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AGRONOMIC QUALITIES OF GENETIC PYRAMIDS OF COMMON BEAN DEVELOPED FOR MULTIPLE-DISEASE-RESISTANCE

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ABSTRACT

Multiple co-infections by different pathogens on common bean (*Phaseolus vulgaris* L.) affect its productivity and cause complete crop loss in susceptible varieties. Therefore, gene pyramiding using marker assisted selection (MAS) and backcrossing, provide alternative cost-effective control measures to bean diseases. However, in the process of developing pyramids, linkage drags were likely to affect the qualities of progeny lines, hence, special attention was paid to this situation. The objective of this study was thus to assess the agronomic qualities of advanced genetic pyramids developed from a four-way cross for multiple disease resistance. The disease resistance genes (R) pyramided from four parents were: *Co4*[2] and *Co-5* from G2333; *Phg-2* from MEX54; *Pythium ultimum* Dennis from MLB49-89A and *I* & *bc3* from MCM5001. The progeny lines were planted in an incomplete block design, and replicated thrice for two seasons (2015A and 2015B) in fields at CIAT, Kawanda in Uganda. Agronomic traits were highly heritable (0.6), except number of pods per plant (< 0.3). Backcrossing generated high-yielding bean lines, with 270 - 290 seed per plant and early maturity (95-100 days). Nine superior lines with desirable qualities, such as earliness (95 days), high seed rate (290 seeds per plant), and climbing ability, were obtained. Pyramiding R genes did not affect yield traits, except time to flowering and number of flower buds per plant due to transgressive segregation.

Key Words: Backcrossing, marker assisted selection, transgressive segregation

RÉSUMÉ

Les co-infections multiples par de différents pathogènes sur le haricot commun (*Phaseolus vulgaris* L.) affectent sa productivité et causent la perte totale des variétés susceptibles de la culture. Par conséquent, la pyramide des gènes en utilisant la sélection assistée par des marqueurs (MAS) et le rétrocroisement, fournissent des mesures alternatives de contrôle moins chères des maladies du haricot. Néanmoins, dans le processus du développement des pyramides, les poids des liaisons affectent probablement les qualités des lignées de progénitures, de ce fait,

une attention particulière était portée à cette situation. L'objectif de cette étude était d'évaluer les qualités agronomiques des pyramides génétiques avancées développées d'un croisement de quatre parents pour la résistance aux maladies multiples. Les gènes pyramidés de résistance (R) à la maladie de quatre parents étaient : *Co42* et *Co-5* de G2333; *Phg-2* de MEX54; *Pythium ultimum* Dennis de MLB49-89A et *I* & *bc3* de MCM5001. Les lignées de progénitures étaient plantées en arrangement de block incomplet et répliqué trois fois pendant deux saisons (2015A et 2015B) dans les champs à CIAT, Kawanda en Ouganda. Les traits agronomiques étaient hautement héritables (0,6), à l'exception du nombre de gousses par plant (<0.3). Le rétrocroisement a généré des lignées d'haricot à haut rendement, avec 270-290 graines par plant et à maturité précoce (95-100 jours). Neuf lignées supérieures avec des qualités désirables, telles que la précocité (95 jours), taux de graines élevés (290 graines par plant) et l'habilité grimpante, étaient obtenues. La pyramide des gènes R n'avait pas affecté les traits liés au rendement, à l'exception de la période de floraison et le nombre de bougeons de fleurs par plant dû à la ségrégation transgressive.

Mots Clés: Rétrocroisement, ségrégation transgressive, sélection assistée de marqueurs

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is one of the most important sources of protein, micronutrients, vitamins, and dietary fiber for the human diets especially in Sub-Saharan Africa (Bennink, 2005). However, multiple pathogen co-infections on common beans cause complete crop losses in susceptible bean varieties (Wortman *et al*., 1998; Mahuku *et al.,* 2002).

Combinations of cultural and chemical control have been used, but have limited success against many diseases. Hence, genetic resistance is the most cost-effective and environmentally friendly control measure at farm level (Busogoro *et al*., 1999; Odogwu *et al*., 2017). Broad resistance genes are generally bred into popular crop varieties, to protect them from new emerging pathogen races and prevent epidemics (Agrios, 2005).

Recurrent backcrossing transfers alleles at one or more loci, from donors to an elite variety (Allard, 1960); while forward selection with markers, also referred to as marker-assisted selection (MAS), only introduces that specific region. But by chance other, unlinked regions in the genome will also be introduced and this will create more variations through hybridisation upon which selections could be based (Kelly *et al*., 2003; Miklas *et al*., 2006; Collard and Mackill, 2008). Pyramiding bean disease resistance genes through MAS and backcrosses, could provide a better strategy for developing durable disease resistances.

Previously, four bean parents with resistance to major diseases, namely Angular leaf spot (ALS), Anthracnose (ATH), bean common mosaic and necrosis virus (BCMNV); and *Pythium ultimum* (*P.ult*) root rots were combined into the same genotype at CIAT, a process referred to as pyramiding. Common bean genetic pyramids could, therefore, offer long-term strategies for managing major common bean diseases. However, in the process of developing pyramids, co-transmissions of loci that affects the overall quality of progeny lines is likely to occur. Hence, special attention needs to be paid to this condition.

