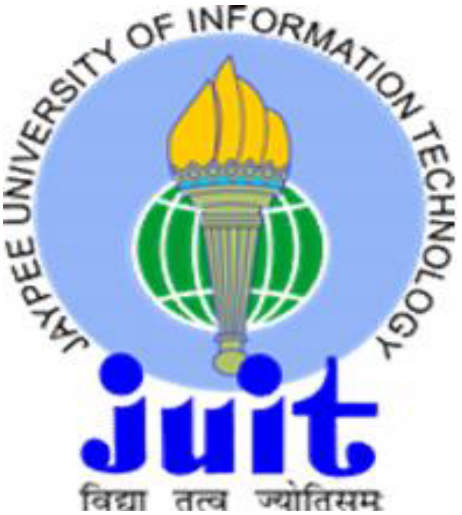
AGRO-MORPHOLOGICAL, MOLECULAR AND NUTRITIONAL CHARACTERIZATION OF INDIGENOUS POPULATION OF *ATRIPLEX HORTENSIS* L. FROM TRANS HIMALAYAN LADAKH REGION OF INDIA

Thesis submitted in fulfilment of the requirements for degree of

DOCTOR OF PHILOSOPHY

By

TSEWANG RINCHEN



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DECLARATION BY THE SCHOLAR

I hereby declare that the work reported in the Ph.D. thesis entitled **"Agro-morphological, molecular and nutritional characterization of indigenous population of *Atriplex hortensis* L. from trans-Himalayan Ladakh region of India"** submitted at **Jaypee University of Information Technology, Waknaghat, India,** is an authentic record of my work. I have not submitted this work elsewhere for any other degree or diploma. I am fully responsible for the contents of my Ph.D. Thesis.

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SUPERVISOR'S CERTIFICATE

This is to certify that the work reported in the Ph.D. thesis entitled **"Agro-morphological, molecular and nutritional characterization of indigenous population of *Atriplex hortensis* L. from Trans Himalayan Ladakh region of India"** at **Jaypee University of Information Technology, Waknaghat, Solan Himachal Pradesh, India,** is a bonafide record of his original work carried out under our supervision. This work has not been submitted elsewhere for any other degree or diploma.

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MY WIFE AND DAUGHTERS

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Tsewang Rinchen

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LIST OF ABBREVATIONS

%	Percentage
ACCESSION NO.	.Number of samples
Al	Aluminium
ALT	Altitude
AMOVA	Analysis of molecular variance
ANOVA	Analysis of variance
B	Boron
BSI	Botanical Survey of India
Ca	Calcium
CC	Chlorophyll content
CIAT	International Centre for Tropical Agriculture
CTAB	Cetyl trimethyl ammonium bromide
Cu	Copper
DNA	Deoxyribonucleic acid
DW	Dry weight
Fe	Iron
FYM	Farm yard manure
GPS	Global positioning system
HSD	Honestly Significant Difference
ICP-OES	Inductively Coupled Plasma Optical Emission Spectrometry
ICRISAT	International Crops Research Institute for the Semi-Arid Tropics
ILV	Indigenous leafy vegetable
INFLP	Inflorescence length

INFP	Number of Inflorescence
K	Potassium
LA	Leaf area
LAT	Latitude
LEFYP	Leaf yield per plant
LL	Leaf length
LONG	Longitude
LTK	Leaf thickness
LW	Leaf width
M AMSL	Meter above mean sea level
MCT	Moisture content
MDS	Multidimensional scaling
Mg	Magnesium
Na	Sodium
NBPGR	National Bureau of Plant Genetic Resource
NOLP	Number of leaf per plant
P	Phosphorous
PBP	Number of Primary branches per plant
PCA	Principal component analysis
PCR	Polymorphic chain reaction
PH	Plant height
PL	Petiole length
RAPD	Randomly amplified polymorphic DNA
RBD	Randomized Block Design
S	Sulphur

SD	Shoot diameter
SD	Standard deviation
SE	Standard error
Se	Selenium
Si	Silicon
SPSS	Statistical Package for Social Sciences
SS	Seed size
SUB-POP	Sub population
SWT	1000 seed weight
SYP	Seed yield per plant
TSS	Total soluble solids
UPGMA	Unpaired Group Method with Arithmetic mean
Zn	Zinc

CHAPTER 1
INTRODUCTION

1.1 Introduction

Trans Himalayan Ladakh region of the Indian state of Jammu and Kashmir is high altitude area with altitudinal between 2550-8000 meters. The climate of Ladakh is characterized by extreme harsh, snowy winters and intense sunlight, high wind speed, and relatively higher temperature fluctuations between day and night during the summer season. So a limited number of winter crops can be grown in the summer season in this region and nothing can be cultivated in winter months. Most of the winter crops grown also do not perform optimum due to short crop growing season and reversed the order of weather cycle i.e., onset of the winters just before crop maturity. Therefore, there is always a shortfall of fresh vegetables in the region. Local population and heavily deployed army establishment are dependent on the outside supply of fresh agricultural commodities particularly fresh vegetables, from distant places. The long-distance transportation, snags in transportation due to difficult geography and unfavourable climatic conditions results in a gap between demand and supply and increased cost. The problem can be obviated to some extent by identifying indigenous plants used traditionally by local people as fresh vegetables and developing a cultivation system to enhance their production. The hypotheses here are that indigenous plants are growing in the region for ages and would have been well adapted to the peculiar prevailing climatic and weather conditions. This requires long term efforts starting with documentation of traditional knowledge, validating it through scientific studies, selection of better-performing species, their populations and genotypes, and finally development of packages of practices for cultivation. This will also set a stage for the further improvement of selected plants and their genotypes through breeding and selection. The current study is one of such endeavour in which we have explored the traditional knowledge concerning use of indigenous leafy vegetables (ILV) in the region and further characterized most potent *Atriplex hortensis* L. populations and genotypes.

The vegetation in Trans Himalayan Leh-Ladakh is dominated by annual and perennial herbs and shrubs instead of trees. Traditionally, people living in the cold desert of Ladakh depend on wild edible plants, including indigenous leafy vegetables (ILV) to meet their dietary requirements (Figure 1.1.1). They collect such plants from mountain slopes and around agricultural fields. The indigenous leafy vegetables (ILV) of Ladakh region include *Amaranthus cruentus, Lepidium latifolium, Rhodiola heterodenta, Fagopyrum tataricum, Fagopyrum esculentum, Atriplex hortensis, Malva verticillata, Rumex patientia* etc., which are used by

different tribal people of the region [1]. The indigenous plants do not require intensive care and can grow in marginal land and on less fertile soil. Besides, these can withstand harsh climatic conditions due to tolerance to abiotic and biotic stresses, which is inherent in their genetic makeup by years of natural selection. However, such plants grow quite sparsely in nature and their production is very low [2]. Increasing their production by developing production systems and their inclusion in commonly grown vegetable types in the remote regions would contribute towards nutritional security in the mountainous region. Among these wild edibles, species found in Ladakh *A. hortensis* is the first green to appear after the prolonged winter [3]. Despite its potential as leafy vegetable there has been limited or no study on agro-morphological characterization, genetic diversity, phytochemical and nutritional aspects of this indigenous plant. Therefore, it is very important to document the species, their populations in different localities of the region based on agro-morphology, nutritional and genetic diversity to establish production, cultivation system and initiate crop improvement programs.

Figure 1.1.1 Collection of indigenous vegetable from cultivated field and processing for winter storage

The literature on related edible plants of Ladakh and their indigenous uses are widely explored. But it was observed that only partial ethno-botanical investigation had been carried out in the region despite of the huge potential of edible plants as bio-resource. A list of some different ethnobotanical surveys on edible indigenous vegetables conducted in Ladakh is presented in Table 1.1.

Table1.1 Ethnobotanical surveys carried out in Ladakh, India

Investigator	**Plants information**	**Study site**	**Observations**
Ballabh et al., 2007 [4]	44 species of 20 families	Five valleys of Ladakh Zanskar, Drass, Suru, Indus, and Changthang valleys	Raw edibles used by ethnic people of Ladakh
Murugan et al. 2010 [5]	27 plant species from 5 genera of 18 families	Nubra valley	Edibles plants used in different Ladakhi dishes
Juliane Dame and Marcus Nusser. 2011[6]	Cabbage, cereals, potatoes, turnip, green leafy vegetables and carrots, onions	Five valleys of Ladakh Drass, Zanskar, Indus, Changthang valleys and Suru	The plants are being used in different parts of Ladakh
Alessandro Boesi 2014 [7]	12 plants species from 11 genera of 10 families	Sapi (hotspot) region Ladakh	The plants are being used by different tribes of the region as edibles with different recipe

Atriplex hortensis L. belongs to family Chenopodaceae. It is an annual herb known as mountain spinach, garden orach, sea purslane, and locally known as *Phaltora* in the Ladakh region [1]. The plant is known to have originated from Europe and Siberia. [8]. In traditional medicine, Atriplex is used as a health tonic, it helps in nutrition absorption, digestion and

enhances the metabolism. Besides vitamins and proteins, the leaves of Atriplex are also rich in calcium, fat, total carbohydrate, fiber, β-carotene, saponin [9] [10]. Leaves are diuretic, emetic, purgative, and efficacious when used externally in the treatment of gout. *Atriplex hortensis* is also characterized by a high content of flavonoid, mineral components, and amino acids [11] [12] [13] [14]. Liniment prepared from the whole plant is used in folk medication such as indurations and tumour [15].

Agro-morphological traits are a very common way to estimate relationships between and among genotypes [16]. Knowledge of existing diversity in any plant resources is useful for the selection of better performing genotypes, conservation of germplasm and selection of parents for further improvement through breeding and hybridization. Basant and Chaurasia [17] reported that indigenous leafy vegetable *A. hortensis* is being used by local people in the Ladakh region. However, a comprehensive study on agro-morphological traits of *A. hortensis* (Figure 1.1.2) related to its populations is still not done so far. Thus one of the objectives of the current study was to identify and evaluate agro-morphological traits of *A. hortensis* and its available germplasm in the Ladakh region.

Figure 1.1.2 *Atriplex hortensis* growing in the field (A), Flowering stage (B), Seed stage (C).

Minerals are essential for a wide range of physiological functions in higher animals. Fourteen trace elements including iron have been identified as essential to humans [18]. The most widely recognized micronutrient inadequacies are iron, zinc, and iodine, yet certain populations experience the ill effects of deficiencies of magnesium, calcium, and selenium. It has been estimated at world level there are almost 3.7 billion individuals are iron deficient (60%) out

of which 54% have severe inadequacy [19]. There are various reports on studies conducted on mineral composition of different species of Atriplex worldwide viz., [20] [21] [22] [23]. However, mineral compositions of *A. hortensis* and variations in mineral composition in different regional populations have not been investigated in previous studies. Therefore, the present study was aimed to estimate the mineral content of *A. hortensis* and its populations in the Ladakh region. Such selected populations may serve as potential genetic resources for a selection of genotypes with higher essential mineral nutrients which could be directly recommended for cultivation or may be included in future breeding programs.

Molecular markers (DNA) are now adays are being routinely used in the assessment of genetic variability of both animal and plant species [24] [25]. Many authors worked on the assessment of genetic diversity in Ladakh primarily on trees, shrubs, and medicinal plants such as, Korekar et al. [26] on *Hippophae rhamnoides*, Bajpai et al. [27] on *Morus alba.* The assessment of the genetic diversity of this indigenous plant species in a different population of the Ladakh region would also facilitate in devising better conservation strategy and utilization of genetic resources in future breeding programs.

The increasing demand for indigenous vegetables could not be fulfilled by harvesting these from sparse wild populations that vary in quality and quantity, but through the cultivation of the selected potential germplasm of the species. Moreover, for the success of commercial cultivations programs, improvement of selected genotypes concerning to important characters that dictate yield and quality is required. This potential of future improvement is determined by the extent of genetic variability available in the germplasm of a species [28] [29]. Therefore, simultaneous employment of morphological, phytochemicals, nutritional characterization and assessment of population genetic diversity would be helpful to recommend potential genotypes / populations with desirable characters for the cultivation as well as to be included in the future breeding programs. Thus considering research and development requirements as highlighted above present study was intended to carry out an extensive field survey, agro-morphological and nutritional characterization and to study the genetic diversity of *Atriplex hortensis.*

Objectives

1. Screening of selected potential indigenous leafy vegetables of the cold desert- Ladakh on the basis of yield parameters and consumer acceptance

2. Agro-morphological characterization of indigenous populations of *Atriplex hortensis* L. in the Ladakh region.

3. Determination of minerals composition of indigenous populations of *Atriplex hortensis* L. in Ladakh region.

4. Assessment of genetic diversity of *Atriplex hortensis* L. among indigenous populations of Ladakh using randomly amplified polymorphic DNA (RAPD) markers.

CHAPTER 2
REVIEW OF LITERATURE

Tribal are distinctive cultural groups that are typically restrained to distinct geographical regions. In India, tribal people live frequently in the hills, plateaus, forests, and isolated natural places. They are known with different names such as Adivasi, Adinmivasi, Aboriginal, Vanvasi, and many more names symbolizing their economical, ecological, historical, or cultural uniqueness [30]. The Jammu and Kashmir state (India) is populated by a huge number of different tribal groups. More than 6 foremost tribal communities known as Bot, Balti, Purik, Dokpa, Dardi, Changpa, etc., with distinct cultural entities, inhabit the region of Ladakh. They have huge information about bio-resource utilization in the region. The utilization knowledge and name of the species of native plants differ between the tribal groups [31]. The trans-Himalayan region of Ladakh supports temperate, sub-alpine, and alpine vegetation. The vegetation of the cold desert comprises of herbs, shrubs, and rarely trees.

2.1 Indigenous vegetables and traditional knowledge

The literature on related edible plants of Ladakh and their indigenous uses are widely explored. But it was observed that very partial ethno-botanical investigation had been carried out in the region despite of the huge potential of edible plants as bio-resource available in the region. Ethnobotany is an important tool for conservation and complimenting management at the local and regional level which is well accepted worldwide scientifically [31] [32] [33]. Modernization of agriculture and the introduction of modern-day cultivated vegetables leads to narrowing the use of locally available crop species [34]. Regeneration, characterization and standardizing agro-practices become necessary steps for further use of ILV plants [35]. Therefore, documentation and collection of diverse local ecotypes become extremely important for ensuring food security in the remote mountainous regions. There is a need for crop improvement through selection of potential indigenous leafy vegetables (ILV) based on indigenous uses, different yield contributing characters and consumer acceptance.

A very limited ethno-botanical surveys have been conducted by some authors. Ballabh et al. [4] described 40 plant species belonging to 15 families in the Ladakh region that is being used as raw edibles by the ethnic people of the Ladakh region. People use various plant parts, viz.,

leaves, rootstocks, bulbs leaves-stalks, fruits and seeds to prepare local dishes. Murugan et al. [5] reported 27 edible plant species and investigated traditional wild edibles plants available in the Nubra valley, used for the preparation of special traditional food items by the local tribal people like Thukpa, Tangtur, Kholak, Paba etc. Alessandro [7] also reported the wild edibles and medicinal plants species from hotspots region of the Ladakh that being used as vegetable and as folk medicines.

2.2. Agro-morphology

Agro-morphological traits are a very common way to estimate relationships between and among genotypes [26]. Traditional knowledge of existing diversity in indigenous species is useful for germplasm management and conservation. Besides, it is also a very important way to select the parent lines having variability within species so that crop improvement becomes more efficient [27]. The characterization and conservation of these genetic resources are also necessary for utilization in different breeding programs to improve yield and tolerance to various biotic and abiotic stresses. Therefore it is important to assess the variations of this germplasm through agro-morphological traits to provide insight into the variation of accessions [28].

The study made by various authors on agro-morphological traits viz., Sabaghnia et al. [29] studied one hundred one spinach landrace collected from the different locations and they were evaluated for their diversity by using several agro-morphological traits. In the same way, many authors studied on different crops to access the morphological diversity viz., Parikh et al. [30] studied variability in aromatic rice germplasm through agro-morphological characterization. Aghaee et al. [31] used pattern analysis to study agro-morphological examination on durum wheat genotypes. Similarly, Sajid et al. and Akaneme and Ani [32] [33] examined the *Oryza Sativa* and *Amaranthus hybridus* germplasm traits respectively. Upadhyaya et al. [34] used different morphological traits to identify and evaluated the germplasm of pigeon pea by analyzing different agro-morphological traits.

