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Research Article

Biotechnological and breeding values from variability studies among Spinach (*Amaranthus hybridus*) collections in Pakistan

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Abstract This work was carried out to assess the biotechnological and breeding values from variation that exist among spinach (*Amaranthus hybridus*) collections. A total of sixty (60) accessions were collected from five (5) different markets and planted in a Randomized Complete Block Design (RCBD) structure. Data was collected on fifty (50) characters (21 qualitative characters and 29 quantitative characters) and subjected to Multivariate Statistical analysis on the Minitab 16.0 software. The results show a wider variety of variability in the majority of the quantitative traits. Percentage germination ranged from 20 to 100% (mean= 51.97±3.83, CV=45.37%). Vigor index ranged from 27.50 to 480.00 (mean =133.70 ± 15.90, CV% = 73.10%). Average number of spikes/plant and spike weight were 135.30 ± 38.60 and 35.70 ± 11.50 respectively. Number of branches, Spike weight and Number of spikes produced are the top three most variable characters based on CV values. Stem pigmentation and spike color gave slight variation among qualitative characters. Accessions with good breeding qualities were selected. Correlation analysis showed relatedness and association among some of the character studied as number of seeds and spikes per plant were highly and positively correlated (r=+0.98). Cluster analysis gave rise to 2 distinct groups with each group demonstrating similarities and differences in characters with a promise for use in cultivar development. The most divergent collections were found in the (AHWD1 and AHWD2) and (AHRM3) markets the distinct groups might serve as propagation lines for improvement of genetic variability in plants through cross-breeding. Thus, the observed variability could be explored in the biotechnological research of the crop in the overall goal of food security.

Keywords | *Amaranthus hybridus*; Biotechnology; Breeding; Improvement; Variability

Keyfindings | The wonderful organizations ought to function breeding traces for genetic development of the plant through pass-breeding. Thus, the observed variability could be explored in the biotechnological research of the crop in the overall goal of food security.

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1. Introduction

Amaranthus (family Amaranthaceae), collectively called amaranth, is a sophisticated genus of annual or brief-lived perennial plants, consisting of approximately 60 species, which consistent with the uses for human intake may be divided into grain and vegetable amaranths (Mlakar*et al*., 2010). The plant commonly called Spinach is an annual herb or forbs and as expected, the plant is in great abundance during the rainy season and it is one of the weeds of *amaranthus* species (Das, 2012) that is generally used as a leafy vegetable (Mobina and Jagatpati, 2015). *A. hybridus*, a weed of great important particularly in Pakistan where it has been domesticated by almost all homes and has a long history of cultivation as a food crop that is still sometimes cultivated on a small scale for its edible leaves and seeds (TPD, 2019). It belongs to C4 plant and widely grown in the tropics (Ebert *et al*., 2011), a comparatively drought-tolerant crop (Olufolaji*et al.,* 2010) And as a leafy vegetable, it can be produced in regions and seasons wherein different vegetation are inaccessible, on the grounds that they develop in poor soil situations and tolerate temperatures up to 40ºc (Ebert *et al*., 2011). *A. hybridus* is a monoecious plant having staminate and pistillate flowers on the same plant, it is self-fertilized as a means of reproduction, each type of flower has five (5) cream sepals and no petals, each staminate flower has five (5) stamens, while each pistillate flower has an ovary with three (3) styles, (Bayer, 2013) and the flowers are wind pollination. The plant is generally considered a weed in North America and is commonly known as smooth amaranth, though it is consumed as a staple food in other areas of the world such as Asia, Africa the Caribbean and part of Europe (Abbasi*et al*., 2013).

