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RESISTANCE OF ANDEAN BEANS AND ADVANCED BREEDING LINES TO ROOT ROTS IN UGANDA

W. AMONGI, F. KATO, A. MALE, S. MUSOKE, S. SEBULIBA, B. NAKYANZI, C. NALUWOOZA, C. ACAM and C. MUKANKUSI

International Center for Tropical Agriculture (CIAT), P. O. Box 6247, Kampala, Uganda
Corresponding authour: w.amongi@cgiar.org

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ABSTRACT

Root rots cause severe yield losses of up to >70% in common bean (*Phaseolus vulgaris* L.) in most parts of the world, with devastating effects on the major commercial bean cultivars in East and Central Africa. Increased intensity of droughts and rains, and higher temperatures influence the occurrence and distribution of root rots, resulting in increased epidemics. The causal pathogens tend to occur in a complex, and since most cultivars do not have broad resistance, adverse effects continue to occur. The objective of this study was to evaluate the levels of dual resistance of new breeding lines (BL) developed for root rot resistance, as well as Andean (ADP) genotypes, for resistance to Fusarium (FRR) and Pythium root rots (PRR). Altogether, 316 new BL developed for root rot resistance and 295 ADP bean genotypes were evaluated at Kawanda in Uganda. There were significant differences ($P<0.05$) among genotypes for both root rots and yield. Thirty and 1.9 percent of the BL and ADP genotypes expressed resistance to both root rots. In addition, more than 80% of ADP genotypes showed susceptibility to both root rots. Yield was generally poor with means of 458 kg ha^{-1} for ADP, and ranging from 949 to 1075 kg ha^{-1} for the BL groups. Nonetheless, the 0.3 and 2.4% of the ADP and BL that yielded >2000 kg ha^{-1} expressed high yield potential, considering that majority of the genotypes yielded below 1000 kg ha^{-1}.

Key Words: Dual resistance, Fusarium, *Phaseolus vulgaris*, Pythium

RÉSUMÉ

Les pourritures des raciness de haricot (*Phaseolus vulgaris* L.) causent plus 70% de pertes de rendement allant dans la plupart des régions du monde, avec des effets dévastateurs sur les principaux cultivars de haricots commerciaux en Afrique orientale et centrale. L' augmentation de l' intensité des sécheresses et des precipitations et les températures plus élevées influencent l'apparition et la distribution des pourritures des racines, ce qui entraîne une augmentation des épidémies. Les agents pathogènes causaux ont tendance d' apparaître dans un complexe, et comme la plupart des cultivars n'ont pas une large résistance, des effets indésirables continuent d' apparaître. L'objectif de cette étude était d'évaluer les niveaux de double résistance des nouvelles lignées de sélection (BL)

développées pour la résistance à la pourriture des racines, ainsi que les génotypes andins (ADP), pour la résistance à la pourriture des raciness causée par Fusarium (FRR) et Pythium (PRR). Au total de 316 nouveaux BL développés pour la résistance à la pourriture des racines et 295 génotypes de haricot ADP ont été évalués à Kawanda en Ouganda. Il y avait des différences significatives (P <0,05) entre les génotypes pour les pourritures des racines et le rendement. Trente et 1,9 pour cent des génotypes BL et ADP ont montré une résistance pour deux raciness pourries. De plus, plus de 80% des génotypes d'ADP ont montré une susceptibilité pour deux raciness pourries. Le rendement était généralement médiocre avec des moyennes de 458 kg ha^{-1} pour l'ADP, et allant de 949 à 1075 kg ha^{-1} pour les groupes BL. Néanmoins, les 0,3 et 2,4% d'ADP et de BL qui ont donné plus de 2000 kg ha^{-1} ont montré un potentiel de rendement élevé, étant donné que la majorité des génotypes ont produit moins de 1000 kg ha^{-1}.

Mots Clés: Double résistance, Fusarium, *Phaseolus vulgaris*, Pythium

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is widely consumed for calories, protein and micronutrients (Myers and Kmiecik, 2017), especially in Africa where over 400 million people consume it (CIAT, 2018) because it is cheap and has a long shelf life. However, many economically important diseases such as root rots (*Fusarium solani* f.sp. *phaseoli*, *Pythium ultimum*, *Rhizoctonia solani, Sclerotium* species) threaten bean production (Mukankusi, 2008; Paparu *et al*., 2014). Root rot is a worldwide challenge, especially in Brazil, Central America and African countries (Schneider *et al*., 2001; Macedo *et al*., 2017) where climate change has greatly influenced the occurrence and distribution of this disease (Macedo *et al.,* 2017; Paparu *et al*., 2017); thereby aggravating its effects.

Root rots are particularly devastating on major commercial and adapted bean cultivars in eastern and central Africa, where different casual pathogens; majorly *Pythium* spp., *Fusarium* spp., *Sclerotium rolfsii* and *Rhizoctonia solani* were described to occur in the same field (Tusiime, 2003; Mukankusi, 2008). *Fusarium solani* species complex that have been reported to cause root rot in common bean include *F. brasiliense*, *F. virguliforme* and *F. cuneirostrum* (O'Donnell *et al*., 2008; Aoki *et al*., 2014).

In Uganda, *F. cuneirostrum* has been identified as the causative agent (Sang *et al*., 2018). These complexes present more challenges to disease management through agronomic practices and chemical treatments. In a study conducted by Naseri *et al*. (2016), high incidences of Fusarium root rot, and consequently seed yield losses, were associated with furrow irrigation, farm machinery, and lack of fertiliser and herbicide use. Recommended practices for root rot control include seed dressing with appropriate fungicides, efficient weed management, rotation of beans with appropriate crops, shallow seed planting, other agronomic practices that promote plant root development (Naseri and Hemmati, 2017), and sowing on raised beds (Naseri and Moradi, 2015; Habtegebriel and Boydom, 2016).

Evidence based studies on the relevance of some of these practices in controlling root rots in bean production were documented by several authors (Naseri, 2013; Naseri and Moradi, 2015; Naseri *et al*., 2015; Kalantari *et al*, 2018; Naseri and Veisi, 2019). The use of some of these practices (e.g. seed dressing with appropriate fungicides and planting on raised beds) is limited to small farms that dominate eastern and central Africa bean production areas. Besides, alternative hosts crops like maize and groundnuts, which are commonly rotated with beans in Uganda, promote the spread of southern blight (*Sclerotium rolfsii*) (Paparu *et al*., 2017). Use of resistant varieties would represent an easier and more efficient management option for the

smallholder farmers, who dominate the production segment of the common bean value chain (Navarro *et al*., 2003; Abawi *et al*., 2006; Habtegebriel and Boydom, 2016).

