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Diversity analysis in *Plectranthus edulis* (Vatke) Agnew collection in Ethiopia

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Thirty six (36) accessions of *Plectranthus edulis* were evaluated to assess the extent of genetic diversity within the collected accessions using 16 characters. Analysis of variance for each characters indicated highly significant (p<0.01) variation among the accessions for all characters except tuber length. The accessions under study were grouped into six clusters. Clusters I and II contained the maximum number of accessions (12 accessions each) and cluster VI contained the minimum number of accession (one accession). The maximum distance was observed between clusters IV and V (D2 =348.67), while the minimum was between clusters II and IV (D2 =32.69). The present study indicates a considerable amount of variability for the majority of the characters of interest in *P. edulis* for exploitation. Nevertheless, the need for confirmation of genotypic-environmental interaction, conventional characterization approach through advanced tools of biochemical and molecular approaches and widening of the genetic base for strengthening *P. edulis* improvement strategy are suggested.

Key words: Cluster analysis, D2 analysis, genetic variability, *Plectranthus edulis*.

INTRODUCTION

Plectranthus edulis is an indigenous annual tuber crop grown widely in the central, southern, western, northwestern and southwestern parts of Ethiopia (Uphof, 1968; Westphal, 1975; Zeven and Zhukovsky, 1975; PGRC/E, 1986; Edward, 1991; Edossa, 1996; Abdissa, 2000; GRIN, 2005). It is a dicotyledonous plant and belongs to the family Lamiaceae/Labiatae; subfamily Nepetoideae and tribe Ocimeae (GRIN, 2005).

In different growing areas of Ethiopia, different vernacular names are used for *P. edulis.* Among these are '*Dinicha Oromo*' in Oromia, meaning "potato of the Oromo people" (Abdissa, 2000), '*Wolaita Dinich*' (potato of the Wolayita people) around Wolaita (Endale, 1997), '*Agew Dinch'* (potato of the Agew people) in the northwest and '*Gurage Dinich'* (potato of the Gurage people) around Gurage zone (Westphal, 1975). For generations, farmers in different parts of the country have been cultivating *P. edulis* primarily for its edible tuber. The leaves are also eaten by human as green vegetable in some regions (Abebe, 1988). Moreover, the edible tubers are good for people with asthma (IAR, 1980) and because of its abundant nectar, the plant is a good source for honey bee (Reinhard and Admasu, 1994).

Despite its importance for food security and medicinal value, only limited research has been conducted on the crop (Abebe, 1988). On the other hand, changes in agricultural practices and environmental degradation are causing genetic loss in the local gene pool of this crop (Amsalu and Tesfaye, 2004). It is well known that adequate genetic diversity is necessary in breeding program for the development of high yielding varieties. The utility of multivariate analysis and the use of the

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generalized distance (D^2) as quantitative measure of genetic divergence are well illustrated. Moreover, information on the extent of genetic diversity in *P. edulis* germplasm is absolutely essential in parental selection to start hybridization. It is generally believed that crosses between parents with maximum genetic divergence are likely to produce desirable segregation and recombination in progenies (Reddy, 1988). Although there has been collection of *P. edulis* from different parts of the country, there is minimum information on the extent of genetic diversity to determine breeding values of the collected accessions. Admasu (2002) indicated that lack of knowledge about the genetic diversity of the enset (*Ensete ventricosum*(Welw.) E.E. Cheesman crop complicated the conservation, improvement and utilization by farmers, conservationists and breeders. He also noted that knowledge on clonal diversity allows the selection of clones prioritized for conservation, by removing duplication and optimizing genetic diversity and hence optimizing cost benefit ratio in maintaining the crop germplasm. Hence, this experiment was designed to cluster 36 accessions into different diversity classes and thereby estimate the extent of genetic distance between clusters and to choose and recommend genetically divergent parent for hybridization.

