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Mineral content estimation in *Atriplex hortensis* L., an indigenous vegetable of Trans-Himalayan region of Ladakh, India

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ABSTRACT

The present study was aimed at estimating the mineral contents and elucidating nutritious importance of this traditionally grown vegetable, *Atriplex hortensis* L. from Ladakh region of India. Altogether 70 accessions of 14 populations (five individuals from each population) of *A. hortensis* L. were collected from different regions. The collected accessions (seeds) were sown in randomized block design (RBD) with three replications at research field of Defence Institute of High Altitude Research (DIHAR, DRDO) during the cropping season of the year 2015-16. Plants were evaluated for mineral content by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). Statistical analysis was carried out for analysis of variance and principal component analysis (PCA) using SPSS ver 21.0 and XLSTAT, 2017. The results demonstrated a high level of variation in 13 estimated mineral elements including Ca, Mg, Zn, Na, K, S, B, Cu ($P \leq 0.01$) and Si ($P \leq 0.05$). Sulphur (S) was found to be the highest and ranged from 53.090±0.020 to 81.433±0.025 mg/g in the study samples, whereas selenium (Se) was present in very low concentration and ranged from 0.004±0.0001 to 0.089±0.003, respectively. The PCA suggested that Mg, Na, Zn, Si and Ca were highly correlated to the first component (27% of the variability); K, Cu and P to the second component (17% of the variability), and Al and S to the third component (15% of the variability). Altogether 59% of variability was accounted for the first three components (PCA) in the study. The study indicated that Trans-Himalayan vegetable, *A. hortensis* L. was rich in mineral content and hence had considerable nutritional value. This vegetable is naturally supported by the harsh climate of Ladakh region, therefore, could augment appreciably to the fresh ration requirements of troops and local population.

Key words : *Atriplex hortensis*, Ladakh, mineral content, Trans-Himalayan region

INTRODUCTION

The significance of mineral components in human, animals and plant sustenance has been notable, as many minerals have been associated with the nutrition of living organisms (Underwood, 1971; Darby, 1976). Mineral deficiency may cause lack of nutrition leading to a range of diseases that can emerge in different ways (Gordon, 1977). Mineral elements have been reported to play crucial roles in disease states and overall health of humans and domestic animals. Studies have demonstrated that iron deficiency anaemia and goitre due to iodine deficiency are prominent problems of public health importance in many communities (Deosthale and Belavady, 1978). Furthermore, many trace elements such as zinc and selenium are of a significance to people suffering with HIV. Selenium has been reported as an excellent antioxidant that increases immune function against various infections. Zinc, usually taken to stimulate the immune system, has been reported to weaken immune system function and lower calcium (Ca) levels in HIV infected individuals (Connor, 1995; Wood, 2000). Similarly, other minerals such as phosphorus (P), sulphur (S), copper (Cu), sodium (Na), potassium (K), etc. have also been associated with many diseases that could be fatal to human and plants.

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Many vegetables and plants are rich sources of minerals and their products and derivatives have been used historically to fulfil the mineral elements requirement. However, to understand the ampleness of mineral in plants and vegetables, it is equally important to estimate their mineral contents and create information of their mineral richness (Rao and Rao, 1981; Simsek and Aykut, 2007). Although vegetables are rich source of micronutrients and antioxidants, but many traditional and indigenous vegetables that are grown in remote areas of the country have not been studied for the mineral element richness. In fact, many of them such as spinach, kale and cabbage, in the recent studies, have been found to contain very high level of elements compared to exotic varieties (Orech *et al.*, 2007).

A. hortensis L. (Family : Chenopodaceae) is abundant in Trans-Himalayn Ladakh region (annual temperature range : -17° to 30°; 2500 to 4100 m msl) of India and popularly known as Phaltora. The plant is probably one of the oldest wild edible plants and rich source of protein and vitamins. *A. hortensis* L. has been historically used as food and health supplement by various tribes such as balti, purik, bodh and dardi, etc. Broad green leaves of this plant have been shown effective in the treatment of gout, induration and tumor. This plant is the first green vegetable to appear after the end of long winter season.