2.3. Mineral composition

Minerals are essential for a wide range of body functions in higher animals. Fourteen trace elements including iron have been identified as essential to humans and animals [35]. Mineral elements play a major role through vitamin and enzyme systems in maintaining the

health of human beings [36] [37]. Many authors studied mineral composition in many crop's germplasm including genus Atriplex spp. Pinheiro et al. [38] investigated the variation in seeds mineral composition of *Phaseolus vulgaris* accessions. Karakoy et al. [39] studied the diversity of mineral nutrients in the seeds of *Lens esculent*. Carlson and Hallqvist [40] studied for the mineral composition of leaves compared between *Atriplex hortensis* and *Spinacia oleracea* cultivated under different conditions. Carlson and Clarke [41] investigated the production of leaf protein concentration and mineral nutrients in *Atriplex hortensis.* Khalil et al. [42] studied Atriplex leaves from Saudi Arabia on mineral composition and reported that leaves of Atriplex are good source of mineral nutrients for livestock forage. Niekerk et al. [43] studied the mineral composition of *Cassia sturtii and* Atriplex spp., the results show Atriplex spp., have higher macro and trace mineral concentrations. Abu-Zanat et al. [44] studied macro and micro element assessment in *Atriplex nummularia* and *Atriplex halimus* and found sufficient mineral content in the Atriplex species. Kachout et al. [45] also studied the accumulation of Pb, Cu, Zn, and Ni in the salt-tolerant plant of Atriplex grown on polluted soil that also helps in rehabilitation of soil. Above cited literature revealed that attempt has been made to study minerals content in many Atriplex species, and to the best of our knowledge not a single study was found on population-wise estimation of mineral content of Atriplex species particularly in Ladakh region and in other locations of the world.

2.4 Genetic diversity

Genetic diversity in plant species is the basis and plays an important role in their survival and adaptation to counter evolutionary forces and directly or indirectly for human survival is also dependent on it [46]. It can offer information about the distribution of populations in nature along with their reproductive success [47] [48] [49]. Bouda et al. [50] examined the genetic variability in genus *Atriplex* using RAPD markers, results in 95% genetic diversity in Atriplex species which indicating that RAPDs are an abundant source of polymorphic markers in Atriplex species. Besides many authors studied the genetic diversity of plant species in Ladakh region include Korekar et al. [25] investigated, genetic diversity among thirty six genotypes of *Hippophae Salicifolia* of Uttarakhand region and *Hippophae rhamnoides* subsp . Turkestanica of Ladakh region using simple sequence repeat (SSR) markers.

Prabodh et al. [26] studied genetic diversity of *Morus alba* by using SRAP markers (Sequence-related amplified polymorphism) from trans-Himalaya of Ladakh. Sampling was conducted in three valleys viz., Suru, Indus and Nubra valleys. It was found that genetic diversity was 80% within *Morus alba* populations where as it was 20% among the populations. They argued that there is gene flow between Suru and Indus population while geographical barrier between Nubra and Indus-Suru population efficiently obstruct the gene flow.

The assessment of genetic diversity of plant species in a different population of the Ladakh region would facilitate in devising better conservation strategy and utilization of genetic resources in future breeding programs such study would help in the selection of superior genotypes having higher value qualitative or quantitative characters by using developed molecular-based markers.

Molecular markers are important tools to evaluate genetic diversity. Molecular markers occupy specific genomic locations among chromosomes referred as loci. There are 3 kinds of molecular markers such as DNA markers, morphological markers, and biochemical markers. Morphological markers are phenotypic characterized such as flower colour, seed shape, growth habits. Biochemical markers are differing in enzymes, detected by specific staining and electrophoresis [46]. The major drawbacks of these 2 markers are viz., narrow in number, and are affected by different environmental conditions [47]. However, DNA markers are boundless and are not affected by different environmental conditions. DNA markers have numerous applications in plant breeding programs and to evaluate the genetic variability within and among germplasm [48] [49] [50] [51]. Based on method of detection deoxyribonucleic acid (DNA) markers differentiated into 3 classes: DNA sequence-based, hybridization-based and PCR based [52] [53] [54] [55].

DNA markers are widely being as important tools in crop improvement programs of many crops viz. O*ryza sativa* [56] [57], *Triticum aestivum* [58] [59] [60], *Zea mays* [61] *Hordeum vulgare* barley [62], [63], pulses [64], tuber crops [65], oilseeds [66] crop species of horticultural importance [67] and fodder species [55]. The molecular markers would play a major role in boosting global food production by improving the efficiency of conventional plant breeding programs [68] [69]. Therefore, many institutions working on breeding programs in plants have accepted to improve and develop the facility for molecular marker [70] [71]. Many

types of molecular markers used for genetic studies in crop plants including RFLPs, RAPDs, AFLPs and SSRs [72].

2.4.1 RAPD molecular markers

RAPD was introduced after the development of Polymerase Chain Reaction (PCR) and considered as markers of economic class [73]. This marker used to estimate genetic relationships and fingerprinting accessions in germplasm of plant species. The advantages of RAPD were their simplicity and capability to detect little quantity of genetic diversity [74] [75]. However, there are some limitations in RAPD markers that include reproducibility of bands, rigorous standardization of reaction, and dominant inheritance [76] [77]. Despite these limitations, the random polymorphic DNA has the potential for generating huge numbers of markers and used in a several crop plants to analyze genetic variability at population and species level [78] [79].

Zeng et al. [80] studied *Betula alnoides* (Birch) from China using RAPD data, it was observed 90% of genetic variance within the population. Weng et al. [81] analysed 131 Zoysia spp. from 59 locations in Taiwan and its surrounding areas using RAPD to reveal their genetic variation in germplasm of 18 cultivars of cocoyam and 2 cultivars of Taro. Schnell et al. [82] estimate the genetic diversity within accessions of cocoyam cultivars, the genetic similarity was observed ranged from 86% to 97%. Ochiai et al. [83] analyzed the variations in *Colacasia esculenta* using RAPD markers accessions collected from the Yunnan. The study conducted by Pillai and Sreelekha on Taro cultivars using RAPD markers showed quite similar genetic relationships within cultivar of Taros, forty-five accessions of taro, showed distinct morphology traits evaluated using eleven RAPD primers [84]. The study showed that the genetic relatedness between accessions is useful to develop a breeding program and DNA fingerprinting can be used to indicate the uniqueness of a variety. Colombo et al. [85] studied 126 accessions of cassava spread at four locations. Samples included native accessions, ethno cultivars, local 58 cultivars, and representative accessions from the International Centre for Tropical Agriculture (CIAT) gene bank. Results showed a weak genetic structure of the cassava.

CHAPTER 3

SCREENING OF SELECTED POTENTIAL INDIGENOUS LEAFY VEGETABLES OF COLD DESERT LADAKH

Abstract

In the present study seven indigenous leafy vegetables (ILV) that can grow in high altitude harsh climatic conditions with minimal care and that are being used by native people of Ladakh as a vegetable, were evaluated for their potential for yield, early harvesting stage for consumption and conducted a sensory evaluation for consumer acceptance. Among all the seven ILV the highest yield (1.80±0.06 kg/m2), minimum days to harvest and highest hedonic points were observed in *A. hortensis*. The study suggested that *A. hortensis* has high yield high early harvesting stage and consumer preference.

3.1 Introduction

The high altitude region of Ladakh of India state Jammu and Kashmir is characterized by harsh snowy winters, high range of day and night temperature fluctuation in summers, high wind velocity, low precipitation, low atmospheric pressure, higher intensity of UV-radiation fragile ecosystem and sparse plant density The temperature drops down to -30 º C in winter Long harsh winters reduces the total crop growing season to just 5-6 months in a year. The region remains cut off for over six months in a year due to very heavy snowfall in winters and crops can be grown in this region during the summer season only [86]. As mentioned earlier most vegetable crop varieties suitable for other region of the country do not produce an optimum yield in this region due to many factors mainly short cropping season and reversed the order of weather cycle i.e., the onset of winters just before crop maturity etc. The limited indigenous production of fresh vegetables and difficulties in transportation due to geographic, harsh climatic conditions result in a short fall in supply of the fresh vegetables to the local population and the deployed army in this region. The availability of fresh vegetables significantly decreases during the winter months, also result in marked seasonal differences in dietary intake, diet being generally unbalanced during winters [87].

The demand for fresh vegetable produce is also ever-increasing due to the population increase, an increase in income of people, urbanization, and heavy deployment of the army. Therefore, meeting the increasing requirements of fresh vegetables in this remote mountainous area is a difficult challenge. Traditionally, people living in the cold desert of Ladakh depended on wild edible plants, including indigenous leafy vegetables (ILV), to meet their dietary requirements. They collect such plants from mountain slopes and around agricultural fields. The indigenous plants do not require intensive care and can grow in less fertile soil. Besides, these withstand harsh climatic conditions due to abiotic or biotic stress tolerance, which is inherent in their genetic makeup by years of natural selection. However, such plants grow quite sparsely in nature and their production is very low [86]. So, the first step to increase the availability of fresh vegetables and nutritional security in the region, based on traditional knowledge would be documentation and scientific validation of the traditional knowledge of indigenous inhabitants [88]. Another reason for such documentation may be that the indigenous traditional knowledge among farmers, on use of such plants is fast declining due to easy availability of modern-day

conventional vegetable types [89]. Moravian missionaries introduced vegetables such as potatoes, spinach, cauliflower, radish, green beans, kohl rabi, brussels sprout, and tomatoes in last quarter of the nineteenth century in Ladakh [90]. Defence Institute of High Altitude Research, introduced and distributed seed and seedlings of beans, beet root, cabbage, cauliflower, carrot, lettuce, peas, onion, spinach, tomato, turnip, okra, knol-khol, leek, radish, sugar beet, chillies, coriander, cucumber, Chinese cabbage, mint, brinjal, garlic, methi, pumpkin among farmers in Ladakh between 1965-1975. Modernization of agriculture and the introduction of modern modern-day cultivated vegetables leads to the narrowing of the genetic diversity of the indigenously used plant species [91]. There is a dire need to identify indigenous plants species, characterize their germplasm, and develop cultivation package and practices. The hypothesis here is that indigenous plant species are well adapted to the climatic conditions and could perform better if their germplasm selected on a scientific basis. Therefore, documentation and collection of myriad local genotypes become extremely important for ensuring food security in the remote mountainous regions. So in this part of the research program we surveyed the Ladakh region and selected seven plant species which are frequently used as vegetables and further screened these based on yield potential and consumer acceptance.

3.2 Materials and Method

3.2.1 Ethnobotanical survey

An ethnobotanical survey was conducted in three valleys viz., Nubra, Indus and Suru valley of Ladakh region, the documentation was done by open-ended questionnaire and filling the passport data book (Figure 3.2.1) standardized by National Bureau of Plant Genetic Resources, NBPGR India. Around twenty-seven species were recorded that are being used as an indigenous vegetable in the region. Among twenty-seven plant species, seven were found promising and the potential to be cultivated in the region (Figure 3.2.2). Therefore-these seven plant species were further evaluated to select the one of most potential indigenous vegetables.

Figure 3.2.1 Ethnobotanical survey in Ladakh

3.2.2 Plant material and cultivation practices

Seeds of seven selected plant species based on ethnobotanical survey. viz., *F. tataricum, R. patientia, F. esculentum, A. cruentus, M. verticillata, L. latifolium* and *A. hortensis* collected from farmers in Ladakh and were sown in a randomized complete block design in the year 2015 at the experimental field of DIHAR Leh, (elevation 3500 m amsl).

Farm yard manure (25t.ha^{-1}) was applied at the time of field preparation. Flat bed 2 m wide and 2 m long were prepared. Seeds were mixed with sand in a 3:1 ratio and sown in rows 20 cm apart. Plant to plant distance was maintained at 12 cm by thinning. Irrigation was done by flooding immediately after sowing followed by seven days interval at later stages. Weeding was done manually. Additional farm yard manure (FYM) (1.5 kg.m^2) was applied at the time of hoeing after the first picking of the leaf. The leaves were harvested when they are young and tender during the vegetative stage. Leaf harvest was made by cutting the plant a few centimetres above the ground except in the case of *M. verticillata* where it was harvested by topping leaves in the middle of the petiole.

Fagopyrum esculentum ***Fagopyrum tataricum*** ***Rumex patientia*** *Atriplex hortensis*

Amaranthus cruentus ***Lepidium latifolium*** ***Malva verticillata***

3.2.3 Observation of agro-morphological characters

Agro-morphological characters were recorded (Figure 3.2.3.1) as per the descriptor developed by the National Bureau of Plant Genetic Resource (NBPGR) [92] with slight modification. The data were recorded taking three replications of each accession for nine characters. Qualitative traits were recorded for a variation after every 10 days interval at the vegetative and flowering stages. Leaf area was recorded using portable laser leaf area meter (CI-201, CID Bioscience). Petiole length was recorded by cutting the portion of the leaf from the base of the leaf blade, and leaf thickness was measured using a digital vernier calliper (MITUTOYO, Japan). Plant height was measured from base of the plant to the tip of the leaf. Shoot diameter was recorded by taking three readings from the lower, middle, and top of the shoot. Leaf chlorophyll contents were measured with a chlorophyll content meter (CCM-

200plus, Opti-Sciences, Inc., USA). The yield of the plants was measured using a digital weighing balance.

Figure 3.2.3.1 Data recording during field study

3.2.4 Organoleptic test

Forty-five trained, healthy, and non-smoker subjects of two groups, army, and local people, in the age bracket of 20 to 55 years, comprising of 20 females and 25 males were selected to participate in the organoleptic test trials (Figure 3.2.4.1). The subjects were asked to refrain from eating or drinking for a minimum of one hour before their testing sessions. On the day of testing, freshly harvested leaves were washed and trimmed before preparation. It was

cooked and a pinch of salt was added. A stir bar was used to mix the salt until it was completely dissolved, and then promptly stored in a container at room temperature. Each subject was asked to score the samples on 9-points hedonic scale on paper ballot [92]. In between each sample, panellists were asked to rinse their mouth at least twice with water during one-minute break. A five-minute break was given between testing blocks to prevent fatiguing.

Figure 3.2.4.1 Evaluation of organoleptic test conducted on Army subjects at DIHAR Research laboratory.

3.2.5 Data analysis

One-way analysis of variance (ANOVA) was used to analyze data to figure out differences among different treatments. Correlation and 2-sided Tukey's Honestly Significant Difference (HSD) at $p \leq 0.05$ was carried out using Statistical Package for Social Sciences, SPSS version 17.0.

3.3 Results and discussion

3.3.1 Crop yield potential

The yield potential of ILVs is presented in Table 3.3.1.1. A significant difference in yield potential was observed among seven plant species under study which ranged from 0.45 to 1.86 kg/m^2. *A. hortensis* showed the highest yield (1.86 kg.m^2) followed *F. tataricum* (1.75 kg.m^2) and *R. patientia* (0.86 kg.m^2). The crops attained harvesting stage between 25 to 85 days after sowing which was minimum (25-30 days) in the case of *A. hortensis*. Crop reaching to harvesting stage earlier would be advantageous as it would ensure the supply of fresh harvest for longer duration in its growing season. Beside early maturity is also beneficial when crop growing season is short in the Ladakh region due to harsh environmental conditions. Thus based on of yield and minimum days required to harvest it may be concluded that *A. hortensis* is the best plant species among the seven species under the study.

Table 3.3.1.1 List of indigenous leafy vegetables and their days to harvest and yield

Botanical name	**Vernacular name**	**Common name**	**Days of harvesting**	**Yield (kg.m^2)**
Amaranthus cruentus	Khi snama	Amaranth	60-70	0.65±0.38^{b}
Atriplex hortensis	Phaltora/Phaltor	Mountain orach	25-30	1.80±0.06^{d}
Fagopyrum esculentum	Tayat/bro	Buckwheat	45-50	0.75±0.03^{bc}
Fagopyrum tataricum	Tayat/Kho bro	Buckwheat	50-55	1.75±0.04^{d}
Malva verticillata	Sochilik	Chinese mallow	75-85	0.64±0.35^{ab}
Rumex patientia	Shoma	Garden patience	70-80	0.86±0.33^{c}
Lepidium latifolium	Shang sho	Pepper weed	55-68	0.45±0.43^{a}

Different lowercase letters indicate significantly different yield at 5% level of significance

3.3.2 Agro-morphological characters

Significant variation in plant morphological characters was observed between the seven ILVs studied (Table 3.3.2.1). Plant height ranged from 9.0 to 19.5 cm depending on the crop. The mean number of leaves ranged from 6.3 to 14.7 per plant. The chlorophyll contents ranged from 28.9 to 49.4 mg. g^{-1}. Leaf characteristics and petiole length showed significant differences among the seven ILVs studied. We observed that a leaf character was found highest in *A. hortensis* which contributed to the yield of the plants. Therefore, the highest yield was also observed in *A. hortensis*.

