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Genetic interrelationship among tepary bean (*Phaseolus acutifolius* A. Gray) genotypes revealed through SSR markers

Zinhle Babongile Mhlaba[1], Beyene Amelework[2], Hussein A. Shimelis[1,2], Albert Thembinkosi Modi[1], Jacob Mashilo*[1,2]

[1]University of KwaZulu-Natal, School of Agricultural, Earth and Environmental Sciences, Discipline of Crop Science, Private Bag X01, Scottsville 3209, Pietermaritzburg, South Africa
[2]University of KwaZulu-Natal, African Centre for Crop Improvement (ACCI), Private Bag X01, Scottsville 3209, Pietermaritzburg, South Africa

***Corresponding author: jacobmashilo@yahoo.com**

Abstract

Tepary bean (*Phaseolus acutifolius* A. Gray) is one of the useful plant genetic resources possessing novel genes for abiotic and biotic stress tolerance. It is largely used in the breeding of common bean or related tropical legumes. The objective of this study was to determine the genetic interrelationship present among 20 diverse tepary bean genotypes using 10 selected and polymorphic simple sequence repeat (SSR) markers and to identify genetically unique parental lines for developing breeding populations. The SSR markers amplified a total of 57 putative alleles with a size range of 102 to 304 base pairs. Number of alleles ranged from 2 to 16 with a mean of 5.7 per locus. Number of effective alleles ranged from 1.5 to 11.6 with a mean of 4.32. The mean observed and expected heterozygosity were 0.45 and 0.51, respectively, reflecting moderate level of genetic interrelationship among the tested genotypes. The mean polymorphic information content (PIC) of the SSR loci was 0.50 suggesting that the selected markers had high discriminatory power for genetic diversity analysis of tepary bean. Genetic distances varied from 0.18 to 0.64 with a mean value of 0.42, signifying relatively low levels of genetic diversity among the studied genotypes. Cluster analysis grouped the genotypes into three distinct genetic groups. Unique genotypes such as G40201, G40237, G40158, G40157 and G40158 from Cluster I, G40084 and G40033 from Cluster III were selected. These are distantly related parents and recommended for further breeding or genetic recombination to broaden the genetic basis of tepary bean or related legumes.

Keywords: breeding**,** legume, *P. acutifolius,* tepary bean, microsatellite markers.
Abbreviations: CTAB_mixed alkyltrimethyl-ammonium bromide protocol; H_o_observed heterozygosity; H_e_ expected heterozygosity; SSRs_simple sequence repeats; UPGMA _unweighted pair group method using arithmetic averages.

Introduction

Tepary bean (*Phaseolus acutifolius* A. Gray) belonging to the genus *Phaseolus* is a diploid (2n = 2x = 22) and predominantly self-fertilizing crop (Schinkel and Gepts, 1988). Tepary bean is originated from arid and semi-arid regions of north-western Mexico and south-western United States (Blair et al*.,* 2002; Garvin and Weeden, 1994). Tepary bean has unique genetic attributes such as tolerance to drought, heat and salt stress making it an ideal crop for cultivation in arid and semi-arid environments (Rao et al*.,* 2013; Porch et al., 2013a). Four botanical varieties (var.) of tepary bean are recognized including var. *acutifolius*, var. *latifolius,* var. *tenuifolius* (Buhrow, 1983; Blair et al., 2012; Gujaria-Verma et al., 2016). Further, the species *P. parvifolius* is reportedly genetically related to *P. acutifolius* and morphologically identical to var. *tenuifolius* (Freytag and Debouck, 2002; Muñoz et al., 2006). The cultivated tepary bean is believed to be a descendant of the wild var. *acutifolius* (Muñoz et al., 2006). The grain of tepary bean contains high protein content of 24%, essential mineral elements (e.g. Ca, Mg, Cu, Fe, K, Mn, S, Zn), oil and 33% saturated fatty acids, 67% unsaturated fatty acids, 24% monounsaturated fatty acids and 42% polyunsaturated fatty acids (Amarteifio and Moholo, 1998; Bhardwaj and Hamama, 2004; 2005). In some instances, the crop is grown as leafy vegetable and the haulms are used for animal feed (Molosiwa et al*.,* 2014). Tepary bean has high level of resistance to bean weevil caused by *Acanthoscelides obtectus* (Kusolwa and Myers, 2011), common bacterial blight [*Xanthomonas campestris* (axonopodis) pv. *Phaseoli*] (Singh and Muñoz, 1999; Miklas et al., 2006), rust (*Uromyces phaseoli*), anthracnose (*Colletotrichum lindemuthianum*), angular leaf spot (*Isariopsis griseola)* (Pratt and Nabhan, 1988; Thomas et al., 1983) and bean golden mosaic virus (Miklas and Santiago, 1996). Owing to the various desirable attributes, tepary bean genetic resources are recommended for production under harsh growing environments and some useful genes are transferred into common bean (Tar'an et al., 1998; Liu et., 2008; Beebe et al*.,* 2013). It has been

