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Studies on different genotypes of Indian bathua (*Chenopodium album*) for thier yield, quality and antioxidant activities

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ABSTRACT

Sixteen genotypes of *Chenopodium* (*Chenopodium album*) were evaluated for desired horticultural traits, viz. plant height (cm), leaf colour, leaf length and width, days to bolting, dry matter (%) and yield/plant (g); and their important quality traits, total carotene, ascorbic acid, total phenolic content, Cuprac ion Reducing Antioxidant Capacity (CUPRAC) and Ferric Reducing Antioxidant Power (FRAP) were also estimated during *rabi*/winter season of 2010 and 2011 at Division of Vegetable Science, IARI, New Delhi. Among the genotypes plant height ranged from 54.7 cm (Desi bathua) to 223 cm (Bathua 2). Yield/plant was recorded maximum in Bathua 13 (280 g) followed by Bathua 12 (277 g). Local Bathua (Desi type) recorded lowest yield 45.1 g/plant; however, it recorded maximum and significantly high dry matter content (16.5 %). Considerable variability was recorded in total carotenoids which ranged from 30.7 mg /100 g (Bathua 7) to 89.2 mg /100 g (Bathua 10). Bathua 10 also recorded maximum value of ascorbic acid (157.8 mg /100g) which was significantly higher than other genotype. High variability was recorded in total phenolic content, which ranged from 276.87 µg gallic acid equivalent (GAE)/g (Bathua 6) to 893.83 µg GAE/g (Bathua 13). Antioxidant activities recorded by both CUPRAC and FRAP method was found high in Bathua 13, Bathua 12 and Desi Bathua. On overall basis, it was concluded that Bathua 13, Bathua 12 and Bathua 14 could be the desirable genotypes for increasing yield and quality of *Chenopodium*. However, Bathua 10 was found rich source of carotene and ascorbic acid and Desi Bathua for high antioxidant activity. These genotypes can be further utilized to develop nutritionally rich leafy vegetables.

Key words: Antioxidant activity, Ascorbic acid, Carotenoids, *Chenopodium album,* Green leafy vegetables, Phenolic content, Yield and Yield attributing traits

Green leafy vegetables (glvs) in our country are consumed by all classes of people as they are known to be the cheap source of several vital nutrients. Glvs also contain an immense variety of bioactive non-nutritive health promoting factors as antioxidants, phytochemicals, essential fatty acids and dietary fibres which provide health benefits beyond basic nutrition. In india and other developing countries a large portion of the population are suffering from malnutrition due to less availability of vitamins and minerals rich diet, since wheat and rice are the principal food crops. Leafy vegetables not only supply the protective nutrients and add variety to a monotonous diet, but also have an alternative taste, pleasing appearance and aroma. Majority of the Indian population is vegetarian and daily intake of at least 100 g of fresh green leafy vegetable is recommended by the nutrition experts (Reddy 1999). Among Glv, *Chenopodium* commonly known as bathua (*Chenopodium album*) is a store house of vitamins such as carotene (precursor of vitamin A), ascorbic acid (precursor of vitamin C), folic acid and riboflavin as well as minerals such as iron, calcium and phosphorous. *Chenopodium* 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).This underutilized crop does not require high inputs and can be easily grown on agriculturally marginal lands (Partap *et al*. 1998, Bhargava *et al*. 2003). Seeing the potential of *Chenopodium* 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, since very limited information is available on *Chenopodium*. To identify the potential of different genotypes of *Chenopodium* available in India diverse genotypes of bathua were collected from various parts of country and evaluated for their yield and yield contributing traits. Total antioxidant activity assessed by Ferric reducing antioxidant power (FRAP), Cupric ion reducing antioxidant capacity (CUPRAC), total phenols, ascorbic acid and total carotenoid content 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. Sixteen diverse genotypes of bathua were used in this study. All the genotypes were grown in three replication in Completely Randomized Block Design (RBD) at research farm, Division of Vegetable Science, IARI, New Delhi for 2 consecutive years November 2010 and 2011. The standard recommended practices of growing bathua were followed. The maximum temperature ranged from 13.6°C to 28.5°C and minimum temperature ranged from 0°C to 15.3°C and the weather remained cool 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. Five plants from each replication were selected randomly for recording varius qualitative and quantitative data. The edible portion was harvested at marketable stage and fresh leaf samples were washed under running tap water followed by double distilled water. They were drained completely, dried over filter paper and analysed for total carotene, ascorbic acid, polyphenols, antioxidant activities and dry matter contents.

