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CHARACTERISATION OF *Phaseolus coccineus* INTERSPECIFIC GERMPLASM ACCESSIONS FOR DISEASE RESISTANCE, GRAIN MARKET CLASS AND YIELD ATTRIBUTES

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ABSTRACT

Sister species of the common bean (*Phaseolus vulgaris* L.) are an attractive genetic resource to broaden the genetic base of this crop, especially for adaptation to extreme environments. The runner bean (*Phaseolus coccineus)* in particular, has been shown to contribute to disease resistance and tolerance to low soil fertility, and has been used to introduce these traits into the common bean. The objective of this study was to identify germplasm with agronomic traits suitable for cultivation from an interspecific population (*Phaseolus coccineus* G35346 *x P. vulgaris;* SER 16) of 186 bush lines. The lines, coded ALB and one yield check, CAL96, were field evaluated for three rain seasons; 2011a (March-June), 2011b (September-November) and 2012 (March-June) at the National Agricultural Research Laboratories (NARL)-Kawanda, Uganda. Artificial inoculum of bean common mosaic virus (BCMV) was applied to the field experiment in 2011a. The morphological (seed type and growth habit) and agronomic attributes (plant vigour, days to physiological maturity; DPM and days to 50% flowering; DF), reaction to occurring diseases and yield performance were monitored. The lines including root rot resistant and susceptible checks; MLB-49-89A, RWR 719 and CAL 96, were also subjected to inoculum of two major root rot pathogens; *Fusarium solani f.sp. phaseoli* (isolate FSP3) and *Pythium ultimum* (isolate MS61) under screen house conditions to select for root rot resistance. Results indicated significant differences ($P<0.05$) among the interspecific lines for the parameters measured. Days to flowering and to DPM ranged from 31-39 and 81-86, respectively. Field disease pressure was generally low, but in general, 50% of the lines had yield above the mean; while 8% maintained above average yield in all seasons. The superior lines included; ALB169 (mean yield 2,564 kg ha^{-1}), ALB214 (mean yield 2,125 kg ha^{-1}), ALB196 (mean yield 2,084 kg ha^{-1}), ALB5 (2,062 kg ha^{-1}), ALB152 (2,016 kg ha^{-1}), and ALB179 (2011 kg ha^{-1}), compared to the check CAL96 (1,607 kg ha^{-1}). These lines, except ALB169 and ALB179, were resistant root rot. More lines expressed resistance to Fusarium than to Pythium root rot, with 21.5% showing resistance to both root rot isolates. Over 91% of the lines were small or medium seeded (< 35.0 g per 100 seeds), with red monochrome seed pattern; characteristics that are important for farmer acceptance.

Key Words: Fusarium, *Phaseolus coccineus, Phaseolus vulgaris,* root rot

RÉSUMÉ

Les espèces sœurs du haricot commun (*Phaseolus vulgaris* L.) sont une ressource génétique attractive pour accroître la base génétique de la culture, spécialement pour adaptation aux environnements extrêmes. Le haricot rampant (*Phaseolus coccineus)* en particulier, contribue à la résistance contre la maladie et à la tolérance à la faible fertilité du sol de, et a été utilisé pour introgresser ces traits dans le haricot commun. L'objectif de cette étude était

d'identifier le germplasm avec des traits agronomiques favorable à la culture d'une population interspécifique (*Phaseolus coccineus* G35346 *x P. vulgaris;* SER 16) d'une série de 186 lignées. Les lignées le ALB codé sur le rendement de contrôle, CAL96, étaient évaluées pendants trois saisons pluvieuses 2011a (Mars-Juin), 2011b (Septembre-Novembre) et 2012 (Mars-Juin) aux laboratoires Nationaux des Recherches Agricoles (NARL)-Kawanda, Ouganda. L'inoculation artificielle du virus mosaïque (BCMV) du haricot commun était appliquée aux expérimentations du champ en 2011a. Les attributs morphologiques (type de graines et l'habitude de croissance) et agronomiques (vigueur de plant, le nombre de jours de maturité physiologique, DPM et le nombre de jours de 50% de floraison ; DF) ; la réaction à l'apparition de maladies et la performance de rendement étaient suivis. Les lignées comportant des contrôles résistants et susceptibles à la pourriture des racines; MLB-49-89A, RWR 719 et CAL 96, étaient aussi sujets à l'inoculum de deux pathogènes majeurs causant la pourriture des racines ; *Fusarium solani f.sp. phaseoli* (isolat FSP3) et *Pythium ultimum* (isolat MS61) sous les conditions de la serre afin de sélectionner pour la résistance contre la pourriture des racines. Les résultats ont indiqué de différences significatives ($P<0.05$) entre les lignées interspécifiques pour les paramètres mesurés. Le nombre de jours de floraison et DPM ont varié entre 31-39 et 81-86, respectivement. La pression des maladies du champ était généralement faible, mais en général, 50% des lignées ont eu un rendement au-dessus de la moyenne ; alors que 8% ont maintenu leur rendement au-delà pendant toutes les saisons. Les lignées supérieures comprenaient ; ALB169 (rendement moyen 2 564 kg ha-1), ALB214 (rendement moyen 2 125 kg ha-1), ALB196 (rendement moyen 2 084 kg ha-1), ALB5 (2 062 kg ha-1), ALB152 (2 016 kg ha-1), et ALB179 (2011 kg ha-1), comparées aux contrôles CAL96 (1 607 kg ha-1). Ces lignées, à l'exception d'ALB169 et ALB179, étaient résistantes à la pourriture de la racine. De nombreuses lignées ont exprimé leur résistance à la pourriture de racines due au Fusarium qu'au Pythium, avec 21.5% montrant leur résistance aux deux isolats de pourriture de racines. En plus, 91% des lignées ont de petites ou moyennes graines (< 35.0 g par 100 graines), avec une monochrome rouge de motif germinal ; des caractéristiques qui sont importantes pour l'acceptation du producteur.

Mots Clés: Fusarium, *Phaseolus coccineus, Phaseolus vulgaris,* pourriture de racine

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.), plays a significant role in human nutrition and livelihood (Akibode and Maredia, 2011, CGIAR, 2017) by feeding over 100 million people in Africa (Buruchara *et al*., 2011). Beans contain two to three times as much protein as cereals, and can reduce the risk of chronic diseases such as diabetes, heart disease, and cancer. Common beans are important sources of micronutrients, such as iron and zinc (US Dry Bean Council, 2011). In addition to their nutritional benefits, they are important components of African cropping systems, because they participate in nitrogen fixation in the soil (Manrique *et al*., 1993; Giller, 2001).