successfully used to develop advanced breeding lines of common bean with enhanced level of drought tolerance through genomic introgression. So far, 25% of the tepary genome has been introgressed into common bean (Muñoz et al. 2004).

Tepary bean is cultivated in South Africa but is a neglected and under-researched crop compared to other legumes such as common bean, cowpea, and Bambara groundnut. There is no dedicated tepary bean breeding program in South Africa to release varieties with tolerance to drought, pest and disease resistance. The crop has considerable potential as an alternative future food security crop and can be exploited by legume breeding programs in South Africa. To cultivate tepary bean in semi-arid and unfavourable environments outside of the centre of diversity, legume breeders at the International Center for Tropical Agriculture (CIAT)-Columbia are developing candidate genotypes. These genotypes can be selected in target growing environments to identify well-adapted and high yielding candidates. An attempt was made to introduce and select heat and drought tolerant tepary bean genotypes developed by CIAT in South Africa to design, develop and deploy improved cultivars. Well-characterised and genetically unique tepary bean germplasm is a pre-requisite to develop locally adapted and superior cultivars.

Genetic diversity analysis is an important step in plant breeding programs to identify genetically unique and complementary parental genotypes. These will also enable to create variability for further selection and to introgress desirable genes from diverse germplasm into the cultivated genotypes. Effective genetic grouping requires utilization of robust and informative DNA markers. Several studies have determined the level of genetic diversity in tepary bean. Muñoz et al. (2004) used amplified fragment length polymorphism (AFLP) markers and reported that *P. parvifolius* was genetically distinct from *P. acutifolius*. Blair et al. (2012) assessed genetic diversity in tepary bean accessions using SSR markers and reported that genetic differentiation was relatively higher in wild than cultivated tepary beans. Gujaria-Verma et al. (2016) reported genetic diversity using single nucleotide polymorphism (SNPs) markers.

There are limited genomic resources currently available for genetic analysis of tepary bean and only 54,917 tepary bean expressed sequence tags (EST) are currently available publicly (Gujaria-Verma et al., 2016). Further, only two genetic linkage maps based on AFLP and SNPs have been developed for tepary bean (Muñoz et al. 2004; Gujaria-Verma et al., 2016). There are no SSR markers developed specifically for the crop; however, SSR markers developed for common bean serve as important genomic resources for genetic diversity analysis and breeding in tepary bean (Blair et al., 2003; 2012). Simple sequence repeats (SSRs) markers are currently the most-suitable markers for germplasm characterization (Varshney et al*.,* 2005), genome-wide analysis (Blair et al*.,* 2012), gene tagging and gene mapping (Peng et al*.,* 1999) in diverse crop species. SSR markers are PCR-based, co-dominant, locus-specific, highly reproducible, polymorphic, informative and relatively simple to use (Xu et al., 2014). The objective of this study was to determine the genetic interrelationship present among 20 diverse tepary bean genotypes using 10 selected and polymorphic simple sequence repeat (SSR) markers and to identify genetically unique parental lines for developing breeding populations.

