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Studies on diverse genotypes of amaranth (*Amaranthus* species) for their antioxidant and nutritional activities

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ABSTRACT

An investigation was carried out with eight diverse genotypes of amaranth (*Amaranthus* species) to estimate the biochemical composition (including anthocyanin, total carotenoids, ascorbic acid, antioxidant, i e Ferric Reducing Antioxidant Power (FRAP) and Cupric ion Reducing Antioxidant Capacity (CUPRAC) and total phenolic content) at IARI, New Delhi, during 2011. Among genotypes, anthocyanin ranged from 5.33 mg/100 g of fresh sample (Renu Sree) to 40.81 mg/100 g (Pusa Lal Chaulai). Maximum ascorbic acid was recorded in Co 1 (81.2 mg/100 g), followed by Pusa Kirti (78.4 mg/100 g).Total carotenoids were maximum in Pusa Lal Chaulai (69.41 mg per 100 g). Total antioxidant activity measured by CUPRAC and FRAP showed that three genotypes, Pusa Lal Chaulai, Krishna Sree and Co 1, had higher value of antioxidant in CUPRAC method, however in FRAP method highest antioxidant activity was recorded in Pusa Lal Chaulai (13.157 µmol ascorbic acid equivalent, AAE/g), followed by Pusa Kirti (13.033 µmol AAE/g). Total phenolic content was recorded maximum in Co 1 (1 411.62 µg gallic acid equivalent (GAE) per g), followed by Pusa Lal Chaulai (1 138.5 µg GAE per g). By considering all, it was observed that Co 1, Pusa Lal Chaulai, Pusa Kirti and Renu Sree were potential genotypes and hence may be utilized for improvement of amaranth for high nutritional activity.

Key words: *Amaranthus* species, Antioxidant activity, Ascorbic acid, Carotenoids content, Green leafy vegetables (GLV), Phenolic

Amaranth (*Amaranthus* species) is consumed as a green leafy vegetable has wide source of different bioactive compounds which show extra-nutritional constituents that typically occur in small quantities in foods and its intake usually regarded as beneficial to health. It can be used as a potential food crop for diversification of agriculture to newer areas, environmental sustainability and for combating the nutritional deficiency in many parts of the world (Jacobsen 2003, Bhargava *et al.* 2006). *Amaranthus* species has been cultivated since centuries as a leafy vegetable and subsidiary grain crop in different parts of the world (Risi and Galwey 1984). Amaranth constitutes a single group of leafy vegetables, which has attained commercial significance in India and its cultivation is widespread in South India. Because of its low production cost and high yield, amaranth is considered to be one of the cheapest leafy vegetables in the market and it could be rightly described as 'poor man's vegetable'. Presently it is mainly cultivated in Tamil Nadu, Andhra Pradesh, Karnataka, Kerala, Uttar Pradesh, Bihar, Maharashtra and Odisha at large scale. Seeing the potential of amaranths as a cheap source of antioxidants and other nutrients, identification of nutritionally rich genotypes with good quality components is the main objective of this study, which will help the breeder to use these genotypes in breeding programme for high nutraceutical. To identify the potential of different genotypes of amaranths available in India as antioxidants, methanolic extracts of amaranth was studied in different systems at multiple concentrations. Total antioxidant activity assessed by Ferric reducing antioxidant power (FRAP), Cupric ion reducing antioxidant capacity (CUPRAC), total phenols, ascorbic acid, total carotenoids and anthocyanin contents were also analysed.

