



Biochemical Analysis of 25 genotypes of Grain Amaranths (*Amaranthus hypochondriacus* L.)

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Keywords : *Amaranthus hypochondriacus*, *Biochemicals*, *Chlorophyll*, *Vitamins*.

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Biochemical Analysis of 25 genotypes of Grain Amaranths (*Amaranthus hypochondriacus* L.)

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Abstract - Biochemicals such as Chlorophylls (a & b), Carotenoids, Protein, Vitamin C, Phenol, Niacin, Vitamin B1, Vitamin B2, Moisture analysis for 25 genotypes of *Amaranthus hypochondriacus* L. has been worked out at Division of Genetics and Plant Breeding and Agrotechnology, National Botanical Research Institute, Lucknow in 2011. The analysis results with some genotypes with highly nutritious value. Out of the 25 genotypes studied, different genotypes having highest values for different biochemicals. AG 828, SKNA 211 and SKNA 21 are the best genotypes which will help in formation of products with superior nutritional quality. Thus the data emanating from the present study indicated the scope for utilizing best nutritional yielding lines for healthcare edible products and as the base material for developing nutraceuticals. The present investigation will also fill the gap regarding the processing of amaranth seeds for the development of superior quality edible food products for infants and also used in fast days. The highly nutritious lines of this crop are so high promising for supplemented nutritive food amelioration of nutritional deficiency.

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I. INTRODUCTION

Grain amaranths of the genus *Amaranthus* (Family – Amaranthaceae) embraces about 60 species wild (weedy) and cultivated types as an important subsidiary food crop for the people inhabiting to tropical and sub-tropical highlands of South and Central Americas and Asia (Sauer, 1967). There are mainly four species of grain amaranths viz. *Amaranthus caudatus* L., *A. cruentus* L., *A. edulis* Speg., *A. hypochondriacus* L. among which the later is extensively cultivated in Mexico and in the Himalayas of India from Kashmir to Arunachal Pradesh. It is an outbred-inbred crop upto 40% crossing varying in the different genotypes and geographical regions (Hauptli and Jain 1985; Jain *et al.* 1982; Pal and Khoshoo 1974;

Vietmeyer 1980; Walton 1968). The seed of grain amaranth is small but rich in protein (14-19 %), nutritionally more balanced because of better amino acid profile than other improved cereals, contains more lysine, 5- 8 % (Downton 1973; Misra *et al.* 1985; National Academy of Sciences 1975; Vietmeyer 1980; Pisarikova *et al.* 2005). *Amaranth* is a highly nutritious food. The

leaves, shoots and tender stems are eaten as a potherb in sauces or soups, cooked with other vegetables. Biochemicals such as Carotenoids, ascorbic acid, phenols, Vitamin B1, B2 and niacin are important for human nutrition since some of Carotenoids serve as precursors of vitamin A while others have been shown to function as antioxidant (De Pee *et al.*, 1995; Krinsky, 1989; Palozza and Krinsky, 1992). Ascorbic acid functions as antioxidant and anti cancer agent (Shibata *et al.*, 1992). In stomach, ascorbic acid act as a scavenger of nitrites and free radicals formed during metabolic process (Cameron and Pauling, 1979). Phenolic compounds, exhibited the best antioxidant activity. Phenols are the organic acids which defend the plants from pest and diseases. Riboflavin (vitamin B2) is manufactured in the body by the intestinal flora and is easily absorbed, although very small quantities are stored, so there is a constant need for this vitamin. Niacin is needed for energy metabolism, proper digestion, and healthy nervous system. All B vitamins help the body convert food (carbohydrates) into fuel (glucose), which is used to produce energy. These B vitamins, often referred to as B complex vitamins, also help the body metabolize fats and protein. B complex vitamins are needed for healthy skin, hair, eyes, and liver. They also help the nervous system function properly, and are needed for good brain function. The aim of the present study was evaluation of the intra-species variation of grain *amaranth* by comparing the leaf biochemical components such as chlorophyll a and b, total chlorophyll, carotenoids, phenol content, leaf moisture, leaf protein content and Vitamins (C, B₁, B₂, B₃)

II. METHODS AND MATERIALS

In the present investigation the material were seeds of 25 accessions (i.e. AG-303, AG141/1, SANA-20, AG828, AG198, AG114, AG198/2, AG301, AG21BB, AG306, GA-2, SKNA211, APOLO LOKI, SKNA21, SKNA-21-1, GA-1, BGA-2, AG-114 (NBPGR), RMA-2, RMA-3, IC-120588, SKNA-23, RSUNA4, BGA-3, GA-2) of grain *amaranth*, which are being maintained at experimental field of Cytogenetics Laboratory of CSIR-National Botanical Research Institute, Lucknow. The leaf samples were cleaned and properly dried as required before analysis.

Chlorophyll (a, b and total) Carotenoid were estimated following Arnon (1949) methods.

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a) Reagent preparation

80% acetone - 80 ml acetone in 100 ml (80 ml acetone + 20 ml distilled water)

Sample – Fresh leaves sample weight 250 mg or 0.25g.

b) Procedure

The fresh leaves of all the given twenty genotype were weighted 0.25gram using Digital Analytical Balance C.165 citizen which was transferred to make homogenize with mortar and pestle with addition 10ml of 80% acetone, paste was made Chlorophyll extract was poured in a funnel having

wattmaan filter paper and collect in volumetric Flask, collect all extract from the mortar, the green extract is gradually attained by adding 2-3 ml of acetone every time. Washing 3-4 time are giving and extraction continued until become colourless make volume to extract 25ml with 80% of acetone. Since the extract is subject to photo-oxidation. It is kept away from direct sunlight and store in the dark place. The O.D. of the chlorophyll extract is recorded on spectrophotometer using wavelength on 480, 510, 645, 652 and 663 nm continuously. The O.D. show the absorbance of solution.

