Plant Omics Journal POJ

POJ 3(4):129-134 (2010) ISSN:1836-3644

Genetic studies for biochemical and quantitative characters in grain amaranth (*Amaranthus hypochondriacus* L.)

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Abstract

Twenty six accessions of grain Amaranth (*Amaranthus hypochondriacus* L.) were evaluated for salient biochemical and quantitative traits particularly reference to chlorophyll a and b, total chlorophyll, phenol content, leaf moisture, leaf protein content, test weight and yield plant^{-1}. Genetic divergence and association among these traits were analysed. Chlorophyll a, chlorophyll b, total chlorophyll and phenol content showed significantly higher values for all the accessions studied indicating thereby that these accessions can be successfully utilized for the improvement of these characters in this crop. Leaf protein content revealed exceptional attributes for ameliorating protein deficiency strictly in the diet of vegetarian people. Leaf protein content was noted significant in four accessions, namely AG-67/1 (3.152 mg g^{-1}), AG-21 (2.452 mg g^{-1}), AG-306 (2.101 mg g^{-1}) and AG-1175 (2.101 mg g^{-1}). Accessions with more leaf protein have potential to increase nutritional value and can be utilized for vegetable purposes. However, knowledge about amaranth leaf composition is still marginal. Using Euclidean cluster analysis 26 accessions were distributed in 3 clusters (at 9.0 euclidean distance) of which cluster I contained maximum (13) accessions, cluster II (10) and cluster III (3) accessions. The determination of chemical composition of leaf is necessary for variety evaluation, on the basis of high nutritive value for human diet. Biochemical characters had no significant genetic association with grain yield plant^{-1} which revealed that biochemical traits can be improved without altering grain yield. Cluster I and III were found more diverse than others and therefore can be used for developing recombinants.

Keywords: *Amaranthus hypochondriacus*, biochemical traits, correlation, genetic diversity

Introduction

Amaranthus (Family *Amaranthaceae*) includes over 75 wild and weedy species native to tropical and temperate regions of the world (Sauer, 1993). Presently, grain amaranths are cultivated in many parts of the world, including Central and South America, Africa, India and China. Scientists working on nutrition have recognized three groups: i) cereals and tubers rich in carbohydrates; ii) legumes and other sources of plant protein; iii) fruits and vegetables rich in iron and vitamins, especially A and C. Amaranths come into all the three groups and its cultivation and use depend on seeds which, contain carbohydrates and proteins (12-16 %), with high lysine content. The major grain-producing species are *Amaranthus caudatus* L., *Amaranthus cruentus* L. and *A. hypochondriacus* L. Amaranth uses the C_4 pathway and has a high efficiency of CO_2 utilization, high photosynthesis rate at high temperature and drought tolerance (Williams and Brenner, 1995), which make it possible to be grown in areas not suitable for other crops (Breene, 1991 and Lehman 1996). The nutritional value of Amaranth has been extensively studied (Becker et. al., 1981; Teutonico et. al., 1985; Martirosyan, 2001, 2003). Grain amaranths have higher protein having significantly higher lysine content than other cereal grains (Bressani, 1989; Lehman, 1989). Amaranth leaves are an excellent source of protein at its blossoming phase for various samples (Kadoshnikov et. al., 2005). Chlorophyll a and b present in leaves of higher plants are the main pigments of photosynthesis in the chloroplasts and have important functions in the absorption and exploitation of light energy, thereby influence photosynthetic efficiency **(**Pan; Dong, 1995). Chlorophyll content is positively associated with photosynthetic rate which increases biomass production and grain yield (Araus et. al., 1997; Thomas et. al., 2005). Therefore, understanding the genetic mechanism of chlorophyll content would be very important for yield improvement. Significant relationships between chlorophyll content and yield and yield components facilitate selection of high yielding genotypes (Singh, 2001). Phenols are the organic acids which protect the plants from pests and diseases. Grain yield is a complex quantitative trait, considerably affected by environment; therefore, selection of genotypes based on yield is not effective. Higher yield can be achieved by improving its component traits. Correlation studies alone are not indicative of interrelationships among heritable traits this may lead to negative results (Bhatt, 1973). On the other hand, path coefficient analysis measures the direct and indirect effect and permits the separation of the correlation coefficients into components of direct and indirect effect

