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EVALUATION OF COMMON BEAN (*Phaseolus vulgaris* L.) GENOTYPES AGAINST ANTHRACNOSE (*Colletotrichum lindemuthianum* Sacc. and Magn.)

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ABSTRACT. Twelve genotypes of common bean were evaluated against anthracnose under natural epiphytotic conditions. This study was carried out in a randomized complete block design with three replications at the research field of Agriculture Research Station (ARS), Vijaynagar, Jumla, Nepal from June to September 2018. The area under the disease progress curve (AUDPC) and disease severity were calculated. In laboratory conditions, artificial inoculation was carried out on detached leaves of twelve genotypes using a pure culture suspension of *Colletotrichum lindemuthianum* (1.2×10^6 conidia ml^{-1}) in a completely randomized design with three replications. The results showed that bean genotypes varied significantly for disease severity both in the field and laboratory conditions. In the field, bean genotypes showed resistance to highly susceptible reactions. Their AUDPC value ranged from 120.55 to 502.31. The lowest mean AUDPC value was recorded in KBL-1 (120.55) followed by KBL-3 (123.79) and KBL-2 (124.44). Similarly, the lowest severity value was recorded with KBL-1 (0.51), KBL-2 (0.52) and KBL-3 (0.53). Detached leaf assay in laboratory experiment showed that the lowest mean AUDPC was found in KBL-2 (16.67) and KBL-3 (16.67). Therefore, KBL-2 and KBL-3 could be utilized as resistant varieties to anthracnose disease under Jumla and similar field conditions.

Introduction

Beans are a good source of protein with 2–3 times the amount of cereal grains (Siddiq *et al*., 2010). Starch, dietary fibre, minerals and vitamins are all abundant in foods with a high dry matter content (Kutos *et al*., 2003; Costa *et al*., 2006). Beans also include a wide range of phytochemicals, antioxidant activity and a wide range of flavonoids, including anthocyanins, flavonoids, proanthocyanidins, flavonols, phenolic acids and isoflavones (Beninger, Hosfield, 2003; Choung *et al*., 2003; Granito *et al*., 2008; Lin *et al*., 2008). Dry edible beans are grown and eaten in large quantities all over the world (Sathe, 2002). Bean is an indigenous crop of the Jumla and Karnali zones of Nepal (Subba *et al*., 2016). It can also be interpreted as local Rajma. Jumli simi, the indigenous bean's name, is gaining popularity.

Indigenous bean plants come in a wide range of colour, shape, size and growth habit (Subba *et al*., 2016). Local Jumli simi cultivars are high in minerals and have a high antioxidant potential. Because of its high polyphenol content, Jumli simi is a nutritious food. When compared to light-coloured beans, dark-coloured beans are found to have higher phytochemicals and antioxidant activity (Subba *et al*., 2016).

Both biotic and abiotic constraints are responsible for reducing the production of common beans. Among them, anthracnose caused by *Colletotrichum lindemuthianum* (Sacc. & Magn.) is an important disease of the beans (Muimba-Kankolongo, 2018). It is the major yield-limiting disease impacting seed quality and marketability (Schwartz, Pastor-Corrales, 1989). The disease can cause 95–100% yield loss when the infected seed is used for cultivation and favourable weather conditions occur during the crop cycle (Yesuf, Sangchote, 2007). The disease is particularly important in relatively cool, wet areas of tropical and temperate regions (Schwartz, Pastor-Corrales, 1989). In Nepal, it causes a huge loss in the temperate and sub-tropical areas (Manandhar *et al*., 2016). The disease symptoms are seen in all the above-ground parts including leaf, stem, pods and seeds (Agrios, 2005). On leaves, symptoms generally occur on the underside as linear, dark brick-red to black lesions on the leaf veins. As the disease progresses, discolouration appears on the upper leaf surface. Leaf symptoms often are not obvious and may be overlooked when examining bean fields (Kelley, Vallejo, 2004). The most striking symptoms develop on the pods. Small, reddish-brown to black blemishes and distinct circular, reddish-brown lesions are typical symptoms on bean pods. Since, the disease is mostly visible in the fields from flowering (R6) to pod filling stage (R8), disease scoring is mostly recommended during this stage (Manandhar *et al.*, 2016).

