



Multiple Race Inoculation as an Option in Breeding for Resistance to *C. Lindemuthianum* In Common Beans

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Abstract: Bean anthracnose caused by *Colletotrichum lindemuthianum* causes severe common bean (*Phaseolus vulgaris* L.) yield losses of up to 100% worldwide. Breeding for resistance is the best method to mitigate this problem. The objective of this study was to investigate the appropriateness of *C. lindemuthianum* multiple race inoculation in breeding for resistance to *C. lindemuthianum* in common bean. Seven parents with varying reactions to *Colletotrichum lindemuthianum* were mated in all possible combinations to generate forty-two progeny crosses. These crosses together with their parents were evaluated in the green house for their reaction to *C. lindemuthianum*. The experiment was laid out following a Completely Randomised Design (CRD) with four replications. The treatments used were: (1) inoculation with race 54; (2) inoculation with race 311 and (3) multiple inoculation of race 54 X race 311. The mean genotypic score among treatments were found to be 1.76, 2.62 and 3.06 for treatments 1, 2 and 3 respectively. There were significant differences ($P < 0.01$) among genotypic responses to *C. lindemuthianum* with respect to race 311. The *t*-test analysis revealed that multiple race inoculation (Treatment 3) had a higher mean disease severity expression than those of singly race inoculations (Treatment 1 and Treatment 2) ($P < 0.01$). The results suggest that multiple infection had a synergistic effect, indicating its suitability for screening resistant genotypes in the breeding program.

Keywords: *Colletotrichum lindemuthianum*; Beans; Races; Inoculation.

1. Introduction

Common beans (*Phaseolus vulgaris*.L.) are an important grain legume in human consumption in the whole world. The world's annual production is about 12 million tons with latin America being the largest producer at 5.5 million tons. Africa is the second largest producer contributing about 2.5 million metric tons [1, 2] with Zambian production standing at an average of 50,398 metric tons [3]. In Zambia resource poor farmers and other households rely on the production of beans as an important source of income and protein. It also plays a role in improving soil fertility status through nitrogen fixation [4, 5]. Common bean in Zambia is hampered by several biotic and abiotic stresses. The low productivity of the crop is caused by abiotic stresses such as; low soil Phosphorous (P) levels, droughts, floods, poor agronomic practices and biotic stresses such as pests and diseases. Diseases are usually triggered by use of recycled seed, unfavourable weather patterns and/ or use of poor field sanitation practices. Among the diseases that affect beans are fungal diseases, anthracnose, caused by *Colletotrichum lindemuthianum*, is an important disease and can cause great yield losses of up to 100 percent [6-9].

A number of control measures such as application of fungicide, use of disease free certified seed, crop rotation and field sanitation have been used to control *C. lindemuthianum* but to a limited success [8, 10, 11]. Alternatively the use of resistant common bean genotypes to *C. lindemuthianum* have been found to be the most effective, efficient and affordable for resource poor farmers [12, 13]. Generally, in breeding for resistance to *C. lindemuthianum* in beans, plant breeders have employed single race inoculations or have paid attention to specific races [14-16]. However, information generated could be misleading as races do not necessarily occur in isolation. This may explain why it has been difficult to breed for durable resistance which is effective across environments [17]. It should however be noted that multiple infection of these races may occur, which may lead to different effects when compared to a challenge with single race infection. This study therefore sorts to investigate the appropriateness of multiple race inoculations in breeding for resistance to common bean.

2. Materials and Methods

2.1. Germplasm and Pathogenic Races Used in the Study

Seven common bean parents (Table 1) and 42 progeny crosses previously raised from mating seven parents in all possible combinations were used in the study. With regards to pathogens, two races, race 54 and race 311 of *C. lindemuthianum* previously characterised and identified at the University of Zambia, Plant Pathology Laboratory were used in the experiments.

2.2. Disease Assessment for Single and Multiple Inoculations

Seven parents and their 42 progeny crosses were grown in plastic pots in the green house at the University of Zambia in May 2015. The treatments used were: Treatment 1 (Inoculation with single race 54), Treatment 2 (Inoculation with single race 311) and Treatment 3 which constituted inoculation with multiple race. A haemocytometer was used to count the number of conidia for each race and the conidia concentration of the races for each treatment was standardised to 1.2×10^6 . Multiple race inoculum was achieved by adding 500 mls each of race 54 and race 311 to constitute a final volume of 1000 mls which had a concentration of 1.2×10^6 conidia/ml.

The experiment was laid out as a Completely Randomised Design (CRD) with four replications. The inoculation of the specific treatment was done thirty days after genotypic emergence and the genotypes were inoculated with single and multiple race using a one litre hand sprayer until run off. Each treatment was inoculated on one specific set of genotypes and placed in different mist chambers. The plants were then incubated and maintained in mist chambers for 96 hours, at 23°C and 90 – 100 % relative humidity. Disease assessment of the genotypes' reaction to infection for each respective treatment was done seven days after inoculum inoculation. Four individual genotypes for each of the seven parents and the 42 progeny crosses were scored visually for the disease symptoms using a 1 to 9 scale. Two distinct plant reactions were considered using binary system that is Resistant (R) phenotype was assigned to plants with no or limited symptoms (Scores 1 to 3); whereas plants graded 4 or greater were considered to be susceptible (S).