MATERIALS AND METHODS

The study was carried out at Jimma Agricultural Research Centre, located at 7°46' N and 36° E with an altitude of 1753 m above sea level. The soil type of the experimental area is Eutric Nitosol (reddish brown) with a pH around 5.2. The area receives mean annual rainfall of 1536 mm with a mean annual maximum and minimum temperature of 25.9and 11.2°C, respectively (IAR, 1997). A total of 36 *P. edulis* accessions collected from six different regions of the country by Jimma Agricultural Research Centre and the Institute of Biodiversity Conservation (Table 1) were grown in plots during the 2005 main cropping season (April-October) in 6 x 6 simple lattice design. Each row was 3.5 m long with a space of 1 m between rows. Plants were spaced 50 cm apart within the row. Tubers that have just started sprouting were used as planting material. Planting was done at the beginning of the rainy season (April) in well-drained, loose soil on flat ground. Three kg/plot of farmyard manure (8.571 t/ha) was applied along the rows. One month later, when the crop was well established, earthing up with loose soil was undertaken. Hand weeding was conducted as required to keep plots weed-free. A total of 16 quantitative traits were recorded on plant basis on five selected plants. An average of five plants was used for statistical analysis. Days to flower initiation and days to 50 percent flowering were recorded on plot basis. Collected traits includes : Plant height (cm), stem girth (cm), number of tubers per hill, tuber length (cm), tuber diameter (cm), number of nodes, internodes length (cm), tuber weight per hill (kg), tuber dry matter content (%), number of stems per hill, days to initiation of flowering, days to flowering, number of primary branches, leaf length (cm), leaf width (cm) and flower length (cm).

Analysis of variance was done by the normal procedure. All the collected data were subjected to Analysis of Variance to determine whether there was any difference between the analyzed accessions. Clustering was performed to group accessions into homogenous classes by average linkage methods and number of cluster was chosen by examining Pseudo F statistics and Pseudo t^2 statistics using SAS version 8.2(SAS,2001).

Genetic distance between clusters was calculated using the generalized Mahalanobis's D^2 statistics: $D^2_p = (X_i-X_j)\ ^1\ _S{}^{-1}\ (X_i-X_j)$ where, D^2_p = total generalized distance based on p characters. X_i and X_j are the p mean vectors of accessions i and j, respectively. S = p x p pooled error variance- covariance matrix (Mahalanobis, 1936). D^2 value for pairs of clusters was considered as the calculated value of Chi-square (χ^2) and was tested for significance at the required level of probability against the tabulated values of χ^2 for p degrees of freedom, where p is the number of characters considered (Singh and Chaudhary, 1985). SAS (2001) was employed for the analysis.

RESULTS AND DISCUSSION

Analysis of variance

The analysis of variance for each character revealed highly significant (P<0.01) difference among the accessions for all the characters examined except tuber length, indicating the presence of considerable amount of variability for the characters. Amsalu (2003) and Baye et al. (2005) also reported similar results for the majority of the characters in potato and cassava, respectively.

Cluster analysis

Accessions of *P. edulis* were grouped into six distinct clusters of different sizes (Table 2). The dendrogram for clustering activity is presented in Figure 1. Moreover, in order to identify characters, which discriminate among the six groups of populations, means of the traits were calculated for each cluster separately (Table 3). Clustering pattern indicated that the number of accessions in each cluster/group varied from 1 in cluster VI to 12 in clusters II and I. Clusters I and II consisted of a maximum number of accessions, each of them accounting for about 33.33% of the total accessions (Table 2). Cluster I includes seven accessions from Jimma, two each from Illubabor and Wolaita, and one from Yem areas. Accessions in this cluster were characterized predominantly by higher leaf length.

On the other hand, cluster II includes seven accessions from Jimma, three from Illubabor, and two from Hawi. Lower plant height and short internodes length characterized accessions in this cluster (Table 3). Cluster III includes two accessions from Jimma and one from Illubabor. Accessions in this cluster were characterized by higher stem girth along with higher number of primary branches as well as maximum tuber diameter and early in days to flower initiation and lower tuber dry matter percentage. Similarly, cluster IV includes three accessions (8.33%) each from Wolaita, Hawi and Gedio and was characterized by maximum flower length and leaf width and lower stem girth, number of nodes, number of stems per hill, tuber weight per hill, number of tubers per hill and days to 50% flowering (Table 3).