Assessing the morpho-agronomic qualities of genetic pyramids is necessary and fits in the cyclic, recurrent breeding process of developing progeny lines with superiority to local checks (Allard, 1960). In addition, transgressive segregation is another source of genetic variability to look out for among genetic pyramids, since it causes individuals in a segregating population to express traits of interest outside the boundaries of the parents (Arama *et al*., 2000; Schwarzbach *et al*., 2001; Acquaah, 2007).

The objective of this study was to assess the agronomic qualities of advanced genetic pyramids developed from a four-way cross [(G2333 x MCM5001) x (MEXICO-54 x

MLB49-89A)] at CIAT (Fig. 1) and relate genotypic information with field disease resistance among superior pyramided lines identified prior to multi-location evaluations.

MATERIALS AND METHODS

Common bean populations. The common bean populations evaluated (Table 1) were categorised as (a) parents: G2333, MEXICO 54, MLB49-89A and MCM5001, (b) genetic pyramids (Fig. 1) and blank sister lines to pyramids, which lacked the combined genes; and (c) large seeded commercially released checks (NABE12C, NABE29C and K132). NABE 12C and NABE29C were picked due to their climbing ability in relation to climbing pyramided lines (Table 1). K132 a bush bean was included as a local check due to its complete susceptibility to the targeted diseases and early maturity. All the bean populations evaluated were climbers except one donor parent, MCM5001.

Field experiment. The experiment was conducted on-station, in the fields of the National Agricultural Research Laboratories (NARL) at Kawanda in central Uganda (latitude: 0.4223° N, longitude: 32.5415° E; altitude 1178 m above seas level). The experiment was conducted for two seasons in 2015 (April - July and September - December). The bean populations were evaluated in four randomised incomplete blocks, to increase homogeneity of soils within blocks; and replicated three times. The available seed of each entry was divided into two volumes to allow for set-up of the experiment for two seasons.

Thirty seeds were planted per entry in plots of two rows, measuring three meters long, with spacing of 20 cm x 60 cm within and

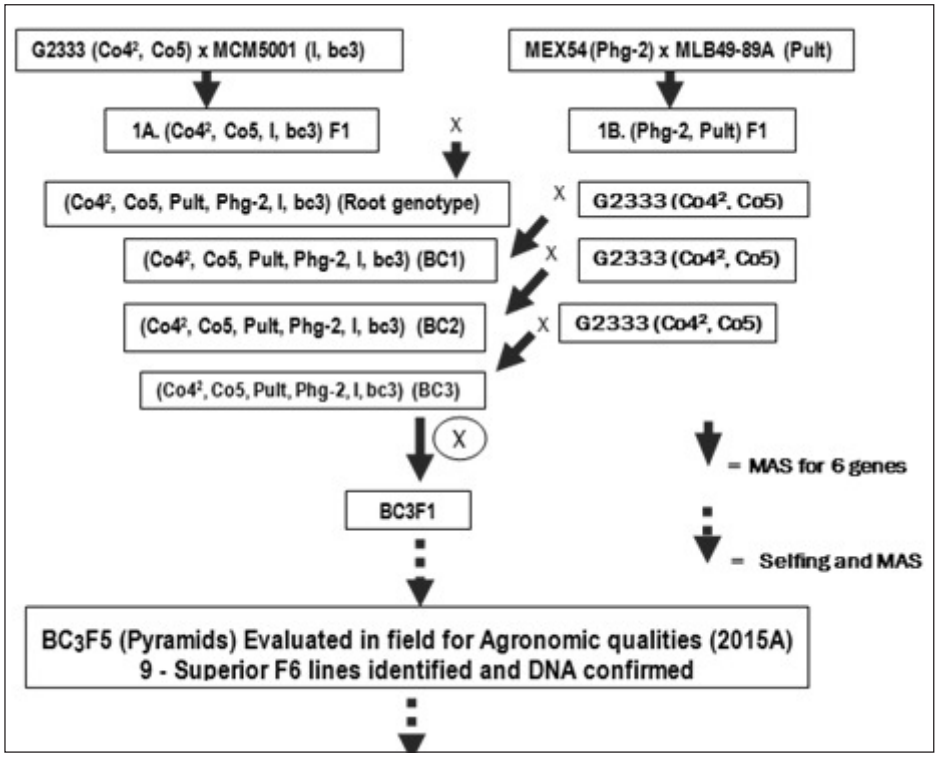

Figure 1. A simultaneous gene pyramiding scheme derived with SCAR markers since 2010 at CIAT, showing, parents with disease resistance genes donated in brackets, three backcrosses of pyramid/root genotype to G2333 and subsequent selfing to fix genes pyramided.