Table 3.3.2.1 Agro-morphological characters of indigenous vegetables

Characters	*F. esculentum*	*A. hortensis*	*F. tataricum*	*M. vertisilester*	*R. patientia*	*L. latifolium*	*A. cruentus*
Plant height (cm)	19.50±2.02^{c}	12.40±0.55^{ab}	16.87±0.03^{bc}	13.40±0.95^{ab}	9.00±0.58^{a}	13.00±0.58^{ab}	11.33±0.88^{a}
No. of leaf/plant	7.33±0.33^{ab}	14.00±2.00^{bc}	8.00±2.31^{abc}	6.33±1.20^{a}	14.67±1.45^{c}	9.76±0.88^{abc}	14.67±1.45^{c}
Leaf length (cm)	2.86±0.49^{a}	4.18±0.87^{ab}	3.61±0.32^{abc}	4.19±0.17^{ab}	6.49±0.32^{bc}	7.72±1.26^{c}	3.89±0.90^{ab}
Leaf width (cm)	2.33±0.44^{ab}	3.92±0.97^{abc}	5.87±0.23^{c}	2.66±0.12^{bc}	2.43±0.19^{a}	2.98±0.17^{ab}	2.43±0.19^{a}
Leaf thickness (mm)	0.48±0.11^{a}	0.50±0.13^{a}	0.48±0.02^{a}	0.32±0.01^{a}	0.24±0.03^{a}	0.49±0.14^{a}	0.30±0.03^{a}
Petiole length (cm)	3.97±0.26^{bc}	1.83±.75^{ab}	7.90±1.30^{c}	5.77±0.93^{bc}	3.33±1.42^{ab}	0.10±0.00^{a}	1.30±0.35^{a}
Leaf area (cm^2)	6.67±1.47^{a}	10.27±4.41^{a}	16.58±7.06^{a}	12.64±1.06^{a}	11.43±1.27^{a}	14.98±3.16^{a}	8.77±0.41^{a}
Chlorophyll ($mg.g^{-1}$)	28.90±0.89^{a}	55.33±2.02^{d}	38.67±2.70^{ab}	42.67±3.28^{bc}	49.37±2.18^{cd}	45.00±1.15^{bcd}	49.37±2.18^{cd}

Values of quantitative traits are represented as mean ± SD, different super scripts in the columns indicate that differences are statistically significant at 5% level

3.3.3 Correlation among variables

Table 3.3.3.1 representing the correlations among agro-morphological variables recorded in case of *A. hortensis*. Significant correlation was observed between plant height with leaf length (r = 0.438), leaf width (r = -0.435) and yield (r = -0.540). Number of leaves per plant is significantly correlated with leaf thickness (r = 0.537) and chlorophyll contents (r = -0.789). Leaf

length is positively correlated with chlorophyll contents (r = 0.592), and negatively correlated with leaf width (r = -0.509). Leaf thickness is positively correlated with petiole length, (r = 0.671), leaf area (r = 0.515) and yield (r = 0.549). The above results shows that the leaf thickness, petiole length and leaf area is significantly correlated with the yield. Therefore, it may be concluded that these three parameters viz., leaf thickness, petiole length, and leaf area are yield contributing characters of *A. hortensis* and can be used for a further selection of germplasm.

Table 3.3.3.1 Pearson's correlation coefficients of morphological characters, chlorophyll contents and yield of indigenous leafy vegetable *A. hortensis*

Variables	Plant height	Leaf/ plant	Leaf length	Leaf width	Leaf thickness	Petiole length	Leaf area	Chlorophyll content	Yield
Plant height	1	0.356	0.438*	-0.435*	-0.338	-0.410	0.129	0.429	-0.540*
Leaves/plant		1	-0.335	0.325	0.537*	0.418	-0.081	-0.789**	0.141
Leaf length			1	-0.509*	-0.095	-0.412	-0.069	0.592**	0.112
Leaf width				1	-0.091	-0.170	0.616**	0.297	-0.099
Leaf thickness					1	0.671**	0.515*	-0.172	0.549**
Petiole length						1	-0.097	-0.240	0.235
Leaf area							1	-0.372	0.367
Chlorophyll content								1	0.139
Yield									1

*Significant at $p \leq 0.05$, **Significant at $p \leq 0.01$

3.3.4 Organoleptic test

Overall consumer preference for ILVs based on organoleptic test is presented in Table 3.3.4.1, figure 3.3.4.1 and group-wise preference was presented in figure 3.3.4.2 and 3.3.4.3. The results show that *A. hortensis* was preferred by most of the consumers over other indigenous

leafy vegetables in terms of taste, flavour, palatability, and overall acceptability. Therefore, *Atriplex hortensis* need to be selected for further detailed studies and for promotion as potential ILV among native people of the Ladakh region.

Table 3.3.4.1 Acceptability of indigenous leafy vegetables with respect to Taste, Flavour, and Palatability in organoleptic test of both groups

Vegetable	Code	Taste	Flavour	Palatability	Overall Acceptability
Amaranthus crunteus	V1	7.33 ± 0.49^{bc}	7.17 ± 0.48^{bc}	7.17 ± 0.48^{bc}	7.00 ± 0.45^{bc}
Fagopyrum tataricum	V2	7.33 ± 0.75^{bc}	6.83 ± 0.40^{bc}	6.50 ± 0.50^{abc}	6.67 ± 0.61^{bc}
Malva verticillata	V3	6.17 ± 0.21^{b}	5.67 ± 0.61^{ab}	5.67 ± 0.92^{ab}	5.17 ± 1.11^{ab}
Fagopyrum esculentum	V4	6.00 ± 0.60^{b}	5.83 ± 0.48^{ab}	5.83 ± 0.60^{abc}	5.83 ± 0.60^{abc}
Atriplex hortensis	V5	8.67 ± 0.45^{c}	8.33 ± 0.21^{c}	8.33 ± 0.33^{c}	8.33 ± 0.33^{c}
Rumex patientia	V6	5.83 ± 0.33^{b}	5.33 ± 0.76^{ab}	5.50 ± 0.67^{ab}	5.50 ± 0.67^{abc}
Lepidium latifolium	V7	3.33 ± 0.27^{a}	4.00 ± 0.26^{a}	4.33 ± 0.33^{a}	3.12 ± 0.60^{a}

Scores of sensory evaluation parameters presented as mean ± SD, different super scripts in the columns indicate that differences are statistically significant at 5% level.

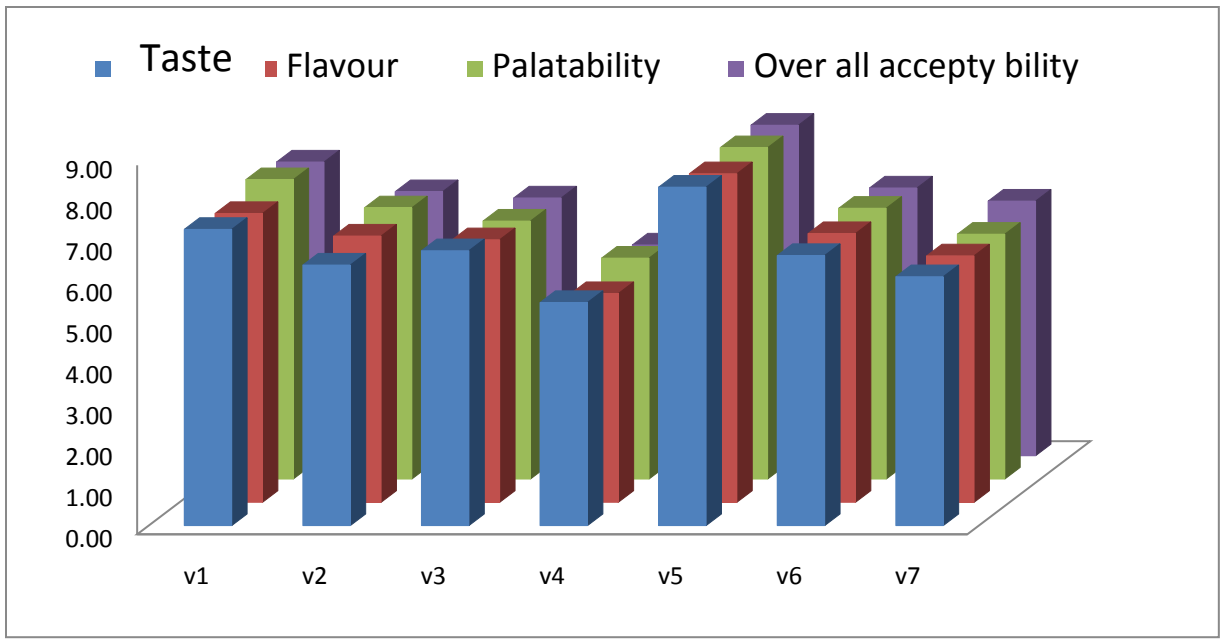


Figure 3.3.4.1 Acceptability of indigenous leafy vegetables with respect to Taste, Flavour, and Palatability in organoleptic test of both groups shown as bar diagram

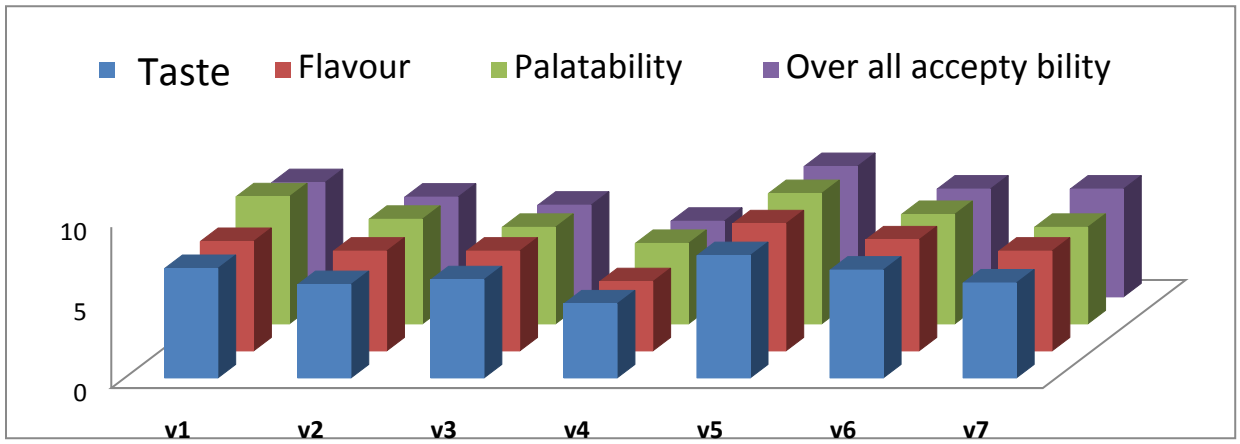


Figure 3.3.4.2 Acceptability of indigenous leafy vegetables with respect to Taste, Flavour, and Palatability in organoleptic test on army subjects

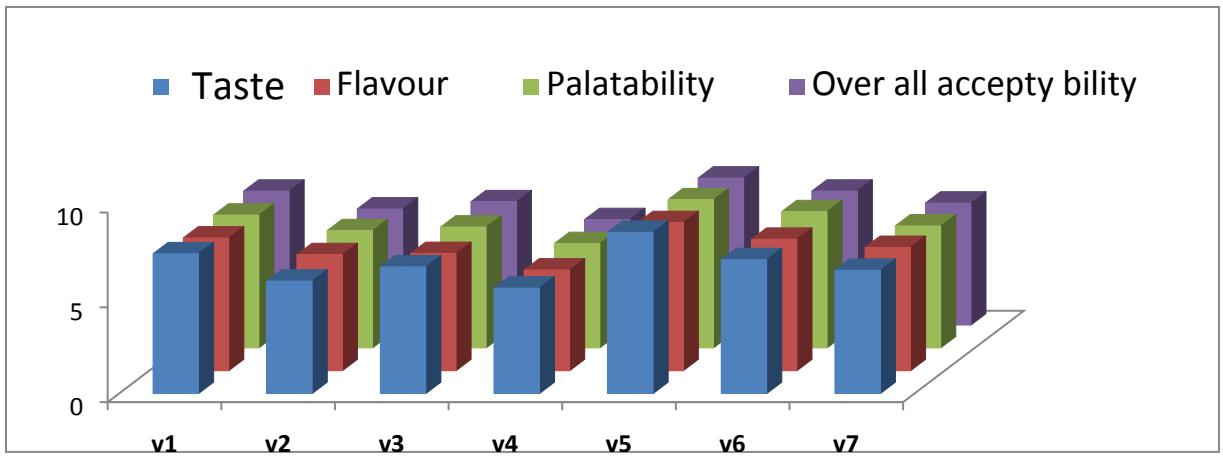


Figure 3.3.4.3 Acceptability of indigenous leafy vegetables with respect to Taste, Flavour, and Palatability in organoleptic test on local subjects

3.4 Conclusion

The yield of *A. hortensis* was observed highest and the number of days required to achieve the full potential of yield was minimum than other indigenous vegetables. The study also found that *A. hortensis* have high consumer acceptance and preferred for consumption by local people as well as by Army personnel. Therefore, it is suggested that a detail study on *A. hortensis* required.

CHAPTER 4

AGRO-MORPHOLOGICAL CHARACTERIZATION IN *ATRIPLEX HORTENSIS* L. AN INDIGENOUS VEGETABLE FROM COLD DESERT OF TRANS-HIMALAYAN REGION OF LADAKH, (J&K), INDIA

Abstract

In the current study, a total of 132 accessions of indigenous vegetable *A. hortensis* L. collected from 15 diverse geographical locations of the Ladakh region were evaluated for morphologically i.e., six qualitative and fifteen quantitative agro-morphological parameters standardized by the National Bureau of Plant Genetic Resources (NBPGR). To examine whether these populations are similar or diverse, principal component analysis (PCA), multivariate analysis, multi-dimensional scaling (MDS), and group analysis were carried out using agro-morphological data. More than 60% of diversity among the population could be attributed to the first two principal components. The outcome of the principal component analysis and multidimensional scaling was comparable to that of cluster analysis. It was observed that agro-morphological traits of Atriplex accessions showed considerable diversity which signifies high diversity in populations from the different geographical regions of Ladakh.

4.1 Introduction

As mentioned in the previous chapter, identification, characterization, and promoting the cultivation of traditional plant species used as a vegetable can partly obviate the problem of shortage of fresh vegetables to army establishments and local inhabitants in the Ladakh region. Towards this endeavour we evaluated seven plant species traditionally used as a vegetable for agro-morphological traits yield and consumer acceptance. The study suggested that yield potential of *A. hortensis* was significantly higher and yield contributing characters were recorded highest in Atriplex. It was found as an early maturing crop amongst seven plant species under study. It was also found to be most preferred by the consumer with respect to the most palatability parameters. Thus, to extend the idea of characterizing indigenous plant species as vegetable crops in the Ladakh region, *Atriplex hortensis* was selected for further studies.

Atriplex hortensis L. member of family Chenopodaceae, locally known as *Phaltora* in Ladakh region popularly known as salt bush, garden orach, and mountain spinach is an annual herb [93]. The plant is known to have originated from Europe and Siberia [8]. It is one of the known leafy vegetables [94]. The species is known for its source of protein [95] and vitamins [96] [97]. It is the first green herb to appear after prolonged winter in Ladakh and used as a leafy indigenous vegetable. In traditional medicine, it is used as a health energizer to help in nutrition absorption, enhance metabolism, and digestion [98]. Leaves are emetic, purgative, diuretic and efficacious. It is used externally in the treatment of gout, ointment prepared from the plant is said to be folk remedies for indurations and tumour [99]. It is also characterized by a high content of flavonoid mineral components [100] and amino acid [101]

Crop improvement mostly depends on the accessibility of genetic variation in the plant species. The genetic variations if available and documented well concerning important traits, then yield of crops can be increased by selecting seeds from desirable populations and or selecting individual genotypes [102]. It is likely that diversity in *A. hortensis* with respect to leaf colour, leaf width, leaf length, chlorophyll content, leaf area, leaf per plant, diameter of the shoot, leaf yield, seed weight, and leaf moisture is available in the region due to occurrence of

the plant in the different geographical region of Ladakh but as of now, there is no record of studying and documentation of this diversity. The existence of genetic diversity in a crop and its proper documentation is also pre requisite for starting a breeding program for the improvement of the crop. Given the above gaps, and the overall objective of increasing yield and promoting indigenous crops in the Ladakh region, in this part of the study, the objective was to characterize and evaluate *A. hortensis* germplasm.