Over the years, several studies have been undertaken on the mechanism of inheritance of root rots, caused by *Fusarium solani* (*Fusarium cuneirostrum*) and *Pythium ultimum* in crosses of Andean and Mesoamerican genotypes. For *F. solani*, quantitative inheritance and high environment interaction, which tend to delay germplasm development, have been reported (Schneider *et al*., 2001; Roman-Aviles and Kelly, 2005; Mukankusi *et al*., 2011).

Mukankusi *et al*. (2011) found two to four additive genes, modified by dominant epistasis in a full diallel mating of 12 Andean and Middle American genotypes. In addition, Ongom *et al*. (2012) reported one to three partially dominant loci, modified by epistasis in a full diallel mating of five Andean and Middle American genotypes. Several quantitative trait loci have been reported and validated for marker-assisted breeding for FRR resistance in crosses of Andean and Middle American genotypes (Schneider *et al*., 2001; Roman-Aviles and Kelly 2005; Kamfwa *et al*., 2013; Wang *et al*., 2018). Abawi and Pastor-Corrales (1990) also reported quantitatively controlled resistance for *Pythium ultimum*. However, a single dominant gene governing resistance in the Andean and Mesoamerican breeding lines MLB-49-89A, AND1062 and RWR719 was suggested by Otsyula (2010).

Considering this information, several Fusarium or Pythium resistant lines with single or multiple genes have been developed, within and across *Phaseolus* species (Tusime, 2003; Mukankusi *et al*., 2010; Obala, 2012; Binagwa *et al*., 2016; Kyomugisha, 2018; Mukankusi *et al*., 2018). However, due to the diverse nature of the root rot pathogens, and changing weather conditions that favour a complex occurrence of multiple species, disease resistance is often broken down over time (Brown, 2015). The objective of this study was to evaluate the levels of dual resistance of new breeding lines (BL) developed for root rot resistance, as well as Andean (ADP) bean genotypes, for resistance to Fusarium (FRR) and Pythium root rots (PRR).

MATERIALS AND METHODS

Experimental site. The study was conducted in the screen house and experimental fields at the International Centre for Tropical Agriculture (CIAT) at the National Agricultural Research Laboratories (NARL), Kawanda. The site is located at 0°252 N, 32° 31'E and at an elevation of 1190 m above sea level.

Germplasm description. Three hundred and sixteen advanced bush bean breeding lines of *Phaseolus vulgaris,* developed for root rot resistance by the common bean research team at Michigan State University, were used in this study (Fig. 1). Additionally, 295 genotypes, coded ADP (Andean diversity panel), which were part of the global collection of Andean germplasm from North, Central, and South America, Africa, Europe and Asia (Cichy *et al*., 2015), were also included in the study.

The panel comprised of landraces, breeding lines and varieties from public and private breeding programmes, making a representative sample of the genetic diversity present in the Andean gene pool (Fig. 1). In addition, 14 commercial cultivars and other 3 genotypes were included as control lines. Overall, 628 genotypes belonging to 12 market classes (Fig. 1) were evaluated twice in the screen house, for resistance to the most virulent isolates of *Fusarium cuneirostrum* (FSP-3) identified by Mukankusi (2008) and *Pythium ultimum* (MS61). Yield related attributes were phenotyped in the field for two seasons. The new breeding lines (BL) were received in four sets that were evaluated separately for both root rot resistance and yield performance.

Resistance to *Fusarium cuneirostrum*. Isolate FSP-3 stored on Agar slants at NARL-Kawanda was sub-cultured onto potato dextrose agar (PDA) plates. These were grown

