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CLUSTERING COMMON BEAN MUTANTS BASED ON HETEROTIC GROUPINGS

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ABSTRACT

Common bean (*Phaseolus vulgaris* L.), is an important legume crop in sub-Saharan Africa (SSA). Most of the local varieties are favoured for their colour and taste, but have a low yield potential of 0.3 to 0.5 metric tonnes per hectare. Mutation breeding is a crop improvement tool in beans that can create new alleles, which when advanced beyond mutated generation 5 (M5), produces stable mutants, which may possess desirable characteristics. These mutants may result in rapid genetic advance and help address issues of low bean productivity. The objective of this study was to cluster bean mutants from a bean mutation breeding programme, based on heterotic groupings. This was achieved by genotyping 16 bean genotypes, using 21 Simple Sequence Repeats (SSR) bean markers. From the results, three different clusters A, B and C, were obtained suggesting great genetic diversity among the bean mutants and these cluster groups (A, B and C) can be taken as heterotic groupings. Depending on the phenotypic trait under consideration, crossing of two distinct genotypes from different cluster groups may lead to hybrid vigour. Furthermore, to create genetic variability for further bean improvement, the breeder can cross distinct genotypes from different cluster groups, which after several generations of selfing accompanied by selection may lead to desirable progenies

Key Words: Genetic variation, mutation breeding, *Phaseolus vulgaris*

RÉSUMÉ

Le haricot commun (*Phaseolus vulgaris* L.), est une légumineuse très importante en Afrique au Sud du Sahara (SSA). La plupart des variétés locales sont favorisées pour leur couleur et goût, mais elles sont potentiellement de faibles rendements allant de 0,3 0,5 tonnes par hectare. La sélection par mutation est un outil d'amélioration variétale utilisée pour le haricot, cet outil peut créer de nouveaux allèles qui, lorsqu'ils sont avancés au-delà de la génération mutée 5 (M5), produisent des mutants stables, qui peuvent posséder les caractéristiques désirées. Ces mutants peuvent contribuer à une avancée génétique rapide et aider à résoudre les problèmes de faible rendement. L'objectif de cette étude était de classer les haricots mutants, en se basant sur les groupements hétérotiques. Ceci a été réalisé en utilisant 21 marqueurs des répétitions de séquences simples (SSR) pour le genotypage de 16 écotypes de haricot. Des résultats obtenus, il ressort trois différentes classes A, B et C, ce qui suggère une grande diversité génétique entre les haricots mutants, ces groupes (A, B et C) peuvent être considérés comme des groupements hétérotiques. Selon le trait phénotypique considéré, le croisement de deux des génotypes appartenant à des groupes différents peut générer de la vigueur hybride. Par ailleurs, pour créer une variabilité génétique dans un but d'amélioration ultérieure, le sélectionneur peut croiser des écotypes appartenant à des groupes différents, ce qui après plusieurs générations d'auto pollinisation suivie de sélection pourra générer des descendants ayant les traits désirés.

Mots Clés: Variation génétique, sélection par mutation, *Phaseolus vulgaris*

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) are cultivated widely in the tropics, the sub-tropics and temperate regions of the world. Because of this wide cultivation, they are subjected to varying environmental conditions in terms of rainfall, soil fertility and acidity. Farmer preference for bean varieties also varies (Buruchara *et al.,* 2011; Asfaw *et al.,* 2013).

Bean is a cheap source of mainly high quality protein and valuable micronutrients. Most of the local varieties that are grown by the farmers in SSA are preferred for their colour and taste, but have a low yield potential of 0.3 to 0.5 tonnes per hectare (Mwale *et al.,* 2008). Considering the current trends in population growth and bean consumption demand, which is expected to grow to unprecedented levels well in the next century, there is need for research to address this concern. The availability of genetic variation is critical for increased performance of new bean varieties.

One method through which genetic variation can be availed is mutation breeding. This is a novel method that makes use of irradiation or chemicals to obtain new variants without resorting to hybridisation. Mutation breeding in beans creates new alleles, which when advanced beyond M5, produce stable mutants, which may possess desirable characteristics compared to the parents. It has been used in beans to initiate different bean characteristics, as well as for use in forward and reverse genetic studies (Blair *et al.,* 2007a; Porch *et al.*, 2009). Little has been reported on cluster analysis of the bean derived mutants; yet this can help generate information essential for utilisation in a bean improvement programme.