Results

Polymorphism and allelic diversity of SSR markers

The estimated genetic parameters are presented in Table 3. The SSR markers detected a total of 57 putative alleles with a size range of 102 to 304 base pairs (bp). The number of alleles (N_a) detected per locus across the 20 tepary bean genotypes ranged from 2 (for marker BMD28) to 16 (PV-AT007), with a mean of 5.7 alleles per locus. Three out of the ten SSR primers (BM053, BM152 and BM210) were monomorphic. The number of effective alleles per locus ranged from 1.54 to 11.59 with a mean of 4.32 alleles per locus. The lowest number of effective alleles (N_e) was observed for loci BMD053, BM152 and BM210 (1.00) and the highest for PV-AT007 (11.59). Fixation index (F_{IS}) exhibited contrasting values ranging from -0.12 to 1.00 with a mean of 0.06. F represents the average deviation of the population's genotypic proportions from Hardy–Weinberg equilibrium for a locus. A negative F value represents an excess of heterozygotes. The polymorphic information content (PIC) ranged from 0.35 for BMD28 and 0.97 for PV-AT007. The mean PIC of the seven polymorphic primers was 0.73. In the present study six SSR markers used had PIC values > 0.60, suggesting adequate discriminatory power of the SSR loci used in the current study. These were further classified as informative markers to establish the genetic relationship among tepary bean genotypes. Observed heterozygosity (H_o) ranged from 0.00 (GATS91) to 1.00 (BMD1, BM154 and BM199), with a mean of 0.45. Gene diversity (H_e) ranged from 0.36 (MBD28) to 0.94 (PV-AT007) with a mean of 0.51, suggesting that 51% of the individuals are expected to be heterozygous at a given locus under random mating conditions. Major alleles ranged from 102 for marker BM152 to 289 for marker BM199. Major allele frequency ranged from 0.13 for marker BM199 to 1.00 for markers BM152 and GATS91.

Genetic relationships among tepary bean genotypes

To evaluate the genetic relationships among the tepary bean genotypes, a dendogram based on neighbor-joining algorithm using the unweighted pair group method using arithmetic average was constructed (Fig. 1). The tested tepary bean genotypes were grouped into three distinct clusters. The distinctiveness of the clusters was confirmed by a highly significant cophenetic correlation coefficient (r = 0.96; P < 0.001). Cluster I comprised of 8 genotypes which originated from Mexico except G40201. Two of the genotypes (e.g. G40157 and G40158) in Cluster I have a prostrate-indeterminate growth habit, while the rest are climbing types. Cluster II comprised of three genotypes that have climbing growth habit with white and opaque seed. Cluster III comprised of 9 genotypes which originated from Mexico and the United States and have a climbing growth habit. The seed coat colours of the genotypes belonging in Cluster III are either white or cream except for genotypes G40068 (yellow) and G40066A (pink). Genetic distance (GD) measured among the genotypes based on Jaccard's genetic distance revealed large genetic variations among the genotypes (Fig. 2). The genetic distance values showed a normal distribution with 68% of the values lying between ± 1 standard deviation. The GD values varied from 0.18 to 0.64,

with a mean value of 0.42, suggesting moderate genotypic differentiation (Table 4). The highest genetic distance was found between G40084 with G40158 (GD = 0.64), G40237 (GD = 0.63), G40148 (GD = 0.60), G40201 (GD = 0.63) and G40157 (GD = 0.62). G40084 was grouped in Cluster III, whereas the rest of the genotypes were grouped in Cluster I. Genotype G40033 was genetically distant from G40201, G40157 and G40066A with GD values > 0.50. G40033 was grouped in Cluster III; whereas, G40201 and G40157 were grouped in Cluster I, except G40066A which grouped in Cluster III. Genotype G40084 was distant from G40066A with a GD value of 0.63 (Table 4).

Discussion

Tepary bean is an important but under-utilized legume crop possessing novel genes for abiotic and biotic stress tolerance breeding in common bean or related tropical legumes (Souter et al., 2017). Understanding genetic relationships among diverse tepary bean genetic resources is essential to facilitate effective breeding. The present study examined genetic relationships among 20 tepary bean genotypes using SSR markers in order to identify genetically unique parental lines for developing tepary bean breeding populations. Initially 50 diverse tepary genotypes were acquired from International Center for Tropical Agriculture (CIAT)-Columbia. Preliminary field and greenhouse evaluations (data not presented) under South African conditions indicated that some 20 genotypes were agronomically suitable with better yield and yield-related traits. These genotypes were selected and subjected to genetic analysis to select genetically distinct ones for effective breeding.