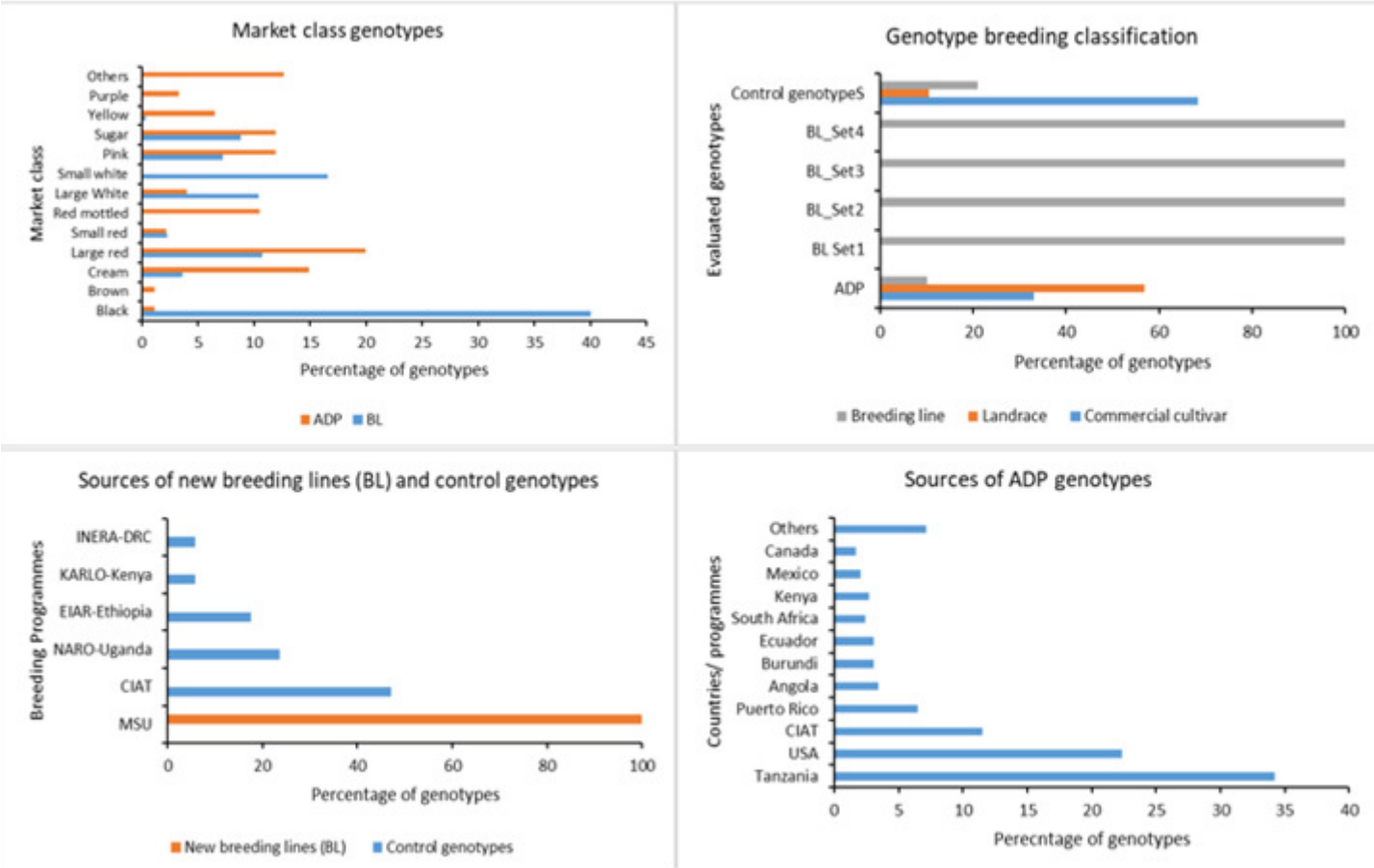

Figure 1. Percentage of the evaluated 628 genotypes belonging to the different market classes of common bean, and their breeding classification and source. ADP = Andean diversity panel, BL = new breeding lines, MSU = Michigan State University, CIAT = International Center for Tropical Agriculture, NARO-Uganda = National Agricultural Research Organisation, EIAR-Ethiopia = Ethiopian Institute of Agricultural Research, KARLO-Kenya = Kenya Agricultural and Livestock Research Organization, INERA-DRC = The National Agricultural Study and Research Institute - Democratic Republic of Congo, USA = United States of America.

for 21 days, for 12:12 light and darkness photoperiod, on laboratory benches at room temperature (22 ± 2 °C). Inoculum was prepared on steam-sterilised sorghum (*Sorghum bicolor*. L) grains as described by Mukankusi (2008). Pathogen colonised grains were mixed thoroughly and added to wooden trays (0.74 m x 0.42 m x 0.115 m) filled to 2/ 3 capacity with steam-sterilised loamy sand soil at a rate of one 500 ml bottle of inoculum per tray, before planting. Each tray was covered with polyethylene bags for 7 days to facilitate sporulation, before repeatedly planting the susceptible control line, CAL96, in the soil to increase inoculum levels prior to planting the experiment. When a score of 9, on a 1 to 9 scale was attained, CAL96, was uprooted, the soil from all trays were poured onto a clean plastic sheet, mixed and redistributed into the trays (Mukankusi, 2008), and the experiment was laid out in a randomised complete block design using trays. The experiment was replicated three times during each of the two screening cycles in 2016/ 2017. Each replication contained 10 plants of the same genotype. In each tray, a row of each of the two control genotypes, MLB-49-89A (resistant) and CAL96 (susceptible), was planted.

Disease severity was assessed at 21 days after planting (Abawi and pastor-Corrales, 1990). For this, plants were carefully uprooted and the hypocotyls and roots washed using tap water to remove soil, before visually rating the lower hypocotyl discoloration on a 1 to 9 scale (Abawi and Pastor-Corrales, 1990; IBP, 2013).

Resistance to *Pythium ultimum*. Isolate MS61, stored at NARL-Kawanda was reactivated by sub-culturing onto corn meal agar (CMA) media. A method described by Mukankusi *et al*. (2018) was used to prepare the inoculum. Finger millet (*Eleusine coracana*) grains were used as a medium for fungal growth and 300 g per bag were placed in several plastic autoclavable polyethylene bags. To each bag was added 300 ml of tap water prior to double sterilisation in an autoclave at 121 °C for 60 minutes. Each bag was inoculated with 3 - 4 discs of actively growing agar blocks bearing a species culture, by placing the discs at different positions in the finger millet bag.

The bags were incubated in a sterile environment in darkness at room temperature (22 ± 2 °C), for at least 12 days to allow uniform growth over the millet grains. After incubation, the colonised millet grains with Pythium inoculum were mixed in steam-sterilised soil, at a ratio of 1:8 v/v inoculum to soil and then placed in wooden flat trays of 0.74 m x 0.42 m x 0.115 m, and left to stabilise in the soil for 7 days. Prior to planting test genotypes, the susceptible control variety (CAL96) was planted in the soil to increase inoculum levels. This process was repeated until a score of 9 on a 1 to 9 scale was obtained. Thereafter, CAL96 was uprooted and the experiment was laid out in a randomised complete block design (RCBD), with two replications, in wooden trays during two screening cycles in 2016/ 2017.

Each tray was planted with 10 test and 2 control genotypes, a resistant (RWR719) line and a susceptible (CAL96) commercial cultivar. Ten plants of each genotype were established in each row. After three to five days after planting, the trays were flooded with water and this was maintained for about 10 days to create a favourable microclimate for the pathogen to move through the soil pores and infect the seedlings. In the 3rd week, the soil water level was slowly reduced by decreasing the frequency of watering to approximately 3 times per week. Pythium root rot symptoms evaluation was done at the end of the 3rd week, by uprooting the genotypes, washing the roots with tap water, and then scoring the disease symptoms using a 1-9 scale (Abawi and Pastor-Corrales, 1990; IBP, 2013).

Field trials. The field experiment was established during the first (A) and second (B)

rainy seasons during April to July and September to December in 2014 and 2015. The experiment was laid out in an alpha lattice design, with two replications. Each group of genotypes, (ADP, BL_*Set1*, BL, *Set2* and BL_*Set3*) was randomised and planted separately. Plots representing each genotype within a replication were of 3 rows by 3 m in length, with row and plant spacing of 50 cm by 10 cm, respectively.

Each setup was weeded thrice and an insecticide, Dimethoate and two fungicides (Mancozeb and Ridomil), were applied weekly, until flowering. The recommended manufacturer's rate of each pesticide was used. Granular N:P:K 17:17:17 fertiliser was hand-applied just before planting, at the rate equivalent to 125 kg ha^{-1}.

Data collection and analysis. Days to flowering (DF) was recorded as the number of days from planting to the day when 50% of plants had at least one flower. Physiological maturity (DPM) was recorded as the number of days from planting to the day when the first pods began to discolour in 50% of the plants (CIAT 1987; IBP, 2013). Growth vigour was recorded on 1-5 scale; where 1 = Excellent, 2 = Good, 3 = Intermediate, 4 = Poor, 5 = Very poor (IBP, 2013). Seed collection for yield began when 90% of the pods had changed colour to yellow (Munoz-Perea *et al.,* 2006). The seeds were sun-dried before recording seed weight and moisture content (MC) per plot. The MC was obtained using a SINAR Model 6095 AgriPro Moisture Analyzer and the weights were adjusted to 13% MC for yield analysis.

