

# Agronomic Performance, Stability Analysis and Evaluation of Anthracnose Disease Resistance of Common Bean Lines Derived by Marker-Assisted Backcrossing in Uganda

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**How to cite this paper:** Nkalubo, S.T., Namayanja, A., Namusoke, A., Mukabaranga, J., Shakirah, N., Nkuboye, A., Gepts, P. and Jebesa, W.T. (2024) Agronomic Performance, Stability Analysis and Evaluation of Anthracnose Disease Resistance of Common Bean Lines Derived by Marker-Assisted Backcrossing in Uganda. *Agricultural Sciences*, 15, 376-397.

<https://doi.org/10.4236/as.2024.153022>

**Received:** December 7, 2023

**Accepted:** March 26, 2024

**Published:** March 29, 2024

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## Abstract

The present study focused on evaluating the agronomic performance, stability, and anthracnose resistance of common bean lines derived through Marker-Assisted Backcrossing in Uganda. Eight marker-assisted selection (MAS) backcross-derived bush bean lines with red seed types, alongside two checks, were evaluated in a randomized complete block design replicated two times in five locations for three consecutive crop-growing seasons in 2021 and 2022. The study aimed to identify lines with both high stable yields and enhanced resistance to anthracnose disease for potential release and utilization in future bean varietal development in Uganda. Agronomic traits, including days to 50% flowering, days to 90% physiological maturity, seed yield, seed yield components, and anthracnose disease reaction under natural infestation were assessed. The response to anthracnose disease was further assessed using six isolates of *Colletotrichum lindemuthianum* representing six different races. Results indicated that the agronomic performances of the MAS backcross-derived bush bean lines were statistically comparable to the recurrent parent NABE14. Specifically, six lines exhibited statistically equal to or higher performance than NABE14 in terms of seed yield, total number of seeds and number of pods per plant. The combined analysis of variance for seed yield showed significant ( $p < 0.05$ ) effects for all the sources of variation except genotype  $\times$  location interaction. Genotype main effect plus genotype  $\times$  environment interaction (GGE) biplots explained 86.56% of the total observed variation for the seed yield. Within this, 64.12% was attributed to the first principal component (PC1), while the second principal component (PC2) ex-

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plained 22.44%. UGKT-B157-4 emerged as the top-performing genotype, being both high-yielding and stable. Positioned closer to the “ideal genotype” in the GGE biplot, UGKT-B157-4 outperformed others, winning in four out of the five test environments. Furthermore, UGKT-B157-4 exhibited resistance to anthracnose under both natural field infestation and artificial inoculation. The observed resistance pattern was similar to that of G2333, the donor parent in the backcross indicating the presence of the *Co-4*<sup>2</sup> and *Co-5* anthracnose resistance genes in the derived line. In conclusion, UGKT-B157-4, identified as the best-performing and stable genotype, demonstrates promise for release and use in future bean varietal development in Uganda, offering a combination of high yields and enhanced anthracnose disease resistance. The study provides valuable insights into the potential of Marker-Assisted Backcrossing in improving common bean varieties in the region.

### Keywords

*Colletotrichum lindemuthianum*, Genotype by Environment Interaction, GGE Biplot, MAS

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## 1. Introduction

Common bean (*Phaseolus vulgaris* L.) holds global significance as the most crucial legume for direct human consumption offering a balanced nutritional source that sustains individuals for extended periods [1] [2]. This versatile crop contributes vital nutrients, including protein, complex carbohydrates, fiber, calcium, iron, and zinc, making it an essential component of the human diet [3]. Highest consumption is primarily in Latin America and Africa, where beans serve as a dietary staple [2] [4]. In Uganda, common bean is not only a staple [5] but also a key cash crop prominently exported to South Sudan, Kenya, and the Democratic Republic of Congo (DRC) [6].

However, the potential yield of common beans worldwide faces challenges from various diseases, particularly those caused by fungal pathogens [7] [8]. Anthracnose, a major fungal disease in Uganda, exhibits varying incidences, severity, and variability across different agroecologies, potentially leading to total crop loss [7] [9] [10]. Given the critical role of beans in food, nutrition, and income, the development of genotypes combining high seed yield with resistance to major diseases like anthracnose is essential for enhancing productivity.

In the development of disease-resistant high-yielding dry bean genotypes, backcrossing stands as a key breeding approach [11]. Traditionally, backcrossing involves time-consuming processes dependent on weather conditions for disease development. To expedite this, molecular marker-assisted selection (MAS) is employed, leveraging molecular markers linked to disease resistance genes. MAS backcrossing has successfully yielded new common bean genotypes with heightened disease resistance and superior seed yield [11] [12].

The expression of quantitative traits, such as seed yield, is intricately influenced by genotype  $\times$  environment interaction (GE), posing challenges in identifying superior genotypes [13]. In common bean, genotype by environment interaction (GEI) significantly impacts seed or grain yield [14] [15]. Therefore, stability and adaptability analyses become crucial, involving the assessment of genotype performance and stability for yield and related traits. Statistical methods such as genotype by environment interaction (GGE) biplot analysis and Additive Main effects and Multiplicative Interaction (AMMI) analysis are commonly employed for stability analysis in multi-environment trials [13] [16] [17].

The NaCRRRI African Bean Consortium (ABC) breeding project utilized MAS backcrossing to introduce anthracnose resistance genes (*Co-4*<sup>2</sup>, *Co-5*) from the donor parent G2333 into the susceptible bush bean variety NABE14. This effort resulted in promising MAS backcross-derived bush bean lines with selections made from the BC<sub>4</sub>F<sub>2.3</sub> plants genotyped by the SCAR molecular markers SH18 [18] and SAB3 [19] linked to the *Co-4*<sup>2</sup> and *Co-5* anthracnose resistance genes, respectively. By the F<sub>7</sub> generation in 2020, the selected lines were ready for evaluation across diverse environments to assess their performance, including yield stability and reaction to anthracnose disease under field and controlled conditions.

This study, therefore, aimed to i) evaluate the performance of common bean lines derived by MAS backcrossing, ii) estimate the effects of genotype by environment (G  $\times$  E) interaction and stability for seed yields, and iii) identify lines with high stable yields combined with improved anthracnose disease resistance for release and incorporation into future bean varietal development efforts in Uganda and beyond.

## 2. Materials and Methods

### 2.1. Evaluation Environments

Multi-environment trials were conducted in three districts of Uganda (Sheema, Kabale and Rakai) during the 2021 first cropping season (2021A), the 2021 second season (2021B), and the 2022 first season (2022A). Geographically, these districts represent mid-Southwestern, Southwestern, and South-central Uganda, respectively (Table 1). Each trial location's geographical details, including latitudes, longitudes, and altitudes are outlined in Table 1.