One of the reasons why there has been recent interest in Amaranth is because of its nutritional qualities. The leaves, stems and heads of the plant are reportedly high in protein (15 – 24% on a dry matter basis) and the plant produces 30 – 60 t/ha of silage (80% moisture) (DAFF, 2010). Nutritional composition of this vegetable reveals high amount of protein, fat, fiber, ash, mineral elements, vitamins and amino acids (Mofunanya and Soonen, 2017). The Leaves of the plant contains high quality protein with relevant levels of lysine (Andine*et al*., 2013) and are used as leafy vegetables similar to spinach and can be made into tea that is purported to have astringent properties and to cure dysentery, diarrhea and ulcers (Paul *et al*., 2018), they possess antibacterial effect, cleansing effect and also help to reduce tissue swelling (Singh and Sheoran, 2011) and hence, they have been used traditionally for the treatment of liver infections and knee pain and for its laxative, diuretic, and cicatrisation. The plant constitutes a major part of the diet of people in Pakistan. (Mofunanya and Sonnen, 2017)

Amaranth seed and amaranth seed oil is excessive in Vitamin E and squalling, which can be useful for humans laid low with high blood pressure or cardiovascular disorder (Anjalt*et al*., 2013). Chemical composition analysis of the grains of the plant confirms their high potential for human nutraceutical uses (Das, 2016). As summed up by Fernard *et al*.(2012), grains of amaranth are a healthy and nutritious food crop that could benefit people if it was produced and consumed in greater quantities. Morphological traits which can be the phenotypic expression of genetic statistics are grouped into : quantitative and qualitative tendencies, the latter is controlled by using one or few genes at the same time as the previous is controlled by way of interactions of many genes known as polygenes (Aguoru*et al*., 2015). Characters interrelate to make a contribution to the general feature of plant and they'll relate undoubtedly or negatively (Aguoru *et al*., 2015). The diploma of relationship may be explored as morphological markers to display inheritance or likely look of different characters because fowl characters are carefully related, improving one of the characters may have an effect on the other person (Singh *et al*., 2016). The crop deserves attention in crop improvement program and biotechnology to address threats to productivity. This work was carried out to assess the biotechnological and breeding values from variations that exist among spinach (*Amaranthus hybridus*) collections.

2. Materials and Methods

Seed Collection: The seeds of spinach (*Amaranthus hybridus*) were obtained from three (3) different locations in five (5) different markets.

Seed Identification: Seeds were identified and authenticated by Department of Botany, Shah Abdul Latif University, Khairpur and were packaged in small white envelops which was appropriately in-scripted in order to remember the market of purchase as well as the locations within the markets.

Site inspection and Preparation: An area was picked at the botanical garden site due to its proximity to water for irrigation as the rains was yet to be stable and constant with due consideration given to soil type. The land was cleared and total of (15) ridges of 160cm long were made with an average height of 15cm from the ground to accommodate (4) accessions per ridge.

Planting: The seeds of *A.hybridus* were with 4 accessions planted per ridge and plant spacing of 28cm allowed between the accessions. Sign post bearing all the accession codes were made out of wood and Polyvinyl chloride (PVC) material and were placed against the accessions based on their order of randomization and planting in the field for easy identification.

Management practices: After planting, management practices had to be carried out to ensure the effective growth of the plants. Watering of the plants was done on occasions when the rains were not forthcoming to prevent death of plants due to desiccation or water loss, weeding was done at regular intervals and ash was applied on the plants to prevent infestations by insects that feed on their leaves.

Data collection: A total of 50 characters were evaluated consisting of 21 qualitative characters that were assigned unique codes and values to indicate presence or absence (Table 2) and 29 quantitative characters (Table 3) that were assigned unique codes and their values were obtained using meter rule. Data collection on growth parameters commenced at day 15 after planting (DAP) and was done on a weekly basis till the 9^{th} week after planting.

Data Analysis: Data computation was done in an Excel work book and later imported into Minitab 16.0 software for Multivariate Statistical analysis. The relationships among the quantitative and qualitative characters were established using Pearson's Product Moment Correlation method. Cluster analysis was carried out using the Average Linkage method to generate a Dendrogram.