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MATERIALS AND METHODS

Experimental material and growing conditions

The present investigation was carried out at Indian Agricultural Research Institute, New Delhi situated at 28.08° N and 77.12°E. The height above the mean level being 228.61 m. The climate is subtropical and semi-arid. Eight diverse and popularly cultivated genotypes of amaranth namely Krishna Sree (red colour), Renu Sree (light green colour), Kunnur Local (red colour), MZ 8 (redish green colour), Pusa Kiran (green colour), Pusa Lal Chauli (red colour), Pusa Kirti (green colour) and Co 1 (dark green

colour) were used in this study. All the genotypes were grown at research farm, Division of Vegetable Science, IARI, New Delhi during March-June 2011. The standard recommended practices of growing amaranth were followed. The maximum temperature ranged from 21.5°C to 43.6°C and minimum temperature ranged from 9.3°C to 29.0°C and the weather remained hot and dry during whole growing period. The nitrogen (N), phosphorus (P) and potassium (K) content of experimental soil was 280.5, 15.2 and 380.3 kg/ha respectively. Organic content (%) was 0.65. The edible portion was harvested at marketable stage and fresh leaf samples were washed under running tap water followed by bi-distilled water. They were drained completely, dried over filter paper and analysed for ascorbic acid, polyphenols, total carotene, antioxidant activity and anthocyanin pigments content.

Determination of anthocyanin, total carotene, ascorbic acid, poly phenols, and antioxidant activities

Anthocyanin was determined with ethanolic HCl and wavelength was measured at maximum absorption (Ranganna 1986). Total carotene was determined by using acetone and petroleum ether as extracting solvents and ascorbic acid was estimated titrimetrically using 2, 6-dichlorophenol indophenol (Ranganna 1986). Total polyphenols content in ethanol extracts was determined with Folin-Ciocalteau reagent using gallic acid as a standard (Singleton and Rossi 1965). Cupric ion reducing antioxidant capacity (CUPRAC) was a widely applicable antioxidant activity, utilizing the copper (II)-neocuproine [Cu (II)-Nc] reagent as the chromogenic oxidizing agent. Reducing ability of polyphenols to copper (II) (or cupric) ion was measured. The method was named by a research group (Apax *et al.* 2004) ″cupric reducing antioxidant capacity" abbreviated as the CUPRAC method. Ethanolic extract (100 µl) of respective sample was taken. Neucuproine (1 ml), 1 ml ammonium acetate, 1 ml $CuCl_2$ and 1 ml double distilled water were added and absorbance was recorded at 450 nm by using UV-VIS double beam PC 8 scanning Auto Cell Spectrophotometer (Model UVD-3200; Labomed, Inc., Culver city, CA, USA). Antioxidant activity was measured by following formula:

$$\mu \text{ moltrolax per gram} = \left(\frac{AF}{ETR}\right)\left(\frac{VF}{VS}\right)\left(\frac{Vcup}{m}\right) \times r \times 1000$$

where, AF, Absorbance; ETR, absolute factor = 1.67×10^4; VF, final volume (4.1 ml), VS, sample volume taken (0.1 ml); r, dilution factor if spectrophotometer reading > 1, then sample is diluted with bi-distilled water; V_{cup}, initial volume; m, weight of sample (g).

Ferric ion Reducing Antioxidant Power (FRAP) assay was measured according to Benzie and Strain (1999). FRAP assay uses antioxidants as reductance in a redox-linked colorimetric method, employing an easily reduced oxidant system present in stoichiometric excess. For that Frap reagent was prepared by adding acetate buffer (300 mM, pH 3.6), Tripyridyl triazine (TPTZ 10 mM) and $FeCl_3$ (20 mM) in the ratio of 10: 1:1. Then 100 µl ethanolic extract of respective genotype was mixed with 3 ml Frap reagent and kept for 30 min. Absorbance was recorded at 593 nm by using UV-VIS double beam PC 8 scanning Auto Cell Spectrophotometer. The calibration curve was prepared with ascorbic acid.

Statistical analysis

The experiment was conducted in Randomized Block Design. Three replicates of each sample were used for statistical analysis. Differences between samples were tested by analysis of variance (ANOVA) followed by Duncan's new multiple range test (MRT) to assess differences groups means. $P \leq 0.5$ were considered significant.

RESULTS AND DISCUSSION

Data presented in Table 1 showed that among different genotypes the highest content of anthocyanin was found in Pusa Lal Chaulai (40.81 mg/100 g) and the lowest content was found in Renu Sree (5.33 mg/100 g). Pasko *et al.* (2009) reported 103.6 mg of cyanidine-3-glucoside equivalent (CGE) per 100 g dry weight in *Amaranthus cruentus* v. Aztec.

