

## Morphological characterization of indigenous vegetable (*Atriplex hortensis* L.) from trans-Himalayan region of Ladakh (Jammu and Kashmir), India

Tsewang Rinchen\*, Narendra Singh, Samar Bahadur Maurya, Vikas Soni, Manisha Phour and Bhuvnesh Kumar

Defence Institute of High Altitude Research (DIHAR), Leh, Ladakh-194101-Defence Research and Development Organisation (DRDO), India

\*Corresponding author: tsewangchol@gmail.com

### Abstract

In the present study a total of one hundred thirty two accession of indigenous vegetable (*Atriplex hortensis* L.) collected from fifteen different Trans-Himalayan geographical regions of Jammu and Kashmir, India, were morphologically characterized for 6 qualitative and 15 quantitative traits following descriptors developed by the National Bureau of Plant Genetic Resource (NBPGR), New Delhi, India, during the year 2014 - 2015. Multivariate analysis, principal component analysis (PCA), multidimensional scaling (MDS) and cluster analysis were performed using morphological traits to determine whether these populations are reliably similar or diverse. The first two principal components encompass more than 60% variation among population. The results of PCA and MDS analysis were comparable to the cluster analysis, which shows considerable phenotypic variation. Study of morphological characteristics of the accessions showed considerable variations which signify rich diversity within populations from different regions of cold desert.

**Keywords:** *Atriplex hortensis* L; morphological characteristics; multi-dimensional scaling; principal component analysis; Trans-Himalayan region.

**Abbreviation:** ANOVA\_analysis of variance; MDS\_multidimensional scaling; PCA\_principal component analysis.

### Introduction

*Atriplex hortensis* L. a member of chenopodiaceae, popularly known as *Phaltora* in Ladakh region is an annual herb (Rinchen and Narendra, 2015) and internationally known as Garden orach, mountain spinach, sea purslane, and salt bush. The plant appears to have originated in Europe and Siberia, and is considered to be one of the oldest wild edible plants, valued primarily for its leaves (Stevens, 1994). The plant is a rich source of protein (Carlsson, 1975), vitamin A and C (Siddiqui et al., 1994; Steinbach, 1996). The region is a cold desert (annual temperature range: -17 to 30) and highly elevated between 2500m asl to 4100 m asl, inhabited by many tribes including bodh, balti, purik, dardi etc.,. Since time immemorial these tribes were settled in different parts of Ladakh Trans-Himalayan region (Jammu & Kashmir, India). Their settlement pattern, socioeconomic status shows a harmonious co-existence with nature. These tribes are the primary source of traditional knowledge in terms of use of plant resources and conservation as they depend largely on the indigenous vegetables and wild edibles, such as, *Atriplex hortensis*, *Fagopyrum esculentum*, *Fagopyrum tataricum*, *Chenopodium album*, *Capiris spinosa*, *Rhodiola* spp., etc for their livelihood. Among these wild edibles, *Atriplex hortensis* is used as an indigenous vegetable in Ladakh region and is the first green to appear as vegetable after the prolonged winter.

In traditional medicine, it is used as a health tonic, helps in nutrition absorption, digestion and enhance the metabolism (Sarwa, 2001). Leaves are diuretic, emetic purgative and efficacious when used externally in the treatment of gout. Liniment prepared from the whole plant is said to be folk

remedies for indurations and tumour (Polunin, 1969; Grieve, 1930). It is also characterized by a high content of flavonoids, (Steinbach, 1996; Wieslawa et al., 2001) mineral components (Sarwa, 2001) and amino acids (Hegnauer, 1989; Nicol, 1994; Siddiqui et al., 1994).

The improvement of the crop is mostly depends on the availability of genetic variability. The variants of *A. hortensis* with different leaf colour, leaf length, leaf width, leaf area, chlorophyll content, number of leaf per plant, leaf yield per plant, shoot diameter, leaf moisture content and seed weight are available in the region. Morphologically indistinguishable type represented from diverse ecological regions have variable chemical constituents (Ali and Ali, 2012). Despite the age old cultivation history of *A. hortensis* no study has been conducted, which could shed light on the morphological diversity of this useful plant. Lack of awareness on nutritional importance and multiple use, the crop remains underutilized in the region. In view of the above gap, the present study was undertaken with the objective of identifying and evaluating *Atriplex* germplasm with traits of vegetable type to enhance its utilization by scientists in crop improvement and direct utilization by the farmer. Further, more intensive scientific efforts are needed for its assemblage, conservation, valuation and standardized cultivation technique which could be utilized for the development of cultivars with high nutraceutical values coupled with high yield and resistance to biotic and abiotic stresses (Rinchen and Narendra, 2015).