The SSR markers generated a total of 57 putative alleles of different fragment size ranging from 102 to 304 base pairs (Table 3). The number of alleles ranged from 1 to 16 with a mean of 5.7 alleles per locus. This was lower than the 8.3 alleles detected by Blair et al. (2012) when evaluating 140 tepary bean genotypes using 20 SSR markers. Further, Blair et al. (2006) reported a mean value of 7.8 alleles per locus in 43 common bean genotypes which was higher than the current findings. The number of effective alleles ranged from 1.0 to 11.59, with a mean value of 4.32. The polymorphic information content which is a measure of allelic diversity based on allele frequency (Smith et al., 2000) ranged from 0.00 to 0.97 with a mean of 0.52 (Table 3). The mean PIC value in the current study was comparable to those reported in other cultivated legumes including common bean (Blair et al*.,* 2006), soybean (Chauhan et al., 2015), cowpea (Ali et al., 2015), groundnut (Otenga-Frimpong et al., 2015) and faba bean (Rebaa et al., 2017). Three of the genomic microsatellites (e.g. BM053, BM152 and BM210) amplified only one band suggesting these gene-based markers were highly monomorphic (Table 3). However, by disregarding the monomorphic genomic SSRs, the mean PIC value for 70% of the markers was 0.71, suggesting adequate discriminatory power of the common bean SSR markers useful to detect differences among the tested tepary bean genotypes. The discriminatory power of these loci has also been reported previously in common and tepary bean (Blair et al., 2006; 2012; Diaz and Blair, 2006). A mean heterozygosity (H_o) value of 0.45 was recorded in this study suggesting that almost half of the loci were heterozygous and the remaining half of the loci reached acceptable level of homozygosity. However, the mean gene diversity (H_e) was 0.51, reflecting moderate level of genetic interrelationship among the tested tepary bean genotypes. The relatively low genetic diversity observed in this study agrees with previous reports that suggested tepary bean has a narrow genetic base (Blair et al*.,* 2012). This is partly attributed to natural and artificial selection exerted during long-term domestication of a limited genetic of pool of the crop (Schinkel and Gepts, 1988). In the past, tepary bean production was practiced in its centre of origin or diversity mainly in isolated geographic regions notably in drier parts of Mexico and the USA. This has probably localised its centre of domestication and hence rendered low adaptation and genetic differentiation of the crop (Freytag and Debouck, 2002). Further, tepary bean has low out-crossing rate with limited natural cross pollination and genetic recombination. This has probably partially contributed to the low genetic variation and limited genetic base of the crop (Blair et al*.,* 2012).

The UPGMA derived dendogram using SSR markers classified the tepary bean genotypes into three main genetic clusters (Fig. 1). A cophenetic correlation coefficient between the genetic similarity matrix and the cluster analysis was 0.96, indicating a distinct clustering structure and a very high goodness of fit of the clustering based on the original distance matrix (Dias et al., 2008; Mujaju et al., 2011). The current study revealed that most tepary bean genotypes were grouped based on their region of origin and morphological traits suggesting that the test genotypes share similar genetic backgrounds. For example, genotypes in Cluster I originated from Mexico, whereas those in Cluster III originated from Mexico and the USA. Further, most of the genotypes in Cluster I have a prostrate-indeterminate growth habit, while the rest are climbing types. Genotypes in Cluster III were either white or cream except for genotypes G40068 and G40066A which have a yellow and pink seed coat colours, respectively. Schinkel and Gepts (1988) reported similar pattern of genetic grouping based on geographical origin and morphological traits. In their study, tepary bean genotypes with and without basal lobed leaflet type originated from the USA and Mexico, respectively and were grouped independently. Tepary bean is originated from the USA and Mexico and results of the current study confirmed the possibility and agreeing with Gujaria-Verma et al. (2016) who reported that tepary bean genotypes were grouped based on geographic origin.