TABLE 1. Summary of agronomic and yield traits, disease scores and heritability among common bean populations evaluated in 2015A

Populations	DFLO	Nodeno	Flobuds	Npodsplt	Sdplt	100 ws (g)	ALS	ATH	BCMNV	*P. ult*
1. Pyramid [S]	43-47	18	7-9	38-47	247-290	24.6-28.8	1-7	1-8	1-8	-
2. Blank[S]	46-58	14-25	4-12	10-70	217-256	19.2-30.8	4-7	4-7	4-6	
3. G2333[P]	39	22	10	44.5	361	26.8	1	1	5	3
4. MEXICO54[P]	40	14.5	5	34	137	34.6	1	3	7	4
5. MLB49-89A [P]	39	19	6.5	40	140	37.2	8	9	5	1
6. MCM5001 [P]	45	4	5	24	120	21.2	8	3	2	7
7. NABE12C [R]	50	14	8	21	213	44.0	8	7	4	7
8. NABE29C [R]	48	14	8	17	199	38.4	4	3	5	5
9. K132 [R]	36	4	6.5	18	86	47.3	9	7	5	7
Mean	45±0.3	18±0.3	7.6±0.2	41±1.3	263±10.3	27.8±0.7	2.2±0.04	1.5±0.1	4.3±0.16	-
CV (%)	8.3	6.9	6.4	11.22	14.2	12.8	5.8	8.3	4.7	-
Heritability	0.73	0.83	0.68	0.14	0.56	0.69	0.74	0.38	0.84	-

Populations, 1 and 2 are segregating progenies, genotype 3 – 6 are parents of the pyramids; and 7-9, released commercial varieties. All populations are climbing, except 6 (MCM5001) and 9 (K132). DFLO = days to flowering, Nodeno = number of nodes on the main stem, Flobuds = number of flower buds formed per inflorescence, Npodsplt = number of seeds per plant, Sdplt = number of seeds per plant; and 100ws weight of 100 seeds per plant. Diseases, ALS – angular leaf spot, ATH = anthracnose and BCMNV = bean common mosaic necrosis virus

between rows, respectively. Climbing beans were supported by wooden stakes. Traits evaluated were days to 50% flowering (DFLO), number of internodes (Nodeno), flower buds (Nflobuds), pods per plant (Npd_splt), seeds per plant (Sd_plt) and weight of 100 seeds (100Ws). Data were recorded following guidelines in the Bean trait Dictionary (http://www.cropontology.org/ontology/CO_335/Common%20bean, accessed April 2015).

Foliar diseases in the field were scored as: <3 = resistant, 3.1 - 6 = moderately resistant, 6.1-9 = susceptible, from flowering to maturity for each plant that survived. *Pythium ultimum* root rots disease (symptoms like plant wilting) were absent in the field and, therefore, further uprooting and sampling of *P. ultimum* pathogens was not necesary at this stage, since root rot evaluations would require destructive uprooting of potential superior bean lines whose seed would need multiplication for use in future evaluations. Common bean qualitative traits observed included: plant type, flower colour, seed shape, and seed colour. Superior progeny lines previously, tagged in the field with resistance to diseases and desirable agronomic qualities, were harvested using hands, put in paper envelopes and labelled accordingly and kept as germplasm.

Marker assisted selection

Polymerase chain reaction (PCR) and electrophoresis. The total genomic DNA of 345 plants in the field, from pyramided populations, was isolated in the molecular laboratory facility at CIAT, Uganda, according to Mahuku (2004), and kept in Eppendorf tubes at -20°C for further analyses. DNA samples for amplification through PCR, were diluted to a factor of 1 in 30 µl solution and sorted out according to the field plan. The PCR reaction mix had 5 µl of the *Accu-Power PCR* premix composed of DNA polymerase, dNTPs, reaction buffer, blue tracking dye and patented stabiliser. One microlitre of DNA, 0.3 µl of each primer, and 3.4 µl water was added to the premix to make a total reaction volume of 10 µl.

The 345 sample tubes were placed in a thermocycler (MyGenie, Daejeon) for the PCR reaction cycle consisting of 95æ%C for 5 minutes followed by 35 cycles of (94°C for 20 seconds, primer annealing temperature for 40 seconds, extension at 72°C for 1minute), and final extension at 72°C for 7 minutes. The PCR products of markers used (Table 2 and, http://phaseolusgenes.bioinformatics.ucdavis.edu, accessed March, 2015, for more marker details) were separated on 1.2%

TABLE 2. Properties of molecular markers used in selection

No.	Marker	Genes	Expected size (bp)	Targets	References
1	SAB3	Co-5	400	Anthracnose	Vallejo and Kelly (2001)
2	SAS13	Co4^2	950	Anthracnose	Awale and Kelly (2001), Kelly *et al*. (2003)
3	PYAA19	*P.ult*	750	*Pythium ultimum*	Mahuku *et al.* n.d (Unpublished)
4	SN02	Phg-2	700	Angular leaf spot	Sietsche *et al*. (2000)
5	SW13	I	690	BCMV	Kelly *et al*. (1995), Melotto *et al.* (1996)
6	ROC11	bc-3	420	BCMNV	Johnson *et al*. (1997)
7	Phs	Phaseolin	Multiple	Gene pools	Kami *et al*. (1995)

Foliar diseases are, Diseases, n mosaic and necrosis Angular leaf spot, anthracnose and BCMV and BCMNV Bean common mosaic and necrosis viruses), while *P.ultimum* causes root rot diseases. Markers 1 -6, are SCAR = sequence characterized amplified-region, while *Phs* is a sequence tag site (STS) marker for gene pool determination. Marker sizes are in bp = base pairs. Source: Marker Database (http://phaseolusgenes.bioinformatics.ucdavis.edu)

agarose gels ran for 30 minutes, at 140 voltages and stained in ethidium bromide (10 mg ml^{-1} solution) for visualisation under UV light, in Gel Snap (Syngene, India). Similarly, PCR products amplified with the phaseolin marker were separated as in Kami *et al.* (1995), using 6% acrylamide gels due to its higher resolution.