Furthermore, cluster V includes five accessions (13.89%); four from Illubabor and one from Yem and was

Table 1. *Plectranthus edulis* accessions and their area of collection.

Accession Code	Accession Number	Area of collection	
		Zone	District (Wereda)
1	028/02	Jimma	Seka
2	076/03	Illubabor	Metu
3	106/03	Yem	Yem
4	073/02	Jimma	Sekoru
5	066/02	Jimma	Setema
6	102/03	Illubabor	Metu
7	107/03	Yem	Yem
8	082/02	Jimma	Kersa
9	010/02	Jimma	Dedo
10	018/02	Jimma	Seka
11	049/02	Illubabor	Chora
12	067/02	Illubabor	Denbi
13	022/02	Jimma	Kersa
14	099/03	Illubabor	Darimu
15	052/02	Illubabor	Bedele
16	235969	Wolaita	Damota gale
17	044/03	Illubabor	Metu
18	003/02	Jimma	Dedo
19	063/02	Illubabor	Denbi
20	079/02	Jimma	Omonada
21	064/02	Illubabor	Denbi
22	011/02	Jimma	Kersa
23	235976	Wolaita	Sodo zuria
24	046/02	Jimma	Dedo
25	014/03	Illubabor	Alle
26	242494	Hawi	Dangila
27	071/02	Illubabor	Denbi
28	004/02	Jimma	Dedo
29	242493	Hawi	Dangila
30	Lu-bo	Jimma	Choche
31	113/03	Jimma	Dedo
32	045/02	Jimma	Dedo
33	041/02	Jimma	Gera
34	235975	Wolaita	Sodo zuria
35	235978	Gedio	Wenago
36	242491	Hawi	Metekel

characterized by maximum tuber weight per hill and number of tubers per hill (Table 3). Finally, cluster VI had one accession (2.78%) from Illubabor and was characterized by higher values in respect of majority of the character such as plant height, number of nodes, number of stems per hill, days to flower initiation, days to 50% flowering, internode length and tuber dry matter content. Generally, genetic diversity is associated with geographical diversity but it is not necessary directly related with geographical distribution. Because there was no definite association between geographical distance and genetic diversity since accessions from different places of collection fell in the same cluster and accessions from the same place fell in to different cluster. This indicates that the geographical and genetic distribution did not follow the same trend which might be due to continues exchanges of genetic material among *P. edulis* growing areas of the country or accession from the same origin might have different genetic background and vise versa. Similar result was reported by Alom et al. (2003) on maize and Gemechu et al. (1997) on groundnut. Clusters contributing maximally to divergence were given greater emphasis for deciding the type cluster for the purpose of further selection and the choice of parents

Table 2. Distribution of 36 *Plectranthus edulis* germplasm accessions in six clusters.

Cluster	Number of accessions	Accessions name
I	12	028/02,076/03,106/03,073/02,010/02,022/02,235969,003/02,046/02,071/02,045/02 and 235975
II	12	066/02, 099/03, 052/02, 079/02, 011/02, 014/03, 242494, 004/02, Lu-bo, 113/03, 041/02 and 242491
III	3	082/02, 018/02 and 064/02
IV	3	235976, 242493 and 235978
V	5	102/03, 107/03, 049/02, 067/02 and 063/02
VI	1	044/03