c) Calculation

Following formula are used for the calculation

$$\text{Chlorophyll a (mg/g)} = \frac{12.7(\text{O.D.663}) - 2.69(\text{O.D.645}) \times V}{W \times 1000}$$

$$\text{Chlorophyll b (mg/g)} = \frac{22.9(\text{O.D.645}) - 4.68(\text{O.D.663}) \times V}{W \times 1000}$$

$$\text{Total Chlorophyll (mg/g)} = \frac{\text{O.D.652} \times 1000 \times V}{34.5 \times W \times 1000}$$

$$\text{Total carotinoids (mg/g)} = \frac{7.6(\text{abs480}) - 1.49(\text{abs510}) \times \text{final volume}}{1000 \times \text{fresh weight}}$$

Determination of Crude Protein has been done by following Kjeldahl methods

*d) Material Required***Alkali-40%NaOH**

Receiver solution- Make 4% Boric acid solution with distilled water (warm) cool the solution at room temperature and add indicators-100ml of Bromocresol green and 70ml methyl red.

Kjeltabs- K_2SO_4 (3.5g) and CuSO_4 (0.4gm)

Digestor-2006 Digestor foss tector

Distillation unit -2200 Kjeltac Distillation unit.

Titration- 0.1N HCL burette etc.

Volumetric flask –according to sample.

e) Digestion

The fresh leaves sample of all given genotypes were weighed 0.25 grams using electronic digital analytical balance which was quantitatively transferred to the 250 ml Kjeldahl tube containing 12 ml of concentrated H_2SO_4 in which one kjeltab was added to each sample and was kept on FOSS Tecator Digestion unit at 380°C temperature for about 40-45 minutes. To evacuate the fumes coming from the digest and also prevent excessive acid losses, fume exhaust manifold was used. Placed the samples 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. A clear solution of ammonium sulphate was obtained as an indicative of complete digestion of samples.

f) Distillation

All digested samples were distilled using Auto-Kjeltac Distillation Unit. All samples after the digestion formed ammonium sulphate $(\text{NH}_4)_2\text{SO}_4$ which was used as a standard to check the recovery of the distilling units. The distillation principles converted ammonium (NH_4) into ammonia (NH_3) by using an alkali (NaOH) and there after steam distill it into a receiver flask containing boric acid. A light green color solution is obtained after distillation.

g) Titration

The solutes were titrated against N/10 HCL for the detection of colorimetric end point until the color of the solution turns from light green to light pink. The observations were taken for each sample as the amount of N/10 HCL is consumed to end point. Similarly, the

blank was also run and titrated with N/10 HCl for the detection of end-point to avoid any error occurring while conducting the experiment.

h) Calculation

Lastly the nitrogen content in each sample were calculated as under;-

$$N_2 = \frac{(Tml-Bml) \times N \times 100 \times 14.01}{W}$$

Where, T = Titration volume for sample

B = Titration volume for blank in (ml)

N = Normality of acid

W = weight of sample

Determination of Protein: - % Protein = %

Nitrogen x F (6.25)

Where,

F = Conversion factor (6.25)

Finally the protein content is obtained in each accession.

Estimation of Vitamin C

i) Reagent preparation

0.1% starch- 1g starch + 100ml distilled water and heat at 100°C for 2min and cool then use.

0.01M Iodine and KI - Dissolved 0.5076g of I₂ and 0.332KI in 120 ml water, Dilute with 200ml water.

j) Procedure

All fresh leaves sample of all given genotypes were weighed 0.25 grams using electronic digital analytical balance accurately and transfer for homogenized solution with mortar and pestle with 25ml distilled water, filter that solution with Funnel and wattmaan filter paper then collected in conical flask and after few minute added 10, 10 drops of 0.1% starch solution, as indicator. The solution obtain light green colour, then titrated with 0.01M solution of Iodine and KI standard solution and count drop of standard solution which was titrated the leaves solution until change the light green colour solution in dark blue colour solution. It was measured the ascorbic acid in mg/g leaves solution with standard solution.

k) Calculation

Final vitamin C obtain in mg/g by the formula = Drop of standard solution x average of standard solution.

Estimation of total Phenol

l) Reagent preparation

Folin Ciocalteu reagent- 5ml (1:10 diluted with distilled water).

Galic acid or methanolic (methanol:distilled water -50:50) compound- 0.5 ml of 1:10 g/m for standard phenolic compound.

Aqueous sodium carbonate- 4ml 1M- solution.

Fresh leaves sample- .25g.

m) Procedure

All fresh leaves sample of all given genotypes were weighed 0.25 grams using electronic digital analytical balance accurately and transfer for making homogenized solution with mortar and pestle with 5ml Folin Ciocalteu reagent and add 0.5 ml Galic acid or methanolic compound then after 15 min maintain volume 4ml Aqueous sodium carbonate of 1M solution add measure at 765nm wavelength with spectrophotometer in the against of standard Phenolic compound. We use the wavelength for check the absorbance of solution, measured the phenol volume in the leaves sample. Calculate Phenol,

Final Phenol content was obtain in mg/g by the formula = O.D. at 765nm standard solution x average of standard solution x 100.

Estimation of Moisture content in the plant leaves

n) Procedure

All fresh leaves sample of all given genotypes were weighed by using electronic digital analytical balance accurately and write on then write pad, and then put in oven at 60°C for dry to loss the moisture for 24 hours and took weight and calculated the percent of moisture contain with these formula.

$$\% \text{ moisture} = \frac{\text{Freshweight} - \text{dryweight} \times 100}{\text{Freshweight}}$$

Estimation of Vitamin B₁

o) Reagent preparation

0.1M Perchloric acid.

Acetic anhydride.

Anhydrous Formic acid.

