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Genetic variation, Heritability estimates and GXE effects on yield traits of Mesoamerican common bean (*Phaseolus vulgaris L*) germplasm in Uganda

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Abstract

Germplasm of common beans from the Mesoamerican gene pool races: Durango, Jalisco, Mesoamerica and Guatemala have highest genetic variation for the crop's improvement. The objective was to assign 50 common bean germplasm in Uganda into its gene pool races based on analyses of population structure. Secondly, to estimate heritability and effects of genotype × environment (GXE) interaction on common bean agronomic and yield traits in space and time. Sample genomic DNA was amplified in 2011 with 22 Simple sequence repeat markers (SSRs) and alleles separated using capillary electrophoresis. Field evaluations were conducted in 2010 and 2011 at NaCRRI and 2015 at CIAT – Kawanda. Multivariate analyses of SSRs data identified four subgroups within the germplasm: K4.1–K4.4, with corresponding Wrights fixation indices (F_{ST}) as 0.1829 for K4.1, 0.1585 for K4.4, 0.1579 for K4.2 and least for K4.3 at 0.0678. Gene pool race admixtures in the population (14%) were notable and attributed to gene flow. Four superior parents currently used in improving resistance to major diseases grouped as; Jalisco for MLB49-89A; Mesoamerica for MCM5001 and G2333; Durango for MEXICO 54. Heritability values for yield traits estimated using phenotypic data from above fixed parents, was above 0.81. Season and location had significant effect ($P < 0.05$) on numbers of: flower buds per inflorescence, pod formation and weight of 100 seeds. The findings will improve understanding of co-evolutionary relationships between bean hosts and pathogens for better disease management and will broaden the germplasm base for improving other tropical production constraints.

Keywords: SSRs, Mesoamerica, races, diseases, heritability, yield, variations

Introduction

Common bean (*Phaseolus vulgaris* L.) is a true diploid ($2n = 22$) with a small genome (650 Mb) (Broughton

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et al., 2003) and the most important grain legume for human consumption in developing countries (Buruchara *et al.*, 2011). Common bean comprises of Mesoamerican and Andean gene pools, further sub-divided into races. Mesoamerican races include: Durango (D), Jalisco (J) and Mesoamerica and Guatemala (Gepts and Bliss, 1988; Singh *et al.*, 1991; Beebe *et al.*, 2000). This report was limited to the Mesoamerican gene pool due to its wider adaptation and additional genetic diversity (Singh and Muñoz, 1999; Beebe *et al.*, 2000).

Population sub-divisions arose in wild common bean approximately 110,000 years ago followed by a bottleneck before domestication, and later, a domestication bottleneck reduced genetic diversity (Mamidi *et al.*, 2013). Improvement programmes of common beans can be envisioned in evolutionary perspectives to fit Darwin's theories, which include: selection, heredity and variations into the plant-breeding cycle (Acquaah, 2007; Hallauer *et al.*, 2010). Natural and heritable variation accounts for most of the responses made in plant breeding (Caligari, 2001). While highly heritable traits are subject to large genetic gains then phenotypic selection is done per generation (Hallauer *et al.*, 2010).

Population structure analyses helps to detect levels of allelic variability, which is critical for discovery of quantitative trait loci (QTLs) in admixed populations and impacts association mapping, which exploits recombination events that have occurred in genotypes (Myles *et al.*, 2009; Mamidi *et al.*, 2013). Population structure needed to be determined among common bean germplasm collection because it causes spurious association between molecular markers used in selection and QTLs (Yu and Buckler, 2006) of economic importance such as yields.

To improve common bean productivity in Eastern Africa; yield traits need to be selected for in addition to pest and diseases, which cause bean production loses (Abebe *et al.*, 1996; Wortmann *et al.*, 1998).

Previously, core germplasm maintained in Uganda was characterized with Microsatellite markers (SSRs) which found high genetic variations in the germplasm for use in breeding (Okii *et al.*, 2014b). Performance of developed bean progenies can also be predicted using well-characterized parents selected (Muthomi *et al.*, 2011). For example, interracial transfer of economically important traits was successful among bean gene pool races (e.g. Kelly, 2004; Pathania *et al.*, 2014). Four parental germplasm later detailed under Materials and methods section required more focus, because they are popular source of resistance to major diseases in East Africa. Identification and selections of high-yielding recombinant bean lines with desirable seed types, however, remains a challenge for crosses derived from parents from the same gene pool race (Singh *et al.*, 1991a; Singh *et al.*, 1993). The study objectives were, first, to assign 50 Mesoamerican germplasm into respective gene pool races using both SSRs and phenotypes and comparisons to Mesoamerican bean races attributes in previous reports (Singh *et al.*, 1991; Beebe *et al.*, 2000; Díaz and Blair, 2006; Blair *et al.*, 2006a). Secondly, to estimate the heritability of yield its for predicting its genetic gains in bean populations and progenies derived from the available Mesoamerican germplasm.

Materials and methods

Parental genotypes and field evaluations in 2015

Thirty seeds of parental genotypes: G2333, MCM5001, MEX54 and MLB49-89A were planted in plots of three rows measuring 3 m long, with spacing of 20 cm between plants and 60 cm for climbers between rows. The field trials were done for two seasons in 2015 with three replications at CIAT, Kawanda. Climbers were supported on wooden stakes and the following traits were evaluated from plant emergency to post-harvest using the bean trait dictionary (http://www.cropontology.org/ontology/CO_335/Common%20bean); days to 50% flowering (DFLO), numbers of: internodes (Nodeno), flower buds (Flobuds) and pods per plant (Npdsplt), seeds per plant (Sdplt) and weight of 100 seeds (HWS). Descriptive traits observed among germplasm were plant types and seed colours.

Genotype G2333 (Colorado de Teopisca) from Mexico (Young and Kelly, 1996) is grown in East Africa and has broad resistance to severe races of *Colletotrichum lindemuthianum* (Pastor-Corrales *et al.*, 1994; Miklas *et al.*, 2006). G2333 was released in Rwanda as Umubano, as Lyamungu 85 in Kenya and as NABE10C in Uganda. G2333 belongs to the Mesoamerican gene pool race Guatemala (Blair *et al.*, 2006a, b) with climbing growth type IV, with shiny red seeds preferred in local markets, tolerant to low soil fertility and yields highly with more than 40 pods per plant.

