Original Research Article

Diversity in phenotypic traits in fluted pumpkin (*Telfairiaoccidentalis*)

ABSTRACT

Fluted pumpkin (*Telfairiaoccidentalis*) is an important indigenous leaf and seed vegetable of West Africa. It is among the neglected and underutilized crops with high nutritional, medicinal and industrial potentials. Twenty five genotypes of fluted pumpkin collected from eleven states in Nigeria were planted in the rainy seasons of 2012 and 2013 in Abeokuta and Akure to determine the genetic diversity in phenotypic traits among the genotypes. Randomized Complete Block Design (RCBD) was used and data were collected on growth and yield characters. Principal Component Analysis (PCA) and Single Linkage Cluster Analysis (SLCA) were employed to analyse the magnitude and pattern of diversity among the genotypes. Efficiency of the techniques in classifying these genotypes was also examined.The first eightPCA axes captured 90.77% of the total variance. The PCAidentified marketable leaf yield, vine length, leaf fresh weight, vine fresh weight, leaf area, number of leaves, fruit weight, fruit length, number of seeds and seed weight as most important characters in discriminating the 25 fluted pumpkin genotypes. The genotypes were grouped into six clusters based on their level of similarity by the SLCA. These clusters displayed a wide range of diversity for most of the traits.SLCAprovedtobeaneffectivemethodingroupingthe genotypes for efficient breeding programmes. The diverse genotypes identified in the different clusters could be used as parents and when crossed heterosiscould be achieved which would translate higher seed and leaf yield.

Keywords: *Telfairiaoccidentalis*; SLCA; PCA; genetic diversity

INTRODUCTION

The genus *Telfairia*(family *Cucurbitaceae*) has two main species:*Telfairiaoccidentalis*Hook. F. endemic to West and Central Africa and *Telfairiapedata*(Smith ex Sims) Hook –the oyster nut which is native to mainland Tanzania and northern Mozambique and the isles of Zanzibar and Pemba (Okoli, 2007). The two main species of *Telfairia*are dioecious and perennial. This genus is

characterised by large droopy pepo fruit weighing up to 15kg and it has one of the largest seeds among the cucurbits (Odiaka and Schippers, 2004; Okoli, 2007). Out of the two species of the genus fluted pumpkin (*Telfairiaoccidentalis*)is the most important economically and relatively the most cultivated.Fluted pumpkin is a multipurpose vegetable, cultivated for itsseed, leaves and tender shoot. The crop gained importance as a promising vegetable crop owing to itsperennial nature that ensures all year round availability, ability to alleviate poverty, nutritional and medicinal values of its leaves and seeds and its industrial potential as vegetable oil crop (Akoroda, 1990; Ehiagbonare, 2008; Fayeun*et al*., 2012).

In spite of the outstanding qualities of this crop, due attention has not been given towards a decisive crop improvement programme. This neglect may be as a result of some identified drawbacks. The restriction of the fluted pumpkin to West and part of Central Africa made it foreign to people outside these regions. The seed is recalcitrant in nature (Akoroda, 1990) and easily loose viability due to its bulkiness and high moisture content and hence cannot be stored for long. This problem is even compounded by the propensity of the seeds to germinate *in situ* while in fruit. The dioecious nature of the plant, inadequate knowledge about its mechanisms of pollination and floral biology coupled with long maturation period and cost of staking deprived the crop of needed breeding researches.Notwithstanding the identified problems, efforts need to be made to address the demand for genetic improvement this crop so as to enhance its leaf and seed yield.The availability of genetic variation among different strains and within strains provides great scope for crop improvement through judicious selection and breeding to develop the desired genotype (Shukla *et al*., 2009).Assessment of genetic diversity is invaluable in crop breeding programme, as it helps in the identification of diverse parental combinations to create segregating progenies with maximum genetic variability (Barrett and Kidwel, 1998) and facilitates introgression of desirable genes from diverse germplasm into the available genetic base (Thompson *et al*., 1998). Multivariate statistical methods are employed as tools for to assessing genetic diversity in crops. Some of the examples of