Fresh leaves sample.

p) Procedure

All fresh leaves sample of all given genotypes were weighed 0.14 grams using electronic digital analytical balance accurately and transferd for made homogenized solution in mortar and pestle with 5ml of anhydrous formic acid R for total thiamin part extraction filter the sample's all extract from mortar with waatmaan filter paper and funnel then collect in volumetric flask sample colour obtained light colour and added 50ml of acetic anhydride our solution colour obtained violet blue colour, then titrated whole solution with 0.1M perchloric acid until the colour change in light pink colour. Note the titrated end point from the burette compared with blank and then find out the vitamin b₁ content from the leaves sample solution. 1ml of 0.1M Perchloric acid is equivalent to 16.37 mg of C₁₂H₁₇N₅O₄S.

Estimation of Niacin**q) Reagent preparation**

0.1M Sodium Hydroxide-

Phenolphthalein

Fresh leaves sample

r) Procedure

All fresh leaves sample of all given genotypes were weighed 0.25grams using electronic digital analytical balance accurately and transferred for homogenized solution with 5ml distilled water in mortar and pestle, filtered the sample's all extract from mortar with filter paper and funnel then collected in volumetric flask then add 10ml of Phenolphthalein as indicator and then titrated whole solution with 0.1M Sodium Hydroxide until the sample solution colour change in light pink colour. Note the titrated end point from the burette compare with blank and then find out niacin the from the sample solution. Calculate niacin 1ml of 0.1M Sodium Hydroxide is equivalent to 12.31 mg of $C_6H_5NO_2$.

Estimation of Vitamin B₂**s) Reagent preparation**

Sample- fresh leaves = 0.2g

Glacial acetic acid R = 0.2ml diluted with 50ml distilled water.

Sodium acetate R = 0.10g diluted with 10 ml distilled water

t) Procedure

All fresh leaves sample of all given genotypes were weighed 0.2 grams using electronic digital analytical balance accurately and transferd for homogenized solution in mortar and pestle with 3ml distilled water, then filtered the sample's all extract from mortar with filter paper and funnel then collected in volumetric flask, add 0.2ml Glacial acetic acid; it was change sample colour and added 7ml sodium acetate solution sample colour obtained light colour. And measure the absorbance at 444nm. and calculates the specific absorbance at 328nm.

III. RESULT

Chlorophyll a, chlorophyll b, total chlorophyll and carotenoids content were estimated and presented in Table 1 and figures-1&2 for all 25 accessions showed AG-141/1 had maximum chlorophyll a content was 24.47 mg g⁻¹ followed by RSUNA4 3(21.79 mg g⁻¹), SKNA23 (19.75 mg g⁻¹). The maximum chlorophyll b content was SKNA211 (15.55mg g⁻¹) followed by AG-303 (12.21 mg g⁻¹) and BG1(8.78 mg g⁻¹). The maximum total Carotenoid content was GA-1(12.49 mg g⁻¹) followed by AG306 (12.37mgg⁻¹) and AG-198 (12.35 mg g⁻¹). The highest protein content (%) of grain *amaranth* leaves are 3.50, 3.15, 3.15 and 2.45 respectively for

genotypes AG-198, AG-301, AG-306, AG-21BB and AG-114 NBPGR, SKNA-21 (figure-3). Vitamin C (Ascorbic acid) showed maximum values for RSUNA4 (8.40 mg/g) followed by BGA-2(6.63 mg/g) and SKNA -21-1(6.19 mg/g) and minimum for AG-301(1.83mg/g)(figure-4). In the present case accessions such as GA-2, RMA-2, AG-141/1, AG-306, AG-301, phenol content was 6.83, 5.97, 5.96, 5.65 mg g⁻¹ respectively in high content. The lower phenol content SKNA 21, SKNA 21-1, APOLOA LOKI 1.66, 2.36 2.74, mg g⁻¹(figure-5(a)& (b)). The highest moisture content was noted in % Genotype AG-141/1(82.9) followed by Genotype AG-303,(82.6), AG-306 (81.9) and the lowest moisture content was RMA-3(65.10), GA-2(68.38), GA-1(68.89), APOLOALOKI (70.07)(figure-6). The maximum vitamin B₁ content was observed 116.33 mg/g (SKNA-211), 64.07 mg/g (AG-198), 53.95 mg/g (IC-120588) 52.7 mg/g (AG-141/1) and minimum content 8.43 mg/g (AG-114 (NBPGR), 18.55 mg/g (RMA-2), 28.66 mg/g (GA-2) (figure-7). Higher Vitamin B₃ (niacin) content for AG-828 (73.86mg/g) followed by genotype AG198/2 (52.93 mg/g) and SKNA-211(49.29 mg/g) and the lower content was genotype IC120588 (12.31 mg/g) followed by genotype RMA-2(14.77 mg/g) BGA-3(16.00 mg/g) (figure-8). Vitamin B₂(Riboflavin) content was maximum for genotype SKNA-20 (4.72mg/g) followed by genotype AG198 (4.35 mg/g) and AG198/2(3.00 mg/g) and the minimum content was for genotype of AG-114 NBPGR (1.20mg/g) followed by genotype BGA-3 (1.71 mg/g) SKNA-21(1.79 mg/g) (figure-9).

Cluster analysis was also performed with the help of WARD Clustering Method with 0.05 level of significance in order to find out the variation level. From the dendrogram, three main clusters were observed, out of which one cluster comprising of genotypes shows maximum variation. These genotypes are very distant from the other genotypes which again indicate that there is a possibility of getting good recombinants if these genotypes are crossed with other genotypes.

The hybridization process can improved. The protein and other traits can also be thus improved. This information can be utilized for successful breeding strategies. *A. hypochondriacus* L. using Euclidean clustering analysis, to identify promising genotypes, which can be used in different for genetic improvement program of this crop. There are 23 cluster show in the chart and Accessions with more leaf protein have potential to increase nutritional value and. However, knowledge about amaranth leaf composition is still marginal. Using Euclidean cluster analysis 25 accessions were distributed in 3 clusters (at 9.0 euclidean distance) of which cluster I contained maximum (16) accessions, cluster II (6) and cluster III (2) accessions. The I genotypes cluster euclidean distance with 25 genotypes cluster at 9.0, 24 genotypes cluster at 8.0, 23 genotypes cluster at 7.0, respectively. The

determination of chemical composition of leaf is necessary for variety evaluation, on the basis of high nutritive value for human diet. Cluster I and III were found more diverse than others and therefore can be used for developing recombinants.(figure-10).