Various studies have been conducted on this plant. However, data on mineral content of this plant are still scanty. In the present study, mineral content of 14 different populations of *A. hortensis* L. collected from different sub-regions of Ladakh region has been determined quantitatively.

MATERIALS AND METHODS

Study Area

The study was conducted at vegetable research unit (altitude 3500 m msl, latitude 34°8′16.119″ N, longitude 77°34′19.2216″E) of Defence Institute of High Altitude Research (DIHAR), in Leh-Ladakh, India. The soil texture of the experimental site was silty loam with pH 7.5±0.3 (mean±SD). Organic matter and organic carbon content were 4.4±0.5 and 1.3±0.2%, respectively.

Experimental Design and Sampling

To determine the diversity of mineral accumulation, 14 populations of five accessions each representing population of *A. hortensis* L. from three valleys (Nubra, Indus and Suru) were collected. The samples were carefully collected at a distance of about 2 to 5 km of each accession and about 25 to 250 km from each population. The seeds were sown in DIHAR farms during May 2016 in randomized block design for the production of vegetative part in open condition. The plot size was 2 m^2 and row-to-row and plant-to-plant spacing was 15 and 10 cm, respectively. All recommended agronomic practices were followed. The harvest was carried out approximately after 30 to 45 days when the plants were young and tender and ready-to-consume. Random samples from each set of three plants leaves from each accession and each replication were combined. Each sample was weighed and immediately snap-frozen in liquid nitrogen and stored at -80°C until it was lyophilized. Dry weight measurements were made on lyophilized samples.

Materials

AR grade reagents, distilled water and Borosil glassware were used for the preparation of solutions and sample analysis. For mineral analysis, ICP multi element standard solution IV (Merck, India) was used. All the glasswares were sterilized before use in experiments.

Sample Treatment and Analysis

For mineral analysis, all plant samples were digested on mass to weight basis, using trace metal grade 69% nitric acid (HNO_3), 60% perchloric acid ($HClO_4$) and 35.4% hydrochloric acid (HCl). Samples were digested on 42 blocks using Automated Hot Bock digestion system (Questron Technologies Inc, Canada) and used to estimate the minerals using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) (Optima 7000 DV; Perkin-Elmer Analyst, USA) following standard manufacturer's instructions and according to the standard estimation methods (Kalra, 1998). Plasma conditions of ICP-OES method were standardised for the present experiments as follows : plasma flow 15 l/min; auxiliary gas flow 0.2 l/min; nebulizer gas flow 0.8 l/min; RF power 1300 watt and pump flow rate 1.5 ml/min.

Statistical Analysis

Data have been analyzed using analysis of variance (ANOVA) followed by Tukey's test and considered significant if P≤0.05. Genetic diversity among the populations was studied using generalized distance (D^2). Principal component analysis (PCA) was used as correlation matrix to determine the variables containing maximum possible variance with the number of principal components (Kovacic, 1994). The similarity coefficient was determined by following Gower General Similarity Coefficient that reflects the differences existing among the accessions (Kendall, 1980). All statistical analyses were performed using SPSS version 21.0 and XLSTAT, 2017.

RESULTS AND DISCUSSION

The concentration of 13 important mineral elements, namely, Ca, Mg, Zn, Fe, Na, Al, K, P, S, Si, B, Cu and Se in the leaves of the 70 accessions representing 14 different populations of *A. hortensis* L. of Ladakh region (Fig. 1) have been shown in Table 1. Varying levels of concentration of mineral elements were found in different populations. S, Al, K and Ca were found in high concentration than the other mineral elements (Table 1). A highest concentration of S (81.43±0.025 mg/g) was recorded in population collected from Gonpa area, whereas the lowest was found in the population collected from Udmaru area (53.09±0.020 mg/g). On the other hand, lowest concentration was recorded for Se in the present study. It ranged from 0.004±0.001-0.089±0.003 mg/g in the study populations. Furthermore, Al was also found in considerably high concentration and ranged between 5.26±0.003-12.95±0.006 mg/g in the present study. Among the other elements, K and Ca were present in considerable concentration and their highest concentration of 1.83±0.002 and 1.70±0.002 mg/g, respectively, were recorded in the populations collected from Pakskum.