Adopting various methods such as the use of disease-free certified seeds, use of resistant varieties, crop rotation (of 2 to 3 years), field sanitation and chemical application, this disease can be managed (Tesfaye, Pretorius, 2005). Crop rotation, intercropping, field sanitation and plant debris removal, altering planting dates, applying compost and blending diverse cultivars have all been shown to reduce disease severity (Joshi *et al*., 2009). However, because these treatments have both economic and environmental limitations, the most cost-effective and environmentally sustainable option is to use host plant resistance to reduce anthracnose disease in beans.

Plant resistance is also the most efficient, simple, secure and cost-effective alternative for small, resource-poor farmers (Opio *et al*., 2006), Since, Jumla was declared as an organic district by the District Development Committee of Jumla in 2007, plant resistance can be an effective option for disease management where the use of chemicals is prohibited. In comparison to other management practices, Pastor-Corrales (1995) found that using genetically resistant cultivars is the most effective, least expensive and the easiest method for farmers to implement. Therefore, this study was conducted to determine the level of anthracnose resistance in promising common bean pipeline genotypes appropriate for the high hills of Nepal.

Materials and Methods

Field experiment

Field screening was carried out from June to September 2018 at the research field of Agriculture Research Station Vijaynagar, Jumla (27°38'51.8" north longitude and 84°20'52.5" east latitude with an elevation of 2 370 masl). The experimental site falls in the temperate climate zone of the high hills where the maximum summer temperature goes up to 26 °C, the minimum temperature reaches –6 °C. The experimental location receives the highest amount of rainfall up to 180 mm. The meteorological data during the experiment is given in Figure 1.

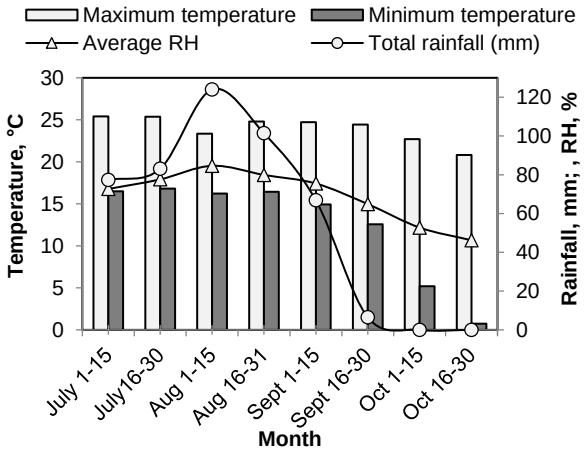

Figure 1. Meteorological data of Jumla during experiment in 2018 at Vijaynagar, Jumla, Nepal (RH – relative humidity)

Twelve bean genotypes were evaluated in a randomized complete block design with three replications in a 6 m^2 plot size with the spacing of 50 cm × 10 cm (row to row and plant to plant spacing) for each entry. The list of genotypes used in this experiment is given in Table 1. The source of these genotypes is Agriculture Research Station (ARS), Jumla, Nepal.

Fertilizer was applied at a rate of 100:60:40 kg N: P_2O_5:K_2O [N (100 kg ha^{-1}); Urea and Di-Ammonium Phosphate, P_2O_5 (60 kg ha^{-1}; Di-Ammonium Phosphate) and K_2O (40 kg ha^{-1}; Murate of Potash)] with the full dose of phosphorus and potassium as a basal dose. Nitrogen was applied in two split with half dose as basal and the remaining half dose was top dressed just after first weeding (one month after sowing). Staking of trailing beans was done according to the need. The crop was grown under rain-fed condition. On the first week of June 2018, the crop was planted. All plots were surrounded by a spreader area of one meter in width planted with the highly susceptible local variety (Farmer's variety).

