Morphological and Molecular Analysis of Intervarietal Variability in Water Chestnut (*Trapa natans* var. *bispinosa* Roxb.) in Central Uttar Pradesh

THESIS

SUBMITTED TO THE
BABASAHEB BHIMRAO AMBEDKAR UNIVERSITY, LUCKNOW

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2020

DEDICATED

To My

Beloved Parents

&

Teachers

Munni . . . .

CERTIFICATE

This is to certify that the thesis entitled **"Morphological and Molecular Analysis of Intervarietal Variability in Water Chestnut (*Trapa natans* var. *bispinosa* Roxb.) in Central Uttar Pradesh"** submitted by **Ms. Munni Goud, Enrolment No. 273/13** is an original research work and has not been previously submitted in part or full for the award of any other degree or diploma to this or any other University.

The thesis submitted to Babasaheb Bhimrao Ambedkar University Lucknow satisfies all the requirements as stipulated in the Doctor of Philosophy (Ph.D.) Regulations, 1999 as amended in 2008/2010/2013 and it is fit for submission and evaluation for the award of the degree of Doctor of Philosophy of the University.

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DECLARATION

I, **Munni Gond, Enrolment No. 273/13,** hereby declare that, I am a candidate for the Degree of **Doctor of Philosophy in Horticulture,** Department of Applied Plant Science (Horticulture), School for Biosciences and Biotechnology, Babasaheb Bhimrao Ambedkar University (A Central University), Vidya Vihar, Rae Bareli Road, Lucknow-226025 (U.P.), India and have carried out my research work entitled **"Morphological and Molecular Analysis of Intervarietal Variability in Water Chestnut (*Trapa natans* var. *bispinosa* Roxb.) in Central Uttar Pradesh"**. The thesis has been submitted for the award of the degree of Doctor of Philosophy in Horticulture and is my original research work.

I do also hereby undertake that the thesis is essentially free from any kinds of plagiarism.

Munni Gond

Date: 24-02-2021 **(Munni Gond)**

Place: Lucknow **Research Scholar**

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List of Abbreviations

cm	:	Centimeter
CD	:	Critical Difference
C.R.D.	:	Complete Randomized Design
cv.	:	Cultivar
d.f.	:	Degree of Freedom
et al	:	Et Alii
g	:	Gram
Kg.	:	Kilogram
MT	:	Metrication
ha	:	Hectare
i.e.	:	That is
ml	:	Millilitre
µ	:	Micron
pH	:	Puissance de Hydrogen
R. H.	:	Relative Humidity
S.E	:	Standard error of mean
S.S	:	Sum of Square
Viz	:	Videlicet
%	:	Percent
PCV	:	Phenotypic coefficient of variatio
GCV	:	Genotypic coefficient of variatio
h^2	:	Heritability
GA	:	Genetic advance
GAM	:	Genetic advance as percent of mean
CV	:	Coefficient of variation
SEM	:	Scanning Electron Microscopy
AEZ	:	Agri-export Zone
ddH_2O	:	Double distilled water
SDS-PAGE	:	Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis
SDS	:	Sodium Dodecyl Sulphate

Chapter -I

INTRODUCTION

Water chestnut (*Trapa natans* var. *bispinosa* Roxb.) belonging to family Trapaceae is an annual, aquatic, herbaceous and angiospermic nut crop, popularly known as *Singhara* or paniphal in India (Karmakar *et al*., 2011). It is a free-floating plant grown in shallow water fields, ponds or swampy lands contain with a pH range of 6.7 to 8.2 an alkalinity of 12 to 128 mg/l of calcium carbonate (Anonymous, 2003 and Bhatiwal *et al*., 2012) in rainy season (June to July) for its fresh, edible and valuable fruits, considered a minor crop because of its low yield and lack of suitable cultivation techniques since there is paucity of information on its cultivation and performance (Arima *et al.,* 1999). Yet it is an alternative crop since it has potential for cultivation in waterlogged wastelands (Dwivedi *et al*., 2011a). It is utilised as a fresh fruit where mature seeds are eaten boiled or roasted. Dried fruits are ground into fine flour, used as a substitute for cereals in India, especially during certain religious ceremonies and used also to make colours, powder and dye (Hoque *et al.,* 2001). It has two forms, one is red (leaf, petiole and fruit) and the other is green (leaf, petiole and fruit) each bearing fruit large in size having two dull spines (Faruk *et al.,* 2012) while elsewhere, they are categorised by O'Neill (2006) into three types: completely green, completely red and green blended with red.

Water chestnut is distributed ubiquitously in tropical, subtropical as well as temperate zones of the world such as Pakistan, Sri Lanka, Indonesia, Africa, South- Eastern countries of Asia and U.S.A. Currently it is also cultivated in India, Japan, Taiwan, China, Thailand as well as Australia. In India, it is extensively cultivated in Uttar Pradesh, Madhya Pradesh, Bihar, Tamilnadu, West Bengal, Assam, Orissa and Jammu and Kashmir, where high rainfall is conducive to successful cultivation (Chakor, 1974; Little, 1979; Mazumdar, 1985 and Bhatiwal *et al.,* 2012).

The fruits of water chestnut are very delicious and contain carbohydrates, proteins, vitamin and essential minerals (Agrawal and Ram, 1995 and Singh *et al.,* 2010) and are also said to have cancer-preventing properties and used medicinally to treat poisonous animal bites and amoebic dysentery. The rind of the fruit has been discovered to have antibacterial activity and is primarily effective against gram negative bacteria (Parekh and Chanda, 2007). Traditionally the plant has been used in India for several important medicinal purposes such as nutritive, appetizer, astringent, diuretic, aphrodisiac, cooling,

antidiarrheal and tonic (Prajapati *et al.,* 2003) and also useful in lumbago, sore throat, bilious affections, bronchitis, fatigues and inflammation (Karmakar *et al.,* 2011). Its fruits are also used in making liniments for the cure of rheumatism, sores and sunburn and stem used in the form of juice in eye disorders and ophthalmic preparations also consonance to several workers (Nadkarni, 1994; Rahman *et al*., 2001; Anjaria *et al.,* 2002; Anonymous, 2003 and Kirtikar and Basu, 2006). The medicinal values of the whole herb and fruit have long been recognized in folklore medicine as a cure for various diseases (Rahman *et al.,* 2001) and the dried kernels of its fruits are recommended for use in bleeding disorders, threatened abortion, dysuria, polyuria and oedema (Rahman *et al*., 2000; Khare, 2007 and Song *et al*., 2007). Previous researchers have reported analgesic and psychopharmacological activities of its roots (Agrahari *et al.,* 2010 and Panda *et al*., 2010), antibacterial and antifungal activity of its fruit peel (Parekh and Chanda, 2007 and 2008) and also a drug of good reputation in Yunani and Ayurvedic medicine in Indian subcontinent (Razvy *et al.,* 2011). Despite this, it is highly neglected and no standard cultivars or agronomic practices have been developed for the crop.

Water chestnut has a flexuous ascending stem and green photosynthetic submerged root, leaving simple alteration, crowded at the upper part of the stem in rosettes, rhomboidal, apex triangular, irregular inciso–serrate in the upper part a dark green above, radish purple beneath, petioles dilated near the apex into a large spongy float, flowers white, opening above the surface of water in the afternoon, axillary and solitary. Fruits are ovoid bony, angular with short conical beak in the centre at the apex and a spreading, flattened, very sharp spinous horn on the other side, indehiscent, one seeded, seeds while starchy (Prajapati *et al.,* 2003).

Suriyagoda *et al.* (2007), Babu *et al.* (2011) and Deb *et al*. (2013) based on a survey in water chestnut germplasm reported wide variability in the performance of plant and fruit morphology in India as also other countries such as Japan, China, Sri Lanka etc. Variability in germplasm has also been reported in other horticultural crops like mango (Begam *et al.,* 2014a) and in Salparni (Manivel *et al.,* 2019). Morphological analysis of inter varietal variability based on 17 fruit characters detected prominent variation in the landraces ‘Banganapalli’, ‘Langra’, and ‘Dashehri’ and some variation in the cultivar ‘Mallika’ (Singh, *et al*., 2009). However, number of these traits is limited, they are unstable and they are not always able to distinguish between closely related accessions or cultivars.

Germplasm identified by the characteristics combination of properties such as plant architecture, fruit size, colour, taste, flavour etc. (Pandit *et al.,* 2007) in mango and some characteristics like fruit physico-chemical properties (Swamy *et al.,* 2017) in jamun. Similarly genetic diversity earlier studies in banana (Kuddu *et al.,* 2018), even in fennel on the basis of these morphological characteristic such as number of primary branches, number of umbels per plant, number of umbellate per umbel, number of seed per umbellate, test weight (g) and seed yield (Kumar *et al.,* 2017).

This diversity of characters, with a continuous variation in each one, creates extreme complexity in the identification and classification of the morphotypes. Although, morphological and molecular diversity analysis have helped in significantly in morphotypes identification in water chestnut (Hoque *et al*., 2005; Kim *et al.,* 2010 and Li *et al.,* 2017), in mango (Ravisankar *et al.,* 2000 and Karihaloo *et al.,* 2003) and in banana (Sawant *et al.,* 2016 and Das *et al.,* 2018). Intervarietal variability has been characterized based on morphological traits and genetic markers in fruit crops (De Souza and Lima, 2004; Diaz-Matallana *et al*., 2009 and Rocha *et al*., 2012) in mango and (Ahmed *et al.,* 2017) in citrus. Thus, morphological characterization is traditionally the most common method used. Until recently, morphology based methods have been used for the characterization of intervarietal variability in water chestnut (Babu *et al.,* 2011; Babu and Dwivedi, 2012), in Barahal (Shukla *et al.,* 2008) and in mango (Singh *et al*., 2009) which has been reported even in water chestnut in respect of fruit shape, colour, shape and size of the leaves and other characteristics, variability in fruit biochemical parameters and its capacity for bioaccumulation of heavy metals in water chestnut (Arima *et al.,* 1999; Dwivedi *et al*., 2011a; Babu and Dwivedi, 2012); in Barhal (Shukla *et al.,* 2008 and Dwivedi *et al*., 2011b) and also in Ber (Shukla *et al.,* 2012). The prime advantage of morphological traits is simple and rapid, inexpensive assay techniques. However, morphological characters may be controlled by epistatic and pleiotropic gene affects which affects heritability and they may be prone to error due to environmental effects, influences may affect expression of these characteristics because of which they suffer from lack of decisiveness in fruit crops (Begum *et al.,* 2014b; Anu *et al.,* 2015 and Kishor *et al*., 2019). Therefore, morphological characters may not adequately represent the genetic heterogeneity among accessions of a morphotypes. Hence, characterization of intervarietal heterogeneity based on morphological traits needs complementation with scanning electron microscopy and molecular.

Stomata are any of the minute pores in the epidermis of the leaf or stem of a plant, forming a slight of variable width which allows movement of gases in and out of the intercellular spaces which is measured by Scanning Electron Microscopy (SEM). Generally, stomatal initiation is controlled by both environmental and genetic factors (Casson and Hetherington, 2010). Stomatal characteristics (i.e., size and density) are highly variable depending on the genetic background of the plants as well as on the growth conditions or the leaf ontogeny (Masarovicova, 1991) in oak leaves. Stomatal density has been shown to vary significantly within individuals, cultivars or ecotypes of a single species, (Afas *et al.,* 2006) as well as within a community (Jones, 1992). Within the *Populus* genus, a wide interspecific as well as inter clonal variation in stomatal density, dimension and stomatal index has already been observed (Pallardy and Kozlowski, 1979 and Ferris *et al.,* 2002) as also in wheat (Shahinnia *et al*., 2016).

Molecular markers have diverse application in crop improvement, particularly in the areas of genetic diversity and varietal identification studies, gene tagging, disease diagnostic, pedigree analysis, hybrid detection, sex differentiation and marker assisted selection. DNA marker can be used to diagnose the presence of the gene without having to wait for gene effect to be seen in apple (Botez *et al*., 2009) and in pears (Sisko *et al*., 2009). However, Li *et al.* (2017) detected that in water chestnut the genetic relationship and diversity among three common *Trapa species* in the area were evaluated using amplified fragment length polymorphism (AFLP) markers.

Plant gene pools are reservoirs of variations, which provide the raw material for crop improvement. Samples in the form of seeds representing the spectrum of genetic variation within cultivated species and their wild relatives. Each variety or a group of varieties exhibits characteristic banding pattern. On the basis of these patterns they can be identified accordingly. Electrophoretic patterns of the protein fractions are directly related to the genetic background of the proteins and can be used to certify the genetic makeup. SDS-PAGE is increasingly used to describe the genetic structure of crop germplasm identification (Asghar *et al.,* 2004). SDS-PAGE is an economical, simple and extensively used technique for describing the seed protein diversity of crop germplasm (Fufa *et al*., 2005). Furthermore, seed proteins, used as genetic markers convey greater precision to measures of genetic diversity because they are the primary products of structural genes (Srivalli *et al*., 1999). Protein markers are widely used to reveal seed

protein and isozyme variation. They operate at the gene product level where the environment has very little influence (Manikandan *et al.,* 2012).

Considering immense potential of this crop for cultivation in the water logged areas and is fast emerging as an alternative fruit crop although it is still unexploited (Babu and Dwivedi, 2012b).There is a need for its improvement and to develop varieties suited to specific agro-ecological conditions and also for specific end use. A thorough knowledge regarding the amount of genetic variability existing for various characters is essential for initiating the crop improvement program. Development of high yielding varieties and improvement of fruit quality depend on genotypic worth of gene pool. Thus, systematic studies to document variability in this germplasm for its conservation become imperative although they are still lacking (Babu *et al.,* 2011) and character to be standardized for selection of the crop for further breeding programme. Species identification is not only a primary requirement for the breeding of these plants, but also the foundation for detecting any emerging hybrids in the natural habitat (Hoque *et al.,* 2009). There are difficulties, however, with the identification and analysis of hybridization among the species of water chestnut. These taxonomic difficulties are due to the limited number of diagnostic characters. For example, fruit size and the number of horns have been mainly used as the diagnostic characters (Kadono, 1987 and Choi *et al.*, 2000).

Keeping the view of the above facts, after an exhaustive literature search, the present study entitled, "**Morphological and Molecular Analysis of Intervarietal Variability in Water Chestnut (*Trapa natans* var. *bispinosa* Roxb.) in Central Uttar Pradesh"** was carried out at Department of Applied Plant Science, Babasaheb Bhimrao Ambedkar University Lucknow, under taken with the following objectives.

OBJECTIVE

1. Survey and collection of germplasm of water chestnut from the various blocks of district Lucknow.
2. To evaluate the intervarietal variability in water chestnut on the basis of botanical descriptors i.e. root, leaf, stem, flower and fruits.
3. To establish intervarietal variability in water chestnut on the basis of physico-chemical characteristics of the fruits.
4. Molecular characterization of intervarietal variability in water chestnut.

Chapter -II

REVIEW OF LITERATURE

An attempt has been made in this chapter to critically examine the available research findings pertaining to the topic of study and review briefly the salient features pertaining to the present investigation in a summarized and classified manner, so as to use it for designing the experiments and to understand and discuss the results obtained in the present study in the context of background literature.

Water chestnut *(Trapa natans* var. *bispinosa* Roxb.*)* is an aggressive, annual, aquatic plant, belonging to the family Trapaceae, dicotyledonous herb with a floating rosette of leaves around a central stem (Ding and Blossey, 2005). It is best grown in shallow, nutrient rich lakes, rivers and ponds. It is generally found in waters with a pH range of 6.7 to 8.2 and alkalinity of 12 to 128 mg/L of calcium carbonate (Naylor, 2003). Species exhibiting rosettes respond to water movements and buoyant tissues in the stem and leaves maintain stability on the surface of the water. The spongy inflated leaf petioles of water chestnut also help the rosette to float (Groth *et al.,* 1996). However, the crop contributes significantly to the food of poor rural masses of these districts and has the potential for nutritional security and can also improve the socio-economic condition of the local farmers with the increasing markets for the crop. Despite this, availability of this underutilized crop is poor owing to lack of standard varieties, cultivation practices, postharvest management etc.

Genetic diversity assessed the certain genotypes of water chestnut and other horticultural crops in the world has been characterized based on morphological traits and genetic markers in water chestnut (Arima, *et al.,* 1999; Hoque *et al.,* 2005 and Babu *et al.*, 2011), in Barahal (Shukla *et al.*, 2008), in mango (Singh *et al.*, 2009 and Rocha *et al.*, 2012) and also in Ber (Shukla *et al.,* 2012). Traditional methods for germplasm identification are based on the observation of phenotypic characteristics as they aid fast and simple evaluation of variability and hence, are considered as an effective means of preliminary evaluation of assessing genetic diversity among morphologically distinguishable accessions in Jamun (Rumana *et al.,* 2016). Furthermore, morphological characterization is the official method accepted for registration and protection of new cultivars (Ertan, 2007).

However, morphological diversity is often limited, characters may not be obvious at all stages of the plant development and appearance may be affected by environment. Nowadays, a variety of different genetic markers has been proposed to assess genetic variability as a complementary strategy to more traditional approaches in genetic resources management. Identification and characterization of germplasm diversity is an essential prerequisite for formulating strategies for plant improvement and conservation of genetic resources. Molecular markers represent a powerful and rapid tool for characterizing diversity within the target species (Kachare *et al*., 2013).

Thus, the previous research works carried out by different workers in relation to the present investigation have been reviewed on the basis of following objectives of respective experiment:

Experiment I

2.1 Survey and collection of germplasm of water chestnut from the various blocks of district Lucknow.

A survey is defined as a research method used for amasses data from a pre-defined group of respondents to gain information and insights on various topics of interest. Surveys have a variety of purposes and can be carried out in many ways depending on the methodology chosen and the objectives to be achieved.

Lucknow has 4 tehsil, 8 blocks and 823 village. The total wetland area in the district is 9607 ha. Lake / pond, river/ stream ox-bow lake/ cut-off meanders are the major natural wet lands. Water logged and tank / pond are the major man made wetland of the district. In addition, 775 small wet lands (<2.25ha) mainly ponds are identified. Aquatic vegetation was observed in many wetland and covered an area of 3573 ha in post-monsoon season while 1963 ha in pre monsoon season. Water spread area in post monsoon season is 3573 ha. Whereas in pre monsoon season the water spread area is 1363 ha. Low turbidity is observed in most of the wet land.

Wetlands are some of the most important ecosystems on Earth. They play a key role in alleviating floods and filtering polluted water and also provide habitats for many plants and animals. Wetlands also interact with climate change. Over the past 50 years, wetlands have been polluted and declined dramatically as land cover has changed in some regions. Remote sensing has been the most useful tool to acquire spatial and

temporal information about wetlands. Its classification, habitat or biodiversity, biomass estimation, plant leaf chemistry, water quality, mangrove forest, and sea level rise (Guo *et al.,* 2017).

Babu *et al.* (2011); Suriyagoda *et al.* (2007) and Deb *et al*. (2013) detected wide variability in water chestnut germplasm, based on a survey reported in the performance of plant and fruit morphology in world such as India, Japan, China and Sri Lanka, etc. Variability in germplasm has also been reported in other horticultural crops like mango (Begam *et al.,* 2014a) and in Salparni (Manivel *et al.,* 2019).

Along similar lines, Dwivedi *et al.* (2011a) have also conducted a preliminary survey, collection and evaluation of different cultivars of water chestnut for their physical and biochemical parameters in order to explore the possibility of genetic variability in water chestnut. Suriyagoda *et al.* (2007) reported that survey and collection of water chestnut germplasm on the basis of yield performance of 17 water chestnut lines and the morphological characters of their fruit were analyzed. Similarly, Arima *et al.* (1999) was conducted that the morphological and yield performance of water chestnuts which distributed in Japan and China were examined for 12 local lines of 6 species i.e. 5 small and 2 medium fruit lines from Japan and 5 large fruit lines from China. The materials were transplanted and grown in a flooded field usually used for rice cultivation in Saga City in 1995. Earlier exploration was conducted forty-three germplasm of Salparni were amassed and regenerated *ex situ* for conservation and evident diversity based on morphological traits such as leaf size, leaf shape, leaf weight and plant height (Manivel *et al.*, 2019). Based on survey 23 genotypes of barahal were collected from 17 different village of block kasia in district Kushinagar and physico-chemical analysis were studied (Dwivedi *et al.,* 2011b). Mukherjee, (1985) was reported a survey in the mango growing belts in West Bengal and described several clones of important mango varieties. An internationally accepted descriptor list for characterization of mango germplasm was also prepared by Mukherjee, (1985) which facilitated uniform description at the global level. It contains characterization and evaluation descriptors covering morphological features of leaf, inflorescence, fruit and tolerance to biotic and abiotic stress conditions. Similarly preliminary exploration was conducted for compilation of multi-crop germplasm to exploring diversity in two special trips, in state of Mizoram. During the exploration trips, 344 germplasm of different agri-horticultural crop were

collected from the six districts, i.e. Kolasib, Aizawl, Serchhip, Lunglei, Lawngtlai and Saiha (Rathi *et al.,* 2013).