IV. DISCUSSION

Amaranth is useful in preventing retarded growth and improving health and strongness in children. Nutrition" (also called nourishment or aliment) is the provision, to cells and organisms, of the materials necessary (in the form of food) to support life. Many common health problems can be prevented or alleviated with a healthy diet. The human body contains chemical compounds, such as water, carbohydrates (sugar, starch, and fiber), amino acids (in proteins), Chlophylls, Carotenoid, Phenol, Vitamins (DNA and RNA). These compounds in turn consist of elements such as carbon, hydrogen, oxygen, nitrogen, phosphorus, calcium, iron, zinc, magnesium, manganese, and so on. All of these chemical compounds and elements occur in various forms and combinations both in the human body and in the plant and animal organisms that humans eat. Chlorophylls and Carotenoids, Protein, VitaminC, Phenol, Neacin, VitaminB1, VitaminB2, Moisture analysis in the present study has shown some genotypes with highly nutritious value. Out of the 25 genotypes studied, AG 828, SKNA 211 and SKNA 21 are the best genotypes on which further work can be done in order to improve the quality of these genotypes. This will help in formation of products with superior nutritional quality. Thus the data emanating from the present study indicated the scope for utilizing best nutritional yielding lines for healthcare edible products and as the base material for developing nutraceuticals which will certainly help in stylizing cottage or small-scale industries and will create more jobs to villagers for improving economic condition of the rural people highly dependent on agriculture.

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Table 1 : Biochemical Analysis for 25 genotypesof grain amaranths.

Genotypes	Chlorophyll a mg g ⁻¹	Chlorophyll b mg g ⁻¹	Total Chlorophyll mg g ⁻¹	Total Carotenoid mg g ⁻¹	Phenol (mg/g)	Niacin (mg/g)	Vitamin C (mg/g)	Vitamin b1 (mg/g)	Vitamin b2 (mg/g)	Protein	Moisture %
AG-303	18.16	12.21	30.38	7.32	5.29	24.62	1.77	38.78	2.9	0.7	82.6
AG-141/1	24.47	1.28	25.75	9.33	5.97	24.62	2.21	52.27	2.93	3.15	82.9
SANA-20	17.82	7.61	25.43	4.59	4.41	24.62	3.1	33.72	4.72	1.75	75.07
AG-828	19.59	8.31	27.91	5.74	4.96	73.86	3.1	64.07	2.31	2.8	76.32
AG-198	13.91	4.95	18.86	12.35	5.92	14.77	2.65	37.09	4.35	3.5	79.03
AG-114	13.56	2.87	16.43	12.17	5.54	36.93	2.65	35.41	2.7	2.1	72.9
AG-198/2	11.34	6.07	17.41	11.81	5.32	52.93	1.77	35.41	3	1.75	72.8
AG-301	10.3	8.23	18.53	11.85	5.65	39.39	1.33	47.21	2.59	3.5	77.9
AG-21BB	9.69	2.83	12.52	10.97	3.9	22.16	3.54	65.75	2.04	3.15	77.6
AG-306	13.33	3.3	16.63	12.37	5.96	34.47	3.98	52.27	2.83	3.15	81.9
GA-2	19.27	4.44	23.72	5.15	6.83	27.08	2.21	37.09	2	0.7	68.38
SKNA-211	8.06	6.52	14.57	2.78	5.42	49.24	1.77	116.33	2.39	0.35	75.98
APOLOA-LOKI	8.07	2.57	10.64	3.18	2.74	33.24	2.65	32.03	2.41	1.4	70.07
SKNA-21	9.48	2.33	11.82	3.22	1.66	27.08	4.42	52.27	1.79	2.1	72.61
SKNA-21-1	12.61	15.55	28.16	4.23	2.36	24.62	6.19	23.6	2.43	2.1	73.5
GA-1	17.32	8.78	26.1	12.49	5.34	23.39	3.1	37.09	2.72	2.1	68.89
BGA-2	7.78	3.88	11.66	3.09	4.6	27.08	6.63	42.15	2.02	1.4	71.7
AG-114 (NBPGR)	9.64	4.34	13.97	12.16	4.87	23.39	3.98	8.43	1.2	2.45	71.3
RMA-2	13.72	2.68	16.4	3.38	6.1	14.77	3.98	18.55	2.47	2.45	77.86
RMA-3	17.68	5.67	23.35	6.1	4.81	23.39	3.54	37.09	2.01	1.4	65.1
IC-120588	15.46	6.37	21.83	5.45	4.27	12.31	4.87	53.95	3.65	1.05	75.7
SKNA-23	19.75	7.99	27.74	6.85	4.6	27.08	3.98	40.46	1.99	1.75	71.23
RSUNA-4	21.78	0.04	21.82	2.83	4.34	36.93	8.4	35.41	2.14	0.35	73.4
BGA-3	11.78	6.37	18.15	4.44	5.32	16	4.87	38.78	1.71	2.1	76.67
GA-2	14.24	3.23	17.47	3.7	5.85	28.31	3.54	28.66	1.79	1.4	73.56
Average	14.35	5.54	19.89	7.1	1.66	12.31	1.33	8.43	1.2	0.35	65.1
Maximum	24.47	15.55	30.38	12.49	6.83	73.86	8.4	116.33	4.72	3.5	82.9
Minimum	7.78	0.04	10.64	2.78	4.88	29.69	3.61	42.55	2.52	1.95	74.6