Production of common bean is constrained by a number of biotic and abiotic factors (Beebe *et al*., 2013), among which are bean root rots caused by a complex of pathogens, *Pythium spp*., *Fusarium spp. Sclerotium rolfsii* and *Rhizoctonia solani* (Tusiime, 2003, Mukankusi, 2008). Yield losses of up to 100% have been reported due to root rots (Otsyula *et al*., 2003; Spence, 2003; Otsyula and Ajanga, 2004). This, usually occurs when susceptible varieties are grown under environmental conditions favourable for pathogen development; namely high levels of humidity and low temperatures ranging from 14 to 17 ^{0}C (Buruchara and Rusuku, 1992). Root rots are particularly severe in poor soils, that are common in farmers' fields in most of Sub-Saharan Africa due a probable imbalance between the beneficial and disease-causing organisms in the soil (Abawi and Widmer 2000).

Though root rot pathogens occur in a complex, small differences in the microclimate result in one pathogen dominating over the other. Resistant varieties are a key and practical component of an integrated management package for bean root rot, especially for small-scale farmers (Abawi *et al*., 2006). Most of bean varieties, currently grown in East Africa, are susceptible to root rot (Buruchara *et al*.,

2014); but varieties resistant to Pythium root rot have been released by the bean programme in Kakamega, Kenya (PABRA report, 2015) and by NACRRI bean programme in Uganda in 2003 (Namayanja *et al*., 2003). Resistance to the different pathogens causing root rot has been shown to be genetically different (Ongom *et al*., 2012), thus necessitating identification of germplasm with combined resistance to the complex of pathogens. The wild relatives of the common bean, such as runner bean (*P. coccineus*), are an attractive option to source for genes for important traits to broaden the genetic base of common bean, especially for adaptation to extreme environments (Porch *et al*., 2013; Redden *et al*., 2015), including root rot resistance.

Over 30 species of *Phaseolus* have been reported (Debouck, 1991; 1999) and of these, five; common bean (*Phaseolus vulgaris* L.), yearlong bean (*Phaseolus polyanthus* Greenman, also known as *Phaseolus dumosus*), runner bean (*Phaseolus coccineus* L.), tepary bean (*Phaseolus acutifolius* A. Gray) and lima bean (*Phaseolus lunatus* L.) are domesticated (Gepts and Debouck, 1991; Debouck, 1999). The common bean (*P. vulgaris*) possesses, by far, the widest use of all *Phaseolus* spp.; representing over 85% of the area planted to cultivated species in this genus worldwide (Singh, 2001).

The wild ancestors of the common bean and its relatives evolved on the margins of forests in which competition for light was intense; where drought was occasional, and nutrients in soils with high organic matter were typically not critically limiting (Gepts and Debouck, 1991; Debouck, 1999). These sister species, hence, represent a wealth of adaptive capacity for the improvement of common bean as a results of their evolutionary origins (Porch *et al*., 2013); and are, hence, a target for introduction into common bean through standard genetic improvement and wide-crossing/introgression.

The sister species, notably runner and tepary bean, have always existed as weeds or "escapes" in forests, hedges; and in a few instances, especially in the West African countries, as a source of food with no major research support. However, they are slowly gaining attention as both food and cash crops, mainly due to their hardiness in changing and extreme weather conditions (PABRA, 2013). The runner bean is an important source of disease resistance, cold tolerance and lodging resistance, which are useful traits to improve common bean (Schwember *et al*., 2017). Use of wild relatives to crop species has been limited mainly due to the possible challenges of cross incompatibility, sterility, reduced recombination, and negative linkages to negative traits (Yadav *et al*., 2015).

The Bean programme of the International Centre for Tropical Agriculture (CIAT) has made use of these species to improve pest and disease resistance, drought and heat tolerance, low soil phosphorus availability and tolerance to aluminum toxicity of the common bean (Beebe *et al.*, 2011). This study sought to evaluate the field performance and seed characteristics of an interspecific population developed from *P. coccineus* line (G35346) and *P. vulgaris* (SER 16), developed at CIAT, and identify lines resistant to root rot and Bean Common Mosaic Virus (BCMV).

MATERIALS AND METHODS

Experimental site. This field experiment was conducted at the International Centre for Tropical Agriculture (CIAT) Uganda station, based at the National Agricultural Research Laboratories (NARL), located at Kawanda in central Uganda. NARL is located 32° 31'E, 0°252 N with an altitude of 1190 masl; and is characterised by a bimodal rainfall pattern. The study was repeated during three rainy seasons namely; 2011a and b, and 2012a, where "a" is the first rainy season (March-June) and "b" is the second rainy season (September-December). Soil properties of the experimental sites are summarised in Table 1. Soil was generally low in available phosphorus and

TABLE 1. Soil characteristics of two different field sites utilized in each of the three experimental seasons (2011b and 2012a) at the national Agricultural Research Laboratories, Kawanda, in Uganda

Season	pH	OM	N	P	Ca	Mg	K	Fe	Zn
		%				ppm			
2011b	5.3	9.8	0.4	5.9	1716.9	361.8	240.4	147.7	4.9
2012a	5.1	5.3	0.3	4.8	1888.2	522.0	214.3	80.6	4.0
Critical values	5.2	3.0	0.2	5.0	350.0	100.0	150.0	-	-
Sufficient levels	5.2-7.0	6.0	0.3	20.0	2000.0	600.0	500.0	50.0	20.0

OM = Organic matter, N = Nitrogen, P = Phosphorus, Ca = Calcium, K-Potassium, Fe = Iron, Zn = Zinc

exchangeable potassium, but with sufficient nitrogen. The screen house trial was conducted at the same location in the CIAT disease screen house.

