Himachal Journal of Agricultural Research 46 (1): 56-61 (2020)

Introgression of anthracnose resistance gene from common bean land race KRC-8 into elite cultivar Jawala

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Manuscript received: 19.03.2020; Accepted: 25.06.2020

Abstract

Bean anthracnose is one of the major diseases leading to a significant yield losses in susceptible cultivars. Hence, development of widely adaptable anthracnose-resistant cultivars using land races is of immense importance to maintain the production and productivity of the crop in disease prone areas. Keeping this in view, the present investigation was planned to introgress resistance gene from a well known Himalayan land race KRC-8 (Baspa) in the background of high yielding susceptible variety Jawala, both belonging to Andean gene pool. The hybridization, advancement with single seed decent approach and phenotypic screening of $F_{2:8}$ recombinant inbred lines (RIL) against anthracnose resulted in identification of fifteen potential lines showing resistance to anthracnose. Evaluation of agronomic traits of these lines revealed JB-3 to be significantly better than the parental genotype Jawala for number of pods per plant (18.67), number of seeds per pod (6.67), pod length (13.9 cm) and leaflet length (15.50 cm). Similarly, weight for 100 seeds, which directly contributes towards seed yield of beans, ranged from 48.6g in JB-5-2-2 to a maximum of 52.1g in JB-16-2 which is almost at par with that of Jawala (52.3g). Hence these RILs can be used as resistant donor germplasm for future breeding endeavours.

Key words: Bean anthracnose, Jawala, KRC-8, Baspa, agronomic traits.

Common bean (*Phaseolus vulgaris* L) is one of the essential components of the food and nutritional security system providing necessary dietary proteins to the logarithmically growing population. Under the pressure of increasing production, the beans are widely cultivated including humid, cool and temperate climatic conditions exposing the plants to tremendous attacks of pathogens including viruses, bacteria and fungi. The cultivation conditions for beans also prop up the pathogens thus posing a great challenge to the farmers to contain them (Peloso 1992). *Colletotrichum lindemuthianum* is one of the most devastating pathogens, causing bean anthracnose, sometimes leading to 100 percent yield loss of the susceptible cultivars (Rodriguez-Guerra *et al.* 2003; Gonzalez-Chavira *et al.* 2004; Sharma *et al.* 2007). The prominent approaches to contain the pathogen include use of chemical fungicides, crop rotation, improved agronomic practices and cultivation of resistant varieties (Odogwu *et al.* 2017). Due to their long term ecotoxicological effects and production cost enhancement, chemicals are less desirable among the marginal and poor farmers. The development of resistant cultivars is the appropriate strategy to reduce the cost of disease management without affecting the productivity of crop. Moreover, it significantly contributes towards sustainable improvement in agricultural practices. To develop resistant cultivars, breeders aim at identifying donor genotypes, cross them with the recipient cultivar and then evaluate the progeny for potential genotypes which are not only resistant but are also agronomically equivalent or superior to the parents. The preferable donor genotypes are the ones lying in the primary gene pool so that the barriers leading to infertility of the progeny may be excluded. Land races grown in specific areas may provide a great opportunity for that as these are less exploited (Fioreze *et al.* 2018).

Following the aforesaid approach of crop improvement, a common bean land race of Himalayan region, KRC-8, identified as resistant to a number of races of *C. lindemuthianum* by Sharma *et al.* (1999) and Katoch *et al.* (2019) was utilized as the donor parent. The large seeded, maroon to purple coloured in a

mosaic pattern, KRC-8 recommended for cultivation as variety Baspa but is not preferred over variety Jawala, another commercially cultivated variety in North Western Himalayas with shiny red coloured large seeds but highly susceptible to bean anthracnose (Sharma *et al.* 2000; Pathania *et al.* 2006). Both genotypes belong to Andean gene pool possessing large seeds. Hence in this study and attempt has been made to introgress the resistance from landrace KRC-8 into cv. Jawala and identify potential genotypes with resistance to anthracnose and high yielding.

Materials and Methods

The present research work was initiated at MAREC, Sangla, Kinnaur (H.P.) and further carried out in the Department of Plant Pathology, CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur, Himachal Pradesh during 2017-2020.

Plant material and artificial hybridization procedure

Seeds of cv. Jawala and KRC-8 (Baspa) were used in the hybridization programme at MAREC, CSK HPKV Sangla, Kinnaur. The cv. KRC-8 is comparatively late maturing than Jawala, hence to concur the flowering of both parents, staggered sowing was done in $2m^2$ plots (40 seeds each) at an interval of 10-15days in the experimental farm of the CSK HPKV, MAREC, Sangla, Kinnaur.

The stigma becomes receptive at least 2 days before and one day after normal anthesis. Buds which were showing colour and were about to open the following day were chosen for the female parent. The wings were carefully removed with the forceps to expose the coiled keel. The keel was removed to allow access to the anthers. A small incision was made near the top of the keel with the point of the forceps. When opened, the upper lip was grasped with the forceps and peeled up the back. The stamens (counting 10 in number) were removed with the forceps. Pollination was done by hooking method (Bliss 1980; Buishand 1956). In this method, the pollinated stigma was removed from the male flower with a forceps and rubbed against the stigma of the female flower. The stigma of the male flower was left in the female flower by hooking it through the style of the latter near the stigmatic surface. About 300-400 flower buds were emasculated to get sufficient F_1 seeds as this crop has very low rate of seed setting (Pathania *et al.* 2006).

