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Antioxidant and mineral studies in different genotypes of Indian bathua (*Chenopodium album*)

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ABSTRACT

Twelve genotypes of bathua (*Chenopodium album* L.) were evaluated for their yield and important mineral content, viz. iron, zinc, calcium, sodium, potassium, manganese and magnesium alongwith quality traits namely, total carotenoids, β-carotene, ascorbic acid, total phenolic content, Cuprac Ion Reducing Antioxidant Capacity (CUPRAC) and Ferric Reducing Antioxidant Power (FRAP). Leaf yield per ha was recorded maximum in Bathua Sel-2 (333q/ha) followed by Pusa Bathua-1 (300 q/ha), while Desi Bathua (local weed type) recorded lowest leaf yield of 65.1 q/ha. Considerable variability was recorded in total carotenoids which ranged from 32.39 mg/100 g (Bathua-6) to 89.2 mg/100 g (Bathua-7). Bathua-3 recorded maximum value of ascorbic acid (89.5 mg/100g) which was significantly higher than other genotype. High variability was recorded in total phenolic content, which ranged from 270.87 µg gallic acid equivalent (GAE)/g (Bathua-6) to 820.47 µg GAE/g (Bathua Sel-8). Antioxidant activities recorded by both CUPRAC and FRAP method was found high in Desi Bathua followed by Pusa Bathua-1. However, Bathua Sel-7 was found rich source of carotene β-carrotene i.e. 89.24 and 10.80 mg/100g respectively. Desirable sodium to potassium ratio (<1) was recorded in almost all the genotypes. Desi Bathua recorded maximum calcium, iron and zinc content. Therefore it was found superior from nutritional viewpoint, however, Bathua Sel-2 and Pusa Bathua-1 were found high leaf yielder and fairly superior in nutritional quality. These genotypes can be further exploited to develop nutritionally rich and high leaf yielding bathua genotypes.

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

Green leafy vegetables (GLVs) in our country are consumed widely 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 GLVs, *Chenopodium* commonly known as bathua (*Chenopodium album* L.) is a store house of vitamins such as carotene (precursor of vitamin A), ascorbic acid (precursor of vitamin C) as well as minerals such as iron, calcium and potassium. *Chenopodium* can be used as a potential food crop for diversification of agriculture and for combating the nutritional deficiency in human being 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 (Pratap *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. The information thus generated 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 mineral content, yield along with total antioxidant activity assessed by Ferric reducing antioxidant power (FRAP), Cupric ion reducing antioxidant capacity (CUPRAC), total phenols, ascorbic acid, total carotenoid content and β-carotene.

MATERIALS AND METHODS

The present investigation was carried out at Indian Agricultural Research Institute, New Delhi situated at

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28.08^0 N and 77.12^0 E. The height above the mean sea level being 228.61 m. The climate is subtropical and semi-arid. Twelve 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 2012-13 and 2013-14. 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 0.0°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 281.5, 15.4 and 382.53 kg/ha respectively. Organic content (%) of experimental soil was 0.63. Five plants from each replication were selected randomly for recording various 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 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-dichlorophenolindophenol (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:

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

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) assaywas 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), Tripyridyltriazine (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.

Solution having carotenoids was passed through column containing Hyflosupercel and magnesium oxide (3:1, v/v) followed by petroleum benzene and acetone. Separation took place with two different bands one is orange in colour, i.e. β carotene.

The orange colour β carotene collected through column chromatography was then dried using vacuum dryer and was then mixed with 3 ml hexane. The sample was then filtered through 0.45 µm Millipore filter. The injection volume of 1 µl was used for the run.

Edible portion of different leafy vegetables were washed and cleaned then oven dried at 50˚C for 24 hours. The dried sample was then pulverized into fine powder in a mortar pestle. About 0.5 g dried sample was extracted with 10 ml of nitric acid at different temperature stages by using digestion unit. Once cooled, the solution was diluted to 50 mL distilled water then volume was made to 100 ml with distilled water. No further sample preparation was required. 10 ml solution was taken from 100 ml volume for MP-AES (microwave plasma- atomic emission spectrometer). The injection volume of 1 µl was used for the run.