2.3. Data analysis

Analysis of variances (ANOVA) was used to evaluate the mean genotypic responses of beans to different races of *C. lindemuthianum* for each treatment and it was performed in GenStat 17th edition [18]. Estimates of heritability for resistance to *C. lindemuthianum* were performed using mid-parent offspring regression and was computed as $b_{po} = \frac{V_a}{V_p} = h^2$ where b_{po} is the regression coefficient, V_a and V_p are additive and phenotypic variance components respectively, and h^2 is the narrow sense heritability estimate.

Mean disease severity score comparisons among treatments (Single race and multiple race infection) were done using student *t*-test using in Excel.

3. Results

3.1. Appropriateness of *C. Lindemuthianum* Multiple Race Inoculation on Genotypic Response in Common Bean

There were significant differences ($P < 0.01$) for disease severity score among genotypes for treatment 2 only (Inoculation with single race 311) (Table 2).

The genotypic means of all the genotypes in the study with respect to inoculation with race 311 were computed (Table 3). Results showed that parents AB136 with a disease severity mean score of 1.25 had the lowest genotypic mean performances and Perry marrow with a disease severity mean score of 4.0 had the highest. The parent Kabulangeti showed moderate mean performance for disease severity with a score of 3.75. The F1's progeny crosses, [G2333 X Solwezi], [AB136 X G2333], [Kabulangeti X AB136], [Mbala X G2333] had the lowest mean disease severity score of 1 while [Solwezi X AB136] had the highest disease severity mean scores of 5.25. The rest showed moderate genotypic mean performance to disease severity with scores ranging from 3.0 - 5.0. The narrow sense heritability estimate for treatment with race 311 (with significant genotypic responses) was found to be 0.03. The overall mean genotypic disease severity reaction for inoculation with single race 54, race 311 and multiple inoculation with *C. lindemuthianum* were 1.76, 2.62 and 3.06 respectively. The use of student *t*-test for evaluating response among the races (Table 4), indicated that the disease severity mean score of 1.76 for treatment 1 (Inoculation with single race 54) was significantly ($P < 0.001$) lower than the mean severity score of treatment 3 (Inoculation with multiple race). Similarly the mean severity score of 2.62 for treatment 2 (Single race inoculation with race 311) was significantly ($P = 0.015$) lower than the mean severity score of 3.06 for treatment 3, inoculation with multiple race. The mean severity score of 2.62 for treatment 2 (Inoculation with single race 311) was significantly ($P < 0.001$) different to the mean severity score of 1.76 for treatment 1 (Inoculation with single race 54). It is therefore evident that the single race inoculation and multiple inoculations exhibited different degree of pathogenicity virulence with multiple race infection being the most virulent.

4. Discussion

There were significant difference ($P < 0.01$) for disease severity mean score among the genotypes (Table 2). The genotypic responses considered were only for race 311 whose treatment exhibited significant differences among

genotypes (Table 3). Lack of significance in genotypic responses within treatments, race 54 (treatment 1), and multiple race inoculation (treatment 3) could probably be due to differences in virulence among *C. lindemuthianum* races used in the study [14, 19]. There has been evidence of different *C. lindemuthianum* races existing around the world [9, 20-22]. There were significant differences among genotypes with regards to disease severity caused by *C. lindemuthianum* within treatment 2 (inoculation with race 311). Genotypes G2333, PI206262, AB136 exhibited high levels of resistance to race 311 which correlated with other studies where they were screened with race 521 and 25 [9, 14, 23]. On the other hand Solwezi, previously reported susceptible to race 65 and race 342 by Zulu (2005) was found resistant to race 311, with a mean score rating of 2.75. This implies that cultivars may respond differently to different races of *C. lindemuthianum*. The narrow sense heritability estimate with regards to treatment with race 311 was found to be low ($h^2 = 0.03$), implying that early generation selection is not an appropriate breeding strategy in selecting for this race. Previous studies have found high heritability values [24-26]. The differences could be due to differences in the material under study. There is therefore need to employ different germplasm with a view of gaining further information on implications of heritability when genotypes are singly or multiple inoculated.

There were differences among treatments with regards to overall mean disease severity among treatments (Table 4). From these results it was deduced that multiple race infection had higher disease severity expression than those of single race infection. Other researchers found out that when one or multiple physiological race(s) of *Phytophthora capsici* in *Casipicum annuum* was inoculated on a single plant, the effect and extent of disease infection did not differ [27]. While other researchers reported synergistic effects resulting in increased disease symptoms [28]. Results in this study are similar to those found by Aliyu, *et al.* [28], who established that multiple infection of Blackeye Cowpea Mosaic Virus and Cowpea Yellow Mosaic virus in cowpea were more virulent than those of single infection. Therefore due to these synergistic interactions in common bean, multiple race infection should be used to screen for resistant to *C. lindemuthianum*. However where a particular race is prevalent in a locality, breeding for single race resistance can be taken as a priority.