The genetic distance in the current study varied from 0.18 to 0.64, with a mean value of 0.43, suggesting moderate genetic differentiation among the studied tepary bean genotypes. Previous studies using SSR markers and SNPs have focused on genetic differentiation (e.g. population structure analysis) among different varieties of tepary bean (Blair et al., 2012; Gujaria-Verma et al., 2016) with very little information reported on genetic distances between genotypes. Such information is important to identify genetically distant parental lines that can be used to conduct crosses aimed at developing novel breeding populations. In the current study, genotypes G40084 and G40158, G40237 G40148 and G40157 displayed the highest genetic distance and were grouped in different clusters (Table 4; Fig. 1). Further, genotype G40033 allocated in Cluster III was distantly related to G40201 and G40157 allocated in Cluster I. The identified genotypes especially from unrelated genetic groups exhibiting high genetic distances will aid in the

Table 1. Description of tepary bean genotypes used in the study with geographic origin, growth habit and grain characteristics.

No	Genotype	Country of origin	Growth habit	Seed colour	Seed brightness
1	G40033	Mexico	Climbing	Cream	Opaque
2	G40001	Mexico	Climbing	White	Opaque
3	G40084	Mexico	Climbing	Cream, Brown	Opaque
4	G40111	Mexico	Climbing	Black, Cream	Opaque
5	G40125	Mexico	Climbing	White	Intermediate
6	G40135A	Mexico	Climbing	White	Opaque
7	G40144A	Mexico	Climbing	Cream	Intermediate
8	G40173A	Mexico	Climbing	Yellow	Opaque
9	G40237	Mexico	Climbing	Cream	Intermediate
10	G40148	Mexico	Prostrate-Indeterminate	White	Opaque
11	G40157	Mexico	Prostrate-Indeterminate	White	Opaque
12	G40158	Mexico	Prostrate-Indeterminate	White	Opaque
13	G40023	United States of America	Climbing	White	Opaque
14	G40042	United States of America	Climbing	White	Opaque
15	G40063	United States of America	Climbing	White	Opaque
16	G40066A	United States of America	Climbing	Pink	Opaque
17	G40068	United States of America	Climbing	Yellow	Opaque
18	G40069	United States of America	Climbing	White	Opaque
19	G40201	Costa Rica	Prostrate-Indeterminate	Black, Cream	Opaque
20	G40064	United States of America	Climbing	White	Opaque

Table 2. Descriptions of the simple sequence repeat (SSR) markers used for genetic differentiation of tepary bean genotypes.

Marker name	Primer sequence	Marker type	PIC
PV-AT001	F: GAGGGTGTTTCACTATTGTCACTGC	Gene-based	0.94 [(1)]
	R: TTCATGGATGGTGGAGGAACAG		
PV-AT007	F: AGTTAAATTATACGAGGTTAGCCTAAATC	Gene-based	0.94
	R: CATTCCCTTCACACATTCACCG		
BMD1	F: CAAATCGCAACACCTCACAA	Gene-based	0.94
	R: GTCGGAGCCATCATCTGTTT		
BMD28	F: TGCATCAACTTTAGGAGCTTG	Gene-based	0.87
	R: TCTTGTCTTATCAGCAGGTGGA		
BM053	F: TGCTGACCAAGGAAATTCAG	Genomic	0.92
	R: GGAGGAGGCTTAAGCACAAA		
BM152	F: AAGAGGAGGTCGAAACCTTAAATCG	Genomic	0.89
	R: CCGGGACTTGCCAGAAGAAC		
BM154	F: TCTTGCGACCGAGCTTCTCC	Genomic	0.94
	R: CTGAATCTGAGGAACGATGACCA		
BM199	F: AAGGAGAATCAGAGAAGCCAAAAG	Genomic	0.91
	R: TGAGGAATGGATGTAGCTCAGG		
BM210	F: ACCACTGCAATCCTCATCTTTG	Genomic	0.88
	R: CCCTCATCCTCCATTCTTATCG		
GATS91	F: GAGTGCGGAAGCGAGTAGAG	Genomic	0.91
	R: TGTCACCTCTCTCCTCCAAT		

[(1)] Blair et al. (2006); F: Forward primer; R: Reverse primer; PIC: Polymorphic information content.

