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Diversity in the morphology of Amaranth (*Amaranthus* sp.) germplasm Collection in the Philippines

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ABSTRACT---- ***The diversity in the morphological characters of 18 accessions of amaranth germplasm collection at National Plant Genetic Resources Laboratory (NPGRL) was investigated. This study will provide valuable information regarding the potential of the germplasm collections as well as identify gaps for future collecting missions. These germplasm materials have been collected from 10 different provinces in the Philippines. Morphological characterization was conducted using standard descriptors published by International Board for Plant Genetic Resources (1981). A total of 34 characters comprising 22 qualitative and 12 quantitative data were observed. Based on Shannon-Weaver Diversity Index, 17 characters (50%) were found to have high diversity (>0.67) while two characters were invariant. Using Gower's coefficient at 0.7 r^2, five clusters were generated from all the morphological characters. Following the taxonomic key, 4 species A. spinosus, A.gracilis, A. hybridus, and A. tricolor were identified in the 18 accessions used in the study. The results of the taxonomic identification and cluster analysis agree with each other.***

Keywords--- Amaranth, morphology, characterization, taxonomy, clustering

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1. INTRODUCTION

Amaranth (*Amaranthus* sp.) is a tropical plant which is not fully exploited but its value in the modern world is now being recognized. Tucker (1986) described amaranth as "the once and future crop" while Cai et al. (2004) cited that it is a rediscovered "new" crop. Osava (2010) mentioned that the US National Academy of Sciences declared amaranth as "the best food of plant origin for human consumption." Amaranth species have been reported to be distributed and utilized for food as a vegetable worldwide (Muyonga et al. 2008). It is the most popular leafy vegetable in Malaysia and Indonesia aside from kangkong (Grubben, 1993). In the Philippines, it is locally known as *kulitis, uray, aya, duot, lasian, pupay,* and *kadiapa* among others. The leaves may be eaten as a vegetable, while the seeds can be used as breakfast cereal and whole grain flour. The leaves of the young plants grown for grain can be used as both human and animal food. Muyonga et al. (2008) mentioned that the seeds or flour from grain amaranth can be used to make products such as cookies, cakes, pancakes, bread muffins, crackers, pasta, and other bakery products. Some species are used as ornamental plants, source of a deep red dye, and as exclusive food source of some Lepidopteran species. Amaranth is rich in vitamins and minerals, as well as antioxidants linked to the promotion of good health. Antioxidants are one of the body's defense mechanism against diseases, and they work by scavenging free radicals that cause DNA damage.

Amaranth is a C4 plant which efficiently uses atmospheric carbon dioxide which occurs in a few plants such as sorghum, corn, and sugarcane. Grubben (1993) cited that amaranth is probably the highest yielding leafy vegetable of the tropics with excellent nutritional values and a cheap green vegetable in the markets. It produces a large amount of biomass in a short period of time (Kauffman and Weber, 1990) and grows very rapidly, particularly under bright sunlight, high temperature, and dry soil. There are also other genotypes that can tolerate unfavorable soil conditions like high salt, acidity, or alkalinity (Tucker, 1986). These are just some of the characteristics of amaranth that makes it a good crop in the future because of our changing climate. It has a potential to contribute as a cheap source of essential proteins and an increase in world food production.

NPGRL holds 54 accessions of amaranth collected from different parts of the Philippines. Theseaccessions have already been collected and stored for a long period of time hence this study will also help regenerate them. These materials need to be characterized and evaluated to properly assess their full potential (for human consumption, productivity, and many others). Assessment of genetic diversity may also provide information to facilitate the identification of gaps for future collecting expeditions.

The study was conducted to assess the genetic diversity of amaranth germplasm collections in the Philippines and to identify gaps for future collecting missions.

2. MATERIALS AND METHODS

Source of Materials

The different accessions of amaranth were obtained from the National Plant Genetic Resources Laboratory (NPGRL) seed storage rooms of the Crop Science Cluster-Institute of Plant Breeding, UP Los Baños, Laguna. NPGRL holds 54 local collections of amaranth. However, only 13 accessions had good seed germination. Six accessions came from Ifugao, two from Abra, and one each from Surigao del Norte, Nueva Ecija, Bohol, Marinduque, and Camarines Sur.To increase the number of accessions for the study, five accessions were collected from Laguna (4) and Pampanga (1) in 2009.