MEXICO 54 is a climbing genotype of growth type III, with medium sized brown seeds, and is a major source of resistance to most African isolates of Angular leaf spot (Mahuku *et al.*, 2002; Namayanja *et al.*, 2006). MLB 49-89A is a climber of type III and medium seeded black seeds and resistant to root rot disease (Otsyula *et al.*, 1998) in fields of Eastern Africa. The black colour of MLB49-89A however has low preferences in local markets but it is highly demanded due to early maturity, fast cooking and good taste (Otsyula *et al.*, 2004; Buruchara, 2009). MCM 5001 (released as K 131 in Uganda) is a CIAT bred genotype of bush type with cream speckled small seeds, with resistance to BCMNV (Bean common mosaic necrosis virus) and was derived from crossing genotypes: IVT 831629 and BAT 1554 (Sengooba *et al.*, 1997).

Field evaluations and SSRs genotyping in 2010–2011

Phenotypic and molecular datasets of 50 Mesoamerican genotypes in the present analyses was obtained from datasets of 100 core Andean and Mesoamerican genotypes previously characterized (Okii *et al.*, 2014b). The 50 genotypes comprised of 39 landraces from Uganda and 11 exotic bean lines including the four parents: G2333, MCM5001, MEXICO 54 and MLB49-89A selected to develop progeny lines with major disease resistance, which are in advanced stages of evaluations at CIAT Uganda. SSR allele fragments at 22 marker loci of the 11 chromosomes of common bean were obtained using the ABI PRISM 3730 genetic analyser instrument (Applied Biosystems, USA) at the Veterinary Genetics laboratory at University of California, Davis in 2011. Plant type, seed size and 100 seed weight, which strongly depicts the crops domestication syndrome (Koinange *et al.*, 1996) and SSR allele sizes of the 50 genotypes in storage was analysed in the present study.

Data analysis

Allelic diversity among genotypes

The following genetic diversity parameters: allele number and frequencies, gene diversity, heterozygosity and polymorphism information content (PIC) were calculated for each SSR marker among the 50 Mesoamerican bean germplasm using Power Marker software version 3.25 (Liu and Muse, 2005).

Race structure determination using multivariate analyses

In order to identify the race structure among the 50 Mesoamerican genotypes; its genotypic data was analysed using two programs according to similar studies (for example, Díaz and Blair, 2006 and Blair *et al.*, 2006a). An unweighed neighbour-joining (NJ) phylogenetic tree was generated using the Darwin program (Perrier and Jacquemoud-Collet, 2006). While, the amounts of genetic differentiation (F_{ST}) among presumed sub-populations (K) was analysed using the STRUCTURE program (Pritchard *et al.*, 2000). The minimum number of K (1) and maximum K (4) were set in STRUCTURE given existence of a single Mesoamerican gene pool with four distinct races. The analysis had 10 simulations per K using 5000 replicates for burn-in and for analysis 50,000 iterations. The number of sub-groups or clusters was determined using the highest estimated probability among 50,000 runs using the software program STRUCTURE (Pritchard *et al.*, 2000; Díaz and Blair, 2006). The 'true' number of sub-populations (K) was confirmed according to Evanno *et al.* (2005) using the STRUCTURE Harvester (Earl and Vonholdt, 2012), available online (http://taylor0.biology.ucla.edu/struct_harvest/) for visualizing outputs. The race admixtures were identified for genotypes using the shared membership coefficients (%) among differently coloured sub-groups in the STRUCTURE bar plot (Díaz and Blair, 2006).

Determination of phenotypic variations and heritability

The level of phenotypic variations among the 50 Mesoamerican genotypes was determined with a classical and non-parametric linear dimensionality reduction technique (Jolliffe, 2002) of principal component analysis (PCA) in SAS program (SAS Institute Inc, 2011) to show genetic relationships. Phenotypic data, depicting the crops domestication syndrome (Koinange *et al.*, 1996) specifically plant type, 100 seed weights and seed colours from our previous study (Okii *et al.*, 2014b) was used in PCA to establish, the relationships between groups generated from DNA molecules and phenotypes.

Heritability values for six agronomic traits were regarded as narrow sense due to fixed nature (Acquaah, 2007) of the four parental populations evaluated in the Breeding-View program (Release 18.0, VSN International Ltd, Hemel Hempstead, UK).

Genotype × Environment (GXE) analysis using populations of four parents

Agronomic traits of selected parents (G2333, MCM5001, MEX54 and MLB49-89A) was planted in 2010/2011 at NaCRRI (environment 1) and 2015 at CIAT-Kawanda (environment 2) using Randomized incomplete block designs with three replicates was tested using *t* tests, to predict the effects of GXE interactions on bean agronomic traits of genetic pyramided lines derived from these parents, which are in advanced stages of evaluations towards development of germplasm with multiple disease resistance. Data of the four parents evaluated in 2010/2011 (Okii *et al.*, 2014a) were compared as part of the analysis.

Results

Genetic variations within the genotypes

The level of genetic variation was considerable among the germplasm for utilization in developing superior bean lines (Table 1). Alleles in parents ranged from one to six with a mean of four alleles per locus. Marker PVBR107 on linkage group (LG) two had the highest number of alleles. Heterozygosity (Ho) ranged from zero to 0.8, with mean Ho of 0.5 for the markers tested. All the SSR markers were

Table 1. Genetic diversity parameters among 50 common bean germplasm in Uganda

Marker	Linkage group	Major allele frequency	Alleles	Gene diversity	Heterozygosity	PIC
BMd10	1	0.5	3.0	0.6	0.3	0.5
BM200	1	0.4	4.0	0.7	0.5	0.6
BM156	2	0.8	2.0	0.4	0.0	0.3
PVBR107	2	0.3	6.0	0.8	0.8	0.8
BM159	3	1.0	1.0	0.0	0.0	0.0
BM197	3	0.5	3.0	0.6	1.0	0.6
BM171	4	0.5	2.0	0.5	0.0	0.4
PVatgc002	4	0.5	4.0	0.7	0.3	0.6
BMd28	5	0.4	5.0	0.8	0.8	0.7
BM175	5	0.3	6.0	0.8	0.5	0.8
BM187	6	0.3	5.0	0.8	0.5	0.7
BMd37	6	0.3	6.0	0.8	0.5	0.8
BM183	7	0.8	2.0	0.4	0.0	0.3
BM160	7	0.8	3.0	0.4	0.5	0.4
BM189	8	0.4	5.0	0.8	1.0	0.7
BM211	8	0.4	5.0	0.8	1.0	0.7
BM141	9	0.5	4.0	0.7	0.3	0.6
PVat007	9	0.4	5.0	0.8	0.5	0.7
GATS11B	10	0.4	6.0	0.8	0.8	0.8
BMd42	10	0.3	6.0	0.8	0.8	0.8
BMd41	11	0.5	4.0	0.7	0.3	0.6
BM205b	11	0.3	5.0	0.8	0.5	0.7
Mean		0.5	4.0	0.6	0.5	0.6

able to distinguish parental lines except marker BM159 on common bean LG three. The mean polymorphic information content (PIC) of markers within this Mesoamerican germplasm was 0.6 and ranged from 0.0 for BM159 to 0.8 for PVBR107, BM175, BMd37, GATS11B and BMd42.