Table 1: Qualitative Characters Evaluated and their assigned codes and values

S/No	Symbols	Qualitative Characters	Codes
1	PHB	Plant habit	1 = Annual, 2 = Biennial
2	P	Phyllotaxy	1 = Opposite, 2 = Alternate
3	LS	Leaf shape	1 = Entire, 2 = Serrate
4	LM	Leaf margin	1 = Entire
5	SP	Stem pigmentation	1 = Reddish stem, 0 = Greenish stem
6	SPS	Stem hairiness/ pubescence	1 = Pubescence, 2 = No pubescence
7	LPS	Leaf hairiness/ pubescence	1 = Pubescence, 2 = N0 pubescence
8	SLC	Sepal colour	1 = Cream, 2 = Red
9	LT	Leaflet texture at days 60	1 = Soft, 0 = Hard
10	FS	Floral symmetry	1 = Bilateral/Actinomorphic,2 = Radial/Zygomorphic
11	ST	Stamen type	1 = United
12	AF	Anther fixation	1 = Free/Dithecous
13	PT	Placentation type	1 = Basal placentation
14	WA	Whorl adhesion	0 = No adhesion, 1 = Adhesion present
15	WC	Whorl cohesion	0 = No cohesion, 1 = Cohesion present
16	PS	Petal shape	0 = No petal

17	FF	Floral formula	1 = Br Brl⊕ $0^{\nearrow}_{+}$ $P_{4-5}A_{4-5}C_{(2-3)}$
18	SPC	Spike colour	1 = Green, 2 = Green & Red, 3 = Red
19	SDC	Seed colour	1 = Blackish Red
20	ST	Seed type	1 = Dry, Utricle
21	SHT	Shell texture	1 = Soft, 2 = Hard, 3 = Very hard

Table 2: Quantitative Characters Evaluated and their units of measurement

S/No	Symbols	Quantitative characters	Units of measurement
1	DOG	Day of germination	
2	NOG	Number of germination	Head count
3	%G	Percentage germination	%
4	PS	Plant spread	cm
5	VI	Vigor index (percentage germination × seedlings length)	
6	DOM	Day of maturation	
7	IL	Internode length	cm
8	NOSP	Number of spikes	Head count
9	FL	Flower length (Calculated as pedicel length + length of floral whorl)	cm
10	NOS	Number of sepal per flower	Head count
11	NOP	Number of petal per flower	Head count
12	NOB@30	Number of branches at day 30	Head count
13	NOB@60	Number of branches at day 60	Head count
14	PH@30	Plant height at 30	cm
15	PH@60	Plant height at 60	cm
16	LL	Leaf length	cm
17	LW	Leaf width	cm
18	WLW	Wet leaf weight	g
19	LPL	Leaf petiole length	cm
20	SC	Stem circumference	cm
21	NOS/P	Number of spikes/plant	Head count
22	SL	Spike length	cm
23	SW	Spike width	cm
24	SWT	Spike weight	g
25	NOSD/P	Number of seeds/ plant	Head count
26	NOSD/S	Number of seeds/spike	Head count
27	SDL	Seed length	cm
28	SDWT	Seed width	cm
29	SDW	Seed weight	g

3. Results

The results obtained for germination studies showed that a total of 38 accessions germinated while 22 accessions did not germinate out of the 60 accessions planted. The highest number of germination was observed in the accession (AHWD3) while accession AHNB1, AHRM3 and AHMM1 had the lowest numbers of germination. Descriptive statistics of the quantitative characters studied are shown in table 4. Day of germination had a mean of 4.74± 0.35, CV% of 44.90 and ranged from day 2 to day 10. Earliest