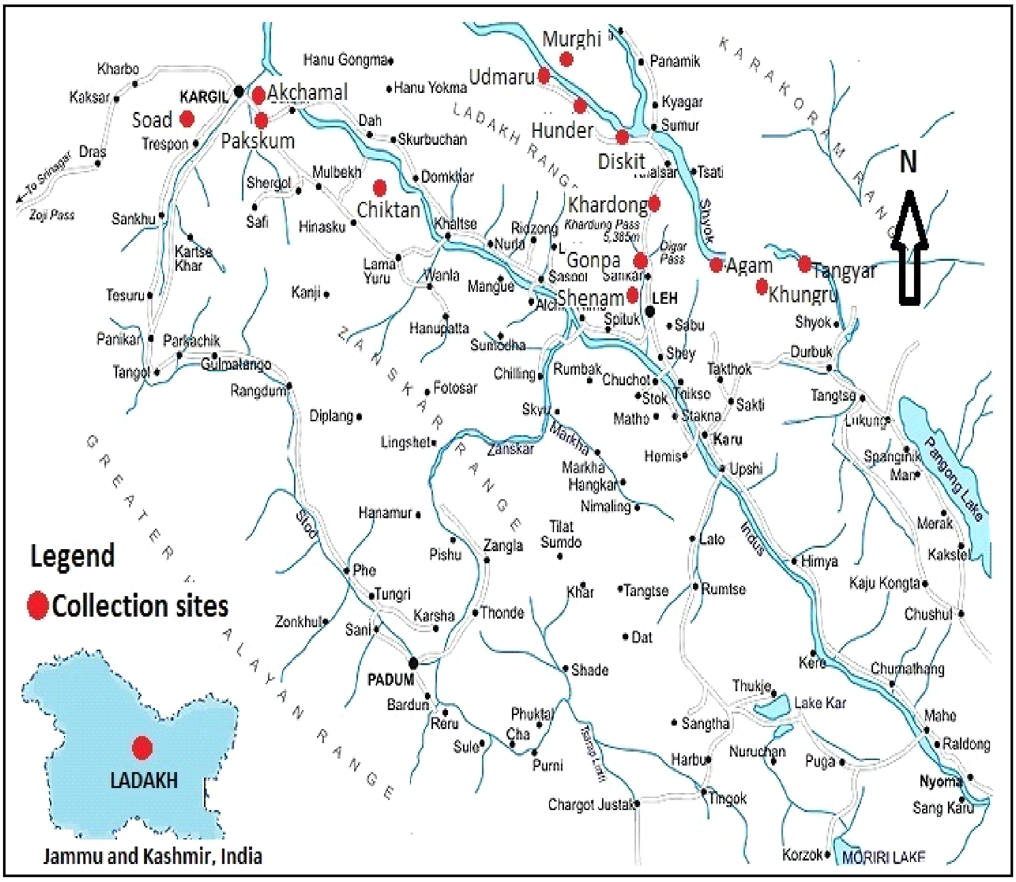

Fig. 1. Map of Ladakh region showing the collection sites (marked in red) of *A. hortensis* L. accessions for the present study (Not to be scaled).

Table 1. Mineral elements (mg/g) estimated in the study population of *Atriplex hortensis* L.

