Journal of Plant Breeding and Crop Science

Volume 6 Number 12 December 2014

ISSN 2006-9758

Academic Journals

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Journal of Plant Breeding and Crop Science

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Vol. 6(12), pp. 179-184, December 2014
DOI: 10.5897/JPBCS2014.0481
Article Number: 1F187B648117
ISSN 2006-9758

http://www.academicjournals.org/IPBCS

Journal of Plant Breeding and Crop Science

Full Length Research Paper

Combining ability for yield and yield components in six parents and their 15 F_1 hybrids of sesame (*Sesamum indicum* L.) in half diallel mating design

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Received 4 August, 2014; Accepted 25 September, 2014

Combining ability estimates were studied for seed yield, yield components and other morphological traits in six sesame parental lines and their 15 F_1 hybrids crossed in half diallel for two consecutive seasons 2012/2013 - 2013/2014 at Gadaref University Farm, Gadaref, Sudan. Combining ability analysis revealed that both additive and non additive types of gene actions were important in the studied traits. For days to 50% flowering and days to maturity, Khidir was the only parent that scored negative general combining ability (GCA) effects in both seasons. Therefore it was desired to be selected for earliness. For seed yield kg/ha and the yield related characters viz., 1000- seed weight and the yield per plant, significant positive SCA effects were observed by the crosses, Kenana-2 X Gd 002SPSN-12 and Promo X Gd2002SPSN.12, whereas, negative significant effects were showed by Gadarif-1 XUmshagera. The rest of the crosses combinations were inconsistent across the seasons, some of them recorded a positive value in one season and a negative values in another one. Khidir and Promo recorded a positive significant GCA effects for the yield and its components at least in one season. Moreover, Promo was the best combiner with other parental lines for earliness since it recorded negative SCA values. Therefore, Khidir, Promo and Gd2002SPSN.12 could be recommended to produce progeny having high yield and early maturing hybrids, through recurrent selection or reciprocal cross.

Key words: Combining ability, sesame hybrids, sesame yield, yield components.

INTRODUCTION

Sesame (*Sesamum indicum* L.), commonly known as *gingelly, til, benniseed, simsim* is a member of the order Tubiflorae and family Pedaliaceae. It is probably the most ancient oilseed known and used by man and its domestication is lost in the mists of antiquity (Weiss, 1983). Although originated in Africa, it spreads early through West Asia to India, China and Japan which themselves became secondary distribution centers (Weiss, 1983). It is called the "Queen of oil seeds" because of its excellent qualities of the seed, oil and meal. Sesame is highly nutritive (oil 50%, protein 25%) and its oil contains an antioxidant called sesamol which

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imparts a high degree of resistance against oxidative rancidity. Sesame cake is nutritious feed for dairy cattle and it can also be used as fertilizer (Ashri, 1989).

Sesame is one of the most important oilseed crops in Sudan for both local consumption and for export (Ahmed, 2008). It is widely grown under rain-fed conditions; in Gadarif, Damazin, Kordofan and Darfur. Recently, sesame has been grown on small scale in River Nile State under pump irrigation (Abdelkarim and Sulieman 2008). Sesame ranks third after sorghum and millet area wise. It was grown on about 820,260 ha, and produced about 187,000 tons of seed (covering about 4% of the total world production), with average seed yield of 228 kg/ha. The world average seed yield is 511 kg/ha (FAOSTAT, 2014).

In an often cross-pollinated crop like sesame there is a good scope for exploitation of heterosis. Further, an understanding of the combining ability and gene action is a prerequisite for any successful breeding programme. For breaking the yield barrier and evolving varieties with high yield potential, it is desirable to combine the genes from genetically diverse parents. There are several techniques for evaluating the varieties or cultivars or lines in terms of their combining ability and genetic make up, of these, Diallel, partial Diallel and line X tester techniques are in common use.

The concept of combining ability analysis gives precise estimates of the nature and magnitude of gene actions involved in the inheritance of quantitative characters, which facilitate the identification of parents with good general combining ability (GCA) effects and crosses with good specific combining ability (SCA) effects.

Many researchers studied the concept of the combining ability for yield and yield related characters in sesame. Thiyagarajan and Ramanathan (1995) reported that the influence of non-additive gene action was observed for number of branches /plant, number of capsules /plant, 1000 seed weight and seed yield. The predominance of additive gene action was observed for days to50% flowering and plant height. Zhong (1999) reported that the additive gene actions were predominant in controlling most of the characters studied although non-additive gene were also important for height to first capsule, 1000-seed weight, days to maturity and seed yield per plot. He reported that the (GCA) estimates revealed that the parent Zhongzhi 10 was the best general combiner for plant height, days to maturity and seed yield.

Saravanan et al. (2000) reported that the (SCA) variance was higher than (GCA) variance for seed yield per plant, they reported that the mean degree of dominance was less than unity for all the traits studied except 1000-seed weight. Saravanan and Nadarajan (2003) reported that the variance due to general combining ability (GCA) and the specific combining ability (SCA) were significant for all characters studied and the (GCA) variances were greater than the (SCA) variances.

The present study was set up to estimate the combining ability in sesame among 6 parents and their 15 F_1-hybrids of sesame designed in a half- Diallel fashion under rain-fed conditions of Sudan.

MATERIALS AND METHODS

Site description

Gadarif is located in Eastern Sudan, 12° 17" N to 34° 36" E and altitude of about 600 m (a.s.l), the soil is heavy cracking clay soil (vertisol) with a very low organic matter and nitrogen (0.70 , 0.03) respectively, and available phosphorus(3 mg/kg soil) and approximate pH value of 7.8. The minimum average temperature is about 17°C in January and the maximum is about 47°C in April to May. The annual rainfall is about 600 mm in the southern region, 450 mm in central region and 300 mm in the northern region. The relative humidity is about 33% in January and about 71% in August.

Plant materials

The plant materials used in the study were 5 locally developed parental lines, and one an introduced line, the parental names, designation, origin and description were presented in Table 1.

Experimental procedures, data collection and statistical analysis

Experimental procedures

For crossing, the six parents were grown in three rows of 5 m length and 0.8 m apart for each genotype. Thinning was done after two weeks from sowing and the area was weeded after three weeks from emergence. All parents were crossed manually in all possible combination in a half diallel fashion (excluding reciprocals). Thus 15 F_1hybrids were produced and then six parents and their 15 hybrids were sown in a randomized complete block design with three replications for two consecutive seasons 2012/2013 and 2013/2014, each entry was grown on two rows of 2.5 m long and 1.6 m apart. The 21 genotypes were sown by hand on 11/7/2012 and 28th July 2013 for the first and the second seasons, respectively. To raise healthy crop, all cultural practices were carried out as recommended by the Agricultural Research Station.

The data were collected on the following parameters:

1. Number of days to 50% flowering (NDTFPF).
2. Number of days to maturity (NDTM)
3. Plant height (PHT) (cm).
4. 1000-seed weight (1000-SW) (g).
5. Seed yield/plant (SYPP) (g).
6. Seed yield (kg/ha) (SY/Ha).

Statistical analysis for estimation of combining ability

Analysis of variance was used for each season for the data to test significant differences among the genotypes. Griffing (1956) method II was used to estimate the general and the specific combining ability (GCA and SCA). Two steps were involved in the analysis; the first one was analysis of data for testing significant differences among the genotypes. The second step was carried out to estimate the combining ability shown following.

Estimation of sum squares and means squares is as follows:

Sum of squares due to general combining ability (gca) = $1/n+2\{\sum (Yi+Yii)^2 - 4/n\ Y^2\ldots\}$

Table 1. Parental name, designation, origin and description of the plant materials used in the study.

Parents	Designation	Origin	Description
Khidir	Kh	GARS#	A white seeded locally developed variety and released in 1998.
Kenana-2	K2	GARS	A white seeded variety selected from an African introduction and released in 1991
Promo	P	GARS	A variety selected from introduction materials of temperate origin (Greece) characterized by high branching medium duration, even maturity and delayed shattering (Ahmed, 1997; Ahmed, 2008)
Gadarif -1	Gd-1	GARS	A variety selected from segregated materials of crosses between temperate and tropical cultivars, it is characterized with non-branching habit late duration to flowering and good vigorous (Ahmed et al., 2003)
Gadarif 2002 single plant selection line number 12	Gd 2002 SPS-12	GARS	Advanced line (Khalafalla and Ahmed, 2003, 2004, 2005) has very long capsule
Um shagera	Um	GARS	A variety which has white and large seeds medium duration to flowering, maturity, and high yielder

#, Gadarif Agricultural Research Station.

Sum of squares due to specific combining ability (sca) = $\sum \sum Yij^2$ - $1/n+2\sum (Yi+Yii)^2 + 2/(n+1)(n+2) Y^2$...

Check treatment S.S = r (S.S due to gca + S.S due to sca)

Sum of squares due to error = Total sum squares - Genotype sum square – Replication sum square

Mean square for general combining ability (gca)
Mean square for specific combining ability (sca)
Mean square for error

For testing the significance due to general and specific combining ability analysis Griffings method II model I was applied.

Estimation of genetic components is as follows:

Component due to GCA = $1/n-1 \sum gi^2 = Mg-Me'/n+2$

Component due to SCA = $2/n(n-1) \sum i<j \sum sij^2 = Ms-Me'$

The ratio of GCA variance to SCA variance was calculated as follow:

$$1/n\text{-}1 \sum gi^2 \Big/ \frac{2}{n(n-1)/\sum i<j \sum sij^2}$$

Estimation of GCA effects is calculated is as follow:

$$gi = \frac{1}{n+2}\{\sum(Yi+Yii) - 2/n\, Y..\}$$

Estimation of SCA effects are calculated as follow:

$Sij = Yij - 1/n+2 (Yi - Yii + Yj + Yjj) + 2/(n+1)(n+2) Y..$

Standard errors are calculated as follow:

$S.E.(gi) = \{(n-1)\sigma^2e/n(n+2)\}^{1/2}$

$S.E.(gi-gj) = \{2\sigma^2e/(n+2)\}^{1/2}$

$S.E.(sij) = \{n(n-1)\sigma^2e/(n+1)(n+2)\}^{1/2}$

$S.E.(sii-sjj) = \{2(n-2)\sigma^2e/(n+2)\}^{1/2}$

Where S.E. (gi) is standard error for GCA effects of the parents; n is the number of parents included in the analysis; σ^2e is the expected error mean square in the combining ability analysis; S.E. (gi-gj) is standard error difference for GCA effects between the i th and j th parent; S.E. (sij) is standard error for SCA effects of the ith and jth parent; S.E. (sii-sjj) is standard error of difference for SCA effects between the i th and j th crosses.