Data collected for both screen house and field study components were analysed separately using unbalanced designs option in GenStat (VSN International, 2019) to assess variability. Data for disease resistance from single plants were averaged prior to analysis of variance (ANOVA).

RESULTS

The genotype responded significantly ($P< 0.001$) to PRR and FRR in the Andean Diversity Panel (ADP) and in most of the four groups (*BL_Set1, BL_Set2, BL_Set3 and BL_Set4*) of the new Breeding lines (BL) (Table 1). The interaction of genotype by disease screening cycle (Genotype x Screening) or yield trial season (Genotype x Season) was significant ($P <0.001$) in PRR, FRR, DF and DPM in the ADP. The Genotype x Screening (Season) interaction was also significant in all the four groups of BL in at least two variables (Table 1).

Plant parameters. The majority of the genotypes had an intermediate vigour in both ADP (67.2%) and the new BL (57.9%); and less than 3.0% of genotypes had excellent or good vigour in both groups (Fig. 2). Although 24.1% of ADP genotypes flowered in less than 35 days (early), non-reached physiological maturity in less than 60 days. For the new BL, 62.5% flowered after 40 days (late) and 92.3% matured after 70 days (late). Above 85% of genotypes yielded less than 1500 kg ha^{-1} in both ADP and BL; 0.3 and 2.4% of ADP and BL yielded higher than 2000 kg ha^{-1} (Fig. 3).

Resistance to Pythium (PRR) and Fusarium root rot (FRR). Generally, there were more genotypes resistant to FRR than to PRR in the screen house (Fig. 4). The majority (>80 %) of ADP showed susceptibility to both root rot pathogens, with 3.1 and 10% expressing resistance to PRR and FRR, respectively. For new breeding lines, 46.1 and 51.7%, were resistant to PRR and FRR.

Table 2 shows the response of a set of genotypes that expressed resistance to Pythium and Fusarium root rot, in comparison with the control genotypes. Of the 14 commercial cultivars included as control genotypes, only

TABLE 1. Analysis of variance for assessed variables during two screening activities in the field and screen house

Change	d.f.	PRR	FRR	PLNTVIG	DF	DPM	YDHA
ADP							
Screening (Sea)	1	0.24*	0.52**	11.42***	11088.3***	7258.6***	9050612.0**
Rep/ Screening (Sea)	2	0.02	0.37**	0.87	77.2***	14.2	1204643
Genotype	298	11.84***	22.76***	0.48***	18.2***	17.8***	1123076
Genotype x Screening (Sea)	189	0.14***	0.09***	0.34	20.9***	14.6***	990055
Residual	435	0.06	0.07	0.33	11.2	8.2	1092044
Total	925	3.02	5.73	0.4	27.7	20.8	1081903
BL Set1							
Screening (Sea)	1	1.31	2.64	31.55*	1011	17400.8**	87052
Rep/ Screening (Sea)	2	0.31	13.41***	0.78***	1815.5	22.3	1392564.5 **
Genotype	88 (76)	17.95***	10.5***	0.28	1210.8	32.6	456300.9
Genotype x Screening (Sea)	88 (76)	1.58***	1.21	0.22***	1173.2	26.9***	351525.3**
Residual	176 (152)	0.24	1.15	0.08	1126.8	7.9	223339.6
Total	355 (307)	4.96	2.81	0.24	1155.8	62.3	276673.9
BL_Set2							
Screening (Sea)	1	1.86	0.16	4	334.6*	4595.9**	5956338*
Rep/ Screening	2	4.67***	2.36	3.30***	1	17.9	220593.5
Genotype	54 (56)	23.32***	17.5	0.33	46.3***	93.3*	769104.9*
Genotype x Screening (Sea)	54 (56)	0.32	0.25	0.46**	19.5***	50.4***	387104.4*
Residual	108 (112)	0.44	1.07	0.26	2.6	6.8	290221.6
Total	219 (227)	6.1	4.93	0.34	17	54.2	415590

TABLE 1. Contd.

Change	d.f.	PRR	FRR	PLNTVIG	DF	DPM	YDHA
BL_Set4							
Screening (Sea)	1	0.4	0.12	1.58	644.4	5233.2*	108939412.0**
Rep/ Screening (Sea)	2	0.61***		0.95*	147.4***	221.5***	407707
Genotype	102 (126)	2.14***	1.26***	0.72	8.4	15.9	681371.4
Genotype x Screening (Sea)	102	0.29***		0.54***	9.5	16.3**	702924.1***
Residual	204 (126)	0.04	0.59	0.22	7.4	9.2	352162.6
Total	411 (253)	0.63	0.92	0.43	10.4	26.2	782850
BL_Set3							
Screening (Sea)	1	6.15	1.18				
Rep/ Screening (Sea)	2	20.23***	1.71***				
Genotype	76	12.78***	11.74***				
Genotype x Screening (Sea)	76	1.1	0.18				
Residual	152	1.39	0.2				
Total	307	4.28	3.06				

ADP = Andean diversity panel, BL = new breeding lines, Sea = Season, Rep = Replication, d.f. = Degree of freedom (d.f. in parenthesis is for screen house evaluation), PRR = Pythium root rot in the screen house, FRR = Fusarium root rot in the screen house, PLNTVIG = Plant vigour in the field, DF = Days to 50% flowering, DPM = Days to 50% physiological maturity, YDHA = Yield (kg ha^{-1}), *, **, *** significant at 0.05, 0.01 and 0.001 probability level respectively

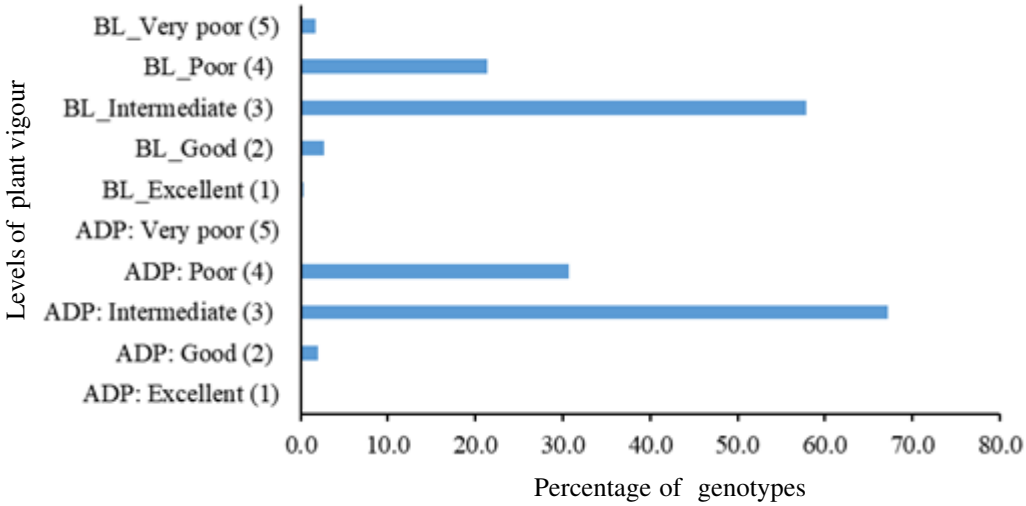

Figure 2. Percentage of 628 genotypes for plant vigour rated on a scale of 1 to 5 for Andean Diversity panel (ADP) and new breeding lines (BL) in during field trials in 2014 and 2015.