According to Deb *et al.* (2013) a survey was conducted in three different districts of Nagaland, India (Mokokchung, Wokha and Zunheboto) by interacting with the people about the plants/parts used by them. Information was also collected on the purpose of use, source of the plants/parts, season of collection, preparation process. Some of the selected plant species were collected from the wild and established in the Departmental experimental garden where they are propagated. The information collected are compiled according to category like, wild, cultivated and used for vegetable, medicines and others.

Begum *et al.* (2013) reported in mango samples of fruit and leaf from the 16 trees of Panchadarakalasa' (PK Acc-l to PK Acc-16) spread over the three eco-geographical regions (Coastal Andhra, Rayalaseema and Telangana) of the state were collected in summer season, which were subjected to *in-situ* morphological and *ex-situ* microsatellite analysis, respectively to identify whether there is variability in the plants grown in the state. Explore the intra-varietal variability based on survey by several researchers, Begum *et al.* (2014a) explored three eco-geological regions of the state and the thirty morphotypes of mango cv. 'Cherukurasam' (CKR Acc-l to CKR Acc-30) were collected of fruit and leaf samples to existence the intra-varietal diversity through classical and molecular analysis.

Survey was conducted three eco-geographic regions of the state, 31 morphotypes of mango cv. Beneshan (BN Acc-l to BN Acc 31) were collected from the fruit and leaf to examined intracultivar heterogeneity based on morphological fruit traits and microsatellite markers, respectively Begum *et al.* (2014b).

Petrović *et al.* (2016) investigated bioaccumulation of the ecotoxic metals Cd, Pb and Cr from sediments of Skadar Lake in the aquatic macrophyte *Trapa natans* L. Samples of sediment and plants were collected at nine locations covering all major water inputs to the lake as well as locations where contamination could be expected. The results indicate that *Trapa natans* L. may be a very promising bioindicator of trace metal contamination in Skadar Lake.

Experiment II

2.2 Intervarietal variability in water chestnut on the basis of botanical descriptors i.e. root, leaf, stem, flower and fruits

2.2.1 Botanical descriptors

Botanical classification of this plant has always provided better contribution to conserve existing plant names, for the benefit of researchers, botanists, taxonomists, pharmacognocists and other users. A concise botanical classification of water chestnut (*Trapa natans* var. *bispinosa* Roxb) as is descried in chapter 4.

Previously studied in water chestnut Hoque *et al.* (2007) identified genotypic variation as an important factor affecting organogenesis in more than 18 different *Trapa natans* genotypes drawn from all over the world. However, the current tendency is to consider the family Trapaceae as a single monogeneric group containing two species that exhibit high genetic and morphological variation.

Çakılcıoğlu *et al.* (2010) analysed 41 medical plants species have collected belonging to 17 families were identified in Yazıkonak and Yurtbası Districts of Elazığ Province, Turkey and were prepared herbarium materials and the specimens were nomenclature for botanical study. The special characteristics of both surfacing and submerged leaves of this plant are described and discussed. Water chestnut has previously been classified as one polymorphic group or as one genus having up to around 20 different species such as *Trapa maximouswiezii, T. acornis, T. quadrispinosa, T. bicornis, T. bispinosa, T. incisa, T. japonica, T. psedoincisa and T. potanini* detected prominent to (Jiang- weimei *et al.*, 2004) which may have different chromosome numbers (2n = 96 and 2n = 48) Takano and Kadono (2005). According to Hummel and Findlay (2006) the variety *T. natans* var. *natans,* with its four-spined nut is widely distributed in Eurasia, Africa and the north-eastern United States, whereas *T. natans* var. *bispinosa* a two-spined variety, cultivated in China, Japan, India and Southeast Asia.

Trapa kashmirensis, a new species from Wular Lake in Kashmir, is described and illustrated. It differs markedly by its fruit morphology from all other members of the genus, primarily by the characteristic long upper horns and strongly reflexed lower horns, the longest known in the genus.

Roots

According to Sculthorpe (1967) water chestnut roots have contain chlorophyll which has often misled people to think they were submerged leaves with segments comparable to

the terrestrial roots. *Trapa* has no primary root system, just the adventitious roots that extend from the hypocotyls and Prajapati *et al.* (2003) have been detected that green photosynthetic submerged root of water chestnut. The lateral roots contain only one strand of xylem and phloem. Although the most important function of the roots is to absorb nutrients, they also provide an anchor for plant, but the developmental origin of the roots is unclear. Water chestnut has adventitious roots that develop in pairs on either side of the leaf scars at lower nodes of the floating stem. The roots are feathery and can often reach to the sediment, but usually remain suspended in the water column (Groth *et al.,* 1996). Its main root system adheres in the muddy soils at the bottom of the pond and it is connected with floating leaves by herbaceous stems in water body (Singh *et al*., 2010).

Stem

Prajapati *et al.* (2003) reported *Trapa* are an aquatic floating plant with flexuous ascending stem, produced several branches, each terminating in a rosette. Hummel Kiviat (2004), Adkar *et al*. (2014), Bercu (2004) have reported that stem is flexible and from 1 to 5 m long, nodes of the stem have slender linear roots while the plant is anchored in the sediment by the lower roots that emerged from propagating seed. Nodes of the lower stem also bear slender, unbranched roots in the substrate and in the water above the substrate (Crow and Hellquist, 2000; Hummel and Kiviat, 2004).

Leaves

Sculthorpe (1967) screened the water chestnut plant are produce floating leaves and arranged in a rosette. Leaves are forced to physiologically deal with being exposed to air and water simultaneously and gases move through stomata in the upper epidermis. Floating leaves will usually take a circular peltate form. Individually, the 24 cm (0.75-1.5 in.) long upper leaves are slightly rhombic to rhombic ovate and are sharply dentate along the leaf margins (Groth *et al.*, 1996 and Naylor, 2003). Prajapati *et al.* (2003); Chandana *et al.* (2013) were observed that leaf margin leaving simple alteration, crowded at the upper part of the stem in rosettes, rhomboidal, apex triangular, irregular inciso-serrate in the upper part a dark green above, radish purple beneath, petioles dilated near the apex into a large spongy float. Karmakar *et al*. (2011) screened that the reddish green leaves are villous on the dorsal side and about 5 to 8 cm long, have hairy petioles 10 to 15 cm in length and also Cozza *et al.* (1994) was reported to plant produces new leaves from a central terminal meristem in the rosette near the surface of the water.

Surfacing leaves are triangular with toothed edges and an inflated petiole and form a rosette on the water surface. Submerged leaves are feather-like, each leaf is divided into segments that are whorled around the leaf stem. White flowers form in the axils of the surfacing leaves in July (Bercu, 2003). Adkar *et al*. (2014) obtained leaves are alternate, crowed on the upper part of the stem; 3.8-5 cm long, rhomboid, somewhat truncate at the base, irregularly inciso- serrate, radish purple beneath petiole dilated near the apex.

Flowers

Flowers were inconspicuous, bisexual, white colour, opening above the surface of water in the afternoon, axilary and solitary consist of four 8 mm (0.3 in.) long, white petals and four green sepals, and are located in the centre of the rosette detected by several researchers (Naylor, 2003; Prajapati *et al.,* 2003; Karmakar *et al.,* 2011; Chandana *et al.,* 2013) and raised in early June continues until the plants are killed by frost and the nuts will mature approximately a month later (Groth *et al.,* 1996). Flower had a two-chambered ovary, four stamens, four petals, and four sepals that eventually become the spines of the fruit. The flowers are generally cross pollination, but self-pollination may occur before the flower opens by screened (Hummel and Kiviat, 2004). Arima *et al.* (1999) reported that water chestnut flower has no inbred whorl of stamen, ovary is half inferior, bilocular with single pendulous ovule in each locule. According to Chakor, (1974) was reported that pollinated flower developed into a fruit are ready for picking within 10-15 days.

Fruits

Based on husk colour Faruk *et al.* (2012) detected that two types of water chestnut fruit one is red (leaf, petiole and fruit) and the other is green (leaf, petiole and fruit) each bearing fruit large in size having two dull spines. The fruit is a one seeded, top shaped drupe, the fleshy pericarp of which is delicious and covers a large two or four horned, stony endocarp (Pyxena). Similarly Wójcicki (2001) and O'Neill (2006) were observed water chestnut is three types: completely green, completely red and green blended with red. Fruits ripen in about a month and can remain viable for up to about twelve years. Each seed can give rise to ten to fifteen rosettes and each rosette may produce as many as twenty seeds. When mature, the fruits fall from the plant and sink to the bottom of the body of water. Seed dormancy can be from four months to twelve years (Naylor, 2003). Prajapati *et al.* (2003) also reported that fruits are ovoid bony, angular with short conical

beak in the centre at the apex and a spreading, flattened, very sharp spinous horn on the other side, indehiscent, one seeded and seeds while starchy. Seed production and maturation continues until senescence. In dense growths of *Trapa*, as many as 170 seeds per m^2 have been documented (Methe *et al.*, 1993). According to Hoque *et al.* (2001) water chestnut plant is propagated mainly through seeds. A single seed can give rise to 10 to 15 plant rosettes. The seeds can stay viable for up to 12 years. Cozza *et al.* (1994) reported that when the fruits are mature, the fruits fall from the plant and sink to the bottom of the water body. Seed germinates after completing dormancy period of four months. Karmakar (2011) studied that the fruit is about 2 cm in diameter. The fruit is a bony one-seeded nut having very unequal cotyledons and a tope-shaped drupe. The fleshy pericarp covers a large 2-4 horned, stony endocarp. When ripe, the nuts fall to the bottom of the pond where they remain all winter as they must be kept moist to retain their viability. The fruits mature from mid-July into September and are released by the rotting peduncles were earlier detected by many researchers (Smith, 1955; Countryman, 1978; Cronk and Fennessy, 2001). Each rosette produces 10 to15 nuts, each ca. 6 g wet mass (2.1 g dry mass) and 2 to 4 cm wide (excluding the barbed portions of the horns) at maturity (Countryman, 1978; Gleason and Cronquist, 1991; Kiviat and Beecher, 1991). Singh *et al.* (2010) observed that water chestnut kernel is covered with a thick jet-black outer pericarp shaped like a horn protruding from the head of a buffalo.

2.2.2 Anatomical and stomatal studies:

Plant anatomy is the term for the study of the internal structure of plants. Originally it included plant morphology, the description of the physical form and external structure of plants, but since the mid-20^{th} century plant anatomy has been considered a separate field referring only to internal plant structure (Raven *et al.,* 2005 and Hagemann, 1992*)*. Plant anatomy is now frequently investigated at the cellular level and often involves the sectioning of tissues and microscopy (Evert and Esau, 2006)

Preliminary studied was conducted in water chestnut by Bercu (2004) who detected anatomical and histological features of the leaves of *Trapa natans* a free-floating hydrophyte, living in the Danube Delta channels. The special characteristics of both surfacing and submerged leaves of this plant are described and discussed. Sculthorpe (1967) and Naylor (2003) have reported gaseous movement through stomata in the upper epidermis of the leaves surface which are absent on the adaxial surface. Leaves had little or no lignin and the vascular tissues were generally poorly developed in the leaves and buoyant tissues in the stem were found (Groth *et al.*, 1996) and upper stem swelling had

a lacunate pith and four or five rings of air spaces in the cortex whereas the remaining pith is compact having only two rings of cortical lacunae in the lower stem were reported (Sculthorpe, 1967).

Scanning Electron Microscopy (SEM) is an ideal technique for examining plant surface, germplasm identification and morphological characterization at high resolution along with other research tools such as molecular markers (Pathan *et al.,* 2008). Stomatal traits are highly variable depending on the genetic background of the plants as well as on the growth conditions or the leaf ontogeny (Masarovicova, 1991 and Jones, 1992).
Stomata are any of the minute pores in the epidermis of the leaf or stem of a plant, forming a slit of variable width which allows movement of gases in and out of the intercellular spaces which is measured by Scanning Electron Microscopy (SEM). Generally, stomatal initiation is controlled by both environmental and genetic factors (Casson and Hetherington, 2010).

In twelve poplar (*Populus*) clones which belonged to different species and interspecific hybrids, were studied stomatal characteristics, as stomatal density and stomatal length, for fully expanded leaves from all clones and at two canopy positions (upper and lower canopy) (Afas *et al.,* 2006).

Masarovicova (1991) studied stomatal characteristics (i.e., size and density) which are highly variable depending on the genetic background of the plants as well as on the growth conditions or the leaf ontogeny. Stomatal density has been shown to vary significantly within individuals, cultivars or ecotypes of a single species, as well as within a community (Jones, 1992). Within the *Populus* genus, a wide interspecific as well as inter clonal discrepancy in stomatal density, dimension and stomatal index has already been observed (Ferris *et al*., 2002). Stomatal traits have already been suggested as criteria for clonal discrimination in the genus (Ceulemans *et al*., 1988). Such an eventual use of stomatal features requires a detailed knowledge of the stomatal characteristics of the most frequently used poplar species and hybrids.

Orlovic *et al*. (1998) observed a strong correlation between adaxial stomatal density and biomass in *Populus* hybrids and this correlation was proposed to be used in the selection of nursery stock for biomass production.

Some species have been reported as possessing generally high heritability (i.e., less sensitive to environmental change) in their stomatal characteristics (Sharma and Dunn, 1969; Orlovic *et al.,* 1998), while others have been reported as being more sensitive to environmental factors (Schoch *et al.,* 1980).

Several studies have been performed to understand the genetic basis for stomatal-related traits. In Arabidopsis, natural variation in stomatal responses to environmental changes has been reported (Aliniaeifard and Meeteren, 2014; Takahashi *et al.,* 2015), as well as for stomatal density (Delgado *et al.,* 2011).

Delgado *et al.* (2011) studied genetic variation for stomatal development was observed, as well as an indication of relationships among stomatal traits and extreme or uncommon morphotypes as resources for the genetic dissection of stomatal development.

Using a subset of a population of *Solanum pennellii* introgression lines, Fanourakis *et al.* (2015) assessed variation in stomatal responsiveness to desiccation and gs-related anatomical traits under well-watered conditions. Considerable differences with respect to stomatal size, density, distribution between the leaf adaxial and abaxial sides, as well as pore area per stomatal area were observed.

Marron (2005) postulated that stomatal traits could be used as early indicators of growth potential in poplar as well as criteria for clonal discrimination in the genus and stomatal density is reported to differ significantly even among clones belonging to different parentages, between different canopy positions and on leaf surfaces besides varying within leaves, plants, and individuals of a single species (Afas *et al.,* 2006). Stomatal length has also been reported to correlate with genome size (Xu and Zhou 2008). Therefore, the genetic and developmental basis for high stomatal density and stomatal conductance and its application in germplasm studies is a research priority in plant physiology, agriculture, and paleo-biology (Wang *et al.,* 2015).

Riaz and Chaudhary (2003) observed genotypic and phenotypic coefficient of variation (7.43 and 7.29%) for stomatal size indicated that all of the variation for the trait was due to genetic causes. The maximum value of heritability (97.50) and genetic advance (20.86) were found for stomatal density.

Examination of diversity in stomatal traits such as stomatal density (SD), stomatal length (SL), stomatal width (SW) and stomatal surface (SS) on the basis of changes in the

vegetative parameters in different growing media in corn. All growth parameters studied were analyzed significant differences between combination rates in both of growing media. The increase in leaf growing parameters led to increase in SW, SL and SS values but decrease in SD. It can be said that the SS of corn plant leaf set by changing both of the SW and SL sizes, acting positively on growth conditions. It can be suggested to combine the results obtained from maize with other uninvestigated plant and growth condition to clarify mechanisms of stomatal which are under control of genetic and environmental factors (Orcen *et al.,* 2013).

Xu and Zhou (2008) reported that stomatal length correlates with both of genome size and water conditions. Besides, stomatal density is genetically determined as a quantitative trait (Gailing *et al.,* 2008). The relative importance of gene versus environment in determining stomatal density or stomatal length and its interspecific variation have not yet been estimated under a unified framework. A wider diversity of models for the genetic and environmental control of stoma should be considered (Zhang *et al.,* 1999).

Camargo and Marenco (2011) reported large variation in both stomatal density (S_D) and stomatal size (S_S) among species. S_D ranged from 1.10-2 mm in Neea altissima to 846 mm^{-2} in Qualea acuminata. However, in most species S_D ranges between 271 and 543 mm^{-2}, with a negative relationship between S_D and S_S. who also found a positive relationship between S_D and tree height ($r2 = 0.14$, $p < 0.01$), but no correlation was found between S_D and leaf thickness. The most common stomatal type was anomocytic (37%), followed by paracytic (26%) and anisocytic (11%). Conclude that in Amazonian tree species, stomatal distribution on the leaf surface is a response most likely dependent on the genetic background of every species, rather than a reaction to environmental changes, and that somehow S_D is influenced by environmental factors dependent on tree height.

Stomatal density has been shown to vary significantly within individuals, cultivars or ecotypes of a single species, as well as within a community (Jones, 1992). Within the *Populus* genus, a wide interspecific as well as interclonal diversity in stomatal density, dimension and stomatal index has already been observed (Ferris *et al.,* 2002). Stomatal traits have already been suggested as criteria for clonal discrimination in the genus (Pallardy and Kozlowski, 1979 and Ceulemans *et al.,* 1988).

Boso *et al.* (2016) reported that a number of studies have highlighted differences in the density of stomata between *Vitis* species, but few have examined differences between varieties of *V. vinifera*. The density and size of the stomata in the lower epidermis of leaves belonging to 12 grapevine varieties, a direct producer hybrid (DPH) involving a *V. vinifera* and a non-*vinifer*a parent. These prints were then examined under a light microscope and the number of stomata in a unit area of 0.196 mm^2 counted. Image analysis software was then used to measure the length and width of all those counted. Rootstock 'SO4', 'Chasselas Dorée', 'Albariño' and 'Cabernet Sauvignon' had the highest stomatal densities (all > 34 stomata per unit area), while 'Castañal', 'Torrontés' and 'Caiño Blanco' and 'Jacquez' (DPH), had the smallest (all < 26.50 stomata per unit area). 'Treixadura' and 'Caiño Blanco' had significantly longer and wider stomata than all the other varieties examined; the DPH 'Jacquez' had among the shortest and narrowest. No relationship was seen, however, between mean varietal leaf size and the stomatal density or stomatal size; nor was any seen between the variables examined and the condition of belonging to *V. vinifera* or not.

Sreelakshmi *et al.* (2014) determined that stomata are apertures in the epidermis, each bounded by two guard cells. Their main function is to allow gases such as carbon dioxide, water vapours and oxygen to move rapidly into and out of the leaf. In green leaves they occur either on both surfaces (amphistomatic leaf) or on one only, either the upper (epistomatic leaf) or more commonly the lower i.e., hypostomatic leaf. Four types of stomata are recognized such as anomocytic, paracytic, diacytic, and anisocytic from the materials under study such as *Manihot esculenta* Crantz, *Colocasia esculenta* (L.) Schott, *Maranta arundinacea* L., *Annona squamosa* L., *Artocarpus heterophyllus* Lam., *Passiflora edulis* Sims., *Curcuma longa* L., *Mangifera indica* L. and *Garcinia cambogia* (Gaertn.) Desr. Among all of the stomatal types paracytic type is dominated. The guard cell of *Colocasia esculenta* is kidney or bean shaped. But in other monocotyledonous plants under the present study *Curcuma longa* and *Maranta arundinacea* are with dumbbell shaped guard cell. The aim of the present study is to correlate between foliar characters and stomata in leaf (Lawren and Thomas, 2016). Equations for stomatal density and maximum theoretical stomatal conductance as functions of stomatal initiation rate, epidermal cell size, and stomatal size enable scaling from development to flux.

Camargo and Marenco (2011) documented how stomata are distributed on the leaf surface and to determine if there is any significant variation in stomatal characteristics

among Amazonian tree species, and finally to study the relationship between stomatal density (SD) and tree height. Thirty five trees (>17 m tall) of different species were selected. Stomatal type, density (SD), size (SS) and stomatal distribution on the leaf surface were determined using nail polish imprints taken from both leaf surfaces. Irrespective of tree species, stomata were located only on the abaxial surface (hypostomaty), with large variation in both SD and SS among species.

2.2.3 Morphological studies:

Arima *et al.* (1999); Babu *et al.* (2011) and Suriyagoda *et al.* (2007) based on a survey in water chestnut growing areas in the world reported a wide variability in performance of the morphotypes of the same species. Further, there are some reports on variability among the morphotypes of the in same species with respect to fruit morphology (Babu *et al.*, 2012; Shukla *et al.*, 2008 and Dwivedi *et al.,* 2011b) which are highly influenced by environment.