(Dewey and Lu, 1959). The aim of the present study was genetic evaluation of the intra-species variation of grain amaranth on the basis of leaf biochemical characters such as chlorophyll a and b, total chlorophyll, phenol content, leaf moisture, leaf protein content and quantitative traits as test weight (1000 grains) and grain yield plant^{-1}. Which in turn was used i)to determine the genetic relationship between bio-chemical component and yield, ii) partitioning of genetic association through path coefficients analysis to assess relative importance of direct and indirect effects of above traits on grain yield plant^{-1}, which are not much explored and iii) to work out the divergence among the 26 genotypes of *A. hypochondriacus* L. using Euclidean clustering analysis to identify promising genotypes, which can be used in different genetic improvement programes of this crop.

Materials and methods

Twenty six accessions of grain amaranth (*A. hypochondriacus* L.) viz AG-21, AG-67, AG-114, AG-198, AG-198/2, AG-301, AG-303, AG-306, AG-67/1, AG-114/1, AG-828-A, AG-821, SKNA-20, AG-901, AG-1117, AG-1119, AG-1121, AG-1122 AG-1134, AG-1135, AG-1137, AG-1138, AG-1149, AG-1172, AG-1173, AG-1175 available in the grain amaranths germplasm comprised the material for the present study. These were grown in three replications at Cytogenetics Experimental Field during 2007-08 at the National Botanical Research Institute, Lucknow. The experimental bed size was 3x 3 meters and spaced 15 cm plant to plant and 45 cm row to row. Twenty plants from each accession of each replication were taken randomly for recording observations on chlorophyll a and b, total chlorophyll, phenol content, leaf moisture, leaf protein content, test weight (1000 grains) and grain yield plant^{-1}.

Biochemical Traits

For biochemical analysis such as estimation of chlorophyll a, chlorophyll b, total chlorophyll, phenol and leaf protein, leaf moisture content multiple leaves of each accession were plucked randomly from multiple plants.

Chlorophyll estimation

Leaves of each accession were properly cut into small pieces and weighed 0.25 g and were taken for chlorophyll estimation. Chlorophyll a, chlorophyll b and total chlorophyll were estimated following Arnon's method (Arnon, 1949). The absorbance of the solution was read at 645, 663 and 652 nm for Chlorophyll a, Chlorophyll b and total chlorophyll.

Calculation

Chlorophyll a (mg g^{-1}) = 12.7 (D663) - 2.69 (D645) × $\frac{V}{1000 \times w}$

Chlorophyll b (mg g^{-1}) = 22.9 (D645) - 4.68 (D663) × $\frac{V}{1000 \times w}$

Total chlorophyll (mg g^{-1}) = 20.2 (D645) + 8.02 (D663) × $\frac{V}{1000 \times w}$

Where D = optical density
V = final volume of 80% acetone (ml)
w = dry weight of sample taken (g)

Phenol estimation

Phenol content was calculated following folin-ciocalteau method (Slinkard and Singleton, 1977). The blue colour developed in solution was read at 650 nm against a blank reagent. The concentrations of phenols were expressed as mg phenol g^{-1} tissue.

