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GENETIC DIVERGENCE IN AMARANTHUS COLLECTED FROM PAKISTAN

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ABSTRACT

In the present study different species (*Amaranthus hypochondriacus* & *A. tricolor*) of amaranthus were collected from different agro-ecological zones of Pakistan and compared for their phenotypic and nutritional traits. A UPGMA cluster, grouped the 13 amaranthus genotypes into two major clusters, I and II, differentiating the ornamental amaranthus cultivars from edible. However, comparative view of the cluster showed that the *Amaranthus hypochondriacus* were closest to the China variety than to the *Amaranthus tricolor* according to their morphological characters. Optimal level of carbohydrates, fats, proteins and moisture contents were observed in 7033 (192.7 mg/ml), 7051(30.68 %) and 7033 (102.7 µg/ml) and 7034 (16 %) respectively, of Gonar, Northern Area and AJK of Pakistan. Accession numbers were allotted to the amaranthus germplasm and preservation of there seeds in the gene bank at PGRI, Islamabad, for future research activities including evaluation, characterization and crop improvement.

Key words: Diversity, phenotype, amaranthus, altitude, nutrition.

INTRODUCTION

Amaranthus belongs to the family Amaranthaceae, collectively known as amaranth, is a cosmopolitan genus of herbs. *Amaranthus cruentus*, *A. hypochondriacus*, and *A. caudatus* are the essential grain species. The plants are characterized by great diversity of species and forms, and green parts of some species are used as a vegetable. Amaranthus shows a wide rage of morphological diversity among and even within certain species. People around the world value amaranths as leaf vegetables, cereals, and ornamentals. *A. hypochondriacus* (prince's feather) and *A. cruentus* (purple amaranth) are commonly grown for grain and *A. tricolor* (tampala) is grown primarily for the leaves. *A. caudatus* (love-lies-bleeding) is a third type of grain species, although is often grown more as an ornamental. When used as a grain type, *A. caudatus* varieties are best adapted to the tropical highlands. Other vegetable amaranths are represented by *A. dubius*, *A. blitum* and *A. cruentus* (purple amaranth); weedy species are represented by *A. retroflexus* (redroot pigweed), *A. albus* (tumbleweed) and *A. spinosus* (spiny amaranth). There are not many genetic differences in amaranth species. They are easily cross-bred, and even weedy types will cross with the intended crop if not rogued from the field (Brien and Price, 2008).

The genetic diversity of amaranth is widely related by archaeological, historical and morphological data from the agricultural and commercial point of view, the characterization of germplasm is based on high and low hereditability characteristics, with environmental influence as the main obstacle. It is difficult to taxonomically classify, since it would consider characteristics such as pigmentation that presents wide segregation and the size of the plant; that depends on the hours of sunlight and other environmental variables. Finally, the amaranth plant has high plasticity (Espitia, 1986).

The amaranth grain is one of the valuable foods as it has high protein content and well balanced amino acid profile (Gamel, *et al*. 2006). Chemical composition and nutritional value of amaranth grain (Bressani, *et al*. 1987; Dodok, *et al*. 1997; Andrasofszky, *et al*. 1998) confirms its high potential for use as human and animal nutrition as well as medicine (Oke, 1983; Teutenico and Knorr, 1985). With the increasing need of exploring alternate sources of food, it is necessary to accelerate and expand the production of amaranthus. The objective of this study was to evaluate the phenotypic diversity of amaranthus germplasm and to search alternative rich nutritional source to face the challenges of food constrains.

MATERIALS AND METHODS

Amaranthus seeds were collected from different agro-ecological zones of Pakistan with altitudinal variation from 710 to 2433 miles at sea level (m.a.s.l) coded with collection number as 7029 (710 m.a.s.l; NWFP Mansehra), 7030 (1470 m.a.s.l; NWFP Mansehra), 7033 (1500 m.a.s.l; Nortern Area, Gilgit/Chilas), 7034 (1560 m.a.s.l; Northern Area, Gilgit), 7036 (1500 m.a.s.l; Northern Area, Gilgit), 7039 (1575 m.a.s.l; Northern Area, Garuch), 7041 (1950 m.a.s.l; Northern Area, Gilgit), 7043 (2120 m.a.s.l; Northern

Area, Gilgit), 7047 (2433 m.a.s.l; Northern Area, Gilgit), 7051 (396 m.a.s.l; AJK/ New Mirpur), 7058 (885 m.a.s.l; AJK/ Punch Rawalakot),7065 (1550 m.a.s.l; AJK/ BAGH) and one variety acquired from China was also included in the present study. All the samples collected for the study belonged to the species *Amaranthus hychondriaccus* (7029, 7030, 7033, 7034, 7036, 7039 7051, 7058 and 7065) and *Amaranthus tricolor* (7041, 7043, 7047 and China).