4.2 Material and methods

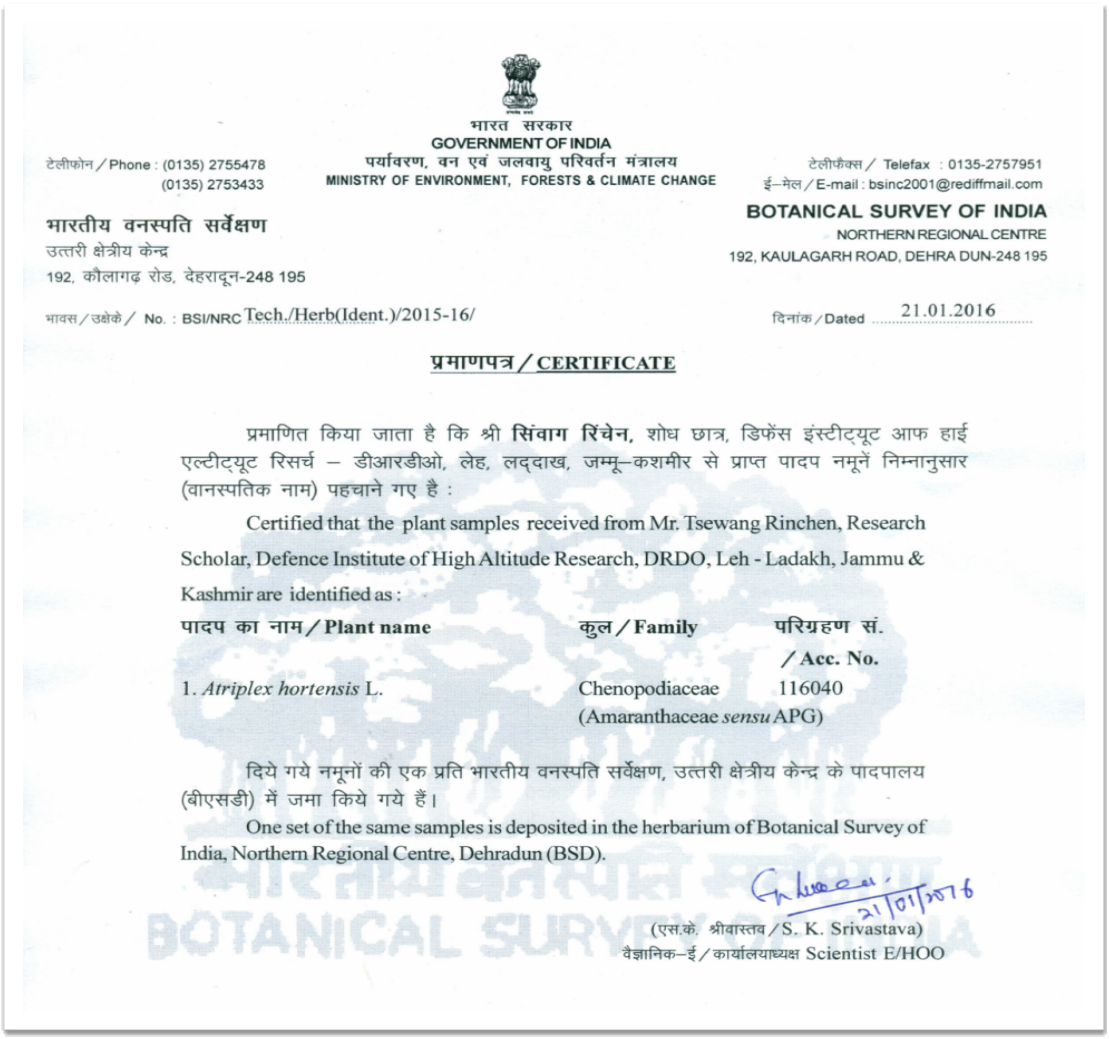
4.2.1 Pant Material

Representative germplasm of *A. hortensis* (N=132) collected from wild and cultivated field, representing three populations from 15 different locations spread across Suru, Indus, and Nubra valleys of the Trans-Himalaya region in Ladakh during 2013-2015 was used in this investigation. (Table 4.2.1.1). 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 Global positioning system GPS, (GARMIN 72, Olathe, Kansas USA). To ascertain the authenticity of plant species was verified by Botanical Survey of India (BSI), Dehradun, India (Figure 4.2.1.1) a herbarium of *A. hortensis* L. representative samples collected from three valleys was prepared and the voucher specimens were submitted to BSI.

Table 4.2.1.1 Geographical regions and sampling locations of *Atriplex hortensis* germplasm collection from trans-Himalayas of Ladakh.

Valleys	Sub population	Number of Accessions	Altitude (m amsl)	Latitude (N)	Longitude (E)
Nubra	Khardong	6	4109	34º 33.822	077º 39.487
	Tangyar	10	3915	34º 15.168	077º52.259
	Khungru	9	3623	34º 17.003	077º 26.556
	Agham	11	3335	34º 19.706	077º 49.877
	Murghi	5	3201	34º 45.516	077º 32.643

	Panamik	5	3184	34º 46.963	077º 32.304
	Hunder	20	3169	34º 35.213	077º 27.688
	Udmaru	5	3129	34º 37.627	077º 26.180
	Diskit	5	3117	34º 33.210	077º 32.686
Indus	Gonpa	10	3666	34º 11.026	077º 35.560
	Shenam	11	3477	34º 09.550	077º 34.766
Suru	Chiktan	11	3252	34º28.166	076º30.432
	Achakmal	6	2888	34 º33.220	076º09.449
	Pakskum	11	2881	34 º33.120	076º09.443
	Soad	7	2860	34 º33.974	076 º 10.305



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भावस / उक्षेके / No. : BSI/NRC Tech./Herb(Ident.)/2015-16/

दिनांक / Dated 21.01.2016

प्रमाणपत्र / CERTIFICATE

प्रमाणित किया जाता है कि श्री **सिंवाग रिंचेन,** शोध छात्र, डिफेंस इंस्टीट्यूट आफ हाई एल्टीट्यूट रिसर्च – डीआरडीओ, लेह, लद्दाख, जम्मू–कशमीर से प्राप्त पादप नमूनें निम्नानुसार (वानस्पतिक नाम) पहचाने गए है :

Certified that the plant samples received from Mr. Tsewang Rinchen, Research Scholar, Defence Institute of High Altitude Research, DRDO, Leh - Ladakh, Jammu & Kashmir are identified as :

पादप का नाम / Plant name	कुल / Family	परिग्रहण सं. / Acc. No.
1. *Atriplex hortensis* L.	Chenopodiaceae (Amaranthaceae *sensu* APG)	116040

दिये गये नमूनों की एक प्रति भारतीय वनस्पति सर्वेक्षण, उत्तरी क्षेत्रीय केन्द्र के पादपालय (बीएसडी) में जमा किये गये हैं।

One set of the same samples is deposited in the herbarium of Botanical Survey of India, Northern Regional Centre, Dehradun (BSD).

21/01/2016

(एस.के. श्रीवास्तव / S. K. Srivastava)
वैज्ञानिक–ई / कार्यालयाध्यक्ष Scientist E/HOO

Figure 4.2.1.1 Certificate provided by BSI for the authenticity of the *Atriplex hortensis* L.

4.2.2 Experimental site and field layout

The experiments were conducted in the field of Defence Institute of High Altitude Research (DIHAR) Leh, Ladakh. Collected accessions of *A. hortensis* were grown in the summer season of the year 2014 - 2015 with three replications established in randomized blocks, a total of three hundred ninety-six experimental beds were prepared of size $2m^2$ (1m x 2 m) spacing of 15 cm between plants and three rows with spacing of 30 cm plant to plant. The crop received a basal dose of locally available compost and irrigations (Figure 4.2.2.1). The crop was protected from weeds by weeding. Three representative plants were selected arbitrarily for each accession for recording data (Figure 4.2.2.2) on twenty-one parameters. Precipitation of the study region is less than 200 mm of which more than 70% is in the form of snow [104].

Figures 4.2.2.1 Seeds of *A. hortensis* sown at experimental field collected from different location of Ladakh

Figure 4.2.2.2 Recording the agro-morphological data in the field during the seed stage

4.2.3 Quantitative and qualitative characters

Data on agro-morphological parameters were recorded by taking three replications of each accession for twenty-one parameters i.e., six qualitative traits and fifteen quantitative traits (Table 4.2.2.1**)**. To study the morphological characters, we used the parameters standardized by National Bureau of Plant Genetic Resources (NBPGR) [105]. All the parameters were recorded for variation in characters at the peak of the vegetative stage and flowering stage. Data were analysed based on of agro-morphological data. Vegetative data collected in 25-30 days when the plant were ready to consume, flowering data collected after 55-65 days, and at the fruiting stage of 85-95 days old plants. Data were recorded from three replications and analysed statistically. Width of the leaf, length of leaf and area of leaf were recorded using portable laser leaf area meter (CI-201, CID Bio-Science), length of petiole by cutting the leaf from the base attached shoot, leaf thickness by using 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, leaf per plant and weight per plant were recorded at the vegetative stage when the plant was ready to harvest for consumption, moisture content was recorded by using formula of a wet the weight of 100 g minus dry weight using digital electronic weighing balance to an accuracy of 0.01 g, height of the plant was measured from the base to tip

of the plant 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 digital vernier calliper (MITUTOYO, Japan), total soluble solid (TSS) concentration of freshly-chopped extracted juice of representative leaf samples was examine by using a digital hand refractometer (Atago, ATC-1E, Kyoto, Japan) [106]. The leaf chlorophyll content of the species was obtained by collecting three-leaf samples of the same size with three replications using chlorophyll content meter (Opti-science, CCM-200 plus).

Table 4.2.2.1 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 0.71mm^2)	2	Shoot diameter (mm)
3	Moisture content (%)	3	Leaf length (cm)
4	Leaf colour (colour chart)	4	Leaf width (cm)
5	Dry weight (g)	5	Leaf area (cm^2)
6	Weight of 1000 seeds (g)	6	Leaf thickness (mm)
		7	Length of petiole (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

4.2.4 Statistical analysis

Statistic of multivariate and univariate was used to analyse the data recorded during study using SPSS, Statistical Package for Social Sciences, version 17.0. ANOVA, Analysis of variance

was carried out and the outcome was comparable to Duncan's multiple range tests at the significance level of 5 percent. To analyse the associations among the characters, correlation analysis was carried out by Pearson's. Principal component analysis (PCA,) and Multi dimensional scaling (MDS), was used to analyse the variance and co-variance among characters within and among populations [107]. Normal euclidean distance intended for each population of Atriplex. A distance matrix was used to construct a phonetic dendrogram using an average linkage procedure [108].

4.3 Results

4.3.1 Diversity in Agro- morphological traits

Considerable variations were observed in various Agro-morphological characters of *A. hortensis* collected from the different geographical regions of Ladakh. These variations could be attributed to the phenotype expression of the population and environmental factors [109]**.** Leaf colour 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 agro-morphological characters (Table 4.3.1.1) recorded in the study showed that the mean plant height ranged from 15.36 to 27.35 cm (P = 0.001), while mean leaf length ranged from 6.39 to 11.46 cm (P = 0.002), mean leaf width ranged from 4.82 to 6.46 cm (P = 0.013), mean leaf area was from 20.22 to 46.28 cm^2 (P = 0.001), mean number of leaf ranged from 18.27 to 37.93 (P = 0.013), mean chlorophyll content ranged from was 43.19 to 70.60 SPAD units (P = 0.005), mean dry weight was 11.09 to 12.64 g (P≥ 0.026), mean shoot diameter varied from 4.07 to 5.38 mm (P = 0.026), mean petiole length ranged from 2.47 to 3.76 cm (P = 0.003), range of mean moisture content was 87.36 to 88.91 % (P = 0.02), mean number of inflorescence per plant ranged from 20.17 to 29.47 (P = 0.017), mean seed yield per plant ranged from 32.25 to 67.39 g (P = 0.26) and mean 1000 seed weight was ranged between 1.91 to 2.20 g (P = 0.001) in the study area. The highest mean values of agro-morphological traits vis a vis populations of *A. hortensis* are presented in Table 4.3.1.2. From the above observations it can be concluded that four populations viz., Hunder, Udmaru, Chiktan and Soad were better in terms high values of traits.

Table 4.3.1.1 Duncan's test for comparisons of mean of agro- morphological characters of *Atriplex hortensis* populations

Population	**Mean total soluble solids (SE) brix % P≤0.05**	**Mean chlorophyll content (SE) SPAD P≤0.01**	**Mean number of leaf per plant (SE) P≤0.01**	**Mean Plant height (SE) cm P≤0.05**	**Mean leaf area (SE) cm2 P≤0.05**	**Mean leaf length (SE) cm P≤0.01**	**Mean leaf width (SE) cm P≤0.01**
Khardong	9.77b (0.51)	53.27abc (5.13)	23.67abc (2.49)	24.74d (1.73)	25.17ab (2.49)	7.80abc (0.53)	5.25ab (1.37)
Tangyar	8.87abc (0.40)	53.45abc (3.45)	27.17abc (1.93)	21.51bc d (1.50)	29.59ab c (2.09)	8.90bc (0.45)	5.56abc(1.21)
Gonpa	9.22ab (0.29)	47.25bcd (3.84)	24.00bcd (3.19)	18.48d (1.40)	23.83bc (2.15)	6.78abc (0.48)	4.82abc(1.38)
Khungru	8.56abc (0.31)	51.03ab (3.31)	26.64abc (2.25)	23.15ab c (1.52)	29.89ab (2.13)	8.63ab (0.43)	5.60a (1.38)
Aghyam	9.04ab (0.67)	52.25abc (3.36)	27.48abc (3.72)	23.37cd (1.82)	31.42ab c (3.59)	8.51abc (0.72)	5.89abc (1.60)
Chiktan	8.48abc (0.55)	**70.60bcd (4.67)**	**37.93bc** (5.43)	25.97bc d (2.25)	46.28bc (8.48)	**11.46bc (1.32)**	6.43abc (2.14)
Shenam	8.69abc (0.20)	52.67bcd (1.95)	24.91ab (1.48)	23.74d (1.20)	28.32ab c (1.75)	8.56 abc(0.62)	5.43abc (1.42)
Murghi	8.22abc (0.30)	52.87abc (5.38)	32.87abc (4.07)	27.35cd (2.43)	33.09bc (2.79)	8.60 abc (0.42)	6.30abc(1.33)
Panamik	9.87ab (0.91)	43.19d (4.47)	18.27d (1.75)	15.36d (1.97)	20.22ab c (3.20)	6.39 d (0.60)	4.97c (2.35)
Hunder	8.43ab (0.33)	58.09abc (3.65)	30.03abc (2.62)	25.30cd (1.95)	31.77ab c (2.47)	8.69 abc (0.52)	5.89abc (1.60)
Udmaru	8.85a (0.33)	59.51abc (3.71)	23.18cd (1.90)	25.46d (1.24)	28.06dc (2.32)	8.17 abc (0.50)	5.45bc (1.40)
Diskit	9.11bc (0.30)	57.50a (4.13)	28.77a (2.76)	21.87a (1.48)	30.64a (3.35)	8.74a (0.64)	5.61a (1.97)

Ackchamal	8.75ab (0.37)	53.78abc (5.96)	22.50ab (2.89)	16.44ab (1.54)	23.41ab (2.32)	7.53abc (0.52)	4.85a (1.23)
Pakskum	9.25abc (0.50)	65.16cd (4.50)	26.34abc (3.08)	23.60cd (1.44)	28.81abc (3.10)	8.18abc (0.59)	5.45abc(1.88)
Soad	10.25c (0.51)	55.24abc (4.62)	28.71bc (2.13)	26.68d (2.17)	36.22c (4.14)	9.61cd (0.71)	**6.46c (1.85)**
Population	**Mean leaf thickness (SE) mm P≤0.05**	**Mean dry weight (SE) g P≤0.01**	**Mean shoot diameter (SE) mm P≤0.01**	**Mean petiole length (SE) cm P≤0.01**	**Mean leaf yield per plant (SE) g P≤0.01**	**Mean primary branches per plant (SE) P≤0.05**	**Mean moisture content (SE) % P≤0.01**
Khardong	0.60a (0.02)	12.46bc (0.56)	4.66abc (0.25)	2.59a (0.16)	16.33abc (2.24)	26.72bc (1.67)	87.54ab (0.56)
Tangyar	0.64a (0.02)	12.04abc (0.20)	4.87abc (0.21)	2.80ab (0.12)	18.91abc (2.11)	26.23abc (1.38)	87.96abc (0.20)
Gonpa	0.64a (0.08)	12.27ab (0.36)	4.07c (0.23)	2.58ab (0.12)	13.99c (2.02)	23.44abc (1.55)	87.73bc (0.36)
Khungru	0.62a (0.03)	11.74bc (0.16)	4.67a (0.18)	2.58a (0.11)	18.82ab (1.90)	24.64ab (1.49)	88.26ab (0.16)
Aghyam	0.71a (0.09)	12.31abc (0.28)	4.83abc (0.39)	2.62a (0.20)	20.58abc (3.33)	29.47abc (1.13)	87.69abc (0.28)
Chiktan	0.61a (0.06)	11.95abc (0.47)	4.85abc (0.31)	**3.76ab (0.50)**	19.60abc (2.89)	20.40abc (2.01)	88.05abc (0.47)
Shenam	0.64a (0.02)	11.94c (0.18)	4.80abc (0.14)	2.78ab (0.09)	18.54abc (1.68)	22.98ab (0.97)	88.06a (0.18)
Murgi	0.60a (0.03)	11.96bc (0.34)	5.08abc (0.33)	3.04ab (0.14)	23.44abc (4.03)	23.00c (2.99)	88.04ab (0.34)
Panamik	0.67a (0.05)	12.63abc (0.24)	4.27abc (0.33)	2.47c (0.24)	11.61abc (1.98)	23.93a (1.62)	87.37abc (0.24)
Hunder	0.66a (0.04)	11.50abc	**5.38abc**	2.81ab	**26.84ab**	24.53ab	88.50abc