Data analysis. Statistics from the evaluated bean population were summarised using GenStat 14th Edition (Payne *et al.*, 2011). Phenotypic correlations among traits were determined using the PROC CANCORR subprogramme of SAS (2011); and genetic correlations were computed using the Breeding View programme (VSN International Ltd, UK).

The assumption of equal variances among treatments required for mean comparisons of treatments in the general linear model (GLM), was not appropriate in these analyses due to segregation of bean progenies (pyramids and blank), which were regarded as random variables. Agronomic qualities and yield trait means were, therefore, compared using linear mixed models of the REML algorithm (McCarville *et al.*, 2014), using the Breeding View programme, where the fixed part of the model for the phenotypic traits (Y) was the overall mean (μ), plot and blocks; while the main effects of seasons and interactions were random variables. The random model terms were the main effect of the genotypes, assigned to incomplete blocks within whole plot and error using the Equation below.

$$Y = \mu + \text{genotype} + \text{plot} + \text{block} + \text{season} + \text{genotype} * \text{season} + \text{interactions} + \text{error}$$

To test the effect of pyramiding on agronomic traits, a student t test was conducted among pyramided lines and blanks as a control. This was guided by the hypothesis that "the six pyramided disease resistance genes did not affect yield traits of pyramided bean populations".Observed transgressive traits segregation was computed by subtracting the mean of the four parents from that of pyramided bean populations.

RESULTS

Agronomic qualities, yield traits and transgressive segregation. The agronomic and yield traits performance, heritability and disease scores among common bean populations are presented in Table 1. Pyramids segregated with varying levels of resistance to foliar diseases (ALS, Anthracnose and BCMNV), which enabled identification of nine superior lines with desirable qualities such as increased pod load per plant. For blank genotypes, levels of resistances to the three foliar diseases ranged from moderate to susceptibility; while differences in BCMNV disease scores among pyramids and checks was significant ($P<0.01$).

Among yield traits, only days to flowering and number of flower buds formed per inflorescence, were significant ($P<0.05$) among pyramids and blank bean lines at BC3F6 generation (Table 3). The means of the pyramided progeny bean lines deviated from means of the four donor parents; there was notable increase in flowering time (five days), one flower buds per inflorescence, 10 pods per plant and 88 seeds per plant; while weight of 100 seeds reduced by 3.1 grammes.

Correlations between agronomic traits and diseases. The relationship between disease scores and agronomic traits for bean populations are shown in Table 4. Number of nodes per plant had a strong negative correlation with Angular leaf spot ($r = -0.8$, $P<0.01$) and Anthracnose ($r = -0.76$, $P<0.01$). BCMNV had strong negative correlations with weight of 100 seeds per plant ($r = -0.54$, $P<0.01$); while the correlation between flower buds and weight of 100 seeds per plant was negative ($r = -0.67$, $P<0.01$).

The number of seeds per plant had a strong correlation with flower buds ($r = 0.58$, $P<0.01$)

TABLE 3. Yield related traits among pyramided genotypes and their deviations from donor parental means to show transgressive segregation

Trait	Genotype	Mean	Deviation from parental mean	P value
Days to flowering	Pyramids	46	+5	0.0014*
	Blanks	52	+11	
Flower buds per inflorescence	Pyramids	8	+1	0.043*
	Blanks	7	0	
Nodes on the main stem	Pyramids	19	+4	0.3316
	Blanks	21	+6	
Pods per plant	Pyramids	46	+10	0.5051
	Blanks	40	+4	
Seeds per plant	Pyramids	278	+88	0.9197
	Blanks	284	+94	
Weight of 100 seeds in grammes	Pyramids	26.9	-3.1	0.2222
	Blanks	25.7	-4.3	

Genotypic results were used to disaggregate bean progenies into pyramids and blanks for comparing their phenotypes through t tests; asterisk (*) shows significance at 95% confidence interval. Positive or negative deviation from parental mean shows the direction of segregations of pyramided genotypes from the mean of the four donor parents

TABLE 4. Phenotypic correlations of common bean agronomic traits and disease scores in Uganda

	HWS	ALS	ATH	BCMNV	DFLO	Flobuds	Nodeno	Npods
ALS	0.03							
ATH	0.40	**0.76**						
BCMNV	**-0.54**	0.22	-0.16					
DFLO	-0.12	-0.47	-0.36	-0.24				
Flobuds	**-0.67**	-0.34	-0.45	0.19	0.17			
Nodeno	-0.27	**-0.80**	**-0.76**	0.04	**0.54**	0.48		
Npods	-0.12	0.22	0.24	0.15	-0.05	0.44	-0.12	
Sdplt	-0.08	-0.39	-0.19	-0.23	0.48	**0.58**	0.42	**0.55**

Bold values are significance of P set at 0.05 and 0.01. HWS =100 seed weight (g), ALS = Angular leaf spot, ATH = Anthracnose, BCMNV = Bean common mosaic necrosis virus, DFLO = Days to flowering, Flobuds = flower buds, Nodeno = number of nodes on the main stem, Npods = number of pods per plant and Sdplt = seeds per plant

and seed per plant and pods per plant (r = 0.55, P<0.01). Days to flowering and nodes formed on the main stem were strongly correlated (r = 0.54, P<0.01).