**Table 1.** Geographical information of the trial locations used during the three cropping seasons.

Region	District	Sub county/division	Village	Environment code	Latitude	Longitude	Altitude (m.a.s.l)
Mid-southwestern	Sheema	Kagango	Rubare	E1	-0.6239800	30.4128190	1440
	Sheema	Kabwohe	Nyabishera	E2	-0.5754260	30.3716830	1426
Southwestern	Kabale	Kamuganguzi	Nyakyonga	E3	-0.6545775	31.4113437	1857
South Central	Rakai	Lwanda	Kamengo	E4	-0.670064	31.464009	1366
	Rakai	Kitonezi	Nsozi biri	E5	-0.6445950	31.4178930	1275

m.a.s.l = Meter above sea level.

## 2.2. Experimental Genotypes

The genotypes evaluated comprised advanced backcross-derived bush bean lines with red seed types originating from the MAS backcrossing program involving NABE14, as the recurrent parent, and G2333, as source of the *Co-4<sup>2</sup>* and *Co-5* anthracnose resistance genes. Initially, 33 advanced backcross-derived bush bean lines were selected based on their field reaction to anthracnose disease and agronomic performance. Subsequently, eight lines exhibiting superior agronomic performance and field resistance to anthracnose were consistently evaluated across the three districts over three consecutive crop-growing seasons (2021A, 2021B and 2022A). These lines included UGKT-B73, UGKT-B93, UGKT-B133, UGKT-B119, UGKT-B157-4, UGKT-B157-7, UGKT-B160 and UGKT-B264-3. These lines had been previously genotyped by SCAR molecular markers SH18 and SAB3 linked to the *Co-4<sup>2</sup>* and *Co-5* anthracnose resistance genes, respectively (Table 2). By 2021A, these lines had reached the F<sub>8</sub> generation. In this study, genotypes NABE14 and G2333 were included as checks.

## 2.3. Experimental Design and Field Data Collection

The experimental setup employed a randomized complete block design (RCBD) with two replications. Each experimental plot consisted of 2 rows of 1 m length, or 5 rows of 1 m length spaced at 10 cm within and 50 cm between rows. Agronomic practices including weeding, were implemented throughout the crop growth periods under rain-fed conditions.

Data was recorded for agronomic traits including days to 50% flowering and days to 90% physiological maturity. Anthracnose disease reactions were visually assessed at flowering and mid pod filling to pod stage towards physiological maturity using CIAT's 1 - 9 scale; where 1 = no visible symptoms and 9 = severely

**Table 2.** Molecular characterization of the NABE14/G2333 backcross-derived lines, NABE 14 and G2333 for SH18 and SAB3.

Genotype	Description	Markers linked to the resistance genes	
		SH18 ( <i>Co-4<sup>2</sup></i> )	SAB3 ( <i>Co-5</i> )
UGKT-B73	NABE14/G2333 backcross-derived line	+	-
UGKT-B93	"	+	+
UGKT-B133	"	+	+
UGKT-B119	"	+	-
UGKT-B157-4	"	+	+
UGKT-B157-7	"	+	+
UGKT-B160	"	+	+
UGKT-B264-3	"	+	+
NABE14	Recurrent parent: bush, large-seeded red	-	-
G2333	Resistant parent: climbing, small-seeded red	+	+

+ and - indicate presence and absence of the marker respectively.

diseased [20]. Additionally, seed characteristics including 100 seed weight (gm), total number of seeds harvested and seed yield (gm) per plot were documented. The seed yield (gm) per plot was used to calculate yield per hectare. In later seasons (2021B and 2022A), data on the number of pods per plant and the number of seeds per pod were also collected.

#### 2.4. Anthracnose Resistance Evaluation under Controlled Conditions

Basing on the observed resistant reaction to anthracnose disease under natural infestation and high seed yield in the field, the backcross-derived lines UGKT-B157-4, UGKT-B133, UGKT-B160, and UGKT-B157-7 were selected for artificial inoculation with *C. lindemuthianum* under controlled conditions. Genotypes G2333 and NABE14 were included as resistant and susceptible checks respectively. Four seeds of each of the backcross-derived lines and the checks were planted in 1 litre transparent plastic pots filled with loam soil and sand in the ratio of 3:1. This was replicated two times, giving a total of eight plants. The plants were kept in the screen house at room temperature (24°C - 26°C) for 14 days until inoculation. Six isolates of *Colletotrichum lindemuthianum* belonging to six different races previously characterized and preserved at NaCRRI by Nkuboye [21] including: 204A (Race 863), 168A (Race 10), 087A (Race 15), 217A (Race 64), 178-2A (Race 254) and 055A (Race 111) were used for inoculation. These were selected based on the race characterization studies by Nkuboye [21], where all the six selected isolates were avirulent on the resistance source G2333 and virulent on NABE14, the susceptible recurrent parent. The procedure of preparing inoculum followed previously published procedures [22]. The whole plant or seedling method of inoculation was used. At the primary leaf stage, the eight plants for each backcross-derived line and the eight plants each for the parents G2333 and NABE14 were inoculated with spore suspensions ( $1.0 \times 10^6$  spores per ml) of the six *C. lindemuthianum* isolates. All plants were sprayed until there was a visible runoff of the suspension on plant surfaces using a sterile hand sprayer. Control plants were sprayed with sterile distilled water. To avoid cross-contamination, a different set of eight plants for each backcross-derived line and eight plants each for G2333 and NABE14 were used for each isolate. Inoculated bean plants were then covered with transparent 2 kg-capacity polythene bags to maintain high humidity (approximately 95%), then kept in a humid chamber at 18°C - 22°C. After 96 hours, the polythene bags were removed and plants transferred to a screen house for three days for symptom development. Seven days after inoculation, disease severity was assessed visually on each inoculated plant using the severity scale of 1 - 9 proposed by Pastor-Corrales as stated by Ragagnin *et al.* [23]: 1 (plants with no visible symptoms), 3 (few isolated small lesions with a high frequency on mid-veins in the lower leaf surface), 5 (many small lesions scattered on the mid- and secondary veins), 7 (large lesions on leaves, stems and petioles), and 9 (severely diseased or dead).

Plants with scores of 1 - 3 were considered as resistant and 4 - 9 as susceptible.

## 2.5. Statistical Analysis

The statistical analyses were conducted using the General Linear Model (GLM) Procedure of GenStat Fourteenth Edition (VNS International Hempstead, UK). To verify the normal distribution and constant variance assumptions on the error terms for each response variable, a normal probability plot of the residuals and a plot of the residuals vs. fitted values were created, respectively. The independence assumption was met through randomization of the treatments within each block. The data was subjected to analysis of variance (ANOVA) to assess genotype-by-environment ( $G \times E$ ) interaction. Subsequent mean separation utilized Fisher's protected least significant difference test at the 5% level of significance to generate letter groupings. The seed yield data for the eight backcross-derived lines and the recurrent parent NABE14 underwent further analysis using Genotype main effect plus genotype-by-environment interaction (GGE) biplot. Various types of GGE biplots were generated using the metan R package version 1.18.0, to identify high-yielding and stable genotypes across environments [24] [25].