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

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. 100 µl ethanolic extract of respective sample was taken. 1 ml neucuproine, 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:

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

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 average value of 3 replications of 2010 and 2011 were used for statistical analysis. Differences between samples were tested by analysis of variance (ANOVA) to assess differences group means. P values ≤ 0.5 were considered significant.

RESULTS AND DISCUSSION

The mean value of quantitative and qualitative traits presented in Table 1 shows that there was significant variability among 16 *Chenopodium* genotypes for plant height, leaf length, leaf width, leaf weight, days to bolting, dry matter content and yield/plant. Plant height at flowering ranged from 54.7 cm (Local Bathua) to 223 cm (Bathua 2). Several genotypes namely, Pusa Bathua 1, Bathua 2, Bathua 9, Bathua 12 and Bathua 13 recorded plant height more than 2 meter, which was significantly high than other genotypes. All these genotypes were multi-cut type also. Leaf length varied from 6.5 cm (Local Bathua) to 18 cm (Bathua 6). Significant differences among genotypes were recorded for leaf length. Similarly leaf width also ranged from 4.1 cm to 9.33 cm. Average leaf weight, which played a major role in total yield of a plant, ranged from 0.24 g (Local Bathua) to 0.96 g (Bathua 13). Genotypes namely, Bathua 12 (0.88 g), Bathua 8 (0.70 g), Bathua 9 (0.68 g), Bathua 2 (0.65 g) and Bathua 10 (0.65 g) also recorded high average leaf weight, which was significantly higher than other genotypes. Yield/ plant in single harvest/pulling was recorded maximum in Bathua 13 (280 g) closely followed by Bathua 12 (277 g), Pusa Bathua 1 (263 g) and Bathua 2 (243 g). However, lowest yield/plant, i e 45.1 g was recorded in Local Bathua at marketable stage. Most of the high-yielding genotypes also had green to dark green leaves which is considered desirable trait from consumer's view point. Earliest bolting was recorded in Local Bathua followed by Bathua 3 and Bathua Lal 15. Bathua 14 (152 days), Bathua 10 (152 days), Bathua 11 (146 days), Bathua 7 (143 days) and Bathua 5 (143) were found late bolters, which is a desirable character

Table 1 Leaf colour, plant height, leaf length, width and weight, days to bolting, dry matter and yield/plant of 16 *Chenopodium* genotypes (values are mean ±SD)