Ascorbic acid content was found to be highest in Co 1 (81.2 mg/100 g), which was significant high with all other genotypes, except Pusa Kirti. High value of ascorbic acid was also recorded in Pusa Kirti, Renu Sree and Pusa Kiran and the value was significantly different with each other (Table 1). The lowest value of ascorbic acid was found in Kunnur Local (5.6 mg/100 g). It was observed that genotypes having green leaves have higher ascorbic acid content than red leaves. Green amaranth is moderately rich in vitamin C (Ogunlesi *et al.* 2010). Gupta and Prakash (2009) reported the ascorbic acid content of the green leafy vegetables (GLV) ranging between 15.18 mg/100 g for *Centella asiatica* to 101.36 mg/100 g for *Triggonella foenum graecum* and it was significantly different among different vegetables.

Table 1 Anthocyanin, ascorbic acid and total carotenoid content in different amaranth genotypes

Genotype	Anthocyanin (mg/100 g FW)	Ascorbic Acid (mg/100 g FW)	Total carotene (mg/100 g FW)
Krishna Sree	5.8_d	8.4_e	45.13_d
Renu Sree	5.3_d	73.0_b	44.91_d
Kunnur Local	17.0_b	5.6_f	54.01_c
MZ 8	13.3_c	11.2_d	33.96_e
Pusa Kiran	5.2_e	67.2_c	59.04_b
Pusa Lal Chaulai	40.8_a	$7.3e_f$	69.41_a
Pusa Kirti	5.7_d	78.4_a	58.62_b
Co-1	5.5_d	81.2_a	55.31_c
CD (P = 0.05)	0.6	3.1	2.56
SEM	0.2	1.01	1.03

Mean value (n = 3) in each column followed by a different lower case letter indicate significant differences between genotypes at P≤05. FW is fresh weight of sample.

It was evident from the data (Table 1) that genotypes varied significantly with regard to total carotenoids contents ($P \leq 0.5$). The highest total carotenoids contents (TCC) was measured in Pusa Lal Chaulai (69.41 mg/100 g) followed by Pusa Kiran (59.04 mg/100 g) which was at par to Pusa Kirti (58.62 mg/100 g). The lowest value was recorded in MZ 8 (33.96 mg/100 g). Gupta and Prakash (2009) reported that GLV are also good sources of total carotene and β-carotene (precursor of vitamin A). *Murraya koenigii* was found to be the richest source of total and β-carotene (64.51 and 8.85 mg/100 g, respectively) among the GLV analyzed followed by *Centella asiatica* > *Amaranthus s*p. > *Triggonella foenum graecum*, respectively.

Phenolics are aromatic secondary plant metabolites and associated with colour, sensory quality and nutritional and antioxidant properties of food. The antioxidant activity of phenolic compound is mainly due to redox properties which allows them to act as reducing agents, hydrogen donors, singlet oxygen quenches, heavy metal chelators and hydroxyl radical quenchers (Kaur and Kapoor 2002). It was concluded from the Fig 1 that genotypes differed significantly with respect to Total phenolic contents ($P \leq 0.5$). Genotype Co 1 had the highest Total phenolic contents (1 411.6 µg gallic acid equivalent (GAE) per g) followed by Pusa Lal Chaulai (1 138.5 µg GAE/g) which did not show any significant difference with Pusa Kirti (1 134.1 µg GAE/g). While lowest value was recorded in MZ 8 (687.1 µg GAE/g). Kaur and Kapoor (2002) reported the total phenolic content of amaranth species to be 158.3 mg of tannic acid per 100 g of fresh vegetable. The total polyphenol content of some common Indian leafy vegetables was found to be in the range of 5–69.5 mg of tannic acid/g of extract (Shyamala *et al*. 2005). Therefore it can be said that the total polyphenol content of vegetables varies widely depending on the variety of vegetable and a comparison is difficult, as different standard compounds have been used for their analysis. Fig 1 revealed highly significant differences in the total polyphenol content of the amaranth genotypes analyzed ($P \leq 0.5$).