commonly multivariate statistical methods used include canonical analysis, cluster analysis, factor analysis, discriminant analysis and principal component analysis.Many researchers have used thesemethods in classifying, summarizing and describing variation patterns in populations of crop genotypes (Rhodes and Martins, 1972; Sharma and Rana, 1986; Ariyo, 1987; Raji, 2003; Nassir and Ariyo, 2007; Shukla *et al*., 2009; Makinde and Ariyo, 2010;Fayeun and Odiyi, 2012; Odiyi*et al*., 2014). The use of more than one technique to investigate genetic diversity in crops by many researchers is common because of complementary effects of the techniques on the analysis and allows comparison among the techniques to know which one captures most of the variation and provide clearer and informative display of the relative positions of the genotypes (Odiyi*et al*., 2014). However, reports on diversity analysis using multivariate statistical methods in fluted pumpkinare inadequate.Odiaka (2005) used PCA and metroglyph to analyse variation on nine accessions of fluted pumpkin collected in Markurdi and found some levels of variation. Fayeun and Odiyi, 2012 used cluster analysis to group 35 genotypes of fluted pumpkin collected from Southern Nigeria into four clusters based on their level of similarity. While Odiyi*et al*. (2014) employed PCA and factor analysis to assay genetic diversity among some selected genotypes of fluted pumpkin and they found out that the two methods though different identified similar characters that discriminated the genotypes.All the authors above carried out their studies at a single location. Therefore, the present study was conducted to evaluate the diversity in phenotypic traits in fluted pumpkin at two diverse locations.

MATERIALS AND METHODS

This experiment was conducted at the Teaching and Research Farm Directorate of Federal University of Agriculture, Abeokuta (FUNAAB) (7º25'N, 03º25'E) with sandy loam soil and Teaching and Research Farm of Federal University of Technology, Akure (FUTA) (7º16'N, 05º12'E) with sandy clay loam soil. FUNAAB and FUTA belong to derived savanna and rainforest agro-ecologies respectively. Twenty five (25) matured fluted pumpkin fruits of different genotypes

of were collected from 11 states in Nigeria in 2012. The description of the fluted pumpkin genotypes based on states of collection in Nigeria is presented in Table 1. The 25 genotypes were evaluated for two consecutive years (2012 and 2013) at each location. Land preparation involved manual clearing within the sites. The experiment was conducted in a randomized complete block design (RCBD) with three replications. Each replication had 25 plots of 2meters x 2meters, with 1meter inter-plot spacing. Seeds were first raised in the nursery using saw dust as growth medium as suggested by Akoroda andAdejoro (1990). Seedlings were transplanted directly on flat ground two weeks after planting. One seedling wastransplanted per hole at a spacing of 1 meter by 1 meter resulting in 9 plant stands per plot. Trellises of 2meters x 2meters x 1.5meters high were constructed on each plot to support the vines. Manual weeding was done at 3 weekly intervals to keep the field weed-free. There was no application of fertilizers and pesticides throughout the experimentation.Growth and yield data were collected on four competitive plants on each plot at each site on each genotype using appropriate equipment. Data on number of branches,vine length andnumber of leaveswere collected four weeks after transplanting (4WAT). Also at firstmarketable leaf yield harvest (8WAT)the following data were collected: number of branches, vine length,number of leaves, vine girth, petiole length, leaf length, leaf width, Internode length,leaf fresh weight, vine weight,marketable leaf yield and leaf area. The leaf area was measured in centimetre square by scanning leaf samples with HP scanner and the area of scanned images were determined using Compu Eye, Leaf and Symptom Area software of Bakr, 2005. Also the following data were collected on fruit: fruit length, fruit circumference, fruit diameter, fruit weight, number of seeds per fruit, seed length, seed girth, seed thickness and seed weight.The raw data were compiled by taking the means of all plantstaken for each treatment and replication for different traits inboth locations and experimental years. The data were subjected to analysis of variance and covariance (SAS 2000). Mean data were standardised and subjected to multivariate analysis. Principal

Component Analysis and single linkage cluster Analysis were performed using the SAS package (SAS, 2000).