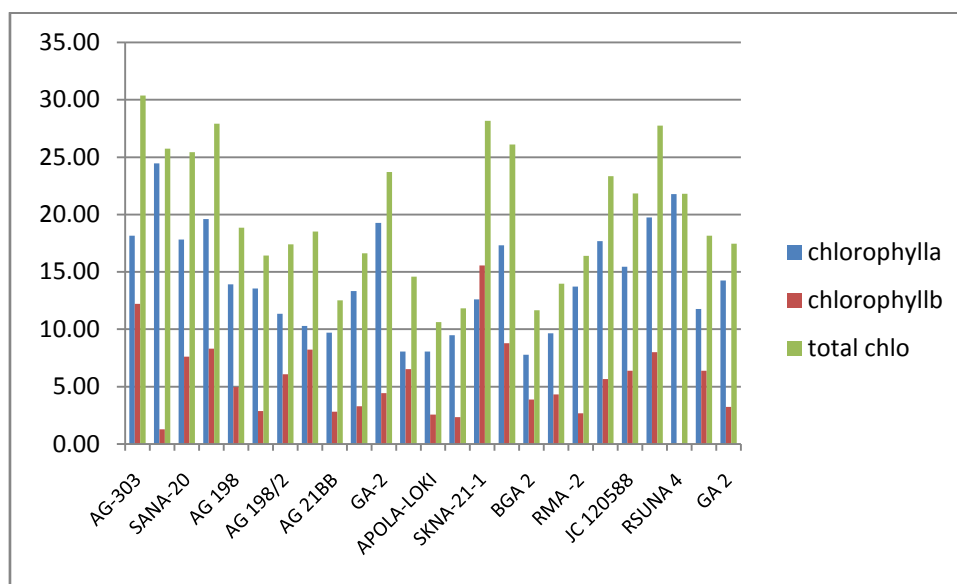


Figure 1 : Chlorophyll (mg/g) for twenty five accessions of grain amaranth (*Amaranthus hypochondriacus* L.).

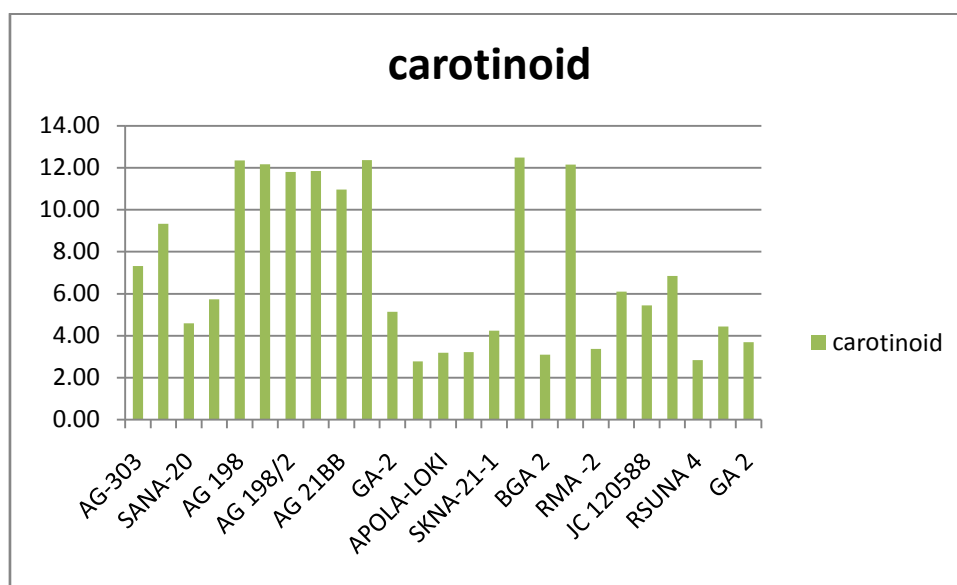


Figure 2 : Carotenoids (mg/g) for twenty five accessions of grain amaranth (*Amaranthus hypochondriacus* L.).

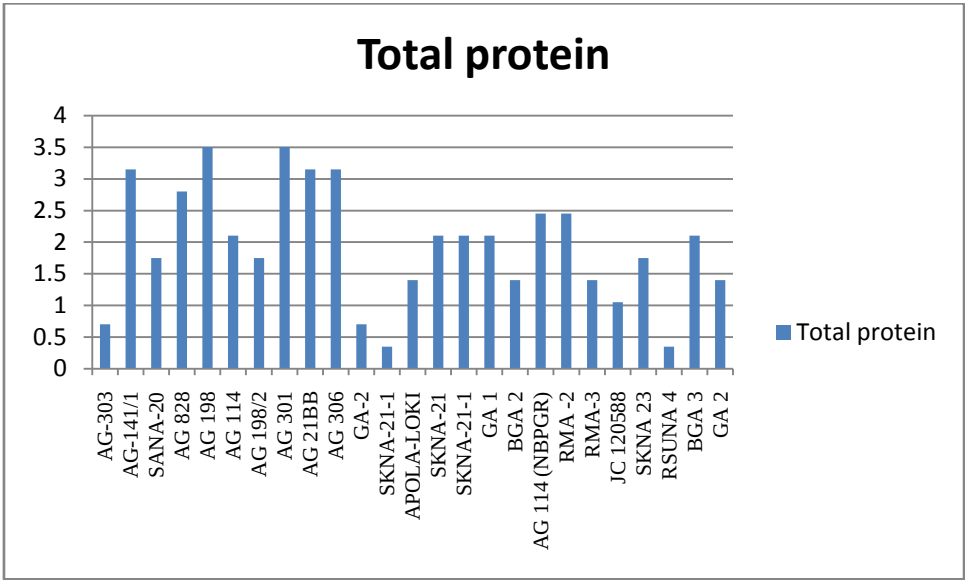


Figure 3 : Protein content (%) for twenty five accessions of grain amaranth (*Amaranthus hypochondriacus* L.).