Leaf protein estimation

Protein content was determined by using Kjeltec Auto Distillation Unit. The collected leaves of each accession were properly cut into small pieces and weighed 0.25 mg using electronic analytical balance (Metler) accurate to 0.1 mg which was quantitatively transferred to the 250 ml Kjeldahl tube in which one Kjeltab was added to each sample. To evacuate the fumes coming from the digest and also prevent excessive acid losses, fume exhaust manifold was used. The samples were placed on the digester with exhaust manifold on top with water aspirator at full flow for the first five minutes of the digestion to evacuate moisture etc. and after five minutes the aspirating effect was essentially decreased with the help of flow regulator. The controlled temperature of the digester was maintained at 380 ^{0}C for 40 minutes. A clear solution was obtained which was indicative of complete digestion of the samples. All samples after the digestion formed ammonium sulphate $(NH_4)_2SO_4$which were used as a standard to cheek the recovery of the distilling units. The distillation principles converted ammonium (NH_4) into ammonia (NH_3) by using an alkali (NaOH) and thereafter steam distilled it into a receiver flask containing boric acid and titrated with N/10 HCL solution using colorimetric endpoint. Similarly, the blank was also run and titrated with N/10 HCL for the detection of end-point. The observations were noted for each sample as the amount of N/10 consumed to end- point and the nitrogen content in each sample was calculated as under:
Nitrogen = (T-B) x N x 1.401 / wt. of the sample in mg
1.401 x N/10 (T-B)/wt. of the sample
% Protein = % Nitrogen x F
Where, T = titration volume for sample (ml)
B = titration volume for blank (ml)
N = Normality of acid
F is conversion factor for nitrogen to protein (6.25)
Finally, the protein content was obtained in each accession.

Leaf moisture estimation

The leaf moisture content was determined in each accession by wet-weight method using following formula.

Moisture % = $\frac{\text{Fresh weight-Dry weight}}{\text{Fresh weight}}$ x 100

Table 1. Mean values for some biochemical and quantitative traits in 26 accessions of *Amaranthus hypochondriacus* L.

S. No	Characters/ Accessions	Chlorophyll a (mg g^{-1})	Chlorophyll b (mg g^{-1})	Total chlorophyll (mg g^{-1})	Phenol (mg g^{-1})	Leaf moisture (%)	Leaf protein (%)	1000-grain weight (g)	Grain yield/plant (g)
1	AG-21	1.546	0.627	2.173	10.216	78.28	2.452	0.845	37.4
2	AG-67	1.117	0.561	1.678	9.503	78.56	1.401	0.795	25.6
3	AG-114	1.437	0.522	1.959	11.503	80.60	1.401	0.839	37.8
4	AG-198	1.147	0.562	1.709	10.216	80.08	0.700	0.800	31.4
5	AG-198/2	1.640	0.938	2.578	9.210	75.18	0.700	0.823	36.8
6	AG-301	1.042	0.530	1.572	6.280	78.40	1.051	0.835	26.8
7	AG-303	0.824	0.325	1.149	15.656	78.38	1.051	0.752	24.2
8	AG-306	1.277	0.837	2.114	10.471	74.92	2.101	0.872	35.8
9	AG-67/1	0.850	0.302	1.152	9.159	77.82	3.152	0.790	37.5
10	AG-114/1	0.967	0.497	1.464	11.987	71.40	1.751	0.770	28.8
11	AG-828-A	1.330	0.736	2.066	12.063	77.50	1.751	0.747	36.6
12	AG-821	1.058	0.633	1.691	6.662	76.30	1.751	0.754	33.6
13	SKNA-20	1.537	0.714	2.251	8.509	76.26	1.401	0.695	23.4
14	AG-901	1.318	0.835	2.153	11.184	75.50	1.751	0.805	30.2
15	AG-1117	1.359	0.744	2.103	11.248	78.82	1.751	0.825	32.0
16	AG-1119	1.342	0.665	2.007	9.222	76.66	1.051	0.775	41.8
17	AG-1121	1.247	0.622	1.869	7.630	74.78	1.401	0.833	35.0
18	AG-1122	1.297	0.784	2.081	7.044	76.84	0.700	0.825	31.2
19	AG-1134	1.214	0.687	1.901	10.292	75.92	1.401	0.778	26.4
20	AG-1135	1.370	0.585	1.955	8.128	76.62	1.051	0.778	32.6
21	AG-1137	1.427	0.919	2.346	11.923	75.72	1.401	0.826	42.8
22	AG-1138	1.607	0.778	2.385	14.942	77.82	1.751	0.813	27.8
23	AG-1149	1.139	0.375	1.514	9.082	77.34	1.401	0.801	30.4
24	AG-1172	1.251	0.699	1.950	7.757	73.30	1.401	0.815	45.8
25	AG-1173	1.043	0.525	1.568	16.216	77.46	1.401	0.785	32.8
26	AG-1175	0.929	0.378	1.307	13.171	77.42	2.101	0.878	28.4
Mean± SE		1.242±0.044	0.63±0.034	1.881±0.075	10.356±0.525	76.84±0.39	1.508±0.109	0.802±0.006	32.80±13.10
CD at 1%		0.090	0.070	0.154	1.081	0.80	0.224	0.012	26.98
CD at 5%		0.122	0.094	0.209	1.464	1.08	0.304	0.016	36.54

Table 2. Correlations coefficient in grain amaranth germplasm.