germination was recorded on day 2 while delayed germination was recorded on day 10. Percentage germination ranged from 20- 100%, CV% of 45.37 and a mean value of 51.97±3.83. Low percentage germination (%G) ranged between 0-40 percent while high percentage germination (%G) ranged between 40%-100%. At day 30, Accession AHMM2A had the minimum plant height of 3cm while the maximum plant height of 49cm was recorded in the accession AHWD1C. Accessions AHWD3B and AHRM1Chad the minimum leaf length of 5cm each, while the maximum leaf length was recorded in accession AHRM2D to be 23.5cm. Accessions AHRM1C, AHMM2A and AHNB2A had the least leaf width of 2cm each while the highest leaf width of 21.5cm was recorded was in the accession AHWD2C. The lowest wet leaf weight of 0.6g was recorded in the accession AHNB3D while the highest wet leaf weight of 7.14g was recorded in the accession AHRM2A. The lowest recorded value of 1.3cm for Leaf petiole length was in the accession AHMM1D while the accession AHWK2C had the highest value of 18.5cm.Accession AHMM2A had the lowest stem circumference of 0.6cm while the highest was recorded in the accession AHRM2B to be 14cm. Lowest Spike width of 1.2cm was recorded in the accessions AHWD1C and AHRM1B while the highest spike width of 15cm was recorded in the accession AHWK2D. Qualitative characters studied showed Plant habit, phyllotaxy, leaf shape, leaf margin, stem hairiness, leaf hairiness, leaflet texture, floral symmetry, stamen type, anther fixation, placentation type, whorl adhesion, whorl cohesion, floral formula, seed color, fruit type and shell texture were all the same in all the accessions except slight variations recorded in Stem pigmentation and spike color (Table 5). Pearson's correlation matrix (Table 6) of some selected quantitative characters showed that the highest correlation was between Number of seeds/plant (NOSD/P) and Number of spikes/plant (NOS/P) (R = 0.98). Number of seeds/spikes highly correlated with seed weight (R = 0.90), Plant height at day 60 highly correlated with leaf length (r= 0.75), Percentage germination had a moderate correlation with plant spread. The results of cluster analysis as represented by the dendrogram which is based on similarity coefficient divided the accessions into two main clusters (cluster 1 and cluster 2) with the level of similarities between the clusters ranging from 99.88 to 70.28 indicating that the accessions studied were similar with little variability existing among them (Figure 1). The most divergent collections were found in the (AHWD1 and AHWD2) and (AHRM3) markets.

Table 3: Descriptive Statistics of Quantitative Characters studied

Variables	Mean	SE Mean	(CV%)	Minimum Range	Maximum Range
Day of germination	4.74	0.35	44.90	2.00	10.00
Number of germination	2.18	0.16	44.98	1.00	4.00
Percentage germination (%)	51.97	3.83	45.37	20.00	100.00
Plant spread(cm)	3.63	0.31	51.79	0.90	8.00
Vigor index(cm)	133.70	15.90	73.10	27.50	480.00
Internode length(cm)	1.62	0.14	51.14	0.30	3.10
Number of spikes	130.00	39.30	183.84	0.00	917.00
Flower length(cm)	9.40	1.87	122.73	0.00	40.00
Number of branches at day 30	1.11	0.35	192.55	0.00	7.00
Number of branches at day 60	2.42	0.81	206.55	0.00	19.00
Plant height at day 30(cm)	16.91	1.56	56.89	3.00	49.00
Plant height at day 60(cm)	68.83	5.71	51.15	17.00	154.00
Leaf length(cm)	15.15	0.99	40.20	5.00	23.50
Leaf width(cm)	8.04	0.74	56.92	2.00	21.50
Wet leaf weight(g)	2.37	0.35	90.27	0.09	8.96

Leaf petiole length(cm)	8.50	0.76	55.45	1.00	18.50
Stem circumference(cm)	6.68	0.52	48.30	0.60	14.00
Number of spikes/plant	135.30	38.60	175.89	0.00	917.00
Spike length(cm)	9.40	1.87	122.73	0.00	40.00
Spike weight(g)	35.70	11.50	198.41	0.00	354.00
Spike width(cm)	3.80	0.71	115.21	0.00	15.00