## 3. Results

### 3.1. Combined Mean Agronomic Performance, Combined Analysis of Variance, and Reaction to Anthracnose Disease under Natural Field Infestation

The mean agronomic performance combined across seasons and locations showed that the backcross-derived lines' performances were generally statistically similar to those of the recurrent parent NABE14 (**Table 3**). In relation to the seed yield, total number of seeds, and number of pods per plant, six of the lines were statistically equal to or higher in relation to NABE14. The maximum seed yield was observed with the backcross-derived lines UGKT-B157-4 (1512 kg/ha), followed by UGKT-B157-7 (1288 kg/ha), and UGKT-B133 (1267 kg/ha), compared with NABE14 (1136 kg/ha). All the lines reached 50% flowering at 43 - 45 days after planting and 90% physiological maturity at 86 - 87 days, which was also statistically the same for NABE14. On the other hand, the most significant difference was observed for yield, total seeds, 100 seed weight, number of pods per plant, and number of seeds per pod between these backcross-derived lines and the donor parent, G2333.

The combined analysis of variance showed that the effects of genotype and season were significant ( $p < 0.05$ ) on all the agronomic and seed trait variables as shown in **Table 3**. The effect of location was significant ( $p < 0.05$ ) on all the agronomic and seed trait variables, except number of seeds per pod. The effect of genotype  $\times$  location was only significant ( $p < 0.05$ ) for total number of seeds and days to 90% physiological maturity. Genotype  $\times$  season interaction showed significance ( $p < 0.05$ ) on all variables. Location  $\times$  season interaction was significant ( $p < 0.05$ ) for all variables except number of pods per plant and number of

seeds per pod. Genotype  $\times$  location  $\times$  season interaction showed significance ( $p < 0.05$ ) on seed yield (gm), total number of seeds, 100 seed weight (gm), number of pods per plant, and anthracnose disease scores at flowering and pod stages.

Generally, the mean values for anthracnose disease scores of the backcross-derived lines under natural field infestation both at flowering and pod stages did not differ statistically from that of G2333 (**Table 3**).

Only lines UGKT-B264-3 and UGKT-B119 and the recurrent parent, NABE 14 differed statistically in relation to G2333. Much as the combined disease expression for NABE14 was low, the disease expression was more important in some seasons and locations than others (results not shown). For example, the

**Table 3.** Mean agronomic performance, reaction to anthracnose disease under natural field infestation and combined analysis of variance for 8 NABE14/G2333 backcross-derived bush bean lines, NABE14 and G2333 evaluated for three seasons across five locations in Uganda.

Genotype	Seed yield (kgha)	Total Seeds	100 Seed weight (g)	No_of_pods	No_of_ seeds	DTF	DTM	Anth 1	Anth 2
UGKT-B73	1092bcd	267bc	38b	7bc	5b	43a	86a	1.1ab	1.5ab
UGKT-B93	1186bcd	339bc	40a	8bc	5b	45a	86a	1.5bc	1.8ab
UGKT-B133	1267bc	348bc	39ab	8bc	5b	44a	86a	1.4ab	2.0bc
UGKT-B119	943cd	222c	40a	6c	4b	44a	86a	1.9cd	2.8c
UGKT-B157-4	1512b	453b	38b	9b	5b	44a	86a	1.3a	1.4a
UGKT-B157-7	1288bc	410bc	38b	9b	5b	45a	87a	1.3ab	1.3a
UGKT-B160	1126bcd	367bc	39ab	8bc	5b	45a	87a	1.2ab	1.3a
UGKT-B264-3	800d	216c	39ab	6c	5b	44a	86a	2.2d	2.7c
NABE14 (recurrent)	1136bcd	365bc	39ab	7bc	5b	45a	86a	2.0d	2.5c
G2333 (Resistant)	1998a	1053a	23c	16a	7a	49b	90b	1.0a	1.0a
<b>Mean</b>	<b>1235</b>	<b>404</b>	<b>37</b>	<b>8</b>	<b>5</b>	<b>45</b>	<b>87</b>	<b>1.5</b>	<b>1.8</b>
<b>s.e.d</b>	<b>231</b>	<b>111</b>	<b>3</b>	<b>1</b>	<b>0.2</b>	<b>1</b>	<b>3</b>	<b>0.5</b>	<b>0.3</b>
<b>LSD (5%)</b>	<b>455</b>	<b>219</b>	<b>5</b>	<b>3</b>	<b>0.4</b>	<b>2</b>	<b>5</b>	<b>0.9</b>	<b>0.7</b>
<b>F probability:</b>									
Genotype	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001
Location	<.001	<.001	<.001	<.001	Ns	<.001	<.001	<.001	<.001
Season	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001
Gen. $\times$ loc.	Ns	<.001	Ns	Ns	Ns	Ns	<.001	Ns	Ns
Gen. $\times$ seas.	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001
Loc. $\times$ seas.	<.001	<.001	<.001	Ns	Ns	<.001	<.001	<.001	<.001
Gen. $\times$ loc. $\times$ seas.	<.001	<.001	<.001	<.001	Ns	Ns	Ns	<.001	<.001

No\_of\_pods = Number of pods per plant; No\_of\_ seeds = Number of seeds per pod; DTF = days to 50% flowering; DTM = days to 90% physiological maturity; Anth 1 = anthracnose disease score at flowering stage; Anth 2 = anthracnose disease score at the pod stage, towards physiological maturity; Gen.  $\times$  loc. = Genotype  $\times$  location; Gen.  $\times$  seas. = Genotype  $\times$  season; Loc.  $\times$  seas. = Location  $\times$  season; Gen.  $\times$  loc.  $\times$  seas. = Genotype  $\times$  location  $\times$  season; Means followed by the same letter within the same column are not significantly different ( $p = 0.05$ ) by Fisher's protected least significant difference test.



**Figure 1.** Resistant reaction to anthracnose on pods of UGKT-B157-4 (left) compared with susceptible disease reaction observed on NABE14 (right) in Kabale-Kamuganguzi under natural infestation during 2021 second season.



**Figure 2.** **Top row:** Susceptible reaction to anthracnose on NABE14 (left) compared with resistant reactions on pods of UGKT-B157-4 (centre) and UGKT-B157-7 (right) at pod filling stage under natural infestation in Kabale-Kamuganguzi in 2022 first season. **Middle row:** Susceptible reaction to anthracnose on NABE14 (left) compared with resistant reaction on pods of UGKT-B133 (centre) and UGKT-B160 (right) at pod filling stage under natural infestation in Sheema-Kagango in 2022 first season. **Bottom row:** Susceptible reaction to anthracnose on NABE14 (left) compared with resistant reaction on pods of UGKT-B157-7 (centre) and UGKT-B157-4 (right) at physiological maturity stage under natural infestation in Kabale-Kamuganguzi during 2022 first season.

backcross-derived line, UGKT-B157-4 and other backcross-derived lines showed clear resistant reactions to anthracnose and yet NABE14 was susceptible as



shown in **Figure 1** during 2021 second season.