		(0.23)	**(0.27)**	(0.16)	**c (5.73)**	(1.17)	(0.23)
Udmaru	0.60a (0.03)	**12.64abc (0.24)**	4.67bc (0.18)	2.98ab (0.17)	17.54bc (1.84)	20.76ab (1.42)	87.36abc (0.24)
Diskit	0.59a (0.01)	12.01c (0.36)	4.83ab (0.25)	2.66a (0.15)	22.25a (3.75)	24.51abc (2.18)	87.99a (0.36)
Ackchamal	0.67a (0.03)	11.08a (0.38)	4.22ab (0.33)	2.71ab (0.21)	15.12ab (2.51)	23.22ab (1.50)	**88.92c (0.38)**
Pakskum	0.64a (0.02)	12.03abc (0.20)	4.67abc (0.28)	2.76ab (0.15)	19.12ab c (2.83)	21.58ab (1.30)	87.97abc (0.20)
Soad	0.62a (0.02)	11.43ab (0.28)	5.28c (0.29)	3.22b (0.27)	22.76bc (2.47)	25.81abc (2.83)	88.58bc (0.28)

Population	**Inflorescence length per plant (SE) cm P≤0.05**	**Number of inflorescence per plant (SE) P≤0.05**	**Seed yield per plant (SE) g NS**	**Seed size (SE) mm P≤0.05**	**1000 Seed weight (SE) g P≤0.01**
Khardong	16.33abc (2.24)	20.17a (2.07)	48.60ab (7.24)	2.05abcd (.06)	2.91ab (.13)
Tangyar	18.91abc (2.11)	22.33abc (1.39)	41.99a (4.12)	1.99ab (.04)	2.56ab (.10)
Gonpa	13.99abc (2.02)	23.15abc (1.16)	41.98ab (6.04)	2.03abcd (.05)	2.89ab (.38)
Khungru	18.82abc (1.90)	25.27abc (1.15)	42.02a (4.63)	1.99abcd (.05)	2.55ab (.08)
Aghyam	20.58bc (3.33)	25.40abcd (1.86)	67.39a (12.22)	2.20ab (.06)	3.12ab (.16)
Chiktan	19.60abc (2.89)	27.07abc (2.07)	45.36a (6.34)	1.91bcd (.07)	2.64ab (.14)
Shenam	18.54abc (1.68)	24.10abc (1.07)	47.56a (3.96)	2.09cd (.03)	2.80b (.07)
Murgi	23.44bc (4.03)	29.47abcd (1.13)	53.88b (9.92)	1.97d (.07)	2.56ab (.16)
Panamik	11.61bc (1.98)	20.40cd (2.01)	32.25a (6.93)	2.07a (.06)	2.66ab (.12)
Hunder	26.84c (5.73)	23.27abc (1.59)	54.09ab (7.53)	2.03bcd (.04)	2.68ab (.09)
Udmaru	17.54d (1.84)	23.18d (1.08)	44.11ab (5.45)	2.19ab (.04)	3.40ab (.11)
Diskit	22.25a (3.75)	23.15ab (1.58)	45.07a (7.42)	2.10abcd (.05)	2.59ab (.10)
Ackchamal	15.12ab (2.51)	24.33abcd (1.63)	36.06a (7.73)	2.02abc (.07)	2.45a (.14)

Pakskum	19.12abc (2.83)	20.33ab (1.35)	38.44a (3.75)	1.98ab (.03)	2.73ab (.18)
Soad	22.76c (2.47)	25.86bcd (2.65)	45.45a (5.56)	2.20d (.07)	**4.15c (.94)**

Values represented as mean±SE. Different superscripts in column, indicate significant differences

Table 4.3.1.2 Highest mean values of morphological traits in *A. hortensis*

Population	**Characters**	**Highest value**
Chiktan	Chlorophyll content (SPAD/CCI unit 0.71mm^2)	70.60
	Number of leaf per plant	37.93
	Petiole length (cm)	3.76
	Leaf Yield/plant (g)	22.76
Soad	Leaf width (cm)	6.46
	Leaf length (cm)	11.46
	Moisture content (%)	88.92
Hunder	Shoot diameter (mm)	5.38
	1000 seed weight (g)	4.16
Udmaru	Dry weight (%)	12.64

Figure 4.3.1.1 shows the character values plotted against the first two-component variants from principal component analysis and each population concerning to their euclidean distance from multi dimensional scaling. Traits were positioned quite close to each other in the axis concerning to their population (four groups) (Figure 4.3.1.2). The closely related samples belong to group II which include Akchamal, Diskit, Tangyar, Agyam, Khungru, while group I include Panamik, Pakskum, Shenam, Chiktan, group III include Soad, Gonpa, Udmaru and group IV including

Khardong, Hunder and Murghi populations. The most significant morphological characters were distinguished from these four groups that are reflected in their loadings characters on the first two components. The first two components include more than 60 % variation among populations. The highest loadings traits were leaf area, number of leaf, height of the plant, yield of leaf, width of leaf, petiole length, shoot diameter and number of inflorescence for principal component 1 whereas seed size and number of primary branches for principal component 2 (Table 4.3.1.3). These loading characters along with the populations with highest values of traits can be used for future breeding programs to improve the quality and quantitative traits of the species

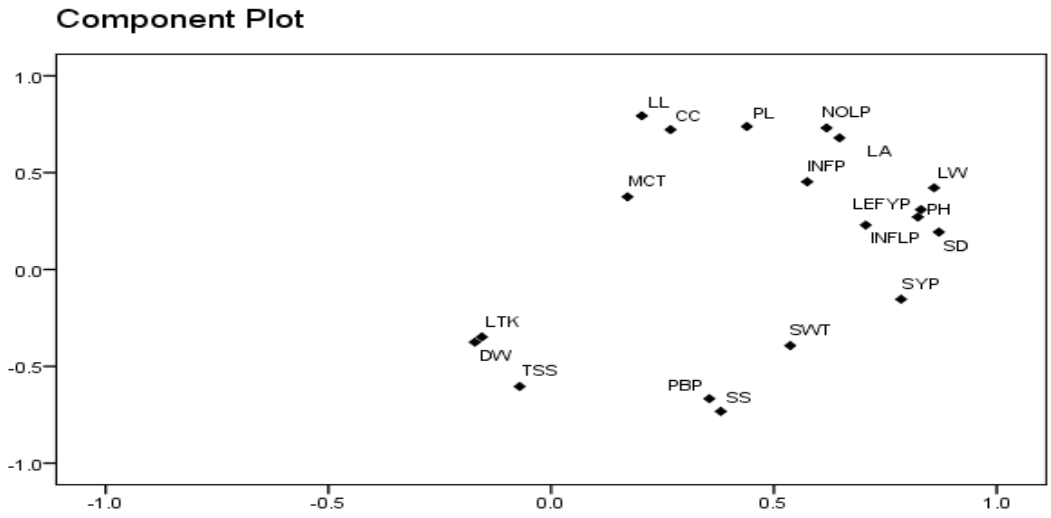

Figure 4.3.1.1 Principal component analysis of morphological data of *Atriplex hortensis* (NOLP: leaf per plant, LA: leaf area, LW: width of leaf, PH: plant height, PL: petiole length, LEFYP: leaf yield per plant, SD: Shoot diameter, INFP: Number of Inflorescence, INFLP: Inflorescence length, CC: Chlorophyll content, LL: Leaf length, TSS: Total soluble solids, DW: dry weight, MC: moisture content, LTK: leaf thickness, SS: seed size, PBP: primary branches per plant, SWT: 1000 seed weight, SYP: Seed yield.

Table 4.3.1.3 Morphological characters of *Atriplex hortensis* and their principal component weightage recorded during the study

Characters	**Characters acronym**	**PC1 (42.50 %)**	**PC2 (16%=59.59)**
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
Primary branches per plant	PBP	-0.159	0.739
Weight of 1000 seeds	SWT	0.156	0.647
Seed yield	SYP	0.501	0.624

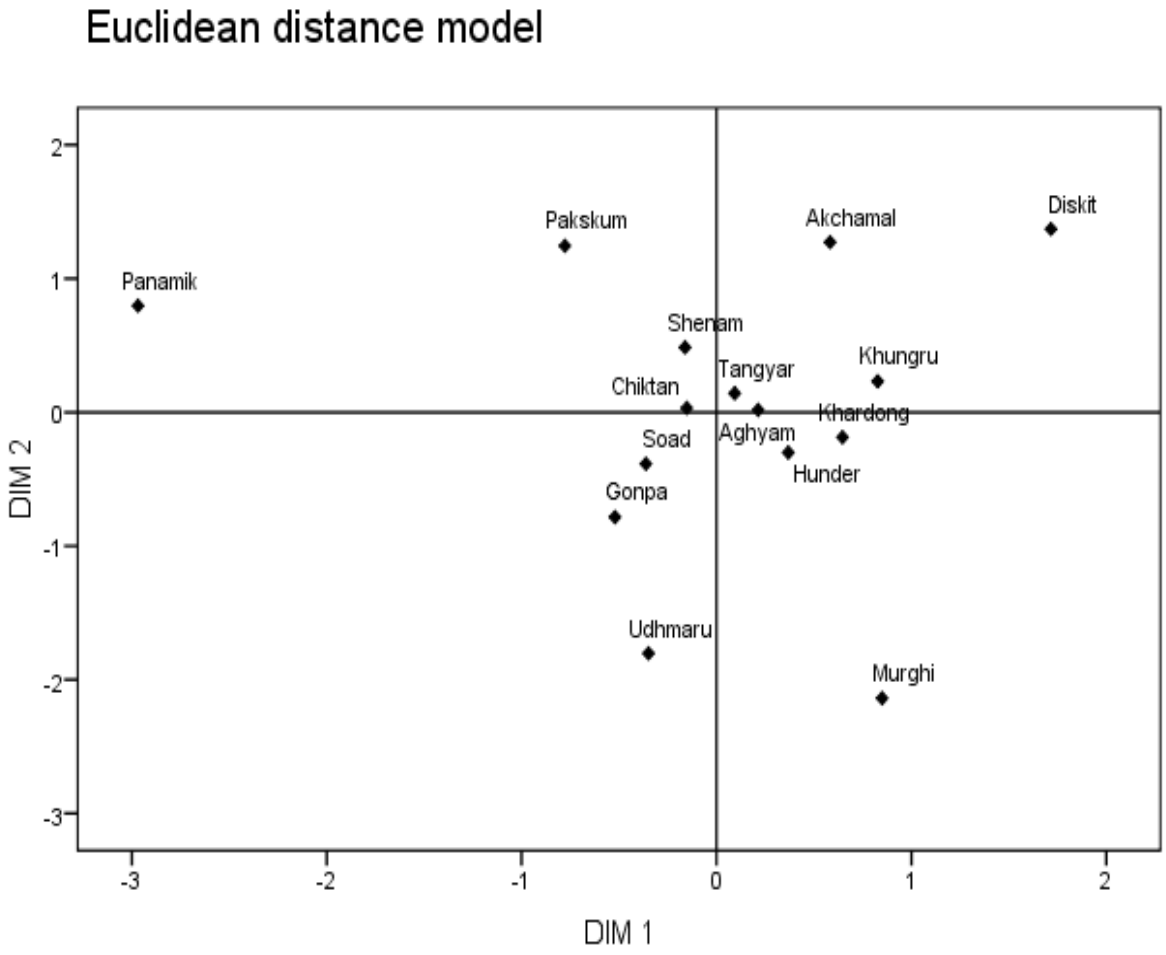

Figure 4.3.1.2 Multidimensional scaling of morphological data of *Atriplex hortensis* with their respective populations.

A dendrogram was drawn to show the phenotypic relations among diverse populations based on Euclidean distances from the morphological data matrix (Figure 4.3.1.3). Dendrogram grouped 396 phenotypes into two main clusters A and B. Cluster A represent the phenotype of Aghyam, Hunder, Tangyar, Chiktan, Pakskum, Gonpa, Khardong, Shenam, Murghi, Soad, Udhamaru, and Panamikwhile, while the cluster B represent the phenotype of Khungru, Diskit, and Akchamal. The results of MDS and PCA analysis were similar to the cluster analysis. The populations with high values of morphological characters were found scattered in clusters which

shows high diversity in the population of *A. hortensis* collected from the different geographical region of Ladakh.

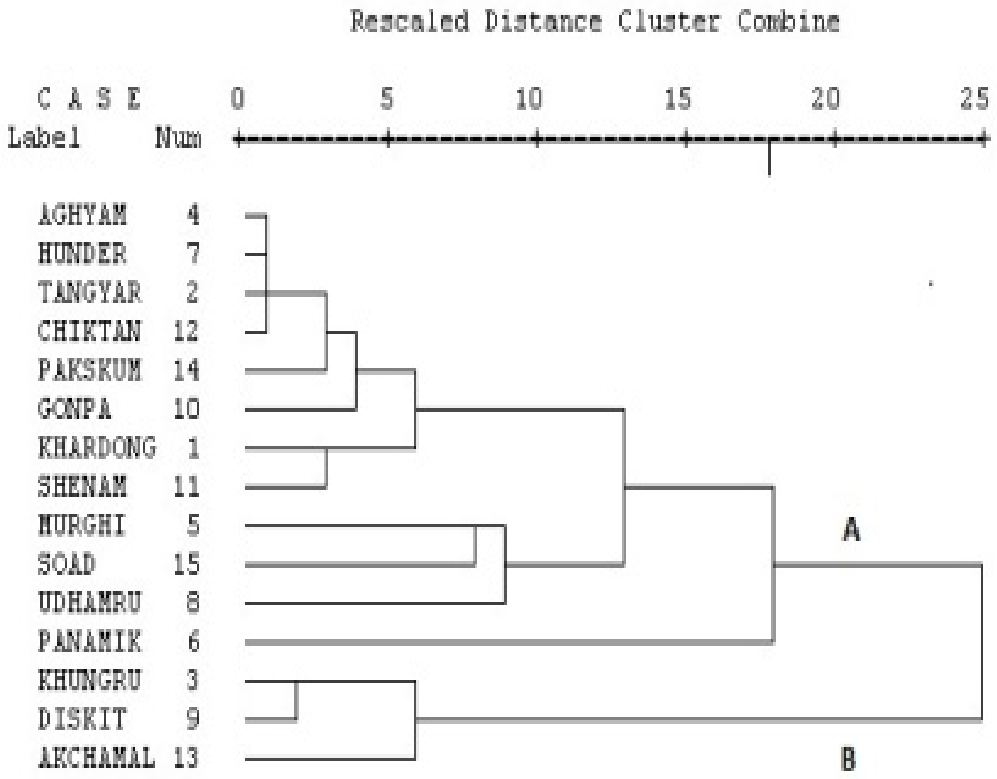

Figure 4.3.1.3 Dendrogram showing the phenetic relationship among 15 populations based on distance from the morphological data matrix. Dendrogram using Average Linkage (Within Group)

4.4 Discussion

Diversity in the plant species plays a major role in the crop improvement program. In the current study, large variations were observed in agro-morphological traits among trans-Himalaya populations of *A. Hortensis*. A similar study also reported by Talamali et al. [110] in the genus Atriplex where variation in floral architecture, vegetative, and fruit morphology in the population was observed. Cluster analysis is based on average linkage between different agro-morphological characters grouped populations in two clusters. High loading agro-morphological characters were found scattered in different clusters. Based on yield data and seed yield genotype from cluster 'A' population (Panamik) and (Murghi) would be better for selecting high yield type for commercial purpose.

Agro-morphological traits of the populations are based on phenotype expression and are influenced by diverse environmental factors [109]. The distinct morphological characters of four populations, Hunder, Udhamru, Chiktan, and Soad were found scattered in different clusters across the phytogeographical regions. Nubra and Indus valley phenotype having potential plant types. For a better understanding of this aspect more accessions representing different phyto-geographical regions especially from Nubra and Indus valley need to be assembled for detailed study on similar lines. Multi-location evaluation of *A. hortensis* populations or genotypes from Hunder, Udmaru, Chiktan and Soad is suggested to identify the potential accessions for cultivation and utilization of parental lines in breeding programs.

4.5 Conclusion

Considerable variation was observed for different morphological traits among the population of *A. hortensis* of Ladakh regions. The distinct morphological characters of four populations viz., Hunder, Udmaru, Chiktan, and Soad were observed scattered in different clusters across the phytogeographical regions. This study would benefit regions like Ladakh (J&K), which remain cut off from the mainland for half of the year and resulting depriving the local people and troops deployed in this area from green vegetables. Multi-location evaluation of selected *A. hortensis* populations or genotypes is suggested to identify the potential accessions for cultivation and utilization of parental lines in breeding programs.