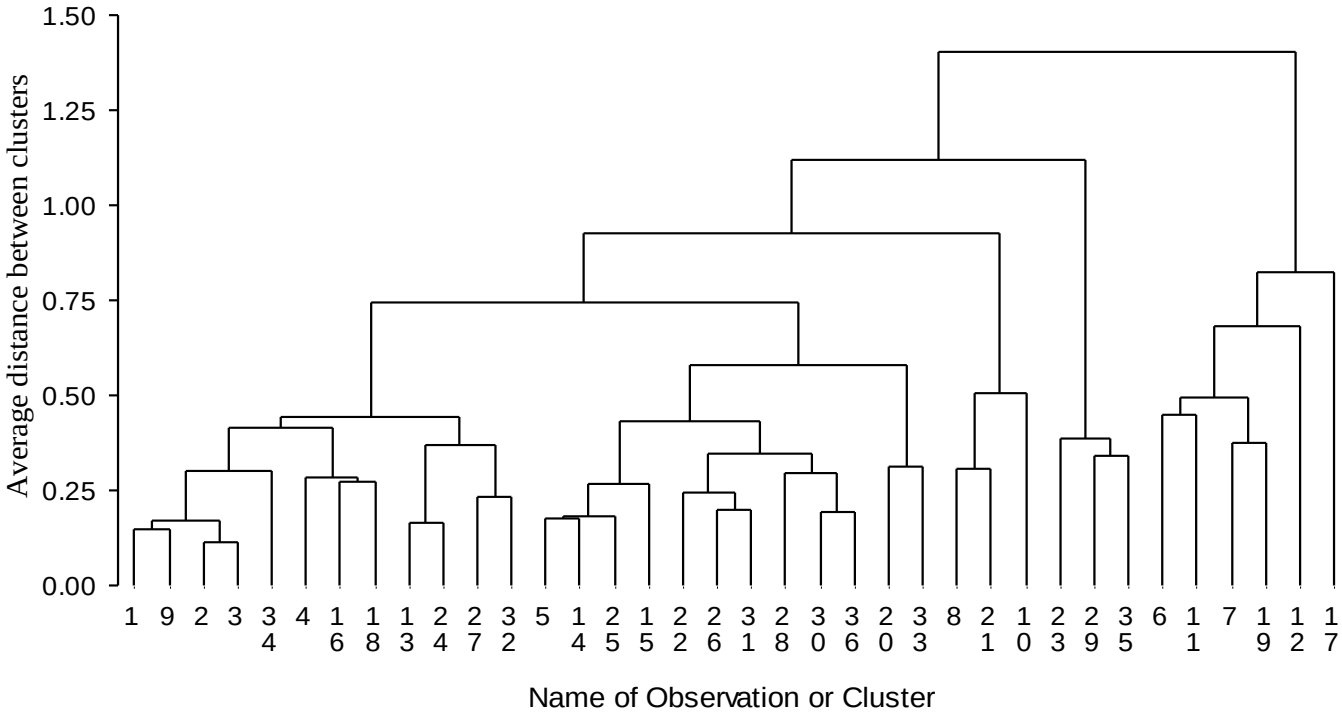

Figure 1. Dendrogram generated using UPGMA demonstrating the genetic similarities between 36 collected accessions of *Plectranthus edulis*. Code used for accessions are found in material and methods.

for hybridization. Accordingly, germplasm accessions falling in cluster III, V and VI showed higher performance for the characters of interest viz., number of tubers per hill, tuber diameter, tuber weight per hill, number of stems per hill and tuber dry matter content. These accessions include, for example 064/02 from cluster III, 102/03 from cluster V and 044/03 from cluster VI based on their higher tuber weight per hill. Furthermore, most of these characters also had positive genotypic association with tuber yield per hill except tuber dry matter content (data not indicated). Hence, their potential as parents in heterotic breeding work seems possible. On the other hand, cluster IV, which consisted of three germplasm accessions was the least in performance for the majority of quantitative characters studied (Table 3). For example, all of the accessions grouped under this cluster gave the least tuber weight per hill and average number of tubers per hill. The result also pointed out that the importance of accessions in cluster IV for their exploitation in tuber yield improvement appeared limited in view of their poor performance for the majority of the characters of interest. This indicates that different clusters have different breeding values that enable breeders to improve different traits and parental selection should be made based on the relative merits of each cluster for each trait depending on the objective of the breeding program. Kumar and Debey (2003) further reported that while selecting genotypes from a particular cluster, the inter cluster distance, cluster mean and *per se* perfor-mance should be taken into consideration.

Table 3. Cluster means for 15 quantitative characters of *Plectranthus edulis* germplasm accessions.