Table 1. List of bean genotypes used in the experiment in 2018 at Vijaynagar, Jumla, Nepal

Genotype	Growth habit	Flower colour	Seed colour	Pod colour	Seed shape
PB0001	Bushy	Light pink to white	Dark red with white streaks	Green	Kidney
PB0002	Trailing	Dark pink	Creamy white with purple streaks	Green with dark-purple streaks	Elongated
PB0048	Trailing	Very light pink to white	Dark red	Green	Elongated
KBL-1	Trailing	Dark pink	Black	Green	Elongated
KBL-2	Trailing	White	Dark red	Reddish green	Elongated
KBL-3	Trailing	Very dark pink	Dark purple with white streaks	Green	Elongated
KBL-4	Trailing	Dark pink	Creamy to very light brown	Green	Flattened, elongated
KBL-5	Bushy	Pink	Light brown	Green	Elongated
KBL-6	Trailing	White	White	Green	Elongated
KBL-7	Trailing	Whitish pink to dark	Creamy to very light brown	Green	Oval
KBL-8	Bushy	Very light pink to white	White with dark red streaks	Green	Kidney
KBL-9	Trailing	Dark pink	Light to dark grey	Green	Elongated

Disease assessment

Disease severity. Disease scoring was done by using a 1–9 scale given by (CIAT, 1987) which was converted to disease severity percentage. Disease severity (Eq. 1) was calculated by using the formula given by (Wheeler, 1969).

$$DS,\% = \frac{SNR}{TNR \times MR} \times 100 \quad (1)$$

where,

DS – disease severity,
SNR – a sum of all numerical ratings,
TNR – total number of plants observed and
MR – maximum rating.

Before analysis severity percentage data were arcsine transformed to improve variance inequality.

AUDPC values. AUDPC value was calculated by using the following formula (Eq. 2) as given by Das *et al.* (1992).

$$AUDPC = \sum_{i=1}^{n-1} [\{\frac{(Y_i + Y_{i+1})}{2}\} \times (t_{(i+1)} - t_i)] \quad (2)$$

where,

AUDPC – area under the disease progress curve,
Y_i – disease severity on the i^{th} date,
t_i – time on which Y_i is recorded and
n – number of time observations were taken.

Based on the mean AUDPC values, the genotypes were categorized into 4 groups of resistance levels as below (Magar *et al*., 2015):

Mean AUDPC value	Resistance category	Code
1–30	Resistant	R
31–60	Moderately resistant	MR
61–90	Susceptible	S
>90	Highly susceptible	HS

Anthracnose disease scoring was carried out on a scale of 1–9 (CIAT, 1987).

Laboratory experiment

Detached leaf assay

A modified detached leaf technique was used to screen the common bean genotypes (Tu, 1986).

Inoculum preparation. A small piece of the infected pod was surface sterilized in 1% sodium hypochlorite solution for 25–30 seconds, washed three times in sterilized distilled water under aseptic conditions, excess water was drained out and the sample was placed in sterilized moist blotter paper and incubated at 22 ± 1 °C for seven days in Biological Oxygen Demand (B.O.D) incubator. A stereomicroscope was used to inspect the incubated materials and a single acervulus was transferred to a Potato Dextrose Agar (PDA) plate in a laminar flow hood. Commercial PDA manufactured by HiMedia Laboratories Pvt. Ltd., Mumbai, India was used for inoculum preparation purposes. The culture was obtained after ten days and then sub-cultured in PDA. Using a hemocytometer, the concentration of the conidial suspension was adjusted to 1.2×10^6 conidia ml^{-1} (Mahuku, Riascos, 2004).

Inoculation. Bean plants of test genotypes grown in a screen house were used for this assay where apical leaflets from one-week-old plants were washed with sterilized distilled water and placed abaxial surface up in Petri dishes containing two layers of water-soaked blotting papers and glass slides (to avoid rotting). The spore suspension was brushed gently onto the leaves as described by (Tu, 1986). The plate was covered with a transparent lid and the inoculated leaflets were incubated at 22 ± 1 °C with 12 hours light cycle. On the 7th day after inoculation, the severity of the disease was scored based on the percentage of veins diseased. The disease scoring was carried out on a scale from 0 to 3 (Table 2).