In many situations, the most easily obtained assessment of genetic variation is that of measuring morphological or phenotypic variation. The sharing of phenotypic characters is interpreted as an indication of relatedness. Morphological traits are often influenced by environmental conditions (Jasienski, 1997; Kercher and Sytsma, 2000), which in turn may influence the estimation of genetic variation and relatedness. Suriyagoda *et al.* (2006) has also reported that seventeen water chestnut lines representing Europe and Asia were studied for their canopy growth and fruit morphology. Leaf area of a single leaf, SLA, LAI at the beginning of flowering was significantly higher for the Chinese lines compared to other Asian and European lines. Based on morphology discrepancy was observed in forty-three accessions of Salparni with respect leaf size, leaf shape, leaf weight and plant height (Manivel *et al.,* 2019).

Diversity study in several fruit crops from India and other countries has been characterized based on morphological traits and genetic markers (De Souza and Lima, 2004; Diaz-Matallana *et al.*, 2009; Singh *et al.*, 2009; Rocha *et al.,* 2012). Morphological characterization is traditionally the most common method used. Until recently, morphology-based methods have been used for the characterization of intervarietal variability in water chestnut and another fruit crops (Suriyagoda *et al.,* 2007; Ertan, 2007; Singh *et al.,* 2009 and Rumana *et al.,* 2016) which have been documented significant variation with regard to fruit morphology. As early as Oppenheinmer, (1956) reported wide variability in the performance of the trees of the same variety in the same

orchard. Further, there are few reports on variability among the trees of the same variety in an orchard with respect to fruit size, shape, color and quality as early as 1971, (Naik, 1971) which are highly influenced by environment. Morphologically, all the six 'clones' of mango studied *viz.,* 'Harummanis', 'Apple', 'Irwin', 'Kent', 'Malgoa' and 'Hj. Bakar' are very distinct with respect to the morphology and the taste of the fruits (Gan *et al.*, 1981; Pandey, 1998) studied different clones of 'Alphonso' and found that they differ from one another in more than one character. Morphological analysis based on 17 fruit characters detected prominent variation in the landraces 'Banganapalli', 'Langra', and 'Dashehri' and some variation in the cultivar 'Mallika' (Singh *et al.,* 2009). In the above cases, the identification of intravarietal variability is based on morphological traits. But number of these traits is limited, they are unstable and they do not always enable to distinguish between closely related accessions or cultivars.

Iyer and Subramanyam (1986) screened the mango varieties at Indian Institute of Horticultural Research, Bengaluru and classified them on the basis of flowering time, sex ratio, fruit retention, harvesting time, fruit weight, TSS, acidity, total sugars and TSS: acid ratio.

The growth and development of a variety having a definite genetic character in particular set of environmental conditions shows positive relation. The variation in vegetative growth characters among mango varieties might be due to variation in genetic makeup. High variability in vegetative growth amongst the mango varieties have also been reported that Singh *et al.* (1998). Until recently, morphology-based methods have been used for the characterization of intra-varietal variability in mango where significant variation among the trees of the same variety in an orchard with regard to fruit shape, size, color and quality of the fruits has been observed which was ascribed to bud mutations (Gan *et al.*, 1981 and Pandey, 1998).

The variations observed in fruiting behaviours may be attributed to the genetic nature of varieties and weather parameter. Fruit drop in mango during initial stages is reported to vary with growth of fruitlets. It is more on number basis at mustard stage and on weight basis at marble stage (active fruiting growth phase). This reveals that the drop of larger fruitlets (those at marble stage) is more associated to ethylene evolution than those of smaller fruitlets (Muhammad *et al.*, 2002 and Kumar and Jaiswal, 2004).

It is a highly cross-pollinated and heterozygous plant, performance of which varies with the climate resulting in a high level of genetic diversity. Most cultivars have arisen through selection of desirable types from naturally produced seedlings (Karihaloo *et al.*, 2003) where each cultivar is identified by the characteristic combination of properties such as plant architecture, fruit size, color, taste, flavor etc. (Anu *et al.,* 2015). Intra-varietal variability of certain varieties of mango from India and other countries has been characterized based on morphological traits and genetic markers (De Souza and Lima, 2004; Diaz-Matallana *et al.,* 2009; Singh *et al.,* 2009; Rocha *et al.,*2012). Morphological characterization is traditionally the most common method used. Until recently, morphology based methods have been used for the characterization of intravarietal variability in mango. Pandey (1998) and Singh *et al. (*2009) observed significant variation among the trees of the same variety in an orchard with regard to fruit shape, size, color and quality of the fruits which was ascribed to bud mutations.

Experiment III

2.3 To establish intervarietal variability in water chestnut on the basis of physico-chemical characteristics of the fruits

Variability studied of water chestnut genotypes from India and other countries has been characterized based on morphological traits and genetic markers (Babu *et al.,* 2011; Dwivedi *et al.,* 2012; Arima, *et al.,* 1999; Hoque *et al*., 2005; Shukla *et al.,* 2008; Singh *et al*., 2009; Rocha *et al*., 2012). Morphological characterization is traditionally the most common method used. Until recently, morphology based methods have been used for the characterization of inter varietal variability in horticultural fruit crops (Suriyagoda *et al.,* 2007; Ertan, 2007; Singh *et al.*, 2009; Rumana *et al.*, 2016) observed significant variation with regard to fruit shape, size, color and quality of the fruits which was ascribed to bud mutations. Very little work has been done on physico-chemical parameters of water chestnut. However, germplasm studied in guava (Ball and Khera, 2005) and in mango (Kishor *et al.,* 2019) indicates clearly that variability can be established on the basis of physical and chemical parameters. Available research finding have been reviewed briefly in the present chapter and the salient finding pertaining to the present investigation have been documented in a classified manner, so as to use it as reference literature. Singh *et al.* (2010) investigated the physico-chemical properties of water chestnut (*Trapa natans* L. var. *bispinosa* Roxb.) contents of moisture, crude lipid, crude fibre, crude ash, and crude protein were 81.12, 0.36,

0.72, 1.33, 1.87%, respectively. Total soluble solids and titrable acidity determined was 7.2 and 0.142%, respectively.

Babu and Dwivedi, (2012b) has analysed that the biochemical property of water chestnut cultivars collected from different sites was comparatively evaluated. The significant variation in TSS, pH, reducing sugar (%), non- reducing sugar (%), total sugar (%), acidity (%), and sugar/acid ratio among the various cultivars collected from the different areas.

Physio-chemical analysis of *Trapa natans* var. *bispinosa* with respect of weight and size and nuts and kernel they content of dry matter carbohydrate, sugar, acidity, amylose, ash, protein ether extractive part and pectin of the kernels belonging to the green and purple husked nut has been reported by (Gani *et al*., 2010)

Shalabh *et al.* (2012) reported that the kernels are delicious to eat and contain carbohydrates, proteins and essential minerals and are reported to be medicinal used. Faruk *et al*. (2012) was screened two varieties (Green and red) of water chestnuts had been selected for their biochemical analysis as well as nutrient composition using standard methods. Kumar and Chopra, (2017) conducted to assess the phytoremediation potential of water caltrop (*Trapa natans* L.) using municipal wastewater collected from activated sludge process (ASP) based municipal wastewater treatment plant. The most contents of Cd, Cu, Fe, Mn and Zn were translocated in the leaves of *T. natans* while the most contents of Cr and Pb were accumulated in the root of *T. natans.* Different biochemical components were recorded in the order of total sugar > crudeprotein > total ash > crude fiber > total fat in *T. natans* after phytoremediation of municipal wastewater.

Sixteen germplasms of *Musa* sp. (sub group- AAB) were assessed with an aim to describe the phenotypic diversity and the heterogeneity within morphological parameters, yield and quality attributes. A close relation between selection and uses of cultivars with the morphological, physico-chemical quality attributes and rheological specificities were highlighted for these germplasms and a significant variation among them was highlighted through Cluster analysis, Proximity Matrix for characterization of variables to identify major characters responsible for grouping of homogeneous cultivars. Dudhsagar genotype was the best for its high economic yield, high TSS and good sugar: acid ratio (Kundu *et al.,* 2018)

Ball and Khehra, (2005) was conducted twenty five fruits germplasm of guava were collected at ripening stage on basis of physio chemical parameters and observed

variability in fruit weight (95-248g), number of seed (92-481), seed weight (1.7-5.8g), T.S.S (9.5-126%), acid (0.3-0.60%) and ascorbic acid (118-272 mg/100g pulp).

Anu *et al. (*2015) detected that significant variation exists among the clones of 'Langra' mango with respect to fruit shape, size, colour, quality and taste. The clonal variability exit in some 'Langra' mango using morphological, biochemical and molecular markers. These results provide evidence that a significant level of genetic variation exits among 10 clones of 'Langra' mango which can be used for mass multiplication of superior clone(s) and can be further utilized in breeding programs. According to survey three regions of the state31 accessions of 'Beneshan' (BN Acc-l to BN Acc-31) were selected and their fruit and leaf samples were collected to study intra-cultivar heterogeneity based on morphological fruit traits and microsatellite markers, respectively. There was a wide range of intra-varietal heterogeneity (up to 50%) indicating that 'Beneshan' whatsoever cultivated throughout the state is not pure clone, which allows the genetic breeding of this cultivar by means of mass selection (Bebum *et al.*, 2014a).

Bisla and Daulta 1988) was conducted in ber to attribute with the presumption that such information would be useful to fruit breeder for future breeding programme in ber crop. The heritability was observed to be quite high in different trait *viz.*, acidity (9.161%) ascorbic acid (89.25%) and total soluble solids76.04%), respectively. The genetic advance expressed in percentage ranged from 10.81% for total soluble 204.29% for disease intensity. The genetic advance was quite high for acidity (91.61%) but it was observed in low total sugar (16.70%) and ascorbic acid (35.71%).

Experiment IV

2.4 **Molecular characterization of intervarietal variability in water chestnut**

Electrophoretic protein profiling by SDS-PAGE is used for fractioning total soluble seed protein into different number of bands, varying in their relative mobility and intensity, which marked the heterogeneity among different genotypes. The proteins separated on the acrylamide gel could be distinguished and grouped based on the standard marker. The dissimilarity in the banding pattern showed the polymorphism thus depicting the unique character of the genotype under study. In this study the total soluble seed protein could be fractionated into maximum 26 bands and minimum 17 bands. Polymorphism of around 34.61% was observed. Weak polymorphism was obtained earlier by Mennella *et al.* (2001), Miskoska *et al.* (2008) and Rodriguez *et al.* (2008).

Twenty genotypes of rice (*Oryza sativa* L.) were analysed for total seed protein through SDS-PAGE, to ascertain the extent of genetic variation and its geographical distribution. A considerable variation in protein banding pattern was found which was distributed to various geographical regions. Inter-specific variation was more as compared to intra-specific variation. Inter and intra-specific variation in SDS-PAGE of seed protein in rice *(Oryza sativa L.)* germplasm (Asghar *et al.,* 2004).

Studies was conducted by Yatung *et al.* (2014) thirty chilli (*Capsicum annuum* L) genotypes were amassed from different regions and inter varietal variably estimated for using morphological traits and SDS-PAGE analysis. The result was detected diversity in banding pattern of total protein, which varied from 7-19 numbers of bands. Based on presence (+) or absence (-) of bands, similarity index was analysed and genotypes were grouped in three major clusters which were further sub divided in 9 sub-clusters. The genotype CHFC9 was most distantly related to CHFC18. Hence, genotype CHFC9 and CHFC18 could be utilised for crossing programme to create more genetic diversity or segregates of desired characteristics through chilli breeding programmes. Hence, the result were clearly indicates that variability in genotypes from North eastern hill region of India paves the way for conservation and utilization of genotypes and contributes to the development of systematic breeding programme.

Molecular analysis was conducted in kurz *(Bryophyllum pinnatum* Lam.) separated the protein, through sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) the result was showed that most proteins migrated in the range of 10 kDa to 30 kDa MW. Bands on the gel was then excised and digested with trypsin and subjected to liquid chromatography tandem mass spectrometry (LC/MS/MS) for protein identification. Proteinase K has been identified from the MS/MS data. The protein identified was Proteinase K, which is used commercially in digesting of unwanted proteins liked keratin (Sharma *et al.,* 2014).

Shuaib *et al.* (2010) stated diversity in seed storage proteins by using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) of 15 wheat varieties. Each variety were scored and presence or absence of each band noted and was entered in a binary data matrix. On the basis of SDS-PAGE gels data, cluster analysis was performed to check the variations among varieties. The result was found low degree of heterogeneity however different varieties reveal differential protein banding pattern. It

was observed wheat endosperm protein is useful for existent of genetic variability and cultivars identification that help in wheat breeding program.

SDS-PAGE technique was used for the study of seed protein polymorphism among three genotypes of *Abrus precatorius* with different seed coat colour (Chittora and Purohit, 2012). A total of 44 polypeptide bands were recorded. Out of these 26 were common among all three genotypes and 18 (40.90%) were polymorphic. The data analysis using UPGMA clustering revealed that genotypes with black and white seed coat colour were closer as compared to genotype with red seed coat colour. Each of three genotypes of A. precatorius had some polypeptide bands which were peculiar to them only. This enabled distinguishing all three genotypes on the basis of specific polypeptide fragments using SDS-PAGE analysis.

With the increase in the number of immunocompromised individuals, there has been a consequent rise in the number of opportunistic infections, especially those due to *Candida* sp (Rizvi *et al.*, 2011). A rise in the incidence of antifungal resistance has also been reported. The present study was undertaken to evaluate the incidence of *C. albicans* in superficial and deep-seated infections, to study its antimicrobial susceptibility profile, to analyze the protein-band profile of isolates of *C. albicans* and assess its use as a means of characterizing the yeast, especially in resistant strains. Seventy-six isolates of *C. albicans* from various clinical specimens were identified by standard mycological techniques and further subjected to SDS-PAGE. Molecular weights were calculated with reference to the marker and dendrograms were prepared using the SPSS software. Susceptibility testing of five antifungal agents (fluconazole, clotrimazole, nystatin, amphotericin-B and voriconazole) was done by the disc diffusion/colorimetric microdilution method. On cluster analysis, six types of banding patterns were observed. Maximum resistance (19.8%) was observed against fluconazole. On analysis of the dendrogram cluster groups, the fluconazole-resistant isolates of *C. albicans* formed a separate cluster distinct from those of the fluconazole-sensitive isolates. It was also observed that the specimens from a common site tended to fall close together in the dendrogram pattern. Significantly, high frequency of fluconazole resistance was noticed in this study, which is alarming. In resource-limited laboratories, SDS-PAGE could be used as an alternative method for typing.

Saini and Sarin (2012) reported that electrophoretic protein analysis technique was used, which revealed that some of the protein bands are varied and shown their presence and absence in gel at different stages of gall formation. The amount of total protein increased

during early development and young stages of gall formation and falls down in older stages. It is due to a rapid enzymatic activity in gall tissue during early and mature stages as a response to insect interaction.

Stoyanova and Boller (2010) recorded 45 genotypes (forty genotypes selected within two gene pools and five varieties) of meadow fescue were examined. Genetic diversity was described using ISTA/UPOV methods for crop variety identification. Modifications of acid-PAGE of alcohol-soluble proteins (prolamins), and SDS-PAGE of salt-soluble proteins (globulins) were elaborated for seed analyses of *Festuca pratensis* Huds. The results of this study indicated that the genotypes of meadow fescue could effectively be differentiated on the basis of polymorphism, detected between protein patterns. SDS-PAGE presented a higher differentiation power and better repeatability; thus could be used as a rapid and reliable method for the identification of *F. pratensis* genotypes in breeding programmes and the seed industry.

Singh *et al.* (2017) analyzed variability through seed protein by using SDS-PAGE. Extracting the total seed proteins from 12 cultivars of pea and performed SDS-Polyacrylamide gel electrophoresis. On the basis of banding patterns through SDS-PAGE, indicated that the number of bands found in cultivars ranged from 12 to 19 with Rm value 0.12 to 0.9. Among all the cultivars, the cultivar KPMR-400 had recorded highest number of bands (19) whereas, the minimum number of bands (12) observed in three cultivars viz., KPMR-921, KPMR-902 and KPMR-913. The total seed protein variation were also analyzed using Un-weighted Pair Group Method with Arithmetic Mean (UPGMA) and resultant cluster analysis based on the data of protein profiling, classified twelve cultivars into six major groups. Finally the study concluded that, the protein variability analysis clearly showed that there was sufficient genetic divergence among these cultivars of pea with respect to seed storage protein. Among all the cultivars, the KPMR-906 in cluster IV having wider genetic diversity and suggested to utilize in future crop improvement program.

Aiswariya and Thomas (2016) recorded the genetic variability and relatedness among five cultivars of *Oryza sativa viz.,* Jaya and Uma (improved) and Odachen, Chennellu and Vetteri Black (traditional) cultivated in Kerala using seed protein profiling and morphological characteristics. SDS PAGE of grain protein of five edible rice varieties showed about 50% polymorphism.

Cluster analysis and dendrogram studies

Inter varietal variability were exist among the three *Trapa* species using amplified fragment length polymorphism (AFLP) markers. Variability estimated by dissimilar approaches (NJ tree, STRCTURE, PCA and UPGMA) clearly indicated that all the three *Trapa taxa* formed genetically distinct groups, which confirmed the taxonomic status of the three separate species (*T. quadrispinosa, T. japonica* and *T. bispinosa*). Evident genetic structure was found among populations for each *Trapa taxon*, contributing more than 50% of the total gene diversity (Li *et al*. (2017). Similarly, Hoque *et al.,* 2005 recently studied in water chestnut based on RAPD markers genetic distances among the different varieties were analyzed with a UPGMA-derived dendrogram. Similarity matrix showed the similarities between varieties ranged from 0.25 to 1.0. The dendrogram results suggested that the European varieties were in the same cluster group.

Karibasappa *et al.* (1999) used numerical taxonomic approach of unweighted pair group method using arithmetic average (UPGMA) method for 37 quantitative and 6 qualitative characters in sixty-seven genotypes of mango and identified eleven clusters. They reported that clusters 2, 4, 3 and 10 were most homogenous, whereas cluster 9, 7 and 11 were highly heterogenpus. Cluster 7 (Neelum, Baramasi, Kalepad) and cluster 11 (Batlimavu and Cowasji Patel) were the most divergent followed by cluster 10 (Dophasla, nl.him-46, Neeluddin, Local- 4, Ko-11, Creeping) and cluster 11, while the cluster 3 (Dashehari, Pahutan, nl.him-32, nl.him-33, Local-1, csr.nl, Nekkare-2, Nekkare-1) and cluster 10 were the least divergent.

Kumar (2001) estimated genetic diversity of fifty mango cultivars using RAPD markers in south Indian conditions and found a moderate degree of genetic diversity based on dendrogram study. The hybrids, which had a parent in common, were placed together, whereas alternate bearers and regular bearers formed separate groups in the cluster.

Karihaloo *et al.* (2003) analysed genetic diversity of twenty-nine Indian mango cultivars using RAPD markers. They reported that unweighted pair-group method with arithmetic means (UPGMA) dendrogram had shown the majority of the cultivars from northern and eastern regions of India clustering together and separate from southern and western cultivars. Differences among regions were significant, however, northern and eastern regions formed one zone and western and southern regions formed another zone of mango diversity in India.

Most of the studies related to dendrogram and clustering patterns of various mango germplasm have been done with the help of molecular markers. Such studies are highly useful for confirming the results obtained by conventional methods. Lopez-Valenzuela *et al.* (1997) examined fifteen mango cultivars collected from four different geographical regions using RAPD markers and produced a dendrogram of the genetic relatedness. They reported that the four major bifurcations in the dendrogram clearly separated the genotypes into 4 groups according to their geographic origin Eiadthong *et al.* (1999) evaluated twenty-two mango cultivars for their genetic variation using simple sequence repeat (SSR). Based on dendrogram study, they stated that two Thai mango cultivars Nang Klangwan and Nong Saeng were very far distant of the genetic relationship from the other cultivars and remaining 11 Thai cultivars were classified into three groups.

Begum *et al.* (2014a) constructed a dendrogram and reported that 30 accessions of Cherukurasam mango were divided into four major clusters (cluster-I, II, III and IV). Of these four clusters, cluster-IV was the largest (18 accessions), followed by cluster-I (7 accessions) and cluster-III was the smallest (4 accessions), while cluster- II was solitary (1 accession).

A UPGMA cluster diagram grouped the 31 accessions of Beneshan mango divided into two major clusters (cluster I and II), effectively differentiating the accessions collected from different regions with additional sub-clusters. Cluster-I consisted of 23 accessions, while cluster-II comprised of 8 accessions. Cluster-I was divided into three sub-groups (cluster IA, IB and IC) and cluster II was divided into two sub-groups (cluster IIA and IIB). Of the three sub clusters (cluster-IA, IB, IC) of cluster-I, the sub cluster IA and IC are solitary comprising of single genotype each (Begum *et al.,* 2014b).

Chapter-III

Materials and Methods

The materials used, experimental procedures and techniques adopted during the course of investigation entitled, **"Morphological and Molecular Analysis of Intervarietal Variability in Water Chestnut (*Trapa natans* var. *bispinosa* Roxb.) in Central Uttar Pradesh"** have been described in detail in the present chapter. The investigation was divided into two parts.