Table 4: Data on all the qualitative characters

Characters	Numbers of accessions present
Plant habit	Annual
Phyllotaxy	Alternate
Leaf shape	Entire
Leaf margin	Entire
Stem pigmentation	37 Green, 1 Red
Stem hairiness	Pubescence
Leaf hairiness	Pubescence
Leaflet texture	Soft
Floral symmentry	Bilateral/Actinomorphic
Stamen type	United
Anther fixation	Free/Dithecous
Placentation type	Basal
Whorl adhesion	Absent
Whorl cohesion	Present
Floral formula	Br Brl⊕ 0♃ P_{4-5} $A_{4-5}C_{(2-3)}$
Spike colour	37 Green, 1 Red and Green
Seed colour	Reddish black
Fruit type	Dry/Utricle
Shell texture	Hard

Table 5: Correlation Matrix of Selected Quantitative Character Traits

Characters	**%G**	**PS**	**VI**	**NOB@60**	**PH@60**	**LL**	**WLW**	**NOS/P**	**SL**	**NOSD/S**	**NOSD/P**
PS	0.46										
VI	0.53	0.19									
NOB@60	-0.05	-0.02	0.04								
PH@60	-0.07	0.28	0.33	0.41							
LL	-0.06	0.08	0.40	0.19	0.75						
WLW	-0.02	0.16	0.36	-0.08	0.60	0.53					
NOS/P	0.08	0.13	-0.04	0.25	0.35	0.20	-0.07				
SL	-0.00	0.24	-0.14	0.46	0.47	0.01	-0.00	0.67			
NOSD/S	-0.09	0.06	-0.20	0.05	0.15	-0.19	-0.16	0.51	0.67		
NOSD/P	0.08	0.22	-0.07	0.25	0.37	0.18	-0.09	0.98	0.72	0.55	
SDWT	-0.13	-0.19	-0.19	0.07	0.07	-0.20	-0.20	0.41	0.60	0.90	0.40

*Significant at <0.05 level of probability, PS = Plant spread, VI = Vigor index, NOB@60 = Number of branches at day 60, PH@60 = Plant height at day 60, LL = Leaf length, WLW = Wet leaf weight, NOS/P = Number of spike/plant, SL = Spike length, NOSD/S = Number of seeds/spike, NOSD/P = Number of seeds/plant, SDWT = seed weight.