The seeds were sown in seed boxes in a screenhouse and watered daily. The seedlings were transplanted after 3 weeks in 10 x 10 cm plastic bags and a basal fertilizer of 5 g per plant of urea (46-0-0) was applied. Eighteen accessions and 15 plants per accession were planted for morphological characterization. Watering was done regularly to ensure the normal growth and development of the plants. Weeding was also done whenever necessary. The experiment was conducted at NPGRL, CSC-IPB, UP Los Baños from January to August 2010.

Morphological Characterization

Morphological characterization was carried out using standard descriptor for amaranth, published by International Board for Plant Genetic Resources (1981). Characters found to be necessary in the characterization but not in the list were included. A total of 34 characters were scored including 22 characters for vegetative, 9 inflorescence, and 3 characters for the seed. Twenty two qualitative and 12 quantitative characters were observed. For the quantitative traits, 10 plants per accession were measured and the averages were computed.

Genetic Diversity Analysis

Shannon-Weaver Diversity Index (H', Shannon and Weaver, 1949) was used to analyze genetic diversity of selected amaranth germplasm collection at NPGRL. Standardized H' were classified as low (0-0.33), intermediate (0.34-0.66), and high (0.67-1). Data generated from morphological characterization were analyzed using SAS program. Gower's Coefficient was used to generate clusters all morphological data.

3. RESULTS AND DISCUSSION

Morphological Characterization

Quantitative traits

A total of 12 quantitative traits were observed. The mean, standard deviation, and range of observation from 18 accessions are presented in Table 1. These were composed of plant height at flowering and at maturity, length of top and lateral branches, stem diameter; leaf length and width, petiole length, days to flowering, terminal and lateral inflorescence lengths, and 1000-seed weight. Results from the studies of Varalakshmi (2004) on 46 accessions of amaranth indicated a high range of variability in terms of plant height (31-81.5 cm), length of basal lateral branches (2.3-103 cm), length of top branches (5-58.3 cm), leaf width (3-12 cm), petiole length (3-9 cm), inflorescence length (5-50 cm), inflorescence lateral length (2.5-32.6 cm), length of axillary branches (0.2-5 cm) and days to flowering (29-69). The information generated from this characterization data is important to fully utilize the potential of promising amaranth accessions. Plant breeders interested in developing a cultivar that has desirable traits such as tallness, abundant leaves, and late flowering (Varalakshmi 2004) will already have an idea on which accessions they can select. Tall plants usually take longer to flower; hence, longer vegetative stage and more leaves produced. These accessions can be evaluated further for direct utilization or as parents in their breeding programs.

Principal Component Analysis revealed 10 important quantitative characters contributing 84% of the total variation observed. These include plant height at flowering and at maturity, leaf length, petiole length, leaf width, stem diameter, terminal inflorescence length, days to flowering, and length of top and lateral branches.

Table 1. Mean, standard deviation, and range of quantitative data of the 18 accessions of amaranth

CHARACTER	MEAN	STANDARD DEVIATION	HIGH	LOW
Plant height at flowering (cm)	95.56	32.54	159.10	39.75
Length of basal lateral branch (cm)	23.16	10.02	41.65	9.80
Length of top lateral branch (cm)	6.11	2.89	12.25	1.39
Leaf length (cm)	15.89	6.17	22.74	6.30
Petiole length (cm)	8.23	3.25	12.75	2.72
Leaf width (cm)	8.76	3.01	12.40	3.27
Stem diameter (cm)	1.87	0.60	2.95	0.68
Terminal inflorescence length (cm)	27.87	10.01	42.98	12.50
Lateral inflorescence length (cm)	14.54	4.45	24.10	8.49
Plant height at maturity (cm)	136.27	33.65	178.60	72.67
Days to flowering	53.89	15.11	74	28
1000 seed wt (g)	0.33	0.11	0.67	0.15