In Part I, a survey was conducted in five blocks of Lucknow *viz*., Mohanlalganj, Gosainganj, Sarojini nagar, Bakshi Ka Talab and Malihabad where farmers are growing water chestnut. Plants of the promising cultivars were collected from each pond based on the feedback of farmers and were established and conserved in ponds at Vocational Floriculture Farm, Department of Applied Plant Science (Horticulture), School for Biosciences and Biotechnology, Babasaheb Bhimrao Ambedkar University, Lucknow, Uttar Pradesh, India.

In Part II of the study, inter varietal variability in the germplasm of water chestnut in the district Lucknow was evaluated through botanical descriptors developed, anatomical and morphological studies supplemented with fruit physico-chemical evaluation and molecular studies. A herbarium of plants collected was prepared and botanical descriptors for water chestnut (*Trapa natans* var. *bispinosa* Roxb.) were developed. The collected plants were evaluated for their vegetative performance. Fruit physico-chemical and molecular studies were carried out at the Research Laboratory of Department of Applied Plant Science (Horticulture). Anatomical studies were performed at University Scientific Instrumentation Center (USIC), Babasaheb Bhimrao Ambedkar University, Lucknow which have been described in detail in this chapter under appropriate heads and sub-heads.

PART I

Experiment I

3.1 Survey of water chestnut growing areas in various blocks of district Lucknow and collection of promising cultivars:

Gosainganj

Sarojini Nagar

Mohanlalganj

Bakshi KaTalab

Kewadi

Itaunja

Malihabad (Kasmandi kala)

Malihabad (Kethai para)

Plate 3.1 Survey of water chestnut (*Trapa natans* var. *bispinosa* Roxb.) growing areas in various blocks of district Lucknow

3.1.1 Survey of water chestnut growing areas

A survey was conducted in district Lucknow of central Uttar Pradesh in five blocks *viz.,* Mohanlalganj, Gosainganj, Sarojini Nagar, Bakshi Ka Talab and Malihabad blocks of the areas adjoining to Lucknow (Plate 3.1) during the months of June – July 2016 to study the areas where water chestnut is cultivated on a commercial level in ponds.

3.1.2 Location of survey site

Lucknow, at an altitude of 121m above mean sea level is the capital of the state of Uttar Pradesh It is located roughly in the centre of the state on the banks of Gomati River. The climate here is subtropical which is mild and generally warm. It is located at 26° 51' 0.0000" N longitude and 80° 56' 59.9892" E latitude. Annual precipitation is about 1001 mm / 39.4 inch and the average relative humidity is 52%.

Table 3.1. GPS location, survey and collections site for water chestnut in the present study

Sr. No.	Sampling area	Latitude	Longitude
1	Kewadi	26°50' 21.41" N	80°55' 23.27" E.
2	Gosaiganj	26°50'21.41"N	80°55'23.27"E.
3	Gosaiganj	26°50'21.41"N	80°55'23.27"E.
4	Gosaiganj	26°50'21.41"N	80°55'23.27"E.
5	Sarojini Nagar	26.7472° N	80.8633° E
6	Sarojini Nagar	26.7472° N	80.8633° E
7	Mohanlalganj	26.6676^0N	80.9867^0E
8	BaksiKaTalab	26.70135^0N	81.06145^0E
9	BaksiKaTalab	26.70135^0N	81.06145^0E
10	Kewadi	26°50' 21.41" N	80°55' 23.27" E.
11	Kewadi	26°50' 21.41" N	80°55' 23.27" E.
12	Malihabad (Kasmandi Kala)	26° 55' 26.0616" N	80°42'32.4000" E
13	Malihabad (Kasmandi Kala)	26° 55' 26.0616" N	80°42'32.4000" E
14	Malihabad (Kethai para)	26° 55' 26.0616" N	80°42'32.4000" E
15	Malihabad (Kethai para)	26° 55' 26.0616" N	80°42'32.4000" E
16	Itonja	27.0800^0N	80.9200^0E
17	Itonja	27.0800^0N	80.9200^0E
18	Mohanlalganj	26.6676^0N	80.9867^0E
19	Mohanlalganj	26.6676^0N	80.9867^0E
20	Sarojini Nagar	26.7472° N	80.8633° E

Plate 3.2 Map of experimental site for water chestnut in various blocks of Lucknow

3.1.3 Collection of germplasm:

Twenty superior germplasm on the basis of yield and fruit quality based on the feedback of the farmers were collected in the month of July to August 2016 in polythene bags along with some water and soil from the ponds. These were brought to the University campus for establishment in the experimental ponds. The details of the local varieties collected from the different ponds from the different locations is presented below (Table 3.2).

Table: 3.2 Collection sites of different morphotypes of water chestnut (*Trapa natans* var. *bispinosa* Roxb.) from the five blocks of district Lucknow in central Uttar Pradesh

Germplasm	Source of area	Local name	Farmer name
T_1	Kewadi	Singhree	Ram Pratap
T_2	Gosaiganj	DeshiLalla	PhulchandKashyap
T_3	Gosaiganj	Kadama Green	PhulchandKashyap
T_4	Gosaiganj	Saccharchini	PhulchandKashyap
T_5	Sarojini Nagar	Saccharchini	Ramlal
T_6	Sarojini Nagar	Kadama Green	Ramlal
T_7	Mohanlalganj	Deshi	Chandrashekhar Kashyap
T_8	BaksiKaTalab	Saccharchini	Babuchand
T_9	BaksiKaTalab	DeshiLalla	Babuchand
T_{10}	Kewadi	Saccharchini	Rampratap
T_{11}	Kewadi	Deshi Green	Rampratap
T_{12}	Malihabad (Kasmandi Kala)	DeshiLalla	Harilal
T_{13}	Malihabad (Kasmandi Kala)	Kadama Green	Harilal
T_{14}	Malihabad (Kethai para)	Deshi Green	Shivprakash
T_{15}	Malihabad (Kethai para)	Singhree	Shivprakash
T_{16}	Itaunja	DeshiLalla	Badlu
T_{17}	Itaunja	Kadama Green	Badlu
T_{18}	Mohanlalganj	Kadma	Chandrashekhar Kashyap
T_{19}	Mohanlalganj	Saccharchini	Chandrashekhar Kashyap
T_{20}	Sarojini Nagar	Singhdree	Ramlal

Germplasm described in details T_1 (Selection-1), T_2 (Selection-2), T_3 (Selection-3), T_4 (Selection-4), T_5 (Selection-5), T_6 (Selection-6), T_7 (Selection-7), T_8 (Selection-8), T_9 (Selection-9), T_{10} (Selection-10), T_{11} (Selection-11), T_{12} (Selection-12), T_{13} (Selection-13), T_{14} (Selection-14), T_{15} (Selection-15), T_{16} Selection-16, T_{17} (Selection-17), T_{18} (Selection-18), T_{19} (Selection-19) and T_{20} (Selection-20).

3.1.4 Climate

Babasaheb Bhimrao Ambedkar University is located 10 km away from Lucknow city, which has a subtropical climate with temperatures ranging from 22ºC to 45ºC in summer season and from 3.5ºC to 15ºC in winter.

3.1.5 Topography and weather condition

Geographically Lucknow is situated at an elevation of 121 meter above the mean sea level in the sub-tropical climate of central Uttar Pradesh at 20^0 55′ North latitude and 80^0 52′ East longitude and relative humidity ranging from 60% to 80% in different seasons of the year. Lucknow is characterized by sub-tropical climate with hot dry summer and cold winter. Nearly 85% of the total annual rain fall (750mm) is received during the monsoon.The weather data during experimental period (2016-17 and 2017-18) is appended of Appendix-I.

Experimental field preparation

Preparation of ponds

Ponds having a dimension of 2m in length, 1m in width and 1m in depth were dug manually and filled with water everyday till the water started to be retained in the pond. Subsequently the ponds were replenished with water to maintain its level till the brim.

Application of manures

Farm Yard Manure was applied at the rate of 2 kg per pond after preparing experimental ponds.

Agronomical practices

One plant each from each germplasm was planted in one pond after application of the FYM.

Cultural operations

Water was filled in the pond at regular intervals in order to maintain water till the surface of the pond. Inter culture operations were performed manually as per requirement during cropping period.

3.1.7 Establishment/conservation of germplasm

One plant each of twenty germplasm of water chestnut from various blocks of district Lucknow as cited above were collected in the month of July to August along with some soil and water from the ponds for establishing them in the experimental ponds having a dimension of 2m x1m x1m. One plant of each germplasm was planted in a single pond. Collected plants of each germplasm were thus established on the Vocational Floriculture Farm, Department of Applied Plant Science (Horticulture), School for Biosciences and Biotechnology, Babasaheb Bhimrao Ambedkar University, Lucknow, (Uttar Pradesh) India.

Plate 3.3 Establishment of twenty superior morphotypes of water chestnut at the Vocational Floriculture Farm, Department of Applied Plant Science (Horticulture), Babasaheb Bhimrao Ambedkar University, Lucknow, collected from various blocks of district Lucknow

PART II

Experiment II

3.2 Evaluation of intervarietal variability in water chestnut on the basis of botanical descriptors i.e. root, leaf, stem, flower and fruit

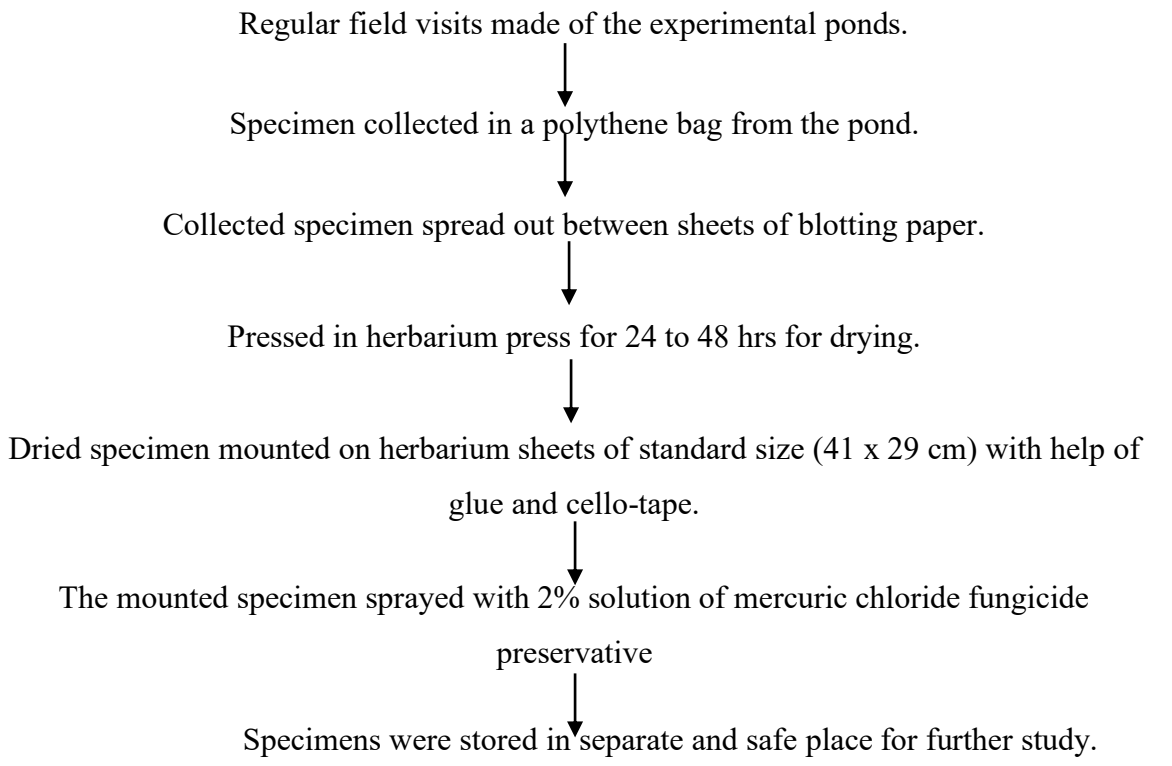
Inter varietal variability in water chestnut was evaluated by studying variability in the vegetative parameters on the basis of botanical descriptors i.e. root, leaf, stem, flower and fruit, through morphological studies of the different plant parts and through stomatal anatomy as per details below:

a. Botanical descriptors through herbarium
b. Anatomical and stomatal studies
c. Morphological studies of vegetative stage of plant.

3.2.1 Botanical description through herbarium

A herbarium is defined as a collection of plants that have been dried, pressed, preserved on sheets and arranged according to any accepted system of classification for future reference and study (Bendre and Kumar, 2016). Twenty superior germplasm established in the experimental ponds as discussed earlier, were utilized for the botanical studies through plant morphological and anatomical characterization. Intervarietal variability on the basis of visual observations through herbarium samples collected from the experimental ponds was done based on the descriptors for different plant parts like leaves, flower, fruit and whole plant. Botanical studies were conducted in consultation with Department of Botany, B. R. D. P. G. College Deoria and National Botanical Research Institute (NBRI) Lucknow and botanical descriptors were subsequently developed.

Figure 3.1 Procedure for preparation of herbarium specimens of water chestnut (*Trapa natans*) plant



Bendre and Kumar (2016)

Method of preparation of herbarium specimens:

Field visits and specimen collection

A complete specimen possesses all parts including root, stem, flowers and fruits which develop in the plant at different stages of plants life cycle. Therefore, regular field visits were necessary to obtain information at every stage of growth and reproduction of a plant species. Regular visits were made of the experimental ponds. The different plant parts to be described were excised from the parent plant using scissors and forceps. These were then collected in a polythene bag for collection of plant part and data was recorded in a field note book.

Pressing and drying

The specimens collected were spread out between sheets of blotting paper and pressed in herbarium press for 24 to 48 hrs for drying.

Mounting

The dried specimens were mounted on herbarium sheets of standard size (41 x 29 cm) with help of glue and cello-tape.

Preservation

The mounted specimens were sprayed with 2% solution of mercuric chloride fungicide.

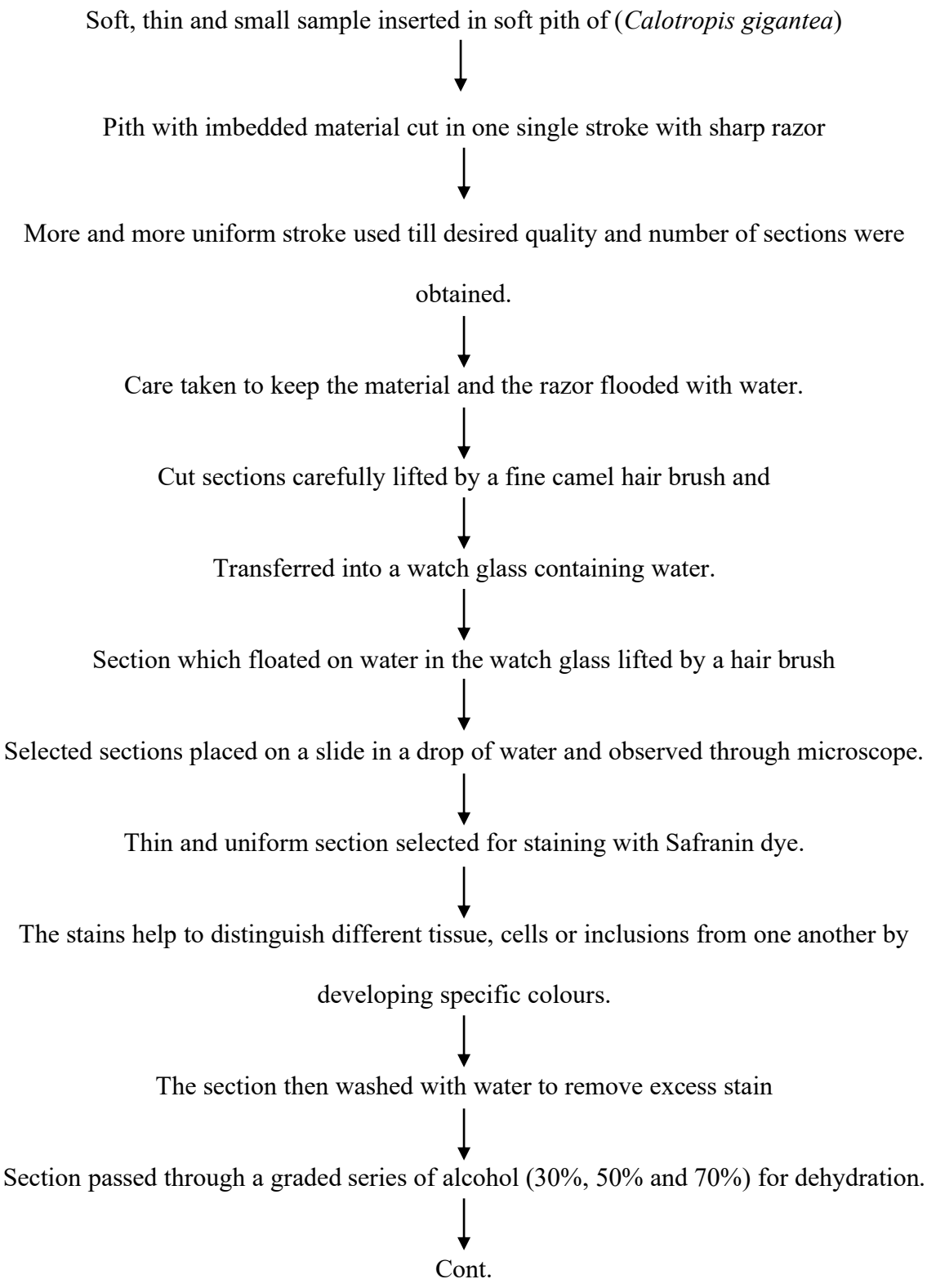
Labelling

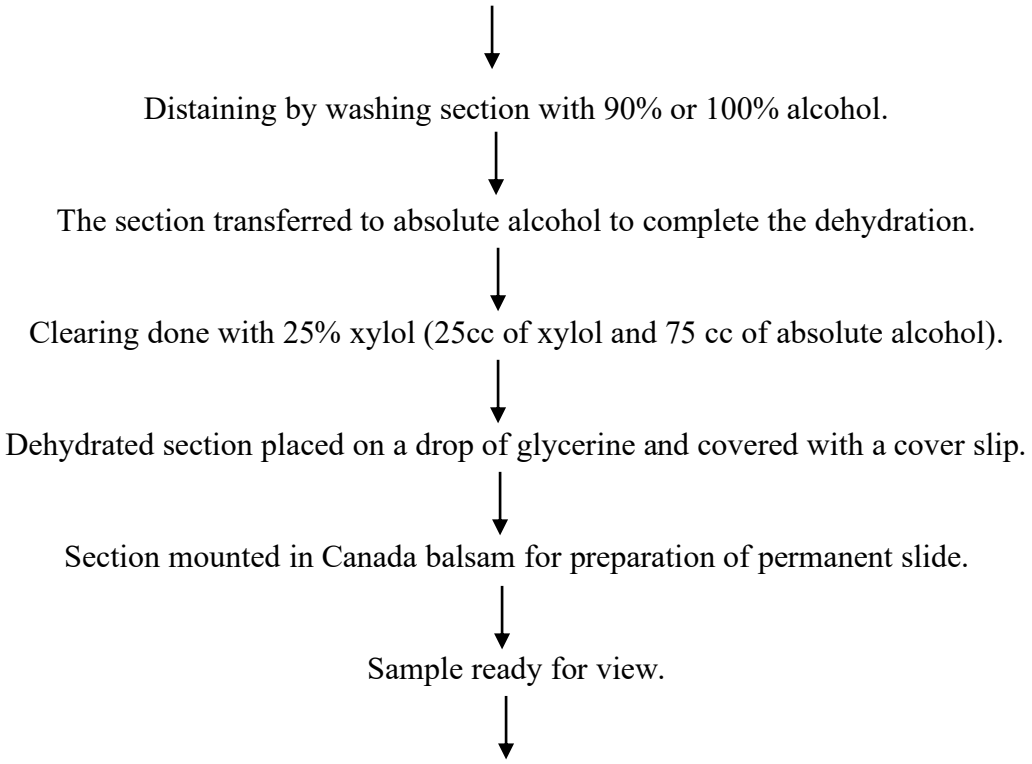
A label was pasted or printed on the lower right hand corner giving the information about the locality, altitude, habit, date and time of collection, name of collector, common name, complete scientific name etc.

3.2.2.1 Anatomical and stomatal studies

Sections of fresh plant material *viz.,* root, stem and leaf, were cut in transverse plane using a sharp razor for histological and anatomical studies in the microscopy laboratory of University Scientific Instrumentation Center, Babasaheb Bhimrao Ambedkar University, Lucknow using a Digital Binocular Microscope (BR Biochem Life Sciences Pvt. Ltd.). The procedure followed is presented in Fig 3.2.