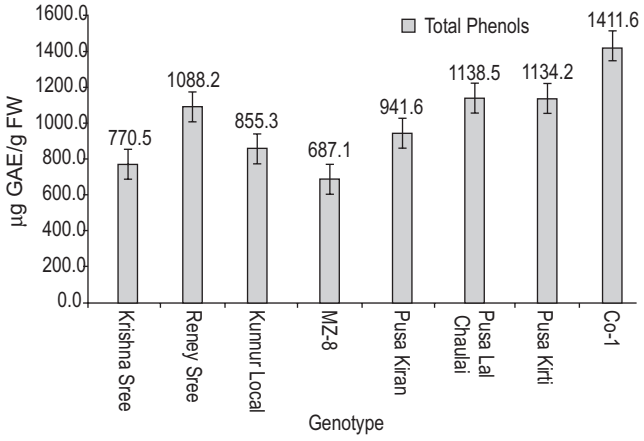

Fig 1 Total phenols content (gallic acid equivalent (GAE)/g) in different genotypes of amaranth. Vertical bars indicate ± SE. LSD (P=0.05) value for total phenols = 4.4, (n=3).

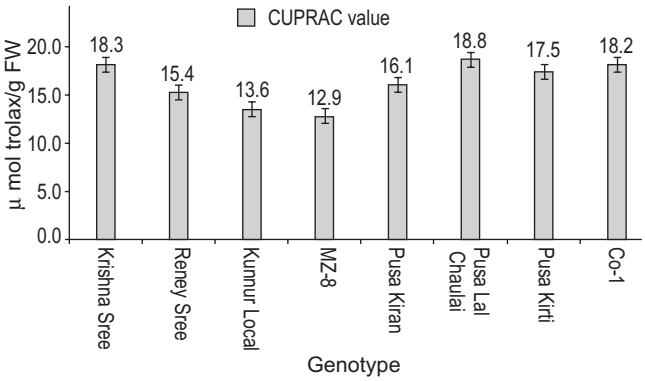

Fig 2 CUPRAC assay in different genotypes of amaranth. Vertical bars indicate ± SE. LSD (P=0.05) value for CUPRAC = 1.4, (n=3).

Among all the cultivars highest antioxidant activity by CUPRAC method was found in Pusa Lal Chaulai (18.8 µ moltrolax/g), which was at par with Krishna Sree and Co 1 (18.2 µ moltrolax/g) and the lowest was recorded in MZ 8 (12.9 µ moltrolax/g) (Fig 2). Gorinstein *et al*. (2008) observed antioxidant activity of *Amaranthus cruentus* (3.94 µ MTE/g of dry weight) by CUPRAC method.

It was clear from the Fig 3 that among different genotypes highest antioxidant activity by FRAP method was found in Pusa Lal Chaulai (13.16 µ mol ascorbic acid equivalent (AAE)/g fresh weight of sample) and lowest in Kannur Local (7.89 µ mol AAE/g). Pasko *et al*. (2009) reported antioxidant activity, i e 3.37 mM Fe^{+2}/Kg DW by FRAP method in *Amaranthus cruentus*. Very high antioxidant activities were found in intensely coloured genotypes (Pusa Lal Caulai and Co 1). Similar observations were also recorded

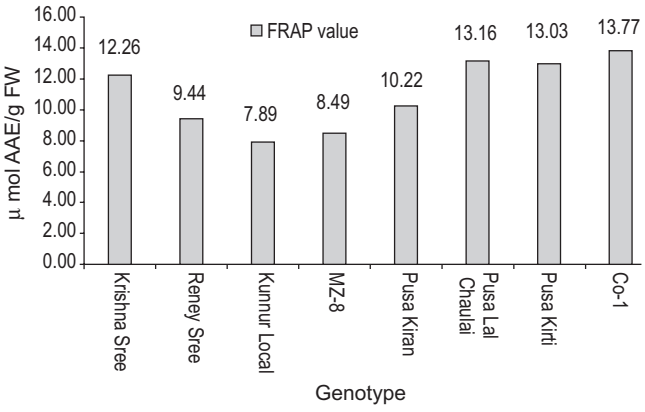

Fig 3 FRAP assay (ascorbic acid equivalent (AAE) per g fresh weight of sample) in different genotypes of amaranth. Vertical bars indicate ± SE. LSD (P=0.05) value for FRAP = 1.3, (n=3).