Qualitative traits

Out of the 22 qualitative traits observed, two descriptors were found to be invariant. These include growth habit and leaf pubescence, where all the accessions were found to be erect and non pubescent leaves, respectively. The predominant stem characteristic in the collection include: all branches are along the stem, non pubescent, green/green with purple stripes pigmentation, and no spines. For leaf characters, most accessions had normal green pigmentation, lanceolate shape, entire margins, obtuse apex, round base, green upper and lower color, and green petiole pigmentation. The distribution of ruggose and smooth characters leaf veins were even, 9 from each character. The terminal inflorescence of most accessions had panicle with short branches shape and erect attitude. More than half of the accessions had no axillary inflorescence and most of the inflorescence were green and dense. Majority of the seeds had brown color and round shape.The diversity in leaves and stem of amaranth is shown in Figure 1. Leaf variations include shapes (lanceolate and elliptic), color (green and purple/red), and sizes (length and width). Differences in stem includes: pigmentation (green, purple/pink, and green with purple stripes), size, and the presence or absence of spines.

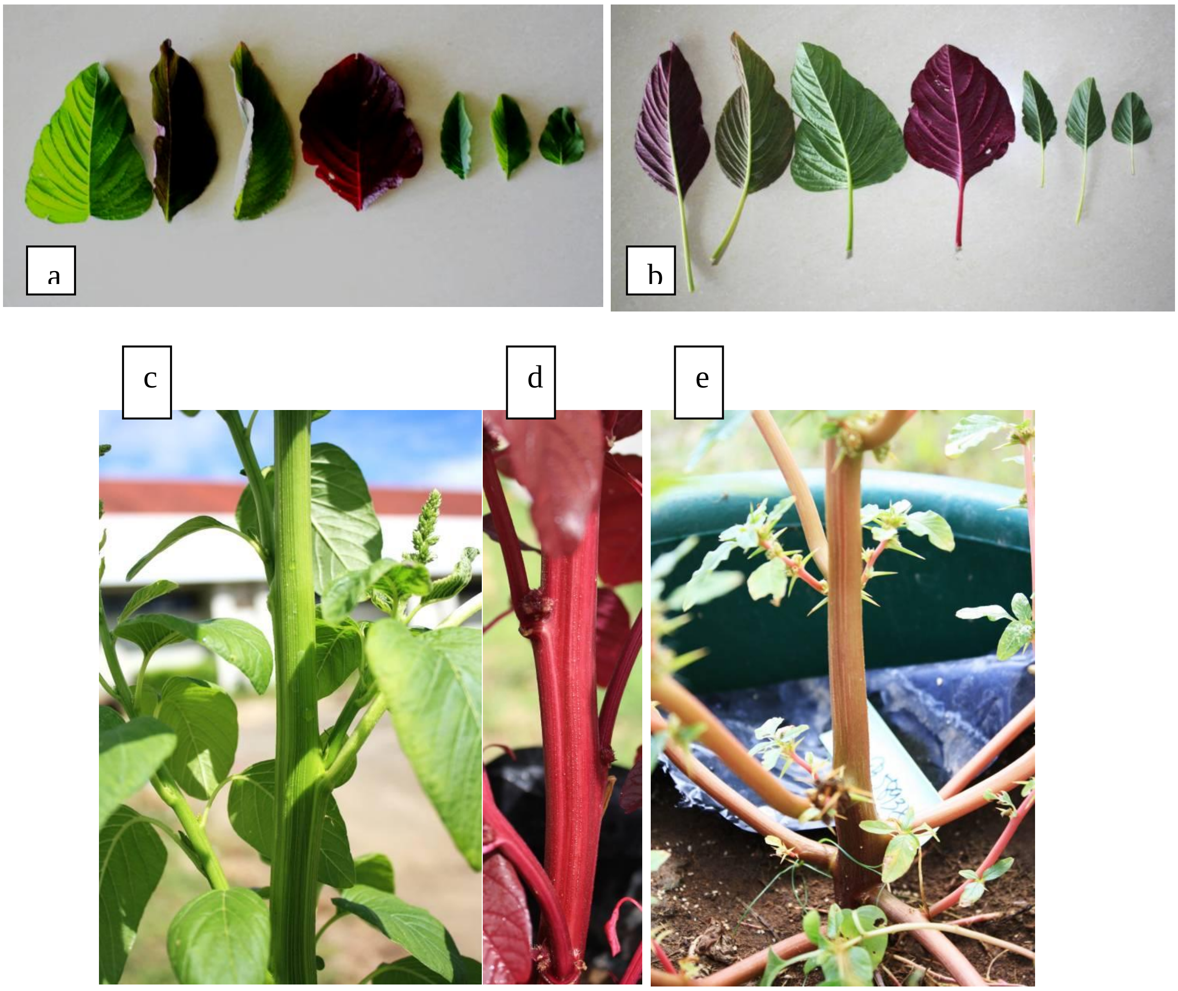

Figure 1. Diversity of amaranth leaves (a,b) and stem (c,d,e) in terms of colors, shapes, and sizes

Diversity Analysis

Shannon-Weaver Diversity Index revealed high diversity (>0.67) in prominence of leaf veins, presence of axillary inflorescence, stem pigmentation, seed shape, length of top lateral branch, stem diameter, inflorescence density, length of basal lateral branch, terminal inflorescence shape, lower leaf color, days to flowering, inflorescence color, leaf length, plant height at flowering, leaf width, terminal inflorescence length, and lateral inflorescence length.