The 2022 first season also favoured anthracnose disease expression under natural infestation especially on the susceptible genotype NABE14 in Sheema-Kagango, Kabale-Kamuganguzi, and Rakai-Lwanda with susceptible scores of 4 - 8 (results not shown). In contrast, the backcross-derived lines (B73, B93, B157-4, B157-7, and B160) did not show any symptoms (**Figure 2**).

### 3.2. Mean Agronomic Performance at Each Location

The mean agronomic performance of each backcross-derived line at each location over three seasons is presented in **Table 4**. The highest seed yield was recorded at Sheema-Kabwohe, followed by Sheema-Kagango, Rakai-Lwanda, Kabale-Kamuganguzi,

**Table 4.** Mean agronomic performance for 8 NABE 14/G2333 backcross-derived bush bean lines, NABE14 and G2333 averaged across three seasons at each of the 5 locations.

Genotype	Seed yield (kg/ha)					100 Seed weight (g)					Days to 50% flowering			
	1	2	3	4	5	1	2	3	4	5	1	2	3	4
UGKT-B73	1240	1152	999	1225	485	35	38	39	40	38	44	43	46	40
UGKT-B93	1229	1309	1167	1334	448	39	39	40	41	38	45	43	47	40
UGKT-B133	1626	1507	1246	859	350	38	41	41	40	37	45	43	47	41
UGKT-B119	1014	1362	808	823	124	37	42	41	42	37	45	43	46	41
UGKT-B157-4	1715	1611	1389	1803	393	36	38	38	42	36	45	43	46	41
UGKT-B157-7	1571	1203	1081	1713	470	36	38	38	39	36	45	43	47	41
UGKT-B 160	1277	1138	1092	1337	317	36	37	42	41	36	45	43	48	41
UGKT-B264-3	1055	984	588	792	131	36	40	42	40	36	45	42	47	40
NABE 14	894	1597	1345	901	326	37	40	40	38	39	45	44	47	40
G2333	2208	2953	1890	1241	336	23	26	21	22	23	50	48	51	45
<b>Mean</b>	<b>1383ab</b>	<b>1482a</b>	<b>1160b</b>	<b>1203ab</b>	<b>338c</b>	<b>35 b</b>	<b>38 a</b>	<b>38 a</b>	<b>38 a</b>	<b>36b</b>	<b>45</b>	<b>44</b>	<b>47</b>	<b>41</b>
<b>s.e</b>	<b>103</b>	<b>102.9</b>	<b>103</b>	<b>126</b>	<b>178</b>	<b>0.822</b>	<b>0.79</b>	<b>0.845</b>	<b>0.99</b>	<b>1.49</b>	<b>0.32</b>	<b>0.32</b>	<b>0.32</b>	<b>0.56</b>
Genotype	No_of_pods_plant					No_of_seeds_pod					Days to 90% physiological maturity			
	1	2	3	4	5	1	2	3	4	5	1	2	3	4
UGKT-B73	7	8	8	7	7	4	5	4	5	5	83	87	94	73
UGKT-B93	7	10	8	7	8	4	5	5	5	5	83	87	95	73
UGKT-B133	8	9	9	6	7	4	5	5	4	5	84	86	94	76
UGKT-B119	5	7	6	7	4	4	5	4	5	4	84	86	94	74
UGKT-B157-4	7	9	9	10	10	4	5	5	5	4	85	85	94	76
UGKT-B157-7	8	10	9	9	9	5	5	5	5	5	85	87	94	77
UGKT-B 160	7	9	8	8	6	5	5	5	5	5	85	86	94	78
UGKT-B264-3	5	9	5	6	4	4	5	5	4	4	85	85	94	74
NABE 14	5	9	8	6	4	5	4	5	4	4	84	85	95	75
G2333	18	20	19	10	12	7	7	7	7	7	89	89	98	79
<b>Mean</b>	<b>8</b>	<b>10</b>	<b>9</b>	<b>7</b>	<b>7</b>	<b>5</b>	<b>5</b>	<b>5</b>	<b>5</b>	<b>5</b>	<b>85</b>	<b>86</b>	<b>95</b>	<b>75</b>
<b>s.e</b>	<b>0.73</b>	<b>0.73</b>	<b>0.73</b>	<b>0.73</b>	<b>1.03</b>	<b>0.16</b>	<b>0.16</b>	<b>0.16</b>	<b>0.16</b>	<b>0.23</b>	<b>0.57</b>	<b>0.57</b>	<b>0.57</b>	<b>0.80</b>

1 = Sheema-Kagango; 2 = Sheema-Kabwohe; 3 = Kabale-Kamuganguzi; 4 = Rakai-Lwanda, 5 = Rakai-Kitonezi; No\_of\_pods = Number of pods per plant; No\_of\_seeds = Number of seeds per pod.

and lastly Rakai-Kitonezi. Seed yield at Sheema-Kabwohe ranged from 984 to 2,953 kg/ha, with UGKT-B157-4 (1611 kg/ha) as the best performing backcross-derived line. At Sheema-Kagango, it ranged from 894 to 2208 kg/ha, with UGKT-B157-4 (1715 kg/ha), UGKT-B133 (1626 kg/ha) and UGKT-B157-7 (1571 kg/ha) as the best performing backcross-derived lines. At Rakai-Lwanda, seed yield ranged from 792 to 1803 kg/ha, with UGKT-B157-4 (1803 kg/ha) and UGKT-B157-7 (1713 kg/ha) as the best performing backcross-derived lines. At Kabale-Kamuganguzi, seed yield ranged from 588 to 1890 kg/ha, with UGKT-B157-4 (1389 kg/ha) as the best performing backcross-derived line. The yield (124 - 485 kg/ha) at Rakai-Kitonezi was severely affected by prolonged dry spells experienced soon after planting and at the critical crop growth stages during the season.

On the other hand, for the rest of the agronomic variables including 100-seed-weights, number of seeds per pod, days taken to reach 50% flowering and 90% physiological maturity, the backcross-derived lines at each of the locations performed in the same range of those of the recurrent parent, NABE14 (Table 4). The performance of G2333 for the same agronomic variables was different from that of the backcross-derived lines.