## Results and discussion

### Variation in morphological characters

Considerable variations were observed on various morphological characters of the species collected from different geographical region. Leaf colours of dark green, intermediate and light green were observed at higher, middle and lower altitude accessions respectively. The leaf margins (dentate, smooth) and aestivation (lower opposite decussate and upper opposite) were observed scattered among all the populations. The morphological characters (Table S3) recorded in the study showed that the mean plant height ranged from  $P \geq 0.001$  (15.36 to 27.35), while mean leaf length varied from  $P \geq 0.002$  (6.39 to 11.46), mean leaf width was  $p \geq 0.013$  (4.82 to 6.46), mean leaf area was  $P \geq 0.001$  (20.22 to 46.28), mean number of leaf was  $P \geq 0.013$  (18.27 to 37.93), mean chlorophyll content was  $P \geq 0.005$  (43.19 to 70.60), mean dry weight was  $P \geq 0.026$  (11.09 to 12.64), mean shoot diameter was  $P \geq 0.026$  (4.07 to 5.38), mean petiole length was  $P \geq 0.003$  (2.47 to 3.76), mean moisture content was  $P \geq 0.02$  (87.36 to 88.91), mean inflorescence per plant was  $P \geq 0.017$  (20.17 to 29.47), mean seed yield per plant was  $P \geq 0.26$  (32.25 to 67.39) and mean 1000 seed weight was ranged between  $P \geq 0.001$  (1.91 to 2.20) in the study area. The highest values of mean chlorophyll content, number of leaf per plant, mean leaf area, mean leaf length, mean petiole length were found in Panamik, mean plant height, mean primary branches per plant was found in Udhmaru and mean seed yield per plant, and 1000 seed weight were found in Murghi of Nubra valley collection site. On the other hand mean dry weight and mean shoot diameter were recorded in Shenam and Gonpa sites of Indus valley.

### Variation among populations

The five populations were superior and significantly different as compare to the other populations at 5% level Figure 2 and 5 show the character values plotted against the first two principal component variants from PCA and sample population with respect to their Euclidean distance from MDS. However, the position of variables mean score and Euclidean distance could be seen in the two patterns. Firstly, traits are positioned quite close to each other in the axis with respect to their population and secondly, sample population form four groups. The closely related samples belong to group I which includes Panamik, Pakskum, Shenam, Chiktan, while group II which include Akchamal, Diskit, Tangyar, Agyam, Khungru, group III which includes Soad, Gonpa, Udmaru and group IV which includes Khardong, Hunder and Murghi populations. The most important morphological characters were distinguished from these four groups which are reflected in their loading on the first two principal components. The first two principal components include more than 60 % variation among the populations. The highest loading characters are, number of leaf per plant, leaf area, leaf width, plant height, petiole length, leaf yield per plant, shoot diameter and number of inflorescence for PC 1. Seed size and number of primary branches are for PC 2 (Table 4).

### Phenotypic relationship among populations

Dendrogram was drawn to show the phenotypic relationships among different populations based on Euclidean distances from morphological data matrix (Fig.4). Based on average linkage (within group) Dendrogram group the 396 phenotype into main clusters A and B. Cluster A represent the

phenotype of Aghyam, Hunder, Tangyar, Chiktan, Pakskum, Gonpa, Khardong, Shenam, Murghi, Soad, Udhamaru, and Panamik, while cluster B represent the phenotype of Khungru, Diskit and Akchamal. The results of PCA and MDS analysis are comparable to the cluster analysis.