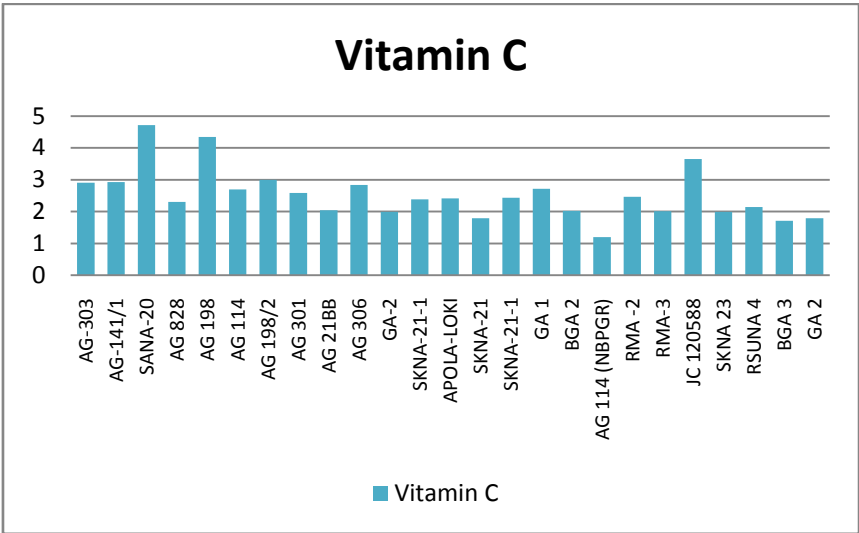


Figure 4 : Vitamin C (mg/g) for twenty five accessions of grain amaranth (*Amaranthus hypochondriacus* L.).

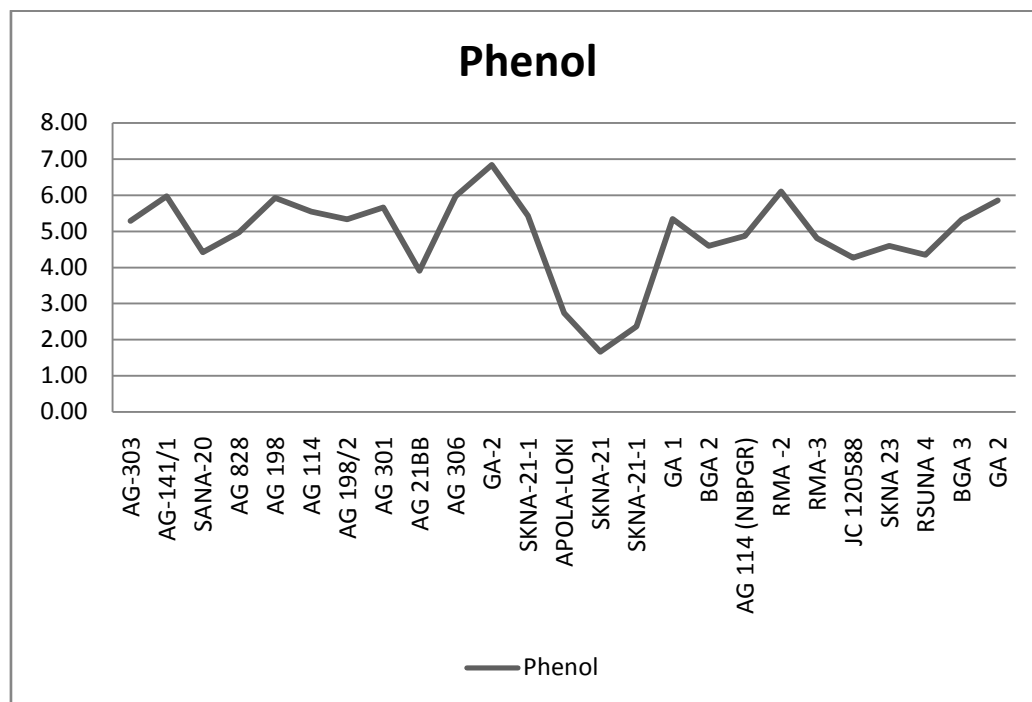


Figure 5 : (a) Phenol content (mg/g) for twenty five accessions of grain amaranth (*Amaranthus hypochondriacus* L.).

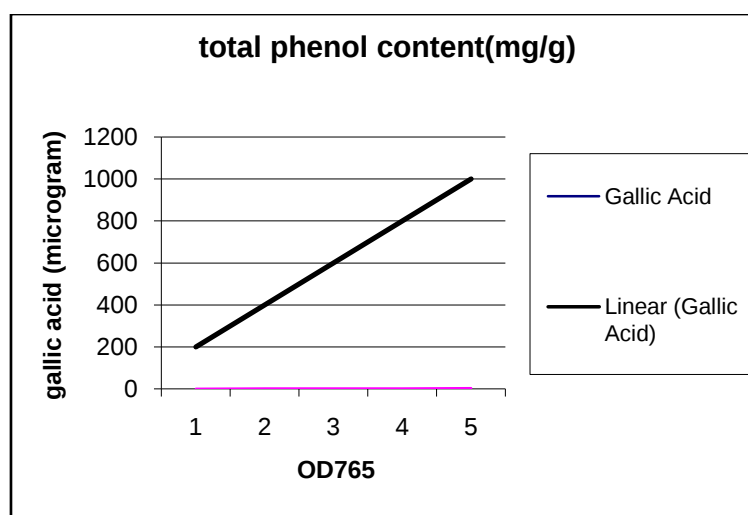


Figure 5(b) : Phenol content (mg/g) for standard.