### 3.3. Stability Analysis for Seed Yield Based on GGE Biplots

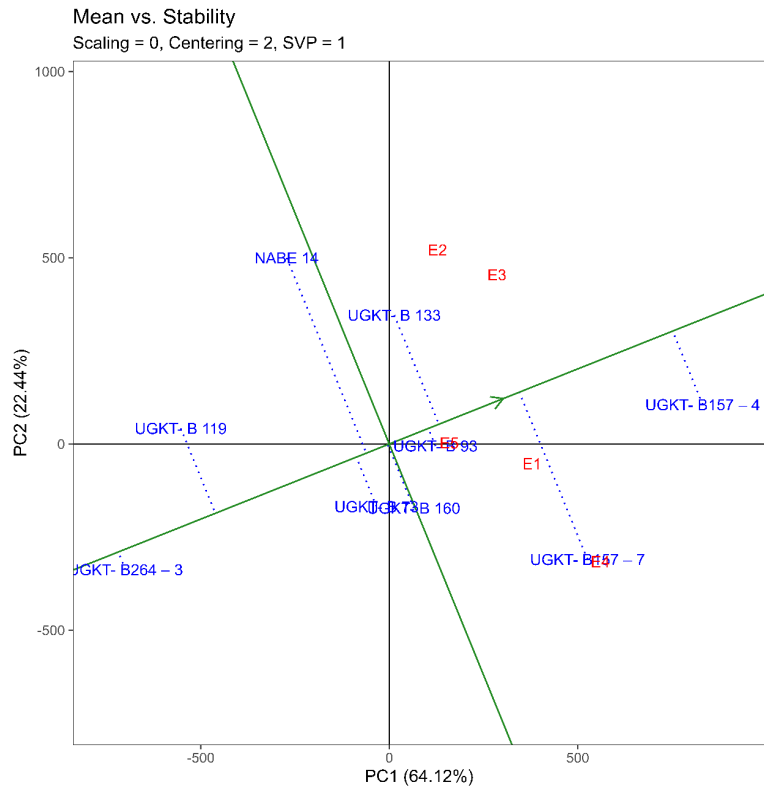
#### 3.3.1. The Total Genotype plus Genotype by Environment (G + GE) Variation

The biplots explained 86.56% of the total variation observed for the seed yield, of which 64.12% was explained by the first principal component (PC1), while the second principal component (PC2) explained 22.44% (Figures 3-6).

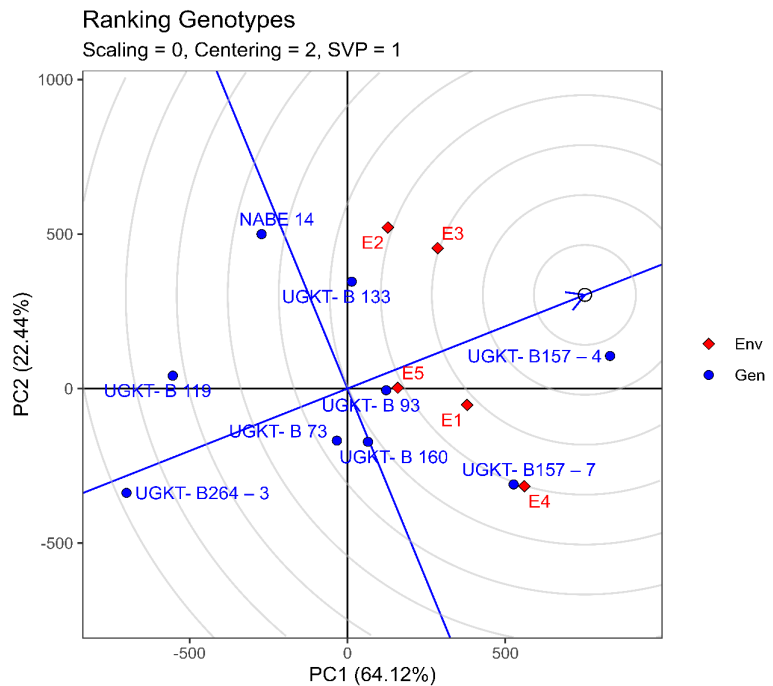
#### 3.3.2. Mean Performance and Stability of the Genotypes

The evaluation of genotypes based on both mean performance and stability across environments is presented as the average-environment coordinate (AEC) view of the GGE biplot (Figure 3). The single-arrowed line on the biplot is the AEC abscissa (or AEA); it points to higher mean yield across environments.

It showed that UGKT-B157-4 had the highest mean yield, followed by UGKT-B157-7 and UGKT-B133, while UGKT-B264-3, followed by UGKT-B119, had the lowest mean yield. The perpendicular line is the AEC ordinate, it points to greater variability (poorer stability) in either direction. Therefore, in terms of stability, UGKT-B93 and UGKT-B264-3 had the smallest projection onto the AEC ordinate and hence were the most stable. Comparing the three highest yielding backcross-derived lines, UGKT-B157-4 had the smallest projection onto the AEC ordinate and thus more stable. UGKT-B157-7 was the second most productive, but had greater projection onto the AEC ordinate and hence great instability. The biplot also revealed that UGKT-B160 had a mean yield similar to the grand mean and small projection making it relatively stable, whereas NABE14 was highly unstable as evident from the greater projection onto the AEC ordinate, though it performed well in some environments.



**Figure 3.** The average-environment coordination (AEC) view to show the mean performance and stability of the genotypes. (Environments: E1 = Sheema-Kagango; E2 = Sheema-Kabwohe; E3 = Kabale-Kamuganguzi; E4 = Rakai-Lwanda; E5 = Rakai-Kitonezi).



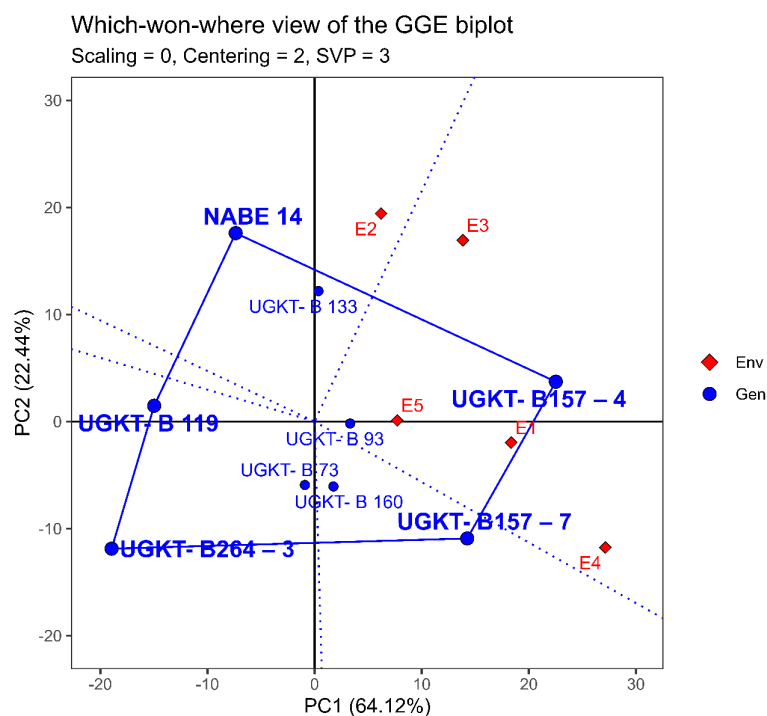
**Figure 4.** The average-environment coordination (AEC) view to rank genotypes relative to an ideal genotype (the center of the concentric circles). (E1 = Sheema-Kagango; E2 = Sheema-Kabwohe; E3 = Kabale-Kamuganguzi; E4 = Rakai-Lwanda; E5 = Rakai-Kitonezi).