The average value of 3 replications was 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 leaf yield and qualitative traits presented in Table 1 shows that there was significant variability among 12 *Chenopodium* genotypes for leaf colour, leaf yield/ha, antioxidant activities and other nutritional traits. Leaf yield was recorded maximum in Bathua Sel-2 (333 q/ha) followed by Pusa Bathua-1 (300 q/ha). However, lowest yield/plant, i.e. 65.1 q//ha was recorded in Desi Bathua at marketable stage. Most of the high yielding genotypes also recorded green to dark green leaves which is considered desirable horticultural trait from consumer's view point. The coloured leaf in Bathua is also significantly important from nutritional point. Bhargava *et al.* (2006) also recorded high variability for foliage yield while studying 13 genotypes of *Chenopodium album.*

Analysis of variance for quality traits such as vitamin, pigments, polyphenols and antioxidant activities showed highly significant difference in all the traits of *Chenopodium* (Table 1). Amongst the quality traits, total carotene is considered important, which ranged from 32.39 mg/100g (Bathua Sel-6) to 89.24 mg/100g (Bathua Sel-7). Significantly high carotene content was recorded in Bathua Sel-2, Bathua Sel-3 and Bathua Sel-4. However, β-carotene was recorded maximum in Bathua Sel-7 (10.8 mg/100g) which was significantly higher than second highest genotype

Table 1 Antioxidant, vitamins, nutrients and yield of *Chenopodium* genotypes (values are mean±SD)

Genotype	Leaf colour	Ascorbic acid (mg/100g)	Total carotenoids (mg/100 g)	β-carotene (mg/100 g)	CUPRAC (µ mol trolax/ g)	FRAP (µ mol AA/g)	Phenols (µg GAE/g)	Yield (q/ha)
Pusa Bathua-1	Red	25.6±0.2	41.95±2.24	4.93±0.35	32.96±1.85	1.80±0.04	587.37±40.26	300.3±12.5
Bathua Sel-2	Green	76.7±1.2	59.00±1.32	5.10±0.20	30.57±1.36	1.90±0.13	798.87±38.00	333.3±2.8
Bathua Sel-3	Dark green	89.5±1.2	58.01±2.32	5.70±0.46	27.43±0.83	1.85±0.06	497.97±26.44	150.0±5.0
Bathua Sel-4	Dark green	83.2±1.3	55.67±0.76	5.90±0.26	19.87±0.71	1.82±0.13	474.17±21.26	162.0±3.0
Bathua Sel-5	Green	56.5±0.5	35.07±1.69	4.63±0.38	23.89±2.26	1.51±0.07	289.90±16.54	149.3±6.1
Bathua Sel-6	Green	40.8±0.6	32.39±2.11	4.00±0.20	24.42±0.59	1.70±0.05	270.87±17.28	160.0±10.0
Bathua Sel-7	Dark green	72.9±1.0	89.24±2.92	10.80±1.05	18.66±0.74	1.90±0.05	673.27±19.67	141.6±10.4
Bathua- Sel-8	Green	68.5±0.7	45.57±0.90	4.97±0.61	32.32±1.92	2.52±0.11	820.47±23.68	277.3±16.6
Bathua- Sel-9	Green	56.2±0.5	41.17±1.01	4.67±0.42	31.88±1.86	2.10±0.10	793.83±22.62	280.0±5.0
Bathua- Sel-10	Green	56.7±0.4	38.06±2.26	4.73±0.21	22.79±2.30	2.63±0.13	278.47±63.74	179.3±4.0
Bathua Sel-1	Dark red	9.5±0.1	41.57±1.29	6.07±0.40	27.97±1.40	2.29±0.44	699.93±15.10	84.0±6.0
Desi Bathua	Red green	60.3±0.5	34.67±3.54	5.03±0.45	34.10±1.00	2.67±0.14	688.50±52.53	65.1±2.0
CD (P=0.05)		5.6	3.32	0.76	2.44	0.22	37.95	19.53
SE(m)		1.45	1.14	0.26	0.84	0.78	13.13	3.31

Bathua Sel-1 (6.07 mg/100g). Therefore, these genotypes can be used for breeding carotene rich varieties.