Table 2. Scoring scale for anthracnose disease based on leaf area affected (Inglis *et al*., 1988).

Scale	Plant parts affected
0	No disease
1	1–10% veins with lesions
2	11–25% veins and veinlets with lesions
3	26% or more veins and veinlets with lesions

Disease severity and AUDPC were calculated s as mentioned previously.

The Pearson correlation coefficient was estimated.

Data analysis

The analysis of variance (ANOVA) was performed for all parameters in GenStat (version 15.0, VSN International Ltd., England & Wales). Duncan's Multiple Range Test (DMRT) was used for mean separation and was performed at a 5% level of significance (Gomez, Gomez, 1984).

Results and Discussion

Field experiment

Disease severity

Twelve bean genotypes varied significantly in the severity of anthracnose at 63, 70, 77, 84 and 91 DAS (Table 3). Anthracnose severity was found 0.51 to 1.27. On 91 DAS, The lowest disease severity was obtained in KBL-1(0.51) followed by KBL-2 (0.52), KBL-3 (0.53) and PB-0001 (0.53) (Table 3).

Table 3. Severity of anthracnose disease on bean genotypes in 2018 under field condition at Vijaynagar, Jumla, Nepal

Genotype	63 DAS	70 DAS	77 DAS	84 DAS	91 DAS
KBL-1	0.35^{a}	0.38^{a}	0.42^{a}	0.46^{ab}	0.51^{a}
KBL-2	0.36^{ab}	0.39^{ab}	0.44^{ab}	0.46^{ab}	0.52^{ab}
KBL-3	0.36^{ab}	0.40^{b}	0.44^{ab}	0.44^{a}	0.53^{b}
PB0001	0.34^{a}	0.40^{b}	0.45^{bc}	0.48^{b}	0.53^{b}
KBL-4	0.38^{bc}	0.44^{cd}	0.47^{cde}	0.52^{c}	0.61^{c}
KBL-6	0.39^{c}	0.42^{c}	0.46^{bcd}	0.52^{c}	0.62^{c}
KBL-7	0.38^{bc}	0.44^{d}	0.48^{de}	0.52^{c}	0.62^{c}
KBL-9	0.40^{c}	0.45^{d}	0.49^{e}	0.54^{c}	0.65^{d}
PB0002	0.38^{bc}	0.59^{e}	0.79^{f}	0.84^{d}	0.97^{e}
PB0048	0.40^{c}	0.59^{e}	0.77^{f}	0.88^{e}	0.97^{e}
KBL-5	0.72^{d}	0.91^{g}	1.03^{g}	1.15^{f}	1.27^{f}
KBL-8	0.72^{d}	0.87^{f}	1.04^{g}	1.15^{f}	1.27^{f}

DAS: Days after sowing, Means in a column followed by different letter's are significantly different at P <0.05. Mean values derived from arcsine transformation of percentage data.

Area Under the Disease Progress Curve (AUDPC)

Resistance category of bean genotypes based on total AUDPC, mean AUDPC and AUDPC day^{-1} are given in Table 4. The genotype KBL-1 had the lowest mean AUDPC value (120.5). Similar observations were recorded for AUDPC day^{-1} values with the lowest AUDPC day^{-1} was observed in KBL-1 (17.2) which was at par with KBL-3 and KBL-2. Among them, four genotypes (PB-0001, KBL-2, KBL-3, KBL-1) were categorized into resistant, four genotypes (KBL-6, KBL-9, KBL-7, KBL-4) into moderately resistant, two moderately susceptible (PB-0048 and PB-0002) and two susceptible (KBL-8, KBL-5) categories based on mean AUDPC values (Table 4).