Germination test was performed with the sterilized seeds of amaranthus according to the paper towel method (Ruiz'SC and Bressani 1990). After a week, the number of seeds germinated was recorded. Seed germination percentage was calculated according to the formula:

Germination % = Number of seed germinated x 100/total number of seed

For morphological characterization, the seeds of amaranthus were multiplied. Sowing of amaranthus seeds was carried out in the month of July 2010, in the germinating trays having sand, clay and peat (1:1:1) as growth medium in the glass house. Seeds emerged within a week. The plants were transferred at 4 leaf stages to the fields of PGRI, NARC, Islamabad, in plots with 45 cm plant to plant and row to row distance 60 cm (plot size 7x8 ft). The experiment was performed in triplicates. Data were recorded for three randomly selected plants for each sample at different growth stages. Quantitative traits including plant height, leaf area, canopy, total branches/plant, number of spikes/branch, stem and yield were recorded. In addition qualitative traits such as stem color, leaf color, seed color and seed shape were also considered. After harvesting, seeds were threshed by hand. The threshed seed was cleaned by winnowing and air dried.

Biochemical characterization of nutritional content of amaranthus seeds was done by estimating total protein, total fats, total carbohydrate content and moisture according to standard methods (AOAC, 2010).

Statistical analyses were performed using Mstat-C and Microsoft Excel software. Analysis of variance and Duncan multiple range test (DMRT) were applied for comparison of different collections of amaranthus with the altitudinal variation.

Unweighted pair group method with an arithmetic average (UPGMA) cluster analysis was used to infer genetic relationships and phylogeny among 13 genotypes of amaranthus by using the NTSYS-pc, Version 2.2 package (Rohlf, 2005; Rabbani, *et al*. 2008).

RESULTS AND DISCUSSION

Thirteen, genotypes of amaranthus were collected from the different agro-ecological zones of Pakistan with the altitudinal variation from 710 to 2433 m.a.s.l. Analysis of variance showed highly significant differences for all the traits studied (Table 1).

The data depicted that germination% ranged from 36 to 96% at 25°C, which was similar to the previous studies as reported by different researchers (Prizster, 1958; Jehlik, 1990; Lanta, *et al*. 2003). The highest germination rate was observed 96.67% in 7058 followed by 7029 (93%).

Plant height ranged from 67cm to 116.7 cm. Highest plant height was recorded for 7033 (116.7 cm) followed by 7058 and 7065 (83.3 cm and 74.33cm, respectively). Greatest leaf area was observed for "7041" (150cm^2) and lowest for "7033" (84 cm^2). Similarly plant canopy ranged from 203 to 253 cm. The largest canopy was exhibited by 7030 (253 cm). These results coincide with earlier findings (Prizster, 1958; Kulakow and Hauptli, 1994; Weber, *et al*. 1990).

The range for number of branches/plant and number of spikes/plant was 5-13 and 1-19, respectively. Highest numbers of branches (19) were observed for amaranthus variety obtained from China while 7065 secured second position for number of branches (10). Lowest number of branches (7) was observed in two samples (7029 and 7030). Maximum average number of spikes/plant (19.6) produced by 7029 while 7041, 7043 and 7047 produced minimum number of spikes/plant (1).

Perusal of the data revealed that highest yield 129.3 g/plant was produced by 7030 and lower levels were observed in 7041 (5 g), 7043 (9 g) and 7047 (13 g). Similar conclusions were also illustrated in different studies (Prizster, 1958; Popenoe, *et al*. 1989; Weber, *et al*. 1990; Gupta and Gudu, 1991; Kulakow and Hauptli, 1994; Jarošová, *et al*. 1998; Lanta, *et al*. 2003) perhaps due to difference in breeding material or variation in environment or interaction.