Unique alleles and potential SSRs for use in selection

For practicability of the high allelic polymorphism for marker-assisted selection (MAS), focus under this section; was shifted to markers on LGs two, six, seven and eight, which have disease resistance genes pyramided as introduced. Each of the four parental lines had a unique allele based on diversity analyses of the 11 bean chromosomes or LGs, presented (Table 2). Similarly, other potential markers in genotype MLB49-89A were SSR allele 148/148 on LG two, at the PVBR107 marker locus and SSR allele 236/236 at marker locus BM156. On LG six examples of polymorphic SSR alleles were: 201/249 in MEXICO54 was at locus BM187 and SSR allele112/160 in MCM5001 at marker locus BMd37. On bean LG seven, heterozygous allele 208/308 was unique to MEXICO54 and located at BM160. On linkage BM189 group eight, two unique heterozygous alleles in genotype MCM5001 were 144/152 and 130/132 located at SSR marker loci BM189 and BM211, respectively.

Consequently, unique alleles observed among parents at different loci and LGs could be amplified in polymerase chain reactions (PCR) and separated in electrophoresis gel systems as markers, if they strongly co-segregated with traits of interest such disease resistance among bean progeny lines. For instance, allele 166/166 in the BM183 locus was present in three parental genotypes MLB49-89A, MEXICO 54 and G2333, which showed resistance to Pythium root rot disease from screen house evaluations in 2016 (results not published). Could allele 208/308 at marker locus BM160 therefore be associated with resistance to *Pythium ultimum* root rot disease in this population and subsequent progeny lines developed?

Allelic dynamics on four LGs with disease resistance genes

As introduced; the four selected parents have broad based disease resistance genes for Anthracnose (*Co4*^{2} *and Co5*), Angular leaf spot (*Phg-2*), *P. ultimum* root rots (*P.ult*) and

Table 2. Allele bins at 22 SSR loci among four targeted parental genotypes

		Genotypes			
Loci	Chromosome	G2333	MCM 5001	MEXICO 54	MLB49-89A
BMd10	1	164/164	164/164	172/172	172/172
BM200	1	**150/172**[a]	**140/172**[a]	**154/170**[a]	**172/172**[a]
BM156	2[b]	244/244	244/244	244/244	**236/236**[a]
PVBR107	2[b]	182/186	**170/170**[a]	180/182	**148/148**[a]
BM159	3	224/224	224/224	224/224	224/224
BM197	3	224/0	220/0	224/0	220/0
BM171	4	160/160	170/170	160/160	**168/168**[a]
PVatgc002	4	176/176	176/176	**164/164**[a]	176/176
BMd28	5	150/172	**142/172**[a]	154/156	172/172
BM175	5	110/112	180/180	**198/200**[a]	182/182
BM187	6[b]	−9	161/161	**201/249**	161/201
BMd37	6[b]	106/106	**112/160**	108/114	−9
BM183	7[b]	166/166	164/164	166/166	166/166
BM160	7[b]	206/208	208/208	**208/308**[a]	208/208
BM189	8[b]	100/106	**144/152**[a]	100/108	100/108
BM211	8[b]	202/204	**130/132**[a]	202/204	130/136
BM141	9	184/184	172/172	184/184	148/214
PVat007	9	**114/226**[a]	234/236	**218/218**[a]	**226/226**[a]
GATS11B	10	**300/300**[a]	**150/160**[a]	**250/300**[a]	**100/140**[a]
BMd42	10	102/107	**180/180**[a]	102/170	**172/172**[a]
BMd41	11	**223/259**[a]	262/62	262/262	**205/205**[a]
BM205b	11	−9	102/102	100/104	104/118

[a]Alleles in bold were polymorphic at a given loci on chromosomes of parental germplasm. Allele number −9 indicates lack of marker amplification or absence of allele in genotype.
[b]Chromosomes – 2, 6, 7 and 8 was tagged to show fungal and viral disease resistance genes in Common beans.

Bean Common Mosaic and Necrosis virus (*I* and *bc3*) pyramided into a single background and CIAT and progeny lines are in advanced stages of evaluations. The six disease resistance genes pyramided are physically located on LGs two, six, seven and eight. Further analyses of molecular data therefore, attempted to find polymorphic SSR markers and its alleles among the four selected parents and relate it to genomic regions earlier mapped in common bean with disease resistance (Miklas *et al.*, 2006) for alternative co-dominant marker discovery.

The most frequent alleles among the four parental genotypes are shown (online Supplementary Table S1). LG two had 57 alleles and the most frequent allele 170/170 bp was homozygous with a frequency of 25% and had 26 alleles at marker locus PVBR107 in genotype MCM5001. LG six; had 86 alleles and the most frequent allele (201/249 bp) was heterozygous with a frequency of 13% and 13 alleles at locus BM187 and found in MEXICO 54. LG seven had 29 alleles detected within the population, with the most frequent allele homozygous (172/172 bp) with a frequency of 46% and 47 alleles at locus BM183. LG eight; had three alleles detected among the four parents, with the most frequent allele heterozygous (212/214 bp) having a frequency of 25% and 19 alleles at locus BM211.