Genetic materials assessed. One hundred and eighty six *P. coccineus* interspecific lines, coded ALB were studied. The lines were developed at the International Centre for Tropical Agriculture (CIAT) in Cali-Palmira, Colombia, from a *Phaseolus vulgaris* accession SER 16, a drought-tolerant and small red bush bean; and a *Phaseolus coccineus* (runner bean) accession, G35346 (medium sized, cream purple speckled seed), a known source of root rot resistance (Boomstra *et al*., 1977; Beebe *et al*., 1981; Silbernagel, 1987). Variety CAL 96 (Calima), bred at CIAT and a commercial variety in many East African countries, was used as a yield check. Lines, RWR 719 (Rwanda accession), a small red bush bean, and MLB-49-89A (Democratic Republic of Congo accession), medium black bush semi-climbing bean, were used as resistant checks for Pythium and Fusarium root rots in the screen house, respectively.

Field experimental design. The experiment was established in an alpha lattice design, with two replications. Each plot comprised of 3 rows of 3-metres length for each entry, with a spacing of 50 cm between and 10 cm within rows.

Weeding was done twice, at V3 stage (when 50% of the plants had the first trifoliate leaf completely unfolded), and the second at R7 (when 50% of the plants present the first pod with the flower's corolla hanging or detached) growth stage. An insecticide, Dimethoate, and two fungicides (Benlate and Ridomil), were applied weekly against insect pests and fungal diseases, until flowering stage. The two fungicides were alternated on a weekly basis. NPK. 17:17:17 fertiliser was hand applied just before planting at the rate of 125 kg ha^{-1}.

Screening for bean common mosaic virus (BCMV) under field conditions. In 2011a, a field experiment was artificially inoculated with Bean Common Mosaic Virus (BCMV) to test for resistance to this disease and its necrotic strain, Bean Common Mosaic Necrosis Virus (BCMNV). Symptomatic leaves from a field grown BCMV susceptible variety, G2333, were plucked and washed with tap water, to remove dirt. The leaves were placed in a refrigerator for at least 4 hours to stabilise the virus (Mills and Silbernagel (1992).

The refrigerated leaves were then ground in a phosphate buffer in proportion of 1:5 (w/v), i.e., 1 g of leaves in 5 ml of the cold buffer to stabilise the virus released in the sap by crushing leaf cells. The buffer was prepared by mixing Potassium phosphate solution (1.36%) with 0.14% Sodium phosphate solution (stock solutions) in the proportion of 30 ml of potassium phosphate solution with 70 ml of sodium phosphate solution in 900 ml of deionised distilled water, as a working

solution and pH adjusted to 7.0 (Mills and Silbernagel, 1992). To inoculate the plants, acid washed sand was added to the buffer to act as an abrasive. After grinding, the sap was filtered to remove leaf debris (Chiumia and Msuku, 2001).Using a multiple needle, holes were punched on the primary leaves, which are critical for virus attack (Larsen *et al*., 2005), of 10 randomly selected plants per plot. The solution was gently rubbed on the entire surface of punched leaves, by rubbing gently with a sterilised sponge dipped in the slurry, 8-14 days after planting. During this process, the sand wounds the leaf cells and facilitates easy entry of the virus into plant cells to initiate infection. The solution and cotton wool were changed for every plant to avoid plant to plant contamination. The inoculum was maintained on ice until the inoculation process was completed.

Screening for root rot resistance under screen house conditions. To identify root rot disease resistant lines, the 186 ALB lines were challenged with *Pythium* and *Fusarium* fungal isolates maintained at CIAT-Uganda, i.e. MS61 for *Pythium ultimum* and FSP3 for *Fusarium solani* f.sp. *phaseoli*.

MS61 cultures maintained at the NARL laboratory were reactivated by sub-culturing onto fresh potato dextrose Agar (PDA) media in Petri dishes in a screen house. Pathogen inoculum was produced on finger millet grain as a medium for fungal growth. Approximately 200 ml of distilled water was added to every 300 g of grain and the mixture placed in polyethylene bags. The millet was double sterilised at 121 ^{0}C for 1 hour in an autoclave and allowed to cool. Each bag was inoculated with 3 - 4 discs of agar bearing the growing cultures, by placing the discs at different positions in the finger millet bag. The bags were incubated in a sterile environment at room temperature for at least 12 days, to allow uniform growth of mycelia over the millet grain. After incubation, the millet with Pythium inoculum was mixed in steam sterilised soil (soil is steam sterilised overnight and allowed to cool for one day) at a ratio of 1:8 v/v inoculum to soil; and then placed in wooden flat trays of 0.74 x 0.42 x 0.115 cm^3, and left to stabilise in the soil for 7 days.

To test the inoculum levels in the soil, a susceptible check variety, CAL96 was planted in the soil and evaluated after 21 days. This process was repeated until a score of 9 (on a 1-9 scale described below) was obtained as a means of increasing inoculum levels in the soil. Thereafter, the susceptible check was uprooted and the test lines planted in a randomised complete block design (RCBD) with two replications. Each tray contained eight test lines, planted in 10 rows; each row representing a unique line of 6 plants and two rows for two check lines; RWR 719 (resistant) and CAL 96 (susceptible).

After three to five days from planting (when <80% of the seedlings had emerged), the trays were flooded with tap water and this was maintained for 7- 10 days to create favourable microclimate for the pathogen to move through the soil pores and infect the seedlings (Mukalazi, 2004). In the 3rd week, the soil water level was slowly reduced by decreasing the frequency of watering, to approximately 3 times a week. The experiment was repeated once.

Similar to Pythium root rot, inoculum of one *Fusarium solani* fsp. *phaseoli* isolate (FSP-3) maintained at NARL, was produced by sub-culturing the fungus stored on Agar (PDA) slants in the laboratory onto potato dextrose agar (PDA) plates; and allowing the fungus to grow for a period of up to 21 days. Borosilicate glass bottles of 500 ml capacity were partially filled with sorghum seed (2/3 capacity) and 150 ml of distilled water was added. The bottles were sealed and autoclaved for 1 hour at 121^{o}C. For each PDA plate bearing the fungal colony, 10 ml of sterile deionised water was added and the fungus scrapped from the media to mix with water and make a slurry. The slurry was then spread evenly onto the surface of the already prepared sorghum medium

within the bottles. The bottles were resealed and agitated to mix the slurry with the sterilised sorghum. The mixture was incubated in the laboratory at 20-28°C for 5 days, to allow the pathogen to grow; after which the bottles were opened. The opening was protected using foil paper to prevent contamination, to allow evaporation of excess moisture and nutrient solution. After 21 days of incubation, the bottles were emptied onto a clean surface, and the medium slowly dried under room temperature to allow for maturation of the fungal resting spores (Tusiime, 2003; Mukankusi *et al.,* 2010).