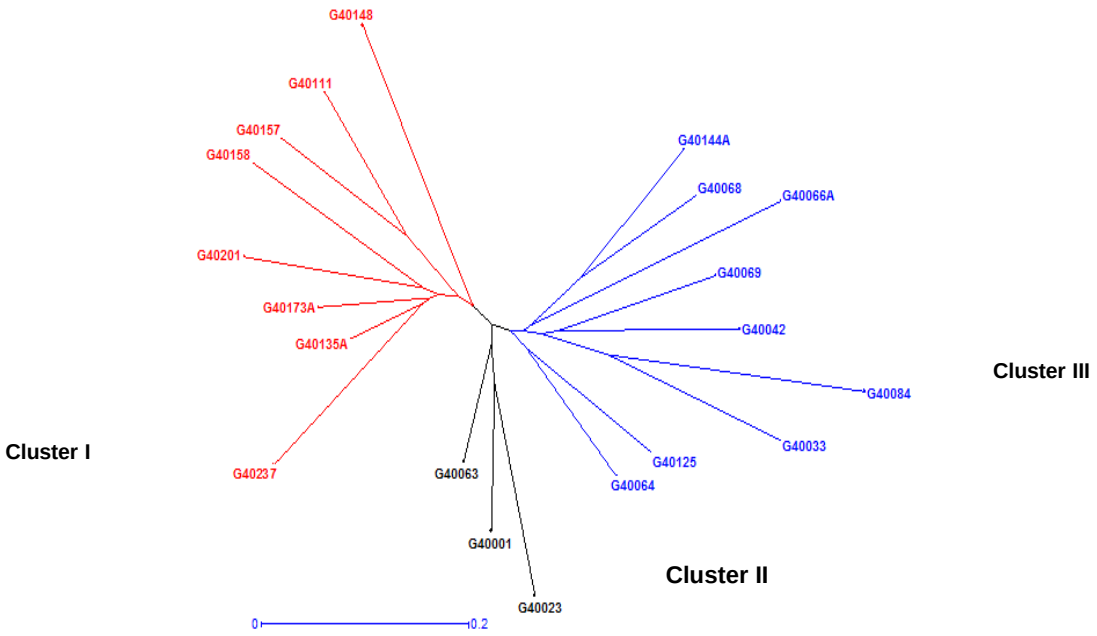

Fig 1. Neighbor joining dendrogram using the unweighted pair group method using arithmetic average based on Jaccard's dissimilarity matrix showing genetic relationships among 20 genetically diverse tepary bean genotypes. Note that different colour represent different groups.

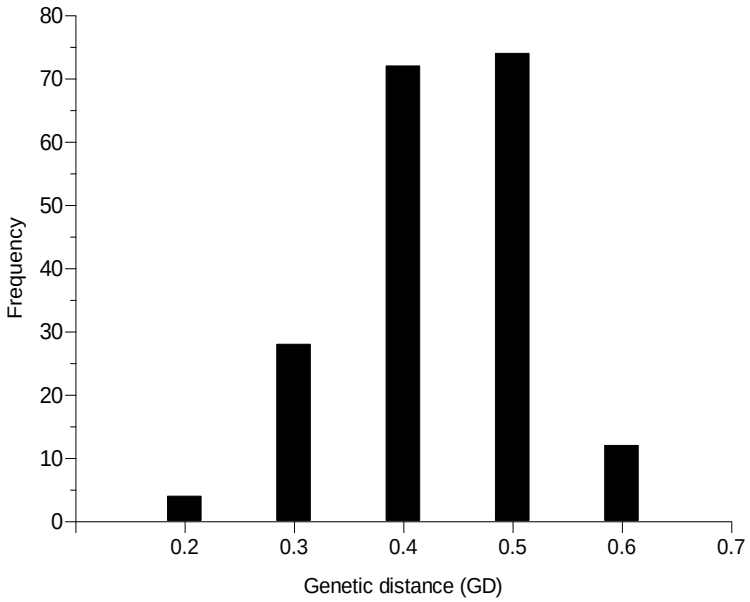

Fig 2. The frequency of tepary bean genotypes (5) based on genetic distance estimates using SSR markers.

Table 3. Genetic parameters estimated by SSR markers among tepary bean genotypes.