Table 4. Resistance category of bean genotypes based on total AUDPC, mean AUDPC and AUDPC day^{-1} in 2018 at Vijaynagar, Jumla, Nepal

Genotype	Total AUDPC	Mean AUDPC	AUDPC day^{-1}	Resistance category
KBL-8	2009.2	502.3	71.7	S
KBL-5	1973.0	493.2	70.5	
PB0048	1292.9	323.2	46.2	MS
PB0002	1265.2	316.3	45.2	
KBL-9	661.1	165.3	23.6	
KBL-7	617.0	154.2	22.0	MR
KBL-4	604.0	151.0	21.6	
KBL-6	598.9	149.7	21.4	
PB0001	513.3	128.3	18.3	
KBL-2	497.8	124.4	17.8	R
KBL-3	495.2	123.8	17.7	
KBL-1	482.2	120.5	17.2	

S – Susceptible, MS – moderately susceptible, MR – moderately resistant, R – Resistant, AUDPC – area under disease progress curve

Grain yield

The effect of anthracnose on the grain yield of different genotypes is given in Figure 2. Grain yield varied significantly among the genotypes. The highest grain yield was found in KBL-9 (6.02 t ha^{-1}) followed by KBL-2 (5.41 t ha^{-1}) while the lowest yield was obtained in PB-0048 (3.46 t ha^{-1}) followed by KBL-6 (3.61 t ha^{-1}) (Fig. 2).

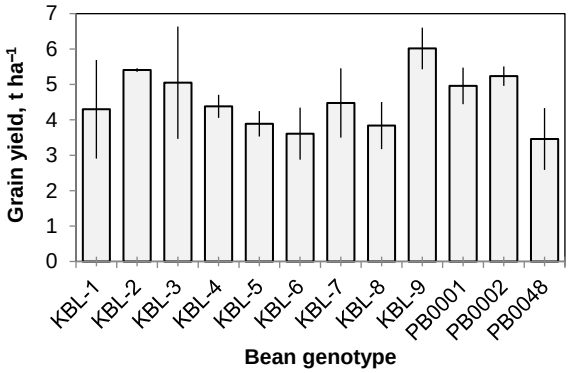

Figure 2. Bar graph illustrating the mean and standard deviation (error bars) of grain yield of bean genotypes in 2018 under field conditions at Vijaynagar, Jumla, Nepal

Laboratory experiment

Final AUDPC and mean AUDPC in detached leaf assay

Final AUDPC and mean AUDPC differed significantly (P<0.01) among genotypes (Table 5). The final AUDPC was found the lowest in KBL-3 (50.0) and KBL-2 (50.0) which was followed by KBL-9 (66.7) and PB-0001(66.7). The minimum mean AUDPC was found in KBL-3 (16.67) and KBL-2 (16.67) followed by PB0001 (22.2) and KBL-9 (22.2) (Table 5).

Table 5. Final AUDPC and mean AUDPC of bean genotypes in detached leaf assay in 2018 at Vijaynagar, Jumla, Nepal

Genotype	Final AUDPC	Mean AUDPC
KBL-5	222.2	74.0
KBL-8	233.3	77.8
KBL-9	66.7	22.2
PB0048	177.8	59.2
KBL-6	144.4	48.1
KBL-4	133.3	44.4
KBL-7	88.9	29.6
PB0002	233.3	77.8
KBL-2	50.0	16.7
KBL-3	50.0	16.7
KBL-1	88.9	29.6
PB0001	66.7	22.2

AUDPC – Area under disease progress curve

Correlation analysis

The correlation studies showed highly significant correlations between different parameters (Table 6). Grain yield showed a negative and significant correlation with mean AUDPC (r = –0.452), disease incidence (r = –0.512) and disease severity (r = –0.442) (Table 6). The correlation values between mean AUDPC and disease incidence (r = 0.960), mean AUDPC and disease severity (r = 0.991), and disease incidence and disease severity (r = 0.959) were significantly positive.