Genotype	Leaf colour	Plant height at flowering (cm)	Leaf length (cm)	Leaf width (cm)	Average leaf weight (g)	Days to bolting	Dry matter containing	Yield/ plant(g)
Pusa Bathua 1	Red	211.3±4.73	16.0±1.00	5.8±0.15	0.61±0.03	123.0±2.6	10.4±0.53	263.3±12.5
Bathua 2	Green	223.3±4.73	13.0±1.00	7.8±0.32	0.65±0.03	134.3±2.5	10.8±0.15	243.3±2.8
Bathua 3	Dark green	152.3±14.19	9.0±1.00	6.2±0.25	0.45±0.04	80.0±2.0	9.0±0.55	50.0±5.0
Bathua 4	Dark green	154.0±9.64	8.0±1.00	5.7±0.25	0.35±0.02	82.3±2.5	10.6±0.53	62.0±3.0
Bathua 5	Green	164.3±7.37	12.8±0.76	7.0±0.06	0.56±0.03	143.0±1.0	10.0±0.25	149.3±6.1
Bathua 6	Green	157.0±11.79	18.0±1.00	8.7±0.25	0.58±0.04	103.0±7.6	9.9±0.26	160.0±10.0
Bathua 7	Green	150.3±11.37	12.3±0.58	7.1±0.36	0.49±0.01	145.6±1.5	10.0±0.38	169.6±5.5
Bathua 8	Green	196.3±11.85	14.1±0.76	8.7±0.20	0.70±0.01	146.5±5.3	10.5±0.31	206.6±7.6
Bathua 9	Green	214.7±8.74	15.0±1.00	7.6±0.17	0.68±0.03	90.4±4.5	10.2±0.45	200.0±5.0
Bathua 10	Dark green	166.3±12.10	15.7±0.58	8.3±0.21	0.65±0.06	152.6±1.5	10.0±0.83	141.6±10.4
Bathua 11	Green	193.0±9.17	17.9±0.90	7.2±0.26	0.64±0.06	146.0±1.0	9.4±0.67	213.6±5.0
Bathua 12	Green	211.3±10.26	12.8±0.76	5.6±0.55	0.88±0.03	119.0±1.0	11.0±0.46	277.3±16.6
Bathua 13	Green	227.0±7.55	11.8±0.29	5.8±0.32	0.96±0.02	129.0±1.0	10.7±0.57	280.0±5.0
Bathua 14	Green	152.0±5.00	15.5±1.32	9.3±0.29	0.58±0.04	152.3±1.5	9.5±0.81	179.3±4.0
Bathua Lal 15	Dark red	111.3±8.74	7.5±0.50	4.1±0.17	0.37±0.02	82.3±2.5	8.6±0.21	54.0±6.0
Local Bathua	Red green	54.7±13.87	6.5±0.50	4.1±0.15	0.24±0.03	76.6±2.1	16.5±0.74	45.1±2.0
CD at 5%		14.33	1.24	0.41	0.04	33.2	0.8	9.53
SEM		4.97	0.43	0.14	0.01	11.42	0.27	3.31

in bathua for leaf yield/cutting point of view. Lowest dry matter content was recorded 8.6% (Bathua Lal 15) while highest dry matter percentage was recorded in Local Bathua (16.5%), which was significantly higher than other genotypes. Bhargava *et al.* (2006) also recorded high variability for foliage yield while studying 13 genotypes of *Chenopodium album.*

Analysis of quality traits such as vitamin, pigments, polyphenols and antioxidant activities showed highly significant difference in all the traits of *Chenopodium* (Fig 1 to 4). Amongst the quality traits, total carotene is considered important, which ranged from 30.7 mg/100g (Bathua 7) to 89.2 mg/100g (Bathua 10) (Fig 1). High carotene content was recorded in Bathua Lal 15, Bathua 3 and Bathua 4 also.