Genetic correlations among agronomic and yield traits are shown in Table 5. Strong positive genetic correlation was observed between seeds per plant (r = 0.71, P< 0.01) and number of pods (r = 0.67, P<0.01). Negative genetic correlations was observed between weight of 100 seeds and days to flowering (r = - 0.58, P<0.01).

Qualitative traits among pyramids and recovery of recurrent parent traits. Progeny lines segregated for all traits, except

TABLE 5. Genetic correlations among bean yield traits in Uganda

No.	Trait	1	2	3	4	5
1	Days to flowering	-				
2	Nodes per plant	0.36	-			
3	Flower buds per plant	0.15	0.45	-		
4	Number of pods per plant	0.26	0.10	0.34	-	
5	Seeds per plant	0.42	0.36	**0.67**	**0.71**	-
6	100 seeds (g)	**–0.58**	–0.43	–0.29	0.20	–0.31

Bold values show strong and significant correlations at probability value set at 0.05 and 0.01

white flower and plant type (Table 6). Red seeds were the most frequent (72%) among progenies of pyramids; followed by black (19%) and brown (9%). Blank progeny lines lacked brown-seeded progenies;while red (43%) and brown (47%) seeds were comparable among pyramids progenies. However, both blanks and pyramids had small and medium seeded progenies. Progenies of pyramids and blanks had predominantly small-seeded lines, with 67% and 57%, respectively.

The predominant seed shape was cuboid, with a frequency of 62% and 57%, respectively.

The recovery of the recurrent parent (G2333) traits in pyramided progeny lines was estimated using frequencies of pyramided phenotypes (Table 7). Climbing ability was fixed; while seed colour and sizes segregated among pyramids.

Days to flowering (DFLO), number of flower buds (Flobuds), and number of nodes (Nodeno) of G2333 were not yet recovered in segregates by the third back-cross. Phenotypic similarity observed between pyramided lines and the recurrent parent, G2333, for the number of pods (12.5%) and weight of 100 seeds (10.7%) were still low.

DISCUSSION

Agronomic qualities, yield traits and transgressive segregation. Generally, gene pyramiding did not affect yield traits (Table 3), presumably due to the independence of six disease resistance genes pyramided, and QTLs for yield traits. Significant transgressive segregations was observed for flower traits, between pyramids (+5 days) and blanks (+11 days) could be due to the effect of recombination in the progeny of the parents hybridised, maternal effects, or genotype by environment interactions.

Extreme phenotypes among segregates, have been reported to be caused by genetic recombination of alleles at several loci, epistasis, and reduced developmental stability in the genotypes (Schwarzbach *et al.*, 2001; Hallauer *et al.*, 2010). Segregations of agronomic qualities and yield traits among pyramided progenies enabled identification of superior lines in the field with desirable qualities such as uniform or columnar pod distributions along the plant (J. Kelly, personal communication, 2015; Michigan State University, Department of Plant, Soil and Microbial Sciences).

The significant difference among pyramided bean populations (Tables 1 and 3) was attributed to trangressive segregations and large environmental effects on yield traits. Transgressive segregation was reported for climbing ability in common beans developed from inter-gene pool crosses (Checa *et al.*, 2006). Transgressive segregation was also found useful in enhancing resistance of wheat to *Septoria tritici blotch* for yield increase (Arama *et al.*, 2000).

The lack of brown seeded blank lines among blank progenies (Table 6) could be due to linkage in *trans* with one of the resistance genes or the relative small progeny sizes.

TABLE 6. Qualitative traits among bean populations evaluated at CIAT, Kawanda in Uganda

No.	Genotypes	Seed attributes (%)			Flower colour	Growth habit	Gene pool
		Colour	Size	Shape			
1	Pyramids	Red (72)	Small (67)	Round (6)	White	Climber	M
		Black (19)	Medium (36)	Oval (18)	-	-	M
		Brown (9)	-	Cuboid (62)	-	-	M
		-	-	Kidney (4)	-	-	M
		-	-	Truncate fastigiate (10)	-	-	M
2	Blanks	Red (43)	Small (57)	Round (0)	White	Climber	M
		Black (47)	Medium (43)	Oval (10)	-	-	M
		-	-	Cuboid (57)	-	-	M
		-	-	Kidney (14)	-	-	M
		-	-	Truncate fastigiate (19)	-	-	M
3	G2333	Red	Small	Round	White	Climber	M
4	MEX54	Cream	Large	Kidney	Purple	Climber	M
5	MLB49-89A	Black	Medium	Round	Purple	Climber	M
6	MCM5001	Cream-mottled	Small	Round	White	Bush	M
7	NABE12C	Cream-mottled	Large	Round	White	Climber	A
8	NABE29C	Red	Large	Round	Pink	Climber	A
9	K132	Red mottled	Large	Kidney	White	Bush	A