Population structure

Majority of genotypes in this study belong to race Mesoamerica (38%), followed by Jalisco (30%), Mesoamerica–Durango hybrids (18%) and interracial-admixtures the least with frequency of 14% (Fig. 1). Identification of race backgrounds of the different single bean genotypes were based on molecular analysis in STRUCTURE (and showed in the bar plot) and related to plant type and seed sizes using the NJ tree. Examples of admixture entries were: 22, 15, 34, 21, 41, 10, 5, 9, 17, 23, 20 and 27 within the red sub-group (Jalisco race) because they drew membership or race background colourations from more than one group. Using the same analogy, admixtures could be identified within race Mesoamerican (green) and Mesoamerica–Durango hybrids. However, the blue race

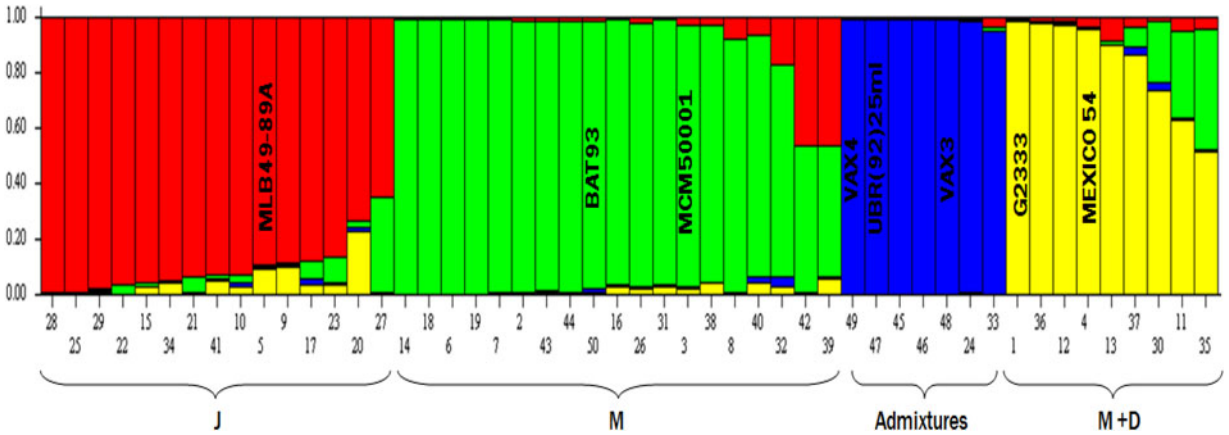

Fig. 1. Bean germplasm was assigned to sub-groups (races) using 50% membership coefficient (y-axis) cut off and races identification (*x*-axis) based the molecular analysis, growth habit, seed attributes and previous reports (e.g. Singh *et al.*, 1991a; Díaz and Blair 2006; Blair *et al.*, 2006a, b). Parents used to develop pyramids were: 5 = MLB49-89A (Jalisco – J), 3 = MCM5001 (Mesoamerica – M), 1 = G2333 (M), 4 = MEXICO 54 (Durango – D) and 50 = Mesoamerican gene pool control (BAT93). The sub-group designated as Admixtures was based on controls in the group such as VAXES derived from inter-specific crosses. *Note:* Individual genotypes within the bar plot above with more than one colour show inter-racial gene flow and were thus also considered race admixtures.

was designated as admixture because of the published pedigrees of control genotypes such as VAX3 and VAX4. Individual bean genotypes were assigned to different sub-populations using controls such as G2333 for the Mesoamerican race and Mexico 54 for race Durango.

The high levels of genetic diversity exhibited by different analyses show that the Mesoamerican germplasm grouped clearly into races, with low marker density. Secondly, NJ tree analyses, complemented STRUCTURE analyses in defining the number of sub-groups in this domesticated bean population.

Fixation indices among four sub-groups (K4.1–K4.4)

The mean fixation index of Wright (1965) shows moderate genetic heterogeneity among subpopulations predicted with STUCTURE as 0.1829 for Fst1 (K4.1 – red sub-group), 0.1579 for Fst2 (K4.2 – green sub-group), 0.0678 for Fst3 (K4.3 – blue group) and 0.1585 for Fst4 (K4.4 – yellow sub-group). High levels of genetic differentiation in the study suggest that this core bean germplasm comprises predominantly interracial admixtures, which depict interracial-gene flow.

More subtle sub-division of the bean population was generated using the NJ tree branches (Fig. 2) coloured based on STRUCTURE bar plot results. Major cluster: C1 categorized as race Mesoamerica had three sub-clusters depicting high levels of germplasm admixtures within this race. Major cluster, C2 was designated as the admixture cluster, predominated by beans of types II and III growth habits. Other major clusters (C3 and C4) were predominated by types III and IV growth habits. A bush and small seeded landrace (No. 42) appeared as an outlier in sub-group C3.

Morphological variability of Mesoamerican races of common beans was reported in Singh *et al.* (1991) to guide germplasm utilizations. For example, beans from race Durango (D) are predominantly type III with small and medium seeds, while Jalisco (J) has predominantly small seeded beans of types I and III. Race Mesoamerica (M) was predominated by small seeded beans of <25 g/100 seeds, with both determinate (type II) and indeterminate beans (types III and IV).

Morpho-agronomic traits and heritability estimates

Agronomic traits were significantly different ($P < 0.05$) among genotypes evaluated across testing regimes (locations and years) as shown in Table 3. Trait means and ranges are presented with parental germplasm (G2333, MCM 5001, MEXICO 54 and MLB49-89A) for developing multiple disease resistance lines with fifteen internodes per plant (Nodeno) ranging from 7.7 to 21.6. The mean flowering time (DFLO) was 40 days (35–46.2), with five flower buds per inflorescence (Flobuds), ranging from 3.8 to 8.1 and seeds of both small and medium sizes weighing 28.4 g/100 seeds and moderately number of pods per plant (Npods_plt), ranging from 20.6 to 44.4 pods. Heritability values estimated were high (≥0.81) for the agronomic traits.

The germplasm growth habit was predominantly of type III (42%), followed by type II (30%) and a 14% frequency