CHAPTER 5

DETERMINATION OF MINERALS COMPOSITION OF INDIGENOUS POPULATIONS OF *ATRIPLEX HORTENSIS* L. IN LADAKH REGION

Abstract

The present study was aimed at estimating the mineral contents and to elucidate the nutritional importance of this traditionally grown vegetable *Atriplex hortensis* L. from Ladakh Region of India. Altogether seventy accessions of five individuals from each population of *Atriplex hortensis* were collected from different regions of Ladakh. The collected accessions (seeds) were sown in Randomized Block Design (RBD) with three replications at the research field of Defence Institute of High Altitude Research (DIHAR,) in the cropping season of the year 2015-2016. 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 version 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 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 significantly correlated to the component first (27% of the diversity); K, Cu and P to the second component (17% of the diversity), and Al and S to component third (15% of the diversity). Altogether 59 % of diversity was accounted for the first three components (PCA) in the study. The study indicated that trans-Himalayan vegetable *A. hortensis* L. is rich in mineral content and hence have considerable nutritional value. This vegetable is naturally adapted to the harsh climate of the Ladakh region, therefore could augment appreciably to the fresh ration requirements of troops and the local population.

5.1 Introduction

Mineral components assume a vital physiological role in humans and plants. The human body needs more than twenty-two minerals that can be provided by a suitable eating habits [110] and the most crucial minerals are Potassium (K), phosphorous (P), calcium (Ca), magnesium (Mg), zinc (Zn), iron (Fe), manganese (Mn) and copper (Cu). Dietary inadequacies in mineral nutrients have noteworthy negative effects, for example, the inability of learning in children, expanded grimness and mortality, low labourer profitability, and high human services costs. The most widely recognized micronutrient inadequacies are of iron (Fe), zinc (Zn), and iodine (I), yet certain population experiences the ill effects of deficiency in magnesium (Mg), calcium (Ca), and selenium (Se). It has been assessed that almost 3.7 billion individuals worldwide are iron (Fe) deficient (60%) out of which 54% have severe inadequacy [19]. There are various reports on studies conducted on the mineral composition of different species of *Atriplex* worldwide viz., [20] [21] [22] [23] [111]. Zinc (Zn) deficiency positions eleventh among the 20 most imperative wholesome inadequacies around the world, and the fifth among the 10 most vital lacks in developing nations [112] [113]. Hotz et al. [114] reported that zinc (Zn) inadequacy influences around 33% of the total population and that its occurrence ranges from 4% to 73% depending upon the Nations. Micronutrient deficiencies mostly result from their low availability in the day to day diet. Most plant nourishments are not adequate to meet daily dietary prerequisites when these foods are consumed in the usual amount. Subsequently, there has been an enthusiasm for expanding the mineral concentration of different crops. Although sustenance supplements were customarily used to treat mineral inadequacies, crop improvement for enhanced micronutrient availability in nourishments advocated as sustainable and long term arrangements.

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 minerals in plants and vegetables, it is equally important to estimate their minerals contents and create the information of their mineral richness [114] [115]. Although vegetables are a rich source of micronutrients and antioxidants, 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 a very high level of elements compared to exotic varieties [116].

Many indigenous vegetables that are grown in remote areas of the Ladakh have not been studied for the mineral element richness. *A. hortensis* L. has been historically used as food and health supplement by various tribes such as Balti, Purik, Bodh, and Dardi, etc [117]. Broad green leaves of this plant have been shown effective in the treatment of gout, indurations, and tumor. Various studies have been conducted on this plant; however data on mineral content on this plant is still scanty. In the present study, the mineral content of 14 different populations of *A. hortensis* L. collected from different sub-regions of Ladakh Region has been determined quantitatively with the hypothesis that different populations of *A. hortensis* growing in Ladakh region may be divergent concerning mineral content. The information generated would be useful to recommend different populations/genotypes of *A. hortensis* for cultivation directly for the enrichment of particular minerals or groups of minerals as well as for the selection of parents in future breeding.

5.2 Materials and methods

5.2.1 Study Area

The experiment was conducted at the Defence Institute of High Altitude Research in Ladakh at an altitude of 3500 meters above mean sea level (m amsl). The soil texture at the experimental site was silty loam and it contained 1.3±0.2% organic carbon and 4.4±0.5% organic matter. The pH of soil was 7.5±0.3 (mean±SD).

5.2.2 Experiment design and sampling

Five accessions each representing a population of *A. hortensis* collected from three valleys viz., Nubra, Indus, and Suru valleys of Ladakh (Figure 5.2.2.1) were used to measure the mineral content of *A. hortensis* leaves. The samples were 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 experimental field during May 2016 in randomized block design for the production of the vegetative part. The plot size was kept $2m^2$ and seeds were sown at a spacing of 15x10 cm. All recommended agronomic practices were followed. The harvest was carried out approximately after 30 to 45 days when the plants were young and tender. Random samples from each set of three leaves from each accession and each replication were combined. The samples were weighed, snap-frozen in liquid nitrogen immediately, and stored at -80ºC until lyophilized.

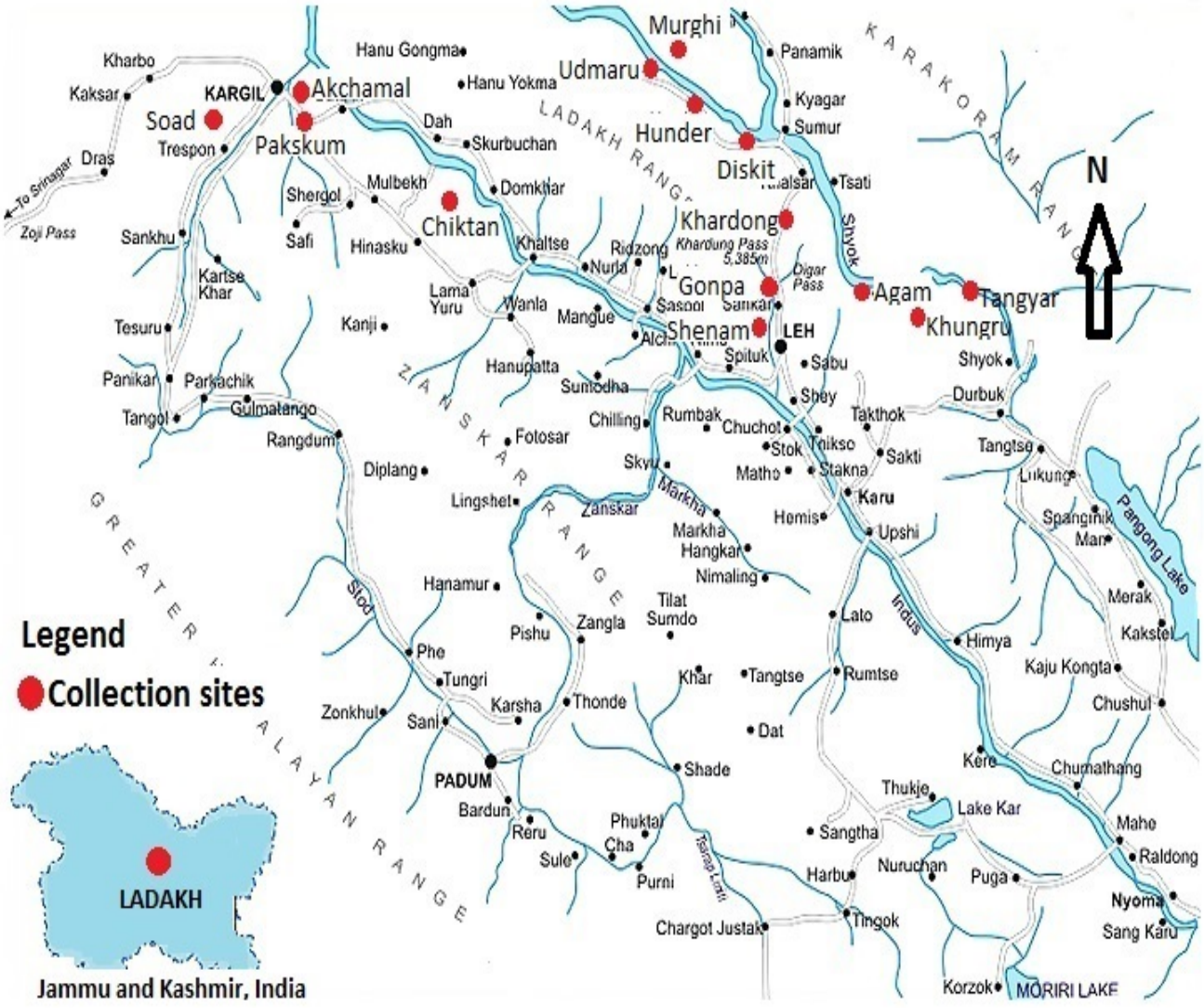

Figure 5.2.2.1 Map of Ladakh region showing the collection sites (marked in red) of *Atriplex hortensis* L. accessions for the present study. (Not to be scaled)

5.2.3 Chemical and reagent

All chemicals and reagents used were of analytical grade. Deionized water (Millipore Water) (RIOs^{TM} type I simplicity, 185 USA) with a resistivity of 18.2MΩ cm and Borosil glassware were used for the preparation of solutions and sample analysis. For mineral analysis, Multi-element calibration standard solution IV (Merck, Darmstadt, Germany) was used. All the glassware and were sterilized before use in experiments.

5.2.4 Sample treatment and sample analysis

Mineralization of all the samples was made by using hot block digester of Questron Technologies Corporation, Canada. In the test tube 0.5mL of samples were taken and 10mL of Nitric acid (70% HNO_3, Merck, Darmstadt, Germany) was added to the test tube. The tubes were left for 30 minutes at room temperature under a fume hood and then heated for 25 min at 70°C temperature. After cooling, 2mL $HCLO_4$ (70%, Merck, Darmstadt, Germany) was added and heated for 25 min at 135°C temperature. After that, 2mL of $HCLO_4$ and 2mL of HNO_3 was added and heated for 25 min at 135°C temperature. Then, 2mL of HNO_3 + 5mL of HCL (37%, Merck, Darmstadt, Germany) were added and the solution was transferred to 50mL falcon tube after cooling. The final volume was adjusted up to a 50mL with distilled water [118]. Thirteen minerals elements were selected for analysis by ICP-OES. Analysis of minerals was done using Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES, Optima 7000DV Perkin Elmer). Multi-element calibration standards were prepared by appropriate dilution of ICP multi-element standard solutions Merck, Darmstadt, Germany. The plasma conditions of ICP-OES method were standardized according to Giri et al. [119] with slight modification.

5.2.5 Data analysis

Analysis of variance (ANOVA) followed by Tukey's test was used to figure out significant differences in different populations of *A hortensis* for mineral content. Genetic diversity among the populations was studied using generalized distance (D2). Principal component analysis (PCA) was used as a correlation matrix to determine the variables containing maximum possible variance with the number of principal components. The similarity coefficient was determined by General Similarity Coefficient that reflects the differences existing among the accessions. All statistical analyses were performed using SPSS version 21.0 (Statistical Program for Social Science, SPSS Corporation, Chicago, Illinois, USA) and XLSTAT, 2017 (Statistical software and data analysis add- on for Excel).

5.3 Results and discussion

The concentration of 12 important mineral elements namely, calcium (Ca), magnesium (Mg), zinc (Zn), iron (Fe), sodium (Na), aluminium (Al), potassium (K), phosphorous (P), sulphur (S), silicon (Si), boron (B), copper (Cu) and selenium (Se) in the leaves of the 70 accessions representing 14 different populations of *A. hortensis*. of the Ladakh region have been shown in Table 5.3.1. Variation in the concentration of mineral elements was found in different populations. Sulphur (S), aluminium (Al), potassium (K), and calcium (Ca) were found in high concentration than the other mineral elements Table 5.3.1. The highest concentration of sulphur (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, the lowest concentration was recorded for Selinium selinium (Se) in the present study. It ranged from 0.004±0.001 -0.089±0.003 mg/g in the studied populations. Furthermore, aluminium Al was also found in considerably high concentrations and ranged between 5.26±0.003 - 12.95±0.006 mg/g in the present study. Among the other elements, potassium (K) and calcium (Ca) were present in considerable concentration and their highest concentration of 1.83±0.002 mg/g and 1.70±0.002 mg/g respectively was recorded in the populations collected from Pakskum.

Overall, there was variation in the concentrations of mineral elements studied in different populations in the present study and found statistically significant. The concentrations of some important mineral elements among different populations varied by 1.5 folds for sulphur S, 2.8 folds for aluminium (Al), 1.5 folds for potassium (K) and 2.4 for calcium (Ca), respectively. Furthermore, taking into consideration the coefficient of variation, the observed variation for micronutrient was 98% for iron (Fe), 94% for boron (B), 30% for zinc (Zn), 10 % for copper (Cu), 54% for selenium (Se), whereas for macronutrients was found to be 24% for magnesium (Mg), 11% for potassium (K), 13% for sodium (Na) and 27% for calcium (Ca) respectively (Table 5.3.1). The above results suggested that overall there was variation in the concentration of mineral elements studied in different populations and found statically significant at $P<0.05$. A high level of variability in Ca, Mg, Zn, Na, K, S, B, Cu ($P\leq0.01$) observed in the collections. This vegetable is rich sources of calcium, magnesium, zinc, sodium, potassium, sulphur, boron

and copper and are present in statistically significant amount and hence could augment to supply the essential mineral requirements of human as per recommended dietary allowance Table 5.3.2.

Table 5.3.1 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±0.0	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	**Paksukum**	**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	**Khung**	1.436±0.0	0.315±0.0	**0.496±0.**	0.716±0.	0.184±0.	7.475±0.	1.250±0.

	ru	02de	02^{h}	**001^{i}**	002^{ab}	002^{a}	003f^{g}	001^{a}
	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

Cont. Table 5.3.1

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	**Paksku m**	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]
	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±SE. Different superscripts indicate significantly difference at $P<0.05$

Table 5.3.2 Recommended dietary allowance comparing with the mineral content in *A. hortensis*

Locations	**Elements**	**Applications**	**mg/g SD**	**RDA**
Pakskum	Calcium	Hormonal regulation of bone formation	1.697±0.002	1000mg/day
	Magnesium	For cell development and other enzymes	0.375±0.001	300mg/day
	Sodium	Regulate blood volume and blood pressure	0.304±0.001	7mg/day
	Potassium	Multiple physiological function like hormonal secretion, blood pressure	1.827±0.002	2800mg/day
Khungru	Zinc	Prenatal and postnatal development in women	0.496±0.001	14.2mg/g
	Boron	Bone and testosterons	0.045±0.001	0.25mg/g
Gonpa	Sulphur	Important for human cell developments	81.433±0.03	850mg/day
Agham	Copper	Key constituent of respiratory enzymes	0.057±0.001	1.9 mg/g

Note: Recommended dietary allowance (RDA) as per World health organisation (WHO), United States Food and Drug Administration (USFDA) 2017.

Pearson correlation analysis (Table 5.3.3) revealed positive correlation among calcium, magnesium, zinc, sodium, potassium, aluminium. Calcium (Ca) shows positive correlation ($P\leq0.01$) with magnesium (Mg), zinc (Zn) and sodium (Na), magnesium (Mg) positive correlated ($P\leq0.01$) with sodium (Na), sodium (Na) positive correlated ($P\leq0.01$) with potassium K and aluminium (Al) positive correlated ($P\leq0.01$) with sulphur (S) and copper (Cu) ($P\leq0.05$), negatively correlated with boron B ($P\leq0.05$).

Table 5.3.3 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

** Significant at the 0.01 (2-tailed)., *. Significant at the 0.05 (2-tailed).

PCA showed that magnesium (Mg), sodium (Na), zinc (Zn), silicon (Si) and calcium (Ca) are highly correlated to first component (27% of the variability), potassium (K), copper (Cu) and phosphorous (P) were correlated to the second component (17% of the variability), and aluminium (Al), sulphur (S) to the third component (15% of the variability) (Figure 5.3.1).

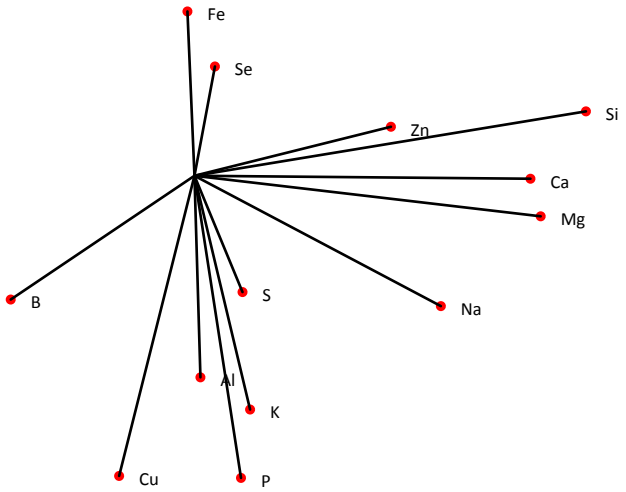

Figure 5.3.1 Principal Component Analysis of the mineral elements in *Atriplex hortensis* L. accessions under the study.