Genetic diversity in the germplasm Accessions is vital for crop improvement programme. In the present study a high morphological variations was observed in *A. hortensis* of trans-Himalaya in terms of number of leaf per plant, leaf area, leaf width, plant height, petiole length, leaf yield per plant, shoot diameter, number of inflorescence, seed size and number of primary branches. Similar study was also conducted by Talamali et al., 2001 and Le Houerou, 1992 in the genus results in comparable variation in floral architecture, vegetative and fruit morphology in the population. Cluster analysis is based on average linkage between different morphological characters used in this study and grouped into two clusters. The SPSS cluster grouping of 132 accessions from different phyto-geographical regions of fifteen populations were grouped into two major clusters, shows that ward's Minimum Variance Dendrogram technique was the most suitable technique for classifying the accessions in discrete and well define clusters. The high loading morphological characters were found scattered in different clusters. On the basis of herbage yield data and seed yield genotype from cluster 'A' population (Pananmik) and (Murghi) would be better for selecting high herbage type for commercial purpose. This indigenous vegetable can be used in place of spinach, as a resilient agriculture with changing environment and for sustainable development of leafy vegetable. Research on augmentation of indigenous germplasm of *Atriplex* with variable traits including cold tolerance and drought tolerance from diverse distribution would be very important.

Morphological traits of the populations are based on phenotype expression and are influenced by diverse environmental factors (Heywood, 2002). Considerable variation was observed for different morphological traits among population of Ladakh regions. The distinct morphological characters of five populations, Panamik, Udhmaru, Murghi, Shenam and Gonpa were found scattered among different clusters across the phyto-geographical regions. Nubra and Indus valley phenotype have potential plant types. For better understanding on this aspect more accessions representing different phyto-geographical regions especially from Nubra and Indus valleys are need to be assembled for detailed study on similar lines. Study on variation pattern based on morphology, phytogeography and linking of morphotypes to their potential value in crop improvement are important element. This study would benefit regions like Ladakh (J&K), which remain cut off from the mainland for half of the year depriving the local people and troops deployed in this area from green vegetables. Multi-location evaluation of selected *Atriplex hortensis* from Panamik, Udhmaru, Murghi, Shenam and Gonpa are suggested to identify the most promising accessions for present and future utilization. Selection of useful accessions by researchers and farmers is necessary for enhancing the utilization of this underutilized indigenous vegetable. Utilization of indigenous vegetable accessions in crop improvement programmes may trigger the release of *Atriplex* variety suitable for varying climates. Indigenous vegetable germplasm accessions are identified and conserved at defence institute of high altitude research Leh, Ladakh.

**Table 1.** Geographical location and sampling sites of *Atriplex hortensis* in the Trans-Himalaya region of Ladakh.

Valleys	Sub Pop (ID)	Accession no. (s)	Alt. (m)	Lat (N)	Long (E)
Nubra	Khardong (KHA)	6	4109	34° 33.822	077° 39.487
	Tangyar (TAN)	10	3915	34° 15.168	077° 52.259
	Khungru (KHU)	9	3623	34° 17.003	077° 26.556
	Agham (AGM)	11	3335	34° 19.706	077° 49.877
	Murghi (MUR)	5	3201	34° 45.516	077° 32.643
	Panamik (PAN)	5	3184	34° 46.963	077° 32.304
	Hunder (HUN)	20	3169	34° 35.213	077° 27.688
	Udmaru (UDH)	5	3129	34° 37.627	077° 26.180
	Diskit (DIS)	5	3117	34° 33.210	077° 32.686
	Gonpa (GON)	10	3666	34° 11.026	077° 35.560
Indus	Shenam (SHA)	11	3477	34° 09.550	077° 34.766
	Chiktan (CHK)	11	3252	34° 28.166	076° 30.432
	Achakmal (AKH)	6	2888	34° 33.220	076° 09.449
Suru	Pakskum (PAK)	11	2881	34° 33.120	076° 09.443
	Soad (SOAD)	7	2860	34° 33.974	076° 10.305