There was significant relationship among all the tested morphological traits except leaf area with plant height which showed non significant relationship (Table 1). Stem, leaf, seed color, and seed shape was also recorded as qualitative traits. Stem color of 7029, 7030, 7033 and 7039 were observed pink. Three collector numbers (7030, 7033 and 7039) had pink colored leaves while all others have green leaf color. Among all the tested collector number variety collected from China exhibited unique leaf (maroon) (Figure 1).

The seed color showed variation from dark brown to black i.e., seeds of collector No.7036 and 7039 were of maroon and dark brown in color while all other collector numbers were found brown. Studies showed that commonly, brown to black color of amaranthus seeds (Lanta, *et al.* 2003). Dark color is distinctive for a hard seed coat which is an imperative trait for long term preservation in seed banks (Barton 1961). Moreover, The anthocyanin (reddish) pigments in amaranth flours and vegetation appear to have great potential for competing with sugar beets as a source of natural, non-toxic red

dyes, could be of value in both food and industrial uses (Myers, 2002). Currently, no variation was observed in seed shape as all were found round shaped.

Nutritional profile of amaranthus seeds showed that highest amount of total carbohydrate, fats, protein and moisture contents were observed in the samples 7033 (190 mg/ml), 7051(31.03%) and 7033 (100 µg/ml) and 7051 (13.75 %) respectively (Table 2). Over all, the nutritional profile of the amaranthus seeds showed high nutritional contents with remarkable differences among the varieties. Similar were the observation regarding nutritional profile in various studies (Pedersen, *et al*. 1987; Aphalo, *et al*. 2009).

Cluster and principal component analysis for the phenotypic relationship among the amaranthus cultivars was assessed by the Unweighted Pair Group Method with an Arithmetic average (UPGMA)

The UPGMA cluster analysis diagram grouped the 13 amaranthus genotypes into two major clusters, I and II, differentiating the ornamental amaranthus cultivars (7041, 7043 & 7047) from edible cultivars having additional sub-clusters within the both clusters (Figure 2). The same two groups have been reported earlier by Ray and Roy (2008) however, other studies were dissimilar with these two groups (Shukla, *et al*. 2010). This difference may be due to their collection from diverse geographical zones.

Group-I consisted of 10 genotypes which showed clear division into two subgroups; I and II. Subgroup I comprised of genotypes numbered as 7029, 7034, 7036, 7039, 7030 and 7051. Genotypes 7033, 7058, 7065 and China were included into sub group II. Group II comprised of a total of 3 cultivars belonging to ornamental amaranthus. The cluster analysis placed most of the tall genotypes with greater number of spikes and high yield together, showing a high level of genetic association among these cultivars.

The dendrogram showed that the genotypes that were derivatives of genetically similar type, clustered together. The genotypes were closely related with each other within the first non ornamental group, whereas relatively diverse within the second ornamental group. The cluster analysis also revealed that the *Amaranthus hypochondriacus* were nearer to the China than to the *Amaranthus tricolor*. The genotypes 7041, 7043 and 7047 mostly shared a high proportion of ancestry and/or agronomic characteristics of *A. tricolor* and Group II cluster belongs to Northern Area, Gilgit, Pakistan. In this study, a number of traditional and improved cultivars originally from various regions, did not form distinct groups. These were interspersed with each another in the cluster analysis, which confirmed no association between the cultivars growth patterns and their geographic origin under investigation.

Euclidean distance among pairs of collector numbers showed highest similarity 93.79% among 7041 and 7029, which belonged to Gilgit, Mansehra and NWFP respectively. Lowest dissimilarity of 1.24 was observed between 7039 and 7036 due to of narrow selection zone. 10-100 % similarity among varieties of *Amaranthus hypochondriacus* (Mandal and Das 2002) reported

Table 1: Mean performance of Amaranthus lines for various quantitative traits.
(abcdefg is statistical data grading)