Figure 3.2 Procedure for obtaining transverse sections of water chestnut (*Trapa natans* var. *bispinosa* Roxb.) plant parts *viz.,* root, stem and leaf





Bendre and Kumar (2016)

3.2.2.2 Stomatal study through Scanning Electron Microscopy (SEM)

Stomata are generally found on the abaxial surface of the leaf (Zaharah and Razi, 2009) and are reported to be under genetic control (Casson and Hetherington, 2010). Hence, the stomatal density and morphology in leaf of water chestnut were studied for further elucidation of the results obtained from morphological analysis of the sample plants. The samples for stomatal studies were prepared at the Ph.D. Laboratory, Department of Applied Plant Science (Horticulture), School for Biosciences and Biotechnology, as per procedure described in (Fig.3.3). Stomatal density and morphology was studied through the Scanning Electron Microscope (SEM) of *AKTA JEOL*, model JSM 6490 at University Scientific Instrumentation Center, Babasaheb Bhimrao Ambedkar University, Lucknow.

3.2.2.3 Stomatal studies for exploring inter varietal variability in water chestnut

Sample collection

Leaves of uniform age and physiological maturity at 6-8 internode from the apex of rosette were collected as per leaf sampling technique (Wolf, 1982) for studying the variations in stomatal study. The Specimen was chopped (2-4 mm) and fixed in 2.5% Glutaraldehyde Karnovsky's fixative for 2-6 hours at 4°C.

Washing

Sample was washed in 0.1 M Phosphate buffer solution for three changes each for 15 minutes at 4°C for removing unreacted fixative.

Dehydration of sample

Samples were dehydrated measured concentration of acetone to remove water from leaves

30% Acetone	30 minute
50% Acetone	30 minute
70% Acetone	30 minute
90% Acetone	30 minute
95% Acetone	30 minute
100% (Dry Acetone)	30 minute

(Dry Acetone = 30% $CuSO_4 .5H_2O$ + 70% absolute acetone)

All steps of dehydration were done at 4°C. After dehydration for protection of samples from excessive fluctuation in relative humidity, kept the sample in dry and dust free environment so for this, kept in desiccators.

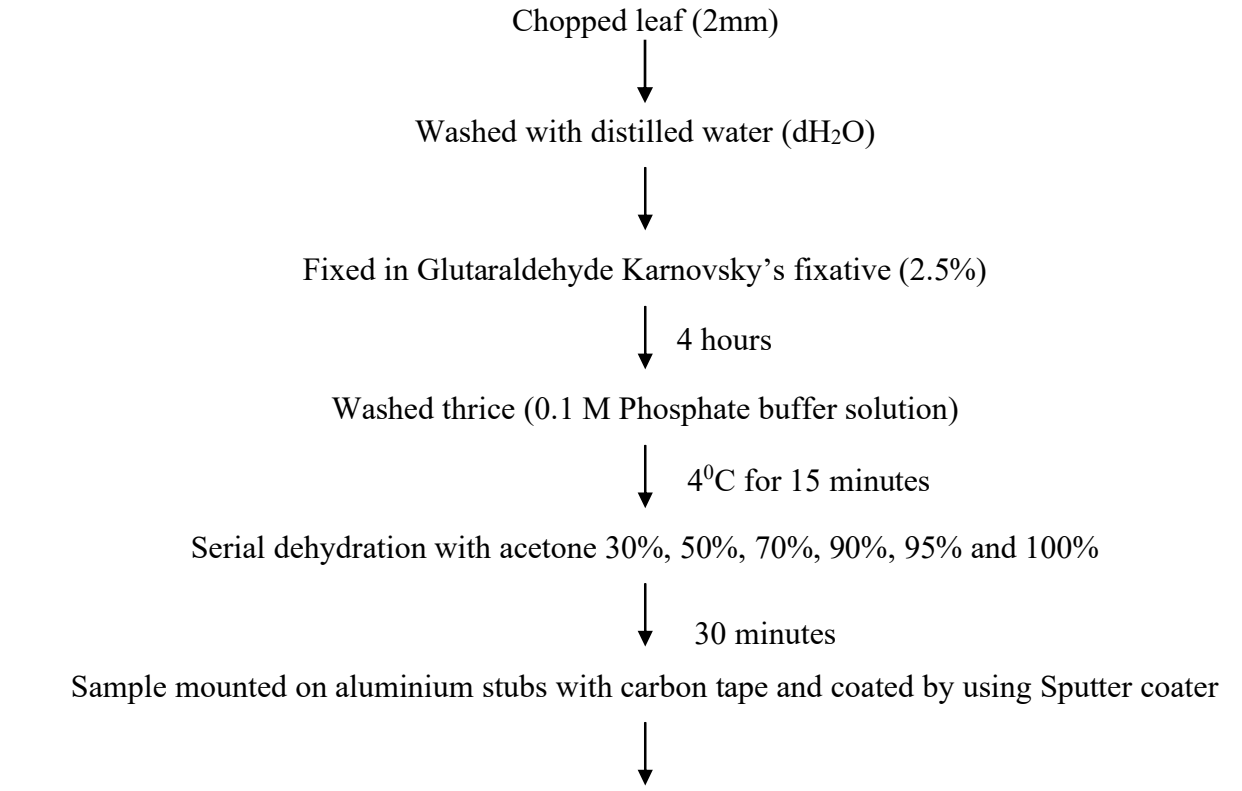
Sample mounting

Samples prepared as above, were mounted to on aluminium stubs with carbon tape.

Coating

Samples were coated by using sputter coater to make sample conductive, and were subsequently observed under Scanning Electron Microscope (*AKTA JEOL*, model JSM 6490) at 500x and 5000x.

Fig. 3.3 Flowchart for Scanning Electron Microscopy (SEM) of leaf water chestnut (*Trapa natans* var. *bispinosa* Roxb.)



(Modified from Fischer *et al.,* 2013)

Data generated was subjected to statistical analysis and the sample population was grouped on the basis of cluster analysis depicted through a dendrogram.

Observations recorded:

Stomatal length (µm)

Length of stomata was estimated to scale using Scanning Electron Microscope and data was expressed in micron (µm).

Stomatal width (µm)

Width of stomata was estimated to scale using Scanning Electron Microscope and data was expressed in micron (µm).

Stomatal pore length (µm)

Length of pore was estimated to scale using Scanning Electron Microscope and data was expressed in micron (µm).

Stomatal pore width (µm)

Width of pore was estimated to scale using Scanning Electron Microscope and data was expressed in micron (µm)

Stomatal density (μm^{-2})

Density of stomata were estimated visually (500 X) using Scanning Electron Microscope and data was expressed in numbers.

3.2.3 Morphological studies:

Studies were conducted on the morphological characters of the water chestnut germplasm collected and conserved in ponds at the Vocational Floriculture Farm, at Babasaheb Bhimrao Ambedkar University, Lucknow in order to explore possibility of the intervarietal variability in the germplasm. Vegetative growth characters *viz*., number of rosette per plant, rosette spread (north-south) cm, rosette spread (east-west) cm, number of leaves per plant, length of leaf (cm), width of lamina (cm), petiole length (cm), leaf pulvinus length (cm) and leaf pulvinus diameter (mm) etc. were measured using standard procedures for the above. Details of observations recorded and the methodology employed are given in detail below.

Observations recorded:

Observations were recorded for vegetative growth, bud initiation, flowering, fruiting and quality of fruits and the data were subjected to suitable statistical analysis.

Experimental details:

Experimental design	:	Randomized block design
Treatments	:	20
Replication	:	3

Vegetative growth parameters

Vegetative growth parameters were recorded as per standard methods which are discussed in detail three plants were tagged for recording observation.

3.2.3.1 Plant morphology:

Number of rosette per plant

Number of rosette were counted manually for replicated plant of each germplasm and average value was noted and calculated and express in per plant.

Rosette spread (N-S) cm

The rosette spread (north-south) cm was recorded by measuring scale for three rosette of each germplasm and average value was calculated and expressed in cm.

Rosette spread (E-W) cm

The rosette spread (east-west) cm was recorded by measuring scale from three rosette of each morphotypes and average value was calculated and expressed in cm.

3.2.3.2 Leaf parameters:

Leaves of uniform age and physiological maturity at 6-8 internode from the apex of rosette were collected as per leaf sampling technique (Wolf, 1982).

Number of leaves per plant

Number of leaves were counted from the apex part of the plant in each germplasm manually and average value were calculated and expressed as in per plant.

Length of leaf (cm)

The leaf length was recorded by measuring scale. Three leaves of each germplasm were selected and measured and average value was calculated and expressed in cm.

Length of lamina (cm)

The length of lamina was recorded by measuring scale. Three leaves of each germplasm were selected and measured and average value was calculated and expressed in cm.

Width of lamina (cm)

The width of lamina was recorded by measuring scale. Three leaves of each germplasm were selected and measured and average value was calculated and expressed in cm.

Petiole length (cm)

The petiole length was recorded by measuring scale. Three leaves of each germplasm were selected and measured and average value was calculated and expressed in cm.

Pulvinus length of leaf (cm)

The pulvinus length of leaf was recorded by measuring scale. Three leaves of each germplasm were selected and measured and average value was calculated and expressed in cm.

Pulvinus diameter of leaf (mm)

The pulvinus diameter of leaf was recorded using digital vernier callipers (Mitutoyo, Japan).Three leaves of each germplasm were selected and measured and average value was calculated and expressed in mm.

Pulvinus: petiole ratio

Pulvinus: petiole ratio was calculated by dividing pulvinus length with petiole length.

$$\text{Pulvinus: petiole ratio} = \frac{\text{Pulvinus length (cm)}}{\text{Petiole length (cm)}}$$

Experiment III

3.3 To establish intervarietal variability in water chestnut on the basis of physico chemical characteristics of the fruits:

Three fruits each were collected from each plant from each germplasm collected and conserved in the ponds at the Vocational Research Farm at Babasaheb Bhimrao Ambedkar University, Lucknow for evaluation of inter varietal variability inwater chestnuton the basis of physico-chemical studies of fruit. These were analysed for the various physical as well as biochemical quality parameters of the fruit as per procedures described below.

Experimental details:

Design	-	Completely Randomized Design (CRD)
Treatments	-	20
Replication	-	3

Observations recorded

3.3.1 Fruit morphological studies

Three fruits of each germplasm were collected from tagged plant in the month of November-December in both the years, from the pond from the selected plant and analysed for the fruit morphological characters *viz.,* number of fruits per rosette, fruit length (cm), fruit width (cm), fruit weight (g), fruit volume (ml), specific gravity of fruit, kernel weight (g), peel weight (g), kernel: peel ratio and peel thickness (mm) etc. in the Ph. D. Laboratory, Department of Applied Plant Science (Horticulture), School for Biosciences and Biotechnology, Babasaheb Bhimrao Ambedkar University, Vidya Vihar, Raebareli Road, Lucknow using standard methods which are described in detail below.

Number of fruits per rosette

Number of fruits per rosette was counted manually in three rosettes of each germplasm and counted and average value was calculated and expressed in per rosette.

Length of fruit (mm)

Three fruits from each germplasm were harvested at harvest maturity stage and length was measured using vernier calliper (Mitutoyo, Japan). Average length was calculated and expressed in mm.

Width of fruit (mm)

Three fruits from each germplasm were harvested at harvest maturity stage and length was measured using vernier calliper (Mitutoyo, Japan). Average width was calculated and expressed in mm.

Cheek diameter of fruits (mm)

Three fruits from each germplasm were harvested at harvest maturity stage and length was measured using vernier calliper (Mitutoyo, Japan). Average cheek diameter was calculated and expressed in mm.

Skin colour of fruits

The skin colour of water chestnut fruits was evaluated by a panel of five judges on visual basis.

Weight of fruits (g)

Three fruits from each germplasm were harvested at harvest maturity stage and weight was measured by digital balance and average weight was calculated and expressed in gram.

Volume of fruit (ml)

The volume of fruit was measured by using water displacement method. A wide mouth jar was filled up to the brim with water. Three fruits were immersed in the jar and the runoff water was collected and measured using measuring cylinder. Volume of water collected was equivalent to the volume of fruits immersed in the water. Average values was calculated and expressed as fruit volume in ml.

Specific gravity of fruit (g/cc)

Specific gravity of fruit was computed by following formula

$$\text{Specific gravity of fruit (g/cc)} = \frac{\text{Fruit weight (g)}}{\text{Fruit volume (ml)}}$$

Thickness of peel (mm)

Three fruits from each germplasm were harvested at harvest maturity stage and length was measured using vernier calliper (Mitutoyo, Japan). Average thickness was calculated and expressed in mm.

Weight of peel (g)

Three fruits from each germplasm were harvested at harvest maturity stage and weight was measured by digital balance and average weight was calculated and expressed in gram.

Length of kernel (mm)

Three fruits from each germplasm were harvested at harvest maturity stage and length was measured using vernier calliper (Mitutoyo, Japan). Average length was calculated and expressed in mm.

Width of kernel (mm)

Three fruits from each germplasm were harvested at harvest maturity stage and length was measured using vernier calliper (Mitutoyo, Japan). Average width was calculated and expressed in mm.

Cheek diameter of kernel (mm)

Three fruits from each germplasm were harvested at harvest maturity stage and length was measured using vernier calliper (Mitutoyo, Japan). Average cheek diameter was calculated and expressed in mm.

Weight of kernel (g)

Three fruits from each germplasm were harvested at harvest maturity stage and weight was measured by digital balance and average weight was calculated and expressed in gram.

Volume of kernel (ml)

The volume of kernel was measured by using water displacement method. A wide mouth jar was filled up to the brim with water. Three fruits were immersed in the jar and the runoff water was collected and measured using measuring cylinder. Average values was calculated and expressed as kernel volume in ml.

Specific gravity of kernel (g/cc)

Stone specific gravity was computed by following formula

$$\text{Specific gravity of kernel (g/cc)} = \frac{\text{Weight of kernel (g)}}{\text{Volume of kernel (ml)}}$$

Colour of kernel

The colour of kernel was recorded on the basis of visual observation by panel of five judges.

Kernel: peel ratio

Kernel: peel ratio was calculated by using the following formula.

$$\text{Kernel: peel ratio} = \frac{\text{Weight of kernel (g)}}{\text{Weight of peel (g)}}$$

3.3.2 Bio-chemical parameters of the fruits

Fruits were harvested in the end of November till mid-December in both the years and were analysed for the bio-chemical parameters *viz.,* total soluble solids (^{0}Brix), pH of the fruit juice, Titrable acidity (%), total sugars (%) reducing sugar (%) and non-reducing sugar (%) at harvest maturity in Ph. D. Laboratory, Department of Applied Plant Science (Horticulture), School for Biosciences and Biotechnology,

Babasaheb Bhimrao Ambedkar University, Vidya Vihar, Raebareli Road, Lucknow, as per standard procedures.

Total soluble solids (^{0} Brix)

The total soluble solids of fruit juice was determined by using a digital refractometer after calibration with distilled water. A small amount of fruit pulp was taken in muslin cloth and crushed. The refractometer was wiped clear with a moist muslin cloth. A drop of juice of crushed pulp was taken on refractometer and the value was recorded and expressed in ˚Brix applying correction factor at 20˚C (Rangana, 2008).

pH of the fruit

pH of the juice was measured with the digital pH meter.

Titratable acidity (%)

The titratable acidity was determined by the method as suggested by Ranganna (2008). 20g of fruit pulp was taken and volume was made up to 100 ml in a volumetric flask by adding distilled water. The content was filtered through Whatman No.1 filter paper. 10 ml of filtered juice titration against N/10 NaOH solution, using phenolphthalein as an indicator. Change of the solution colour to light pink indicated the end point and writes the reading of micro pipette and the titratable acidity was computed by the following formula acidity. The total titratable acidity was calculated in terms of citric acid on the basis of 1 ml of N/10 NaOH equivalent to 0.0064 g anhydrous citric acid. The result was expressed in terms of per

$$\text{Titratable acidity (\%)} = \frac{\text{Titer value x normality of NaOH x volume makeup}}{\text{Alliquot taken x weight of sample x 1000}} \text{ x } 100$$

Sugars were estimated as suggested by Ranganna (2008). The method is briefly explained as follow.

Sample preparation

A 25 g sample was taken and mixed with 100 ml distilled water and neutralized with 1 N NaOH. Two millilitre of lead acetate solution was added and allowed to stand for 10 minutes. Then 1 to 2 ml of potassium oxalate solution was added to neutralize the lead and volume was made up to 250 ml.

Standardization of the Fehling's solution

50 ml each of Fehling's solution A and B were mixed. 10 ml of mixed solution was pipetted into a 250 ml conical flask containing 50 ml of distilled water. Standard invert sugar solution was taken in 50 ml burette and titration was done. Invert sugar solution (18 to 19 ml) was added so that not more than one ml of invert sugar solution was used to complete titration. The flask was heated over a hot plate. Three drops of methylene blue indicator were added and the titration was completed in one minute. The decolourization indicates the end point.

$$\text{Factor of Fehling's solution} = \frac{\text{Titre value}}{1000} \times 2.5$$

Reducing sugar

Ten ml of mixed Fehling's solution was taken into 250 ml conical flask. Burette was filled with the prepared juice sample solution and titration was done. The contents of flask were mixed and boiled for 2 minutes. Then three drops of methylene blue solution were added. Titration was completed within one minute by adding 2 to 3 drops of prepared juice sample solution at 5 to 10 seconds interval until indicator was completely decolorized and ultimately turned into brick red colour. The reducing sugar was computed by the following formula.

$$\text{Reducing sugar (\%)} = \frac{\text{Factor x Dilution x 100}}{\text{Titre value x Wt. of sample or volume}}$$

Non-Reducing sugar

Non-Reducing sugar (%) = Total sugars – Reducing sugar

Total sugars

50 ml of clear juice extract was taken in a 250 ml conical flask. 5 g acid and 50 ml distilled water were added to it. It was boiled gently for 10 minutes to complete the inversion of sucrose into monosaccharides. The solution was neutralized with 1.0 N NaOH and volume was made up to 250 ml by adding distilled water. Fifty ml of aliquot of clarified and de-leaded solution was taken in a 250 ml flask. Ten ml of Hel was added and allowed to stand at room temperature for 24 hours. It was neutralized with concentrated NaOH solution and volume was made up. An aliquot was taken and total sugar was determined.

$$\text{Total sugars (\%)} = \frac{\text{Factor x dilution x 100}}{\text{Titre value x weight of sample or volume}}$$

Experiment IV

3.4 Molecular characterization of inter varietal variability in water chestnut

Intervarietal variability analysis in water chestnut (*Trapa natans* var. *bispinosa* Roxb.) through protein profiling

Inter varietal variability established on the basis of morphological, fruit and stomatal studies was further substantiated through molecular analysis of inter-varietal variability in water chestnut by protein profiling at Ph. D Laboratory, Department of Applied Plant Science (Horticulture), School for Biosciences and Biotechnology, Babasaheb Bhimrao Ambedkar University, Lucknow, Uttar Pradesh, India

3.4.1 Equipments and chemicals

- Power supply with adjustable constant voltage/current (100V, 500 mA)
- SDS-PAGE unit (CAVOY)
- Electronic balance (Instrument limited: CA-224)
- Gel rocker (L1-GR-E-100)
- Multispin Motorless Magnatic Stirrer (Tarsons: CAT. 4060)

- pH meter (Labman Scientific Instruments PVT. Limited: LMPH-10)
- A bench top refrigerated centrifuge (EITEK: 880-2049)
- Transilluminator for gel viewing (GENEI, Bangalore GeneiPvt. Ltd.)
- Refrigerator (Godrej Appliance Limited)
- Water bath (Gupta Scientific Industries)
- Micropipette (10 µl, 20 µl, 30 µl,50 µl,100 µl, 200 µl, 500 µl and 1000µl)

3.4.2 Collection and processing of leaf material

Tender and healthy tagged leaves from each germplasm from the water chestnut pond, were collected and wrapped in aluminium foil and placed in an icebox. Leaves were washed under tap water followed by distilled water, at Ph. D. Laboratory, Department of Applied Plant Science (Horticulture), School for Biosciences and Biotechnology, Babasaheb Bhimrao Ambedkar University, Lucknow, and cleaned with tissue paper. The midribs and thick veins of the leaves were removed and 500 mg leaf lamina sample was weighed on an electronic balance (Instrument limited: CA-224) and again wrapped in aluminium foil and stored at -20˚C temperature overnight. The sample was macerated in a pestle and mortar on the following day in the protein extraction buffer presented as per details below.