Characters	Chlorophyll a (mg g^{-1})	Chlorophyll b (mg g^{-1})	Total chlorophyll (mg g^{-1})	Phenol (mg g^{-1})	Leaf moisture (%)	Leaf protein (%)	1000-grain weight (g)	Grain yield/plant (g)
Chlorophyll a (mg g^{-1})	1.000	0.764**	0.948**	-0.111	0.007	-0.197	0.088	0.280
Chlorophyll b (mg g^{-1})		1.000	0.919**	-0.143	-0.311	-0.247	0.116	0.305
Total chlorophyll (mg g^{-1})			1.000	-0.124	-0.118	-0.222	0.117	0.300
Phenol (mg g^{-1})				1.000	0.172	0.162	-0.004	-0.181
Leaf moisture (%)					1.000	-0.041	0.064	-0.215
Leaf protein (%)						1.000	0.155	0.135
1000-grain weight (g)							1.000	0.297
Grain yield/plant (g)								1.000

** Significant at 1% levels

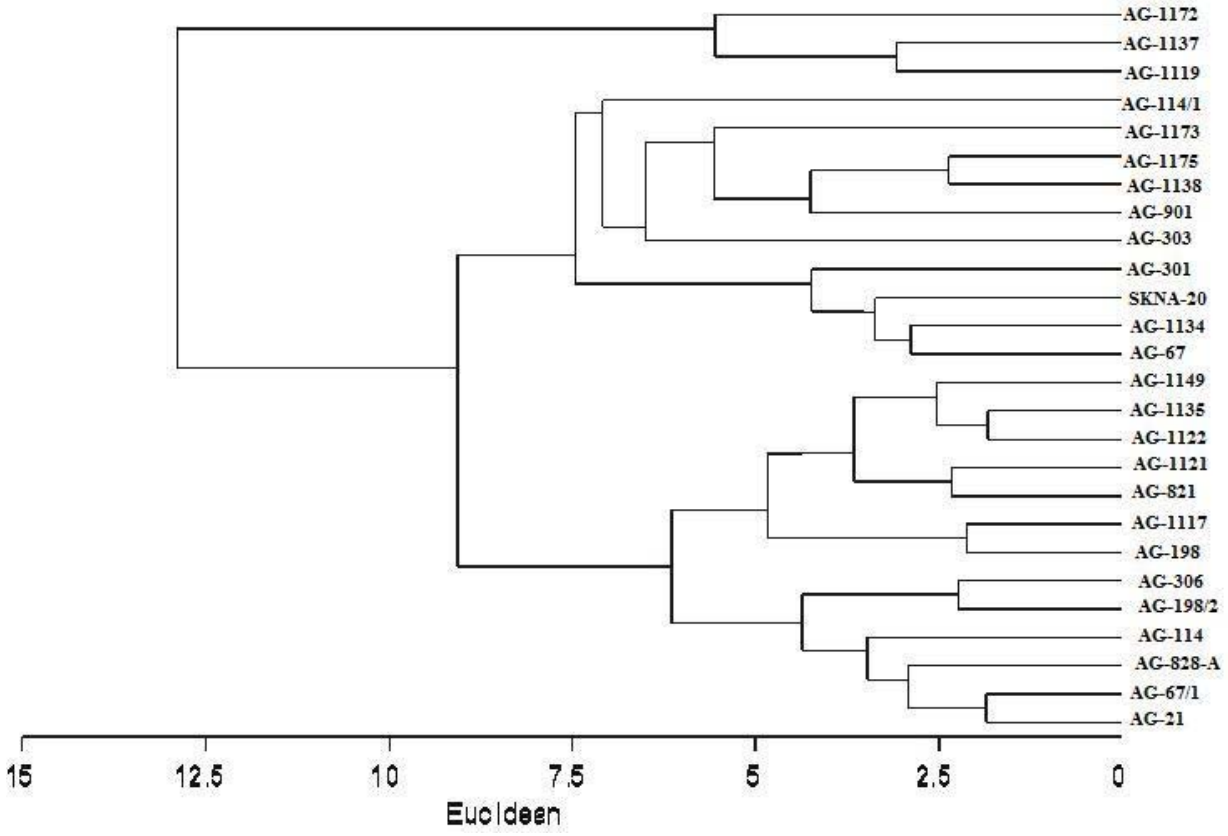

Fig1. Euclidean dendrogram of 26 genotypes of grain amaranth.

Quantitative Traits

Twenty plants from each accession were taken to compile the observations on grain yield $plant^{-1}$. From these five plants were involved for 1000 grain weight as test weight for each accession. Correlation coefficients and path coefficient for chlorophyll a and b, total chlorophyll, phenol content, leaf moisture, leaf protein content, 1000 grain weight and grain yield $plant^{-1}$ were analyzed by the method of Dewey and Lu (1959). Euclidean cluster analysis (Indostat cluster package, 1994) was used for grouping of the accessions in clusters.

Results