The derived bean mutants may have potential to address low productivity through direct use or in hybridisation as parents in a bean improvement programme. At the University of Zambia, School of Agricultural Sciences, bean mutants have been generated which exhibit different seed size and colour, days to maturity, etc., when compared with parental genotypes. Molecular cluster analysis of these mutants may help identify genotypes, which may give highest genetic potential or heterotic vigour, when crossed to each other or offer high genetic variability for desirable traits in their progenies essential for crop improvement. The objective of this study was therefore, to cluster bean mutants based on heterotic groupings so as to identify genotypes which may offer highest genetic potential or heterotic vigour when hybridised with each other.

MATERIALS AND METHODS

Phenotypic variation. Sixteen genotypes (Table 1) were used in this study; constituting of three parental genotypes Calioca (Ca), Sakala (SK) and Solwezi (SZ) with their respective mutants. The two others were Lyambai (LY) bean derived mutants. All the mutants were generated in collaboration with the National Institute for Science and Industrial Research (NISIR) in Zambia. The phenotypic evaluation of generated mutants generally showed variations in terms of leaf size, growth type, seed colour and size (Figs. 1 and 2) and days to maturity compared with their parental genotypes.

Genotyping and cluster analysis. Genotypic DNA from the mutants and respective parents were extracted from young leaves (1 to 2 weeks after seed germination), using the Cetyltrimethy lammonium bromide (CTAB) method (Hoisington *et al.,* 1994). These genotypes were further genotyped using twenty one Simple Sequence Repeats (SSR) markers (Table 2) on GenBank data base (Yu *et al.,* 2000). The SSR primers were used as part of the PCR reaction mixture, whose final concentrations of reaction components were as follows: 0.2 µM each of SSR forward and reverse primers, 0.16 U *Taq* polymerase (BioLabs); 1× PCR, buffer, 2.0 mmol $MgCl_2$ L^{-1}; 0.2 mmol L^{-1} each of dNTP, 30 ng genomic DNA and distilled sterile water to a total volume of 20 µl. The PCR conditions and cycling profiles were done based on Masi *et al.* (2003), and results were visualised on 1% high grade TopVision Agarose (Themo Scientific, USA). The genotyped data were then uploaded in Darwin Software (Perrier and Jacquemoud-Collet, 2006), and analysed using weighted neighbor joining to carry out a cluster analysis.

TABLE 1. Genotypes used in the Cluster analysis evaluated at the University of Zambia, School of Agricultural Sciences- key characteristics of each mutant as compared to its parental genotype

ID No	Genotype	Key characteristics
1	CA_P	Small seeded, Small seed and light green leaf, Indeterminant growth
2	CA_3	Small seeded, Medium leaf size and dark green, EM, High yield, Tolerant to Al toxicity
3	CA_15	Small seeded, Large leaf size and dark green leaf, Tolerant to BSM, HNF, High yield, EM
4	CA_24	Small seeded, Medium to large seed size and dark green, High yield
5	LY2-7-B	Medium seeded, High yield
6	LY2-8-B	Medium seeded, Tolerant to Al toxicity, High yield
7	SK_P	Small seeded ,Light brown seed, Determinant growth
8	SK37-55-5	Small seeded, Light brown seed, Determinant growth, EM, High yield
9	SK44-34-1	Medium seeded, pink seed, Tolerant to BSM, EM, High yield
10	SK44-33-1	Medium seeded, pink seed, Semi climber, Tolerant to BSM, EM, High yield
11	SK47-46-21	Small seeded, light brown seed, Determinant growth, EM, High yield
12	SZ_P	Medium seeded, Indeterminant growth, Round and plump seed, Rose colored seed coat
13	SZ3-1-B-B	Medium seeded, Indeterminant growth, KSS, Moderately tolerant to Al toxicity, High yield
14	SZ3-3-B-B	Medium seeded, Indeterminant growth, KSS, Tolerant to Al toxicity, EM, High yield
15	SZ7-4-B-B	Medium seeded, Indeterminant growth, KSS, Tolerant to Al toxicity, EM, High yield
16	SZ3-1-4B-B	Medium seeded, lighter green leaves, KSS, Tolerant to Al toxicity, EM, High yield

Each genotype is presented with an Identity Number (ID No.). Genotypes 1, 7 and 12 are parental genotypes for Carioca (Ca_P), Sakala (SK_P), and Solwezi (SZ_P) respectively. 2,3 and 4 are derived mutants of genotype 1; 8,9, 10 and 11 are derived mutants of genotype 7 and 13, 14, 15 and 16 are derived mutants from genotype 12. EM- Early Maturing, Al- Aluminium, HNF- High Nitrogen Fixation, BSM- Bean Stem Maggot, KSS- Kidney shaped seed

Figure 1. Differences in seed coat colour and size exhibited by the Parental genotype (Sakala) X with its mutant (SK 44-33-1) Y.