### 3.3.3. Ranking Genotypes Relative to the Ideal Genotype

The average-environment coordination (AEC) view to rank genotypes relative to an ideal genotype is presented in **Figure 4**. An “ideal” genotype is located at the center of the concentric circles and genotypes are ranked based on their distance from the ideal genotype. Genotypes located closer to the “ideal genotype” are more desirable than others. The biplot revealed that UGKT-B157-4 was located closer to the “ideal genotype” and was thus more desirable than the others. UGKT-B157-7 located on the next concentric circle after UGKT-B157-4 was also a desirable genotype. UGKT-B264-3 located the farthest was the poorest genotype.

### 3.3.4. Which-Won-Where?

The “which-won-where” biplot view of the relationship between genotypes and environments is presented in **Figure 5**. Genotypes UGKT-B157-4 and UGKT-B157-7 located on the vertices on one side of the polygon, performed the best, while the genotypes UGKT-B264-3, UGKT-B119 and NABE14 also located on the vertices on the other side of the polygon performed the poorest. The biplot showed that UGKT-B157-4 was the winning genotype in most of the environments, *i.e.*, four environments out of five. The other genotypes (such as UGKT-B133, UGKT-B160) located within the polygon were less responsive than the vertex genotypes. The perpendicular lines to the sides of the polygon divided the biplot into five sectors and two mega-environments namely, mega-environment I (E1, E3, E4, and E5) and mega-environment II (E2).



**Figure 5.** The “which-won-where” view of the GGE biplot showing which genotypes performed best in which environment. (E1 = Sheema-Kagango; E2 = Sheema-Kabwohe; E3 = Kabale-Kamuganguzi; E4 = Rakai-Lwanda; E5 = Rakai-Kitonezi).

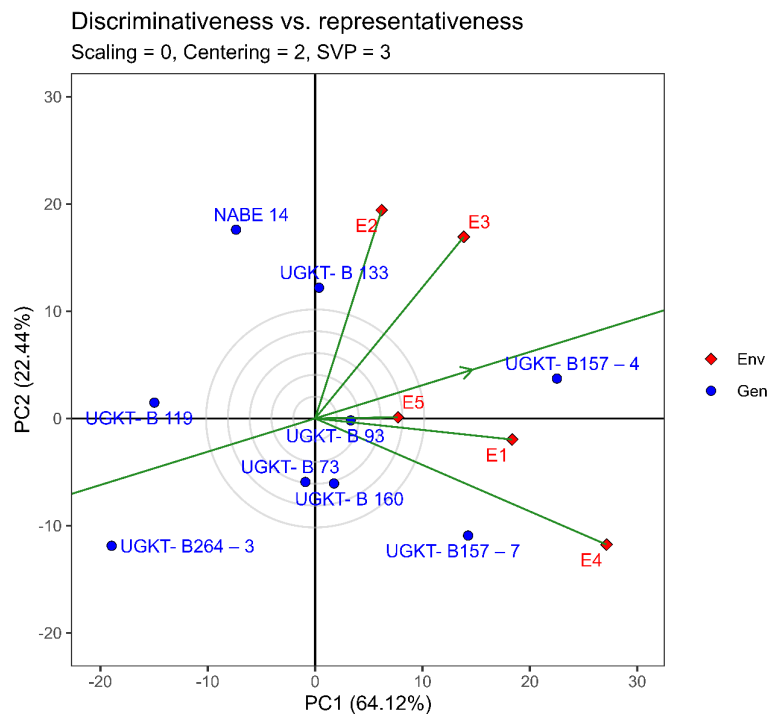
### 3.3.5. Discriminating Ability and Representativeness of the Test Environments

The environment vector plot showing discriminating ability and representativeness of the test environments is shown in **Figure 6**. The biplot revealed that E2 (Sheema-Kabwohe), E3 (Kabale-Kamuganguzi) and E4 (Rakai-Lwanda) with the longest vectors from the biplot origin were the most discriminating (informative) environments, followed by E1 (Sheema-Kagango). E5 (Rakai-Kitonezi) had the shortest vectors from the biplot origin and was the least discriminating test environment.

In the biplot, the Average-Environment Axis (AEA) is the line that passes through the average environment and the biplot origin. The test environment E5 (Rakai-Kitonezi) had a smaller angle with the AEA and was considered as more representative of other test environments, followed by E1 (Sheema-Kagango) and E3 (Kabale-Kamuganguzi); whereas E2 (Sheema-Kabwohe) and E4 (Rakai-Lwanda) that had the largest angles with the AEA were least representative. Therefore, out of the test environments, E3 (Kabale-Kamuganguzi) was both discriminating and representative, whereas E2 (Sheema-Kabwohe) and E4 (Rakai-Lwanda) were discriminating but non-representative test environments.

### 3.4. Anthracnose Resistance Evaluation under Controlled Conditions

Results of artificial inoculation of the backcross-derived lines showed resistant reactions to the six isolates of *Colletotrichum lindemuthianum* (**Table 5**). Genotype

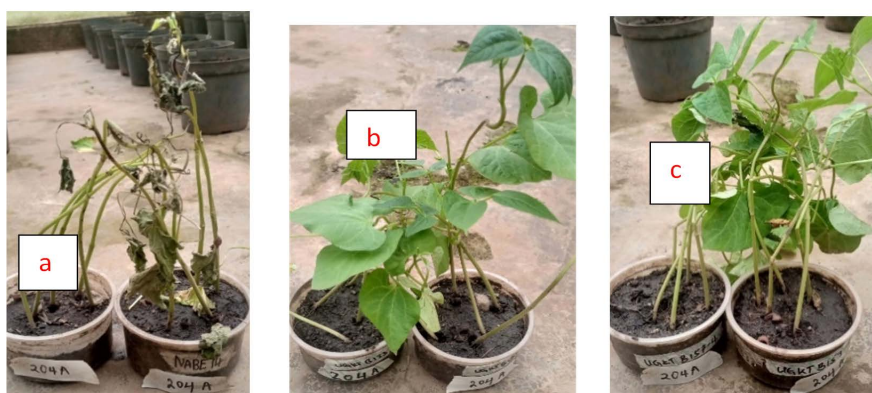
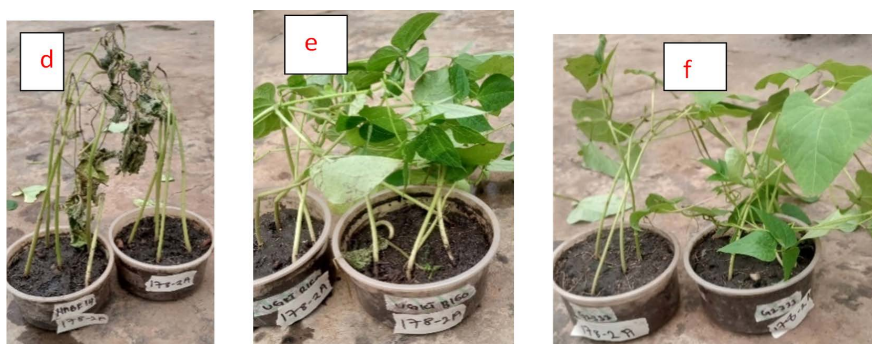


**Figure 6.** Environment vector plot showing discriminating ability and representativeness of the test environments. (E1 = Sheema-Kagango; E2 = Sheema-Kabwohe; E3 = Kabale-Kamuganguzi; E4 = Rakai-Lwanda; E5 = Rakai-Kitonezi).