Ladakh is a cold desert region that 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 6 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 at least during the un-favorable season.

Present results have demonstrated that *A. hortensis* L. is found most of the region and is a source of many crucial micros as well as macro nutrients which are critical components of the human food. Although *A. hortensis* L. does not need much input to cultivate as a 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 a vegetable. The present study has advocated that the rich mineral contents of *A. hortensis* L. make it a very suitable vegetable candidate in the entire Ladakh region. It is also recommended that by selecting the populations of Gonpa, Pakskum, Agham and Khungru area representing high level of calcium (Ca), magnesium (Mg), zinc (Zn), sodium (Na), potassium (K), sulphur (S), boron (B), and copper (Cu) can be used to develop an improved variety of this crop and related species through various breeding programs.

Present results indicate the significant variability in the mineral elements concentration in different germplasm collections of the Ladakh region. This variability could be due to genotypic differences in the species because the genotypic difference in legumes has also been found to vary the concentration and content of mineral in the previous studies [119] [120]. A wide literature survey was made, it was found that mineral concentration on genus Atriplex studied by many authors [121] [122] [123] [124] [125] [126] [127] [128] but, there was not a single study found on diversity in mineral concentration in a particular species of Atriplex till date. Therefore, this study is, the first-hand information that is focused on analysis of mineral content of cold desert indigenous vegetable *A. hortensis* from 14 populations showing its diversity in different mineral composition.

5.4 Conclusion

The present study has demonstrated that *A. hortensis* L. is an important vegetable crop that has many essential mineral elements. There is variation in mineral contents in Atriplex populations, it is and rich sources of potassium, phosphorus, calcium, magnesium, selenium, iron and manganese,- hence could augment to cater to the essential mineral requirements of human and animal diet. It is also recommended that populations of Gonpa, Shenam, Pakskum and Khungru area representing high level of sulphur (S), aluminium (Al), iron (Fe), potassium (K) and calcium (Ca) respectively can be used to develop an improved variety of vegetables through breeding program.

CHAPTER 6

ASSESSMENT OF GENETIC DIVERSITY AMONG INDIGENOUS POPULATIONS OF *ATRIPLEX HORTENSIS* L. FROM LADAKH USING RANDOMLY AMPLIFIED POLYMORPHIC DNA (RAPD) MARKERS

Abstract

Sixty-seven percent of genetic variability was observed among populations and 33% genetic diversity was observed within the population of *Atriplex hortensis*. Presence or absence of intense bands was scored from two hundred forty-six bands for all individuals, Out of the 246 bands, 7% were monomorphic and 93% were polymorphic in Atriplex population. Genetic variability analyzed by using RAPD markers showed the highest values of Shannon information index (0.38), Nei's genetic diversity (0.25), and polymorphic loci (87.37%) accessions from population of Tangyar and least values of Nei's genetic diversity (0.11), Shannon information index (0.17) and polymorphic loci (31.35 %) were observed Akchamal population. As a result the Akchakmal population accessions are diverse in a narrow range while, the populations of Tangyar accessions were more diverse.

6.1 Introduction

Existence, characterization and documentation are pre-requisites for the utilization, conservation and management of plant genetic resources. Further, the information about genetic relationships (similarity or divergence) among the potential breeding material of a plant species could be useful in crop improvement strategies such as hybridization programs [129]. The benefits of hybrid vigour or heterosis and recombination of the traits can be exploited to the maximum extent if breeding stocks are diverse and distinct in the phylogenetic tree. Some research reports genus Atriplex contains a high genetic variability. Our finding of this investigation reported in chapter 4 concludes that there is high variability for agro-morphological traits among different populations of *A. hortensis* from the Ladakh region. But most of such findings reported on genetic variability are based on found based on physiological, morphological, and biochemical analysis, and these characters are often affected by environmental factors[130] [131]. Therefore, many times it becomes difficult to examine character or trait variations have genetic base or due to environmental factors etc. More over there is no report on the assessment of genetic diversity and relatedness among different populations of *Atriplex hortensis* using molecular markers at the global level in general as well as from the Ladakh region so far.

The molecular markers offer an alternative to analyse genetic variability and relationships between germplasm of a plant species [132] and recently these new paradigms are routinely being used to study genetic diversity and genetic relationship among breeding stocks, germplasm or cultivars of crop plants. Myriad of molecular markers such as RFLP, SSR, SCAR, AFLP, ISSR, RAPD, SRAP, have been used to evaluate plant genetic diversity and characterization. All such DNA markers have got their own advantages, disadvantages, and pre-requisites depending on the availability of genomic resources viz. submitted sequences of microsatellites, SSR marker primers, already used markers, and sequence of genes or their tags, etc. of the target plant species.

RAPD markers are fairly good markers to study the genetic diversity of plant species for which such genomic resources (in gene databases or literature) are lacking or scarce. RAPD marker has proved useful in various genetic studies [133] [134] [135]. As far as best of our knowledge no such resources are available with respect to *A. hortensis*. Thus considering the limitation for genomic data we used RAPD markers to assess genetic diversity and relationships

among fifteen populations of *A. hortensis*. This study may provide basic information for conservation, management, restoration of genetic resource, and selection of potential parental lines for an improved variety of the Atriplex species through breeding programs.

6.2 Materials and Method

6.2.1 Study sites and sampling

The study was conducted at DIHAR Leh (Defence Institute of High Altitude Research). A total of forty-five locations were visited to cover diverse growing habitats of the *A. hortensis* in the Ladakh region out of which *A. hortensis* was found growing in fifteen sites (Figure 6.2.1.1). Accessions collected from different cites of Ladakh were sown at DIHAR experimental field and fresh seventy-five individuals of young leaves were collected randomly for molecular analysis form the field. Five samples of *A. hortensis* representing each population were taken (Table 6.2.1.1). The fresh leaves were stored at -80^{o} C until it used for analysis.

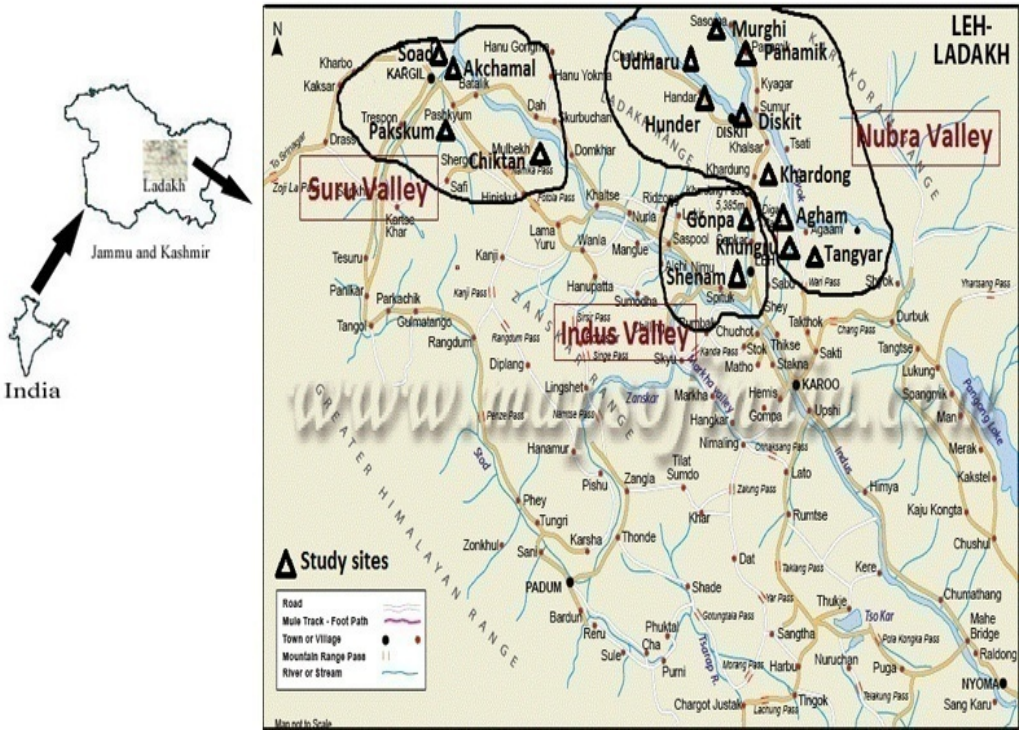

Figure 6.2.1.1 Map of sampling sites of Ladakh region, India

Table 6.2.1.1 Geographical locations and sample sizes of *Atriplex hortensis*

S.No	Population name	Population code	Alt (m amsl)	Longitude (E)	Latitude (N)
1	Khardong	A	4109	077° 39.487	34° 33.822
2	Tangyar	B	3915	077°52.259	34° 15.168
3	Agham	C	3335	077° 49.877	34° 19.706
4	Murghi	D	3201	077° 32.643	34° 45.516
5	Hunder	E	3169	077° 27.688	34° 35.213
6	Udmaru	F	3129	077° 26.180	34° 37.627
7	Diskit	G	3117	077° 32.686	34° 33.210
8	Gonpa	H	3666	077° 35.560	34° 11.026
9	Shenam	I	3477	077° 34.766	34° 09.550
10	Chiktan	J	3252	076°30.432	34°28.166
11	Achakmal	K	2888	076°09.449	34 °33.220
12	Pakskum	L	2881	076°09.443	34 °33.120
13	Soad	M	2860	076 ° 10.305	34 °33.974
14	Panamik	N	3184	077 ° 32.304	34 ° 46.963
15	Khungru	O	3623	077 ° 26.556	34 ° 17.003

6.2.2 DNA Extraction and PCR amplification

6.2.2.1 RAPD analysis

Total genomic DNA of *A. hortensis* was extracted by using the protocol of Cetyl-trimethyl ammonium bromide with minor modifications using young and tender leaves [137]. The concentration of extracted DNA was determined by NanoDrop. A set of 13 random primers (Xcelris Genomic Primex) belonging to A, B, C, D, and E series was used for PCR amplification these primers were already reported to show amplification in genus *Atriplex halimus* by Dorda et al. [138]. RAPD-PCR was performed by using the protocol of Williams *et al.* [139], with slight modification for optimization. Amplifications were done in BIO RAD C 1000 Touch™ 96 wells thermal cycler, each consisting of 94 ° C de-naturation step for 1 min, 36 ° C, annealing

step for 2 min, and a 72 ^{o}C extension step for 1min. Electrophoresis was conducted on amplified products using 1% gel and run at constant 80 Volt in 1x TAE for 3 hours, and then analyzed by staining with ethidium bromide 0.5 μg ml^{-1}. Documentation was done after electrophoresis on a gel documentation system (Alpha-InfoTech, Alpha imager, U.S.A).

6.2.3 Data analysis

The presence (1) and absence (0) of binary data was examined by population genetic software (POPGENE) version 1.31 [140]. The similarity matrix was subjected to cluster analysis by UPGMA [141]. Nei's genetic diversity (H), Shannon's information index (I), and the percentage of polymorphic loci (PPL), were calculated [142]. The analysis of molecular variation, AMOVA [143] was also carried out using squared Euclidean distances for all samples to divide the variability in to two levels of hierarchical population and the individuals.

6.3 Results

Two hundred forty-six bands form seventy-five *A. hortensis* individuals was produced by using 13 RAPD primers with an average of 18.92 bands per primer (Table 6.3.1). The size of the bands ranged from 265 to 2345 base pairs. Out of 246 bands 229 (93%) were polymorphic and 16 (7%) were mono-morphic. The maximum Nei's (I) genetic diversity (0.26), Shannon information index (H) (0.38) and polymorphic loci (PPL) (87.37%) was found for accessions from Tangyar population and lowest values of genetic diversity Nei's (I) (0.11), Shannon information index (H) (0.17) and polymorphic loci (PPL) (31.35 %) in the case of the Akchamal population (Table 6.3.2). Nei's. [144] classified the levels of genetic diversity at less than 0.05 as low, between 0.05 and 0.15 as medium and greater than 0.15 as high. As a result, the Akchakmal accessions were diverse in a narrow range and the Tangyar accessions were more diverse in nature.

Table 6.3.1 Thirteen primers sequences, with scorable number of polymorphic and amplified bands

Primer	Sequence	Amplified bands	Polymorphic bands
OPA02	TGCCGAGCTG	18	17
OPA05	AGGGGTCTTG	17	17
OPA09	GGGTAACGCC	15	14
OPB01	GTTTCGCTCC	20	19
OPB03	CATCCCCCTG	19	18
OPB06	TGCTCTGCCC	17	15
OPC07	GTCCCGACGA	24	22
OPC08	TGGACCGGTG	19	18
OPC15	GACGGATCAG	16	16
OPD08	GTGTGCCCCA	18	17
OPD11	AGCGCCATTG	21	19
OPD15	CATCCGTGCT	29	24
OPE12	TTATCGCCCC	13	13
Range		13-29	13-22
Mean		18.92	17.62
Total		246	229

Table 6.3.2 Estimate of genetic diversity statistics for all RAPD loci of the Atriplex accessions.

Markers	**Sites**	**Size of Sample**	**Nei's Genetic diversity H (mean + SD)**	**Shannon index I (mean + SD)**	**%age of polymorphic loci**
	Khardong	5	0.12 ± 0.17	0.18 ± 0.26	36.72
	Tangyar	5	**0.26 ± 0.17**	**0.38± 0.22**	**87.37**
	Agham	5	0.16 ± 0.18	0.24 ± 0.27	51.41
	Murghi	5	0.19 ± 0.17	0.29 ± 0.26	61.58
	Hunder	5	0.22 ± 0.19	0.36 ± 0.27	68.36
	Udmaru	5	0.20 ± 0.18	0.31 ± 0.26	62.15
	Diskit	5	0.20 ± 0.18	0.31 ± 0.27	62.71
	Gonpa	5	0.17 ± 0.18	0.26 ± 0.26	54.80
RAPD	Shenam	5	0.13 ± 0.18	0.20 ± 0.27	37.29
	Chiktan	5	0.13 ± 0.19	0.20± 0.27	36.72
	Achakmal	5	**0.11 ± 0.16**	**0.17 ± 0.16**	**31.35**
	Pakskum	5	0.22± 0.18	0.34 ± 0. 21	72.41
	Soad	5	0.17 ± 0.19	0.25 ± 0.28	52.42
	Panamik	5	0.20± 0.18	0.30 ± 0.27	62.59
	Khungru	5	0.12 ± 0.17	0.19 ± 0.26	36.28
Mean			**0.17**	**0.28**	**-**

A dendrogram depicting genetic relatedness among different accession of *A. hortensis* based on similarity calculated using UPGMA method from on RAPD data is shown in figure 6.3.1. On perusal of the dendrogram it is evident that there are three main distinct sub-clusters marked as I, II, and III. The sub-cluster I comprises the accessions of populations from Agyam, Hunder, Tangyar, and Chiktan. Sub-cluster II comprises accessions of the population from Pakskum, Gonpa, Khardong, Shenam, Murghi, Soad, Udmaru, Panamik, and Khungru. Whereas accessions from populations sub-cluster Diskit, Akchamal, and some accessions of Khungru forms sub cluster III. It can also be seen that though each sub cluster comprised of accessions

from 2-4 populations, but within each sub cluster the accession from the same population are placed more closely forming an ordered arrangement instead of random distribution. This indicates that there is a noteworthy separation of *A. hortensis* populations, hence variations among the populations of *A. hortensis has a genetic basis*.

Results obtained from AMOVA analysis, also show there were highly significant genetic differences (P <0.001) among the population of *A. hortensis*. 67 % percent of genetic variability was observed among the population and 33 % percent observed within populations (Table 6.3.3, figure 6.3.2).