RESULTS AND DISCUSSION

Combining ability

Table 2 shows analysis of variance of the mean squares due to the genotypes, general combining ability (GCA) and specific combining ability (SCA) and their ratios for all characters in both seasons. The mean squares due to genotypes were highly significant in both seasons for all characters under study. This indicated that an adequate amount of variability is present in the parental material, and thus suggested the effectiveness of selection for the development of new genetic lines possessing improved traits. Variance due to the general combining ability was highly significant for all characters in both season except for the number of days to maturity in the first season and the seed yield per plant in the second season. Dhillon (1975) reported that combining ability of parents gives useful information on the choice of parents in terms of expected performance of the hybrids and their progenies. Like the GCA, the mean squares due to specific combining ability (SCA) was highly significant for the yield and its components, but it was not significant for the days to 50% flowering, days to maturity in both seasons and for the plant height in the first season only.

This indicated that both additive and non-additive gene actions were responsible for the inheritance of the studied traits. This suggests the use of reciprocal recurrent

Table 2. Mean squares for the genotypes, general (GCA) and specific combining ability (SCA) and their ratios for six traits in sesame genotypes seasons 2012/2013 and 2013/2014 grown at Gadaref University Farm under rain-fed condition.

Source of variation	df	NODTFPF		NODTM		PH		1000- SW		SYPP		SYTPH	
		012/013	013/014	012/013	013/014	012/013	013/014	012/013	013/014	012/013	013/014	012/013	013/014
Reps	2	18.79	26.16	83.76	11.29	620.37	69.54	0.05	0.31	1.82	31.53	1589.26	1922.02
Genotypes	20	28.89**	45.39 ***	37.43 N.S	20.12 **	201.58 **	203.31 ***	0.50 **	0.65 ***	128.69 ***	44.24 **	138304.94 ***	50677.32 ***
GCA	5	90.13**	126.84**	50.47 N.S	54.13**	352.31**	372.84**	1.03**	0.65**	128.04**	8.56 N.S	190909.38**	21242.99**
SCA	15	8.47 N.S	18.23 N.S	33.09 N.S	8.79 N.S	151.33 N.S	146.80**	0.33**	0.65**	128.91**	56.14**	120770.12**	60488.76**
Error	40	10.81	10.03	23.10	5.37	83.60	33.66	0.17	0.07	0.71	18.16	1567.52	1507.75
GCA:SCA		10.64	6.96	1.53	6.16	2.33	2.54	3.12	1	0.99	0.15	1.58	0.35

Table 3. Estimates of general combining ability (GCA) effects for six sesame parents grown at Gedarif University Farm, seasons 2012/2013 and 2013/2014.

Parent	NODTFPF		NODTM		PH		1000-SW		SYPP		SYKPH	
	012/013	013/014	012/013	013/014	012/013	013/014	012/013	013/014	012/013	013/014	012/013	013/014
Khidir	-0.286	-1.80	-0.250	-1.06	-2.50	-8.17**	0.28	0.00	1.67**	-4.22	182.18**	-76.99**
Kenana-2	-0.616	0.87	0.500	1.11	-1.85	-5.33	-0.12	0.06	-2.79**	-4.65*	15.16	-195.16**
Promo	-0.119	2.04	1.083	1.11	0.33	5.25	-0.02	0.05	-2.57**	-1.31	-84.76**	105.60**
Gedarif-1	1.964	2.5	2.000	-1.56	-10.1*	-6.08*	0.33	-0.26	-3.68**	-4.32*	-26.09	-148.41**
Gd2002SPSN.12	3.798*	-1.38	5.417*	-2.81*	-11.6*	-12.75**	-0.30	-0.13	-12.86**	-4.52*	-211.17**	65.93**
Umshagera	-0.119	1.87	-0.417	-0.98	-0.85	-1.58	0.52*	0.05	2.35**	-6.45**	-0.09	-78.07**
SE (gi)	1.06	1.02	1.55	0.75	2.95	1.87	0.13	0.09	0.27	1.38	12.78	12.53
SE or SE(gi-gj)	1.64	1.58	2.40	1.16	4.57	2.90	0.21	0.14	0.42	2.13	9.80	19.41
CD ,t 5%	3.32	3.20	4.86	2.34	9.23	5.86	0.41	0.27	0.85	4.31	40.00	39.24
CD,t 1%	4.45	4.28	6.50	3.13	12.36	7.84	0.55	0.37	1.14	5.76	53.53	52.50

*, ** Significant at 0.05 and 0.01 probability levels respectively.

selection for exploiting both types of genetic variances. Similar findings were reported by Zhong (1999) in sesame.

GCA:SCA

The ratio for the general combining ability (GCA) to that of specific combining ability (SCA) were almost more than one for all characters under study, in both seasons, except for the seed yield per plant. This result indicating that the inheritance of these traits were due to general combining ability effects and were mostly controlled by the additive gene actions. The ratios were less than one in both seasons for seed yield/plant and seed yield kg/ha in the second season only, indicating that the inheritance of this trait was due to non- additive gene actions. Thiyagarajan and Ramanathan (1995) reported that the non- additive gene action was observed for seed yield in sesame.

Table 3 shows the estimates of general combining ability (GCA) effects, magnitudes and their directions. The best combiners were (Gd2002sps-12, Gadaref-1), since they recorded significant general combining ability (GCA) effects

Table 4. Estimates of specific combining ability (SCA) effects for yield and yield components of F_1 sesame hybrids grown at Gedarif University season.

Crosses	NODTFPF		NODTM		PH		1000-SW		SYPP		SYKPH	
	012/013	013/014	012/013	013/014	012/013	013/014	012/013	013/014	012/013	013/014	012/013	013/014
Khidir*Kenana-2	-0.12	-0.46	1.29	-0.48	-4.24	5.58	0.11	-0.00	-6.19**	-0.38	163.57**	149.10**
Khidir*Promo	1.46	-0.05	4.25	-1.48	-1.82	0.54	-0.45	0.21	-1.69	5.67	-262.39**	-40.36
Khidir*Gedarif-1	0.67	1.33	0.38	1.02	3.48	4.04	-0.21	0.35	0.85	3.61	-180.06**	176.97**
Khidir*Gd2002SPSN.12	-1.58	1.74	-3.42	3.90	0.23	1.38	0.04	0.55*	-1.54	-3.85	-98.60*	-253.36**
Khidir*Umshagera	0.13	1.04	-2.00	-0.85	7.36	4.79	-0.05	-0.01	5.24**	3.40	13.11	121.64**
Kenana-2*Promo	1.96	1.29	2.79	-0.23	1.51	-3.71	0.21	-1.00	3.05**	0.12	-332.46**	1.39
Kenana-2*Gedarif-1	-0.16	-2.67	-0.75	-0.73	2.20	-2.21	-0.11	0.75**	-0.81	1.75	26.54	-22.61
Kenana-2*Gd2002SPSN.12	-1.08	1.74	-3.88	-0.19	3.48	8.46	0.28	0.41	8.61**	3.87	76.00	138.39**
Kenana-2*Umshagera	0.63	-1.63	-0.45	-0.60	0.75	2.54	-0.25	-0.28	0.91	3.93	36.04	124.05**
Promo*Gedarif-1	-1.58	-3.59	-1.46	0.27	8.89	2.08	-0.15	-0.20	-0.25	-1.24	292.58**	78.93*
Promo*Gd2002SPSN.12	-1.83	-3.17	-6.25	-0.19	-0.83	-1.92	0.31	0.19	6.71**	0.41	363.37**	-134.07**
Promo*Umshagera	0.21	1.45	-1.50	-0.60	-8.43	-7.50	0.12	0.70*	-2.70**	-2.34	108.41**	-117.07**
Gedarif-1*Gd2002SPSN.12	-2.62	3.54	-2.13	0.32	11.93	11.25	0.32	0.17	13.83**	2.62	76.04	76.60
Gedarif-1*Umshagera	-0.24	-3.51	-0.04	2.23	-6.33	-3.00	-0.51	-0.55*	-6.26**	1.90	-162.92**	-13.07
Gd2002SPSN.12*Umshagera	-0.49	-1.09	4.83	1.77	8.35	6.33	-0.35	0.04	-1.89*	6.00	5.54	40.60
SE (sij)	2.41	129.78	3.52	94.97	6.69	237.79	0.30	11.09	0.62	174.65	28.98	1591.55
SE or SE(sii-sjj)	3.29	3.17	4.81	2.32	9.14	5.80	0.41	0.27	0.84	4.26	39.59	38.83
CD,t 5%	6.65	6.40	9.71	4.68	18.48	11.72	0.83	0.55	1.70	8.61	80.02	78.47
CD,t 1%	8.89	8.56	12.99	6.27	24.72	15.69	1.11	0.73	2.28	11.52	107.06	105.00

*,** Significant at 0.05 and 0.01 probability levels respectively.

for most of the traits measured.

For days to 50% flowering and days to maturity, Khidir was the only parent that scored a negative general combining ability (GCA) effects in both seasons. Therefore it was desired to be selected for earliness while the parent Gd2002SPSN.12 recorded a highly positive significant general combining ability (GCA) effects in the first season only. Therefore it was the latest maturing parent. For plant height Gadaref-1 and Gd2002SPSN.12 showed significant negative general combining ability (GCA) effects in both seasons, while Promo showed a positive general combining ability (GCA) effects in both seasons. Therefore, Promo was recommended as tallest parent.

1000 seed weight was the most yield related character. Khidir and Umshagera recorded a positive general combining ability (GCA) effects in both seasons, while Gd2002SPSN.12 recorded a negative general combining ability (GCA) effects in both seasons.

For seed yield/plant and seed yield kg/ha, Khidir and Promo recorded a positive significant general combining ability (GCA) effects in the first and the second season respectively, while Kenana-2 recorded significant negative general combining ability (GCA) effects in both seasons. Therefore, the former parents (Khidir and Promo) were recommended for future breeding program to improve seed yield in sesame.