RESULTS

The scores of the major characters describing the first eight principal component axes are presented in Table 2. Four out of the twenty four principal components had eigen values greater than 2.0. The first eight principal component axes jointly accounted for 90.77% of the total variation among the genotypes studied. The first principal component axis (PC1) accounted for 35.54% of the total variation. It was loaded with marketable yield characters, which included marketable leaf yield (0.29), leaf fresh weight at harvest (0.28) and marketable leaf, vine fresh weight at harvest and vine length at harvest (0.29). This axis is considered themarketable leaf yield component. PC2was positively loaded with leaf related traits such as; leaf length at harvest (0.40) and leaf width at harvest (0.39), petiole length at harvest (0.41) and leaf area (0.42). Whereas PC3 and PC4 were associated with fruit and seed traits respectively. PC3 was positively loaded for fruit weight (0.45), fruit length (0.37) and number of seeds (0.48) and negatively loaded for number of leaves (-0.22) and number of branches (-0.28). PC4 positively loaded seed length (0.58), seed girth (0.49), seed weight (0.20) and negatively loaded for fruit length (-0.24) and number of seeds (-0.20).

Configurations of the 25 genotypes along the first three principal component axes are shown in Figures 1, 2 and 3. The ordination of the genotypes on axes 1 and 2 (Figure 1) shows that Ftm12,Ftd1, Fta39, Ftw21, Fts33 and Fte42 were most distant from all other genotypes. Figure 2 displayed the graphing of axes 1 and 3 andshowed that genotype Ftk16, Ftw20 and Ftg24 were distinct from other genotypes. They were described by characters associated with PC1 and PC3. Figure 3 showsanother configuration of the genotypes (axes 2 and 3),Fte42 distinguished itself from other genotypes as it was described by characters (leaf and fruit size) associated with PC2 and PC3.

Figure 4 shows the dendrogram drawn from Single Linkage Cluster Analysis (SLCA) to illustrate the relationship among the twenty five genotypes. At minimum of 0.00 level of similarity,

all the twenty five genotypes were distinct from one another, while at a distance of 0.71 all the genotypes had formed a single cluster, indicating that each genotype had at least one neighbour with more than 0.71 level of similarity. The dendrogram grouped the twenty five genotypes into six distinct clusters based on their level of similarity. Table 3shows the distribution of the genotypes into six different clusters,out of which fifteenwere grouped in Cluster IV, three in Cluster I, four in Cluster V while others have single genotype each. The clustering pattern showed that the distribution of the genotypes within clusters did not follow geographical distribution.The mean performance of each cluster for all characters is presented in Table 3. The mean values showed a wide distinction among the clusters. There was a very wide range in marketable leaf yield per plant among the Clusters - Cluster III (370.80) and Cluster VI (176.80). Cluster I was distinct for number of branches at harvest, fruit weight and fruit circumference, Cluster II was outstanding for petiole length while Cluster III was noted for marketable leaf yield, internode length and leaf area. Cluster V and VI were distinct for fruit diameter and vine length respectively.

DISCUSSION

Currently, landraces (local genotypes)are used by fluted pumpkin farmers due to lack of improved genotypes.Thus, there is unpredictable and low yields, poor quality and low tolerance to diseases and pests.The fact thatthe genetics of the crop has not been fully understood makes genetic improvement of the crop which is quiteessential to achieve high yield difficult. Hence, there is need to exploit the genetic diversity present in thebase population to breed superior and high yielding genotypes for commercial cultivation in order to meet increasing demand for the crop.In the process of genetic improvement of any crop, genetic diversity among germplasm plays a major role, since it opens the way to determine the most divergent parents based on the contribution of different qualitative and quantitative traits, for further utilisation in any hybridisation programme (Shuklar*et al*., 2009).From the result obtained from PCA, it was clear that though vine length and number of leaves contributed significantly to marketable leaf yield, the negative correlation observed between

fruit/seed traits and number of leaves and branches in PC3 might implies that genotypes with numerous leaves and branches might not be a good fruit and seed yielder. Configuration of the genotypes along the axes of PC1 and PC2, PC1 and PC3 revealed that genotypes Ftd1, Fta39, Ftw21 and Fte42 are high-yielding. This suggests that direct selection could be made on these genotypes.Heterosis could be achieved by crossing between and any of these genotypes.