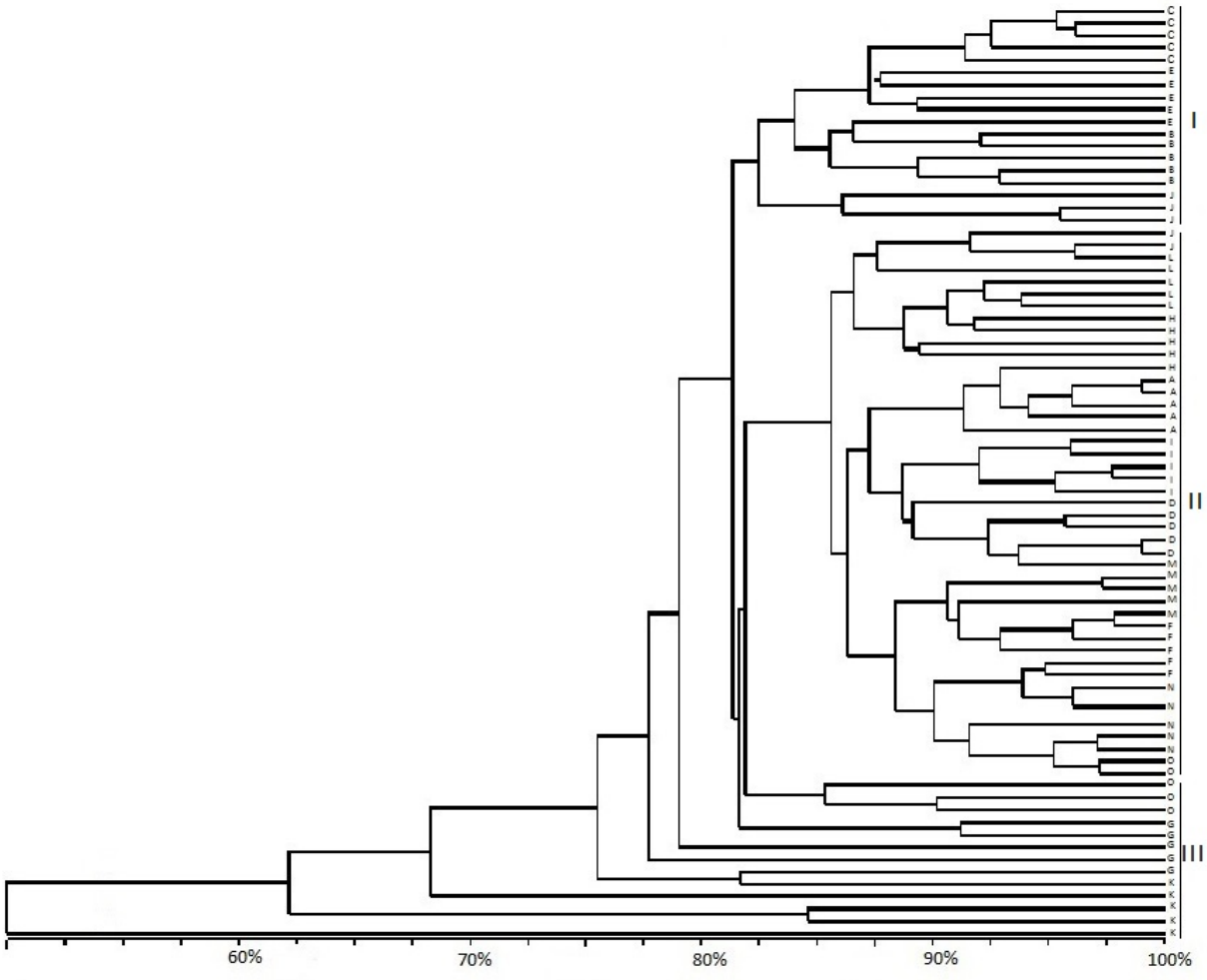

Figure 6.3.1 Dendrogram demonstrated the genetic relationship of 75 *A. hortensis* individuals

Table 6.3.3 AMOVA analysis of *A. hortensis* populations.

Variation	Among populations	Within population	Total
d.f.	14	60	74
Sum of square deviation	46.91	34.00	80.92
Variance	0.52	0.39	0.91
Percentage	67%	33%	100 %
P-value	< 0.001	< 0.001	< 0.001

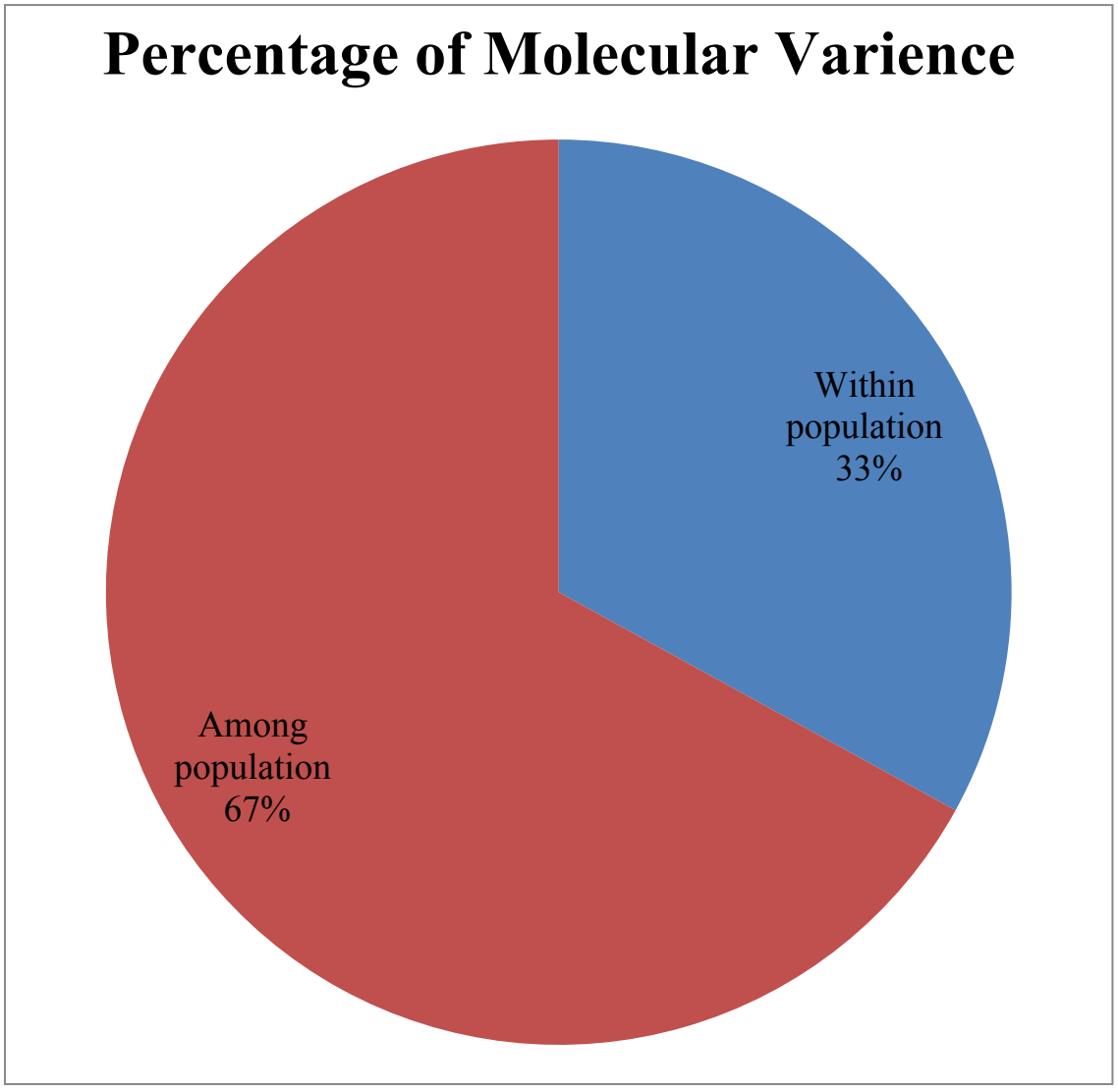

Figure 6.3.2 Total genetic variation analysis using AMOVA in *A. hortensis* from RAPD data

6.4 Discussion

According to Gitzendanner et al. [145], there is a strong relationship between genetic diversity and geographic locations. Mostly the terrestrial annual herb populations are minute and spatially isolated. *A. hortensis* is a terrestrial herb and population of such species, affected by a number of evolutionary factors like gene flow, mating system, random genetic drift, founder effect, and dispersal of seed [146]. AMOVA based on RAPD data shows less genetic variability within populations and high genetic variations among populations of *A. hortensis.* The UPGMA dendrogram proposes that flow of gene is restricted because individuals from the same population clustered together. This could be due to the monoecious character of genus *Atriplex* [147]. In a previous study, we have reported high diversity in *A. hortensis* based on morphological traits as well [3]. So the present work confirms that variation in traits of *A. hortensis* have a genetic basis. Other studies also reported diversity in genus Atriplex using RAPD markers, Bouda et al. [50] studied genetic diversity of eight species of genus Ariplex using RAPD markers, results shows that 42% genetic diversity in the genus Atriplex. Assessment of genetic diversity is pre-requisite for the establishment of appropriate conservation strategies and current breeding programs of plant species. This study is the first-hand information on genetic diversity in *A. hortensis* population in the Ladakh region based on of molecular markers. The present work provides the base line information regarding the genetic diversity in *A. hortensis* which may be helpful for further molecular characterization of the species.

6.5 Conclusion

One the basis of data obtained and its analysis it is concluded there exist high genetic variations among different populations of *A. hortensis* in the Ladakh region and these differences in population have a genetic basis. Therefore, every population of Atriplex deserves specific conservation attention as genetic variation is a prerequisite for good germplasm conservation and useful in breeding programs. This investigation also indicates that RAPD markers analysis was sufficiently informative and powerful to detect genetic variability in indigenous populations of *A. hortensis.*

SUMMRARY

Screening of selected potential indigenous leafy vegetables of cold desert-Ladakh on the basis of yield, earliness and consumer acceptance

The high mountain Ladakh cut off for over 6 months every year as a result of heavy snowfall in winter season and crops can be grown in this region during summer season only. The limited indigenous production of fresh vegetable and difficulties in transportation due to geographic, harsh climatic conditions result in short fall in supply of fresh vegetables to local population and deployed army in this region. Meeting the increasing requirements of fresh vegetable in this isolated high elevated mountainous area is a difficult challenge. The indigenous plants do not require intensive care and can grow in less fertile soil. Besides, these withstand harsh climatic conditions due to its abiotic and biotic stress tolerance. There is a need to identify indigenous plants species, characterize their germplasm and develop cultivation package and practices. We surveyed the Ladakh region and selected seven plants species which are frequently used as vegetables. Further these seven indigenous leafy vegetables (ILV) were evaluated for their potential for yield, early harvesting stage for consumption and conducted sensory evaluation for consumer acceptance. Among all the seven ILV the highest yield (1.80±0.06 kg/m2), minimum days to harvest and highest hedonic points was observed in *A. hortensis.* The study suggested that *A. hortensis* has high yield high early harvesting stage and consumer preference. As a result, detail studies on *A. hortensis* are required and research and extension work is needed for large scale cultivation of the species.

Agro-morphological characterization in *Atriplex hortensis* L. an indigenous vegetable

To extend the idea of characterizing indigenous plant species as vegetable crops in Ladakh region, *Atriplex hortensis* was selected for further studies. *Atriplex hortensis* L. member of family chenopodaceae, locally known as *Phaltora* in Ladakh region It is the first green herb to appear after prolonged winter in Ladakh and used as leafy indigenous vegetable. In traditional medicine it is used as health energizer helps in nutrition absorption, enhance metabolism and digestion.

Crop augmentation mostly depends on accessibility of genetic variation in the plant species and its effective utilization to increase yield by selecting seeds from desirable populations.

Characterization of genetic diversity is pre requisite for selection and starting a breeding program for the improvement of the crop. A total of 132 accessions of indigenous vegetable *Atriplex hortensis* L. collected from 15 diverse geographical locations of Ladakh region were evaluated for agro-morphologically. Considerable variation was observed for different morphological traits among population of *A. hortensis* of Ladakh regions. Principal componentnanalysis (PCA), multivariate analysis, multidimensional scaling (MDS) and group analysis were carried out using agro-morphological data. The distinct morphological characters of four populations viz., Hunder, Udmaru, Chiktan, and Soad were observed scattered in different clusters across the phyto-geographical regions. More than 60% diversity among population could be attributed to first two principal components. Multi-location evaluation of selected *A. hortensis* populations or genotypes is suggested to identify the potential accessions for cultivation and utilization of parental lines in breeding programs.

Determination of minerals composition in *Atriplex hortensis* L. an indigenous vegetable

Mineral components assume vital physiological role in human and plants. The most widely recognized micronutrient inadequacies in human beings are of iron (Fe), zinc (Zn), and iodine (I), Micronutrient deficiencies mostly result from their low availability in the daily diet. Crop improvement for enhanced micronutrient availability in nourishments is advocated as sustainable and long term arrangements. Many indigenous vegetables that are grown in remote areas of the Ladakh have not been studied for the mineral element richness. *A. hortensis*. has been historically used as food and health supplement by various tribes such as balti, purik, bodh, and dardi etc. Various studies have been conducted on this plant; however data on mineral content on this plant is still scanty. The mineral content of 14 different populations of *A. hortensis*. collected from different sub-regions of Ladakh Region has been determined quantitatively with the hypothesis that different population of *A. hortensis* growing in Ladakh region may be divergent with respect to mineral content. The information generated would be useful to recommend different populations/genotypes of *A. hortensis* for cultivation directly for enrichment of particular minerals or groups of mineral as well as for selection of parents in future breeding. There is variation in mineral contents in Atriplex populations, it is and rich sources of potassium, phosphorus, calcium, magnesium, selenium, iron and manganese, hence could augment to cater the essential mineral requirements of human and animal diet. It is also recommended that populations of Gonpa, Shenam, Pakskum and Khungru area representing high level of sulphur (S), aluminium (Al), iron (Fe),

potassium (K) and calcium (Ca) respectively can be used to develop improved variety of vegetables through breeding program.

Genetic diversity of *Atriplex hortensis* L. population in trans-Himalaya of Ladakh investigated by RAPD markers

Existence, characterization and documentation are pre-requisites for the utilization, conservation and managing genetic resources of plants. Further the information about genetic relationships (similarity or divergence) among the potential breeding material of a plant species could be useful in crop improvement strategies such as hybridization programs**.** There are no details on evaluation of genetic diversity and relatedness on different population of *A. hortensis* using molecular markers at global level in general as well as from Ladakh region so for. RAPD markers are fairly good markers to study genetic diversity of plant species for which such genomic resources (in gene databases or literature) are lacking or scarce. We used R A P D molecular markers to measure genetic variation and relationships among of fifteen populations of *A. hortensis*. Sixty seven percent of genetic variability was observed among populations and 33% genetic diversity was observed within population of Atriplex hortensis. Therefore, every population of *A. hortensis* deserve precise preservation attention as genetic variation is prerequisite for good germplasm conservation and useful in breeding programs. The highest values of shannoninformation index (0.38), Nei's geneticdiversity (0.25), and polymorphic loci (87.37%) accessions from population of Tangyar and least values of Nei's geneticdiversity (0.11), Shannoninformation index (0.17) and polymorphic loci (31.35 %) were observed Akchamal population. As a result the Akchakmal population accessions are diverse in constrict range while, populations of Tangyar accessions were high diverse in nature. This investigation also indicates that RAPD markers analysis was satisfactorily helpful and potent to detect genetic diversity in indigenous population of *A. hortensis.*

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PUBLICATIONS IN PEER-REVIEWED JOURNALS

[1] **Tsewang rinchen**, Narendra singh, Samar Bhadur Maurya and Anil Kant. 2019. Mineral content estimation in *Atriplex hortensis* L. an indigenous vegetable of Trans- Himalayan region of Ladakh, India. Research on Crops. 20 (1) : 135-140.

[2] **Tsewang Rinchen**, Narendra Singh, and Samar Bahadur Maurya. 2018. Yield and Palatability of Potential Indigenous Leafy Vegetable of Cold Arid trans-Himalayan Ladakh, India. Defence Life science Journal. 3 (2) 136-139.

[2] **Tsewang Rinchen**, Narendra Singh, Samar Bahadur Maurya, Vikas Soni, Manisha Phour and Bhuvnesh Kumar 2017. Morphological characterization of indigenous vegetable (*Atriplex hortensis* L.) from trans- Himalayan region of Ladakh (Jammu and Kashmir), India. Australian Journal of crop Science. 11(03):258-263.

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[13] Jagdish singh Arya, Narendra Singh, **Tsewang Rinchen** and Samar Bhadur Mouria. 2014. Genotypes, geographical regions and solvents dependent antioxidant activity of *Rumex patientia* L. in cold desert of trans-Himalaya Ladakh, India. Australian Journal of Crop Science. 9(2), 98-104**.**

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Production: A Review. Journal of Agriculture and Veterinary Science.9 (12): pp 90-96.

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1. **Tsewang Rinchen**, Narendra Singh and SB Maurya 2016. Organised by Indian Society of Plant Genetic resource (ISPGR) and Biodiversity international, at National Agriculture Science Complex Pusa New Delhi, Oral Presentstion on *'Phytodiversity and its Conservation Strategies in Permafrost Conditions at Extreme Altitude of Trans Himalaya in Leh Ladakh (India). 1st International Agro-biodiversity congress*.

WORKSHOPS

1. Ten days workshop on **"*Biosystematics of Species Complex*"** organized by Centre for Environment Management of Degraded Ecosystem (CEMDE), University of Delhi, Delhi, and Sponsored by Ministry of Environment & Forests, Govt. of India, New Delhi from February 02-11, 2012 at CEMDE, University of Delhi, Delhi.
2. Seven days training course on **"*Classical and Modern methods in Plant Systematics*"** organised by CSIR-National Botanical Research Institute, Lucknow-226001