Specific combining ability (SCA) effects

Table 4 shows the estimates of specific combining ability (SCA) effects, for 15 sesame crosses. The specific combining ability (SCA) is considered to be the best criterion for selection of superior hybrids. Considerable number of crosses showed

significant specific combining ability (SCA) effects, but they were inconsistence a cross the seasons.

For days to 50% flowering and the days to maturity, the crosses Kenana-2 X Gadaref-1 and Promo X Gd2002sps-12 showed negative specific combining ability in both seasons. On the other hand, Khidir X Gadaref-1recorded positive SCA in both seasons. The other crosses were inconsistence across the seasons regarding the directions of this character. The former crosses (Kenana-2 X Gadaref-1 and Promo X Gd2002sps-12) were good for earliness. The best hybrids combination with negative SCA effect exhibited by these diverge crosses may be due to contribution of favorable alleles by their parents. Singh and Naryanan (1993) stated that unrelated inbreds from different open-pollinated varieties will generally combine to produce high yielding early flowering single crosses than inbreds derived from related parents, which may have more of the same genes in common. This might be the case with this result.

For Seed yield kg/ha and the yield related characters viz 1000- seed weight and the yield per plant, significant positive SCA effects were observed by Kenana-2 X Gd 002SPSN.12 and Promo X 002SPSN.12, whereas, negative significant effects were showed by Gadarif-1 X Umshagera. The rest of the crosses combinations were inconsistent across the seasons, some of them recorded positive value in one season and negative values in another one. From the results of this study it could be concluded that both additive and non- additive gene action were important for improving seed yield in sesame, Khidir and Promo recorded a positive significant general combining ability (GCA) effects in the first and the second season, moreover, Promo was the best combiner with other parental lines for earliness science it recorded negative SCA values. Therefore Khidir and Promo could be recommended to produce progeny having high yield and early maturing hybrids.

Conflict of Interest

The authors have not declared any conflict of interest.

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Vol. 6(12), pp. 185-193, December 2014
DOI: 10.5897/JPBCS2014.0455
Article Number: 3697A0348120
ISSN 2006-9758

http://www.academicjournals.org/IPBCS

Journal of Plant Breeding and Crop Science

Full Length Research Paper

Genetic diversity and adaptation to different tillage and farming systems of cocoyam genotypes (*Xanthosoma sagittifolium* L. Schott) in the Eastern Region of Ghana

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Received 4 April, 2014; Accepted 16 September, 2014

Experiments conducted in Ghana show that cocoyam exists as mixtures of clones in farmers farms. This work aimed to use RAPD markers to determine the extent of diversity in cocoyam genotypes collected from farms at different locations in the Eastern region of Ghana. The study also investigated whether the genotypes have different adaptation to different farming systems (intercropping with plantain and sole cropping) and tillage methods (mounds and flat). The genotypes were grouped into two main clusters at 0.65 similarity coefficient of variation with accessions Pameng Red 3 and Pramkese 2 being the most diverse. The genotypes began separating at 85% similarity index into three discrete groups. Group I, (Pameng 1, Dwenase 2 and 3) did not separate at 100% similarity index. The other two groups consisted of (Pameng 2, Gyampomani 1, Gyampomani 2, Dwenase 1) and (Pramkesse 1 and Gyampomani 3). The analysis of variance of the growth parameters of the genotypes under the tillage and farming systems revealed significant differences. Generally, genotypes in group II grew better under the farming systems and tillage practices studied whiles Pramkesse 2, which did not cluster with any other genotypes in its major cluster, grew poorly under the two farming systems.

Key words: Cocoyam, intercropping, solecropping, mounds, flat, RAPDS.

INTRODUCTION

In Ghana, farmers can identify at least three varieties of cocoyam on the basis of cormel skin colour as follows; mankani-pa, with red skin colour, mankani-fitaa, with white skin colour, mankani-serwaa, with pale skin colour (Karikari, 1971).

Cocoyam contributes significantly to the national food baskets. The FAO estimated that Ghana produced 1,063 tonnes of cocoyam representing about 18% of total world's production (Onwueme and Sinha, 1991). Today the demand for cocoyams has increased both in Ghana and other parts of the world. In Ghana, the high demand is brought about by the establishment of agro-processing companies which use cocoyam as raw material, and other exporters who export chopped cocoyam leaves to Europe.

In spite its importance as a staple food in many countries,

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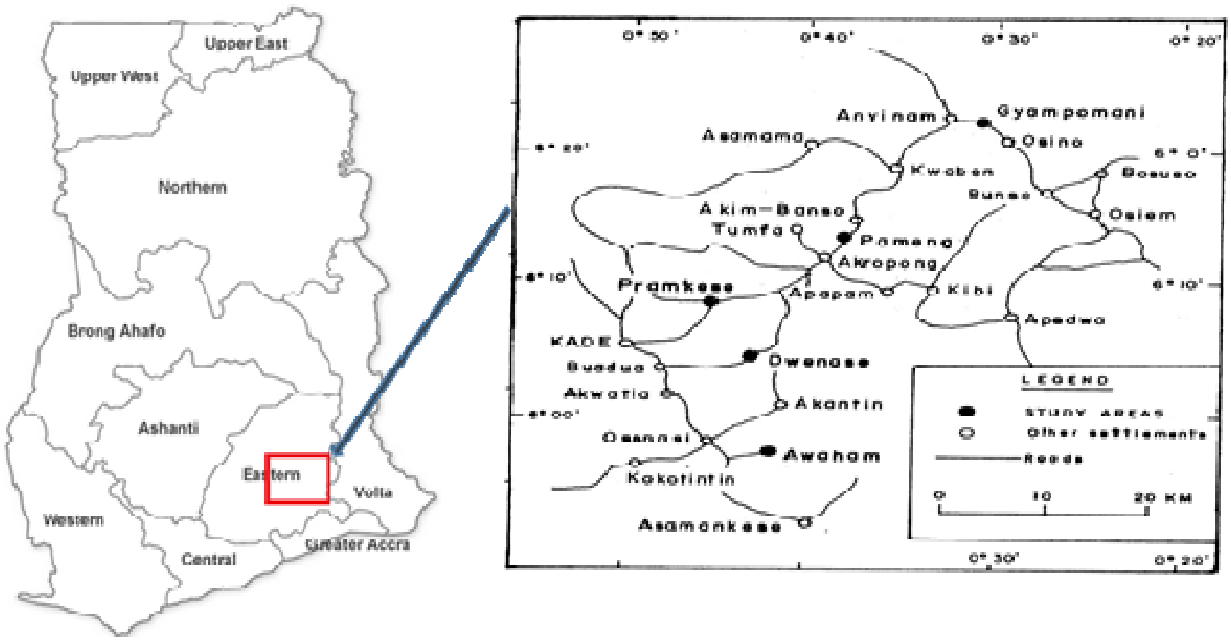

Figure 1. A map showing location of the study areas.

cocoyam has received very little research attention (Goenaga and Hepperly, 1990), and is regarded as an under-exploited and insufficiently studied crop (Nguyen and Nguyen, 1987; Giacometti and León, 1994; Watanabe, 2002).

Previous studies using microsett derived plants from 16 genotypes by Osei and Mintah (2002) indicated that differences exist in growth and yield of different cocoyam genotypes indicating that cocoyam exists as mixtures of clones in farmers' farms.

Hence, there exists a need to assess the extent of genetic diversity to determine these differences.

DNA based markers have become methods of choice in genetic diversity studies, as they analyse variation at DNA level. This excludes all environmental influences and time specificity, since analysis can be performed at any growth stage using any plant part and requires only small amounts of material (Mueller and Wolfenbarger, 1999; Rao, 2004).

Tillage method is considered one of the major factors for increasing the yield of cocoyam on a tuber yield per unit area basis (Ennin et al., 2009). Soils are tilled to create a soil environment favourable for plant growth and development. In general, root and tuber crops do not produce satisfactory yields on compacted or shallow soils (Ennin et al., 2009).

Driven by land economy, most peasant cocoyam farmers in Ghana practice intercropping by utilizing the space under the tree crop canopy for the cultivation of the cocoyam. For this reason, most of the cocoyams are grown under canopies of crops such as cocoa, oil palm and plantains. However few studies have been done to ascertain the agronomic and physiological implications of such intercropping to determine if some cocoyam genotypes are more sensitive to intercropping than others and, if so, could this be a guide in choosing genotypes or cultivars to grow under conditions of low light intensity.

The purpose of this study was:

1) To determine the extent of diversity in cocoyam genotypes collected from different locations in the Eastern region of Ghana.
2) To determine whether the genotypes have different adaptation to different farming systems (intercropping with plantain and sole cropping) and tillage methods (mounds and flat).

MATERIALS AND METHODS

Diversity studies and experimental material

The experiment was carried out at the Biotechnology Centre, College of Agriculture and Consumer Sciences, University of Ghana. Eleven cocoyam genotypes from five towns in the Eastern region of Ghana were used for the experiment. The towns were; Dweanase, Pramkesse, Gyampomani, Awaham and Pameng (Figure 1). The genotypes for the study were labelled as; Pameng 1 and 2, Dwenase 1, 2 and 3, Pramkese 1 and 2, Gyampomani 1, 2 and 3 and Pameng Red 3.

DNA extraction

The young or tender leaves of each genotype were harvested, kept on ice and taken to the laboratory for total DNA extraction. Total DNA was extracted from the leaf tissues using the GenElute™

Table 1. RAPDs (Operon F-series 10-mer) primers.

Primer	Sequence (5'-3')
OPF-08	GGGATATCGG
OPF-09	CCAAGTCTTC
OPF-13	GGCTGCAGAA
OPF-16	GGAGTCTGG
OPF-19	CCTCTAGACC
OPF-20	GGTCTAGAGG
OPF-10	GGAAGCTTGG
RAPD-DCA:OPF-08	GGGATA

Table 2. PCR programme conditions for the DNA amplification.

Programme	Number of cycles	Steps	Temperature (°C)	Hold time (s)
Denaturation	1	1	95	360
Denaturation	45	1(denature)	95	30
Denaturation	45	2(anneal)	35	30
	45	3(extension)	72	60
		4(final extension)	72	600
		Hold step	4	~ ∞

Plant Genomic DNA Miniprep Kit and stored in a freezer at -20°C for subsequent use.