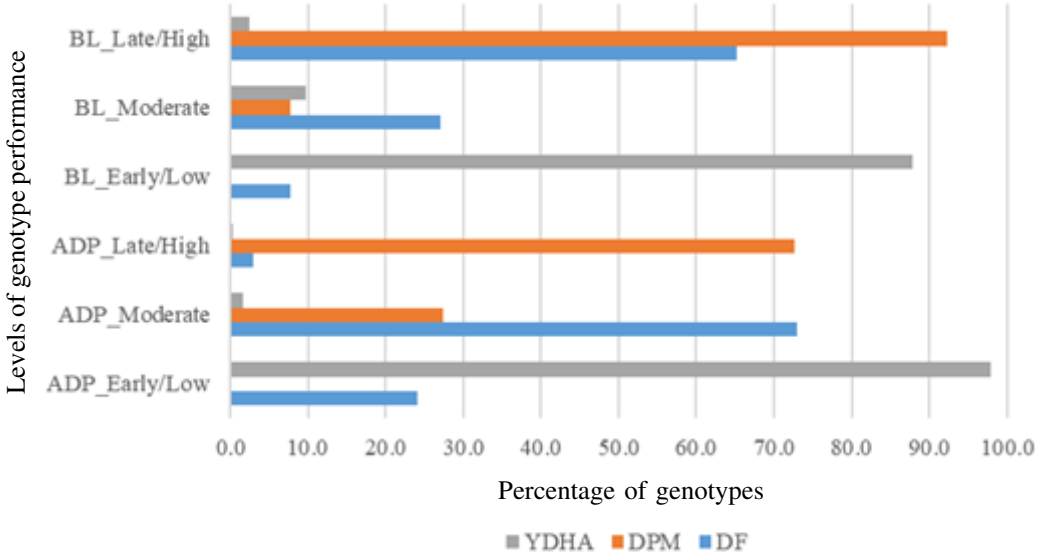

Figure 3. Percentage of 628 genotypes for yield (YDHA), days to flowering (DF) and days to physiological maturity (DPM) in 2014 and 2015. ADP = Andean diversity panel, BL = New breeding lines, DF = Days to flowering: Early = <35, Moderate = 35-40, 60-70, Late = >40, DPM = Days to physiological maturity: Early = <60, Moderate = 60-70, Late = >70, YDHA = Yield): Low = >1500 kg ha^{-1}, Moderate = 1500-2000 kg ha^{-1}, High = >2000 kg ha^{-1}.

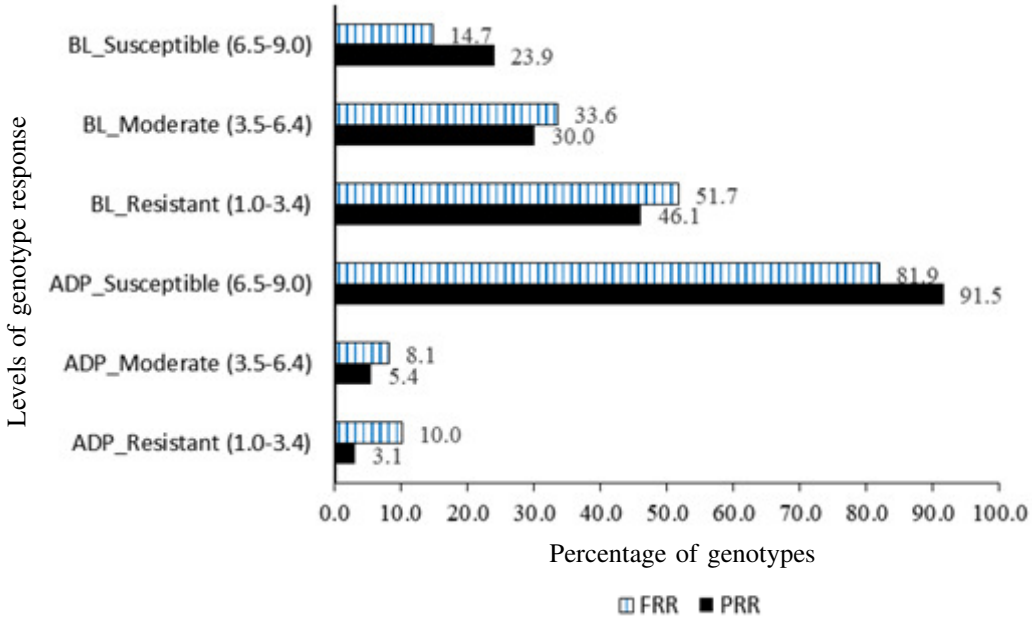

Figure 4. Percentage of 628 genotypes (lines) resistant/susceptible to *Pythium ultimum* isolate MS61 and *Fusarium cuneirostrum* isolate FSP-3. ADP = Andean diversity panel, BL = New breeding lines, FRR = Fusarium root rot, PRR = Pythium root rot.

three were resistant to both FRR and PRR. In total, 1.9 and 30.0% of the ADP and the new BL were resistant to both pathogens, respectively. The seed types of genotypes that expressed dual resistance were cream, red mottled, red kidney and red for the ADP, and cream, pink, sugar, small-medium white, small red and black for the new BL (Table 2). The dual resistance in the new BL was dominated by set 4 that was composed of only black beans; 75.0% of the lines in this set expressed dual resistance (Fig. 5).

Trait association. Most correlation coefficients were significant (P<0.001) and moderate (Table 3). PRR and FRR were positively correlated [0.79***], showing that genotypes with lower severity for PRR tended to have lower severity for FRR. The associations of both PRR [r=0.76***] and FRR [r=0.67***] to weigh of 100 seeds (SW100) showed that small seeded (<25 g) genotypes expressed more resistance to both root rots than large seeded genotypes (>40 g). Similarly, the lower the severity for PRR [r=-0.40***] and FRR [r=-0.32***], the higher the yield (Table 3).