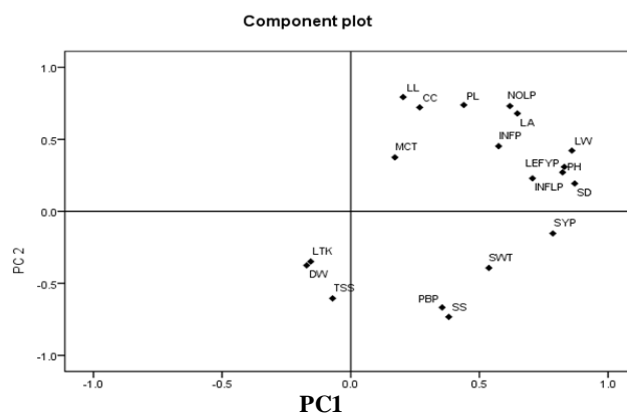
Note: Sub pop-Sub populations; ID-Population ID; No.-Number of samples; Alt-Altitude (m asl); Lat-Latitude; Long-Longitude.



**Fig 1.** *Atriplex hortensis* plants collected from different collection sites grown in experimental field.

**Table 2.** Qualitative and quantitative characters of *Atriplex hortensis* used for data recording in the study.

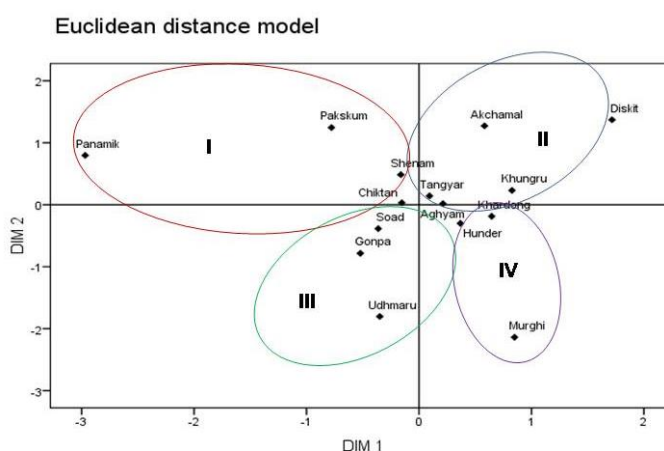
S. No.	Qualitative characters	S. No.	Quantitative characters
1	Total soluble solids (Brix %)	1	Plant height (cm)
2	Chlorophyll content (CCI unit 0.71 mm <sup>2</sup> )	2	Shoot diameter (mm)
3	Moisture content (%)	3	Leaf lamina length (cm)
4	Leaf colour (colour chart)	4	Leaf lamina width (cm)
5	Dry weight (g)	5	Leaf lamina area (cm <sup>2</sup> )
6	1000 seed weight (g)	6	Leaf thickness (mm)
		7	Petiole length (cm)
		8	Primary branches
		9	Number of Inflorescence
		10	Inflorescence length (cm)
		11	Seed size (mm)
		12	Seed yield (g)
		13	Number of leaf per plant
		14	Leaf yield per plant
		15	Leaf aestivation



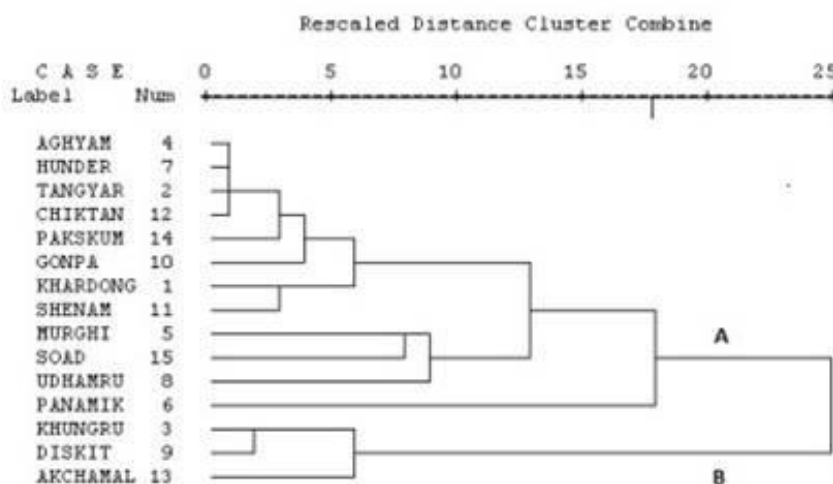
**Fig 2.** Principal component analysis of morphological data of *Atriplex hortensis* (NOLP: Number of leaf per plant, LA: Leaf area, LW: Leaf width, PH: Plant height, PL: Petiole length, LEFYP: Leaf yield per plant, SD: Shoot diameter, INFP: Number of Inflorescence, INFLP: Inflorescence length, CC: Chlorophyll content, LL: Leaf length, TSS: Total soluble solids, DW: Dry weight, MC: Moisture content, LTK: Leaf thickness, SS: Seed size, PBP: Number of primary branches per plant, SWT: 1000 seed weight, SYP: Seed yield per plant).