DNA amplification

A modified protocol was used for DNA amplification, using eight selected RAPD primers (Williams et al., 1990). The (full) list of the eight selected RAPD primers and their respective sequences is presented in Table 1. The amplification mixture contained 1.5 uL PCR buffer, 1uL $MgCl_2$, 0.5 uL dNTP, 2µl primer, 0.5 uL *Taq* polymerase, and 1.5 uL template DNA in sterile de-ionized water. Conditions for the DNA amplification were as stated in Table 2.

Gel electrophoresis and PCR products

The resulting amplicons (amplification products) were taken through gel electrophoresis using 2% agarose gel (molecular biology grade) prepared using 1X TAE (Tris-Acetate EDTA) buffer and stained with ethidium bromide. 7 µl of amplicons were loaded into the wells generated in the agarose gel and run alongside 10 µl of standard molecular weight DNA markers at a constant voltage of 60 V for 21/2 h for all reactions. The products were visualized under UV light in 2% agarose gel stained with ethidium bromide.

Scoring and data analysis

The resulting bands after electrophoresis were scored as binary data with the help of Microsoft Office Excel® indicating the presence of bands as 1 and the absence of bands as 0. A generalized dendrogram was then drawn from the scored bands for analysis using GenStat® computer software, 9th edition.

Evaluation of genotypes under different tillage and farming systems

Experimental site and source of planting materials

The experiment was conducted at the University of Ghana Agricultural Research Centre, Kade in the Eastern Region. Microsett-derived planting materials of the cocoyam genotypes collected from five towns in the Eastern Region of Ghana were used for the study. Ten of the 11 genotypes used for the diversity study were used in the field evaluation. Pameng Red 3 was excluded from the field evaluation because it was collected late.

Field establishment and experimental design

Three months old split-corm derived suckers of local plantain cultivar were planted at a spacing of 3 m x 3 m for the plantain cocoyam intercrop system.

One month after planting the plantain suckers, two months - old microsett-derived planting materials of the different cocoyam genotypes were transplanted at a spacing of 1 m x 1 m in the sole and intercrop systems. The cocoyams were planted 0.5 m away from the plantains.

The experimental design was a split, split plot with the farming systems (sole and intercropping) as the main plot, the tillage system (planting on flat and mounds) as the subplot, and the genotypes as the sub sub plots. Each treatment was replicated three times.

Cultural practices

Compound fertilizer (NPK, 15-15-15) was applied at a rate of 100 g and 200 g per plant to cocoyam and plantain respectively. Watering and weeding were done whenever necessary.

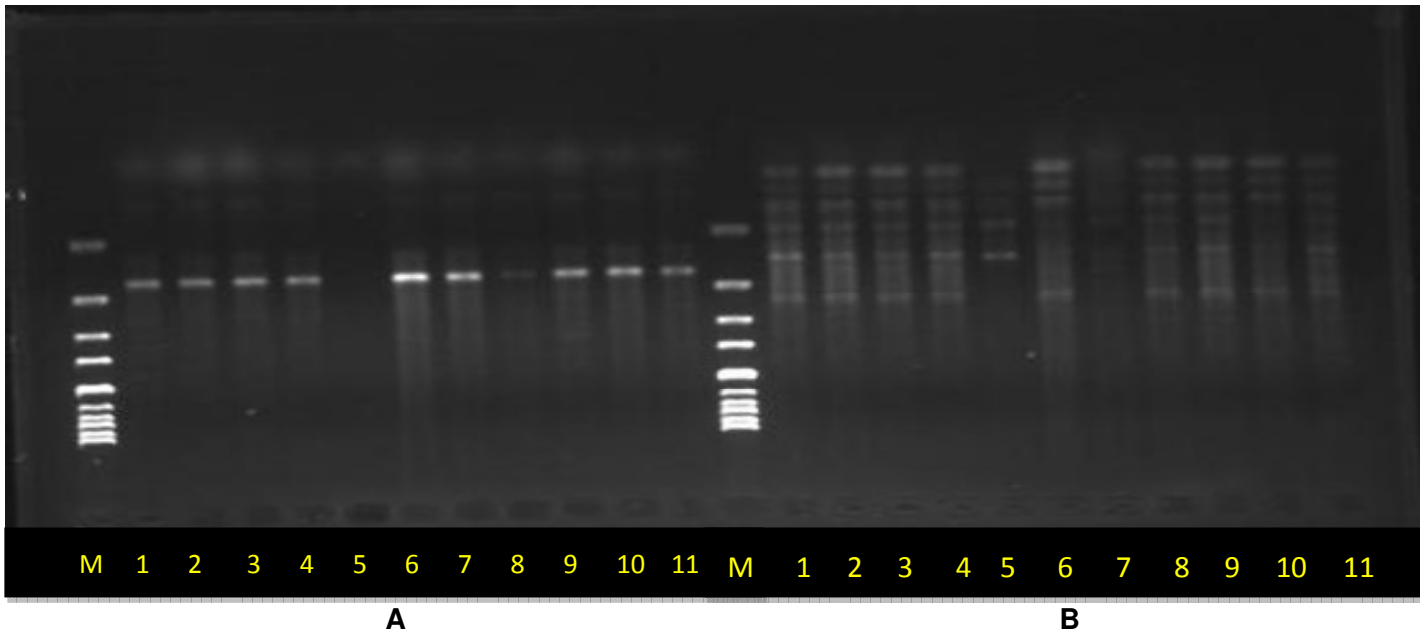

Figure 2. Cocoyam DNA Fingerprints: Amplified DNA bands of eleven cocoyam genotypes obtained after agarose gel electrophoresis of PCR products using primers (A) OPF-19 and (B) OPF-10. *1 = Pramkese 2; 2 = Dwenase 2; 3 = Dwenase 3; 4 = Pameng 1; 5 = Pameng red 3; 6 = Pameng 2; 7 = Pramkese 1; 8 = Gyampomani 3; 9 = Gyampomani 2; 10 = Dwenase 1; 11 = Gyampomani 1.*

Data collection (Growth parameters)

Growth parameters were measured once a month on ten plants per genotype. Data were collected from all ten plants located in the rows of each plot. The parameters evaluated were; plant height, number of leaves, plant girth**,** yield and leaf area. Genstat Discovery Edition 4 was used for the data analysis.

RESULTS

Genetic diversity of eleven cocoyam genotypes

Figure 2 shows the bands of Amplified DNA obtained after agarose gel electrophoresis of PCR products using primers OPF-19 and OPF-10 and total DNA from the eleven cocoyam genotypes. Similar results were obtained with other primers used in the study, except primer DCA-OPF-08 which produced no amplification products.

Cluster analysis

The cluster analysis based on RAPDs from seven primers (Table 2) grouped the genotypes into 2 major clusters. Major cluster 1 contained only Pameng Red 3 at 65% similarity index while major cluster 2 comprised all the other genotypes (Pramkesse 1 and 2, Pameng 1 and 2, Gyampomani 1,2 and 3, Dwenase 1,2 and 3). However major cluster 2 further separated into 2 sub clusters. Sub cluster 1 consisted of only Pramkesse 2 which is named group IV throughout the write up. Sub cluster 2 contained Pameng 1 and 2, Gyampomani 1, 2 and 3, Dwenase 1, 2 and 3. The genotypes in sub cluster 2) began separating at 85% similarity index into three discrete groups. One such group contained (Pameng 1, Dwenase 2 and 3,) which did not separate at 100% similarity index and is named as group I in the write up. The other two groups consisted of Pameng 2, Gyampomani 1 and 2, Dwenase 1 also named group II and Pramkesse 1 and Gyampomani 3 in the other (Group III) (Figure 3).

Effect of two farming systems and two tillage practices on the growth parameters of ten cocoyam genotypes

The results of the growth measurements of the different cocoyam genotypes indicated moderate levels of variability among the genotypes, and also due to their interactions with farming systems and tillage practices.

There were significant ($P<0.05$) differences in all the growth measurements due to genotypes, farming systems, tillage and the interactions except for the interactions between farming systems and tillage for plant height and girth.

Averagely genotypes in cluster II had superior growth than genotypes in clusters I and III in most of the growth parameters measured. Pramkesse 2 in cluster IV grew poorly for almost all parameters measured when compared with the other genotypes.

Generally most genotypes grew better under intercropping than solecropping for most of the parameters

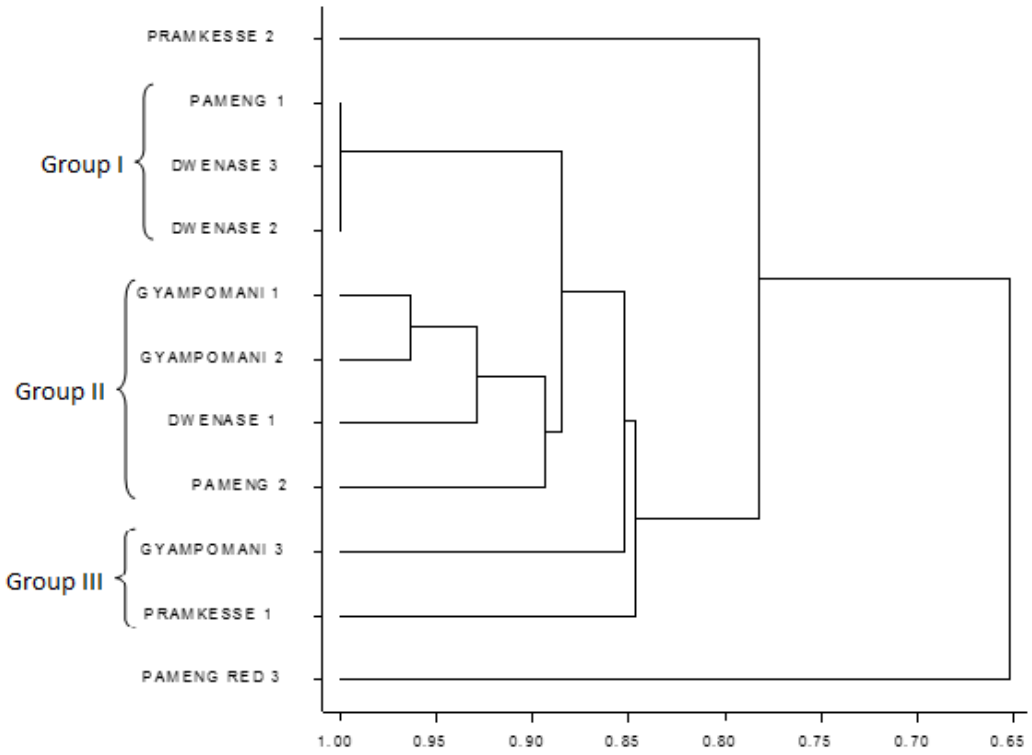

Figure 3. UPMGA cluster analysis for eleven cocoyam accessions: The neighbor-joining analyses revealed close genetic similarities between the cocoyam accessions.