Advancement of generations

The F_1 seeds obtained from artificial hybridization were advanced to $F_{2:8}$ generation by single seed decent approach (Urrea and Singh 1994) both at Sangla and under greenhouse at Department of Plant Pathology, College of Agriculture, CSK HPKV, Palampur. 172 $F_{2:8}$ recombinant inbred lines (RIL) were screened for resistance to anthracnose disease with two races of *C. lindemuthianum* Race 87 and Race 211.

Pathogen race culture, inoculum preparation and method of inoculation

Cultures of race, R-87 and R-211 of the fungus *C. lindemuthianum* differentiating the resistant (KRC-8) and susceptible parents (Jawala) maintained in the Department of Plant Pathology, College of Agriculture, CSKHPKV Palampur, Himachal Pradesh, India were revived on Mathur's medium for sporulation at temperature of 22±2°C in walk-in-incubator facility. Virulence was maintained by periodical inoculation of *C. lindemuthianum* races on the susceptible cultivar Jawala after every third subculture.

Pure cultures of both races were used for the experimental procedures. Conidial suspension was prepared by scrapping the 7-15 days old culture with inoculating needle in sterilized double distilled water. The uniformity of the inoculum was maintained by continuous stirring. The suspension was filtered through the double layered muslin cloth, to free the suspension from agar remnants. Further, heamocytometer was employed to adjust the spore count to final concentration of 1.2×10^6 spore/ml (Bigirimana and Hofte 2001).

Spray inoculation method (Kruger and Hoffman, 1978) was utilized for exposing the population to anthracnose pathogen with first spray at initiation of pod formation stage and second at pod development stage after one week to ensure proper disease pressure. Inoculation was done in the evening time to provide enough time for attachment and germination of spores on the plant tissue. All the plants were kept in well humid ($\geq$90 per cent relative humidity), temperature controlled (20±1°C) conditions with 12hrs photoperiod in the green house facility of the Department. These conditions simulated the natural environment for the development of the disease.

Phenotypic assessment of disease

Upon disease development, the infected plants were evaluated for disease reaction by using the disease scale of 0-9 (Mayee and Dattar 1986) where 0-3 reaction type was categorized under resistant and 5-9 scores were classified as susceptible.

Evaluation of the yield attributes

Among resistant recombinant inbred lines (RILs), fifteen genotypes showing Jawala like seed type and colour along with other morphological traits, were sown along with the parental genotypes in three replicates each in the green house for preliminary assessment of yield related traits. The morpho-physiological traits considered were among those

recommended by IBPGR (1982) and included plant height (cm), number of pods per plant, number of seeds per pod, pod length (cm), leaflet length (cm), seed yield per plant and 100 seed weight (g).

The observed data on various morphological traits was subjected to simple t-test for finding significant differences among the RILs and parents, if any.

Results and Discussion

Common bean has remained the choice of farmers for food, feed and fodder due to high dietary proteins in it and grown across variable climatic conditions inevitably exposing it to a wide array of pathogens. Bean anthracnose is one of those notorious pathogens leading to almost complete yield loss in the susceptible cultivars under cool and humid conditions. Hence, scientific community has joined hands to develop widely adaptable anthracnose resistant cultivars without compromising their agronomic potential. In the present research work also, well known Himalayan land races Jawala and KRC-8 (Baspa) have been exploited to develop recombinants with resistance to anthracnose.

The success rate for the artificial hybridization came out be 7-8 percent as among every hundred flowers pollinated, only seven or eight buds developed F_1 hybrid seeds. The environmental conditions also influenced the hybridization.

Phenotypic screening for disease resistance

The screening for the disease revealed ratio of 1:1

Table 1. Agro-morphological evaluation of potential common bean recombinants derived from the cross Jawala x KRC-8

Genotype	Disease score (0-9)	Plant height (cm)	No of pods/plant	No of seeds/pod	Seed yield/ plant (g)	Pod length (cm)	Leaflet length (cm)	100 seed weight (g)
JB-3	0	72.00	18.67*	6.67*	16	13.90*	15.50*	49.5
JB-5-2-2	1	77.00	11.33	6.00	16	12.57	15.20	48.6
JB-16-2	0	75.33	15.67	5.33	15	13.13	16.17*	52.1*
JB-20	1	81.33	15.67	6.00	24	13.50	15.83*	49.6
JB-42	1	64.00	15.00	6.33	12	13.40	14.40	51.4*
JB-52	1	72.67	12.00	6.00	22	12.63	15.73*	49.8
JB-56b	0	80.00	12.00	5.33	19	12.87	14.37	51.3*
JB-197	1	69.00	11.33	5.67	24	12.33	14.93	49.8
JB-199	0	78.00	16.33	6.00	14	12.20	14.77	50.2
JB-260-1-2	0	80.00	13.00	5.00	19	12.00	14.67	49.7
JB-261	1	85.33*	12.67	5.33	22	11.20	14.70	50.4
JB-264	1	76.00	15.67	5.67	18	13.40	14.63	51
JB-340	0	78.00	14.33	6.00	22	12.67	16.17*	49.2
JB-318	0	81.00	12.00	6.00	23	14.53*	15.43*	49.6
JB-368	0	85.67*	12.00	5.00	18	11.37	15.17	51.7*
Jawala	7	80.67	16.00	5.67	24	13.45	14.87	52.3*
KRC-8	0	102.67	16.00	6.00	20	12.88	16.20	50.4
Mean		78.75	14.10	5.76	19.29	12.83	15.22	50.39
SE		0.151627						