Genotype no, 1- 2 are segregating progenies; 3 - 6 are parents pyramided and 7-9, released commercial varieties. Values in brackets represent frequencies of seed attributes

TABLE 7. Frequencies of traits of G2333 recovered in progeny lines at BC_3F_6 generation

Traits	G2333 (Control)	Frequency (%) (N = 345 progenies)	Recovery (%)
1	Plant type (IV)	100, (345)	100
2	Red seeds	72, (248)	76.8
3	Small seeds (< 26.8 g 100^{-1} seeds)	67, (231)	71.5
4	Days to flowering (39)	0, (43-47)	0
5	Number of flower buds (10)	0, (7-8.5)	0
6	Number of nodes on the main stem (15)	0, (17.9-18.4)	0
7	Number of pods per plant (44)	43, (38.2-46.6)	12.5
8	Number of seeds per plant (361)	0, (247-297)	0
9	Weight of 100 seeds (27.0 g)	37, (24.6-28.8)	10.7

Traits 4-9 are quantitative, with there means in brackets based on the phenotype of G2333. Percentage of trait recovery is the ratio of number of progeny lines observed with phenotypic resemblance to G2333 to all progenies phenotyped. Traits with zero % were not yet recovered by BC3F6 (i.e traits 4 - 6) and had ranges higher than in G2333, while traits, 5 and 8 had lower ranges. Values in brackets represent the actual number of progeny lines with phenotypic resemblance to G2333 for qualitative traits (1-3), and for quantitative traits (4-9) brackets show the range among segregating progenies

Selection for seed colour in common bean was reported to have recovered significant segments of the bean genome after the crop's domestication through linkage drags (Beebe *et al*., 1995), and caused more related genotypes to have seeds of similar colour (Duarte *et al*., 1999), such as red- and black- seeded progenies observed in this study (Table 6).

Correlations between agronomic traits and diseases. A strong negative correlation between days to flowering and 100-seed weight suggests that the small seeded bean lines are late maturing due to increased days to flowering in the bean population studied.

Correlations showed that field diseases negatively influenced bean yield traits (Table 4). For example, the effect of high incidences of BCMNV (a seed-borne pathogen) in the field, on weight of 100 seeds was evident. Strong phenotypic and genetic traits correlations in the present study point to usual correlations among yield traits in common bean (Tanaka and Fujita, 1979). Strong negative correlations among traits generally affect selections for traits of economic importance during crop improvement; while weak correlations cause no difficulties during selection (Hallauer *et al*., 2010).

Indirect selections based on correlations are important for resource and time saving, when they give greater response to selection than direct selections (Hallauer *et al*., 2010). Literature on genetic correlations among agronomic traits and multiple diseases of common beans is still inadequate to enable comparisons within the present study.

Backcrossing pyramids to variety G2333 (Fig. 1), which was the source of the *Co4*^{2} genes for resistance to anthracnose (Pastor-Corrales *et al*., 1994), obviously increased the allele dosages *Co4*^{2} genes in the pyramided background and hence, the increased levels of resistance of pyramided progenies to anthracnose. It was obvious that the increment in allele dosages of *Co4*^{2} genes in the pyramided background would increase the levels of resistance to Anthracnose disease as in Table 5. This was confirmed with the higher frequencies of SCAR marker (SN02) bands (not yet published, Figs. 1 and 2A).

Differential responses of pyramids and blank bean progenies to field diseases shows that enhancing levels of resistance of well adapted bean genotypes through pyramiding multiple disease resistance genes, is possible and can mitigate production losses from field pathogens.

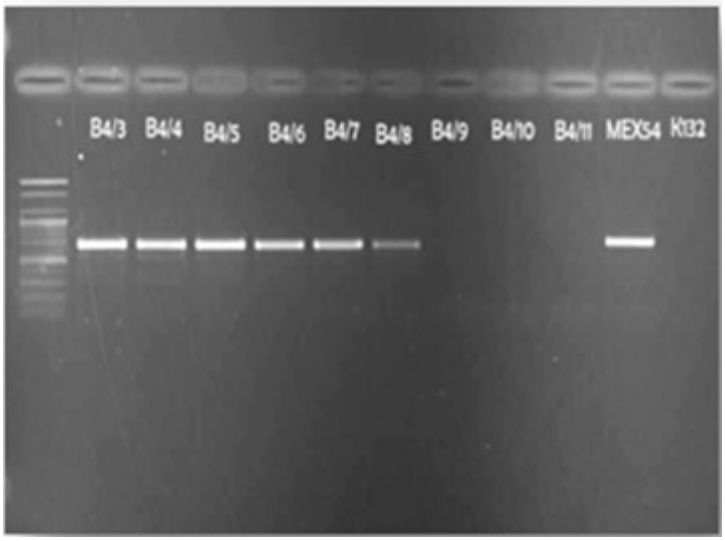

Figure 2A. Agarose gel demo showing presence of positive SN02 marker bands among progeny lines, B4/3-B4/8, and absent in lines, B4/9-B4/11. The ladder used for band sizing is the well in the gel before sample B4/3. Variety Mex54 (the donor for *Phg-2* gene) and K132 (susceptible to Angular Leaf Spot disease), were positive and negative controls, respectively.