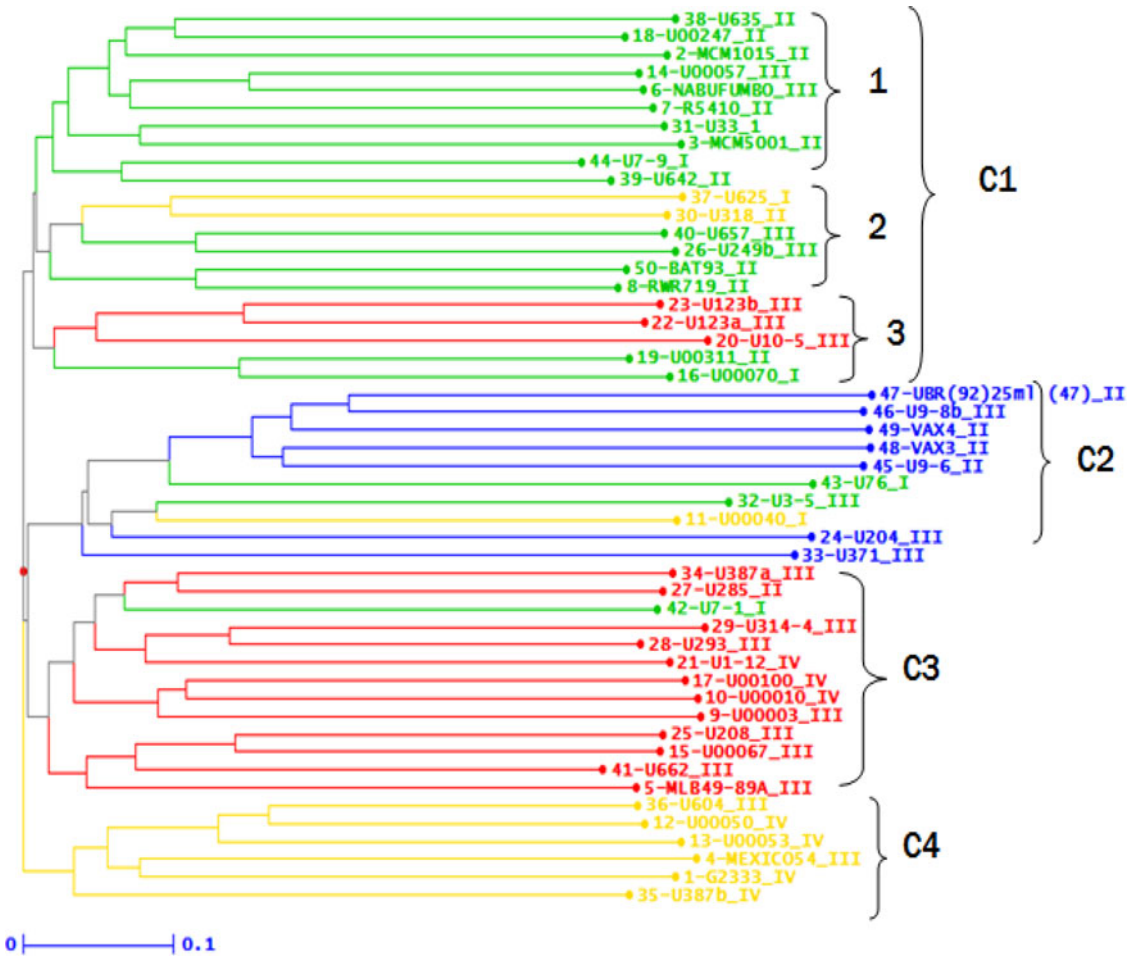

Fig. 2. Un-weighed neighbour-joining tree (NJ) showing Mesoamerican germplasm in Uganda grouped into four major clusters C1-C4. Major group C1 with majority of germplasm was further sub-grouped based on tree branch lengths. NJ tree branches were coloured according to groups identified with STRUCTURE simulations using colour codes in the Power Marker program. There was germplasm membership switching among major clusters; for example genotypes no: 11, 30 and 37 should have been assigned to C4 and 20, 22 and 23 to cluster C3.

for types I and IV. This indicated predominance of climbing beans germplasm (types III and IV) with frequency of 56%, while 44% were bush. The predominant seed colours were cream (12%), mixtures (12%) and red (11%), followed by white (7%), black (4%), maroon (3%) and purple (1%) was the least. The mean seed weight of the germplasm was 25.7 g per 100 seeds.

GXE interactions and correlations among yield traits

The number flower buds per plant, 100 seeds weight and number of pods per plant were significantly affected ($P < 0.05$) influenced by season and environment interactions (Table 3). Strong positive correlations was observed between pod attributes and number of nodes per plant ($r >$ 0.98 and 0.76), number of flower buds and nodes per plant ($r = 0.70$) at different times. Strong negative correlations (Table 4) were observed between flowering time and: number of pods formed per plant ($r = -0.82$ in 2015A/B and $r = -0.28$ in 2010/11) and 100 seeds weight ($r = -0.78$ in 2015A/B and $r = -0.74$ in 2010/11).

The first two principal components explained 80% of the phenotypic variation in the germplasm assembled (online Supplementary Fig. S1). The first component explained up to 46.86% of the variation based on plant type and 100 seed weight. The strongest traits under the second component were seed colour, plant type, and explained 33.2% of the variation among the germplasm.

Discussion

Genetic diversity and population structure

Broad genetic bases of germplasm create opportunities for selection in crop improvement programmes (Hallauer

Table 3. Agronomic trait performance of four selected parents across two environments

Trait	Environment and season	Means	Variance	Range	CV (%)	Heritability (h)	*P* value (<0.001)
Nodeno	1 - 2010/2011	15.5 ± 0.4	8.57	8.70	3.6	0.99	1.066×10^{-74}
	2 - 2015A/B	15.0 ± 0.7	51.51	19.30	6.8	1.00	6.071×10^{-135}
		15.2 ± 0.6	30.04	14.00	5.2	0.99	
Dflo	1- 2010/2011	38.6 ± 1.5	14.91	13.50	5.2	0.89	3.493×10^{-06}
	2 - 2015A/B	41.0 ± 1.2	9.15	8.80	3.8	0.93	8.703×10^{-09}
		39.7 ± 1.4	12.03	11.15	4.5	0.91	
Flobuds	1 - 2010/2011	3.7 ± 0.5	0.68	2.50	16.4	0.81	1.335×10^{-03}
	2 - 2015A/B	6.8 ± 0.3	4.54	6.10	5.4	0.99	2.037×10^{-88}
		5.2 ± 0.4*	2.61	4.30	10.9	0.90	
HWS (g)	1 - 2010/2011	27.0 ± 1.2	31.56	15.94	5.8	0.98	2.235×10^{-33}
	2 - 2015A/B	30.0 ± 0.5	44.63	16.38	2.0	1.00	$0.000 \times 10^{+00}$
		28.4 ± 0.8*	38.09	16.16	3.9	0.99	
Npodsplt	1 - 2010/2011	31.3 ± 1.3	71.15	25.00	5.5	0.99	2.029×10^{-62}
	2 - 2015A/B	35.7 ± 1.2	67.46	22.50	4.2	0.99	2.169×10^{-79}
		33.5 ± 1.2*	69.30	23.75	4.9	0.99	

CV, coefficient of variation, Heritability is narrow sense; Dflo, Days to flowering; Nodeno, Number of nodes on the main stem; Flobuds, Number of flower buds; HWS, weight of 100 seeds; Npodsplt, Number of pods per plant.
Traits with mean performance with asterisk (*) across two environments are significant at *P* value = 0.05, based on t tests. Season 2010/11 and 2015A/B corresponds to the first and second rainy seasons at NaCRRI, Namulonge (environment 1) and CIAT, Kawanda (environment 2) respectively in Uganda.

et al., 2010). In this regard, a major observation was that, germplasm displayed strong considerable intra-gene pool allelic diversity and variation. Moreover, few samples ($N = 50$) were evaluated due to economic reasons, despite larger organized collections in Uganda (Okii *et al.*, 2014a). The four parents selected for developing genetic pyramids drew membership from four sub-populations predicted (Fig. 1) due to prior interracial hybridizations and gene flows. However, genotypes no. 1 (G2333) and no. 4 (MEXICO 54) with distinct seed types, possibly clustered together due to their climbing ability. Previous authors earlier assigned G2333 to race Guatemala (Blair *et al.*, 2006a). For this study, medium seeded genotype MEXICO 54 was assigned to race Durango and MLB49-89A to race Jalisco.