The genotypes were grouped into six clusters, each containing fluted pumpkin genotypes sharing common attributes and being similar to one another. This grouping of the genotypes by SLCA did not follow a particular pattern. Some genotypes from the same source were grouped together while others from different sources were clustered together. This has, in some cases been attributed to lack of similarity between genetic and geographical diversity due to movement of germplasm through seed exchange (Gwanama and Nichterien, 1995; Ntundu, 2002; Fayeun and Odiyi, 2012). According to Fayeun, 2011, exchange of seed is a common practice among African farmers. Odiaka*et al.* (2008) reported that commercial fluted pumpkin growers in the middle belt source their fluted pumpkin seeds from the south eastern states of Nigeria. Similar practices have been reported in Zambia for Zambian cucurbit (*Cucurbitamoschata*Duch.) by Gwanama and Nichterien (1995), and in Tanzania for Bambara groundnut (Ntundu,2002).

Theclear distinction in the mean values of characters across the clusters might make improvement programme in fluted pumpkin through direct varietal selection easy (Fayeun and Odiyi, 2012). In addition clusters that showed some character distinctions could be employed for hybridization purpose.Clusters III and VI for instance, recorded highest marketable leaf yield per plant and largest leaf size but poor seed yield while cluster VI had longest vine length and high seed weight, hence, a high yielding progeny which will have a better combination of vine length, number of leaves, large leaf size and seed weight could be selected from a cross between suitable genotypes in clusters III and VI. Interestingly genotypes from clusters III and VI are from South Eastern

Nigeria where the crop is believed to originate, hence the crop has large diversity in this region (Akoroda, 1990; Odiaka*et al*., 2008; Fayeun, 2011; Fayeun and Odiyi, 2012).

The outcome of single linkage cluster analysis seemed to be consistent with the result obtained from the PCA configurations of the 25 genotypes along the first two principal component axes. Similar finding has also been reported in cowpea *Vignaunguiculata* (L) by Aremu*et al*. (2007), in 'egunsi' melon *Citrulluslanatus* (Thunb) by Idehen*et al.* (2007), and in vegetable amaranth *Amarantustricolor* by Shukla *et al*. (2009).These findings are in concordance with earlier reports that both PCA and SLCAcan disclose complex relationships between taxa in a more understandable way and with equal effectiveness (Rezai and Frey, 1990; Abede and Bjornstad, 1996; Shukla *et al*., 2009). The PCA and SLCAwhen used together proved to be effective methods in grouping the fluted pumpkin genotypes and that may facilitate effective management and utilization of the crop in future breeding programmes (Fayeun, 2011 and Odiyi*et al*., 2014).

In conclusion, the two multivariate statistical techniques (PCA and SLCA) used are appropriate for the classification of diversity among fluted pumpkin germplasm and therefore recommended for future use. The single linkage cluster analysisgrouped the 25 genotypes based on their level of similarity for some characters, thus selection of parents for hybridization can be made from within the clusters.

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268 **Table 1. Descriptions of the 25 fluted pumpkin genotypes based state of collection in Nigeria.**
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S/N	Genotype	State of Collection
1	Ftd1	Edo
2	Ftm11	Imo
3	Ftm12	Imo
4	Ftr13	Anambra
5	Ftk16	Ekiti

6	Ftk17	Ekiti
7	Fta39	Abia
8	Ftw20	Kwara
9	Ftw21	Kwara
10	Ftg22	Ogun
11	Ftg23	Ogun
12	Ftg24	Ogun
13	Ftn44	Ondo
14	Ftn45	Ondo
15	Ftn47	Ondo
16	Ftn43	Ondo
17	Ftn46	Ondo
18	Fte40	Enugu
19	Fte41	Enugu
20	Fte42	Enugu
21	Fty28	Oyo
22	Fty29	Oyo
23	Fty30	Oyo
24	Fts33	Osun
25	Fts34	Osun