Table 6. Pearson's correlation coefficient between parameters of different genotypes of bean grown in the field in 2018 at Vijaynagar, Jumla, Nepal

Parameter	Mean AUDPC	Disease incidence	Disease severity
Mean AUDPC			
Disease incidence	0.960**		
Disease severity	0.991**	0.959**	
Yield	–0.452*	–0.512*	–0.442*

* – significant at P <0.05, ** – significant at P <0.01

Discussion

Common bean provides an important source of fibre, protein and energy to the people of Jumla district. Bean is one of the important pulse crops with good economic value and high export. They are usually used as dal, soup and porridge. In common bean growing areas, the incidence of anthracnose disease has posed a serious threat to bean production (Choudhary *et al*., 2018; Gonçalves-Vidigal *et al*., 2020). Jumla is known as an organic district, the use of pesticides for the control of insect pests and diseases is prohibited in this district. Therefore the development of a disease-resistant variety of beans is important for this district. Agriculture Research Station (ARS), Jumla have been evaluating many local and exotic bean genotypes for disease screening. Due to lack of laboratory and manspower, successful resistant breeding works have not been achieved yet rather than screening works. This study can contribute to developing a potential resistant variety to boost productivity. The leaf detachment assay showed that the final AUDPC and mean AUDPC were found the lowest in KBL-3 and KBL-2. The field screening showed the KBL-2, KBL-3 and PB-0001 had lower disease severity. Variation of the genotypes against anthracnose disease was reported by Prasad *et al*. (2016). The leaf detachment assay could be used to screen beans for anthracnose disease in an efficient and time-saving manner. Detached leaf assay is an alternative to inoculating whole plants that allow breeders to test for diseases or races without destroying the plant, reduces the time between inoculation and disease assessment and confines the pathogen to the lab (Miller-Butler *et al*., 2019).

In the development of resistant plant lines, screening for and selecting resistant plant sources is important (Geetha *et al*., 2013; Sharma *et al*., 2012). In our study, based on mean AUDPC values, genotypes were categorized into four categories; four (PB-0001, KBL-2, KBL-3, KBL-1) were categorized into resistant, four genotypes (KBL-6, KBL-9, KBL-7, KBL-4) into moderately resistant, two moderately susceptible (PB-0048 and PB-0002) and two susceptible (KBL-8, KBL-5) categories.KBL-9 which showed a moderately resistant reaction to the disease produced the highest yield which was also reported as a high yielder by Bhujel *et al.* (2014). In the case of yield, the result wasn't regarding the resistance category as the highest yield was obtained in the moderately resistant one. This might be because, in common beans, yield is a complex character brought about by other pod quality traits as well (Singh *et al.*, 2015). According to Nkalubo (2006), yield differences differed significantly between different accession types but not between resistant classes.

In our study, there was a positive correlation between AUDPC and disease severity. Similar results were observed by (Viriyasuthee *et al*., 2019). A negative correlation between seed yield and AUDPC, disease severity score and percent disease severity was recorded in faba bean (Zebire, Tadesse, 2018). The grain yield showed significant and negative correlations with AUDPC values in Lay Gorebela and Mush in faba bean (Wondwosen *et al*., 2019).

Conclusion

According to the findings, the disease-resistant promising bean genotypes namely KBL-2 and KBL-3 can be recommended for Jumla conditions in terms of both anthracnose disease resistance and grain yield potential. The bean genotypes found promising under Jumla conditions can be considered for multi-location experiments in other high hill areas. This finding will help the bean farmers in enhancing the yield of common beans and minimizing economic loss.

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Conflict of interest
The authors declare that there is no conflict of interest regarding the publication of this paper.

Author contributions
SKC – conceived and designed the experiments; performed the experiments as part of thesis research; analyzed and interpreted the data; wrote the paper.
HKM – guided and helped to design the experiment as a major advisor, provided editorial suggestions in the preparation of manuscript, revision and approval of the final manuscript.
SMS, BA – guided in performing experiments as a member advisor, provided editorial suggestions in the preparation of manuscript, revision and approval of the final manuscript
JS – revised manuscript; wrote the paper.

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