Table 3. Effect of two farming systems (intercrop and sole cropping) and two tillage practices (mounds and flat) on the mean cormel fresh weights (kg/ha) of ten cocoyam genotypes at harvest.

Group No.	Genotype	Intercropping		Sole cropping		Genotypic means
		Flat	Mound	Flat	Mound	
I	PAM 1	6845	11135	5982	6330	7573
	DWEN 3	7120	8670	7035	5155	6995
	DWEN 2	12680	6090	4770	9280	8205
Mean		**8881.7**	**8631.7**	**5929**	**6921.7**	**7591**
II	GYAM 1	7600	8690	9322	7002	8153.5
	GYAM 2	8605	2535	3700	5725	5141.3
	PAM 2	2480	9185	11650	11600	8728.8
	DWEN 1	13500	6040	6728	6080	8087
Mean		**8046.3**	**6612.5**	**7850**	**7601.8**	**7527.7**
III	GYAM 3	4565	5485	5952	6248	5562.5
	PRAM 1	3822	4150	4655	4765	4348
Mean		**4193.5**	**4817.5**	**5303.5**	**5506.5**	**4955.3**
IV	PRAM 2	6690	220	4828	3500	3809.5

s.e.d. of interactions=1902.3 s.e.d. of genotypes=986.4.

measured. Genotypes in cluster II grew highest in plant height, girth, number of leaves, leaf area and cormel fresh weights under intercropping. Genotypes in cluster I followed next in superior growth after genotypes in

cluster II except for number of cormels in which genotypes in cluster I yielded more cormels than genotypes in cluster II on solecropping. However Pramkesse 2 which did not closely cluster with any genotype was more adapted to sole cropping than intercropping for most of the parameters measured. Significant differences were not obtained for interactions between the two farming systems, two tillage practices and farming systems and tillage practices for the mean fresh weight of cormels per hectare after analysis. However, there were significant differences between the ten genotypes as well as interactions between the genotypes, farming systems and tillage practices. The average genotypes in groups I and II produced higher fresh cormel weights per hectare than those in groups III and IV (Table 3). Fresh weights of cormels were higher under intercropping (8881.7 kg, 8631.7 kg) on the flat and on mounds for genotypes in group I than under sole cropping (5929 kg, 6921.7 kg) per hectare respectively. However genotypes in group II produced the highest fresh cormel weights per hectare under sole cropping than under intercropping. Pramkesse 2 in group IV yielded poorly in cormel fresh weights under intercropping particularly on mounds (Table 3). The cormel yield of Prankesse 2 was however better on the flat than on the mound per hectare.

Genotypes (B,C,F,G,H,I) recorded higher cormel fresh weights per plant under intercropping than on sole cropping. However, higher number of cormels per plant were generally recorded in sole cropping than in intercropping (Figure 4).

The genotypes in addition grew better on mounds than on the flat for most parameters with the exception of fresh weight of cormels per plant in which only four (A,D,G,J) out of the ten genotypes grew better on mounds than on flat land.

DISCUSSION

Genetic diversity assessment of eleven cocoyam genotypes collected from five towns in the Eastern region of Ghana

RAPD analysis

Seven out of the eight RAPD primers used in the PCR reactions produced amplification with the DNA of the eleven genotypes collected. Primer DCA-OPF-08 (sequence) did not produce amplification with the DNA of any of the cocoyam genotypes used in this study. This is probably due to the fact that the primer sequence (5'GGGATA3') has no homology with the cocoyam genome or it might be due to manufacturing error.

It is significant to note that the genotypes did not cluster according to their distinct towns of collection. This implies that there has been a significant flow of cocoyam germplasm between the five towns in the Eastern region where the genotypes were collected. The genotypes Pameng 1, Dwenase 2 and 3 were similar at 100% similarity index indicating that these genotypes are probably duplicates grown at different locations. They could have originated in localities different from where they were collected. This suggests that cocoyam genotypes may have been transported between localities as a result of the normal farmer to farmer exchange of planting materials. This exchange of genetic material may have been enhanced by the closeness of the five towns to each other. The clustering of the eleven genotypes into different groups may be due to genetic divergence of cocoyams over the two hundred years since its introduction to Ghana, and to re-introductions or occasional hybridization between clones and thus the crop exists as mixtures of clones in farmers' field. The result of this study is a useful guide in selecting cocoyam germplasm for breeding and conservation. Pameng Red 3 which was the most diverse among all the accessions may have some distinct agronomic characters. It therefore requires further evaluation in the field.

The genetic diversity of cocoyam observed in this work is in agreement with Offei et al. (2004) who used 10 random primers to study the genetic diversity and structure of seventy cocoyam accessions collected in the Eastern and Volta regions of Ghana. The 70 accessions did not cluster into their distinct geographical regions suggesting that there may have been movement of germplasm across the two regions.

Effect of two farming systems (intercropping and sole cropping) on growth components of ten cocoyam genotypes

Most genotypes which grew well under intercropping for plant height, girth, number of leaves and leaf area could be attributed to moisture conservation under intercropping since the plantains provided an amount of shade to the cocoyam therefore reducing the amount of evaporation. This observation agrees with the findings of Goenaga and Chardon (1993) that cocoyam requires moisture throughout its growing season (9 to 12 months).

The high litter fall from both the cocoyam and plantain plants and the activities of soil organisms as a result of the cool environment under the system, maintained soil fertility and this all led to most of the genotypes under intercropping growing superiorly than corresponding genotypes under sole cropping for most of the parameters measured. This observation is also in agreement with Karikari (1971) and Giacometti and León (1994) that cocoyam responds well to organic and chemical fertilization. In fertile soils the crop develops healthy leaves and produces higher yields. Schaffer and O'Hair (1987) also reported that leaves of cocoyam grown under moderate shade appear to be more

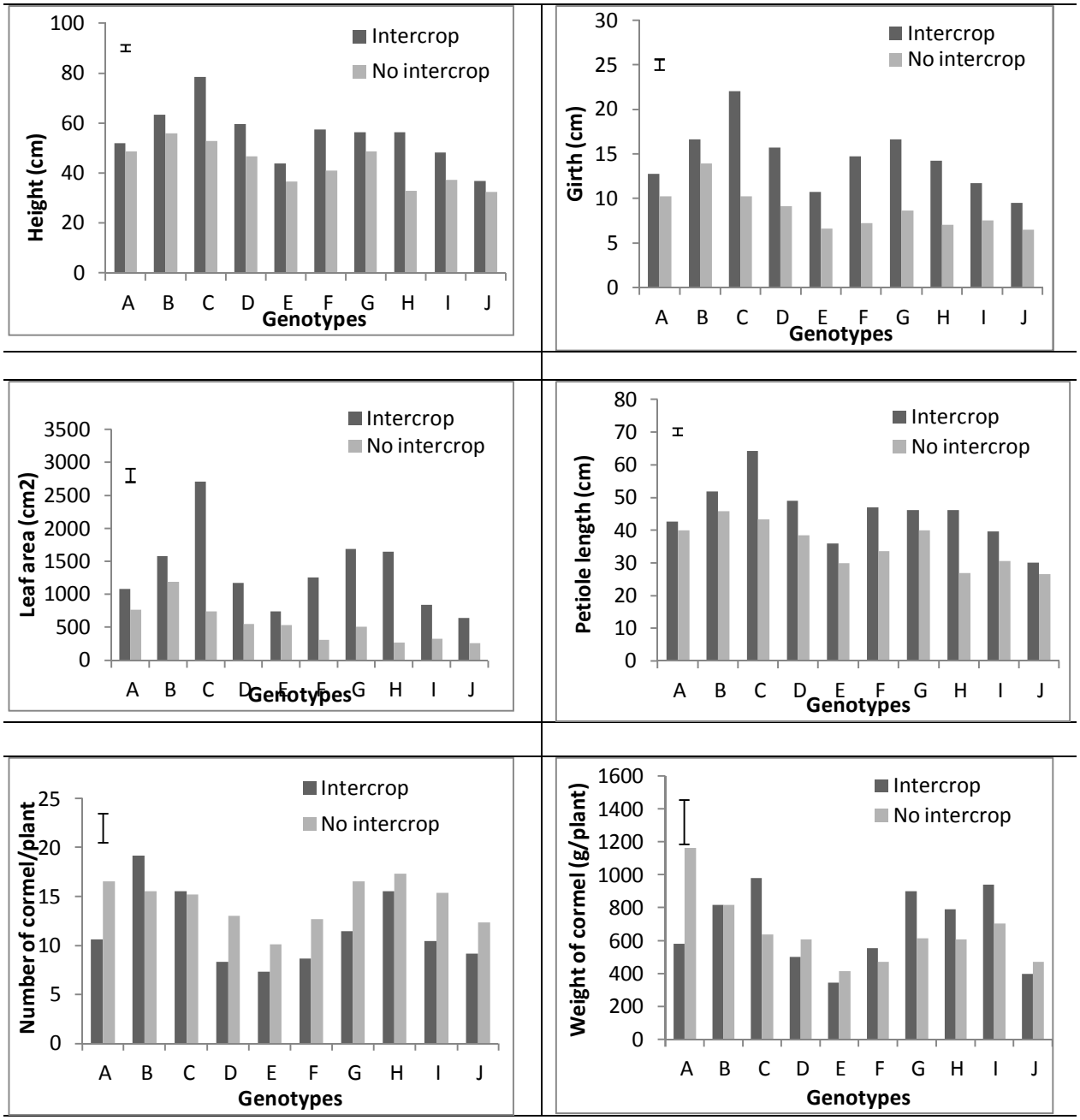

Figure 4. Effect of farming systems (intercrop and no intercrop) on growth and yield of cocoyam genotypes *A-Pameng 2, B-Gyampomani 1, C-Dwenase 1, D-Gyampomani 3, E-Pramkesse 2, F-Gyampomani 2, G-Pameng 1, H-Dwenase 3, I-Dwenase 2, J-Pramkesse 1.*

photosynthetically efficient than leaves grown in full sun. Therefore planting cocoyam as an understory crop in mixed cropping systems may maximize their photosynthetic efficiency. However for number of cormels per plant and cormel fresh weight per hectare of genotypes in group III, most genotypes were more adapted to full exposure or sole cropping than intercropping and this might be due to the different genetic compositions of the genotypes and also competitions between the two intercrops. This indicates that the same farming systems cannot be used for all cocoyam genotypes for optimum growth. This also agrees with the findings of Onwueme and Charles (1994), that yield of cocoyam varies from place to place, depending on the cultivation methods and the environmental conditions.