**Table 5.** Mean disease severity of the NABE 14/G2333 backcross-derived lines, G2333 and NABE 14 seven days after inoculation with six isolates of *Colletotrichum lindemuthianum*.

Line	Mean severity on the 1 - 9 scale when inoculated with six isolates of <i>Colletotrichum lindemuthianum</i>					
	204A (863)	168A (10)	087A (15)	217A (64)	178-2A (254)	055A (111)
UGKT-B157-4	2	1.5	1.3	2.3	1.5	1
UGKT-B160	1.5	1	3	1.8	1.3	1
UGKT-B133	1.5	1	1	2.3	2	2
UGKT-B157-7	1.3	1.3	1	2	1	2
NABE14	6	5	5	5.5	9	5
G2333	1	1	1	1	1	1

1 - 3 = Resistant; 4 - 9 = susceptible.

**Figure 7.** Susceptible reaction on (a) NABE14 and resistant reaction on (b) UGKT-B133 and (c) UGKT-B157-4 seven days after inoculation with isolate 204A.**Figure 8.** Susceptible reaction on (d) NABE14 and resistant reaction on (e) UGKT-B160 and (f) G2333 seven days after inoculation with isolate 178-2A.

G2333 also presented a resistant reaction, while NABE14 was susceptible.

Susceptible and resistant disease reactions after inoculation of the selected lines are illustrated in **Figure 7** and **Figure 8**.

#### 4. Discussion

Generally, the results obtained from this study showed that there was limited

genetic variation among the 8 backcross-derived lines and the recurrent parent, NABE14 for all the tested agronomic variables. This is attributed to the fact that backcrossing results into new lines that are phenotypically identical to the recurrent parent but with the addition of the gene of interest [26]. This also suggested that most of the agronomic characteristics of the recurrent parent NABE14 were fully recovered in the backcross-derived lines. Similarly, the observed none significant differences among the backcross-derived lines themselves for most of the agronomic variables, is also expected given that they have the same genetic background. In relation to seed yield, among the 8 backcross-derived lines, UGKT-B157-4 (1512 kg/ha) had the maximum yield followed by UGKT-B157-7 (1288 kg/ha) and UGKT-B 133 (1267 kg/ha). In relation to the seed type, the backcross-derived lines are the same as NABE14 especially the colour and shape. Their seed sizes also did not differ significantly from that of NABE14. This implies they possess the preferred seed type of NABE14, an important factor for farmer and consumer acceptance. On the other hand, the most significant difference was observed among these backcross-derived lines and the donor parent, G2333 for all the tested agronomic variables.

The presence of significant genotype main effect as well as significant location, season, genotype  $\times$  season, location  $\times$  season, and genotype  $\times$  location  $\times$  season effects for most of the variables suggested differential responses of the backcross-derived lines to environmental changes. The significant genotype  $\times$  environmental interactions (GEI) indicated that there were differences in the relative performance of these lines over locations and seasons. Significant location  $\times$  season interactions reveal that the locations used in the present study differed across seasons. This was expected because of the differences in the weather conditions such as temperatures and altitudes. Rocha *et al.*, [27] reported significant genotype  $\times$  environment interactions for seed yield among common bean families developed for resistance to anthracnose and angular leaf spot assisted by SCAR molecular markers. Similarly, other more recent studies have revealed significant presence of genotype, environment and genotype  $\times$  environmental interactions (GEI) effects for seed/grain yield in common bean [14] [15] [28] [29]. Even in other legume crops such as soybean and mungbean, the presence of important genotype, environment and genotype  $\times$  environmental interactions (GEI) effects for seed yield have been reported [30] [31].

GEI is, therefore, said to occur when different cultivars or genotypes respond differently to diverse environments. GEI associated with each genotype, is a measure of variability or instability of the genotypes [32]. GEI is important only when it causes significant changes in genotype ranks in different environments. From our results given that the GEI was significant, then it was reasonable to proceed with stability analysis to explore such interaction. In common bean, seed yield is an important trait considered by plant breeders as it results in economic benefits to the farmers and the consumers. Therefore, further analysis of GEI was focused only on this particular trait. Good bean genotypes to be rec-

ommended should produce high yields and should remain stable across varying environments. Yan *et al.* [32] recommended that GGE-biplot analysis is the best method for mega-environment analysis and genotype evaluation because it explains more G + GE and is also effective in evaluating test environments. In this research, all the four types of GGE biplots that were considered provided similar results in determining the most outstanding backcross-derived line as UGKT-B157-4. Based on the mean performance vs. stability biplot, UGKT-B157-4 was the highest yielding and also stable as explained by Yan and Tinker [16] and Olivoto and Lúcio [33]. Some backcross-derived lines such as UGKT-B264-3 that were the most stable had the lowest mean yield. UGKT-B93 was also very stable but with a moderate seed yield. Such genotypes were not desirable because according to Yan and Tinker [16], stable genotypes are desirable only when they have high mean performances. On the other hand, the recurrent parent NABE14 was highly unstable as evident from the greater projection onto the AEC ordinate, though it performed well in some environments. This was probably due to its susceptibility to anthracnose disease under natural field infestation in some seasons and locations. NABE14 is a released variety in Uganda for tolerance to root rots [34] [35] and low soil phosphorus [36]; however, during the 2021 second season it showed severe susceptibility to anthracnose disease in Kabale-Kamuganguzi. Similarly, during the 2022 first season, it was susceptible to anthracnose in Sheema-Kagango and Kabale-Kamuganguzi. The Sheema and Kabale districts are hot spots for anthracnose disease evaluation in Uganda. Yan and Kang [13] and Kang [37] stated that differences in insect and disease resistance among genotypes can be associated with stable or unstable performance across environments. Gravois *et al.* [38] also implicated disease resistance or susceptibility as a factor that contributed to GEI.

In this study, the biplot to rank genotypes relative to an ideal genotype further indicated that UGKT-B157-4 was located closer to the “ideal genotype” and was thus more desirable than the other progeny lines. According to Yan and Tinker [16], an “ideal” genotype is located at the center of the concentric circles and is defined to be a point on the AEA (“absolutely stable”) axis in the positive direction. An ideal genotype should have both high mean performance and high stability across environments; genotypes are ranked based on their distance from the ideal genotype. Genotypes located closer to the “ideal genotype” are more desirable than others [16]. Similarly, Olivoto, and Lúcio [33] stated that an ideal genotype has the highest mean yield and is absolutely stable.