High SSR allelic variations within the germplasm are comparable to findings in other studies which involved Mesoamerican common beans (e.g. Blair *et al.*, 2006a; Blair *et al.*, 2010). Our results and similar studies (such as Singh *et al.*, 1993; Duarte *et al.*, 1999; Díaz and Blair, 2006) justifies the selection of parents from only the Mesoamerican gene pool due to its large genetic variations and wider adaptation worldwide (Singh *et al.*, 1993) for developing elite lines, with desirable qualities, such as resistance to major tropical diseases. Literature was limiting, to

Table 4. Correlations of agronomic traits of four selected parents evaluated across different environments and years in Uganda

Traits	Nodeno[a]	DFLO[a]	Flobuds[a]	HWS[a]	Npods_plt[a]
Nodeno[b]		−0.02	0.20	0.14	0.92***
Dflo[b]	−0.84***		−0.03	−0.78***	−0.28
Flobuds[b]	0.70***	−0.42		0.24	0.14
HWS[b]	0.57	−0.74***	−0.15		0.37
Npods_plt[b]	0.98***	−0.82***	0.76***	0.50	

Traits: Nodeno, Number of nodes on the main stem; Dflo, Days to flowering; Flobuds, Number of flower buds; HWS, weight of 100 seeds; Npodsplt, Number of pods per plant.
Significant correlations (***) at $P < 0.001$) for four parental traits evaluated in 2010/11(a) and 2015A/B (b).
[a]first season
[b]second season

compare our results to allele frequencies, unique to the Mesoamerican gene pool of common bean in Eastern Africa.

Allelic dynamics among parental germplasm and linked QTLs

To mark differences between common and rare alleles, threshold is set to consider all alleles with frequencies below 0.05 as rare (FAO, 2009). Rare alleles from parental germplasm will be lost in subsequent progenies due to segregations and selection. Instead, major alleles: 170/170 (PVBR107) in MCM5001 and 201/249 in MEXICO 54 (Table 2) need more investigation, as they will likely be fixed in progenies and could be useful markers for selection. QTLs linked to marker BM187 on LG six are days to flowering, plant height, plant-width and seeds per plant (Blair *et al.*, 2006b).

SSR loci linked to QTLs, revealed significant genotypic and allelic differences associated with common bean agronomic traits suggesting that these markers are potentially useful in selection, at least in the Mesoamerican genetic background described and its progenies.

Genotype membership switching and outliers such as genotype no. 42 based on SSR analyses and NJ tree was possibly due to limited information on the linkage associations between SSR markers and the QTLs for bean seed weight and plant type and environmental effects on the phenotypes. There was however, less evidence for existence of race Guatemala in this germplasm collection possibly due to few samples genotyped. Genotypes belonging to race Guatemala (G) have climbing growth habit and are small seeded genotypes, with resemblance to race Mesoamerica (Beebe *et al.*, 2000; Díaz and Blair, 2006).

High genetic differentiation between race Durango/Jalisco and Mesoamerica based on RAPD markers was earlier reported (Duarte *et al.*, 1999). According to other studies, estimating genetic variations using morpho-agronomic traits does not completely guide the selection of parents especially when some of the genotypes evaluated are not well adapted (Duarte *et al.*, 1999). Nevertheless, the same authors observed that genotypes of Mesoamerican origin tend to separate according to their proposed races, when genotyped with dominant RAPD markers in Brazil. SSRs and other markers in similar studies allowed subpopulation differentiations to be revealed more accurately (Díaz and Blair, 2006).

The numbers of major sub-groups simulated using STRUCTURE (four) and NJ tree (six) differed possibly because STRUCTURE did not assume a particular mutation process during analysis (Pritchard *et al.*, 2000). CIAT lines, such as VAX3 and VAX4 currently used as sources of resistance to Common bacterial blight (CBB) disease, occurred in a separate sub-group (admixtures) because of pedigree; as they were derived from inter-specific crosses of *P. vulgaris* and *P. acutifolius* (Singh *et al.*, 2001).

The identified races of 50 germplasm (which include the four parents used to develop progeny lines for multiple disease resistance at CIAT) will improve our knowledge of host-pathogen co-evolutionary relationships (Guzmán *et al.*, 1995) during evaluation of progeny lines currently under evaluations and other populations derived from this germplasm in the near future. For example, information on virulence and evolutions of major pathogens of beans and periodic follow of this germplasm at farm level is required to compliment efforts for breeding bean for disease resistance.

Persistent seed mixing of beans genotypes by farmers in East Africa (David and Sperling, 1999; Blair *et al.*, 2010), however affects the dynamics of the co-evolutionary relationship. Susi (2014) suggested trade-offs and co-infection to explain host–pathogen evolutionary relationships in plants. The time (400 years) since introduction of common beans into East-Africa (Gepts and Bliss, 1988; Buah *et al.*, 2017), was adequate for emergency of mutant pathogens different from ones in the centre of bean domestications in Latin America (Gepts and Bliss, 1988).

Phenotypic variation, heritability and trait stability

Selection for seed colour was much earlier observed in beans (Beebe *et al.*, 1995) and recovered significant segments of the bean genome, through linkage drags, causing beans with the same colour to be more related under purposive groupings (Duarte *et al.*, 1999). Nonetheless as observed in this study, previous studies found overlaps; between Race Durango and Jalisco in terms of seed colour and growth habit (Díaz and Blair, 2006).

More consistent correlations were observed in different years for: number of, pods per plant and nodes per plant, days to flowering and 100 seed weight. These traits can be evaluated interchangeably during bean breeding. For example, the number of pods per plant showed strong associations with days to flowering and this relationship is critical for future population development and selection of superior bean lines.