DISCUSSION

Disease resistance. All genotypes showed root rot symptom of varying levels, with majority expressing severe symptoms for Fusarium and Pythium root rots (Table 2). BL exhibited more resistance than susceptibility to the two root rots separately; whereas ADP expressed more susceptibility than resistance. This showed that the majority of popular beans in the hands of farmers especially in Africa where Andean beans are most preferred are susceptible to root rots. The levels of resistance observed in BL show significant progress towards improvement of Andean beans of African preference.

In both ADP and BL, more genotypes exhibited resistance to Fusarium than to Pythium root rot (Fig. 4). Literature showed more breeding focus on improvement of

TABLE 2. Response to *Pythium ultimum* isolate MS61 for and *Fusarium cuneirostrum* isolate FSP-3 of a selected set of the ADP and the new breeding lines during screening cycle 1 and 2

Entry	PRR_1	PRR_2	Response	FRR_1	FRR_2	Response	Market class
ADP							
1. ADP-441	2.1	2.1	R	2.0	2.1	R	Small cream
2. ADP-517	2.1	2.3	R	2.2	2.2	R	Medium carioca
3. ADP-438	2.1	2.1	R	3.1	2.4	R	Medium red mottled
4. ADP-58	2.2	2.2	R	2.0	2.1	R	Medium red kidney
5. ADP-445	3.1	3.3	R	2.3	2.3	R	Medium red mottled
6. NABE 6	2.1	2.1	R	2.0	2.0	R	Small white
7. NABE 3	2.2	2.3	R	2.1	2.2	R	Small red
8. Awash Melka	2.2	2.1	R	2.1	2.2	R	Small white
9. K131	2.6	3.5	M	2.7	3.2	R	Small carioca
10. NABE 2	3.5	2.9	M	2.3	2.8	R	Small black
11. NABE 22	3.6	3.2	M	2.5	2.4	R	Medium purple mottled
12. NABE 18	3.9	3.6	M	5.9	5.1	M	Large purple mottled
13. KATB1	9.0	9.0	S	9.0	9.0	S	Medium yellow
14. Masindi yellow long	9.0	9.0	S	9.0	9.0	S	Large yellow
15. Masindi yellow short	9.0	9.0	S	9.0	9.0	S	Large yellow
16. NABE 5	9.0	9.0	S	9.0	9.0	S	Large sugar
17. NABE 19	9.0	9.0	S	9.0	9.0	S	Large red mottled
18. NABE 20	9.0	9.0	S	9.0	9.0	S	Large sugar
19. RANJONOMBY	9.0	9.0	S	9.0	9.0	S	Medium white
CAL96	9.0	9.0	S	9.0	9.0	S	Large red mottled
MLB-49-89A	-	-	-	2.0	2.0	R	Small black
RWR 719	2.1	2.0	R	-	-	-	Small red
Mean	8.3	8.3		7.8	7.8		
CV (%)	3.9	0.5		7.1	7.6		
SE	0.31	0.04		0.53	0.56		
LSD	0.6	0.1		1.1	1.1		

TABLE 2. Contd.

Entry	PRR_1	PRR_2	Response	FRR_1	FRR_2	Response	Market class
BL_Set1							
20. N12468	2.0	2.0	R	2.7		R	Small White
21. N11228	2.7	3.0	R	2.7		R	Small White
22. P11506	2.0	2.0	R	2.8		R	Medium carioca
23. N11277	3.1	2.0	R	2.8		R	Small white
24. N12467	2.9	2.3	R	2.8		R	Medium white
25. I11271	2.0	2.0	R	3.0		R	Small red
26. 108958	3.2	2.8	R	3.1		R	Medium white
RWR 719	2.1	2.0	R	-		-	Small red
MLB-49-89A	-	-	-	2.1		R	Small black
Mean	4.6	4.7		3.8			
CV (%)	6.8	10.5		29.8			
S.E	0.32	0.51		1.15			
LSD (5%)	0.8	1.0		3.2			
BL_Set2							
27. K11714	2.8		R	2.4		R	Large sugar
28. K14703	3.1		R	2.4		R	Medium pink
29. G14507	2.8		R	2.6		R	Small white
30. R12752	3.3		R	2.9		R	
31. R12844	2.1		R	3.2		R	Small red
CAL96	9		S	8.9		S	Large red mottled
RWR 719	2		R	-		-	Small red
MLB-49-89A	-		-	2.2		R	Small black
Mean	5.5			4.1			
CV (%)	21.9			10.8			
S.E	1.21			0.44			
LSD (5%)	3.4			0.9			

TABLE 2. Contd.

Entry	PRR_1	PRR_2	Response	FRR_1	FRR_2	Response	Market class
BL_Set3							
32. ACC:B11625	2.3		R	2.8		R	Small black
33. NE34-12-42	2.2		R	3.3		R	Small black
34. ACC:182054/ ID PUEBLA 152	2.1		R	3.4		R	Small black
CAL96	9		S	8.9		S	Large red mottled
RWR 719	2.1		R	-		-	Small red
MLB-49-89A	-		-	2.4		R	Small black
Mean	5.7			6.5			
CV (%)	12.3			16.6			
S.E	0.70			1.10			
LSD (5%)	1.9			2.1			
BL_Set4							
35. B11604	2	2	R	2.1		R	Small black
36. B11580	2	2	R	2.2		R	Small black
37. B11572	2	2	R	2.2		R	Small black
38. B11539	2	2	R	2.3		R	Small black
39. B11509	2	2	R	2.3		R	Small black
40. B11514	2	2	R	2.4		R	Small black
41. B11588	2	2	R	2.4		R	Small black
CAL96	9	9	S	8.8		S	Large red mottled
RWR 719	2	2	R	-		-	Small red
MLB-49-89A	-	-	-	2.3		R	Small black
Mean	2.2	2.2		3.1			
CV (%)	9.3	2.4		25.1			
S.E	0.25	0.06		0.77			
LSD (5%)	0.7	0.2		1.9			

PRR = Pythium root rot (1= first screening, 2 = second screening), FRR = Fusarium root rot (1= first screening, 2 = second screening), CV (%) = Coefficient of variation, S.E. = Standard error of the mean, LSD (5%) = Least significant difference, R = Resistant (score 1.0-3.4), M = Moderately resistant (score 3.5-6.4), S = Susceptible (score 6.5-9.0)