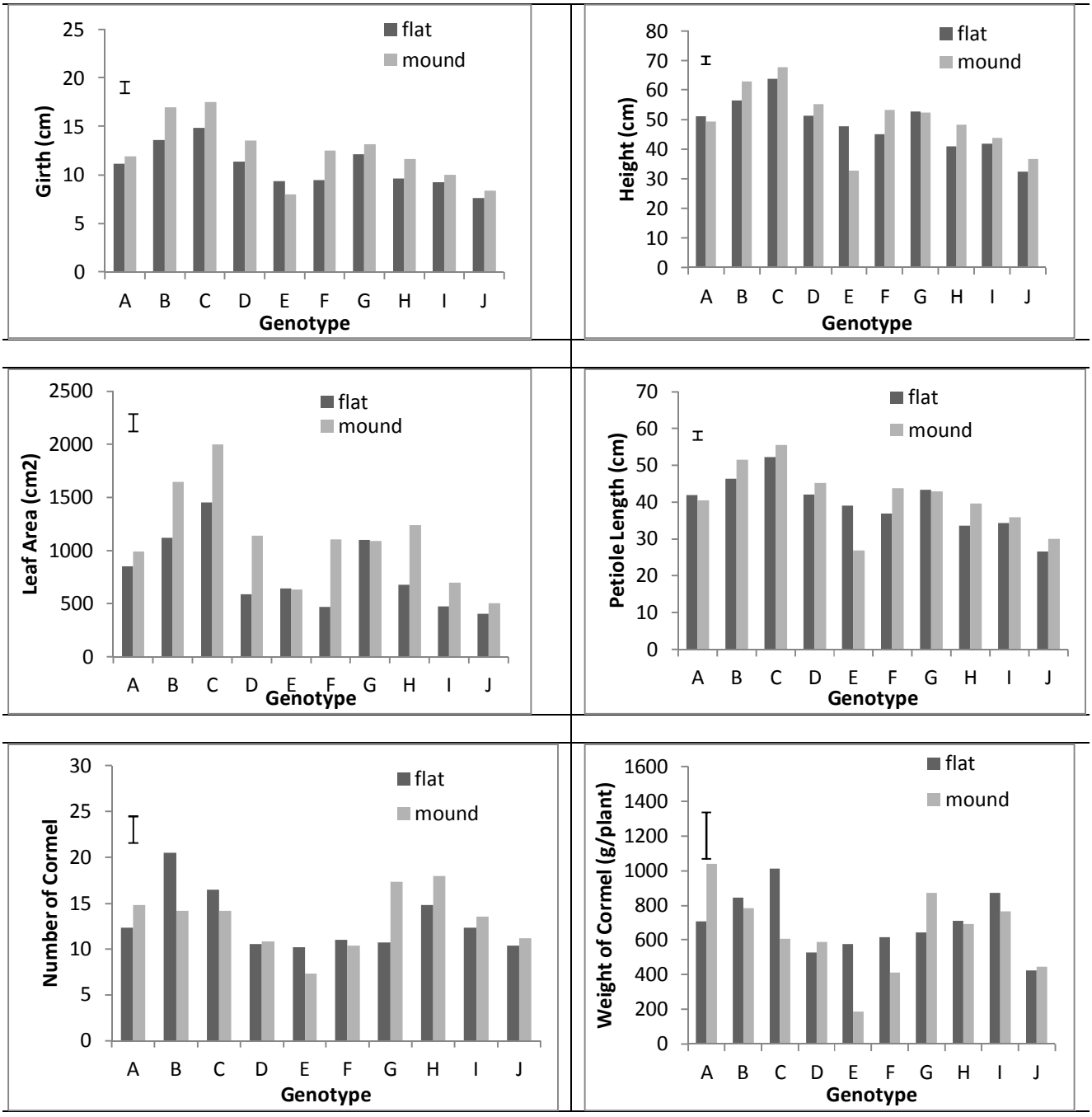

Figure 5. Effect of tillage methods (flat and mound) on growth and yield of cocoyam genotypes. *A-Pameng 2, B-Gyampomani 1, C-Dwenase 1, D-Gyampomani 3, E-Pramkesse 2, F-Gyampomani 2, G-Pameng 1, H-Dwenase 3, I-Dwenase 2, J-Pramkesse 1.*

Effect of two tillage practices (planting on mounds and flat) on growth components of ten cocoyam genotypes

Growing cocoyam on mounds resulted in increased growth in all the growth components measured compared to growing on flat land (Figure 5).

These results may probably be due to the loose nature of soils associated with mounding which enhanced infiltration of water and air, easy penetration of roots and also improved soil water management. This results is comparable to that found by Adekiya et al. (2009) who compared five tillage methods and their effects on growth and yield of cocoyam in the forest savannah transition zone of South West Nigeria and found out that manual mounding produced satisfactory results in mounding.

Conclusions

The seven RAPD markers used in the experiment suggested moderate to low levels of genetic variation (0.65 to 1.00 genetic similarity) among the eleven cocoyam genotypes sampled from the five towns in the Eastern region of Ghana. Clustering was not based on agro-ecological zones but rather dependent on inherent genetic variability. This means cocoyam accessions may have been transported between localities at random as a result of the normal farmer to farmer diffusion of planting materials. This may have been enhanced by the closeness of the five towns to each other.

The cocoyam genotypes showed genotypic differences for most of the growth parameters studied. Genotypes in groups I and II were generally high yielding and were morphologically superior to the other genotypes in the different groups. Genotypes in group III recorded moderate yield and morphological values. However the distantly related genotype Pramkese 2 in group IV in general recorded moderate to low values for most of the parameters that were observed. This suggests that the yield potential of cocoyam genotypes may be deduced from their morphology.

The cocoyam genotypes also showed different adaptations to the two farming systems and two tillage practices. For instance the distantly related genotype, Pramkesse 2 was more adapted to sole cropping and on flat than intercropping on mounds for most of the parameters measured.

This means that to produce optimum yields, different cultural practices maybe required for different cocoyam genotypes.

Conflict of Interest

The authors have not declared any conflict of interest.

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Vol. 6(12), pp. 194-199, December 2014
DOI: 10.5897/JPBCS2014.0458
Article Number: 01F51C448123
ISSN 2006-9758

http://www.academicjournals.org/IPBCS

Journal of Plant Breeding and Crop Science

Full Length Research Paper

Genetic diversity in Tepary bean (*Phaseolus acutifolius*) landraces grown in Botswana

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Received 15 April, 2014; Accepted 1 September, 2014

A field experiment was conducted at Department of Agricultural Research in Sebele in the 2012 to 2013 season using nine accessions that were sourced from the National Plant Genetic Resource Centre (NPGRC), Gaborone, Botswana. Multivariate statistical procedures such as clusters and principal component analysis were used on 15 selected characters to assess agro-morphological variability among tepary bean landraces collected in Botswana. Few characters were statistically significant which suggest lower genetic diversity among the Botswana tepary beans. The first three PCA accounted for 77.12% of accumulated variation. Traits which revealed significant contribution to variation among accessions were number of leaves, plant spread, pod width, 100 seed weight and seeds per pod. The dendrogram results also showed that these characters contributed significantly to the grouping of accessions into three clusters. Three accessions GK011, MTS (Motsumi) and GK012 were separated from the rest of the accessions. However, GK012 and MTS (Motsumi) with highest number of valuable traits are recommended for plant breeders to use as parents in future breeding programs.

Key words: Tepary bean, agro-morphological traits, dendrogram, principal components analysis, multivariate analysis.

INTRODUCTION

The cultivated tepary bean (*Phaseolus acutifolius* A. Gray) is a short life cycle legume originally from the deserts and semi-arid environment of northwestern Mexico and southwestern United States (Nabham and Felger, 1978). It is recognized for its resistance to heat, drought and many diseases (Salgado et al., 1994; Miklas and Stavely, 1998; Rao et al., 2013). These characteristics make it an ideal crop in parts of tropical America, the Caribbean and Africa (Porch et al., 2013) equally so important for Botswana with a semi-arid environment. The crop has no established varieties in Botswana therefore farmers are using landraces which are usually low yielding. Since few famers are involved in planting tepary bean, the development of new varieties could potentially encourage the growing of this crop. The crop is grown in Africa and Middle Eastern countries (Tinsley et al., 1985) where the seeds provide high protein good for human nutrition.

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Table 1. Tepary bean landraces collected from different villages from two agricultural regions of Botswana.

Entry	Accession	Collection sites	Lat.	Long.	District
1	MTS (Motsumi)	Mahalapye	23.108	26.823	Central
2	GK010	Machaneng	23.185	27.483	Central
3	GK012	Sefophe	22.182	27.961	Central
4	E70	Tutume	20.493	27.018	Central
5	GK011	Kubung	24.649	25.303	Kweneng
6	E105	Machaneng	23.185	27.487	Central
7	GK013	Thamaga	24.678	25.531	Kweneng
8	E89	Kgope	24.310	25.940	Kweneng
9	E19	Mahetlwe	24.242	25.684	Kweneng

In Botswana it is commonly known as 'Dibonkise'. It is grown by small scale farmers mainly as a **s**ource of food while the haulms are used as feed for animals. A brief survey by Karikari et al. (1995) in Botswana discovered that its production is lower than that of cowpeas, groundnut and bambara groundnut. Its relatively high protein content recorded at 24% compares well with other *Phaseolus* spp. (Bhardwjal and Hamama, 2004). Tepary beans together with other three underutilized legumes (bambara groundnut, morama bean and mungbean) were assessed for protein and mineral composition. It was found that they are a good source of protein with great potential as food crops which could contribute to improving food security in Botswana (Amarteifio and Moholo, 1998). In addition it is a high value crop as it equally fetches money such as other grain legumes through the Botswana Agricultural Marketing Board (BAMB, 2013).