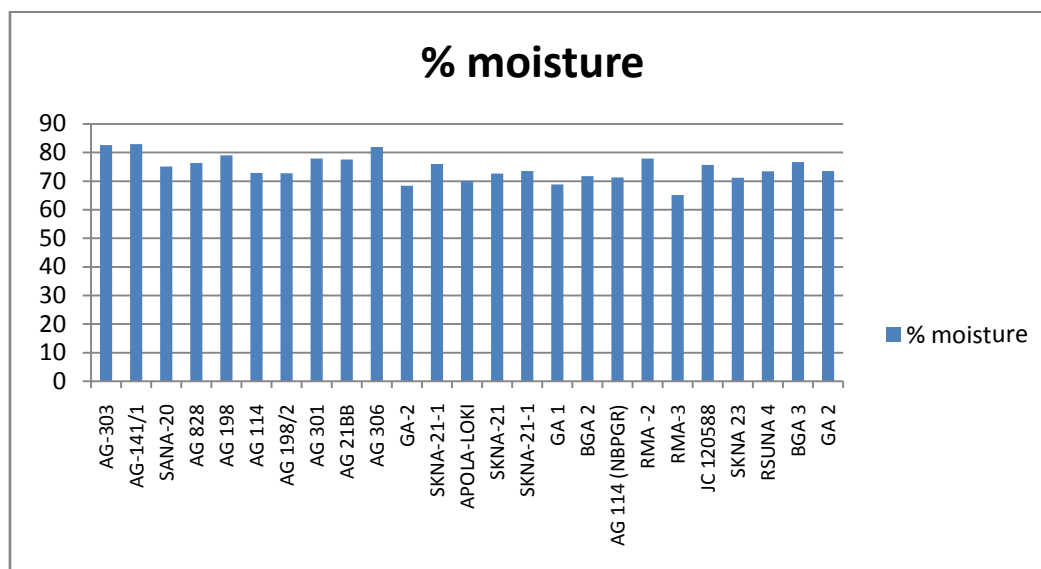


Figure 6 : Moisture (%) for twenty five accessions of grain amaranth (*Amaranthus hypochondriacus* L.)

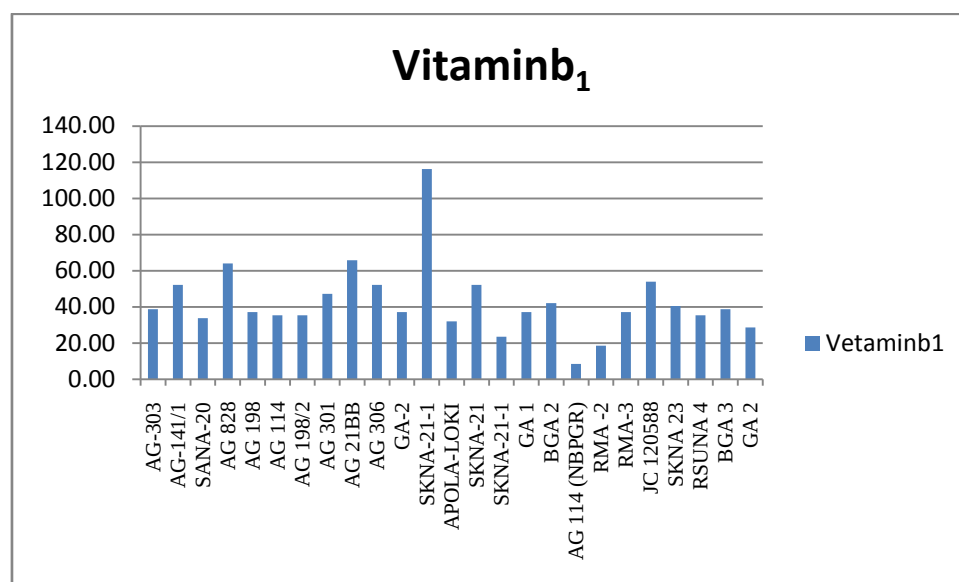


Figure- 7 : VitaminB₁ (mg/g) for twenty five accessions of grain amaranth (*Amaranthus hypochondriacus* L.).

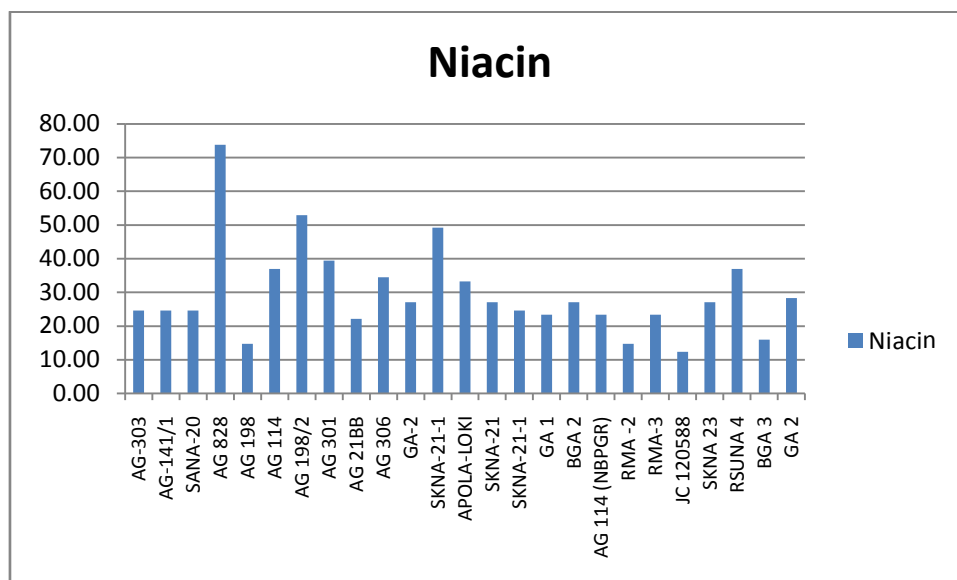


Figure 8 : Niacin (mg/g) for twenty five accessions of grain amaranth (*Amaranthus hypochondriacus L.*)