**Table 4.** Morphological characters of *Atriplex hortensis* and their principal component weightage recorded during the study.

Characters	Characters acronym	PC1 (42.50 %)	PC2 (16%=59.59)
Number of leaf per plant	NOLP	0.944	-0.159
Leaf area	LA	0.933	-0.101
Leaf width	LW	0.929	0.233
Plant height	PH	0.833	0.300
Petiole length	PL	0.812	-0.280
Leaf yield per plant	LEFYP	0.803	0.325
Shoot diameter	SD	0.789	0.414
Number of Inflorescence	INFP	0.731	0.025
Inflorescence length	INFLP	0.687	0.281
Chlorophyll content	CC	0.670	-0.378
Leaf length	LL	0.667	-0.475
Total soluble solids	TSS	-0.444	0.416
Dry weight	DW	-0.373	0.176
Moisture content	MCT	0.373	-0.176
Leaf thickness	LTK	-0.344	0.166
Seed size	SS	-0.182	0.805
Number of Primary branches per plant	PBP	-0.159	0.739
1000 seed weight	SWT	0.156	0.647
Seed yield per plant	SYP	0.501	0.624



**Fig 3.** Multidimensional scaling of morphological data of *Atriplex hortensis* with their respective populations (referred to a table 1).



**Fig 4.** Dendrogram showing the phenetic relationship among 15 populations based on Euclidean distance from morphological data matrix. Dendrogram using average linkage (Within Group).

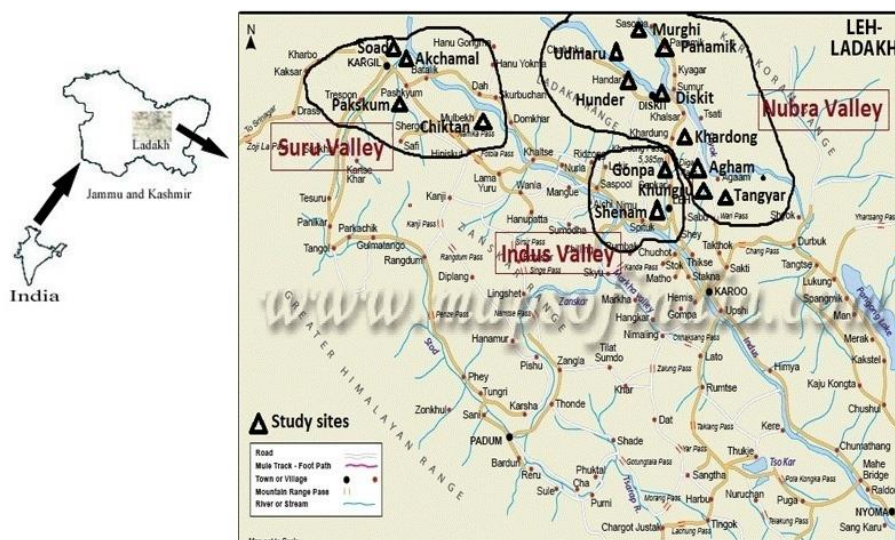


Fig 5. Map of collection sites of Ladakh.