Ascorbic acid (precursor of vitamin C), another important quality traits ranged from 9.5 mg/100g (Bathua Sel-1) to 89.5 mg/100 g (Bathua Sel-3). It was noticed that genotypes having green colour leaves recorded higher ascorbic acid content than red colour. 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 (Table 1) in *Chenopodium* genotypes ranged from 270.87 µg gallic acid equivalent (GAE)/g fresh weight (Bathua Sel-6) to 820.47 µg GAE/g fresh weight (Bathua Sel-8). Variation in total phenolic content was also reported by Shyamala *et al.* (2005) in leafy vegetables.

Antioxidant activities of different *Chenopodium* genotypes studied by CUPRAC and FRAP methods showed significant differences (Table 1). In CUPRAC method, significantly high value of antioxidant was recorded in Desi Bathua (34.10 µ moltrolax/g). High value of antioxidant (> 30 µ mol trolax/g) was also recorded in Bathua Sel-2, Bathua Sel-8, Bathua Sel-9, Pusa Bathua-1 and Desi 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 Sel-1, Bathua Sel-8, Bathua Sel-9, Bathua Sel-10 and Desi Bathua. Similarly Desi Bathua in FRAP method also recorded maximum value of antioxidant activities (2.67 µ mol AAE/g) followed by Bathua Sel-10 (2.63 µ mol AAE/g). Gorinstein *et al.* (2008) observed antioxidant activity of *Amaranthus cruentus* (3.94 µ MTE/g of dry weight) by CUPRAC method, while Pasko *et al.* (2009) reported antioxidant activity of *Amaranthus cruentus* v. 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 significant difference found was among the various *Chenopodium* genotypes. Yadav *et al.* (2013) also reported high antioxidant activities in *Chenopodium album*.

Mineral content in different genotypes of bathua is given in Figs 1-2. The genotypes showed high level of variability for different mineral used for study. Highest calcium content was recorded in Desi Bathua (280 mg/100g) followed by Pusa Bathua-1 (250 mg/100g). All the genotypes recorded more than 100 mg/100g calcium content which showed that Bathua is an important source of calcium and it can be utilized in diet to meet out calcium requirement especially in growing children. Most of the genotypes showed low content of sodium ranging from 43 mg/100g (Bathua Sel-9) to 146 mg/100g (Bathua Sel-7). In contrast to this, potassium content was recorded quite high in most of the genotypes and maximum potassium content was recorded in Bathua Sel-3 (421 mg/100g) closely followed by Desi Bathua (412 mg/100g). Du *et al.* (2014) reported that the sodium-to-potassium ratio was more strongly associated with incident hypertension than either sodium or potassium alone. The sodium potassium ratio should be <1. Similar trends were available in Chenopod genotypes in present study, where most of the bathua genotypes recorded sodium