*=Significant at 0.05 confidence interval

among total 172 F_8 RIL population with 92 genotypes as resistant and 80 lines as susceptible to Race 87 of *C. lindemuthianum*. The ratio remained same for the Race 211 of the pathogen, where among 134 lines, 64 were designated as resistant and 70 as susceptible. The donor parent KRC-8 (Baspa) has been reported to be resistant to few races of anthracnose by different workers (Sharma *et al*. 1993; 2000; Pathania *et al*. 2006). This cultivar has shown high field resistance to the disease (Sharma *et al*. 1993). Whereas cultivar Jawala is susceptible to almost all the known races of *C. lindemuthianum* prevalent in North Western Himalayan region (Pathania *et al*. 2006; Katoch *et al*. 2019)

Morpho-physiological evaluation of RILs

The success of the introgression programme lies in the agronomic performance of the disease resistant recombinants. Seed morphology is an important attribute to the yield of common bean (Dalla Corte *et al.* 2010; Saba *et al.* 2015). As a preliminary study of the outcome, the selected resistant RILs were screened for their seed traits which are of special attention to the consumers. The ones showing similar seed traits (colour, texture and shape) as that of Jawala were selected for their preliminary morpho-physiological evaluation in the green house facility according to the descriptors recommended by IBPGR (1982). Among these, the descriptors related to seed yield of the plant along with RILs, parents were sown for the comparison of agronomic traits directly related to seed yield of the plants. Among all the RILs under evaluation, JB-3 was found to be significantly better than Jawala for largest number of traits including number of pods per plant (18.67), number of seeds per pod (6.67), pod length (13.9 cm) and leaflet length (15.50 cm). But in this line, seed yield and 100 seed weight were found to be 16g and 49.5g, respectively, which were lower than Jawala (Table 1). The plant height of the genotypes under consideration ranged from 64 cm (JB-42) to 102.67 (KRC-8) with an average of 78.75 cm. The assessment of plant height showed JB-261(85.33cm) and JB-368 (85.67cm) to be significantly taller than the female parent, Jawala (80.67cm) but shorter than KRC-8 (102.67cm). In rest of the RILs non-significant differences over the recipient parent were observed for this trait. Similarly, JB-318 was shown to be significantly better for pod length (14.53 cm) and leaflet length (15.43 cm) than Jawala. The high variation among these morphological traits has also been documented by Garcia *et al.* (1997), Gomez *et al.* (2004); Stoilova *et al.* (2005); Meza *et al.* (2013) and Ulukapi and Onus (2014). Larger leaves and heavier seeds are the characteristics of Andean gene pool members (Singh *et al.* 1991). So leaflet length and 100 seed weight were included for morphological characterization of the recombinants. The pods per plant and seeds per plant are the major determinants for enhancement of the seed yield per plant (Hedge and Mishra 2009).

All the genotypes were large seeded like parents which were of the Andean gene pool (Sharma *et al*. 2013). Among all the genotypes, the seed yield per plant varied from 12g (JB-42) to 24g (Jawala) with an average of 19.29g. Seed yield is an important contributor to the crop yield. The present findings were in conformity with those reported by Rana *et al.* (2015). The 100 seed weight in all the resistant RILs as well as parental genotypes (Table 1) ranged between 48.6g to 52.3g with an average of 50.39g. Seed size, weight and colour are the major determinants for the farmers to choose them for sowing (Papa and Gepts 2003). Common beans can be categorized into three groups based on their size and weight: large, with more than 40 g/100 seed; medium, with 25–40 g/100seeds; and small, with less than 25 g/100 seeds (Gepts and Bliss 1985; Khairallah *et al.*1990; Duran *et al.* 2005; Meza *et al.* 2013). The higher seed weight of these elite derivatives justifies the cultivation of Jawala and Baspa in the Hill region and further advocates the use of selected recombinants as potential genotypes for future breeding endeavours. Since the present study was a preliminary experiment in the establishment of agronomic potential of the recombinant inbred lines with the morphological traits of Jawala and disease resistance of KRC-8. Further field assessment in this direction along with marker assisted selection is highly recommended to develop elite common bean cultivars with introgressed resistance to anthracnose. This can lead to the development of elite bean cultivars which can be employed in future crop improvement programmes.

Acknowledgement

The authors are thankful to University Grants Commission (UGC) for the award of RGNF and Department of Science and Technology, Govt. of India for financial assistance.

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