273 Table 2**.** Eigen vector for agronomic characters of the first eight principal components

Characters	**Eigenvectors**							
	PC1	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**	**PC7**	**PC8**
Vine length (cm) 4WAP	**0.22**	-0.13	-0.07	0.07	**-0.36**	**0.42**	**-0.38**	-0.13
Number of leaves 4WAP	**0.29**	0.00	-0.05	-.012	0.14	0.07	**-0.35**	-0.16
Number of branches 4WAP	**0.22**	0.03	-0.12	**-0.28**	**0.35**	-0.07	**-0.30**	**-0.44**
Vine length (cm) at harvest	**0.29**	0.05	-0.17	0.039	-0.14	**0.22**	0.09	-0.01
Number of leaves at harvest	**0.28**	-0.10	**-0.22**	-0.15	-0.02	0.10	0.14	**0.27**
Number of leaves at harvest	**0.22**	-0.05	**-0.28**	**-0.30**	0.12	0.11	**0.30**	0.19
leaf fresh weight (g) at harvest	**0.28**	0.16	-0.08	0.03	-0.04	**-0.36**	0.06	-0.08
Vine fresh weight (g) at harvest	**0.29**	0.16	-0.07	0.05	-0.04	**-0.30**	0.12	0.05
Marketable leaf yield (g)	**0.29**	0.16	-0.08	0.04	-0.04	**-0.33**	0.08	-0.04
Vine girth (cm) at harvest	**0.26**	-0.12	**-0.23**	-0.00	-0.12	-0.06	0.11	0.11
Leaf length (cm) at harvest	-0.02	**0.40**	-0.05	0.03	-0.03	0.14	-0.03	0.09
Leaf width (cm) at harvest	0.06	**0.39**	0.04	-0.04	-0.01	**0.21**	0.09	**0.25**
Petiole length (cm) at harvest	0.00	**0.41**	0.04	0.06	0.04	0.02	**-0.20**	0.13
Internode length (cm) at harvest	0.02	**0.40**	0.07	0.09	-0.07	0.05	0.00	**-0.30**
Leaf area (cm^2) at harvest	0.03	**0.42**	-0.02	0.01	0.02	**0.20**	0.02	0.05
Fruit weight (g)	0.18	0.04	**0.45**	-0.15	-0.13	-0.02	0.13	**-0.25**
Fruit length (cm)	0.15	-0.07	**0.37**	**-0.24**	0.06	**0.31**	**0.39**	-0.13
Fruit circumference (cm)	**0.24**	-0.09	**0.23**	0.01	**0.37**	**0.21**	0.01	-0.05
Fruit diameter (cm)	**0.20**	-0.10	**0.22**	0.15	**0.42**	-0.14	**-0.26**	**0.42**
Number of seeds	0.05	0.08	**0.48**	**-0.20**	-0.04	-0.19	-0.01	**0.25**
Seed weight (g)	**0.23**	-0.07	0.18	**0.20**	**-0.21**	0.09	**-0.34**	**0.25**
Seed length (cm)	0.11	-0.01	0.00	**0.58**	0.18	-0.08	**0.21**	**-0.27**
Seed girth (cm)	0.18	-0.12	0.09	**0.49**	0.06	**0.22**	**0.21**	0.02
Seed thickness (cm)	0.18	-0.11	0.19	-0.04	**-0.50**	**-0.24**	-0.01	-0.06
Eigen value	**8.53**	**5.19**	**2.7**	**2.1**	**1.05**	**0.91**	**0.69**	**0.62**
Proportion of variance (%)	**35.54**	**21.62**	**11.25**	**8.74**	**4.37**	**3.81**	**2.85**	**2.58**
Cumulative variance (%)	**35.54**	**57.17**	**68.42**	**77.16**	**81.53**	**85.34**	**88.19**	**90.77**