## Materials and methods

### Experimental site

The experiments were conducted in the experimental field of Defence Institute of High Altitude Research (DIHAR) Leh, Ladakh, Jammu and Kashmir, India. Representative germplasm of *A. hortensis* (N=132) from random wild and cultivated field, representing three natural populations, were collected from 15 different collection sites spread across Indus, Suru and Nubra valleys of the Trans-Himalaya region in Ladakh during the year 2013-2014 (Table 1, Fig. 5). Collected accessions of *A. hortensis* were grown in summer season in open condition in sandy loamy soil in the year 2014 – 2015 with three replications established in randomized blocks. A total of three hundred ninety six experimental beds were prepared of size 2m<sup>2</sup> (1m x 2 m) with three rows of spacing 30 cm between rows and 15 cm between plants. The crop received a basal dose of locally available compost and irrigations. The crop was protected from weeds by diweeding. Three representatives of plants were selected randomly for each accession for recording observations on twenty one traits. The valleys were considered as separate populations. The altitude of collection sites ranged from 2500-4101m amsl. Altitude and location of study sites were established using GPS (GARMIN 72, Olathe, Kansas USA). Precipitation of the study region is less than 200 mm of which more than 70% of it is in the form of snow (Korekar et al., 2013). A herbarium of *Atriplex* representative samples collected from three valleys was prepared and the voucher specimens were submitted to Botanical Survey of India (BSI), Dehradun, India, to ascertain the *Atriplex* species status.

### Quantitative and qualitative characters

Morphological data were recorded taking three replications of each accession for twenty one characters belonging to six qualitative traits and fifteen quantitative traits (Table 2). To study the morphological characters, we used a descriptors developed by National Bureau of Plant Genetic Resource (NBPGR) (Singh et al., 2003). All qualitative and quantitative traits were regularly recorded for variation in characters after an interval of 10 days at peak of the

vegetative and flowering period. Characterizations of plants were carried out on the basis of recorded data of vegetative and inflorescence characters that recorded both qualitative and quantitative traits at different stages of plant growth. Vegetative data were collected in 35-40 days when the plant is ready to consume, flowering data were collected at 55-65 days and fruiting stage of 85-95 days old plant. Data recorded from three replications and analysed statistically. Leaf length, leaf width, and leaf area were recorded using portable laser leaf area meter (CI-201, CID Bio-Science), petiole length by cutting portion of the leaf from the base of the leaf blade, leaf thickness by digital vernier calliper (MITUTOYO, Japan) measured at lower, middle and top of the leaf blade and average was recorded, leaf aestivation was recorded from vegetative to reproductive stage, number of leaf per plant and weight were recorded at vegetative stage when the plant was ready to harvest for consumption, moisture content was recorded by wet weight of 100 g minus dry weight using digital electronic weighing balance to an accuracy of 0.01 g, plant height was measured from the base of the plant to tip of the leaf using measuring scale, shoot diameter was recorded by taking three readings from the lower middle and top of the shoot using digital vernier calliper (MITUTOYO, Japan), leaf colour was recorded by colour chart, seed size was measured by using digital vernier calliper (MITUTOYO, Japan), total soluble solid (TSS) concentration of freshly- chopped extracted juice of representative leaf samples was determined with a digital hand refractometer (Atago, ATC-1E, Kyoto, Japan) (Ozgen and Sekerci, 2011). The leaf chlorophyll content of the species was obtained by collecting three leaf sample of the same size with three replications using chlorophyll content meter (Opti-science, CCM-200 plus).

### Statistical analysis

Univariate and multivariate statistical procedures were used to analyse the data taken during the study using SPSS 17.0 software. Analysis of variance (ANOVA) was performed and the mean of the results were compared by Duncan's multiple range tests at 5% significance level. To determine the degree of associations among the characters, Pearson's correlation analysis was performed. Principal component analysis (PCA) and multidimensional scaling (MDS) were used to ordinate

population means considering variance and covariance among characters within and among populations (Kim, 1975). Average Euclidean distance was calculated for each population and the resulting distance matrix was used to construct a phenetic dendrogram using average linkage method (Mohammadi and Prasanna, 2003).

## Conclusion

The results showed that there was high genetic diversity with regard to Agro-morphological characters in the *Atriplex* collection. The diversity could mainly be attributed to diverse agro-climatic conditions in the region. Accessions from different regions were closely related and accessions from the same region had different genetic background. The intraregional diversity could be as a valuable source as interregional diversity for *Atriplex* improvement and related genus. The germplasm represents a valuable source of genetic diversity that is expected to be highly useful for future breeding programs. The success in genetic improvement of the crop, however, depends on the availability of genetic resources and their diversity.