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Tables

Table.1. Bean genotypes used in generating crosses at the Seed Control and Certification Institute during the 2014/15 cropping season

Parent	Source	Reaction of parents to <i>C. lindemuthianum</i>	References
G2333	CIAT	R	(Pastor-Corrales, 1991)
PI-207-262	CIAT	R	(Pastor-Corrales, 1991)
AB136	CIAT	R	(Pastor-Corrales, 1991)
Perrymarrow	CIAT	S	(Pastor-Corrales, 1991)
Kabulangeti	SCCI	S / farmer preferred	(Zulu, 2005)
Solwezi	SCCI	Farmer preferred	
Mbala	SCCI	S/ farmer preferred	(Zulu, 2005)

Reaction of bean parents petioles, leaves and stem to *Colletotrichum lindemuthianum* (anthracnose pathogen); Disease severity damage scoring scale (1 - 9), R = Resistant (1 - 3);

S = Susceptible (4 - 9), [29].

Table-2. Mean squares for the F1s genotypic analysis for their reaction, to single and multiple inoculation of *Colletotrichum lindemuthianum* pathogen in common bean evaluated in 2014/15 cropping season at the University of Zambia

Source of Variation	d. f	Disease severity for <i>Colletotrichum lindemuthianum</i> races		
		Race 311	Race 54	^c Multiple inoculated
Replication	3	18.58	0.82	5.92
Genotypes	48	4.94**	2.56	3.60
Error	144	2.65	2.28	3.29

** Significantly different at $P < 0.01$ probability levels e = Multiple inoculation, involved inoculation of the same bean genotype with a mixture of inoculum for race 54 and race 311, each contributed 500ml

Table-3. Bean genotypic means for anthracnose severity measured from *Colletotrichum lindemuthianum* race 311 inoculations on the parents and their F1 progenies evaluated in 2015 at the University of Zambia

Genotypes	^f Disease severity score
G2333	2.00
PI207262	3.00
AB136	1.25
Solwezi	2.75
Kabulangeti	3.75
Mbala	3.00
Perry Marrow	4.00
G2333 X PI207262	1.25
G2333 X AB136	2.50
G2333 X Solwezi	1.00
G2333 X Kabulangeti	3.75
G2333 X Mbala	2.00
G2333 X Perry Marrow	3.75
PI207262 X G2333	3.00
PI207262 X AB136	3.00
PI207262 X Solwezi	1.50
PI207262 X Kabulangeti	2.50
PI207262 X Mbala	1.50
PI207262 X Perry Marrow	2.25
AB136 X G2333	1.00
AB136 X PI207262	2.50
AB136 X Solwezi	4.00
AB136 X Kabulangeti	2.75
AB136 X Mbala	5.00
AB136 X Perry Marrow	4.25
Solwezi X G2333	3.50
Solwezi X PI207262	2.25
Solwezi X AB136	5.25
Solwezi X Kabulangeti	1.25
Solwezi X Mbala	3.00
Solwezi X Perry Marrow	3.50
Kabulangeti X G2333	3.00
Kabulangeti X PI207262	1.75
Kabulangeti X AB136	1.00
Kabulangeti X Solwezi	1.25
Kabulangeti X Mbala	2.25
Kabulangeti X Perry Marrow	2.50
Mbala X G2333	1.00
Mbala X PI207262	2.75
Mbala X AB136	2.50
Mbala X Solwezi	2.50
Mbala X Kabulangeti	4.25
Mbala X Perry Marrow	1.50
Perry Marrow X G2333	1.50
Perry Marrow X PI207262	1.50
Perry Marrow X AB136	3.25
Perry Marrow X Solwezi	4.75
Perry Marrow X Kabulangeti	2.50
Perry Marrow X Mbala	2.75
LSD (P < 0.05)	2.27

LSD- Fishers Protected Least Significant Difference test performed at $P < 0.05$, f = Anthracnose disease severity rating scores on foliage (1 - 9) 1 - 3 resistant, 4 - 6 moderate susceptible, 7 - 9 susceptible. Source: [29].

Table-4. Overall disease severity mean comparisons among single races (race 54 and race 311) and multiple race inoculations (combination of race 54 and race 311) evaluated in the 2014/15 cropping season.

MDSC Comparisons	Student t-test (P-Value)
Race 311(2.62 ^x) vs Race 54 (1.76 ^y)	< 0.001
^e Multiple inoculation (3.06 ^z) vs Race 311(2.62 ^x)	0.015
Race 54 (1.76 ^y) vs ^e Multiple inoculation (3.06 ^z)	< 0.001

MDSC = Mean disease severity score; x = mean genotypic score of race 311; y = mean genotypic score of race 54; z = mean genotypic severity score for multiple inoculations. e = Multiple infection, involved inoculation of the same bean genotype with inoculum for both race 54 and race 311