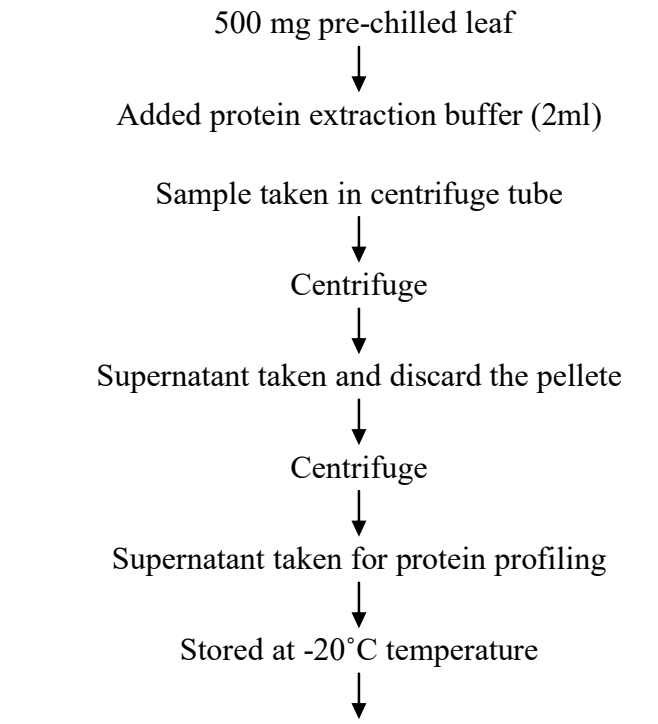
3.4.3 Preparation of protein extraction buffer (pH 8.0)

All chemicals used for the protein extraction were of Analytical grade produced by Genetics Private Limited).

0.1 M Tris HCl	1.576 g
0.01 M $MgCl_2$	0.216 g
Sucrose 18% (w/v)	18.0 g
Sodium dodicylsulfate (SDS) 4% (w/v)	4.0 g
0.4 M β mercaptoethanol	280 µl
Double distilled water	100 ml

Protein of the leaf sample was extracted as per procedure described below (Fig. 3.4)

Fig. 3.4 Procedure for Protein Extraction



3.4.4 Chemicals and preparation of stock solutions/buffers for Sodium Dodicyl Sulphate Poly Acrylamide Gel Electrophoresis (SDS-PAGE)

All chemicals used for SDS-PAGE were Analytical grade and were produced from Genetics, India. Solutions were prepared as per details given below:

Acrylamide/bis-acrylamide (30%)

Acrylamide (29.2g) and bis-acrylamide (0.8g) was mixed and dissolved in double distilled water (ddH_2O) to make volume up to 100ml.

TEMED solution (1ml)

N,N,N,'N'Tetramethylenthyenedaiamine (84µl) was mixed and dissolved in ddH_2O to make volume up to 1000ml.

Ammonium per sulfate solution (1ml)

Ammonium per sulfate (0.125g) was mixed and dissolved in ddH_2O to make volume up to 1.00ml.

Separating buffer (2X) pH (8.8)

Tris base (9.0g) and Sodium Dodecyl Sulphate (SDS) 0.2 g was mixed and dissolved in ddH_2O and pH was maintained at 8.8. Volume was made up to 100ml.

Stacking buffer (2X) pH (6.8)

Tris base (3.3 g) and SDS (0.2 g) was mixed and dissolved in ddH_2O and pH was maintained at 6.8. Volume was made up to 50ml.

Sample buffer (2x) pH (6.8)

Tris base (1.57g), glycine (20.0ml), SDS (4.0g), bromophenol blue (0.002g) and βmercaptoethanol (10.0ml) was mixed and dissolved in ddH_2O and pH was maintained at 6.8. Volume was made up to 10ml.

Commassiae blue staining solution (100 ml)

Methanol (40ml), acetic acid (10ml), commassiae (0.125 g) was mixed and dissolved in ddH_2O and volume was made up to 100ml.

De-staining solution (100 ml)

Methanol (40 ml) and acetic acid (10 ml) was mixed and dissolved in ddH_2O and volume was made up to 100ml

10x running buffer (pH 8.6)

Glysine (144.0g), Tris base (60.4g) and SDS (20.0g) was was mixed and dissolved in ddH_2O and pH was maintained 8.6 and volume was made up to 1000ml.

3.4.5 Preparation of gel for SDS-PAGE

Various solutions required for the preparation of gel were prepared as per details below:

Separating gel (12%): 10 ml

30% Acrylamide	3.96 ml
Separating gel buffer	6 ml
Double distilled water	40 µl

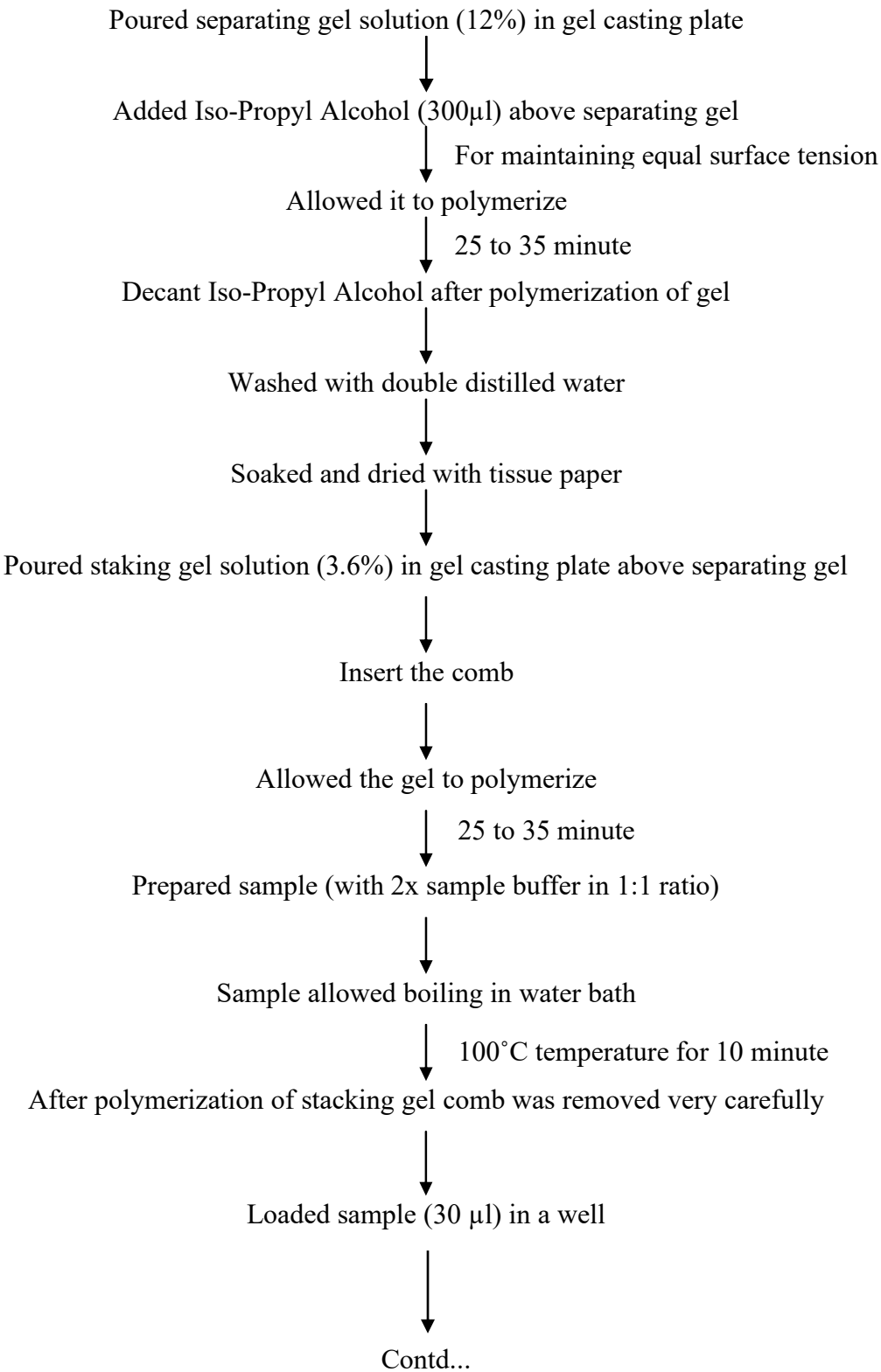
Ammonium per suphate (APS)	100 µl
N,N,N,'N' Tetramethylenthyenedaiamin (TEMED)	50 µl

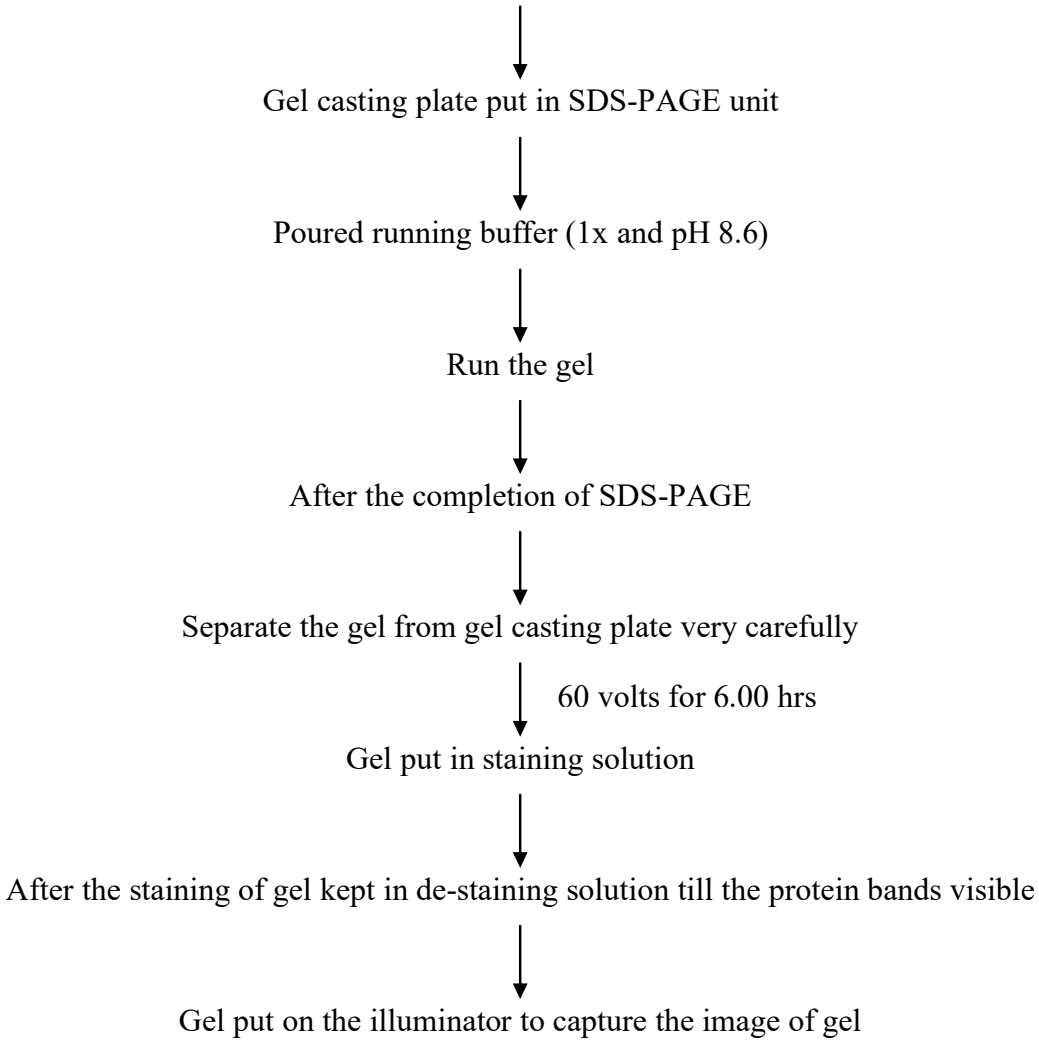
Stacking gel (3.6%): 10 ml

30%Acrylamide	1.2 ml
Stacking buffer	5.0 ml
Double distilled water make up to	3.8 ml
Ammonium per suphate (APS)	100 µl
N,N,N,'N' Tetramethylenthyenedaiamine (TEMED)	50 µl

Performing of SDS-PAGE was done as described by Laemmli (1970) (Fig. 3.5) has been presented in following procedure.

Fig. 3.5 Procedure for SDS-PAGE





The molecular weight was estimated by comparing with standard four colour Prestained Protein Ladder (Puregene, Genetix Biotech Asia Pvt. Ltd.).

3.5 Statistical Analysis

The experimental data was compiled by taking mean values for the various parameters under study recorded from randomly selected plants. These were recorded in respect to various characters *viz.,* vegetative, fruit physico-chemical and stomatal traits. The following statistical parameters were calculated using ICAR-SPAR (Statistical Package for Agricultural Research).

Range

It is the difference of the least and the highest value.

Mean

Arithmetic mean was calculated for each character by using the following formula

$$\overline{X} = \frac{\sum_i^n X_i}{N}$$

Where,

$\overline{X}$ = mean

X_i = value of i^{th} plant for a character

n = number of plants

Variance

It is estimated as mean squared deviation as given below

$$V_X = \frac{\sum X^2 - \frac{(\sum X)^2}{n}}{n-1}$$

Standard deviation

It is square root of variance

$$SD = \sqrt{V_x}$$

Coefficient of variation (CV)

It is percent ratio of SD of a sample to its mean

$$CV = \frac{SD}{\overline{X}} \times 100$$ SD = standard deviation

Standard error

It is the measure of mean difference between sample mean and population mean. It is measure of uncontrolled variation present in sample.

$$SE(m) \pm = \frac{SD}{\sqrt{N}} = \sqrt{V_E\left(\frac{1}{r_1} + \frac{1}{r_2}\right)}$$

V_E = Error variance

r_1 and r_2 = number of observation on which two means are based

If $r_1 = r_2 = r$

$$SE(d) \pm = \sqrt{\frac{2\,V_E}{r}}$$

Critical difference

It is the least significant difference equal to or greater than which all the differences are significant.

$$CD = SE_{(d)} \times t \text{ value (at error d.f.)} = \left[\sqrt{\frac{2V_E}{r}}\right] \times t$$

Analysis of variance

The mean value of the characters from each germplasm in each replication was used for statistical analysis (Sahu and Das, 2014). Data recorded in both the years and pooled data were analysed separately by using Completely Randomized Design (CRD).

Table 3.1 Analysis of variance (ANOVA)

Source of variation	Degree of freedom	Sum of square	Mean sum of square	F-ratio	Tabulated F (0.05)	Tabulated F (0.01)
Treatment	t-1	TrSS	$TrMS = \frac{TrSS}{t-1}$	$\frac{TrMS}{ErMS}$		
Error	n-t	ErSS	$ErMS = \frac{ErSS}{n-t}$			
Total	n-1	TSS				

Biometrical Techniques in Plant Breeding

Biometry or biometrics is the science that deals with the application of statistical procedure to the study of biological problems. Similarly, biometrical genetics is that branch of genetics, which attempt to unravel the inheritance of quantitative traits using statistical concept and procedure it is also known as quantitative genetics for obvious reason. The various statistical procedures employed in biometrical genetics are called biometrical techniques which outline the type of genetic information obtained from each biometrical technique, and the manner in which that information is helpful in plant breeding programmes. Biometrical techniques are useful to the plant breeders in the assessment of genetic variability present in a population and include the followings *viz.,* range, variance standard deviation, coefficient of variation, PCV, GCV, heritability, genetic advance and genetic advance as percent of mean (Singh, 2000).

In the present study heritability (h^2%) was estimated according to Falconer (1989). Phenotypic coefficient of variation (%) and genotypic coefficient of variation (%) to compare the variations among the traits were computed as per the method suggested by Singh and Chaudhary (1985). Genetic advance (GA %) and genetic advance as percent of mean (GAM %) were calculated as per procedure recommended by Singh and Chaudhary (1985) and Allard (1960) for the study conducted during 2016-18 for pooled data.

Cluster analysis was performed (SPSS software) and a dendrogram was prepared on the basis of stomatal and protein profiling parameters using SPSS software for genetic relationships among the germplasm.

4. Experimental Findings

The experimental results of the present investigation entitled **"Morphological and Molecular Analysis of Intervarietal Variability in Water Chestnut (*Trapa natans* var. *bispinosa* Roxb.) in Central Uttar Pradesh''** was conducted in the Vocational Floriculture Farm, Department of Applied Plant Science (Horticulture), Babasheb Bhimrao Ambedkar University, Lucknow, Vidya Vihar, Raebareli Road, Lucknow during the year 2016-2018, over 20 diverse germplasm of water chestnut (*Trapa natans* var. *bispinosa* Roxb.).

The study was divided into two parts. In part I a survey was conducted at the ponds of farmers growing water chestnut in five blocks of district Lucknow viz., Mohanlalganj, Gosainganj, Sarojini nagar, Bakshi Ka Talab and Malihabad. Plants of the promising cultivars were collected from each pond based on the feedback of farmers and were established and conserved in ponds at the Vocational Floriculture Farm, Department of Applied Plant Science (Horticulture), School for Biosciences and Biotechnology, Babasaheb Bhimrao Ambedkar University, Lucknow, Uttar Pradesh, India.

In part II of the study, a herbarium of plants collected was prepared and botanical descriptors for water chestnut (*Trapa natans* var. *bispinosa* Roxb.) were developed. The collected plants were evaluated for their vegetative performance, fruit physico-chemical, anatomical and stomatal parameters as well as molecular studies were carried out at the Research Laboratory of Department of Applied Plant Science (Horticulture). Anatomical and stomatal studies were performed at University Scientific Instrumentation Center (USIC), Babasaheb Bhimrao Ambedkar University, Lucknow. Result have been described in detail in this chapter under appropriate heads and sub-heads. Observations were recorded under experiments for different vegetative parameters, fruit and kernel morphology, fruit biochemical parameters, Scanning Electron Microscopy (SEM) and protein profiling of different water chestnut germplasm. The mean data were subjected to various statistical analyses. The results have been presented clearly through appropriate tables and further elucidated through geographical presentations and plates as required objective wise.

EXPERIMENT I

4.1 Survey and collection of germplasm of water chestnut from the various blocks of district Lucknow.

A survey was conducted in various blocks *viz.,* Mohanlalganj, Gosainganj, Sarojini nagar, Bakshi Ka Talab and Mlihabad of district Lucknow. According to farmer feedback water chestnut is an annual aquatic plant forming dense mats on the surfaces of lakes, ponds and slow moving waters. As most of the cultivation is done by traditional methods, lack of scientific knowledge of cultivation is also considered as one of the major constraints in popularity of this crop. The traditional system of cultivation of water chestnut is very easy and provides the farmers opportunities to cultivate cereals and other field crops in the same fields in a year. The fully mature nuts are placed in container with little water to germinate the seeds. The sprouted seeds are sorted out and broadcast in the nursery tanks. At the beginning of monsoon, the seedlings are lifted from the nursery tanks and planted in pond, when the soil of the pond is fertile. Through this system, the duration of this crop is reduced up to the six months, although this practice by farmers creates a mixture of germplasm in the cultivation ponds because of which there are no standard varieties of water chestnut released till now. Whereas, on basis of peel colour like green, red or purple and a blending of red and green colour are recognized and as well known by the local names given by the farmers. It is propagated mainly through seed. A single seed given 10–15 rosettes and each rosette may produce as many as 20 fruits. Information was collected through the feedback of water chestnut growing farmer during June-July, 2016 to explore the possibility of existence of intervarietal variability in water chestnut. During the exploration water logged condition was prevalent in Mohanlalganj area and most of the locations in the vicinity along the Sharda Canal, which is a manmade canal of the Irrigation Department of the State.

The survey of Mau village revealed that water chestnut was grown in shallow pond because of the water logged areas. A large part of the blocks are covered by pond and lake. There were two types of ponds: (i) shallow/temporary and (ii) deep/ permanent where water chestnut were grown. Three varieties were preferred for cultivation in this area on the basis of colour *viz.*, Kadama (Green), Saccharchini (Green) and Deshi (Red) of water chestnut in Block of Mohanlalganj as shown in Table 3.1.

Similarly, after survey of Gosainganj block, village Bhatwara and Kewadi (Sataikhera) areas were identified. Water logged areas were found and farmer informed that local varieties of water chestnut *viz.,* Deshi Lalla, Kadama Green, Saccharchini, Deshi, Green and Singhree etc. were grown on commercial level. Saccharchini is a sweetest variety of water chestnut.

A survey was conducted in village Nutcut block of Sarojininagar area where water chestnut is grown by farmers in deep/permanent ponds. Based on the fruit colour, quality and yield performance farmers informed that local varieties *viz.,* Saccharchini, Kadama Green and Singhdree etc. were preferred for cultivation in these areas. In block Baksi Ka Talab villages Maghut (Deorai) and Itaunja (Mohara) area were identified where water chestnut farmers grow varieties Saccharchini, Deshi Lalla and Kadama Green. Survey area of Malihabad revealed water chestnut cultivation in village Kasmandi Kala and Kethai para. The farmers preferred some varieties *viz.,* Deshi Lalla, Kadama Green, Deshi Green and Singhree.

EXPERIMENT II

4.2 Inter-varietal variability in water chestnut on the basis of botanical descriptors i.e. root, leaf, stem, flower, fruit and seeds.

Intervarietal variability in water chestnut was evaluated by studying variability in the vegetative parameters on the basis of botanical descriptors i.e. root, leaf, stem, flower and fruit, through anatomical studies of the different plant parts and through stomatal anatomy as per details below:

d. Botanical descriptors through herbarium and anatomy studies.
e. Stomatal anatomy studies through Scanning Electron Microscope (SEM).
f. Morphological studies of vegetative stage of plant.