Acc. No.	Advance lines/ varieties	Germ. (%)	Plant height (cm)	Leaf area (cm^2)	Canopy (cm)	No. of branches/plant	No. of spikes/ plant	Grain yield (g)
24654	7029	93.33^{a}	68.00^{bcd}	31.07^{d}	203.3^{bc}	7.00^{d}	19.67^{a}	66.67^{e}
24655	7030	86.67^{a}	48.00^{defg}	32.50^{d}	253.3^{a}	7.00^{d}	7.00^{d}	129.30^{a}
24656	7033	83.33^{a}	116.70^{a}	84.00^{bc}	150.0^{d}	11.00^{b}	11.33^{b}	118.00^{b}
24657	7034	70.00^{ab}	43.00^{efg}	58.93^{bcd}	150.0^{d}	7.00^{d}	9.00^{c}	27.67^{g}
24658	7036	76.67^{a}	53.00^{cdefg}	28.03^{d}	160.0^{cd}	9.33^{c}	8.33^{cd}	18.00^{h}
24660	7039	70.00^{ab}	63.00^{bcde}	30.25^{d}	206.0^{b}	7.67^{d}	7.00^{d}	33.67^{f}
27372	7041	43.33^{bc}	55.00^{cdef}	150.30^{a}	80.0^{ef}	12.67^{a}	1.00^{e}	5.00^{i}
27373	7043	43.33^{bc}	35.67^{fg}	55.00^{bcd}	70.0^{f}	5.00^{e}	1.00^{e}	9.00^{i}
27374	7047	36.67^{c}	30.67^{g}	97.00^{b}	60.0^{f}	7.33^{d}	1.00^{e}	13.67^{h}
24663	7051	90.00^{a}	61.33^{bcde}	24.00^{d}	200.0^{bc}	7.66^{d}	2.33^{e}	107.3^{c}
24664	7058	96.67^{a}	83.33^{b}	67.33^{bcd}	158.0^{cd}	7.33^{d}	7.66^{cd}	77.33^{d}
24666	7065	76.67^{a}	74.33^{bc}	68.00^{bcd}	116.0^{de}	10.00^{bc}	12.67	117.00^{b}
27371	China	80.00^{a}	59.33^{bcdef}	44.97^{cd}	90.0^{ef}	13.00^{a}	18.33^{a}	70.67^{e}
Variety Mean		1182.4^{**}	1495.2^{**}	3825.9^{**}	10829.2^{**}	17.4^{**}	114.4^{**}	6345.3^{**}
Mean value		72.82	60.87	59.34	145.89	8.61	8.18	61.02
±SE		±5.51	±6.20	±9.89	±16.65	±0.67	±1.71	±12.75
LSD %		26.12	21.45	43.21	41.33	1.402	1.749	4.453
Error CV		21.28	2.90	43.18	16.79	9.66	12.69	4.33

Table 2: Seed nutritional profile of different Amaranthus varieties.
(abcdefg is statistical data grading)

Acc. No.	Collector No.	CHO (mg/ml)	Fats (%)	Protein (µg/ml)	Moisture (%)
24654	7029	152.7^{d}	16.75^{fg}	40.00^{g}	11.73^{bc}
24655	7030	182.0^{b}	19.29^{ef}	95.33^{b}	12.60^{bc}
24656	7033	192.7^{a}	23.27^{cd}	102.70^{a}	13.20^{b}
24657	7034	87.67^{g}	23.69^{c}	86.00^{c}	16.00^{a}
24658	7036	182.3^{b}	15.05^{g}	53.33^{f}	10.17^{cd}
24660	7039	142.0^{e}	18.19^{f}	82.67^{cd}	12.00^{bc}
27372	7041	180.0^{b}	21.07^{de}	35.00^{h}	13.00^{b}
27373	7043	182.3^{b}	28.07^{b}	53.00^{f}	13.00^{b}
27374	7047	153.0^{d}	19.16^{ef}	64.33^{e}	13.00^{b}
24663	7051	153.0^{d}	30.68^{a}	22.33^{i}	13.00^{b}
24664	7058	163.0^{c}	29.86^{ab}	54.67^{f}	12.78^{bc}
24666	7065	104.7^{f}	23.17^{cd}	54.00^{f}	7.17^{e}
27371	China	163.0^{c}	21.82^{cd}	54.00^{f}	9.09^{de}
LSD at 5%	**5.611**	**2.365**	**3.841**	**2.384**	