S. No.	Locations	Ca	Mg	Zn	Fe	Na	Al	K
1.	Chiktan	0.995±0.004^{abc}	0.176±0.002^{a}	0.251±0.003^{de}	0.191±0.003^{a}	0.187±0.002^{a}	4.643±0.560^{a}	1.557±0.050^{f}
2.	Agham	0.983±0.015^{abc}	0.201±0.002^{b}	0.225±0.002^{c}	0.185±0.001^{a}	0.195±0.001^{b}	5.263±0.003^{b}	1.674±0.005^{h}
3.	Soad	1.068±0.004^{abcd}	0.319±0.003^{h}	0.157±0.004^{a}	0.241±0.002^{a}	0.211±0.003^{d}	5.423±0.002^{b}	1.340±0.001^{b}
4.	Khardong	1.110±0.020^{bcd}	0.273±0.003^{g}	0.265±0.003^{ef}	0.219±0.002^{a}	0.211±0.003^{d}	6.883±0.002^{de}	1.445±0.004^{d}
5.	Hunder	1.317±0.001^{cde}	0.222±0.001^{d}	0.354±0.003^{h}	0.214±0.002^{a}	0.213±0.001^{d}	7.121±0.001^{ef}	1.616±0.001^{g}
6.	Akchamal	0.687±0.508^{a}	0.235±0.001^{e}	0.293±0.001^{g}	0.213±0.003^{a}	0.228±0.001^{e}	6.796±0.003d^{e}	1.722±0.002^{i}
7.	Shenam	0.744±0.001^{ab}	0.178±0.002^{a}	0.230±0.001^{c}	1.071±0.795^{b}	0.193±0.004^{b}	6.548±0.005^{d}	1.414±0.003^{c}
8.	Gonpa	0.850±0.007^{ab}	0.247±0.002^{f}	0.199±0.001^{b}	0.208±0.002^{a}	0.211±0.002^{d}	12.947±0.006^{h}	1.643±0.003^{gh}
9.	Murghi	0.966±0.001^{abc}	0.223±0.002^{d}	0.258±0.002^{def}	0.190±0.001^{a}	0.201±0.001^{c}	7.660±0.020^{g}	1.493±0.003^{e}
10.	Pakskum	1.697±0.002^{e}	0.375±0.001^{j}	0.354±0.002^{h}	0.261±0.001^{a}	0.304±0.001^{f}	5.871±0.001^{c}	1.827±0.002^{k}
11.	Udmaru	1.036±0.002^{abcd}	0.203±0.001^{b}	0.274±0.001^{fg}	0.191±0.001^{a}	0.211±0.001^{d}	5.383±0.003^{b}	1.779±0.001^{j}
12.	Diskit	1.128±0.001b^{cd}	0.216±0.002^{c}	0.202±0.001^{b}	0.203±0.002^{a}	0.212±0.002^{d}	7.391±0.007f^{g}	1.381±0.001^{bc}
13.	Tangyar	1.563±0.006^{e}	0.334±0.001^{i}	0.239±0.023^{cd}	0.249±0.001^{a}	0.213±0.002^{d}	6.717±0.001d^{e}	1.717±0.001^{i}
14.	Khungru	1.436±0.002^{de}	0.315±0.002^{h}	0.496±0.001^{i}	0.716±0.002^{ab}	0.184±0.002^{a}	7.475±0.003f^{g}	1.250±0.001^{a}
15.	Total	1.113±0.310	0.251±0.061	0.271±0.083	0.311±0.306	0.213±0.028	6.866±1.940	1.561±0.173

Contd.