## Acknowledgments

The study was supported by Defence Institute of High Latitude Research (DIHAR) – DRDO, Ministry of Defence, Government of India. Tsewang Rinchen is thankful to DRDO for providing financial support by providing Senior Research Fellowship. I would also like to thank the inhabitants of the surveyed areas for their cooperation during my field studies. I am also grateful to Eli Paljor (TO 'D'), Nurbu (STA 'B'), Motup (AMS 'II') and all the field workers of Defence Institute of High Altitude research, Leh for their cooperation. My sincere thanks to Botanical Survey of India, Dheradun for the identification of species. Lastly I would like to acknowledge the reviewers for their most valuable comments.

## References

- Ali A, Ali M (2012) New fatty acid derivatives from *Ocimum sanctum* L. leaves. Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Jamia Hamdard, New Delhi.
- Carlsson R (1975) Selection of centrospermae and other spices for production of leaf protein concentrate. PhD thesis, University of Lund, Lund, Sweden, LUNDBS (NBFB 1004) 1-8. Aliquots.
- Grieve (1930) A Modern Herbal. Temperate plants.
- Hegnauer R, (1989) Chemotaxonomie der Pflanzen, Boston – Berlin: Birkhäuser Verlag, 234–242.
- Heywood VH (2002) The conservation of Genetic and Chemical Diversity in Medicinal and Aromatic Plants, In: Biodiversity: Biomolecular Aspects of Biodiversity and Innovative Utilization. Sener B (Ed.). Springer, Berlin, Heidelberg, pp. 13-22.
- Kim J (1975) Factor analysis. In 'Statistic Package for the Social Sciences', 2<sup>nd</sup>edn, Nie NH, Hull CH, Jenkins JG, Stein brunner K and HD. Bent, McGraw Hill: New York, pp 468-514.
- Korekar G, Dwivedi SK and Singh H (2013) Germination of *Hippophae rhamnoides* L. seed after 10 years of storage at ambient condition in cold arid trans-Himalayan Ladakh region. Curr. Sci. 104 (1):110-114.
- Mohammadi SA and Prasanna BM (2003) Analysis of genetic diversity in crop plants-salient statistical tools and considerations. Crop Sci. 43:1235-1248.

- Nicol J (1994) *Atriplex nummularia*, *Atriplex vessicaria* and other *Atriplex* species. Aust J Med Herbalism. 6, 85–87.
- Ozgen S and Sekerci S (2011) Effect of leaf position on the distribution of phytochemicals and antioxidant capacity among green and red lettuce cultivars. Span J Agri Res. 9(3):801-809.
- Polunin O (1969) Flowers of Europe - A Field Guide. Oxford University Press. ISBN 0192176218. An excellent and well illustrated pocket guide for those with very large pockets.
- Rinchen T and Narendra S (2015) Exploring nutritional potential of *Atriplex hortensis*. Indian Hort. 60: (2): 16-17.
- Sarwa A (2001) Wielki leksykon roślin leczniczych. Książka i Wiedza, lodz.
- Siddiqui BS, Ahmed S, Ghiasuddin M and Khan AU (1994) Triterpenoids of *Atriplex stocksii*. Phytochemistry. 37:1123–1125.
- Singh BM, Mahajan RK, Umesh S and Pareek SK (2003) Minimal discriminator of agri-horticultural crops. Part-iv: medicinal and aromatic plants. National Bureau of Plant Genetic Resources, New Delhi, India.
- Steinbach G (1996) Leksykon przyrodniczy, zioła i owoce lesne. Swiat Ksiazki, Warszawa.
- Stevens JM (1994) Orach-*Atriplex hortensis* L. Fact sheet HS-637. Gainesville, Fla.: Horticultural Sciences Department, Florida Cooperative extension Service, Institute of Food and Agricultural Sciences, University of Florida.
- Talamali A, Dutuit P, Annick LT, Goren flot R (2001) Polygamie chez *Atriplex halimus* L. (Chenopodiaceae). Life Sci. 324:107-113.
- Wieslawan B, Maciej S, Rafal F (2001) Sulphated flavonoid glycosides from leaves of *Atriplex hortensis*. Acta Physiol Plant. 23(3):285-290.