RESULTS AND DISCUSSION

Cluster analysis showed that mutation creates new alleles as evident by mutants, which fell in complete different clusters from their parental genotypes (Fig. 3). For example, parental genotype 7 was in cluster B; while two of its respective mutants 9 and 10 were in cluster C. The distinct groups entail that even if the parental material is the same, the resulting mutants are genetically different from their parents, and this could be seen in their phenotypic differences with their parental genotypes (Figs. 1 and 2). Previous work has equally shown that mutation may cause changes in phenotypic appearance (Sena *et al.,* 1991). Thus, mutation is a desirable approach, as it generates variations in germplasm that may be necessary for plant breeding (Acquaah, 2007). As this tool (mutation) does not involve transfer of genes from one specie to another, it does not create transgenic crops and can be classified as a non-genetically modified organism (GMO) technique.

In this study, we have proved that mutants may actually differ from the parental genotypes (Fig. 3) at the genetic level. Three cluster groups,

Figure 2. Differences in morphology between Parental genotype (Sakala) **X** (determinant) with its mutant (SK 44-33-1) **Y** (Indeterminant).

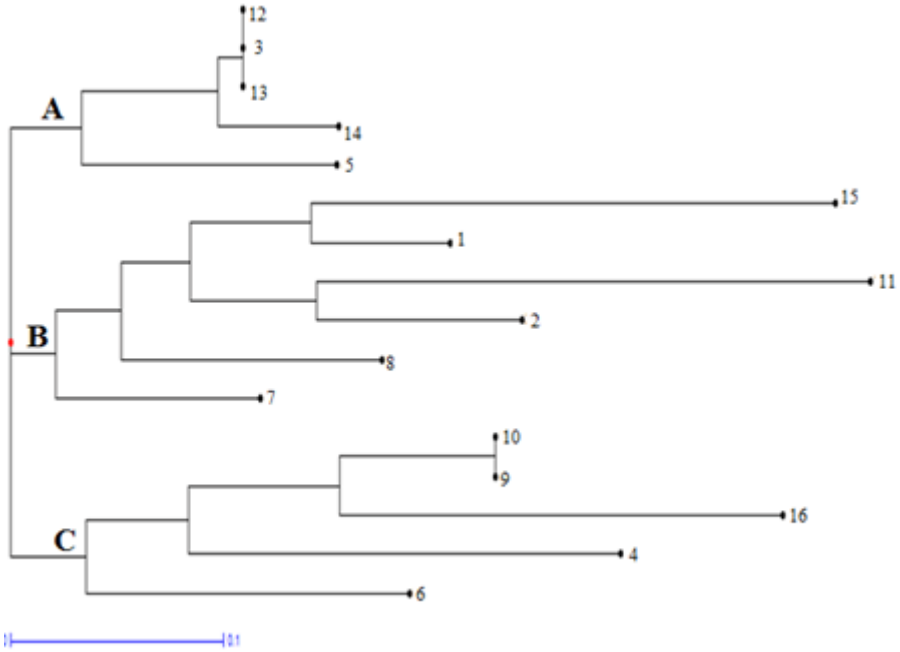

Figure 3. A dendrogram depicting relationships among 16 mutant bean genotypes. Numbers shown from 1 to 16 are identity numbers of the bean genotypes as presented in Table 1. Genotypes 1, 7 and 12 are parental genotypes for Carioca, Sakala and Solwezi respectively. 2,3 and 4 are derived mutants from genotype 1; 8,9, 10 and 11 are derived mutants of genotype 7 and 13, 14, 15 and 16 are derived mutants from genotype 12. A, B and C are clusters.

A, B and C as earlier mentioned, were created and can be taken as heterotic groups. A cross between mutants from two different heterotic groups may generate offsprings which exhibit heterosis. Heterosis is a natural phenomenon, where by offsprings from two diverse parents from the same species exhibit hybrid vigour (Mulungeta *et al.,* 2013). It should be noted, however, that the degree of heterosis obtained between crosses from distinct cluster groups is not quantified at the organism level, but rather on a trait to trait basis. Generally this depends on whether the nature of gene action conditioning inheritance of a particular trait is additive or non-additive (Acquaah, 2007). In beans, like in most self-pollinated crops, heterosis or hybrid vigour

TABLE 2. Twenty-one SSRs identified from bean DNA sequences and used in the cluster analysis