Rectangular wooden trays (0.74 x 0.42 x 0.115m^3) were partially filled (2/3 capacity) with steam sterilised loamy sand soil and the prepared inoculum added to the soil at a rate of 500 ml inoculum per tray, prior to planting. The trays were then covered with polyethylene bags for a week to facilitate sporulation before repeatedly planting the susceptible check CAL96 in the soil till 4 weeks old before uprooting it and planting again until a score of 9 on a 1-9 scale (IBP, 2013 described below) was attained. This acted as a means of increasing pathogen inoculum levels in the soil.

Thereafter, test lines were planted in a Randomised Complete Block Design (RCBD), with three replications. Each tray was planted with five test lines and two checks, MLB-49-89A (resistant) and CAL 96 (susceptible) with each test line having two rows of 10 plants each. The experiment was repeated once (hence two cycles of screening for each root rot pathogen).

Data collection

Field experiment. Data were collected on days to 50% flowering (DF) and days to physiological maturity (DPM). Severity of occurring diseases (angular leaf spot, anthracnose, BCMV, common bacterial blight and rust), and incidence of BCMNV from the field experiments was collected. Severity of Pythium and Fusarium root rot diseases in the screen house experiment.

Days to flowering (DF) was recorded at R6 plant growth stage, as the number of days from planting to the day when 50% of plants had at least one flower. Days to physiological maturity (DPH) was recorded at R9 plant growth stage, as the number of days from planting to the day when the first pod began to discolour in 50% of the plants (IBP, 2013). Growth vigour was recorded on 1-9 scale; where 1 = excellent, 3 = good, 5 = intermediate, 7 = poor, 9 = very poor (IBP, 2013). Severity of field occurring diseases (angular leaf spot, anthracnose, common bacterial blight and rust) was recorded on plot basis at R6 stage using a 1-9 scale for each disease as described in the Trait Dictionary (IBP, 2013).

Data were collected on angular leaf spot (ALSF), angular leaf spot in the field on pods (ALSFP) caused by a fungal pathogen, *Psuedocercospora griseola*, common bacterial blight on leaves (CBBFL), common bacterial blight on pods (CBBFP) caused by *Xanthomonas campestris*, rust on leaves (RUSTF) caused by *Uromyces appendiculatus*, anthracnose on leaves (ANTFL) caused by *Colletotrichum lindemuthianum* and bean common mosaic virus (BCMV) (2012a). In summary; 1 = no visible disease symptoms, 3 = presence of a few small lesions covering appoximately 2% of the leaf surface area; 5 = presence of several small lesions covering appoximately 5% of the leaf or pod area; 7 = abundant and generally large lesions that cover appoximately 10% of the leaf or pod surface area. Lesions may coalesce to produce larger infected areas associated with chlorotic tissue. Lesions may also be found on the stem and branches; 9= 25% or more of the leaf or pod surface area is covered by large and often coalescing lesions. Abundant lesions are present on stem and branches.

The number of plants per plot, showing bean common mosaic necrotic virus (BCMNV) symptoms (black root) was recorded.

Harvesting was done when 90% of the pods changed colour from green to yellow (Munoz-Perea *et al.,* 2006). Plants were cut from the

base and bundled up per plot; and taken to the drying shed to allow for further drying. Thereafter, threshing was done using a locally fabricated single bundle thresher. Threshed seed was winnowed and hand-sorted. The seeds were further sun-dried to about 13% moisture content (measured using a moisture meter), and weighed using an analytical weighing scale. Plot yield per hectare (YDHA) was estimated by calculation (IBP, 2013).

Seed size was characterised based on weight of 100 seeds, seed colour and pattern (IBP, 2013). One hundred seed weight was obtained by taking 100 seeds weight per plot. According to Evans (1973; 1980), genetic diversity in common bean may be organised into three general classes and two genepools; according to seed size namely, the large-seeded (>40 g 100 seed weight^{-1}) Andean genepool and the medium (25-40 g 100 seed weight^{-1}) and small (<25 g 100 seed weight^{-1}) seeded Middle American genepool.

In terms of seed colour, primary seed color, secondary seed colour and seed colour pattern were assessed. Primary Seed colour (PSC) and secondary seed colour (SSC) was assessed using 1-9 scale; where, Primary 1 = white, 2 = cream-beige, 3 = yellow, 4 = brown-maroon, 5 = pink, 6 = red, 7 = purple, 8 = black and 9 = others; Seed colour pattern (SCP) was visually assessed as the distribution of colours on the seeds coat on a 0-6 (O, M, J, P, R, B), where O = no pattern or monochrome, M = mottled, J = speckled, P = pinto, R = striped and B = bicolor.

Growth habit was assessed on a 1-5 scale (IBP, 2013) as follows: 1 = determinate bush, 2 = indeterminate bush habit, erect stems and branches, 3 = indeterminate bush habit with weak main stem; and prostrate stem and branches, 4 = indeterminate climber habit with weak, long and twisted stem and branches and 5 = determinate climber.

Screening for Pythium and Fusarium root rot disease severity under screen house conditions. Root rot severity was assessed at 21 days after planting. All plants were uprooted per test genotypes per replicate. The roots were washed with tap water and the lower hypocotyl discoloration and damage visually assessed using a 1 - 9 scale (IBP, 2013), where 1 = no visible symptoms, 3 approximately 10% of the hypocotyl and root tissues covered with lesions, 5 = approximately 25% of the hypocotyl and root tissues covered with lesions but tissues remain firm with deterioration of the root system, 7 approximately 50% of the hypocotyl and root tissues covered with lesions combined with considerable softening, rotting and reduction of root system; 9 approximately 75% or more of the hypocotyl and root tissues affected with advanced stages of rotting combined with severe reduction in the root system. The mode (most common score) of the root rot disease scores was obtained.

Data analysis. Data for each season were analysed separately to assess within season variability before performing combined analyses to assess variability and stability across seasons. Data were subjected to analysis of variance (ANOVA) using GenStat, Release 16.2, PC/Windows 7; VSN International Ltd. (GenStat. 2013). For all diseases, except for BCMNV, ranking of the lines was 1-3 = resistant: no visible symptoms or very light symptoms, 4-6 = intermediate: visible and conspicuous symptoms resulting only in limited economic damage, 7-9 = susceptible: severe to very severe symptoms causing considerable yield losses or plant deaths.