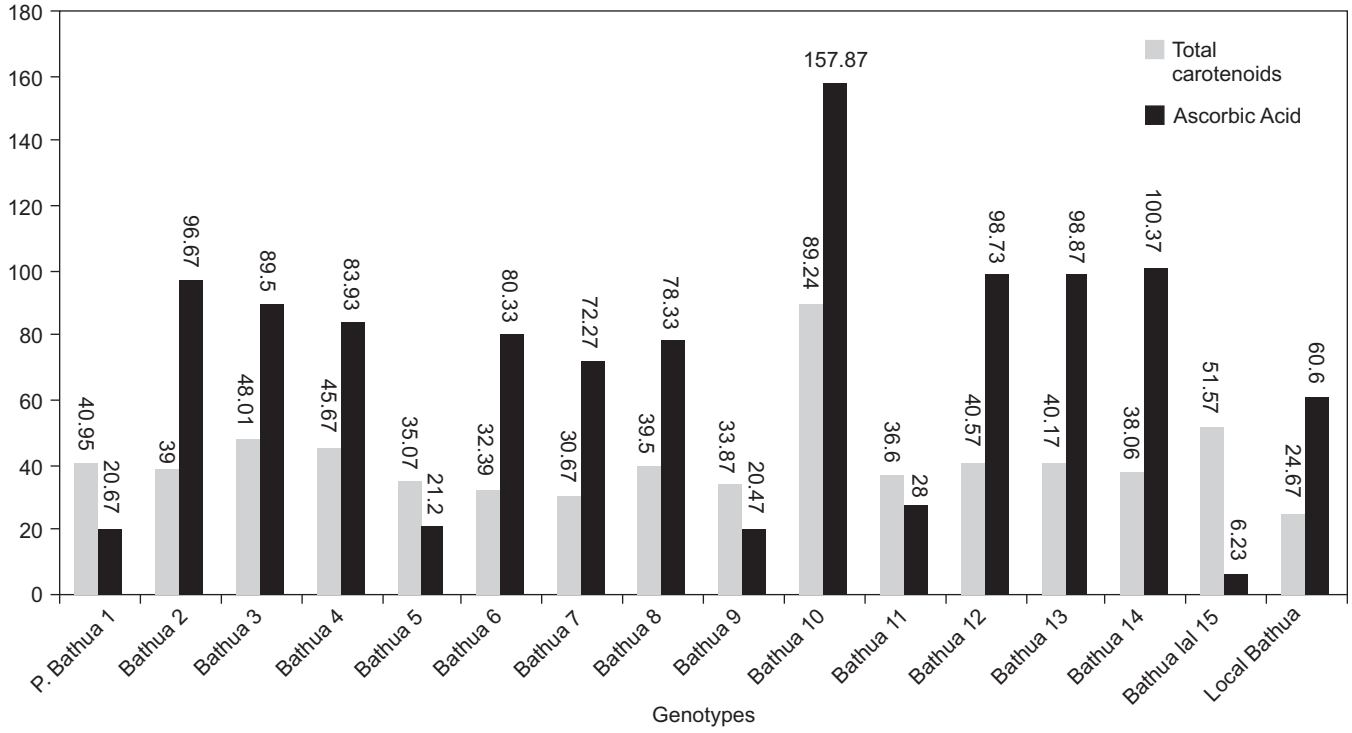

Fig 1 Total carotenoids and vitamin C (mg / 100 g) of 16 *Chenopodium* genotypes.

Therefore, these genotypes can be used for breeding carotene rich varieties. Ascorbic acid (precursor of vitamin C), another important quality traits was found maximum in Bathua 10 (157.8 mg/100g) followed by Bathua 14, Bathua 13, Bathua 12 and Bathua 2 (Fig 1). Singh *et al*. (2009) and Jha *et al.* (2011) also reported high value of ascorbic acid and carotenoids in different green leafy vegetables namely, spinach, coriander, mint, radish and amaranth. Phenolics are aromatic secondary plant metabolites associated with colour, sensory quality and nutritional and antioxidant properties of food. Total phenol content of leafy vegetables varied widely depending on the variety of the vegetables and a comparison is difficult, as different standards have been used for their analysis (Gupta and Prakash 2009). Total phenolic content (Fig 2) in *Chenopodium* genotypes ranged from 276.87 µg gallic acid equivalent (GAE)/g fresh weight (Bathua 6) to 893.83 µg GAE/g fresh weight (Bathua 13). Variation in total phenolic content was also reported by Shyamala *et al.* (2005) in leafy vegetables.

Antioxidant activities of different *Chenopodium* genotypes studies by CUPRAC (Fig 3) and FRAP (Fig 4) methods showed significant differences. In CUPRAC method high value of antioxidant (> 30 µ mol trolax/ g) was recorded in Bathua 2, Bathua 7, Bathua 12, Bathua13 and Local Bathua. Antioxidant activities of *Chenopodium* genotypes measured by FRAP method showed high value of antioxidant (>2 µ mol ascorbic acid equivalent (AAE)/g) in Bathua 13, Bathua Lal 15, Bathua 7, Bathua 12, Local Bathua, Bathua 14. Maximum value of antioxidant in CUPRAC was recorded in Local Bathua (34.20 µ mol trolax/g), however in FRAP method maximum value was recorded in Bathua 14 (2.63 µ