SSR name	Core motifs	Fragment size (bp)	Direction	Primer sequences
J01263	$(ATTCC)_3 (AG)_2 (TAC)_3$	171	Forward	atgcatgttccaaccaccttctc
			Reverse	ggagtggaacccttgctctcatc
J04555	$(CTT)_3(T)_3$	152	Forward	gagggtgtttcactattgtcactgc
			Reverse	ttcatggatggtggaggaacag
K03288	$(ATGC)_4$	126	Forward	tgccaccacagctttctcctc
			Reverse	tatgagagaagcggttggcacg
K03289	$(ATGC)_4$	144	Forward	agctttcacactatgacaccactgg
			Reverse	tgcgacatgagagaaagacacgg
M13968	$(GAA)_3$	182	Forward	acaccttatcatttagaggaaaagaga
			Reverse	acccgaactggctgcaacag
M18093	$(CCA)_6$	151	Forward	ccagctaccatctcctccatcg
			Reverse	tagtggtggaggtggagattt
M18094	$(CCA)_5$	179	Forward	taatttctctcttcccatcccaaac
			Reverse	gtagtaataaggaggaggcggtgag
M68913	$(ATCT)_3$	193	Forward	caattaaaactcaaccaacccaaata
			Reverse	tttcccgccatagaatatgtgaga
M75856	$(GA)_{11}$	157	Forward	caatcctctctctctcatttccaatc
			Reverse	gaccttgaagtcggtgtcgttt
UI0419	$(AAAT)_3$	203	Forward	tggagccatctgtctcttacccac
			Reverse	gagcacgagtcacgtttgcaac
U18349	$(GGC)_5$	238	Forward	ctgaagcccgaatcttgcga
			Reverse	cgcgagaggtgaacgaaagc
U18791	$(TA)_{22}$	239	Forward	gggagggtagggaagcagtg
			Reverse	gcgaaccacgttcatgaatga
U28645	$(CCA)_5$	115	Forward	gcaagagaacactgaagaggatcg
			Reverse	gacattactcatttcatcatctactacacg
U34754	$(AT)_8$	254	Forward	gtttcttccttatggttaggttgtttg
			Reverse	tcacgttatcaccagcatcgtagta
U54703	$(TTA)_4$	106	Forward	cgaggaggaaggagaagacgg
			Reverse	gagggttatcacaaggaagacacg
U70530	$(AG)_7$	144	Forward	cctctctcccgaacttattcatctc
			Reverse	tggccatagattgcatgacaaat
U77935	$(GCCACC)_5$	95	Forward	cgttagatcccgcccaatagt
			Reverse	ccgtccaggaagagcgagc
X02980	$(ATCC)_3(AG)_2(TAC)_3T(CTA)_3$	192	Forward	acttctttcatcatccatccatcc
			Reverse	tatcttggctctcttcctcctcc
X04001	$(AG)_8$	164	Forward	tcacgtacgagttgaatctcaggat
			Reverse	ggtgtcggagaggttaaggttg
X04660	$(AG)_8$	201	Forward	ttgatgacgtggatgcattgc
			Reverse	aaagggctagggagagtaagttgg
X13329	$(GA)_8(A)_2$	139	Forward	gctcacgtacgagttgaatctcag
			Reverse	atctgagagcagcgacatggtag

is associated with traits conditioned by non-additive gene action (Mulungeta *et al.,* 2013). Traits such as 'days to flowering', 'days to maturity' and 'number of pods per plant' are associated with hybrid vigour when obtained from a cross of two diverse parents (Mulungeta *et al.,* 2013). Other traits such as 'phosphorous use efficiency' in beans are associated with complex traits (additive gene action or quantitatively inherited), and hence do not exhibit hybrid vigour (Blair *et al.,* 2007b).

A previous study, on sesame, confirmed that heterotic vigour can be obtained from a cross between two distinct mutants (Praveenkumar *et al.,* 2012). Another breeding approach may involve carefully selected two diverse mutants (especially for traits which are quantitatively inherited) as parental genotypes in crosses in an effort to generate desirable bean lines. In beans, just as in any most self-pollinated crops, several genotypes can be obtained from a cross between two diverse parents after several generation of selfing accompanied by selection (Acquaah, 2007).

CONCLUSION

From our study, three heterotic groups (A, B and C) where generated. Heterotic vigour can be exhibited in the progeny resulting from hybridisation between two distinct mutants from different heterotic groups. Additionally, genetic variability for further bean improvement can be created by crossing distinct genotypes from different heterotic groups. These crosses can be advanced for several generations of selfing and accompanied by selection of desirable progenies until realised progenies are stable.

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