RESULTS

Yield characterisation of *P. coccineus* interspecific lines. There were no significant differences ($P<0.001$) among the lines for yield; however, the lines behaved differently across the three seasons (Table 1). The highest yields were recorded in season 2011b (average yield =1661 kg ha^{-1}); followed by 2012a (average yield = 1558 kg ha^{-1}) and 2011a (average yield = 1180 kg ha^{-1}) (Table 2). In general yield ranged from 100 kg ha^{-1} for

TABLE 2. Analysis of Variance (ANOVA) of reaction to field occurring diseases and yield performance of 186 interspecific lines at the national Agricultural Research Laboratories Kawanda, in Uganda

Source of variation	DF	ALSF	CBBFL	RUSTFL	BR (%)	YDHA (kg ha^{-1})
Season (S)	2	137.44**	71.30**	227.00**	5262.7	19219344.0*
Rep/S	3	3.18**	1.52	2.43**	814.7***	1708993.3***
Entry	187	1.48***	1.68*	1.19**	46.6**	413468.4
Entry x S	374	0.97**	1.29***	0.89***	34.1***	369570.8***
Error	561	0.75	0.92	0.63	25.5	226246.8
Total	1127	1.19	1.30	1.21	43.3	342527.2

Rep = replication, DF = degree of freedom, ALSF = angular leaf spot in the field, CBBFL = common bacterial blight on leaves in the field, RUSTFL = rust on leaves in the field, BR = number of plants with black root expressed as a percentage of germination count, YDHA =seed yield per hectare *, **, *** =significant at $P < 0.05$, $P < 0.01$, $P < 0.001$, respectively

ALB140 in 2011a to as high as 3257 kg ha^{-1} by ALB4 in 2011b. On average, 57 lines had yields higher than the average yield of the commercial check (1,607 kg ha) with the lines ALB169 (2,564 kg ha^{-1}), ALB214 (2,125 kg ha-1), ALB196 (2,084 kg ha^{-1}), ALB5 (2,062 kg ha^{-1}), ALB152 (2,016 kg ha^{-1}), and ALB179 (2011 kg ha^{-1}) being the best performing across the three seasons.

Seed, plant growth and morphological characterisation of *P. coccineus* interspecific lines. Weight for 100 seeds ranged from 19.7 g to 35.6 g, with over 170 lines (91.4%) weighing less than 35 g 100^{-1} seeds. Ninety three lines (50%) were hence grouped as small seeded (<25.0 g) and 76 (40.9%) as medium sized seeds (25.1 g - 35.0 g), all fitting in the Middle American genepool. Seed pattern among the 186 lines was mainly monochrome, but some seeds were speckled. Most lines were red seeded, but black, cream, pink, purple and brown seeds were also present (Table 3). Majority of the lines expressed indeterminate bush growth habit (Type 2), with erect stem and branches (96.8%); and a few (3.2%) determinate bush beans (Type 1). Most of the lines produced white flowers (69.4%); while others had red (23.7%) and lavender (7.0) flower colour. Growth vigour for most lines (96.2%) was excellent (89.2%) and good (10.2%) ranging from 1-4 on 1-9 scale (CIAT, 1987). One line, ALB202, had an intermediate growth vigour (score of 5). Days to flowering and maturity ranged from 31-39 and 81-86. The average days to flowering (DF) and maturity (DPM) were 35 and 85 days, respectively; while the check variety, CAL 96, flowered and matured on day 32 and 81, respectively. Eighty two lines (44.1%) flowered at < 34.8 days and 33.9% of the lines flowered on day 32 or a day earlier or later. Eight percent of the lines matured on the same day as CAL 96, and none was earlier (Table 3).

Response to field diseases of *P. coccineus* interspecific lines. The experiment was affected by five diseases, namely, angular leaf spot, anthracnose, common bacterial blight, rust and BCMV/BCMNV. Natural disease pressure of the occurring diseases was generally through the experimental period. In general, there were significant differences (P=0.05) among the test lines in the severity of these diseases (P <0.001) (Table 1). For all the fungal diseases, scores ranged from 1-3 (on a score scale of 1-9), with no lines having scores of 7-9 for any of the diseases. However, 30 and 40 lines had scores of 4-6 for ALSF and RUSTFL, respectively (Table 3). CBB was the most severe across the seasons with 40%

TABLE 3. Agronomic traits of best yield performing interspecific lines evaluated at the National Agricultural Research Laboratories (NARL), Kawanda, Uganda