Cluster	Character														
	PH	SG	NN	NS	NB	FL	LW	DFI	DF	IL	LL	TD	TW	NT	TDM
I	107.33	1.76	20.19	2.22	16.75	17.83	4.48	145.13	153.29	4.88	14.02	1.92	1.80	127.99	20.97
II	98.49	1.57	19.92	2.28	15.81	17.39	4.11	145.42	157.25	4.53	13.18	1.88	1.19	83.49	20.14
III	103.87	1.79	19.04	2.10	17.63	19.33	4.41	97.17	140.50	4.65	12.89	1.94	1.73	133.09	19.91
IV	100.15	1.35	17.65	1.88	16.49	22.14	4.74	116.00	135.33	4.70	15.05	1.84	0.47	43.12	20.30
V	107.45	1.53	19.95	2.26	16.22	16.85	3.96	137.30	158.50	4.66	13.19	1.70	1.95	194.52	22.10
VI	146.75	1.66	27.00	4.10	12.40	12.82	3.58	159.50	168.00	5.10	11.75	1.56	1.53	167.20	22.50

PH, Plant height; SG, Stem girth; NN, number of nodes; NS, number of stems per hill; NB, number of branches; FL, Flower length; LW, Leaf width; DFI, Days to flower initiation; DF, days to 50% flowering; IL, internodes length; LL, leaf length; TD, tuber diameter; TW, tuber weight per hill; NT, number of tubers per hill and TDM, tuber dry matter content.

Table 4. The pairwise generalized squared distances between six clusters of *Plectranthus edulis.*

Cluster	I	II	III	IV	V	VI
I		36.10**	37.24**	107.44**	87.57**	84.27**
II			102.47**	32.69**	219.35**	178.63**
III				166.00**	55.30**	79.32**
IV					348.67**	290.86**
V						50.27**
VI						

** Significant at 0.01 probability level (x^2_{15} ' 30.58)

Distance analysis

The pairwise generalized square distance (D^2) between the six clusters is presented in Table 4. Distance among all clusters was highly significant (P<0.01) suggesting wide diversity among these clusters. The maximum inter-cluster distance ($D^{2,}$ 348.67) was observed between IV and V followed by cluster IV and VI ($D^{2,}$290.86) and cluster II and V ($D^{2,}$ 219.35) in the order of magnitude. Therefore, crossing of parents selected from cluster V and VI with cluster IV and cluster V with cluster II could produce desirable recombinants in views of the genetic diversity. For example, based on their tuber weight per hill, selecting and crossing of accessions 102/03 from cluster V with accession 044/03 from cluster VI and 102/03 from cluster V with accession 052/02 from cluster II may produce desirable recombinant for tuber weight per hill in view of this study. However, since tuber dry matter content is negatively correlated with tuber weight per hill one has to give care during parent selection. On the other hand, crossing of parents from cluster II and I, I and III and II and IV might not produce desirable recombinants due to the small inter-cluster distance, which indicates close relationship among accessions falling in this cluster. However, Gemechu et al. (1997) reported that selection of parents should also consider the special merits of each cluster and each genotype within a cluster depending on the specific objective of hybridizations.

Nevertheless, the present analysis reveals the existence of sufficient variability and diversity among the tested germplasm accessions. In spite of this fact, it may be of considerable importance to broaden the genetic base either through sustainable germplasm collection throughout the

country and/or induced mutation for genetic improvement of tuber yield in *P.edulis*.

The present study clearly illustrated the existence of a wide range of variation among the germplasm accessions collected from different regions of Ethiopia. This indicates a considerable amount of variability for the different characters. However, the present effort was carried out at single location and season. It is possible that trends could vary across location and need for ascertaining genotypic-environment interaction is highlighted through appropriate studies. The requirement for broadening the genetic base is also emphasized from the point of view of diversifying the prevailing gene pool. *P. edulis* collection representing diverse eco-geographical areas of the country should be organized for diversity analysis to derive some guidelines for conservation activities than reported here. Furthermore, the conventional approaches of characterization as adopted in the present study have certain limitations in identifying duplicates, the use of advanced biochemical (isozyme polymorphism) and molecular [restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), etc] approaches could precisely contribute to the germplasm characterization, management and utilization and are essentially needed for efficient characterization of *P. edulis* which would, in turn, be invaluable for the conservation and improvement of the crop.

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