The “which-won-where” biplot view of the relationship between genotypes and environments showed that vertex genotypes UGKT-B157-4 and UGKT-B157-7, located on one side of the polygon, performed the best [16]. In this study, testing was limited to only three key target districts with five test environments, namely Sheema-Kagango (E1), Sheema-Kabwohe (E2), Kabale-Kamuganguzi (E3), Rakai-Lwanda (E4) and Rakai-Kitonezi (E5). UGKT-B157-4 was the winning genotype in four out of the five test environments. According to Baenziger and



Hain [26], since the recurrent parent is already a proven variety or line, it is usually not necessary to conduct extensive performance trials for the backcross-derived lines once satisfactory introduction of the desired character has been achieved. Sheema-Kagango (E1), Kabale-Kamuganguzi (E3), Rakai-Lwanda (E4) and Rakai-Kitonezi (E5) were grouped into mega-environment I, implying that they were closely related in terms of genotypic yield performance. This implies that the genotypes exhibited similar performance in these different environments. Sheema-Kabwohe (E2) was in mega-environment II. In Yan and Tinker [39], a mega-environment is defined as a group of locations that consistently share the same best cultivar(s). While in Gauch and Zobel [40], a mega-environment is defined as a portion of a crop-growing region with a fairly homogeneous environment that causes similar genotypes to perform best. The practical application of this is that planting the winning genotype in each mega environment optimizes yield.

In the discriminative ability and representativeness biplot, the length of the environment vectors, is a measure of the discriminating ability of the environments [16]. Sheema-Kabwohe (E2), Kabale-Kamuganguzi (E3) and Rakai-Lwanda (E4) with the longest vectors were the most discriminating (informative) followed by Sheema-Kagango (E1). Rakai-Kitonezi (E5) with the shortest vector was the least discriminating. Yan and Tinker [16], stated that test environments that are consistently non-discriminating (non-informative) provide little information on the genotypes and therefore, should not be used as test environments. One reason why Rakai-Kitonezi (E5) could have been the least discriminating is because of the long dry spell that was experienced during the critical crop growth stages.

In terms of representativeness, Rakai-Kitonezi (E5) was most representative followed by Sheema-Kagango (E1) and Kabale-Kamuganguzi (E3); whereas Sheema-Kabwohe (E2) and Rakai-Lwanda (E4) were least representative. Therefore, out of the test environments, Kabale-Kamuganguzi (E3) was both discriminating and representative. Hence good for selecting generally adapted genotypes. This also implies that testing at Kabale-Kamuganguzi is ideal and can save time and resources. This is in agreement with the earlier participatory evaluation trials that were conducted only in the Kabale district and the data/results obtained were sufficient to support the official release of the recurrent parent, NABE 14, for growing in the mid to high altitude regions of Uganda. While the discriminating but non-representative test environments, *i.e.* Sheema-Kabwohe (E2) and Rakai-Lwanda (E4) are useful for selecting specifically adapted genotypes if the target environments can be divided into mega-environments. Discriminating but non-representative test environments are useful for culling unstable genotypes if the target environment is a single mega-environment.

Biotic stresses are a major constraint to crop productivity. In this study, the backcross-derived lines were assessed for reaction to anthracnose disease under natural field infestation and other major common bean field diseases (results not

shown). Then the most promising backcross-derived lines were further subjected to artificial inoculation with six isolates of *Colletotrichum lindemuthianum* belonging to six different races namely 204A (Race 863), 168A (Race 10), 087A (Race 15), 217A (Race 64), 178-2A (Race 254) and 055A (Race 111). These were selected because according to Nkuboye [21], they were all able to phenotypically differentiate between the resistant parent (G2333) and the susceptible parent, NABE14. They occur in the major bean growing regions of Uganda and were previously characterized by Nkuboye [21]. According to race characterization studies by Nkuboye [21], some of them were virulent races with the potential of infecting 50% - 70% of the differential cultivars (*i.e.*, race 111, race 254, and race 863). The results obtained after artificial inoculation were consistent with those of field evaluation for anthracnose resistance under natural infestation. Interestingly, the four most outstanding backcross-derived lines (UGKT-B157-4, UGKT-B157-7, UGKT-B133, and UGKT-B160) were high yielding and were also observed to be resistant to anthracnose both under natural infestation and artificial inoculation. The observed resistant reaction was similar to the reaction of G2333 suggesting that the backcross-derived lines carried the *Co-4<sup>2</sup>* and *Co-5* anthracnose resistance genes given that G2333 was used as the donor parent in the backcross. G2333, a landrace cultivar known as Colorado de Teopisca from Chiapas, Mexico, carries three anthracnose resistance genes, namely *Co-4<sup>2</sup>*, *Co-5* and *Co-7* [41]. Ragagnin *et al.* [23] reported four pyramided lines derived through marker-assisted backcrossing using cultivar Ruda as the recurrent parent and cultivars Ouro Negro, TO, AB 136, and 277 as donor parents. These four lines were high-yielding and with resistance spectra equivalent to those of the donor parents. Similarly, Costa *et al.* [42] reported black bean lines developed in Brazil through a backcrossing program aided by molecular markers that were high-yielding and resistant to the target pathogens tested. Based on this study, G2333 is an excellent source of the *Co-4<sup>2</sup>* and *Co-5* resistance loci. However, to be effective and for more durable resistance, the gene pyramiding strategy must be a continuous effort. Permanent monitoring for the presence of new virulent races in the field and search for new resistance sources are inherent steps in this breeding strategy. It is evident from the recent race characterization studies by Nkuboye [21], that the resistant lines developed from this study possessing the *Co-4<sup>2</sup>* and *Co-5* also would need to be introgressed with the *Co-1<sup>2</sup>* gene from differential cultivar Kaboon.

## 5. Conclusions

In general, the backcross-derived lines were as phenotypically similar and as productive as the recurrent parent, NABE14, indicating that the agronomic characteristics of the recurrent parent were recovered. Their seed type, especially the colour and shape was the same as NABE14. Their seed sizes did not also differ significantly from that of NABE 14.

According to the GGE biplots, backcross-derived line UGKT-B157-4 was

identified as the best genotype. This genotype combined both high mean seed yield and stability performance across the test environments and was characterized as an ideal genotype and, therefore, identified as candidate for possible release. The study also emphasizes the importance of continuous gene pyramiding efforts to enhance resistance against evolving pathogen races.

Given that the studied lines were also previously genotyped by the SCAR molecular markers SH18 and SAB3 linked to the *Co-4*<sup>2</sup> and *Co-5* anthracnose resistance genes respectively in G2333, interestingly, the resistant reactions to anthracnose disease obtained for this genotype both under natural infestation and artificial inoculation were consistent with the molecular characterization.

### Acknowledgements

The authors greatly acknowledge the Kirkhouse Trust for funding all the research activities that were carried out towards the development and testing of the first-ever common bean lines derived through molecular-marker-assisted backcrossing at NaCRRRI in Uganda. The International Centre for Tropical Agriculture (CIAT)-Kawanda provided seeds of the genotype G2333 and technical guidance in pathology related activities for anthracnose. The Kirkhouse Trust consultant, Dr. Robert Koebner, Norwich, UK is greatly acknowledged for constant technical guidance. Ms. Joy Mugisha from the Sheema district, Mr. and Mrs. Biriso from the Kabale district and the staff from the Rakai district were very helpful during the MET trials.

### Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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