Entry	Seed pattern	Market class	100 SW	Average yield (kg ha^{-1})	ALSF	RUSTFL	CBBFL	ANTFL	BR incidence (%)
ALB101	Monochrome	Red	20.9	1647.0	2	1.7	2.3	1.5	3
ALB102	Monochrome	Red	23.2	1694.0	2.7	1.7	3	1.5	6.3
ALB104	Monochrome	Red	23.4	1661.3	2.7	1.7	2.7	1.5	4.3
ALB105	Monochrome	Red	27.6	1688.0	1.7	1.7	2.7	1.5	5
ALB109	Monochrome	Red	24.7	1874.7	2	1.7	3	1.5	2.3
ALB111	Monochrome	Red	21.2	1670.0	2.7	1.7	3.7	1.5	10
ALB112	Monochrome	Red	24.3	1688.3	2	1.7	3.7	1.5	8
ALB113	Monochrome	Pink	27.3	1701.7	2	1.7	2.7	1.5	4.3
ALB117	Zebra stripped	Cream	24.3	1718.0	2.3	2	4	1.5	4.7
ALB124	Monochrome	Red	23.9	1623.0	3.3	2.3	3.3	1.5	2
ALB133	Zebra stripped	Cream	27	1881.3	1.7	1.7	3	1.5	4
ALB143	Zebra stripped	Cream	26.8	1615.3	1.7	1.7	4.3	1.5	8.7
ALB144	Zebra stripped	Cream	25.7	1719.3	1.7	1.7	3.3	1.5	7.7
ALB146	Monochrome	Red	24.2	1882.7	2.3	2	2.7	1.5	2.7
ALB148	Monochrome	Red	25.7	1682.0	2.7	1.7	2.7	1.5	1.7
ALB149	Monochrome	Red	31.4	1724.7	2.3	1.7	2.7	1.5	1.7
ALB150	Monochrome	Brown	21.5	1879.0	2	2	3.7	1.5	8
ALB151	Monochrome	Cream	24	1609.5	3	2.5	4	2	9
ALB152	Monochrome	Red	24	2016.3	2	2.3	2.7	1.5	0.7
ALB160	Monochrome	Red	22.5	1618.3	2.3	1.7	3.3	1.5	5.7
ALB165	Monochrome	Red	26.3	1858.7	1.7	1.7	3.3	1.5	4
ALB166	Monochrome	Red	29.5	1735.0	2	1.7	3	1.5	2.7
ALB167	Monochrome	Red	24.1	1922.7	2	2	3.3	1.5	5.7
ALB168	Monochrome	Red	27.9	1955.3	2	1.7	3	1.5	5.3
ALB169	Monochrome	Red	24.6	2564.0	1.7	1.7	2	1.5	6.3
ALB170	Monochrome	Red	28.6	1792.0	2	1.7	2.3	1.5	6.3
ALB179	Monochrome	Red	21.9	2011.0	2.5	2.5	2.5	2	4
ALB180	Monochrome	Red	26.2	1789.0	2.3	1.7	2.7	2	4
ALB183	Monochrome	Red	22	1776.7	2	2	2.7	1.5	5.3
ALB184	Monochrome	Red	27.2	1710.7	2.7	2.0	2.7	1.5	6.3
ALB186	Monochrome	Red	25.8	1731.7	2.3	1.7	3	1.5	6.3
ALB189	Monochrome	Red	20.8	1759.7	2.7	1.7	2.7	1.5	17.7
ALB19	Monochrome	Red	22.7	1669.7	2.3	1.7	3.3	1.5	6.7
ALB190	Monochrome	Red	20.4	1967.3	1.7	2	3	1.5	1.7
ALB193	Monochrome	Red	25.1	1619.3	2.7	2.3	2.7	2.5	2.7
ALB196	Monochrome	Red	23.1	2083.7	2.7	2	3	1.5	10
ALB204	Monochrome	Red	23.7	1618.3	3	1.7	4.3	1.5	5.3
ALB209	Monochrome	Red	25	1680.7	2.3	1.7	3.3	1.5	4.3
ALB212	Monochrome	Red	25.4	1617.3	2.7	1.7	3	2	5.7
ALB214	Monochrome	Red	25.3	2124.7	1.7	1.7	3	1.5	8.3
ALB24	Monochrome	Red	21.9	1607.7	2.7	2.3	3.7	2	7.5
ALB4	Monochrome	Red	27.1	1909.3	2.3	2	3.3	2	7.3
ALB44	Monochrome	Red	23.7	1611.7	1.7	1.7	2.7	1.5	2.7
ALB48	Monochrome	Red	25.1	1665.7	1.7	1.7	3	1.5	3.7
ALB5	Monochrome	Red	28.6	2061.7	1.7	2	2.7	1.5	1.3
ALB6	Monochrome	Red	27.6	1766.7	2	2	3	1.5	3.7
ALB64	Monochrome	Red	23.8	1820.0	2.7	1.7	2.7	1.5	6.3

TABLE 3. Contd.

Entry	Seed pattern	Market class	100 SW	Average yield (kg ha^{-1})	ALSF	RUSTFL	CBBFL	ANTFL	BR incidence (%)
ALB7	Monochrome	Red	27.1	1961.0	1.7	1.7	3.7	1.5	1.7
ALB74	Monochrome	Black	27.4	1769.0	2	1.7	2.7	1.5	7
ALB79	Monochrome		23.4	1622.7	2	2	3.3	1.5	4.3
ALB8	Monochrome	Red	22.9	1957.3	2	1.7	2.7	1.5	4
ALB85	Monochrome	Red	24.8	1710.3	3	1.7	2.3	1.5	2.3
ALB89	Monochrome	Cream		1672.0	2.7	2	2.7	1.5	4
ALB90	Monochrome	Red	21.6	1617.0	2.7	2.3	3.3	2	3.3
ALB95	Monochrome	Red	19.6	1827.7	2.7	1.7	3.3	2.5	2.7
ALB96	Monochrome	Red	24.1	1729.0	2	1.7	3.3	1.5	5
ALB99	Monochrome	Red	23.1	1763.7	2	1.7	3	1.5	3
CAL96	Mottled	Red	51.2	1607.0	3	3	4	2	1
RWR719	Monochrome	Red	21.8	1375.0	2.5	2.5	3.5	2	12
Mean			25.1	1557.8	2.6	1.9	3.2	1.6	4.5
CV (%)				28.1	33.9	32.9	29.8	37.4	148.5
LSD (5%)				ns	1.9	1.5	1.8	0.7	14.7

*Growth habit for the 33 selected lines is indeterminate bush with erect stem and branches. DF = days to 50% flowering, DPM = days to 50% physiological maturity, 100SW = weight of 100 seeds, CV = coefficient of variation, Se = standard error of the mean, SED = standard error of the difference, LSD = least significant difference, ns = not significantly different, ns = not significantly different

of the lines depicting resistant reaction (scores of 1-3,) and 60% depicting moderately resistant reaction (scores of 3.1-5). In the case of ALS, approximately 90% of the lines showed resistant reactions and 10% were moderately resistant. Ninety seven percent of the lines showed resistant reactions to rust; while 3% were moderately resistant. All the lines, with the exception of ALB29, showed resistant reactions to anthracnose. In general, 39% (73 lines) of the lines had scores less than 3 (on a score scale of 1-9) for all the diseases and included; ALB169, ALB105, ALB44, ALB33, ALB214, ALB48, and ALB169 among others.