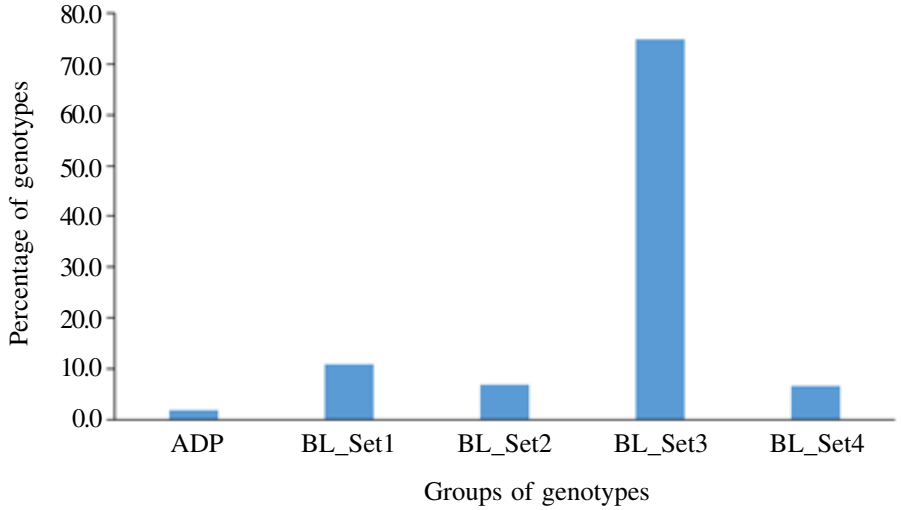

Figure 5. Percentage of 628 genotypes (lines) resistant to both *Pythium ultimum* isolate MS61 and *Fusarium cuneirostrum* isolate FSP-3 in each group.

TABLE 3. Correlation coefficients of the traits in a study of dual resistance to Pythium and Fusarium root rots

	PRR	FRR	SW100	YDHA	DF	DPM
PRR	-					
FRR	0.79***	-				
SW100	0.76***	0.67***	-			
YDHA	-0.40***	-0.32***	-0.33***	-		
DF	-0.61***	-0.52***	-0.55***	0.33***	-	
DPM	-0.68***	-0.63***	-0.67***	0.28***	0.63***	-
PLNTVIG	0.07	-0.06	0.02	-0.31***	-0.03	0.19***

PRR = Pythium root rot (1=resistant, 9 = susceptible), FRR = Fusarium root rot (1=resistant, 9 = susceptible), SW100 = Weight of 100 seeds, YDHA = Yield (kg ha^{-1}), DF = Days to 50% flowering, DPM = Days to 50% physiological maturity, PLNTVIG = Plant vigour. Two-sided test of correlations different from zero *, **, *** significant at 0.001. Number of observations: 471

Fusarium resistance, and thus observed difference in the levels of resistance of genotypes to the two root rots, could be explained by previous breeding efforts. Dual resistance was observed more in the BL (11%) than ADP (2%); the ADP also showed more dual susceptibility (80%) compare to the BL (6%). This revealed the existence of more genotypes that expressed resistance or moderate response to either Fusarium or Pythium root rots in the BL. Such genotypes, especially those that possess attributes of popular African cultivars were identified as candidates for further improvement. However, hybridisation for genotypes possessing dual resistance and similar seed attributes should result in faster genetic progress towards obtaining large seeded resistant genotypes.

The evaluated new breeding lines (BL) were developed by Michigan State University

common bean breeders, with the aim of delivering common bean germplasm with resistance to the major soil borne pathogens (e.g Fusarium and Pythium) in East Africa. About 50% of them were resistant to either Fusarium or Pythium root rot; however, since root rot pathogens tend to occur in a complex, resistance to a single pathogen is not a resilient protection option. The thirty percentage of the lines that expressed resistance to both Fusarium and Pythuim root rot that are presented in part in Table 2, are potential candidates for further evaluation for possible adoption, but majority of them are black seeded beans. Due to a small market share, black beans are not among the priority market classes in the Pan African Bean Alliance (PABRA) programme (Buruchara *et al*., 2011). They are mainly popular in northern Uganda and in South Sudan, along the border, where their promotion would be very important.

Dual resistance was also expressed in one to six lines belonging to some of the preferred market classes, namely, small-medium white, small red, cream, sugar and pink beans (Fig. 1). However, due to the few number of lines within each market class, further selection for other key market traits like foliar disease resistance or cooking time may not be very effective. Thus, they are recommended for the development of new varieties within the market classes. Among the commercial cultivars included as control genotypes, NABE3 (small red), NABE6 (small white) and Awash Melka (small white) were resistant to both root rots. Cultivars, K131 (small carioca), NABE2 (small black) and NABE22 (medium purple mottled) expressed resistance to Fusarium, but moderate reaction to Pythium root rot; whereas Masindi yellow long (large), Masindi yellow short (large), NABE5 (large sugar), NABE19 (large red mottled), NABE20 (large sugar), Ranjonomby (medium white) and CAL96 (large red mottled) were susceptible to both root rots.

Among the evaluated Ugandan commercial cultivars, root rot resistance was more in the cultivars released long ago, compared to the relatively new ones (Table 2). The majority of the evaluated newer releases are large-seeded beans, and introgression of root rot resistance to these backgrounds is highly desirable to achieve better yields in root rot prone areas.

It was observed that very low levels of resistance to both Fusarium and Pythium root rot existed in the Andean/large-seeded bean collection used in the study. This shows the existence of very few resistant large-seeded bean varieties; a challenging situation, especially in East Africa where large-seeded beans dominate the market. Cichy *et al*. (2015) also found limited resistance of Andean beans to root rot caused by *Fusarium solani* and *Macrophomina phaseolina* and suggested that the narrow genetic base in Andean compared to Mesoamerican beans could be one of the causes.

Several genetic studies have identified Quantitative Trait Loci (QTL) in crosses of Andean and Mesoamerica germplam (Mukankusi *et al*., 2011, Ongom *et al*., 2012; Kamfwa *et al*., 2013; Nakedde *et al*., 2016; Wang *et al*., 2018) indicating the efforts towards broadening diversity especially for Fusarium root rot resistance. These studies focused on resistance to single pathogens, yet these root rots tend to occur in a complex. Understanding the association of Fusarium and Pythium root rot resistance at both phenotypic and genetic levels could provide useful information for breeding for dual resistance. The phenotype association of these root rots was strong, positive and significant (Table 3), showing that breeding and selection for dual resistance can be effective. Mukankusi *et al*. (2010) evaluated genotypes resistant to Pythium root rot for Fusarium root rot resistance and confirmed high levels of resistance. They suggested the existence of QTL conditioning resistance to more than one root rot pathogens in the genotypes evaluated.