Loci	N_a [(1)]	N_e	H_o	H_e	F_{IS}	PIC	M_A	A_F
PV-AT001	5	2.7	0.56	0.65	0.12	0.63	173	0.53
PV-AT007	16	11.59	0.7	0.94	0.23	0.97	222	0.15
BMD1	3	2.31	1	0.58	-0.77	0.57	181	0.42
BMD28	2	1.54	0.25	0.36	0.28	0.35	186	0.5
BM053	1	1	0	0	-	0	114	0.78
BM152	1	1	0	0	-	0	102	1
BM154	6	3.86	1	0.76	-0.35	0.75	230	0.35
BM199	10	9.52	1	0.92	-0.12	0.94	289	0.13
BM210	1	1	0	0	-	0	156	0.2
GATS91	12	8.7	0	0.91	1	0.95	165	1
Overall mean	5.7	4.32	0.45	0.51	0.06	0.53	-	-
SE	1.67	1.28	0.14	0.12	0.18	0.13	-	-

[(1)] N_a: number of observed alleles; N_e: number of effective alleles; H_o: Observed heterozygosity; H_e: gene diversity; F_{IS}: inbreeding coefficient; PIC: polymorphic information content; M_A: major allele; A_F: major allele frequency; SE: standard error.

Table 4. Genetic distance estimates among 20 tepary bean genotypes using SSR markers.

Genotype	1 [(1)]	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1																				
2	0.28																			
3	0.48	0.48																		
4	0.42	0.42	0.43																	
5	0.18	0.35	0.42	0.35																
6	0.26	0.41	0.48	0.35	0.26															
7	0.48	0.54	0.56	0.43	0.42	0.48														
8	0.42	0.42	0.36	0.36	0.35	0.35	0.43													
9	0.38	0.39	0.46	0.33	0.32	0.32	0.52	0.46												
10	0.54	0.54	0.43	0.50	0.48	0.48	0.63	0.57	0.46											
11	0.44	0.45	0.52	0.52	0.38	0.44	0.46	0.46	0.43	0.52										
12	0.35	0.47	0.42	0.36	0.28	0.28	0.48	0.36	0.39	0.54	0.45									
13	0.40	0.46	0.42	0.42	0.33	0.40	0.48	0.27	0.44	0.54	0.50	0.35								
14	0.48	0.39	0.50	0.50	0.43	0.48	0.50	0.50	0.42	0.60	0.42	0.53	0.53							
15	0.42	0.53	0.61	0.50	0.36	0.42	0.50	0.50	0.41	0.62	0.28	0.43	0.54	0.45						
16	0.41	0.52	0.59	0.54	0.35	0.41	0.54	0.54	0.50	0.64	0.45	0.41	0.52	0.48	0.43					
17	0.28	0.36	0.48	0.36	0.21	0.28	0.48	0.42	0.39	0.54	0.33	0.30	0.41	0.44	0.24	0.30				
18	0.32	0.45	0.46	0.46	0.25	0.32	0.52	0.33	0.43	0.58	0.37	0.33	0.32	0.42	0.35	0.33	0.20			
19	0.47	0.47	0.53	0.53	0.42	0.47	0.53	0.48	0.50	0.63	0.41	0.47	0.47	0.44	0.44	0.38	0.27	0.30		
20	0.30	0.37	0.58	0.53	0.36	0.41	0.53	0.53	0.45	0.63	0.45	0.42	0.52	0.44	0.43	0.37	0.31	0.29	0.43	

[(1)] 1: G40001; 2: G40023; 3: G40033; 4: G40042; 5: G40063; 6: G40064; 7: G40066A; 8: G40068; 9: G40069; 10: G40084; 11: G40111; 12:G40125; 13: G40144A; 14: G40148; 15: G40157; 16: G40158; 17: G40135A; 18: G40173A; 19: G40237; 20: G40201.

development of superior and high yielding tepary bean lines or for increasing genetic diversity in this and other related legume crops. Crosses conducted between genetically diverse genotypes may result in transgressive segregation because different genotypes can often fix different sets of alleles with complementary effects (Amelework et al., 2015). To date there is a limited number of tepary bean genotypes released for cultivation in dry environments or as source of genes for breeding globally. Only two improved cultivars namely: TARS-Tep 22 and TARS-Tep 32 have been developed and released with resistance to biotic and abiotic stresses in the USA (Porch et al., 2013b). Further, tepary bean introduction lines PI319443 and PI440795 have been widely used as sources of resistance to common bacterial blight in common bean breeding programs (Shi et al., 2011; 2012). Therefore, strategic crosses between selections from the genetic groups established in this study may allow identification of transgressive tepary bean populations.