Severity of BCMV under natural infection was low with 69% of the lines having scores of 1-3, and 31% having scores of 4-6 (Table 3). Majority of ALB lines had less than 10% of plants in a plot, showing black root in all the seasons. Under natural infection, the number of plants per plot, which developed black root (susceptible to BCMNV), ranged from 0 by ALB 189 to 51% in ALB189 (data not shown). In the artificially inoculated trial, the incidence of black root ranged from 0% to 60% (Table 4). Sixty five lines did not express symptoms of BCMNV/ black root; while, 62 lines had 10% of the lines showing symptoms of black root, 31 lines had up to 20% incidence, and 16 lines had incidence of

TABLE 4. Incidence of black root disease in an artificially inoculated field experiment at the National Agricultural Research Laboratories, Kawanda, Uganda

Incidence (%)	Number of lines
0	65
1-10	62
11-20	31
21-30	16
31-40	9
41-50	3
51-60	1

up to 30% (Tables 3 and 4). Only one line (ALB42) showed up to 60% incidence of black root (Table 4).

Resistance to Pythium and Fusarium root rots of *P. coccineus* interspecific lines. Screening cycles in the screen house were statistically different (P<0.05) (Table 5) for both diseases. The lines (Entries) were also significantly different (P<0.01) in response to the two diseases. Entry and screening cycle interaction was only significant (P<0.001) for response to *Fusarium* root rot (FRR). Fifty two lines (28%) showed resistant reactions to *Pythium* root rot (PRR); while 131 lines (70.4%) and 169 (90.9%) turned out resistant to FSP3 for FRR, respectively (Fig. 1). Of the 52 lines that were resistant to PRR, 40 (76.9%) were also resistant to Fusarium root rot in both screening cycles. Several lines like ALB105, ALB153 and ALB164, were resistant to FRR, but susceptible to PRR. The response of some resistant lines, like ALB99, ALB101 and ALB102 to FRR was compared with the reaction of resistant and susceptible checks (Fig. 1).

DISCUSSION

Yield characterisation of *P. coccineus* interspecific lines. Over 30 percent of the evaluated interspecific lines had yield greater than1.5 kg ha^{-1} (Table 3.) and greater than the commercial check (CAL96; 1.6 kg ha^{-1}) on average, positioning them in an attractive yield bracket. Average yield of beans in East Africa has been reported as 850 kg ha^{-1} (FAO, 2015), but may be as high as 1500 kg ha^{-1} in some countries, such as Ethiopia. In Uganda, average on-farm yield of1300 kg ha^{-1} (FAO, 2015) is still less than the potential yield of 1.5–2 tonnes per hectare for new varieties grown under optimum farmer conditions (FAO, 2015). In this study, several lines including, ALB169, ALB146, ALB214, ALB7, ALB152, ALB133 and ALB89, had grain yield higher than the commercial check, CAL96, popularly known as Nambale or Kawomera in Uganda; and also released in Ethiopia and DRC and popular in South Sudan (PABRA, 2013). A yield advantage of 60% over CAL96; and 87% over RWR719 was obtained by the highest yielding line, ALB169. Seventeen lines that yielded

Figure 1. Pictures showing reaction of selected ALB lines to Fusarium root rot in 2015.

TABLE 5. Response to isolate FSP-3 (Fusarium root rot) and isolate MS61 (Pythium root rot) for a selected set of ALB lines in the screen house

Entry	*Pythium* root rot severity (1-9 scale)	Response	Fusarium root rot severity (1-9 scale)	Response
1. ALB5	2	R	2	R
2. ALB6	3	R	2	R
3. ALB10	2	R	2	R
4. ALB24	2	R	2	R
5. ALB25	2	R	2	R
6. ALB31	2	R	2	R
7. ALB34	3	R	2	R
8. ALB36	2	R	2	R
9. ALB41	2	R	2.5	R
10. ALB42	2	R	2	R
11. ALB44	3	R	2	R
12. ALB45	3	R	2	R
13. ALB46	3	R	2	R
14. ALB50	3	R	2	R
15. ALB54	2	R	2	R
16. ALB57	3	R	2	R
17. ALB64	3	R	2	R
18. ALB77	2	R	2	R
19. ALB85	2	R	2	R
20. ALB109	3	R	2	R
21. ALB112	2	R	2	R
22. ALB117	2	R	2	R
23. ALB122	2	R	2	R
24. ALB123	3	R	2	R
25. ALB129	3	R	2	R
26. ALB130	3	R	2	R
27. ALB132	3	R	2	R
28. ALB152	3	R	2	R
29. ALB167	3	R	2	R
30. ALB169	3	R	2	R
31. ALB173	3	R	2	R
32. ALB174	3	R	2	R
33. ALB176	3	R	2	R
34. ALB182	3	R	2	R
35. ALB184	3	R	2	R
36. ALB187	3	R	2	R
37. ALB188	2	R	2	R
38. ALB190	3	R	2	R
39. ALB191	3	R	2	R
40. ALB205	3	R	2	R
41. ALB59	7	S	2	R
42. ALB101	7	S	2	R
43. ALB102	7	S	2	R
44. ALB105	7	S	2	R
45. ALB111	7	S	2	R
46. ALB144	7	S	2	R
47. ALB153	7	S	2	R

TABLE 5. Contd.

Entry	*Pythium* root rot severity (1-9 scale)	Response	Fusarium root rot severity (1-9 scale)	Response
48. ALB164	7	S	2	R
49. ALB165	7	S	2	R
50. ALB199	7	S	2	R
51. ALB30	4	M	5.5	S*
52. ALB40	4	M	5.5	S*
53. ALB60	2	R	6.5	S*
54. ALB67	7	S	3.5	S*
55. ALB71	7	S	4.5	S*
56. ALB74	5	M	4.5	S*
57. ALB78	7	S	3	S*
58. ALB84	7	S	5.5	S*
59. ALB94	7	S	3.5	S*
60. ALB96	7	S	3	S*
61. ALB99	7	S	3	S*
62. ALB136	8	S	4.5	S*
63. ALB139	8	S	9	S*
64. ALB140	8	S	6.5	S*
65. ALB158	3	R	4.5	S*
66. ALB159	7	S	5.5	S*
67. ALB201	5	M	5.5	S*
68. ALB204	6	M	4.5	S*
69. ALB206	5	M	4.5	S*
70. ALB217	4	M	5.5	S*
71. CAL96	9	S	9	S
72. RWR719	2	R		-
73. MLB-49-89A	-	-	2	R
Mean	4.5		2.6	
CV (%)	54.8		52.8	
Se 1.23			0.82	
SED (5%)	1.74		1.16	
LSD (5%)	3		2.5	

CV = coefficient of variation, Se = standard error of the mean, SED = standard error of the difference, LSD = least significant difference, R =Resistant (score 1-3), M-Moderately resistant (score 4-6), S= Susceptible (score 7-9), S*=Differing reaction a result to changes in environmental conditions

greater than 1500 kg ha^{-1} in more than one season were also resistant to both Fusarium and Pythium root.