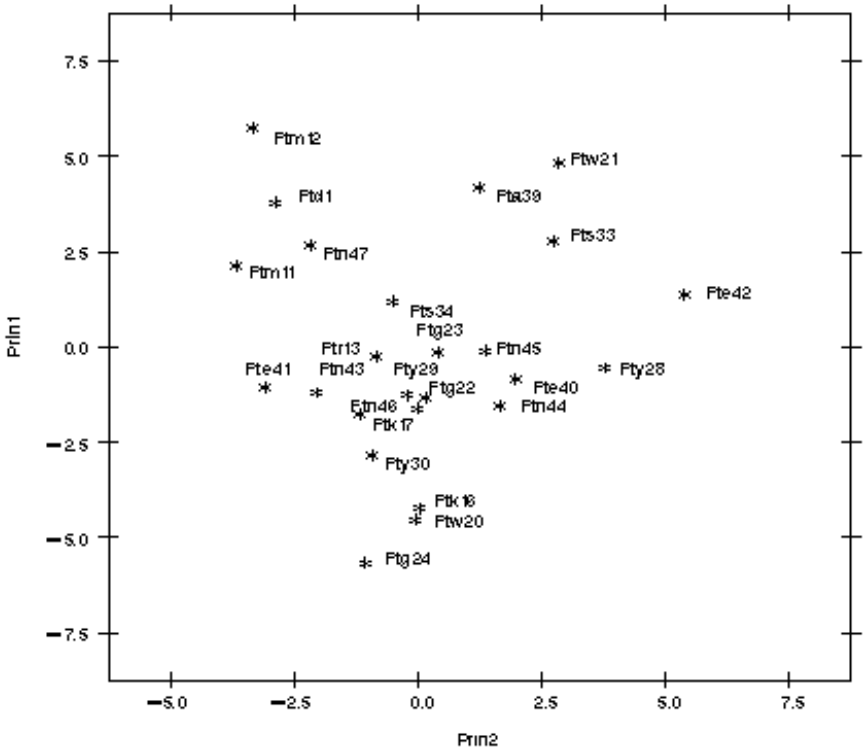

Figure 1. Configuration of twenty five genotypes under axes 1 and 2

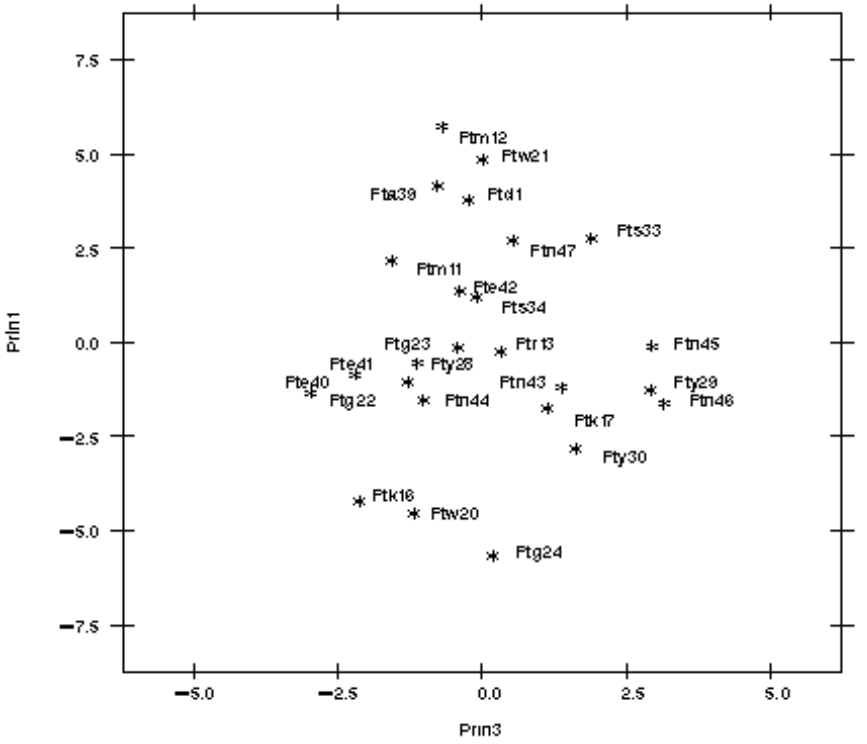

Figure2. Configuration of twenty five genotypes under axes 1 and 3

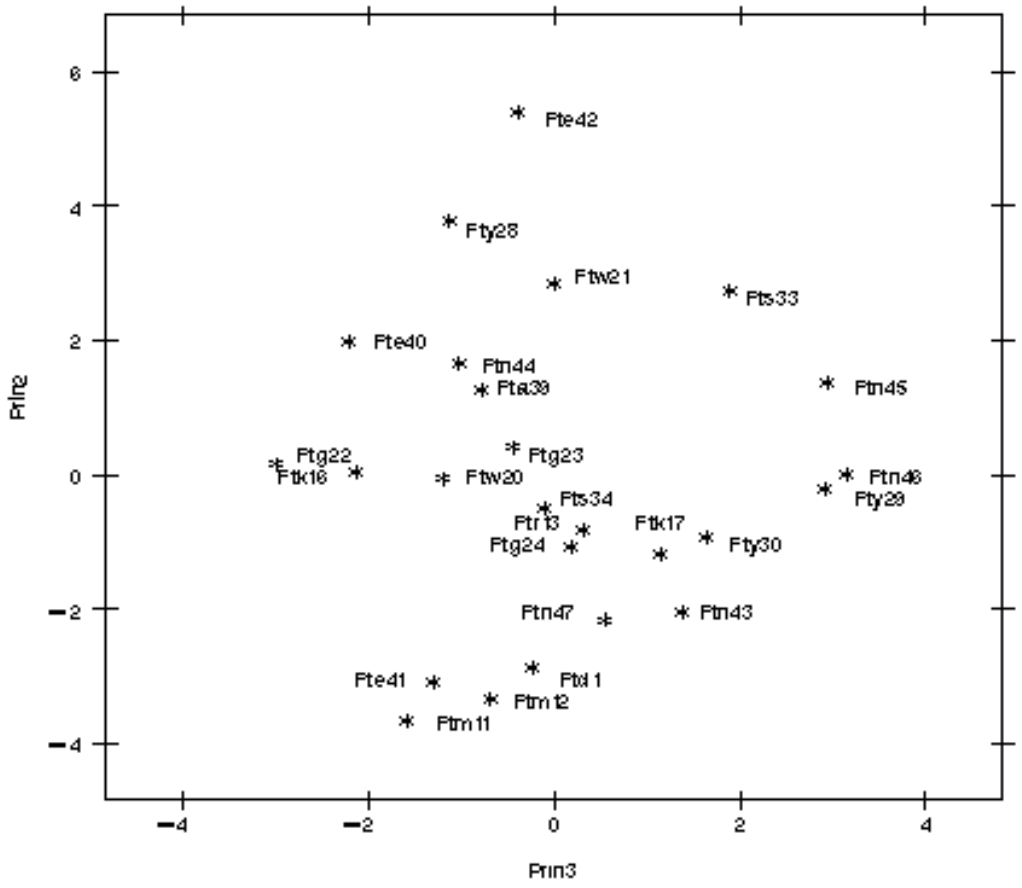

Figure 3. Configuration of twenty five genotypes under axes 2 and 3

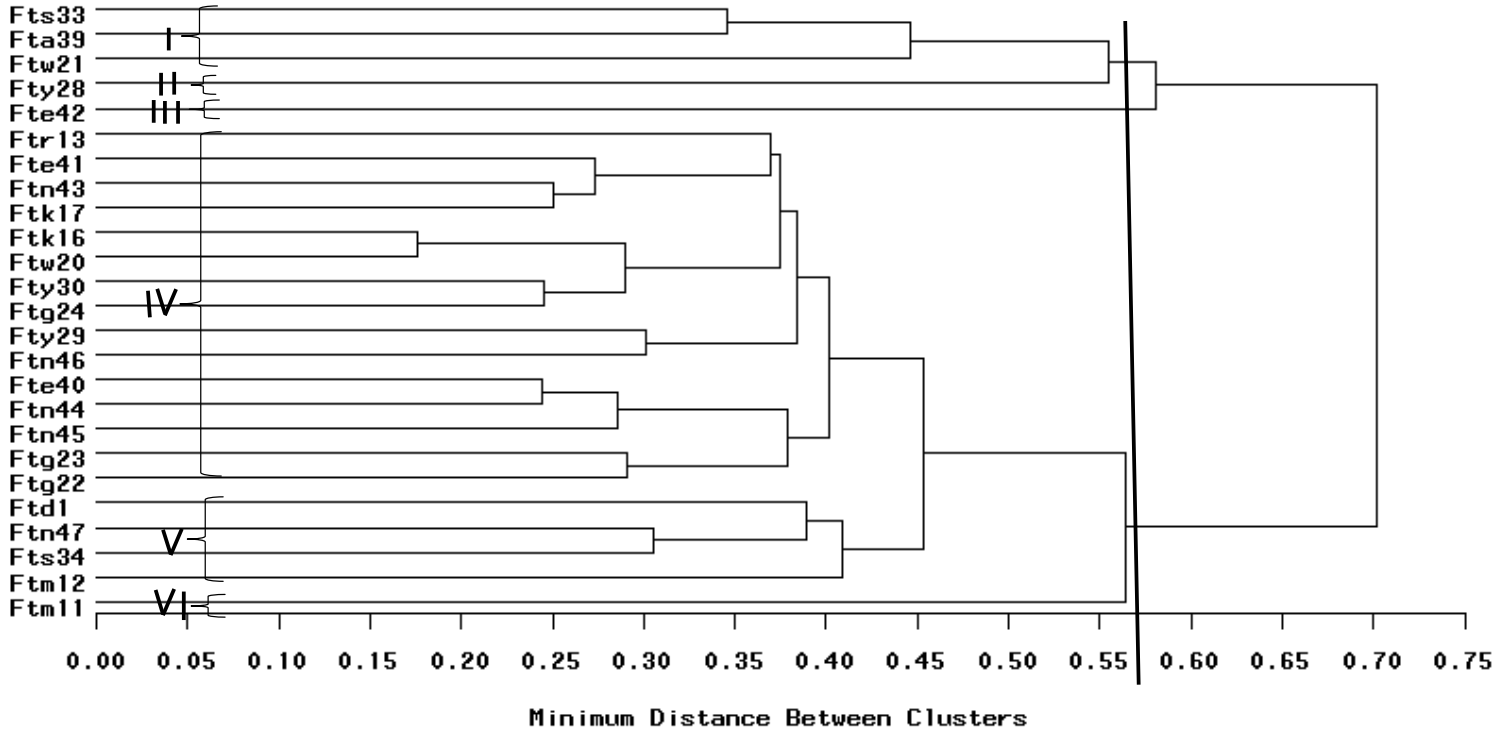

Figure 4. Dendrogram resulting from Single Linkage Cluster Analysis of twenty five genotypes of fluted pumpkin

311 Table 3. Characteristics pattern of clusters of the 25 fluted pumpkin genotypes by single linkage cluster analysis