Table 1 *Contd.*

S. No.	Locations	P	S	Si	B	Cu	Se
1.	Chiktan	0.174±0.006^{a}	69.133±0.025^{i}	0.132±0.003^{b}	0.120±0.026^{f}	0.046±0.003a	0.004±0.001a
2.	Agham	0.395±0.507^{a}	61.817±0.025^{a}	0.057±0.0005^{f}	0.128±0.002^{f}	0.057±0.001^{d}	0.036±0.002^{d}
3.	Soad	0.241±0.001^{a}	62.207±0.025^{d}	0.208±0.003^{d}	0.017±0.002^{abc}	0.049±0.002^{ab}	0.078±0.002^{h}
4.	Khardong	0.157±0.005^{a}	61.887±0.025^{c}	0.166±0.001^{c}	0.005±0.002^{a}	0.051±0.002^{bc}	0.046±0.002^{e}
5.	Hunder	0.244±0.003^{a}	62.847±0.025^{e}	0.222±0.002^{d}	0.012±0.001^{ab}	0.051±0.001^{bc}	0.089±0.003^{i}
6.	Akchamal	0.323±0.004^{a}	62.733±0.15^{e}	0.143±0.006^{bc}	0.014±0.001^{abc}	0.048±0.001^{ab}	0.086±0.000^{i}
7.	Shenam	0.241±0.002^{a}	66.667±0.006^{g}	0.095±0.003^{a}	0.023±0.001^{abcd}	0.045±0.004^{a}	0.036±0.002^{d}
8.	Gonpa	0.324±0.003^{a}	81.433±0.025^{m}	0.137±0.001^{h}	0.024±0.001^{abcde}	0.055±0.001^{cd}	0.019±0.001^{b}
9.	Murghi	0.147±0.001^{a}	68.163±0.021^{h}	0.147±0.002^{bc}	0.029±0.001^{bcde}	0.048±0.001^{ab}	0.060±0.001^{g}
10.	Pakskum	0.304±0.004^{a}	70.937±0.031^{j}	0.289±0.001^{e}	0.026±0.001^{abcde}	0.049±0.001^{ab}	0.025±0.001^{c}
11.	Udmaru	0.191±0.002^{a}	53.090±0.020^{b}	0.131±0.001^{b}	0.035±0.001^{cde}	0.050±0.001^{ab}	0.037±0.001^{d}
12.	Diskit	0.162±0.001^{a}	74.883±0.006^{l}	0.128±0.002^{b}	0.039±0.002^{de}	0.048±0.001^{ab}	0.051±0.002^{f}
13.	Tangyar	0.221±0.003^{a}	63.677±0.006^{f}	0.270±0.001^{e}	0.034±0.001^{cde}	0.047±0.002^{ab}	0.024±0.001^{c}
14.	Khungru	0.316±0.002^{a}	72.987±0.006^{k}	0.275±0.001^{e}	0.045±0.001^{e}	0.047±0.001^{ab}	0.044±0.001^{e}
15.	Total	0.246±0.135	62.196±18.682	4.585±16.072	0.039±0.037	0.049±0.004	0.046±0.025

Values represented as mean±SD. For each column, different superscripts indicate significantly different at P<0.05, as measured by 2-sided Tukey's HSD among 70 accessions of 14 populations (five replications) of *A. hortensis*.

Overall there was variation in the concentrations of mineral elements studied in different populations in the present study and found statistically significant (Table 1). The concentrations of some important mineral elements among different populations varied by 1.5 folds for S, 2.8 folds for Al, 1.5 folds for K and 2.4 for Ca. Furthermore, taking into consideration the coefficient of variation, the observed variation for micronutrient was 98% for Fe, 94% for B, 30% for Zn, 10% for Cu, 54% for Se, whereas for macronutrients was found to be 24% for Mg, 11% for K, 13% for Na and 27% for Ca.

A total correlation matrix analysis (Table 2) revealed two strong sets of correlations, one was found associated with Ca, Mg, Zn, Na, K, S, B and Cu ($P \leq 0.01$), while the other with Si ($P \leq 0.05$). PCA showed that Mg, Na, Zn, Si and Ca were highly correlated to first component (27% of the variability), K, Cu and P were correlated to the second component (17% of the variability), and Al and S to the third component (15% of the variability) (Fig. 2).

Ladakh is a cold desert region which remains mostly inaccessible during the winter season, hence, civil as well military population inhabiting the region remains deprived of fresh vegetables during the season. The region does not support the cultivation of vegetables in more than six months long chilling winters except the few, among which *A. hortensis* L. is considered important. However, the nutritional importance of this vegetable has not been studied properly to make this more popular atleast during the unfavourable season. The present results have demonstrated that *A. hortensis* L. is found in majority of the region and is a source of many crucial micro as well

