 903 was first reported by Sharma *et al.* (2000) and the presence of single dominant gene in KRC-5 (Pathania *et al.* 2006; Katoch 2015) was further confirmed by our group.

The present study established that KRC-5 having monogenic dominant resistance is effective against many races of the pathogen, is one of the important sources of resistance in common bean. The landrace KRC-8 known to possess a recessive gene (Sharma *et al*. 2000) with broader resistance to anthracnose and recommended for cultivation in the state in mid nineties is another very important source for use in crop improvement programs. These two landraces could be utilized in the resistance breeding program of the state of HP after characterizing these genes.

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