Heritability values are classified as low (0–0.29), medium (0.3–0.59) and above 0.6 as high (Dabholkar, 1992). The heritability values determined were narrow sense since the elite parental germplasm in this study generated additive type of variance (Acquaah, 2007).

High heritability estimates for bean agronomic traits presented in Table 3; corroborates values reported elsewhere for architectural traits of dry beans such as 0.85 for

the number of pods in the mid-section of the plant and 0.77 for seed weight in grams per 100 seeds (Acquaah, 2007). High narrow sense heritability was reported in other studies for common bean seed yield using F2 generation families as: 0.69 for pods per plant, 0.67 for 100 seeds and 0.97 for days to flowering (Abebe *et al.*, 1996).

The differences in heritability values in this study in relation to others in literature was attributed to specific focus on: the Mesoamerican gene pool which comprise of small to medium sized seeded beans, sample size, and method used for estimations of variance components of heritability.

Traits with high heritability result in increased population response to selection in the desired direction of change (Acquaah, 2007), suggesting that parents bearing particular measurements reproduce progeny of similar phenotypes. However, despite the high heritability of number of pods per plant observed, with direct effects on bean yield, Muthomi *et al.* (2011) suggested improvement of bean yields, via grain yield selection, due to large effects of the environment on pod number.

The marked difference in performances among parental germplasm across seasons and micro-sites ($P < 0.05$) was due to their inherent genetic differences and phenotypes (small and medium seed types, with prostrate bush types to aggressive climbing abilities in genotype, G2333) and micro-ecological differences such as temperatures, rainfall patterns and soil fertility levels. Climbing lines with resistance to several diseases are of interest because, if planted twice in a year at higher altitudes within East Africa, they have huge yield potentials of up to 5000 kg/ha (Blair *et al.*, 2007).

Conclusions and recommendations

Polymorphic markers such as SSRs, widely distributed in the genome of common bean are consistent in germplasm characterisations, since the environment does not influence them and, are therefore, useful for assessing genetic divergence in populations and selection in breeding. The four CIAT breeding lines (G2333, MEXICO54, MCM5001 and MLB49-89A) selected for development of genetic pyramids had considerable levels of genetic variations for improving agronomic, yield traits and disease resistance. High trait heritability estimates estimated using four selected parents, implies that it is possible to develop superior common beans progeny lines combining disease resistance and desirable agronomic traits. The heritability estimates could be extended to other yield traits to enhance direct-selections of superior lines derived from this Mesoamerican bean population: starting in early generations such as at F2s and F3s to save breeding time and resources in future. Genotype by environment interactions influenced the number of flower buds, weight of 100 seeds and pods per plant, suggesting that instabilities in these traits needs to be considered in future multi-location evaluations of progenies developed from the germplasm in this study.

Future bean germplasm characterisations should consider sequencing the whole genomes of core collections for explicit groupings for use in current and future breeding programmes. Landraces, genetically related to breeding lines, need more evaluations as germplasm using screen houses and field experimentation for multiple tropical constraints.

Supplementary material

The supplementary material for this article can be found at https://doi.org/10.1017/S1479262117000259

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References

Abebe A, Brick MA, Ogg JB and Kirkby JB (1996) Heritability of traits associated with dry bean seed yield. Annual Report of the Bean Improvement Cooperative, vol. 39.

Acquaah G (2007) *Principles of Plant Genetics and Breeding*. UK: Blackwell Publishing, pp. 128–134.

Beebe S, Gonzalez AV, Rengifo J (2000) Research on trace minerals in the common bean. *Food and Nutrition Bulletin* 21: 387–391.

Beebe SE, Ochoa I, Skroch P, Nienhuis J, Tivang J (1995) Genetic diversity among common bean breeding lines developed for central America. *Crop Science* 35: 1178–1183.

Blair MW, Iriarte G, Beebe S (2006a) QTL analysis of yield traits in an advanced backcross population derived from a cultivated andean and wild common bean (*Phaseolus vulgaris L.*) cross. *Theoretical and Applied Genetics* 112: 1149–1163.

Blair MW, Giraldo MC, Buendía HF, Tovar E, Duque MC, Beebe SE (2006b) Microsatellite marker diversity in common bean (*Phaseolus vulgaris* L.). *Theoretical and Applied Genetics* 113: 100–109.

Blair MW, Fregene MA, Beebe SE, Ceballos H (2007) Marker-assisted selection in common beans and cassava. In: Guimaraes E (ed) *Marker-Assisted Selection (MAS) in Crops, Livestock, Forestry and Fish: Current Status and the way Forward*. Rome: FAO, pp. 81–115.

Blair MW, Gonzalez LF, Kimani PM and Butare L (2010) Genetic diversity, inter-gene pool introgression and nutritional quality of common beans (*Phaseolus vulgaris L.*) from central Africa. *Theoretical and Applied Genetics* 121: 237–248.

Broughton WJ, Hernandez G, Blair MW, Beebe SE, Gepts P and Vanderleyden J (2003) Beans (*phaseolus* spp.): model food legumes. *Plant Soil* 252: 55–128.

Buah S, Buruchara R and Okori P (2017) Molecular characterisation of common bean (*Phaseolus vulgaris L.*) accessions from Southwestern Uganda reveal high levels of genetic diversity. *Genetic Resources and Crop Evolution* 1–14.

Buruchara R (2009) How participatory research convinced a sceptic. In Fortmann, L (ed) *Participatory Research in Conservation and Rural Liveliboods: Doing Science Together*. Oxford: Black Well Publishing, pp. 18–33.

Buruchara R, Chirwa R, Sperling L, Mukankusi C, Rubyogo JC, Muthoni Rand Abang MM (2011) Development and delivery of bean research varieties in Africa: the Pan-Africa bean research alliance (PABRA) model. *African Crop Science Journal* 19: 227–245.

Caligari PDS (2001) *Plant Breeding and Crop Improvement. Encyclopedia of Life Sciences*. Nature Publishing Group, Chichester:UK 1–8.

Dabholkar AR (1992) *Elements of Biometrical Genetics*. New Delhi: Concept Publishing Company, pp. 38–165.

David S, Sperling L (1999) Improving technology delivery mechanisms: lessons from bean seed systems research in Eastern and central Africa. *Agriculture and Human Values* 16: 381–388.

Díaz LM, Blair MW (2006) Race structure within the Mesoamerican gene pool of common bean (*Phaseolus vulgaris L.*) as determined by microsatellite markers. *Theoretical and Applied Genetics* 114: 143–154.

Duarte JM, João BS and Leonardo CM (1999) Comparison of similarity coefficients based on RAPD markers in the common bean. *Genetics and Molecular Biology* 22.3: 427–432.