4.2.1 Botanical descriptors through herbarium

Twenty superior germplasm established in the experimental ponds as discussed earlier, were utilized for the botanical studies through plant morphological and anatomical characterization. Inter-varietal variability studies on the basis of visual observations through herbarium samples collected from the experimental ponds were done based on the descriptors for different plant parts like leaves, flower, fruit and whole plant.

Roots: - Roots were found submerged terrestrial assimilatory which contain chlorophyll. These are green, aerial, adventitious roots which prepare food materials through photosynthesis (Table 4.1). *Trapa* has no primary root system, just the adventitious roots that extend from the hypocotyls. Although the most important function of the roots is to absorb nutrients, they also provide an anchor for plant. Its main root system adheres in the muddy soils at the bottom of the pond and it is connected with floating leaves by herbaceous stems in water body.

Stem: - Stem was cylindrical and flexuous, ascending in nature (Table 4.1) and 1 to 5 m long, nodes of the stem have slender linear roots while the plant is anchored in the sediment by the lower roots that emerged from propagating seed hull. The small rosettes remain submerged as the stems elongate to reach the water surface from June to September when the rosettes are present on the water surface.

Leaves: - The leaves were free-floating, hydrophytic and spongy in nature, arranged in a rosette. The leaves were alternate and feather like and long (up to the 11.93 to 19.78 cm were recorded). The average petiole length was recorded from 7.02-13.34 cm as presented in Table 4.2. Generally, leaf shape were found rhombic to rhombic ovate, rhombic deltoid and in some morphotypes found fan shaped. Margin of lamina was noted as serrate inciso and light serrate and abaxial surface of the leaves was found to vary from pale green which was most common, to dark red and dark green and reddish purple beneath is shown in Plate 4.1. A rosette spread north-south (22.10-32.83cm) and east- west (20.72-32.18cm) were recorded on the surface of the water. These submersed leaves drop early and were replaced by pairs of fine, pinnate structures up to 8 cm long.

Flowers: - Inflorescence has inconspicuous, solitary axillary, pedicillate, white in colour, complete flowers were found (Table 4.3 and Plate 4.3) formed in the axils of the surfacing leaves in July, continues until the plants are killed by frost. Four white petals and four green sepals, superior ovary, six androecium and monogynoecious ovary and opening above the surface of water in the afternoon. After pollination, one of two locules and its ovule abort and the peduncle bends down into the water where a unilocular, one-seeded fruit develops.

Fruits: - Flowers were converted into fruits in approximately one week. Fruits were of medium size, triangular in shape, having two spiny horns. 11.67-25.50 fruits were observed per rosette. Fruit length was recorded from 27.51 to 37.45 mm and width

from 27.65 to 36.62 mm as presented in Table 4.3. Fruits colour were showed green to greenish red and red is shown in Plate 4.2. Fruits generally raised in September to November and were released by the rotting peduncles.

Table 4.1 Botanical descriptors for root and stem of 20 superior germplasm of water chestnut (*Trapa natans* var. *bispinosa* Roxb.) collected from various blocks of district Lucknow

Germplasm	Root	Stem	
	Adventitious root	Shape	Nature
T_1	Assimilatory	Cylindrical	Flexuous ascending
T_2	Assimilatory	Cylindrical	Flexuous ascending
T_3	Assimilatory	Cylindrical	Flexuous ascending
T_4	Assimilatory	Cylindrical	Flexuous ascending
T_5	Assimilatory	Cylindrical	Flexuous ascending
T_6	Assimilatory	Cylindrical	Flexuous ascending
T_7	Assimilatory	Cylindrical	Flexuous ascending
T_8	Assimilatory	Cylindrical	Flexuous ascending
T_9	Assimilatory	Cylindrical	Flexuous ascending
T_{10}	Assimilatory	Cylindrical	Flexuous ascending
T_{11}	Assimilatory	Cylindrical	Flexuous ascending
T_{12}	Assimilatory	Cylindrical	Flexuous ascending
T_{13}	Assimilatory	Cylindrical	Flexuous ascending
T_{14}	Assimilatory	Cylindrical	Flexuous ascending
T_{15}	Assimilatory	Cylindrical	Flexuous ascending
T_{16}	Assimilatory	Cylindrical	Flexuous ascending
T_{17}	Assimilatory	Cylindrical	Flexuous ascending
T_{18}	Assimilatory	Cylindrical	Flexuous ascending
T_{19}	Assimilatory	Cylindrical	Flexuous ascending
T_{20}	Assimilatory	Cylindrical	Flexuous ascending

Table 4.2 Botanical descriptors for leaf of 20 superior germplasm of water chestnut (*Trapa natans* var. *bispinosa* Roxb.) collected from various blocks of district Lucknow

Germplasm	Leaves								
	Shape	Margin of lamina	Nature	Average length of leaf (cm)	Average length of lamina (cm)	Average width of lamina (cm)	Leaf petiole length (cm)	Abaxial coloure	Adaxial surface
T_1	Rhombic deltoid	Light serrate	Spongy	16.40	5.62	7.50	11.60	Dark red	Pubescence
T_2	Rhombic ovate	Serrate inciso	Spongy	12.87	4.55	5.09	7.65	Light red	Pubescence
T_3	Triangular	Light serrate	Spongy	13.49	4.54	5.45	9.47	Pale green	Pubescence
T_4	Rhombic ovate	Serrate inciso	Spongy	12.50	4.20	4.22	7.02	Dark green	Pubescence
T_5	Rhombic deltoid	Light serrate	Spongy	16.90	4.73	5.82	12.29	Pale green	Pubescence
T_6	Rhombic deltoid	Light serrate	Spongy	19.78	6.43	8.78	13.34	Dark green	Pubescence
T_7	Rhombic deltoid	Light serrate	Spongy	18.44	5.14	6.87	13.18	Red	Pubescence
T_8	Rhombic	Light serrate	Spongy	17.04	5.12	6.80	12.95	Pale green	Pubescence
T_9	Rhombic	Light serrate	Spongy	17.22	5.55	5.65	12.35	Red	Pubescence
T_{10}	Rhombic ovate	Serrate inciso	Spongy	17.30	5.29	7.69	12.67	Dark green	Pubescence
T_{11}	Rhombic ovate	Light serrate	Spongy	16.20	5.15	5.77	10.28	Dark green	Pubescence
T_{12}	Rhombic	Serrate inciso	Spongy	16.79	5.53	8.53	12.53	Pale green	Pubescence
T_{13}	Rhombic	Serrate inciso	Spongy	13.30	4.22	5.25	9.29	Light red	Pubescence
T_{14}	Rhombic	Serrate inciso	Spongy	13.79	4.99	5.67	9.23	Pale green	Pubescence
T_{15}	Rhombic	Serrate inciso	Spongy	13.14	4.69	5.83	9.35	Dark red	Pubescence
T_{16}	Rhombic ovate	Serrate inciso	Spongy	11.93	4.30	5.43	7.47	Dark red	Pubescence
T_{17}	Rhombic	Serrate inciso	Spongy	14.87	4.55	5.45	9.33	Dark red	Pubescence
T_{18}	Rhombic	Serrate inciso	Spongy	16.17	4.70	5.82	10.67	Pale green	Pubescence
T_{19}	Fan shape	Light serrate	Spongy	17.09	4.78	6.38	12.10	Pale green	Pubescence
T_{20}	Rhombic deltoid	Light serrate	Spongy	17.40	4.52	6.10	12.85	Dark red	Pubescence

Plate 4.1 Inter varietal variability in leaf in twenty germplasm of water chestnut (*Trapa natans* var. *bispinosa* Roxb.) collected from the various blocks of district Lucknow

Table 4.3 Botanical descriptors for flowers of 20 superior germplasm of water chestnut (*Trapa natans* var. *bispinosa* Roxb.) collected from various blocks of district Lucknow

Germplasm	Complete flower							
	Inflorescence	Colour	Pedicellate	Sepals	Petals	Androecium	Gynoecium	Ovary
T_1	Solitary axillary	White	Present	4	4	6	1	Superior
T_2	Solitary axillary	White	Present	4	4	6	1	Superior
T_3	Solitary axillary	White	Present	4	4	6	1	Superior
T_4	Solitary axillary	White	Present	4	4	6	1	Superior
T_5	Solitary axillary	White	Present	4	4	6	1	Superior
T_6	Solitary axillary	White	Present	4	4	6	1	Superior
T_7	Solitary axillary	White	Present	4	4	6	1	Superior
T_8	Solitary axillary	White	Present	4	4	6	1	Superior
T_9	Solitary axillary	White	Present	4	4	6	1	Superior
T_{10}	Solitary axillary	White	Present	4	4	6	1	Superior
T_{11}	Solitary axillary	White	Present	4	4	6	1	Superior
T_{12}	Solitary axillary	White	Present	4	4	6	1	Superior
T_{13}	Solitary axillary	White	Present	4	4	6	1	Superior
T_{14}	Solitary axillary	White	Present	4	4	6	1	Superior
T_{15}	Solitary axillary	White	Present	4	4	6	1	Superior
T_{16}	Solitary axillary	White	Present	4	4	6	1	Superior
T_{17}	Solitary axillary	White	Present	4	4	6	1	Superior
T_{18}	Solitary axillary	White	Present	4	4	6	1	Superior
T_{19}	Solitary axillary	White	Present	4	4	6	1	Superior
T_{20}	Solitary axillary	White	Present	4	4	6	1	Superior

Table 4.4 Botanical descriptors for fruit of 20 superior germplasm of water chestnut (*Trapa natans* var. *bispinosa* Roxb.) collected from various blocks of district Lucknow

Germplasm	Fruits						Seeds/ kernel
	Type	Colour	Average length (mm)	Average width (mm)	Average fruit pedicel length (cm)	Number of spines	Colour
T_1	Nut	Light red	33.87	33.41	4.92	2	Creamy white
T_2	Nut	Dark red	32.23	36.31	4.43	2	Yellowish cream
T_3	Nut	Reddish green	27.51	28.83	5.24	2	Creamy white
T_4	Nut	Dark green	29.39	27.68	4.74	3	White
T_5	Nut	Reddish green	29.19	36.62	4.46	2	White
T_6	Nut	Reddish green	33.84	31.87	4.32	2	Creamy white
T_7	Nut	Dark red	30.43	29.49	4.72	2	Creamy white
T_8	Nut	Pale green	30.97	28.30	4.54	2	Yellowish cream
T_9	Nut	Dark red	37.03	34.24	4.36	2	Creamy white
T_{10}	Nut	Dark green	29.47	27.65	4.18	2	Yellowish cream
T_{11}	Nut	Pale green	27.69	29.30	4.21	2	White
T_{12}	Nut	Reddish green	28.82	28.63	4.47	2	Yellowish cream
T_{13}	Nut	Greenish red	27.96	28.06	4.25	2	White
T_{14}	Nut	Pale green	32.33	29.39	4.10	2	White
T_{15}	Nut	Greenish red	28.97	27.81	4.76	2	White
T_{16}	Nut	Dark red	32.60	31.35	4.46	2	White
T_{17}	Nut	Dark green	37.07	34.37	4.70	2	Creamy white
T_{18}	Nut	Pale green	33.78	32.27	5.16	2	Creamy white
T_{19}	Nut	Reddish green	31.43	31.33	5.13	2	Creamy white
T_{20}	Nut	Greenish red	37.45	35.70	5.17	2	Creamy white

Plate 4.2 Inter varietal variability in fruit of twenty germplasm of water chestnut (*Trapa natans var. bispinosa* Roxb.) collected from the various blocks of district Lucknow

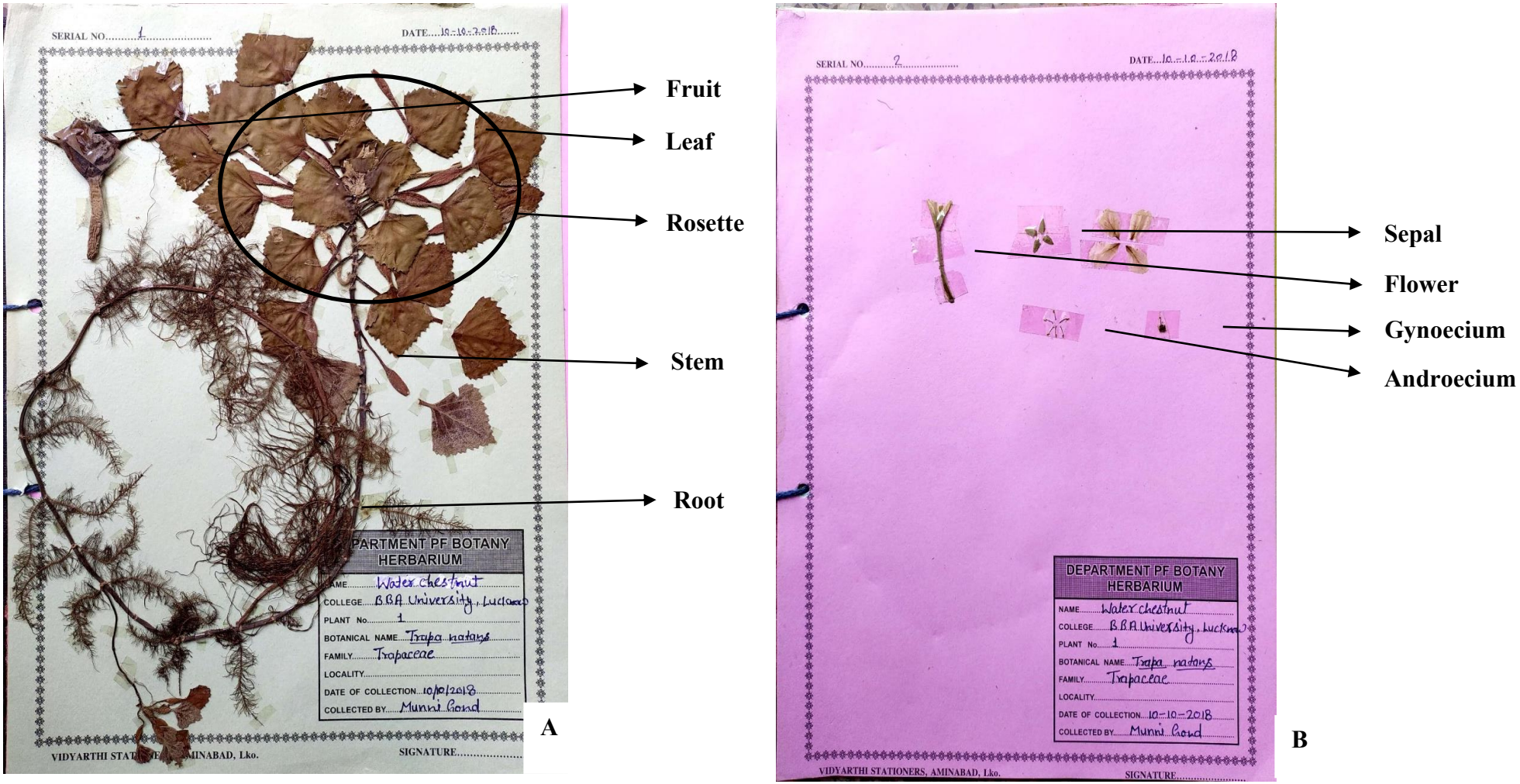
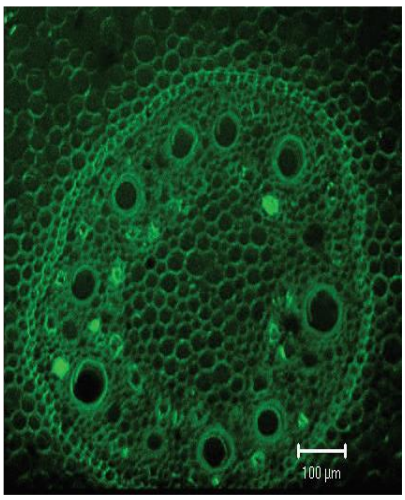


Plate 4.3 A and B herbarium prepared of the water chestnut (*Trapa natans* var. *bispinosa* Roxb.) plant for botanical aspects

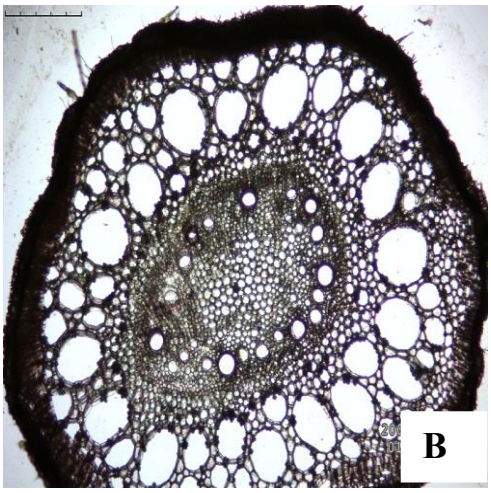
4.2.2 Anatomical and stomatal studies:

Inter-varietal variability studies on the basis of visual observations through herbarium samples collected from the experimental ponds were done based on the descriptors for different plant parts like leaves, flower, fruit and whole plant. Subsequently, in this study anatomical of different plant parts and stomata were done through section cutting of plant part *viz.,* root, stem, leaf and pulvinus and scanning electron microscopy (SEM), respectively. Anatomical studies show that lateral roots contained only one strand of xylem and phloem. The upper stem swelling had a lacunate pith and four or five rings of air apace in the cortex whereas the remaining pith is compact having only two rings of cortical lacunae in the lower stem. In case of leaves stomata were observed in the upper epidermis of the leaves and are absent on the adaxial surface**.** Vascular tissues were generally poorly developed in the leaves. Spongy tissue, air chamber were visible in root, stem and leaf section.

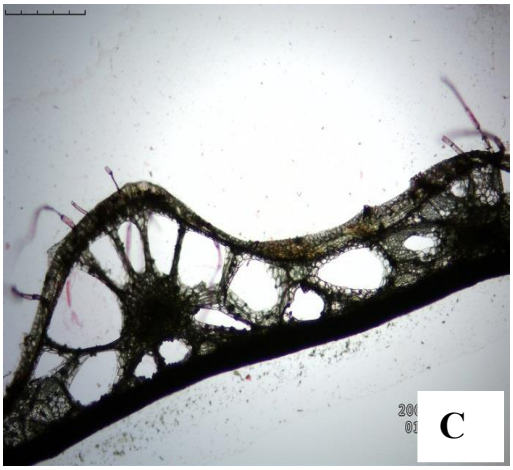


A

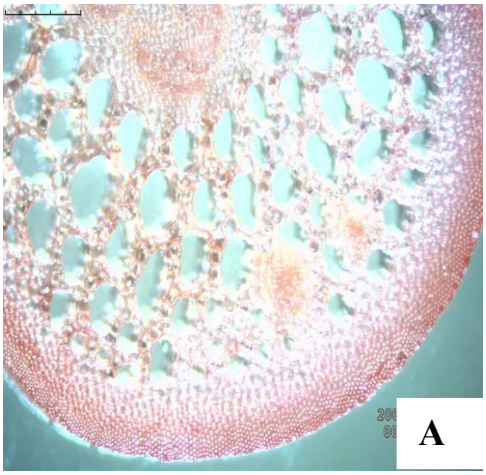
Root



Stem



Leaf



Pulvinus

Plate 4.4 (A, B, C, D) Anatomical structure of root, stem, leaf and pulvinus of water chestnut (*Trapa natans* var. *bispinosa* Roxb.) plant collected from various blocks of district Lucknow

Stomatal data were recorded for analysis of inter-varietal variability study through stomatal characters *viz.,* stomatal density (μm^{-2}), stomatal length (µm), stomatal width (µm), stomatal pore length (µm) and stomatal pore width (µm).

Stomatal length (µm)

A significant variation was recorded in stomatal length of 20 twenty germplasm under the study and varied between 11.48 to17.43 µm with a grand mean 14.12 µm (Table 4.6). However, the maximum stomatal length (17.43 µm) was recorded for T_{19} followed by for T_5 (17.00 µm) and T_6 (16.0 µm). Therefore, the minimum stomatal length (11.48 µm) was recorded from T_{20} followed by T_8 (11.53 µm). Based on stomatal length T_5 and T_{19} were superior among the germplasm and presented in Table 4.5 and Fig.4.1A.

Stomatal width (µm)

Stomatal width showed wide variation among various germplasm under the study and ranged from 4.68 to 8.05 µm with a grand mean 6.25 µm (Table 4.6). The maximum stomatal width (8.05 µm) was observed from T_{14} closely followed by T_{18} (7.52 µm), T_7 (7.36 µm) and T_9 (6.76 µm) while the minimum stomatal width (4.68 µm) was observed for T_3 followed by T_{11} (4.76 µm) as shown in Table 4.5 and Fig. 4.1A.