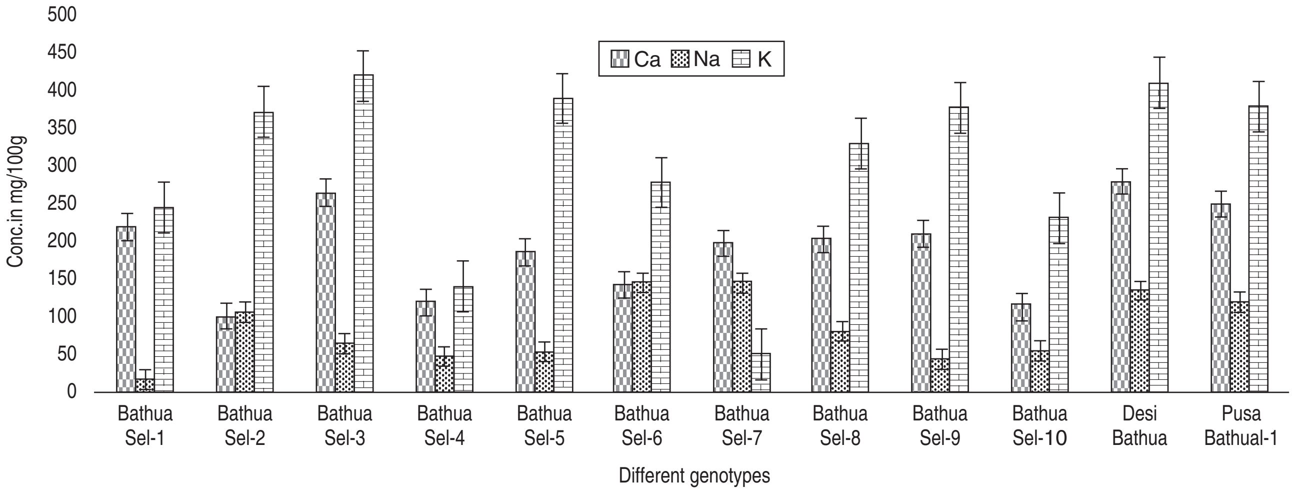

Fig 1 Calcium, sodium and potassium content (mg/100 g FW) in different *Chenopodium* genotypes (LSD for Ca=56.9, Na=16.5 and K=68.3).

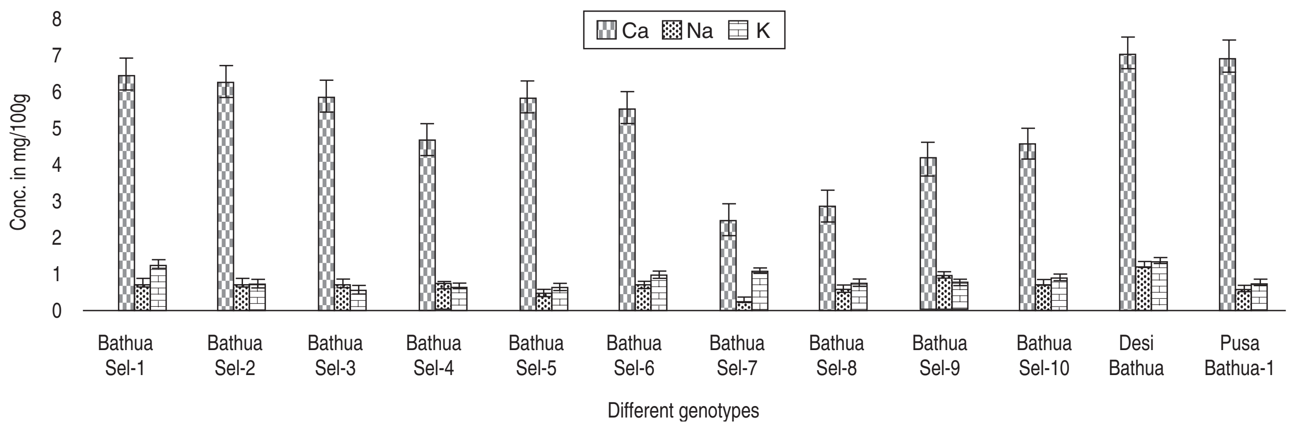

Fig 2 Iron, zinc and manganese content (mg/100 g FW) in different *Chenopodium* genotypes (LSD for Fe=1.6, Zn=0.7 and Mn=0.9).

and potassium ratio less than one.

Iron an important mineral in the diet especially for pregnant and nursing women was recorded maximum in Desi Bathua (7.1 mg/100g) followed by Pusa Bathua-1 (Fig 2). It was evident from the data that high iron content was available in red leaf colour genotypes than green. Zinc another important mineral was estimated maximum in Desi Bathua (1.3 mg/100g) followed by Bathua Sel-9 (1.0 mg/100g). It is evident from the data that Bathua is rich source of iron and zinc also. There was less variability in manganese and magnesium content in different genotypes of Bathua. The finding revealed that Bathua is rich source of iron, calcium, magnesium, sodium and potassium. However, significant genotypic differences within a crop were noticed for mineral content. On overall basis, Chenopod was found rich in most of the mineral under study. Agte *et al.* (2000) analysed various green leafy vegetables for 8 micronutrients and reported higher bioavailability of meals based on leafy vegetables than those based on cereal and legumes.

If the growers are interested to produce high quality leafy vegetables in regard to health benefits, the varieties rich in various antioxidants should be cultivated. Therefore, the results suggested that Pusa Bathua -1 and Bathua Sel-2 can be utilized for high leaf yield and antioxidant nutrients. However, Desi Bathua can be recommended for its high antioxidant, carotenoids and mineral content and may be popularised and made available to the consumers. These genotypes could 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.

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