Table 2. Pearson correlation among mineral elements

	Ca	Mg	Zn	Fe	Na	Al	K	P	S	Si	B	Cu	Se
Ca	1	.697**	.463**	-.076	.442**	-.160	.119	.010	.123	-.114	-.073	-.090	-.158
Mg		1	.309*	-.055	.587**	.048	.046	.115	.252	-.231	-.378*	-.068	.005
Zn			1	.234	.125	-.053	-.052	.128	.159	-.156	-.084	-.179	.088
Fe				1	-.213	.002	-.381*	.059	.143	-.116	-.104	-.342*	-.081
Na					1	-.044	.565**	.102	.169	-.170	-.340*	.019	.001
Al						1	-.059	.096	.449**	-.232	-.382*	.355*	-.064
K							1	.154	-.259	.184	.095	.267	-.208
P								1	-.236	.312*	.119	.271	-.005
S									1	-.933**	-.571**	-.497**	-.033
Si										1	.669**	.584**	-.104
B											1	.144	-.506**
Cu												1	-.010
Se													1

* , ** Correlation is significant at P=0.05 and P= 0.01 levels, respectively (2-tailed).

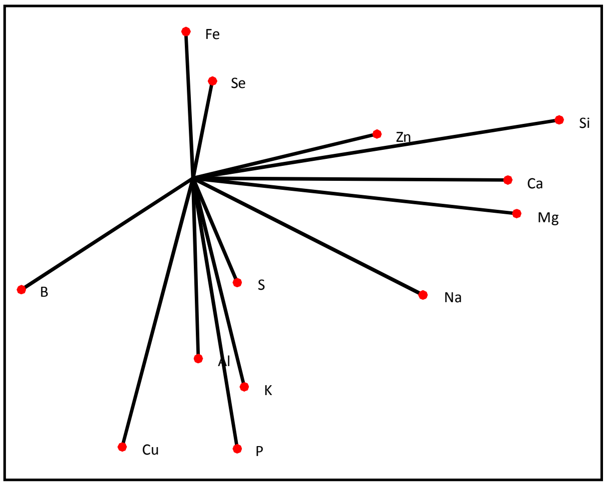

Fig. 2. Principal Component Analysis (PCA) of the mineral elements analyzed in *A. hortensis* L. accessions in the study.

as macro nutrients which are critical components of the human food. Although *A. hortensis* L. does not need much input to cultivate as vegetable still it is not grown in organized farming due to less economic value at present. Therefore, it remains grossly underutilized for human consumption as vegetable. The present study has advocated that the rich mineral contents of *A. hortensis* L. make it very suitable vegetable candidate in the entire Ladakh region.

The present results indicated the significant variability in the mineral elements concentration in different germplasm collections. This variability could be due to genotypic difference in the species. Genotypic difference in legumes was found to vary concentration and content of mineral in the previous studies (Sankaran *et al.*, 2009; Carla *et al.*, 2010).

The mineral concentration on genus Atriplex was studied by many authors (Wilson, 1966; Zid and Boukhris, 1977; Wallace *et al.,* 1982; Hasan, 1983; Khalil *et al.,* 1986; Watson *et al.,* 1994; Smit and Jacobs, 1999; Niekerk *et al.,* 2004) but, there was not a single study on genetic diversity in mineral concentration in a particular species till date. Therefore, this study is the first-hand information which is focused on analysis of mineral content of cold desert indigenous vegetable (*A. hortensis*) from 14 populations showing its diversity in different mineral composition.

CONCLUSION

The present study has demonstrated that *A. hortensis* L. is an important vegetable crop that has many essential mineral elements. This vegetable is grown in large spreads in harsh conditions of Ladakh region hence could be exploited and popularized as vegetable atleast during the long winter season. This vegetable is rich source of potassium, phosphorus, calcium, magnesium, selenium, iron and manganese, hence could augment to cater the essential mineral requirements of human and animal diet. Although there is variation in the elements present in the samples, the vegetable can be categorized as good, fair or rich source of one element or the other. It is also recommended that by selecting the populations of Gonpa, Shenam, Pakskum and Khungru area representing high level of S, Al, Fe, K and Ca, respectively, can be used to develop improved variety of vegetables.

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