Fig. 1. Some of the amaranthus germplasm

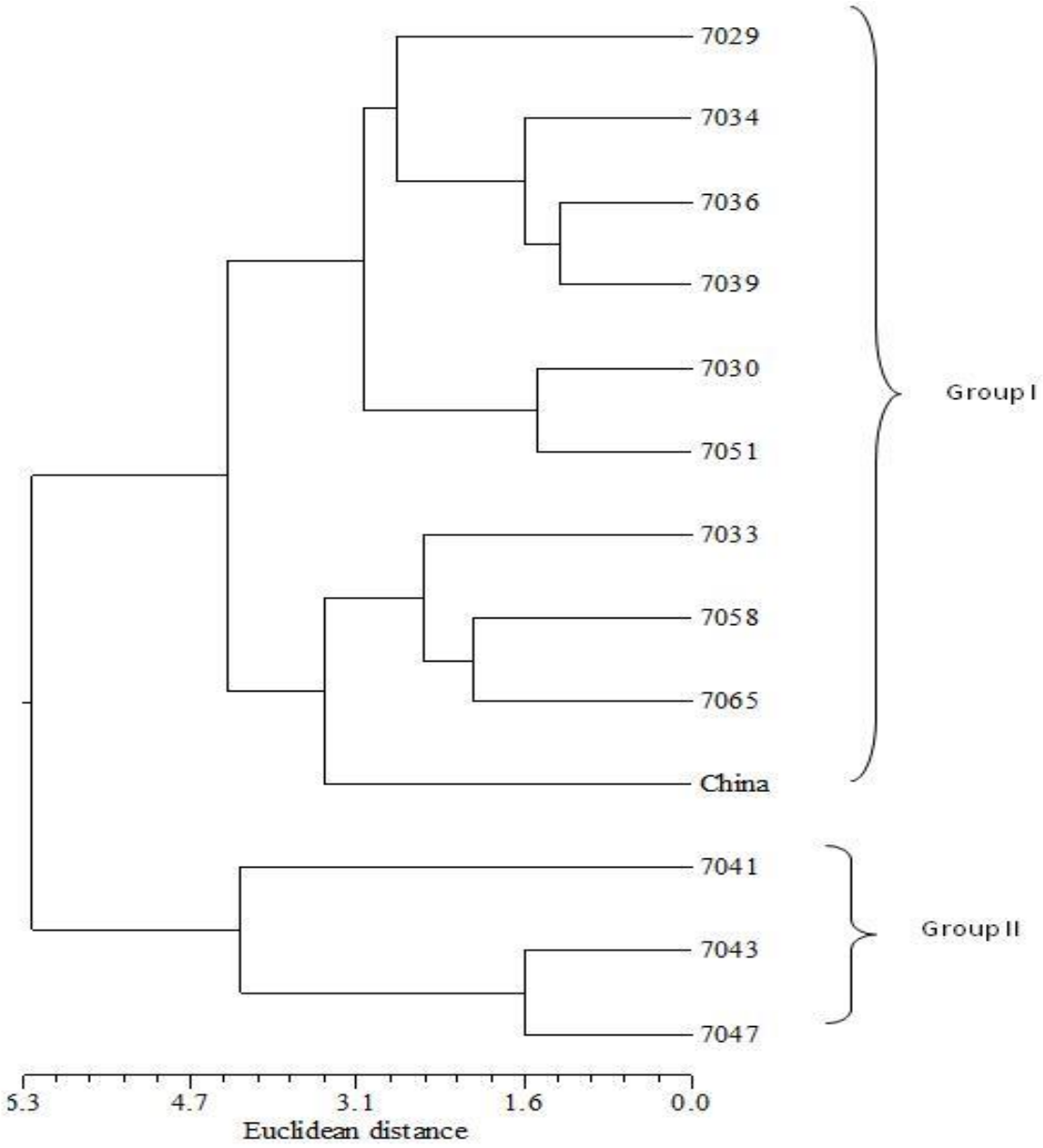

Fig. 2. UPGMA cluster analysis showing the diversity among amaranthus landraces of Pakistan based on morphological traits.

previously. In addition, between *A. hyponchondriacus* and *A. cruentus* there is a relationship, the genetic distance between them being 18-20% (Popa, *et al*. 2010).

The data presented in the current study showed great phenotypic and biochemical diversity among the amaranthus genotypes collected from different agro-ecological zones of Pakistan. Relating to phenotypic diversity to origin/collecting sites of the germplasm indicated the potential for future exploration mission in the area and taking samples with maximum genetic distance to assemble broad based genetic resources of amaranthus for future use. Amaranthus could be a good nutritional alternative to meet the challenges of food constrains.

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