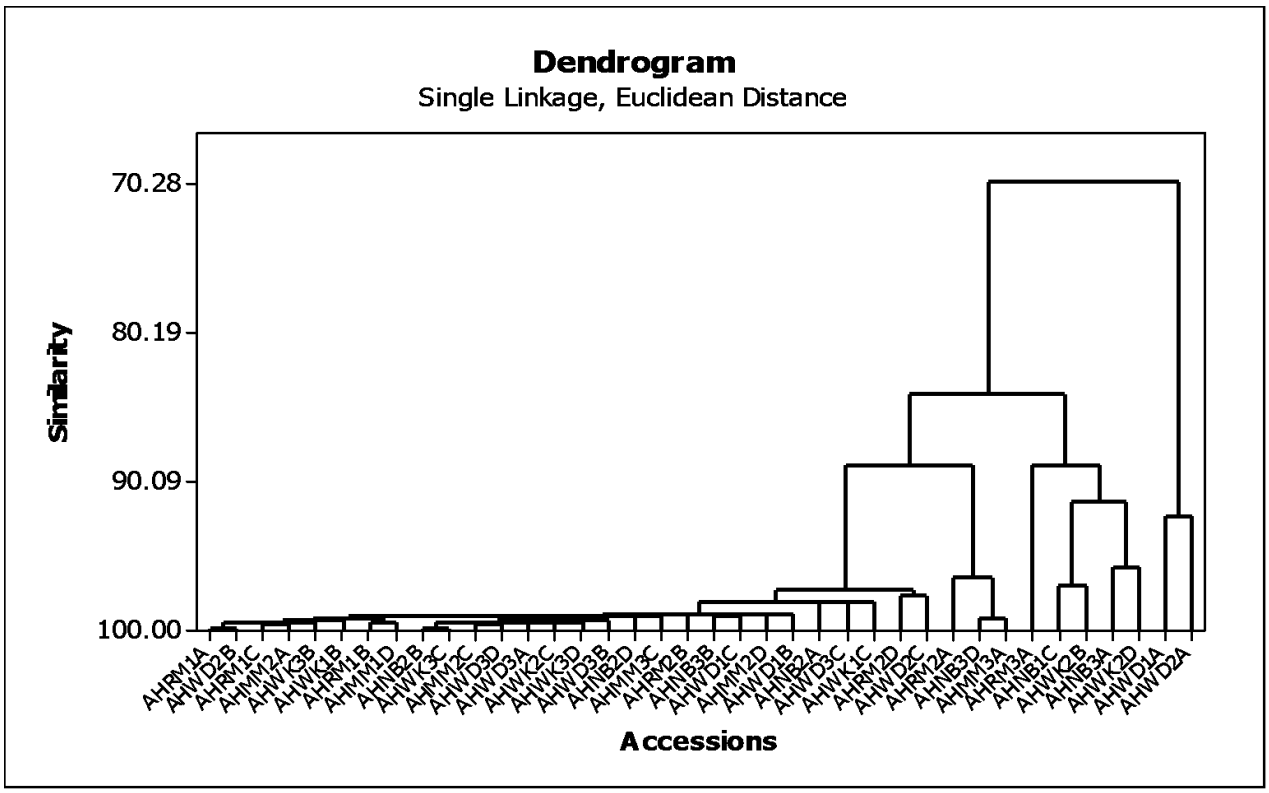

Figure 1: Cluster Analysis Dendrogram of 60 Accessions of *A. hybridus*.

4. Discussion

The value of the Pakistani spinach 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. A detailed breeding and taxonomical knowledge will allow farmers to produce more improved accessions allowing its conservation through use. The wide variability found in spike related tendencies is precious from the perspective of the genetic improvement of Amaranthus hybridus. Wu et al. (2000) in a have a look at of 229 genotypes from 20 Amaranthus species said wide variability which become useful in cultivar development for agronomic traits, a finding that aligned with the work done by Varalaskshmi (2004).

Correlation procedures the strength of association among variables and breeding for yield would be very effective when there is a positive association among characters. In this study,strong positive correlation was observed between many pairs of characters such as between leaf morphology and seed morphology. These findings can be exploited at once or not directly for the genetic improvement of the amaranths as suggested by Hazra and Basu, (2000). Furthermore, Number of seeds per plant depend greatly on number of spikes per plant which signifies that if breeders can genetically improve the genes responsible for spike production, seed production will be greatly and indirectly improved, percentage germination had no significant effect on spike length so breeders who are not after speed or rate of germination can work on accessions with great spike length for improvement, the negative correlation between leaf length and number of seeds produced per spike could also be useful as farmers could harvest vegetables for a long period before harvesting grains especially if the breeding line could be developed that would combine high leaf and grain yield concurrently.

The results from cluster analysis in this study which indicated a very small degree of variability existing among the accessions studied is in tandem with the findings of Oboh (2007) who reported that a great degree of variability exist among the Pakistani accession of *Amaranthus hybridus*. This work goes further to prove the existing report that variability among *Amaranthus* genus is based on anatomical and morphological differences that raise the possibility of further subgenera division (Costea*et al*., 2001).

5. Conclusions

A wide range of variability in the majority of the quantitative traits assessed Number of branches, Spike weight and Number of spikes are the top three most variable characters. Stem pigmentation and spike color gave slight variation among qualitative characters. The most divergent collections were found in the (AHWD1 and AHWD2) and (AHRM3) markets. The wonderful organizations ought to function breeding traces for genetic development of the plant through pass-breeding. Thus, the observed variability could be explored in the biotechnological research of the crop in the overall goal of food security.

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