by Gupta and Prakash (2009) by phosphomolybdenum method who estimated the antioxidant capacities of GLV and the antioxidant capacity of different vegetables were in the order of *Murraya koenigii* > *Trigonella foenum graecum* >*Amaranthus* spp. > *Centella asiatica*.

Hence it can be said that the antioxidant activity can be estimated by different biochemical methods which can investigate the type of phenolic compounds is involved in antioxidant activity. The various biochemical parameters were studied and significant difference was found among the various *Amaranthus* genotypes. Based on the findings it was concluded that the Pusa Lal Chaulai has the highest antioxidant activity followed by Co 1. Pusa Lal Chaulai and Co 1 can be utilized for improving the efficiency of different nutraceutical and pharmacological products. The consumption of these may play a role in preventing human diseases in which free radicals are involved such as cancer, cardiovascular diseases and aging. On the whole it can be said that leafy vegetables apart from being a good source of micronutrients are also excellent antioxidants. Hence leafy vegetables can thus said to be substitutes for synthetic antioxidants.

REFERENCES

Apax R, Guclu K, Ozyurek M and Karademir S E. 2004. A novel total antioxidant capacity index for dietary polyphenols, vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method. *Journal of Agricultural and Food Chemistry* **52:** 7 970–81.

Benzie F F and Strain J J. 1999. Ferric reducing antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Methods in Enzymology* **299**: 15–23.

Bhargava A, Shukla S and Ohri D. 2006. Karyotypic studies on some cultivated and wild species of *Chenopodium (Chenopodiaceae)*. *Genetic Resources and Crop Evolution* **53:** 1 309–20.

Gorinstein P, Lojek A, Milan C Z, Pawelzik E, Delgado-Licon E, Medina J O, Morena M, Salas I A and Goshev I. 2008. Comparison of composition and antioxidant capacity of some cereals and pseudo cereals. *International Journal of Food Science and Technology* **43**: 629–37.

Gupta Sheetal and Prakash J. 2009. Studies on Indian green leafy vegetables for their antioxidant activity. *Plant Foods Human Nutrition* **64:** 39–45.

Jacobsen S E. 2003. The worldwide potential of quinoa *(Chenopodium quinoa* Willd.). *Food Reviews International* **19:** 167–77.

Kaur C and Kapoor HC.2002. Anti-oxidant activity and total phenolic content of some Asian vegetables. *International Journal of Food Science and Technology* **37:** 153–61.

Ogunlesi M, Okiei W, Azeez L, Obakachi V, Osunsanmi M and Nkenchor G.2010. Vitamin C contents of tropical vegetables and foods determined by voltammetric and titrimetric methods and their relevance to the medicinal uses of the plants. *International Journal of Electrochemical Science* **5:**105–15.

Pasko P, Barton H, Zagrodzki P, Gorinstein S, Folta M and Zachwieja Z. 2009. Anthocyanin, total polyphenols and antioxidant activity in amaranth and quinoa seeds and sprouts during their growth. *Food Chemistry* **115:** 994–8.

Ranganna S. 1986. *Handbook of Analysis and Quality Control for Fruit and Vegetable Products*, pp 84-99. Tata Mc Graw Hill publishing company Ltd, New Delhi.

Risi J and Galwey N W.1984. The *Chenopodium* grains of the Andes. *Advances in Applied Biology* **10:** 145–216.

Shyamala B N, Gupta S, Lakshmi J A and Prakash J. 2005. Leafy vegetable extracts-antioxidant activity and effect on storage stability of heated oils. *Innovative Food Science and Emerging Technologies* **6:** 239–45.

Singleton V L and Rossi J A. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagent. *American Journal of Enology Viticuture* **16:** 144–58.