Based on the characterization data, cluster analysis was conducted to group accessions with similar traits. Gower's Coefficient was used in analyzing all morphological traits. This method generated 5 clusters at 0.7 r^2(Figure 2).The cluster means of different quantitative traits of amaranth are presented in Table 2.Cluster 1 is composed of 4 accessions and all the accessions had panicle with long branches terminal inflorescence shape, a trait not found in other clusters. The average of the accessions also had the shortest basal and top lateral braches but they possessed the longest lateral inflorescence length. Cluster 2 had 8 accessions and 7 of which were collected from Ifugao province. The accessions from Ifugao came from the same geographic location, having similar environmental conditions which might have caused their similar traits. This cluster is unique because all the accessions had lower leaf color of green with purple veins and a terminal inflorescence shape of panicle with short branches. The average of the accessions in cluster 2 had the highest in terms of: height at flowering, leaf length, petiole length, stem diameter, terminal inflorescence length, and height at maturity. Cluster 3 is the only cluster where all the three accessions had lax inflorescence. It also had the lowest average heights at flowering and maturity, stem diameter, terminal inflorescence length, lateral inflorescence length, and the earliest to flower. The fourth cluster is exclusive for accessions with spines and they also possessed purple petiole pigmentation, and had the shortest petioles, narrowest leaves, and the smallest seeds. Cluster 5 is composed of only one accession, LSG09-004. It had a unique red/purple leaf color both at the upper and lower sections, its entire lamina was purple/red, and its petiole pigmentation was dark purple. It also had the highest length of basal and top lateral branches, leaf width, days to flowering, and 1000-seed weight.