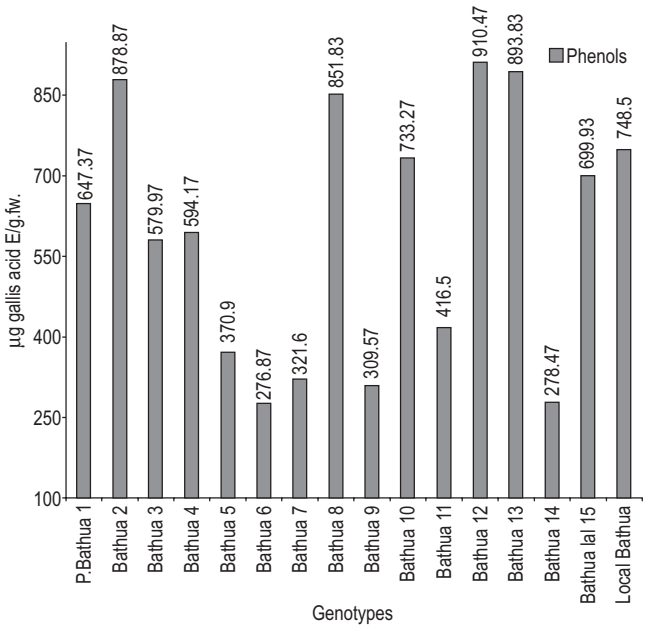

Fig 2 Total phenol (µg gallic acid equivalent (GAE)/g FW) analysis of 16 *Chenopodium* genotypes.

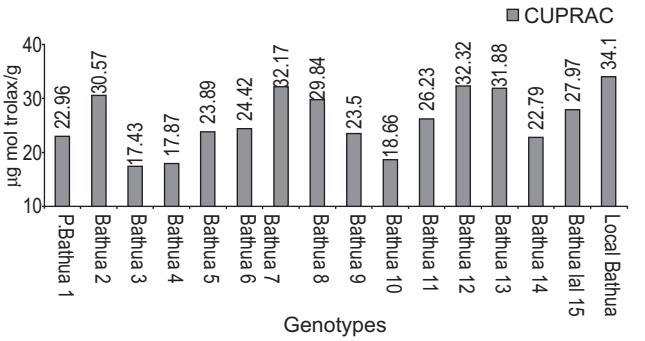

Fig 3 CUPRAC (µ mol trolax/g) analysis of 16 *Chenopodium* genotypes.

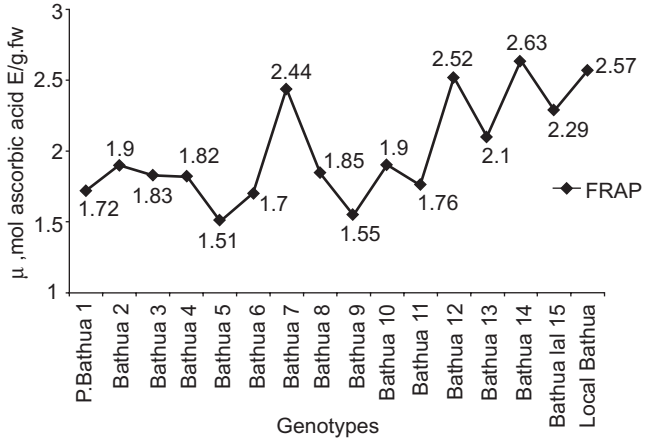

Fig 4 FRAP (µ mol ascorbic acid g/ FW (fresh weight) analysis of 16 *Chenopodium* genotypes.

mol AAE/g) followed by Local Bathua (2.57 µ mol AAE/g). Gorinstein *et al.* (2008) observed antioxidant activity of *Amaranthus cruentus* (3.94 µMTE/g of dry weight) by CUPRAC method, while Pasko *et al.* (2009) reported antioxidant activity of *cruentus*. Aztek (3.37 mM Fe^{+2}/kg DW) by FRAP method. In this study, several genotypes of bathua were found to be rich source of antioxidants, which can be utilized for chenopods improvement. Hence, it can be said that the antioxidant activity can be elucidated by different biochemical methods which can investigate the type of phenolic compounds is involved in antioxidant activity. The various physiological (colour, taste, texture) and biochemical parameters were studied and found significant difference among the various *Chenopodium* genotypes. Therefore, on over all bases Bathua 13 was found best genotype followed by Bathua 12, Bathua 13 and Bathua 14 for improving yield as well as quality of bathua. From carotene and ascorbic acid point of view Bathua 10 was found good. Local Bathua had high antioxidant activities.

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