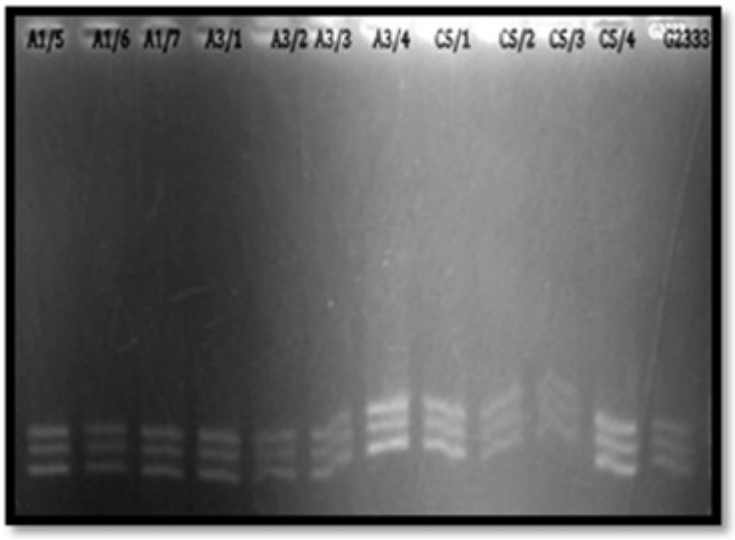

FIgure 2B. Phaseolin marker bands used to identify the gene pools of pyramided lines (A1/5 – C5/4) separated through gel electrophoresis. The band patterns of the Mesoamerican parental and the recurrent parent G2333, was included as the gene pool control.

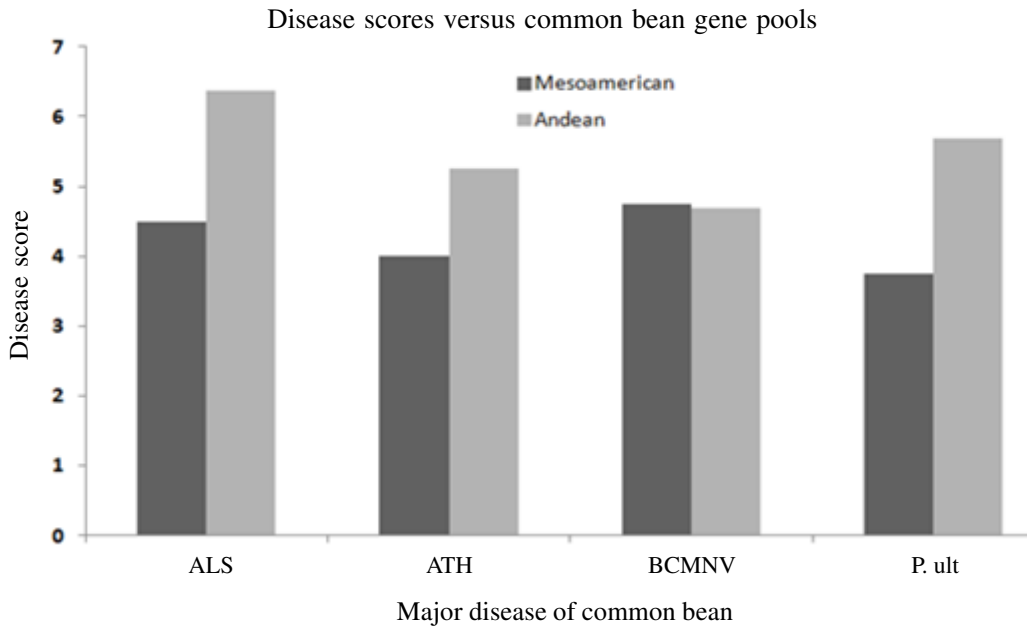

Figure 3. Response of Mesoamerican and Andean beans to foliar and root diseases. ALS = Angular leaf spot, ATH = Anthracnose, BCMNV = Bean common mosaic and necrosis virus and P.ult = *Pythium ultimum* root rot. Data plotted were derived from Table 1, where Mesoamerican beans are parents, while Andeans are the checks.

Disease scores were generally higher for Andean materials compared to Mesoamerican materials, except for BCMNV for which the scores were similar between the two gene pools (Fig. 3). A similar score of BCMNV between the two gene pools shows non-specific interactions of pathogens to the two gene pools. As most of the released beans in Uganda belong to the Andean gene pool, most pathogen strains are thus expected to be of Andean origin. Hence, Andean beans will generally be more susceptible to these strains as reported in other studies for angular leaf spot (Guzman *et al*., 1995) and anthracnose (Balardin and Kelly, 1998).

The predominant seed types and sizes in pyramided populations had characteristic of Mesoamerican beans (Fig. 2B) based on descriptions in previous reports (Singh *et al*., 1991 and Beebe *et al*., 1995). The parents of the genetic pyramids originated from the Mesoamerican gene pool (Okii *et al*., 2014), consisting mainly of small- to medium-seeded bean lines.