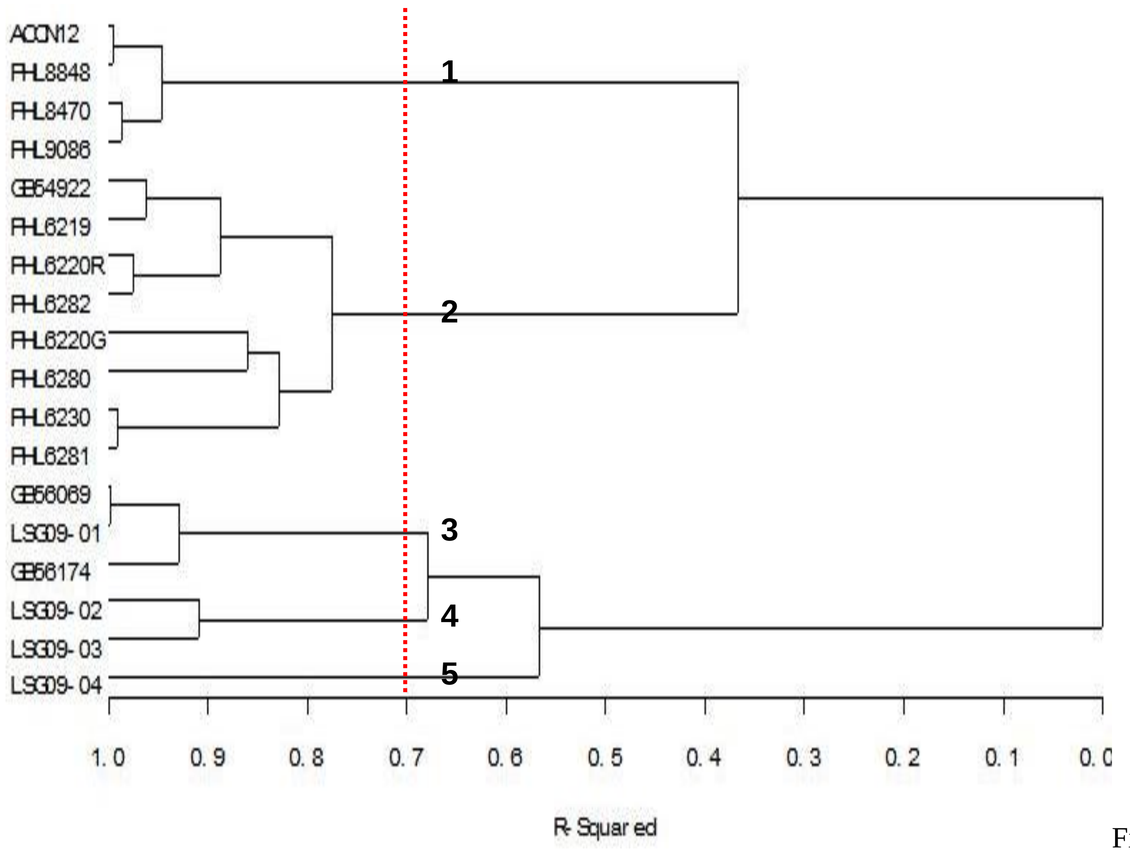

Figure 2.

Dendrogram for all morphological characters using Gower's Coefficient

Table 2. Cluster means of different quantitative traits of amaranth using Gower's Coefficient

CHARACTERS	CLUSTER 1	CLUSTER 2	CLUSTER 3	CLUSTER 4	CLUSTER 5
Plant height at flowering (cm)	100.65	120.17	47.48	54.86	104.00
Length of basal lateral branch (cm)	12.36	20.23	33.96	32.72	38.35
Length of top lateral branch (cm)	2.83	6.58	7.62	5.43	12.25
Leaf length (cm)	15.78	21.21	6.79	7.13	18.70
Petiole length (cm)	7.73	11.16	4.54	3.19	7.90
Leaf width (cm)	11.28	9.81	4.81	3.63	12.40
Stem diameter (cm)	1.83	2.36	1.00	1.20	2.05
Terminal inflorescence length (cm)	31.49	34.93	12.85	14.13	29.50
Lateral inflorescence length (cm)	18.00	16.42	8.90	9.79	12.00
Plant height at maturity (cm)	154.90	158.17	78.64	119.75	.
Days to flowering	51.25	66.13	31.33	36.50	69.00
1000-seed wt (g)	0.30	0.36	0.30	0.16	0.67

Taxonomic Classification

There appears to be a great confusion in the taxonomy of the genus Amaranthus (Grubben, 1993). In the Philippines, Rivero (1996) conducted a comprehensive taxonomic treatment of the family *Amaranthaceae*. The key to the species in the country were described in the paper and written as follows:

1. Nodes armed with spines, stamens 5…………………………………. 1. *A. spinosus*
1. Nodes unarmed, stamens 3.
   2. Flowers in rounded clusters, utricles dehiscent……………….. 2. *A. tricolor*
   2. Flowers in elongated spikes or panicles, utricles indehiscent.
      3. Flowers in panicles, utricles smooth………………….. 3. *A. hybridus*
      3. Flowers in spike, utricles tuberculate…………………. 4. *A. gracillis*

The 18 accessions of amaranth used in the study were not classified in their passport data in terms of their species. Using the taxonomic key as well as the illustrations presented by Rivero (1996), all the four species were recognized among the accessions used in the study.