Earl DE, Vonholdt BM (2012) Structure harvester: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources* 4: 359–361.

Evanno G, Regnaut S and Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology* 14: 2611–2620.

FAO (2009) *Molecular Characterization of Mutant Germplasm*. Manual. Vienna.

Gepts P and Bliss FA (1988) Dissemination pathways of common bean (*Phaseolus vulgaris* Fabaceae) deduced from phaseolin electrophoresis variability. II Europe and Africa. *Economic Botany* 42: 86–104.

Guzmán P, Gilbertson RL, Nodari R, Johnson WC, Temple SR, Mandala D, Mkandawire ABC and Gepts P (1995) Characterization of variability in the fungus *Phaeoisariopsis griseola* suggests coevolution with the common bean (*Phaseolus vulgaris*). *Phytopathology* 85: 600–607.

Hallauer AR, Carena MJ and Filho JBM (2010) *Quantitative Genetics in Maize Breeding, Hand Book of Plant Breeding*. New York: Springer Science and Business Media, pp. 1–60.

Jolliffe I (2002) *Principal Component Analysis*. New York: John Wiley and Sons, pp. 150–166.

Kelly JD (2004) Advances in common bean improvement: some case histories with broader applications. *Acta Hortic (ISHS)* 637: 99–122.

Koinange EMK, Singh SP and Gepts P (1996) Genetic control of the domestication syndrome in common bean. *Crop Science* 36.4: 1037–1045.

Liu K, Muse SV (2005) Powermarker: an integrated analysis environment for genetic marker analysis. *Bioinformatics* 21: 2128–2129.

Mahuku GS, Henríquez MA, Munõz J, Buruchara RA (2002) Molecular markers dispute the existence of the Afro-andean group of the bean angular leaf spot pathogen, Pseudocercospora griseola. *Phytopathology* 92: 580–589.

Mamidi S, Rossi M, Moghaddam SM, Annam D, Lee R, Papa R and McClean PE (2013) Demographic factors shaped diversity in the two gene pools of wild common bean *Phaseolus vulgaris L. Heredity* 110: 267–276.

Miklas PN, Kelly JD, Beebe SE and Blair MW (2006) Common bean breeding for resistance against biotic and abiotic stresses: from classical to MAS breeding. *Euphytica* 147: 105–131.

Muthomi JW, Muimui KK and Kimani PM (2011) Inheritance of resistance to angular leaf spot in yellow beans. *African Crop Science Journal* 19: 267–275.

Myles S, Peiffer J, Brown PJ, Ersoz ES, Zhang Z, Costich DE (2009) Association mapping: critical considerations shift from genotyping to experimental design. *Plant Cell* 21: 2194–2202.

Namayanja A, Buruchara R, Mahuku G, Rubaihayo P, Kimani P, Mayanja S and Eyedu H (2006) Inheritance of resistance to angular leaf spot in common bean and validation of the utility of resistance linked markers for marker assisted selection outside the mapping population. *Euphytica* 151: 361–369.

Okii D, Tukamuhabwa P, Odong P, Namayanja A, Mukabaranga J, Paparu P, Gepts P (2014a) Morphological diversity of tropical common bean germplasm. *African Crop Science Journal* 22: 59–68.

Okii D, Tukamuhabwa P, Kami J, Namayanja A, Paparu P, Ugen M and Gepts P (2014b) The genetic diversity and population structure of common bean (*Phaseolus vulgaris L.*) germplasm in Uganda. *African Journal of Biotechnology* 13: 2935–2949.

Otsyula R, Rachier G, Ambitsi N, Ndiya RJC, Buruchara R and Sperling L (2004) The use of informal seed producer groups for diffusing root rot resistant varieties during period of acute stress. In Sperling L, Remington T, Haugen J and Nagoda S (eds) *Addressing Seed Security in Disaster Response: Linking Relief with Development*. Cali: CIAT, pp. 69–89.

Otsyula RM, Ajanga SI, Buruchara RA and Wortmann C.S. (1998) Development of an integrated bean root rot control strategy for western Kenya. *African Crop Science Journal* 6: 61–67.

Pastor-Corrales MA, Erazo OA, Esrada EI and Singh SP (1994) Inheritance of anthracnose resistance in common bean accession G 2333. *Plant Disease* 78: 959–962.

Pathania A, Sharma SK and Sharma PN (2014) Common bean. In Singh M. *et al*. (eds) *Broadening the Genetic Base of Grain Legumes*. India: Springer, pp. 11–50.

Perrier X, Jacquemoud-Collet JP (2006) DARwin software. http://darwin.cirad.fr/darwin

Pritchard JK, Stephens W and Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics* 155: 945–959.

SAS Institute Inc (2011) *The Statistical Analysis System for Windows V.9*. Cary, North Carolina.

Sengooba TN, Spence NJ, Walkey DGA, Allen DJ and Lana AF (1997) The occurrence of bean common mosaic necrosis virus in wild and forage legumes in Uganda. *Plant Pathology* 46: 95–103.

Singh SP and Muñoz CG (1999) Resistance to common bacterial blight among Phaseolus species and common bean improvement. *Crop Science* 39: 80–89.

Singh SP, Gepts P and Debouck DG (1991) Races of common bean (*Phaseolus vulgaris*, Fabaceae). *Economic Botany* 45: 379–396.

Singh SP, Molina A, Urrea CA and Gutierrez JA (1993) Use of interracial hybridization in breeding the race Durango common bean. *Canadian Journal of Plant Science* 73: 785–793.

Singh SP, Muñoz CG and Terán H (2001) Registration of common bacterial blight resistant dry bean germplasm VAX 1, VAX 3, and VAX 4. *Crop Sci*. 41: 275–276.

Susi H (2014) *Host-pathogen coevolution through trade-offs and coinfection*. PhD Thesis, University of Helsinki, Finland.

Wortmann CS, Kirkby RA, Eledu CA and Allen DJ (1998) *Atlas of Common Bean (Phaseolus Vulgaris L.) Production in Africa*. Cali, Colombia: Centro International de Agricultura.

Wright S (1965) The interpretation of population-structure by F-statistics with special regard to systems of mating. *Evolution* 19: 395–420.

Young RA and Kelly JD (1996) Characterization of the genetic resistance to *Colletotrichum lindemuthianum* in common bean differential cultivars. *Plant Disease* 80: 650–654.

Yu J, Buckler ES (2006) Genetic association mapping and genome organization of maize. *Current Opinion in Biotechnology* 17: 155–160.