There is need to confirm the significance of this association to ascertain its relevance in breeding for multiple resistance to root rots.

In addition, the utilisation of other species of Phaseolus to increase diversity of common bean has proven successful (Mukankusi *et al*., 2018). However, wide crosses are not only resource consuming, but often require modern breeding methods to realise significant genetic gains, thus may not be very practical to initiate in young bean breeding programmes.

Mukankusi *et al*. (2018) evaluated interspecific accessions that expressed dual resistance to Pythium and Fusarium in addition to possessing attractive market attributes including seed colours and high yield. The weights of 100 seeds revealed that all the interspecific lines were small or medium (<36 g) in seed size (Mukankusi *et al.*, 2018). The existence of resistant medium sized seeds, suggests that intentional selection for high weights of 100 seeds during root rot improvement is expected to result in large seeded (> 40 g) resistant genotypes.

In this study, one large white kidney bean (G12903) expressed dual resistance to the root rots; while seven other large seeded genotypes expressed resistant or moderate response to each root rot. Line G12903 is recommended as a parent for hybridisation within large seeded genotypes. The difference in levels of resistance between the small seeded Mesoamerican beans and the large seeded Andean beans is attributed to differences in morphology, biochemical and molecular characteristics (Gepts, 1988; Haley *et al*., 1994). Molecular differences have been exploited over the years to facilitate effective gene transfer between the two gene poles (Schneider *et al*., 2001; Roman-Aviles and Kelly, 2005; Kamfwa *et al*., 2013; Nakedde *et al*., 2016; Wang *et al*., 2018). Also, lines that possessed dual resistance to Fusarium and Pythium root rots were mostly black beans. This is probably because black beans are predominantly small seeded (Sinkovic *et al*., 2019; CIAT-Kawanda Gene Bank information). Higher levels of resistance in black beans to Fusarium root rot have been reported over time (Statler, 1970; Mukankusi *et al*., 2010; Nicoli, 2012). According to Statler (1970), the black pigmentation produces phenolic compounds that inhibit fungal growth during seedling growth. However, there is little information on biochemical pathways associated with root rot resistance in common bean, probably because this approach requires use of more specialised techniques like genome editing that are not popular bean breeding.

Plant parameters and disease resistance. Yield, a key commercial trait, was generally low in both the ADP and the new BL (Fig. 3). Growth vigour was mostly moderate to poor (Fig. 2) perhaps due to excessive flooding during the seasons; thus better yields are anticipated in favourable weather conditions. A small percentage (0.3 and 2.4%) of the ADP and new BL, respectively yielded highly (>2000 kg ha^{-1}). Nonetheless, genotypes from three of BL sets expressed significant differences ($P<0.05$) in yield, indicating diversity. For example, in BL_set2, NE34-12-30 (2109 kg ha^{-1}) yield was significantly different from genotypes that yielded <1093 kg ha^{-1}; while in BL_set3, NABE 2, B11511, B11517A, B11562, and B11503 that yielded 2101 to 2662 kg ha^{-1} were significantly different from all genotypes that yielded < 1273 kg ha^{-1}. Although NE34-12-30 was moderately resistant to Fusarium and susceptible to Pythium root rot, all the other above-mentioned genotypes expressed resistance to both root rots. Such genotypes that combined high yield with dual resistance are very promising, considering that the majority of the lines yielded below 1000 kg ha^{-1}. The potential yield obtainable from new bush bean varieties ranges between 1.5 to 2.0 t ha^{-1} (CIAT, 2008), although up to 4.0 t ha^{-1} can be obtained under experimentation (Beebe *et al*., 2013). The other yield attributes for the new breeding lines like days to flowering (30-47 days) and physiological maturity (67-88 days) (Fig. 3) were within the range for popular common bean varieties like Masindi yellow long (37, 76 days), KATB1 (36, 77

days), Awash Melka (32, 70 days) and CAL96 (37, 73 days). However, none of the BL matured exceptionally early (< 60 days) to be considered early maturing.

The relative magnitude of variance for the BL genotypes in set2 and set3 (Table 1) showed enough genetic variability to make selection for days to maturity unlike in ADP and BL_set3. Recently released bush bean varieties in Tanzania (Binagwa *et al*., 2018) and Uganda (Nkalubo *et al.*, 2016) mature in 67 to 90 days and 58 to 68 days, respectively. The 67 to 88 days to maturity for the BL were within the acceptable range for farmers. Amongi *et al*. (2019) reported a similar range of 62-78 for days to maturity in advanced bush bean lines evaluated at the same location, showing that a particular range of DPM is being targeted during breeding for farmer acceptance.

The positive correlations between yield and DF [0.33***]/ DPM [0.28***] (Table 3) implied that genotypes that flowered and matured late tended to accumulate more dry mass. An early maturity variety might suffer a yield penalty during full rains but this trait allows it to avoid terminal drought and hence maintain its yield potential unlike the late maturing genotypes. Thus, they are preferred in areas prone to droughts that occur late in the growing season.

The moderate, negative and significant correlation coefficients of the two root rots to DF and DPM revealed that late flowering and maturity were associated with less root rot severity. Earlier studies associated resistance to Fusarium root rot to late maturity (Beebe *et al*., 1981; Abawi and Pastor-Corrales, 1990), but the trait was considered undesirable due to preference for early maturing varieties by farmers. The DPM for the new breeding lines were all within the acceptable farmer range, hence resistant late maturing lines from this study are useful for breeding purpose or further evaluation.

CONCLUSION

This study sought to discover new genotypes combining resistance to *Pythium ultimum* and *Fusarium cuneirostrum.* Up to 30 % of the new breeding lines expressed dual resistance for Pythium and Fusarium root rots. These genotypes are recommended for further evaluation for possible adoption in areas that prefer small to medium size beans. Only one large seeded genotypes expressed dual resistance to the root rots although a couple of others expressed resistance to either root rots. While this showed positive progress towards obtaining resistant large seeded genotypes, there remains a huge gap to obtain reasonable number of genotypes that could be further evaluated for possible adoption. Increasing diversity for high weights of 100 seeds (> 40 g) during early breeding for dual resistance to Pythium and Fusarium rot rots is highly recommended. Dual resistance was mainly achieved in the black beans although one to six lines belonging white, red, cream, sugar and pink beans also possessed dual resistance. It may be more relevant to breed within market classes to achieve a higher success for the various market options. The few lines belonging to the preferred market classes in East Africa that expressed dual resistance are valuable for breeding for root rot resistance within the corresponding marker classes.

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