Materials and methods

Plant materials

The study used 20 genetically diverse tepary bean genotypes. The germplasm was acquired form CIAT-Columbia which were originally assembled from different sources. Table 1 summarises the geographic origin, growth habits and grain characteristics of the tested genotypes. Therefore, the 20 genotypes evaluated in the current study were selected based on their diverse region of origin and suitable agronomic traits.

DNA extraction, purification and quantification

Seed of 20 genetically diverse tepary bean genotypes were planted in 4 litre capacity polyethylene pots under glasshouse conditions at the Controlled Environment Facility (CEF), University of KwaZulu-Natal (Pietermaritzburg (29°37'51.75" S; 30°23'59.10" E), South Africa.

Young fresh leaves were harvested from 5 individual plants per genotype four weeks after planting. The leaf samples were sent to INCOTEC PROTEIOS laboratory (Incotech, Pty Ltd, Pietermaritzburg, South Africa) for SSR analysis. DNA was extracted using the standard CTAB protocol (mixed alkyltrimethyl-ammonium bromide) as described by CIMMYT (2005). The concentration of the extracted DNA was determined using 0.7% Tris-Borate-EDTA (TBE) agarose gel. A working concentration of 10 ng μl^{-1} was standardized for all extracted DNA. The samples were bulked and used in SSR amplification.

PCR and SSR analysis

SSR sequences were amplified through polymerase chain reaction (PCR) using SSR primers. Ten selected and polymorphic SSR primers were used for the analysis (Table 2). The markers were selected based on their high polymorphic information content and being effective for common bean and tepary beans (Blair et al., 2003; 2006; 2012). There are no SSR primers developed specifically for tepary bean. The PCR amplification was performed using 25µl containing 25 ng genomic DNA, 0.5µM forward and reverse primers, 25 mM $MgCl_2$, 1 x PCR reaction buffer, 200µM dNTPs and 1 unit of Taq DNA polymerase (Bioline). PCR products were fluorescently labelled and separated by capillary electrophoresis on an ABI 3130 automatic sequencer (Applied Biosystems, Johannesburg, South Africa). The PCR fragment size analysis was performed using GeneMapper 4.1 (Applied Biosystems, Johannesburg, South Africa).

Data analysis

Genetic parameters estimates

Genetic diversity parameters, such as number of alleles per locus (N_a), number of effective alleles per locus (N_e), observed (H_o) and expected (H_e) heterozygosity major allele and major allele frequency were calculated using GenAlex version 6.5 (Peakall and Smouse, 2012) according to the protocol described by Nei and Li (1979). Fixation index or inbreeding coefficient (F_{IS}) was calculated according to Wright's original derivation (Wright, 1951). Polymorphic information content (PIC) was calculated using the formula: $PIC = 1-\Sigma P_{ij}^2$, where P_{ij} is the frequency of j^{th} allele of the i^{th} locus. Pair-wise Nei's unbiased genetic distance was also estimated among the genotypes according to Nei (1978).

Cluster and genetic distance analyses

Genetic relationships among tepary bean genotypes were determined using the neighbor-joining algorithm using the unweighted pair group method of arithmetic mean (UPGMA) in DARwin 6.5 (Peakall and Smouse, 2012). The dendogram was generated based on Jaccard's dissimilarity matrix using binary data (0 = absent and 1 = present) to capture all the alleles amplified. Bootstrap analysis was performed for node construction using 10,000 bootstrap values to estimate the reliability of the clustering pattern. A cophenetic value matrix of the UPGMA clustering was used to test for the goodness-of-fit of the clustering to the resemblance matrix on which it was conducted, by computing the product-moment correlation coefficient (r) with 1000 permutations (Rohlf and Fisher, 1968; Sneath and Sokal, 1973).

Conclusion

The use of common bean derived SSR markers proved highly informative suggesting their effectiveness in detecting genetic differences among the studied tepary bean genotypes. Unique genotypes such as G40201, G40237, G40158, G40157 and G40158 from Cluster I, G40084 and G40033 from Cluster III were selected based on their high genetic distance. These are recommended as parents for further breeding and to broaden the genetic basis of this crop or related legumes.

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