Recovery of the recurrent parent traits and heritability estimates. After three generations of backcrossing, the expected recovery of the recurrent parent (G2333) genome in pyramids would be 93.75% based on the assumptions that the proportion of the recurrent parent genome or genetic background would be recovered at a rate of $1 - (½)^{t+1}$ for each t backcrossing (Babu *et al*., 2004; Semagn *et al*., 2006). While the proportion of donor genomes was expected to reduce by 50% at each advanced generation, if selection was for a few desired traits (Hospital, 2005). Deviation from the expected 93.75% recovery of recurrent parent was attributed to physical linkages and interactions of major quantitative trait loci (QTLs) in donors (pyramid) and nearby genes with effects on agronomic traits (Ribaut and Hoisington, 1998; Semgn *et al*., 2006).

There were many observed deviations from the expected 93.75% among pyramided population (Table 7), because previous marker

assisted selections (Fig. 2A) was only for disease resistance; whereas interactions of other genes in the background shown by trait segregation influenced agronomic traits. Backcross methods are, therefore, only useful to transfer alleles at one or a few loci from a donor genotypes to elite and popular varieties (Allard, 1960; Semgn *et al*., 2006).

Plant type, seed colour and seed size were recovered most frequently (beyond 70%) (Table 6) due to their qualitative nature (Singh *et al*., 1991; Gomez, 2004), which is further demonstrated by their high heritability and low environmental susceptibility. Climbing progeny lines were the most frequent, since only a quarter of the parents in the pyramiding schemes was a bush (MCM5001), locally released as K131 in Uganda. The zero frequency in recovery for polygenic traits, which included earliness (days to flowering <39 d), number of flower buds (zero), and numbers of nodes (zero) compared to the recurrent parent (G2333), was due to high genetic heterogeneity from observed trait segregations. These listed traits, had low recovery despite their high broad sense heritability values estimated (Table 1) and were, thus expected to be highly recovered within pyramided populations.

More investigations are needed to establish the reason(s) why traits with high heritability were not recovered at BC_3F6 generation. One reason is because of high levels of genetic variations and population structure within common bean germplasm domesticated in the Mesoamerican origin (Okii *et al*., 2014). Parents used to develop genetic pyramids (Fig. 1) were actually from this Mesoamerican gene pool (Fig. 2B). Population Structure, in addition to segregations can cause false associations between markers used in selection and desirable genes. Hence attention needed to be paid to this situation, especially when selection is based on molecular markers, unlike conventional selection methods in bean breeding.

Comparable BCMNV scores among large seeded Andean checks, including NABE12C, NABE29C and K132 and Mesoamerican parents and pyramided line (Fig. 3), in a co-evolutionary perspective, suggests that the viral field strains that infected beans are genetically diverse, or that the resistance mechanisms and levels are similar between the two gene pools. Future deployments of Mesoamerican pyramids in Uganda, where commercially released varieties are predominantly Andean (Okii *et al*., 2014; Odogwu *et al*., 2017), will reduce the diverse Andean pathogens and control important diseases fore-casting from co-evolutionary relationships of bean pathogens and the host. For example, resistance genes of Mesoamerican origin were found to be more effective in managing Andean Angular leaf spot pathotypes (Guzman *et al*., 1995; Pastor-Corrales, 1996; Mahuku *et al.,* 2009).

Superior common bean pyramids in this study were still segregating at the BC_3F_6 stage (Table 6) and could be advanced further and fixed to homozygosity in late generations such as BC_3F_{10}. Genotype by environmental and stability of genetic pyramids, could also be tested in disease hotspots to identify superior lines with broader adaptability.

The observed similarity in scores of angular leaf spot and anthracnose disease for pyramids and donor parents, especially G2333 and MEX54; and the strong disease correlation values (Table 4) point to co-segregations of resistance genes for anthracnose (*Co-5*) and angular leaf spot (*Phg-2*) within the pyramided population. This putative linkage of genes of different fungal pathogens on the same chromosome could further be verified with genotypic data to test orientations of linkages in either trans or repulsions for effective gene pyramiding and selection with markers. Co-localisation of resistance genes for angular leaf spot and anthracnose in the same gene block of the beans chromosomes was reported (Miklas *et al*., 2006; Gonçalves-Vidigal *et al*., 2011).

The fourth backcross if done will recover other desirable qualities like early flowering (less than 43 days) and progenies with more

than 290 seeds per plant. There is need to determine the response of agronomic traits to selections to determine genetic gains in this important pyramided populations. We recommend testing for genetic and physical linkages, within the disease resistance genes pyramided and between the same genes and QTLs for flowering and other agronomic traits in common beans.

CONCLUSION

Pyramided bean lines segregate with varying levels of resistance to foliar diseases (Anthracnose and BCMNV). Pyramided disease resistance genes do not affect yield traits, except time to flowering and number of flower buds. Early maturing pyramided lines (flowering in less than 95 days) with high yield potential, with an average of 290 seeds per plant, have been identified among pyramided bean progenies.

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