Chlorophyll a, chlorophyll b and total chlorophyll content were estimated and presented in Table 1 for all the 26 accessions which showed that AG-198/2 had maximum chlorophyll a content (1.640 mg g^{-1}) followed by AG-1138 (1.607 mg g^{-1}) and AG-21 (1.546 mg g^{-1}), AG-1137 had maximum chlorophyll b content (0.919 mg g^{-1}) followed by AG-306 (0.837 mg g^{-1}) and AG-1138 (0.778 mg g^{-1}) and AG-198/2 had maximum total chlorophyll content (2.578 mg g^{-1}) followed by AG-1138 (2.385 mg g^{-1}) and AG-1137 (2.346 mg g^{-1}). The phenol content was also estimated in all the accessions under study and presented in

Table 3. Path coefficient analysis for grain yield/plant in grain amaranth germplasm.

Characters	Direct effect	Indirect effect via						
		Chlorophyll a (mg g^{-1})	Chlorophyll b (mg g^{-1})	Total chlorophyll (mg g^{-1})	Phenol (mg g^{-1})	Leaf moisture (%)	Leaf protein (%)	1000-grain weight (g)
Chlorophyll a (mg g^{-1})	-0.215	-	-0.178	0.668	0.016	0.001	-0.035	0.022
Chlorophyll b (mg g^{-1})	-0.233	-0.165	-	0.645	0.021	0.050	-0.043	0.030
Total chlorophyll (mg g^{-1})	0.700	-0.205	-0.215	-	0.019	0.024	-0.041	0.027
Phenol (mg g^{-1})	-0.144	0.024	0.033	-0.093	-	-0.028	0.029	-0.001
Leaf moisture (%)	-0.158	0.001	0.073	-0.105	-0.026	-	-0.007	0.023
Leaf protein (%)	0.176	0.043	0.058	-0.164	-0.023	0.006	-	0.040
1000-grain weight (g)	0.255	-0.019	-0.027	0.075	0.001	-0.014	0.027	-