Tepary bean possesses considerable variability for yield and yield related traits (Kuruvad and Valdez, 1993; Bhardwj et al., 2002) and is superior in drought tolerance (Mohamed et al., 2005). Compared to common bean, tepary bean was shown to be superior in combining desirable traits that makes it well adapted to drought stress (Markhart, 1985). Tepary bean is a useful genetic donor of important traits such as disease, pest and stress tolerance to improve common beans (*P. vulgaris*) (Schinkel and Gepts, 1988). Little research has been conducted in Botswana environment to ascertain all the useful characteristics of tepary bean crop.

In the US, at least two varieties (TARS-Tep 22 and TARS-Tep 32) have been developed and are available to farmers especially in the production zones prone to abiotic and biotic stress (Porch et al., 2013). In Botswana the crop has not received much attention compared to other legumes probably due to low number of farmers growing the crop. Knowledge of phenotypic diversity of tepary bean accessions grown can be employed in crop improvement and in developing breeding lines (Mohammadi and Prasanna, 2003). In order to improve the use of tepary bean, it is necessary to gain an understanding of its genetic attributes (Schinkel and Gepts, 1988). Lack of information on the genetic diversity of tepary bean has led to poor exploitation of its genetic resources. Therefore the objectives of the project is to study the morphological variability of Botswana tepary bean landraces in order to generate additional information to improve their utilization, and to identify accessions with potential to be exploited by plant breeders.

MATERIALS AND METHODS

The experimental materials for this study comprised nine tepary bean (*P. acutifolius*) accessions that were sourced from the Botswana National Plant Genetic Resource Centre (NPGRC) and originally from nine different villages and two agricultural regions of the country (Table 1). Fifteen agro-morphological characters were used to assess the variability of the accessions: Plant height (PH), number of leaves (NL), leaflet width (LW), leaflet length (LL), plant spread (PS), number of branches (NB), pods per plant (PPP), pod length (PL), pod width (PW), seeds per pod (SPP), pod weight per plant (PWP), seeds per plant (SPP), 100 seed weight (100SW), shoot dry weight (SDW) and yield per m^2 (YIELD) (Table 2). The morphological and agronomic traits selected were chosen from International Board for Plant Genetic Resources, IBPGR (1985) for *P. acutifolius*. Similar traits were considered important for common bean breeding programs (de Lima et al., 2012). All accessions examined were of cream coloured seeds. One of the accessions (GK010) in this project had been sent to Vienna-Austria for mutation experiments.

The experiment was laid out in a randomized complete block design using two replications in the 2012 to 2013 cropping seasons at Sebele Agricultural Research Station. The accessions were sown by hand at a spacing of 75 cm between rows and 30 cm between plants and plot length of 5 m. No fertilizers were applied but, the crops were sprinkler irrigated once a week to ensure proper plant growth. In each accession five representative plants were selected randomly and used for biometric measurements. The agro-morphological mean data were standardized to give equal weighing. The values were used to perform multivariate statistical analysis, using Multivariate Statistical Package (MVSP) software (Kovach Computing Services, UK, 2006) and NTSYS-pc Numerical

Table 2. Mean, range and variance of nine tepary bean accessions assessed based on 15 morpho-agronomic characters.

Accession	PH (mm)	NL	LW (mm)	LL (mm)	PS (mm)	NB	PPP(g)	PL (mm)	PW (g)	SPP (g)	PWP(g)	SPL	100SW(g)	SDW (g)	YIELD (g)
MTS(Motsumi)	350	82	14	37	425	7	38	60	7	5	28	135	11.0	1.1	0.40
GK010	317	77	14	37	356	6	40	61	8	4	24	139	15.5	1.8	0.47
GK012	353	96	15	39	437	7	38	62	8	4	23	130	13.0	2.0	0.77
E70	288	82	13	38	384	7	40	63	8	4	23	115	17.5	1.6	0.42
GK011	261	45	13	36	236	5	17	62	7	4	13	65	14.0	0.6	0.41
E105	281	74	14	38	341	6	28	61	8	4	18	95	11.5	2.2	0.52
GK013	274	74	14	35	277	6	46	61	8	4	23	131	12.5	1.7	0.48
E89	288	69	14	36	299	7	42	63	8	5	20	120	13.0	1.6	0.53
E19	290	63	15	36	305	7	36	61	8	5	24	153	13.0	1.8	0.52
Mean	299	73	14	37	340	6	36	62	8	4	22	120	13.4	1.6	0.51
Minimum	212	44	12	33	160	5	13	57	7	4	10	49	11.0	0.6	0.33
Maximum	421	121	16	44	557	8	58	66	8	5	36	189	19.0	2.8	0.77

Plant height: **(PH),** No. of leaves : **(NL)**, Leaflet width: **(LW),** Leaflet length: **(LL),** Plant spread: **(PS),** No. of branches: **(NB)**, Pods per plant: **(PPP),** Pod length: **(PL),** Pod width: **(PW),** Seeds per pod **(SPP),** Pod weight per plant: **(PWP),** Seeds per plant: **(SPL),** 100 seed weight: **(100SW),** Shoot dry weight: **(SDW),** Yield m^2 : **(YIELD)**

Taxonomy and Multivariate Analysis (Rohlf, 2000). Analysis of Variance (ANOVA) was estimated to calculate the differences on traits using SAS 9.2 (2010) statistical package.

RESULTS

A summary of the results for the mean, range and variances for the 15 characters are presented in Table 2. Number of branches per plant, 100 seed weight and number of seeds per pod were significant at $P < 0.05\%$ probability level while the rest of the characters were not significant. This is an indication of low genetic variability among the traits analyzed for the selected accessions. However, large ranges were observed among some traits, such as in yield m^2 (48 to 254 g), number of seeds per plant (49 to 189), number of leaves per plant (43 to 121) and pod weight per plant (10 to 36 g).

The results presented in Table 2, revealed that accessions GK012 had highest plant height (353 mm), number of leaves (96), plant spread (437 mm) and yield m^2 (178 g). Accession GK011 had the lowest plant height (261 mm), number of leaves (45), number of pods per plant (17), number of seeds per plant (65) and yield per m^2 (74 g) and exhibited a dwarf plant character. Based on cluster analysis (Figure 1) at a demarcated line of coefficient 0.97 the accessions were grouped into three clusters. Cluster 1 and 3 consists of single accessions MTS (Motsumi) and GK011 respectively. The rest of the accessions are grouped in cluster 2, but accession GK012 is separated from the rest of these accessions. The selected traits were not able to distinguish between (E89 and E19) and between (GK010 and E70). A higher genetic difference of 0.69 was observed between the accessions (E89 and E19) at 0.55 and 1.24 of GK011. However, generally a lower difference among most of the accessions was recorded (Figure 1). The dendrogram indicates that the population is mainly influenced by the characters with greater variability.

The Principal component analysis was performed to reveal the phenotypic diversity among the genotypes to identify characters that account for most of the variances. The first three principal components gave an accumulated total variation of 77.12% (Table 3). Axis 1 with 37.89% variability

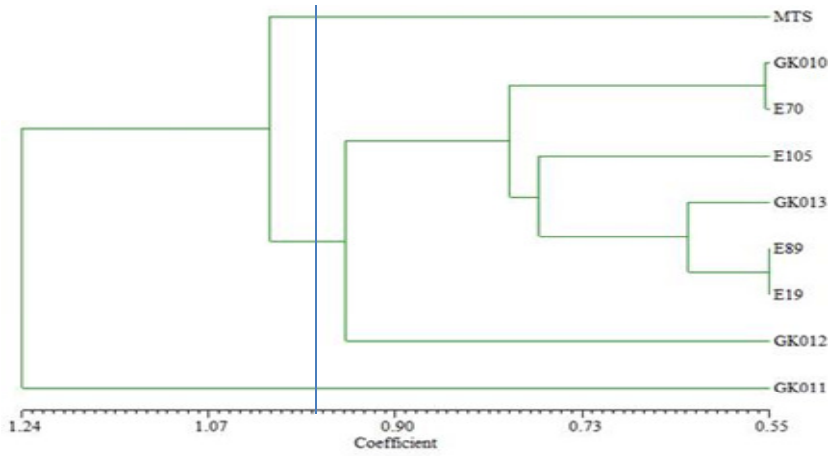

Figure 1. Dendrogram of nine tepary bean accessions showing genetic similarities based on 15 phenotypic traits, using the UPGMA cluster analysis. The test of association: Matrix correlation on NTSYS pc: (r = 0.88).

Table 3. Eigen values and the first three principal component axes in tepary bean diversity analysis.

Parameter	Axis 1	Axis 2	Axis 3
Plant height	0.278	-0.112	0.038
No. of leaves	0.347	0.078	0.013
Leaflet width	0.199	-0.069	-0.280
Leaflet length	0.170	0.160	-0.048
Plant spread	0.301	0.005	0.087
No. of branches	0.345	-0.171	0.135
Pods per plant	0.263	0.009	0.210
Pod length	-0.041	0.150	0.135
Pod width	0.182	0.575	0.068
Seeds per pod	0.245	-0.377	0.505
Pods weight plant	0.196	-0.087	0.174
Seeds per plant	0.253	-0.100	0.115
100seeds weight	-0.054	0.590	0.429
Shoot dry weight	0.294	0.176	-0.263
Yield m^2	0.248	0.159	-0.004
Eigen values	2.832	1.787	1.148
Percentage	37.892	23.909	15.358
Cum. Percentage	37.892	61.800	77.158

had most contributions coming from no of branches (0.345), number of leaves (0.347) and plant spread (0.301). The second variate from axis 2 had higher contributions coming from pod width (0.575), 100 seed weight (0.590), and seeds per pod (-0.377). This indicates the importance of these characters in identifying tepary bean landraces. Principal coordinate analysis (PCoA) clearly demarcated landraces GK011 and MTS (Motsumi) from the rest of the accessions; it also distinguished GK012 from the rest of the accessions better than cluster analysis (Figure 2).