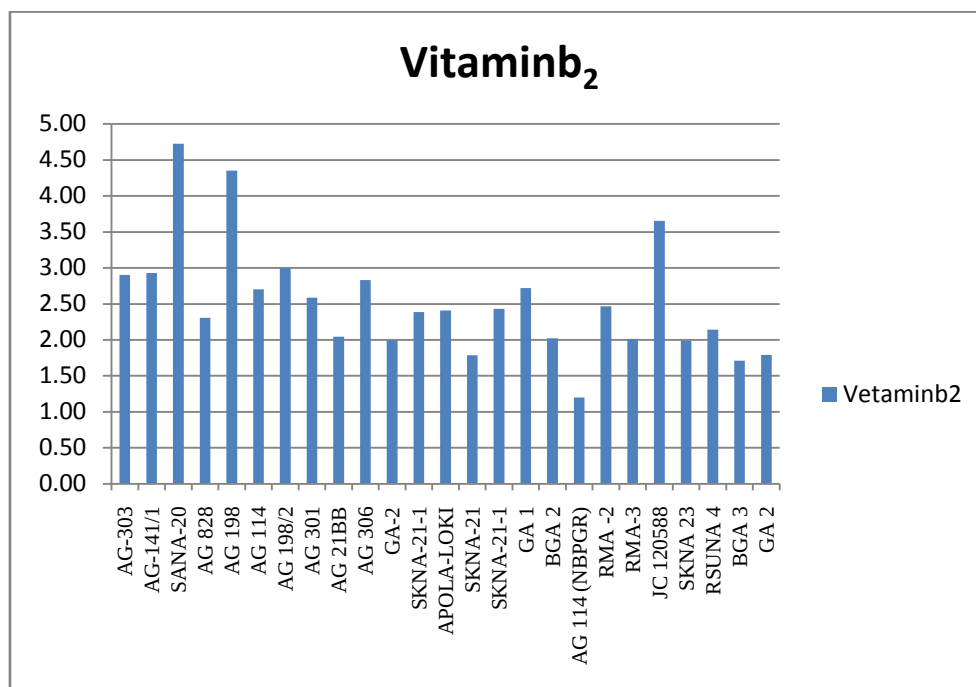


Figure 9 : Vitamin B₂ (mg/g) for twenty five accessions of grain amaranth (*Amaranthus hypochondriacus L.*)

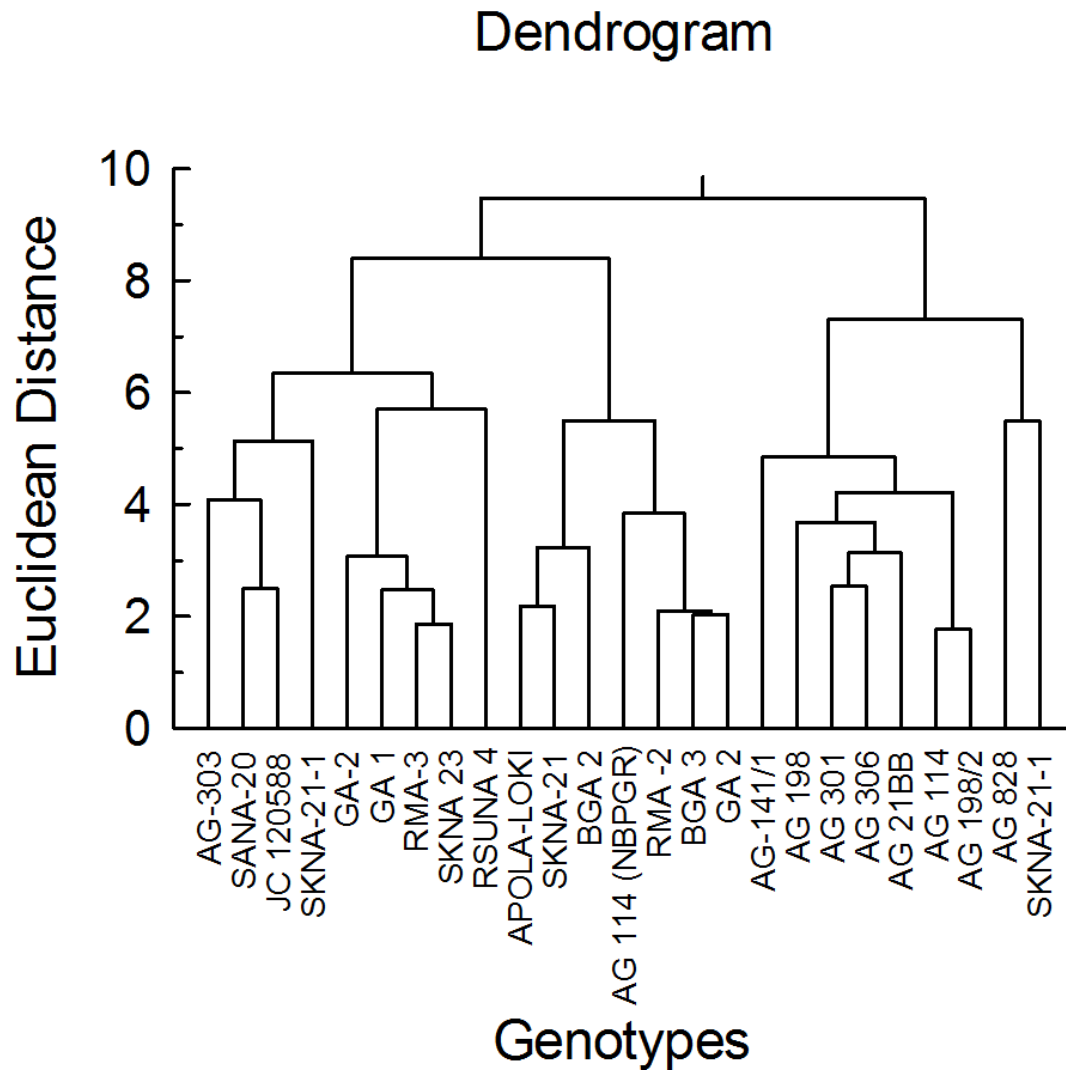


Figure 10 : Cluster analysis of twenty five genotypes of Grain amaranth (*Amaranthus hypocondriacus.L*)