Residual=0.758

Table 1. Phenolic compounds which are present in high concentration in cells of leaves or seeds have been known responsible for the resistance of the young tissues. In the present case in accessions namelyAG-1173, AG-303, AG-1175 and AG-1138 phenol content was 16.216, 15.656, 14.942 and 13.171 mg g^{-1}, respectively. Increasing protein content is an important objective in breeding for high protein varieties in any crop improvement programme. The protein content in leaf of 26 accessions of grain amaranth were evaluated and was found significant in four accessions namely AG-67/1, AG-21, AG-306 and AG-1175 having protein content 3.152, 2.452, 2.101 and 2.101 mg g^{-1}, respectively (Table 1). Test weight is an important yield parameter and high grain weight is a highly desirable character in a cereal. In the present observation almost all the26 accessions showed significant and desirable values for test weight (1000 grains) thereby indicating more desirable yield contributing traits to increase high yield productivity in this crop. Correlation coefficient analysis for these traits presented in Table 2 showed no significant effect on grain yield $plant^{-1}$.Chlorophyll a showed positive and significant correlation with chlorophyll b (0.764) and chlorophyll a and chlorophyll b had positive and significant correlation with total chlorophyll i. e. 0.948 and 0.919, respectively. Path coefficient analysis presented in Table 3 showed that all the traits had no significant direct and indirect effects on grain yield $plant^{-1}$. Genetic diversity among twenty six genotypes of *Amaranthus hypochondriacus* analysed using Euclidean cluster analysis is presented in Fig. 1 which revealed that these accessions were distributed in 3 clusters at 9.0 Euclidean distances. Cluster I had 13 accessions (AG-21, AG-67/1, AG-828, AG-114, AG-198/2, AG-306, AG-198, AG-1117, AG-821, AG-1121, AG-1122, AG-1135 and AG-1149), cluster II had 10 accessions (AG-67, AG-1134, SKNA-20, AG-301, AG-303, AG-901, AG-1138, AG-1175, AG-1173 and AG-114/1) and cluster III had 3 accessions (AG-1119, AG-1137 and AG-1172).

Discussion

As chlorophyll is an important attributes the observations on chlorophyll a, b and total chlorophyll in all the 26 accessions of *A. hypochondriacus* L. have been compared for the yield. Comparison among these characteristics indicated that as such there was no positive association. However, in some accessions viz. AG-1119, AG-1137 and AG-1172 increase in total chlorophyll content indicated an increase in grain yield $plant^{-1}$, suggesting thereby that the chlorophyll content contributed to grain yield $plant^{-1}$ in a positive direction.

Awareness about the grain amaranth has revived the interest in cultivation, evaluation and genetic improvement for grain and protein productivity. There is limited information about leaf chlorophyll content and yield contributing characters of amaranth in the literature. Therefore, further investigation is required on the leaf chlorophyll and other yield contributing characters. The increase in phenolic content may be attributed to the host-pathogen interaction which might have triggered production of more phenols. In other words, tissue offered resistance against further invasion of the pathogen (Vir and Grewal, 1974). In the host-pathogen interaction of fungal diseases phenolic compounds are involved in disease resistance mechanism and they are widely distributed in higher plants (Farkas and Kirlay, 1962). Phenols and their oxidized products are capable of inhibiting spore germination, mycelia growth and sporulation of fungi. Beside these, they can inactivate secreted fungal cell wall enzymes (Mukherjee and Kundan, 1973). In the present study, correlation analysis revealed that the leaf chlorophyll content had no significant relation with grain yield $plant^{-1}$ and the contradictory correlations were found between leaf chlorophyll and seed/grain yield as reported by Feibo *et al*. (1998), Boggs *et al*. (2003) and Reddy and Kumari (2004) who obtained a significant and positive association between chlorophyll index and seed cotton yield. The similar correlations were found between leaf chlorophyll content and yield in wheat by Araus *et al*. (1998), Kabanova and Chaika (2001) and Rodriquez *et al*. (2004) and in rice by Ramesh *et al*. (2002). In amaranths relationship between leaf chlorophyll and seed yield and partitioning through path coefficient analysis are not significant, which revealed that biochemical traits can be improved without altering grain yield. The distance among accessions shows genetic closeness/divergence. The minimum inter-cluster distance between cluster I and II showed that these clusters have maximum common gene combinations and the maximum inter-cluster distance between cluster I and III showed maximum divergence between these two clusters. Clustering patterns indicated that accessions falling in cluster I (AG-21, AG-67/1, AG-828, AG-114, AG-198/2, AG-306, AG-198, AG-1117, AG-821, AG-1121, AG-1122, AG-1135 and AG-1149) and III (AG-1119, AG-1137, AG-1172) can be used in hybridization programme to generate wide range of transgressive segregants in population for high yielding grain amaranth varieties with a better biochemical profile.

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