Seed, plant growth and morphological characterisation of *P. coccineus* interspecific lines. The majority the interspecific lines were small to medium in seed size, and were grouped under Mesoamerican or Durango types, whose 100 seed weights are about 20 and 30 g, respectively (Singh *et al.*, 1991a, b and c). The large seeded Andean beans range from 35–50 g in 100 seed weight. The interspecific lines were diverse in seed colour and were grouped into six market-classes according to seed colour namely, red, black, cream, pink, purple and brown; and according to growth habit into determinate and indeterminate bush beans. This is because seed color, seed size, and plant growth habit play

important roles in farmer, trader, processor and consumer preference (Buruchara *et al*., 2011; Kiwuka *et al.,* 2012). Small to medium seeded varieties are preferred in some parts of Uganda and several others in East Africa.

In Uganda, small red beans are majorly preferred in northern Uganda and eaten in mixtures in central and south western Uganda (Buruchara *et al*., 2011). The small red beans are also, especially preferred by the market in Ethiopia and Kenya. The red kidney beans are popular mainly in south western Uganda and are a major commodity in Tanzania and Malawi (Buruchara *et al*., 2011).

Cream beans are preferred by the market in Kenya and South Africa, and to some extent Uganda (Buruchara *et al*., 2011); while black beans are majorly consumed in northern Uganda and are a niche product in Mozambique (PABRA, 2013). Even though majority of the lines are small or medium seeded, and thus may not be suitable in areas where large seeded varieties are preferred, farmers have been shown to make tradeoffs between preference and food security, when faced with climate uncertainty that requires resilient varieties (Mukankusi *et al*., 2015). This collection of lines, hence provides genetic materials with useful seed grain types that could be adopted by different consumers and also be used by breeders to advance their programmes.

Growth habit was majorly erect indeterminate bush growth habit (Type 2), preferred by most farmers due to ease of applying crop management techniques during the growing phases. Flower colour mostly white, days to flowering and days to physiological maturity were similar to most popular common bean varieties making them suitable for the cropping cycles.

Response of *P. coccineus* interspecific lines to field diseases. The yield loss attributed to naturally occurring field disease infection is probably negligible because of the low disease pressure noted for most field diseases as reflected by low scores obtained in susceptible check, CAL 96 (Table 3.). Nonetheless, some diseases were more severe in some lines compared to CAL 96. Out of 186 test lines, 177 (95%) had at least a plant with black root. Generally, 1-51% of plants in each plot was lost to back root. A comparison of inoculated and non-inoculated plants revealed an unexpected trend, i.e., considering only inoculated plants, 37.4 % of ALB lines did not show necrosis compared to 95% that had necrotic symptoms of BCMNV in the non-inoculated experiment. This possibly suggests existence of external/environmental factors affecting the success of artificial inoculation and disease development.

Of the nine lines (5%), which showed no necrosis in both inoculated and non-inoculated plants, only ALB139 did not show necrosis subsequently. Thus, all these lines, with possible exception of ALB139, may not possess the bc3 gene that confers resistance to BCMNV. This implies that most of the interspecific lines may possess the dominant "I" gene for resistance to BCMV that requires protection by introgressing the bc3 gene in especially the best performing lines. It also implies that these lines could be best utilised as parents at this stage; and not promoted in areas with the necrotic strain of BCMV, before the confirmation of the presence of I and bc3 gene in them.

Resistance of *P. coccinues* interspecific lines to Pythium and Fusarium root rots. This study identified good levels of resistance to Fusarium root rot (*Fusarium solani* fsp. *phaseoli)* compared to Pythium root rot (*Pythium ultimum)* among the interspecific lines and identified those with combined resistance to the two root rot diseases. Preliminary reports of the presence root rot resistance in this population were presented by Beebe *et al*. (2012). Resistance to Sclerotium root rot has also been identified among these lines (Paparu *et al*., 2013; 2014). The ALB lines were characterised as belonging to the Middle American genepool in this study.

The Middle American genepool has been shown to possess resistance to many diseases, in comparison with the Andean genepool (Islam, 2003; Singh, 2013), and this possibly explains the resistance identified in this population.

The identified resistance offers a possible solution to the root rot problems, envisaged to increase in eastern Africa due to increased precipitation that is predicated for this region (Jones *et al*., 2009; Farrow *et al.*, 2011). Some of the lines may also be important under moisture constrained conditions, as they expressed resistance Fusarium root rot which is especially important under conditions of adequate rainfall in the seedling stages and no or little moisture in the proceeding days, a phenomenon that is increasingly becoming common in the East African region.

The lines with combined resistance to Fusarium and Pythium root rot are especially useful in areas where land degradation, as a result of the increasing human population, puts pressure on agricultural land, a factor that is common in most East African countries. Land degradation exacerbates the importance of root rot pathogens (*Pythium* spp, *Fusarium solani* fsp. *phaseoli, Sclerotium rolfsii* and *Rhizoctonia solani*) due to reduction of the soil fertility that renders the plants roots vulnerable to attack hence necessitating the continuous identification of resistant germplasm.

CONCLUSION

This study revealed the potential for utilisation of sister species of *Phaseolus vulgaris* as a source of inherent traits that respond to key bean production constraints especially with the ever changing climate. In this study, the progenies of *P. coccinues* and *P. vulgaris* were tested for seed characteristics, field adaptation and resistance to BCMNV and Pythium and Fusarium root rot. Superiority of some of lines, over the adapted variety, CAL 96 and RWR719 a regional small red variety, in terms of yield and resistance to root rot, was observed. Generally differences occurred among genotypes for the measured variables, suggesting potential for adoption in various areas in Africa. The interspecific lines evaluated in the study exhibited a range of grain market classes that are acceptable in many African countries where particular market classes are preferred. Wider testing of selected lines under farmer conditions is suggested to identify lines that can be adopted by farmers. Introgression of the bc3 gene will be important if they are to be further promoted in BCMNV prone environments as they exhibited levels of susceptibility to this virus.

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