DISCUSSION

In this study we describe for the first time the diversity of tepary bean landraces grown in Botswana. Few morphological characters, number of branches per plant, 100 seed weight and number of seeds per plant exhibit significant variation, which shows low levels of genetic

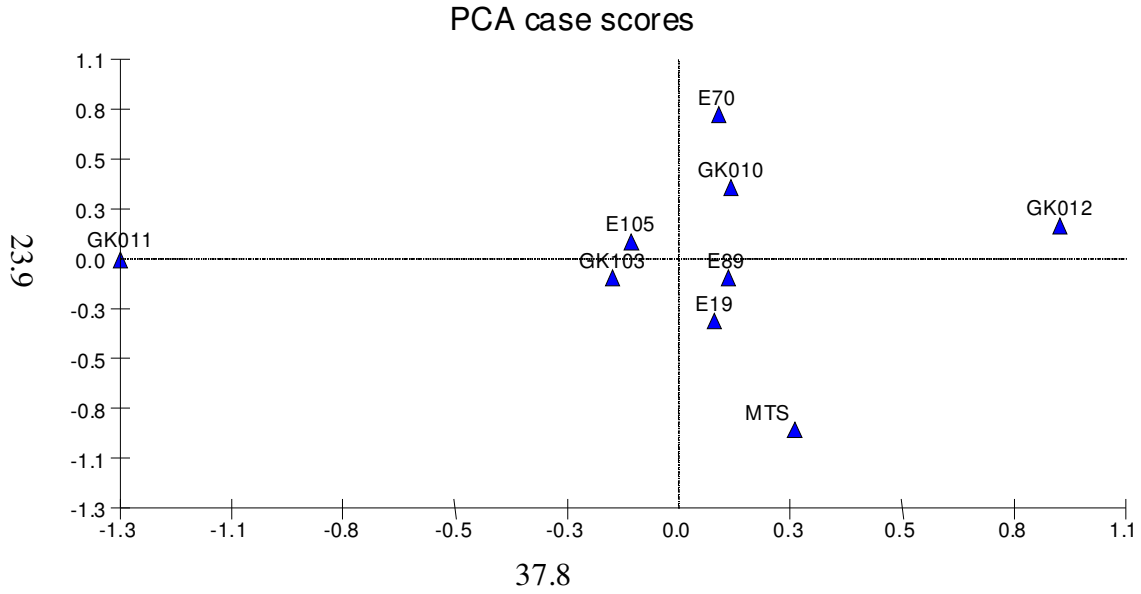

Figure 2. PCO scatter plot for nine tepary bean accessions grown in Botswana from MVSP program with a variation of 61.71%, with Axis 1 contributing 37.8% while Axis 2 explained 23.9%.

variation among the selected genotypes. Similar observations were revealed when using cluster analysis which exhibited lower differences among most of the accessions. The results generally are in accordance with the lower diversity in tepary bean observed by Schinkel and Gepts (1988) when analyzing Phaseolin among 55 wild and 8 cultivated teparies using polyacrylamide gel electrophoresis. Their results showed 15 electrophoretic Phaseolin patterns among wild forms and only one pattern in cultivars which they thought it suggest single domestication in this species.

Characters with greatest variation were yield per m^2, number of seeds per plant, number of leaves and podweight per plant. Similar results were observed in one important yield component in tepary bean of pods per plant with a range of (8.1 to 37.1) among sixteen accessions from five states in Mexico (Kuruvadi and Valdez, 1993). The greater variation appears to indicate that there is a potential for improvement of this crop.

The dendrogram (Figure 1) was divided into three clusters, but with a lower range among most accessions, which still shows a lower genetic diversity among the selected genotypes. The dendrogram is largely in agreement with the PCoA coordinates (Figure 2), which clearly demarcated accessions mostly on the major traits. Principal component analysis revealed those characters that are important in explaining the variation among the selected genotypes such as number of leaves, plant spread, pod width, 100 seed weight and seed per pod. Clusters can be separated mainly based on traits which contribute more variation as observed in barley (Abebe et al., 2010) and in rice (Moukoumbi et al., 2011). The clustering and scatter plots can also have a similar pattern as in pigeon peas (Manyasa et al., 2008) and in *Arachis pintoi* (Carvalho and Quesenberry, 2009).

Morphological character assessment is the first step in characterization of germplasm (Azam-Ali et al., 2001) usually breeding programmes relies on the magnitude of phenotypic variability in crops (Ghafoor et al., 2002). Morphological traits in this study were able to differentiate most of the accessions except in the case of E19 and E89, and E70 and GK10; this suggests that the accessions clearly resemble each other agronomically. Subsistence farmers in Botswana exchange seeds and these could be similar genotypes with different names. Presumably there could be some duplication which can be tested using molecular makers which are better placed to discern the accessions compared to morphological characters which are highly influenced by the environment (Smith and Smith, 1992; Hintum et al., 2000). From ten agricultural districts in Botswana the accessions were sourced from two districts (Table 1), different accessions could be discovered by the National Genetic Resource Centre when they collect more tepary germplasm to improve the genetic diversity. In general, our results revealed that the multivariate analysis used was able to differentiate the nine accessions based on the 15 characters selected. This gives an opportunity for further exploitation of the landrace since the characters with high importance in the characterization of tepary beans has been identified. However, the lower genetic diversity exposed in this study will require further addition of more materials. Currently the Department of Agricultural Research has sourced additional tepary bean lines from CIAT (Centro International de Agricultura Tropical) and a mutation project is on-going with the

Vienna-Sibersdorf laboratory. These initiatives could be useful in improving the diversity among Botswana tepary beans. An initiative has also been taken to source tepary bean multiple stress tolerant germplasm released by (Porch et al., 2012). The promising accessions such as GK012 and MTS (Motsumi) could be used as parental material by plant breeders.

Conflict of Interest

The authors have not declared any conflict of interest.

ACKNOWLEDGEMENT

The authors are grateful to the authorities of the Department of Agricultural Research, Ministry of Agriculture, for funding the research from research and development funds. They extend their thanks to Montisetse Molome and Christopher Rodger for their technical assistance.

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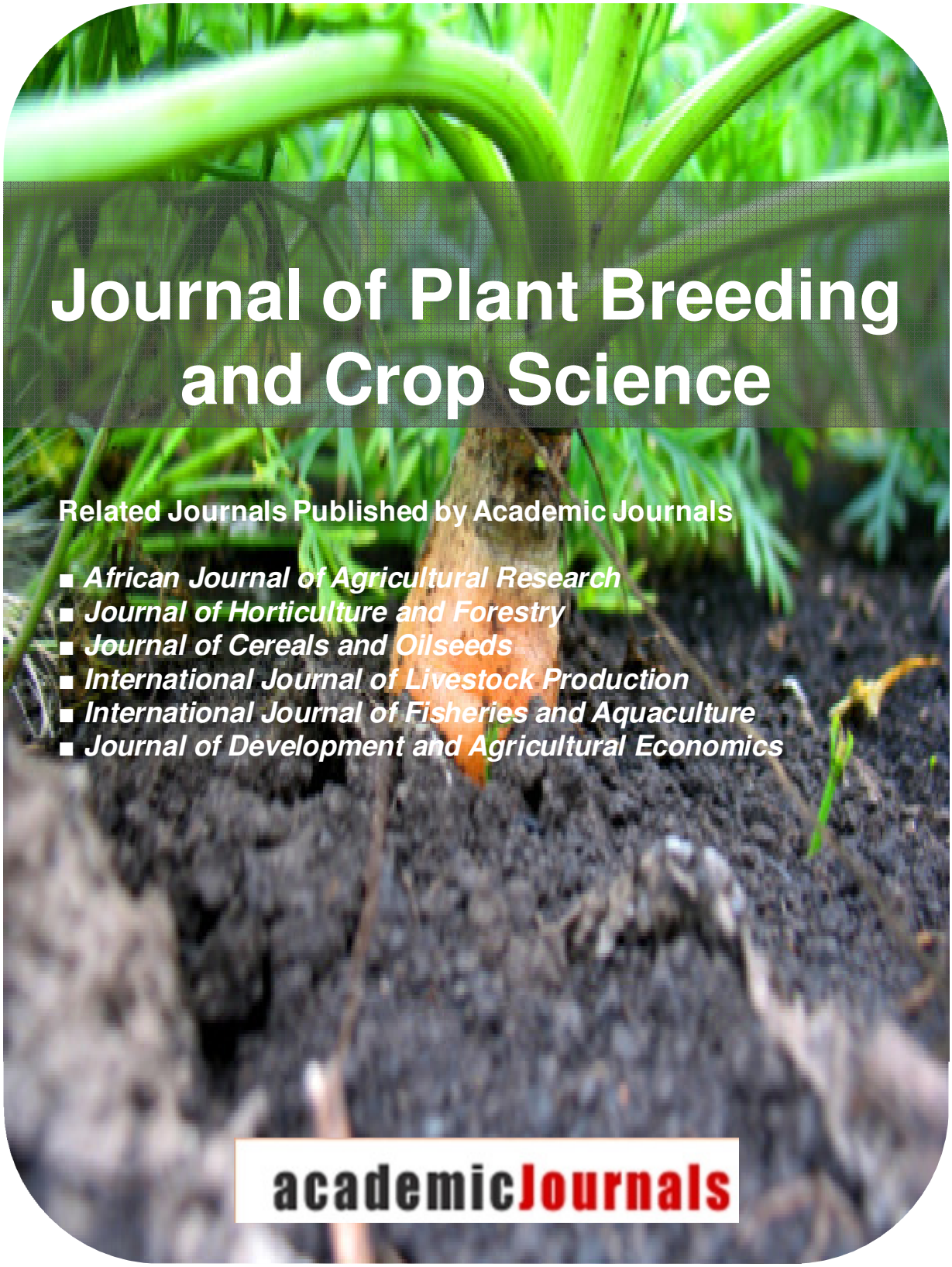

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