LSG09-02 and LSG09-03 were easily identified because their nodes were armed with spines and were therefore classified as *A. spinosus*.

Among the other accessions with no spines, only LSG09-04 had dehiscent utricles. The utricle is the part of the plant that holds the seed. Accessions with dehiscent utricles have high seed shattering characteristic because the utricle split open at maturity which allows the grains to fall away (Legacy, 1990). The flowers of LSG09-04 also had rounded clusters throughout the stem. Hence, it can be classified as *A. tricolor* since its characters matches the species.

The rest of the accessions can be reclassified into 2 groups. The first group had flowers with elongated spikes while the other group had flowers in panicles and indehiscent utricles. GB56069, LSG09-01, and GB56174 had flowers with elongated spikes. This suggests that they belong to *A. gracilis*. All these accessions had lax inflorescence density. They also possessed the lowest average heights at flowering and maturity, stem diameter, terminal inflorescence length, lateral inflorescence length, and the earliest to flower.

All the remaining accessions had no spines, flowers in panicles and indehiscent utricles, hence classified as *A. hybridus*. These accessions include: ACCN12, PHL8848, PHL8470, PHL9086, PHL6219, PHL6220R, PHL6282, PHL6220G, PHL6280, PHL6230, PHL6281, and GB54922. These accessions can however be subdivided into 2 groups. The first group had flowers characterized by panicles with long branches which is composed of ACCN12, PHL8848, PHL8470, PHL9086. The second group, on the other hand, had flowers characterized by panicles with short branches (PHL6219, PHL6220R, PHL6282, PHL6220G, PHL6280, PHL6230, PHL6281, and GB54922).

4. CONCLUSION AND RECOMMENDATION

The value of amaranth is now being recognized, as evident by the rapid expansion in its production in different parts of the world. It is a cheap source of nutrients and antioxidants, easy to grow, and adapted to local growing conditions. Its potential as an alternative source of food is becoming popular because of the increasing demand for healthy foods in the market. Increasing the demand will allow farmers to produce more allowing its conservation through use.

This experiment was undertaken to assess the variability that exist on the selected amaranth germplasm collection at NPGRL in terms of its morphological features. A total of 18 accessions were used in the study, 12 accessions came from the original collection of NPGRL and an additional six accessions were collected. Thirty-four characters (12 quantitative and 22 qualitative) were considered at 2 growth stages: vegetative and reproductive. There were two characters found to be invariant; all accessions had erect growth habit and the leaves were non-pubescent. Fifty percent of the characters had high diversity (>0.67) using Shannon-Weaver Diversity Index. Using Gower's coefficient, the clustering of all morphological characters generated a tree with five clusters at 0.7 r^2. Species identification was done using a taxonomic key and illustrations. These includes *A. spinosus*(2), *A. gracilis*(3), *A. hybridus*(12), *and A. tricolor* (1). These classification fitted the clustering of accessions using Gower's coefficient.

At present, the germplasm collection at NPGRL does not represent the total diversity of amaranth that is available in the field. Collecting missions should be undertaken and priority should be given to descriptor states not found in the germplasm. All accessions should also be evaluated to assess genetic diversity of the amaranth germplasm.Extensive research should be conducted in order to identify the best nutrient and cultural management practices to increase biomass and antioxidant properties of amaranth, as well as specific compounds such as vitamin A, B, C, iron among others.Information on parameters such as number of leaves, yield and sensory evaluation will also be valuable information in identifying suitable uses of each accession. Studies about postharvest handling and the manner of food preparation to make it more tasty and attractive should be conducted to help add value to the crop.

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