Character	**Cluster I** Fts33,Fta39, Ftw21	**Cluster II** Fty28	**Cluster III** Fte42	**Cluster IV** Ftr13, Fte40, Ftw20, Fty30, Ftk16, Ftg22, Ftg23, Ftn46, Ftn46, Fte41, Fty29, Ftn43, Ftg24, Ftk17,Ftn45	**Cluster V** Ftm12, Fts34, Ftn47, Ftd1	**Cluster VI** Ftm11
Vine length 2WAP (cm)	91.63	70.50	67.40	70.98	90.08	**133.10**
Vine length 10WAP (cm)	181.50	148.20	182.30	136.33	170.50	**198.20**
Number of leaves 10WAP	74.40	47.95	48.37	50.33	77.85	**82.68**
Number of branches 10WAP	**6.20**	4.74	4.18	4.74	6.05	5.87
Marketable leaf yield (g)	302.53	240.40	**370.80**	135.31	271.60	176.80
Vine girth 10WAP (cm)	1.53	1.37	1.47	1.36	1.71	1.79
Petiole length (cm)	9.39	**11.31**	10.15	7.68	6.45	5.49
Internode length 10WAP (cm)	9.26	10.13	**11.19**	7.97	6.81	6.64
Leaf area 10WAP (cm^2)	310.63	341.50	**382.60**	217.90	159.75	150.70
Fruit weight (g)	**6.37**	2.75	5.45	4.30	5.60	4.15
Fruit length (cm)	54.67	36.00	36.00	46.80	52.00	47.00
Fruit circumference (cm)	**71.33**	60.00	60.00	61.93	68.50	63.00
Fruit diameter (cm)	14.17	12.50	12.60	12.18	**14.38**	11.50
Seed weight (g)	18.45	18.00	13.20	12.77	20.68	**21.00**
Seed length (cm)	3.42	4.40	4.00	3.42	3.78	3.60
Seed width (cm)	3.86	4.40	3.80	3.63	4.29	4.50