Stomatal pore length (µm)

Stomatal pore length showed significant variation and ranged from 6.64 to 13.52 µm with a grand mean 9.55 µm (Table 4.6). Selection-19 showed the maximum stomatal pore length (13.52µm) was observed for germplasm followed by T_5 (12.81 µm), T_6 (12.78 µm), T_{12} (11.27 µm) and T_{11} (16.78 µm) and the minimum stomatal pore length (6.64 µm) was observed for T_{20} followed by T_7 (6.94 µm). The results showed in Table 4.5 and Fig. 4.1B.

Stomatal pore width (µm)

Stomatal pore width showed significant variation between different germplasm during study and varied between 1.40 to 5.16 µm and grand mean of 2.72 µm (Table 4.6). The widest stomatal pore width (5.16 µm) was recorded from T_6 followed by T_{18} (3.83 µm), T_8 (3.72), T_{16} (3.64) and T_2, T_5 and T_{14} 6.44. However, the lowest stomatal pore width (1.40 µm) was recorded from T_3 followed by T_6 (1.60 µm) as presented in Table 4.5 and Fig. 4.1B.

Stomatal density (µm^{-2})

Stomatal density number of stomata per microscopic field at 500X (magnifications) differed significantly among morphotypes. Stomatal density ranging from 25 to 50 µm^{-2} with a grand mean of 40.43 µm^{-2} (Table 4.6). The highest stomatal density (50 µm^{-2}) was recorded from T_{14} followed by T_{13} (40.00µm^{-2}), T_3 (47.0) and T_{20} (46.0 µm^{-2}). However, the lowest stomatal density was recorded from T_1 (25.0 µm^{-2}) followed by T_5 (29.0µm^{-2}) which was at par with each other and presented and shown in Table 4.5 and Fig. 4.2.

Table 4.5 Inter varietal variability in water chestnut (*Trapa natans* var. *bispinosa* Roxb.) collected from various blocks of district Lucknow on the basis of stomatal parameters

Germplasm	Stomatal density (µm^{-2})	Stomatal length (µm)	Stomatal width (µm)	Stomatal pore length (µm)	Stomatal pore width (µm)
T_1	25	13.73	7.36	9.12	2.48
T_2	38	13.88	6.04	9.91	3.44
T_3	47	12.95	4.68	8.68	1.40
T_4	31	15.24	6.56	8.68	2.20
T_5	29	17.00	6.28	12.81	3.44
T_6	39	16.00	6.68	12.78	5.16
T_7	46	12.55	6.85	6.94	1.84
T_8	44	11.53	6.4	8.08	3.72
T_9	40	14.88	6.76	8.42	3.04
T_{10}	39	14.28	5.29	8.54	1.92
T_{11}	38	14.44	4.76	10.78	1.76
T_{12}	35	15.44	6.76	11.27	1.60
T_{13}	48	11.80	5.44	8.24	2.72
T_{14}	50	13.33	8.05	8.22	3.44
T_{15}	42	15.73	5.48	10.61	2.56
T_{16}	42	13.20	6.52	8.59	3.64
T_{17}	44	13.40	6.56	9.36	6.56
T_{18}	44	14.16	7.52	10.28	3.83
T_{19}	42	17.43	5.53	13.52	1.84
T_{20}	46	11.48	5.92	6.64	2.36
SE(m)±	1.55	0.75	0.36	0.76	1.55
CD (p=0.05)	**2.06**	**1.35**	**0.64**	**1.36**	**2.79**

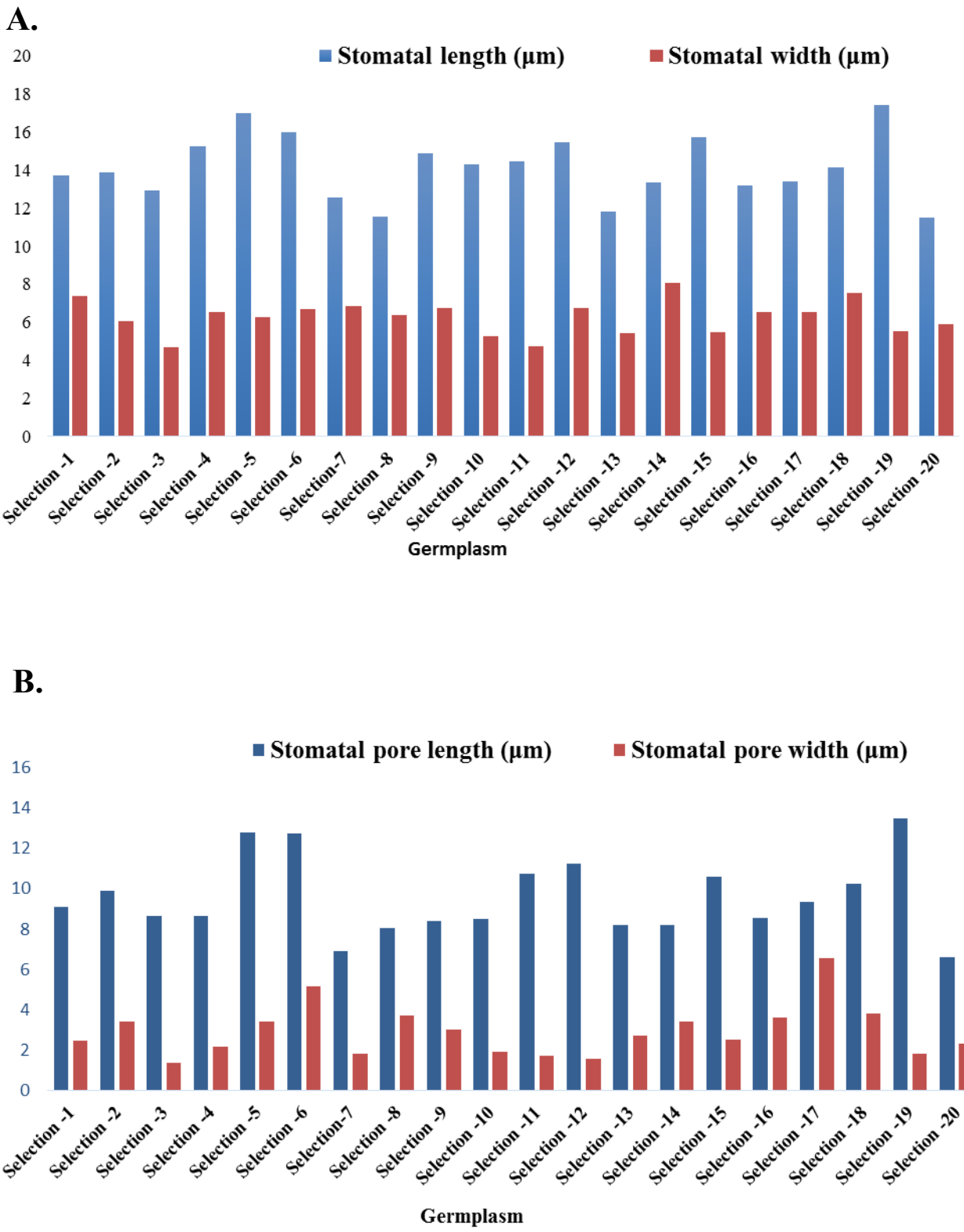


Fig. 4.1 Inter varietal variability in water chestnut (*Trapa natans* var. *bispinosa* Roxb.) collected from various blocks of district Lucknow on the basis of A. stomatal length (µm) and stomatal width (µm) B. stomatal pore length (µm) and stomatal pore width (µm)

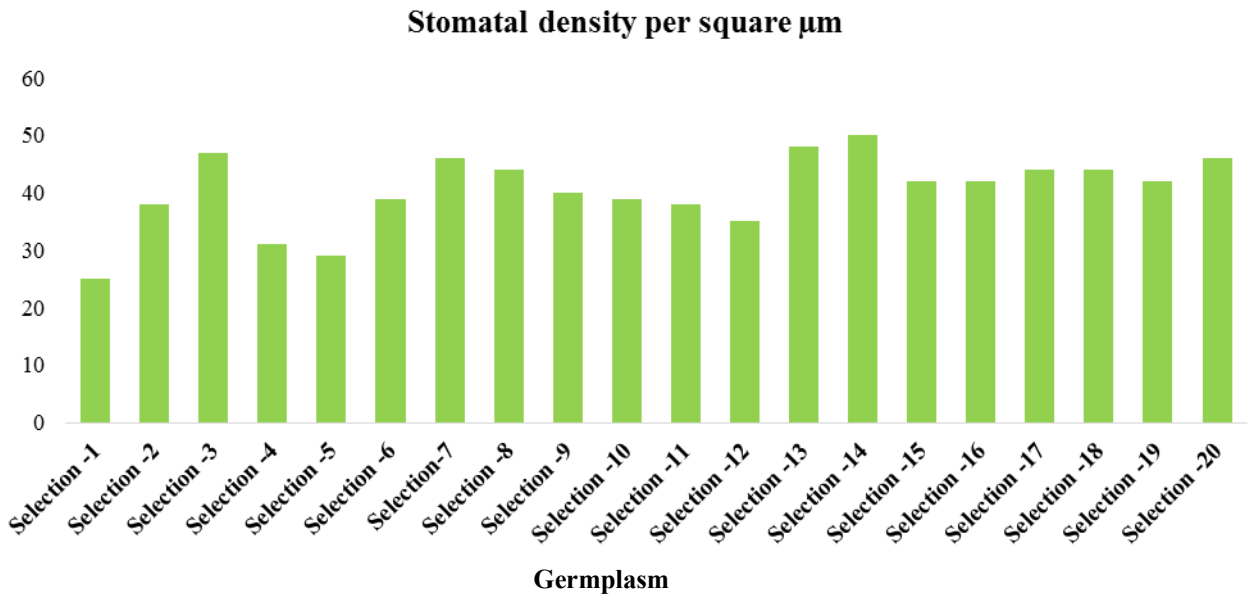


Fig. 4.2 Inter varietal variability in water chestnut (*Trapa natans* var. *bispinosa* Roxb.) collected from various blocks of district Lucknow on the basis of stomatal density (μm^{-2})

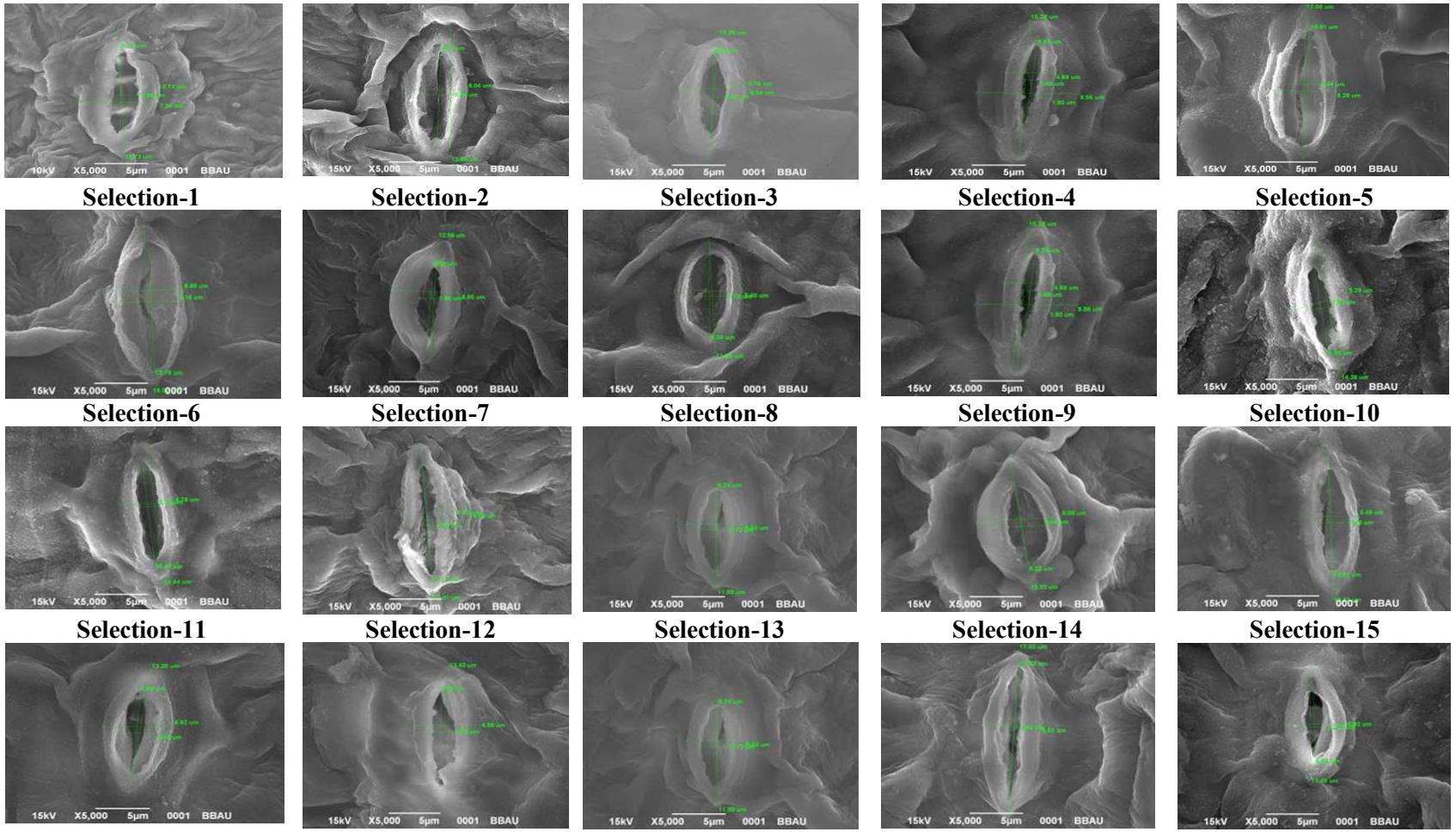


Plate 4.5 Inter varietal variability in stomatal pore size of 20 germplasm of water chestnut (*Trapa natans* var. *bispinosa* Roxb.) collected from various blocks of district Lucknow

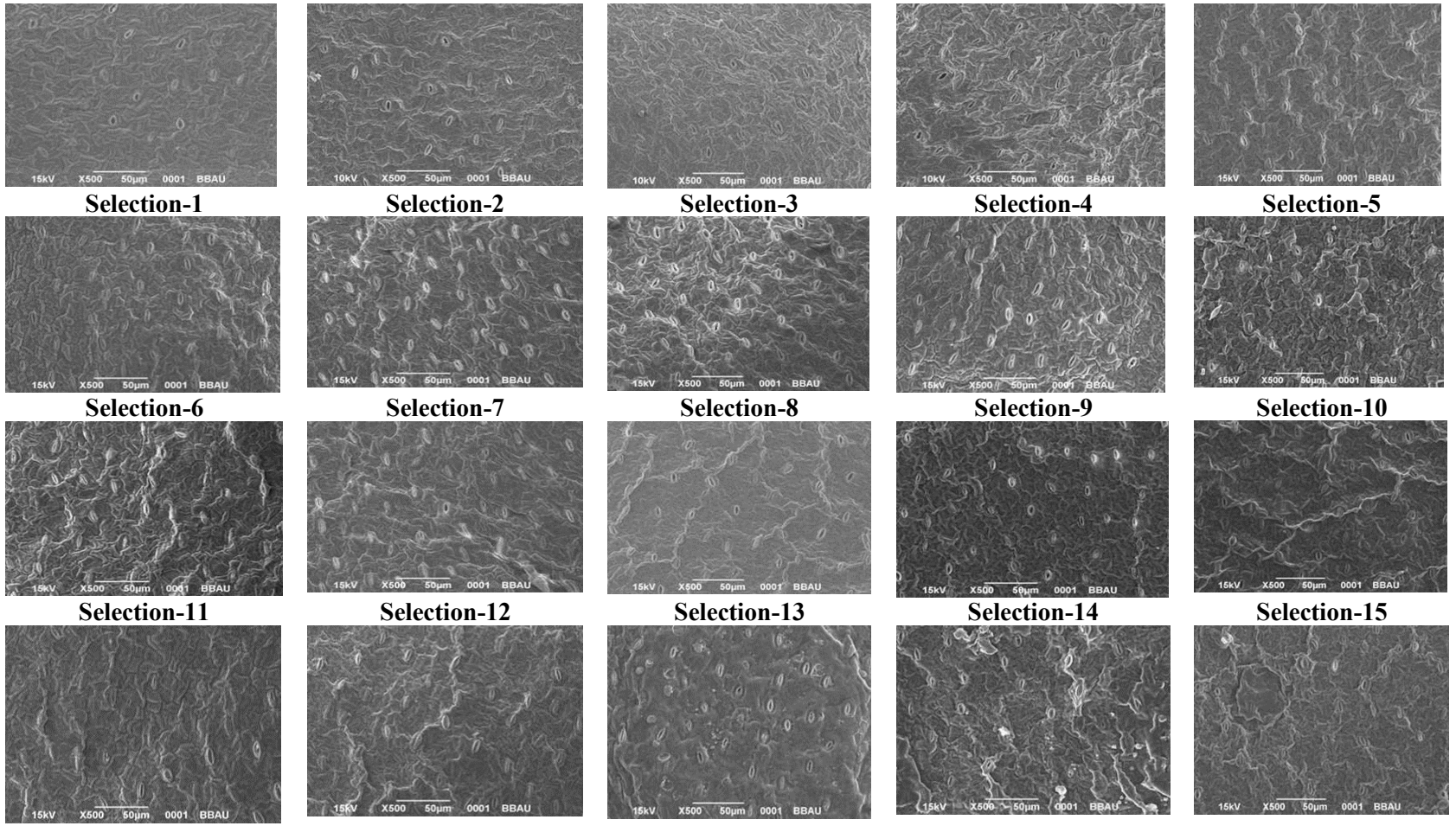


Plate 4.6 Inter varietal variability in stomatal density of 20 germplasm of water chestnut (*Trapa natans* var. *bispinosa* Roxb.) collected from various blocks of district Lucknow

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Biometrical techniques for assessment of inter-varietal variability for fruit stomatal traits of 20 germplasm of water chestnut:

The stomatal morphological data recorded was subjected to biometrical techniques for assessment computed through simple measures of variability viz., range, grand mean and coefficient of variation (CV), genetic variability (PCV% and GCV%), heritability, genetic advance and genetic as percent of mean (%) for further elucidation of the data recorded. Among the stomatal traits the maximum coefficient of variation showed (18.46) in stomatal pore length (µm) (Table 4.6). However, the maximum PCV (35.44%), GCV (35.07%), heritability (97.90%) and genetic advance as percent of mean (147.19%) were recorded for stomatal pore width (µm) while, the highest genetic advance (26.01%) was observed for stomatal density (Table 4.6).

A UPGMA dendrogram was prepared on the basis of stomatal characteristics of 20 germplasm of water chestnut in order to establish their relatedness to each other. The 20 germplasm of water chestnut of under study were noted to be very closely additional sub-clusters, differentiating the germplasm collected from different blocks of district Lucknow. Cluster-I consisted of 17 germplasm which further divided into four sub-groups (cluster IA, IB, IC and ID) while cluster-II comprised three germplasm which was divided into three sub-groups (cluster IIA, IIB and IIIC) (Table 4.7 and Plate 4.7).

Table 4.6 Range (minimum and maximum), grand mean, coefficient of variance (CV), phenotypic coefficient of variance (PCV %), genotypic coefficient of variance (GCV %), heritability h^2 (%), genetic advance (GA) and genetic advance as percent of mean (GAM %) for 5 stomatal characters of 20 germplasm of water chestnut (*Trapa natans* var. *bispinosa* Roxb.) collected from various blocks of district Lucknow

Sr. No.	Characters	Grand mean	Range		CV	PCV (%)	GCV (%)	h^2 (%)	GA	GAM (%)
			Minimum	Maximum						
1	Stomatal density (μm^{-2})	40.43	25	50	4.72	16.51	15.82	91.8	26.01	64.33
2	Stomatal length (µm)	14.12	11.48	17.43	6.56	13.10	11.34	74.9	5.89	41.71
3	Stomatal widths (µm)	6.25	4.68	8.05	7.11	15.23	13.47	78.20	3.15	50.40
4	stomatal pore length (µm)	9.55	6.64	13.52	9.78	21.55	19.20	79.40	6.94	72.67
5	Stomatal pore width (µm)	2.72	1.40	5.16	5.12	35.44	35.07	97.90	4.01	147.19

Whereas, CV: Coefficient of variance, PCV: Phenotypic coefficient of variance, GCV: Genotypic coefficient of variance, h^2: Heritability, GA: genetic advance and GAM: genetic advance as percent of mean

Table 4.7 Non-hierarchical Euclidean Clusters analysis in 20 germplasm of water chestnut (*Trapa natans* var. *bispinosa* Roxb.) on the basis of stomatal characters

Clusters	Germplasm
Clusters I	T_2, T_3, T_6, T_7, T_8, T_9, T_{10}, T_{11}, T_{12}, T_{13}, T_{14}, T_{15}, T_{16}, T_{17}, T_{18}, T_{19} and T_{20}
Clusters II	T_1, T_4 and T_5

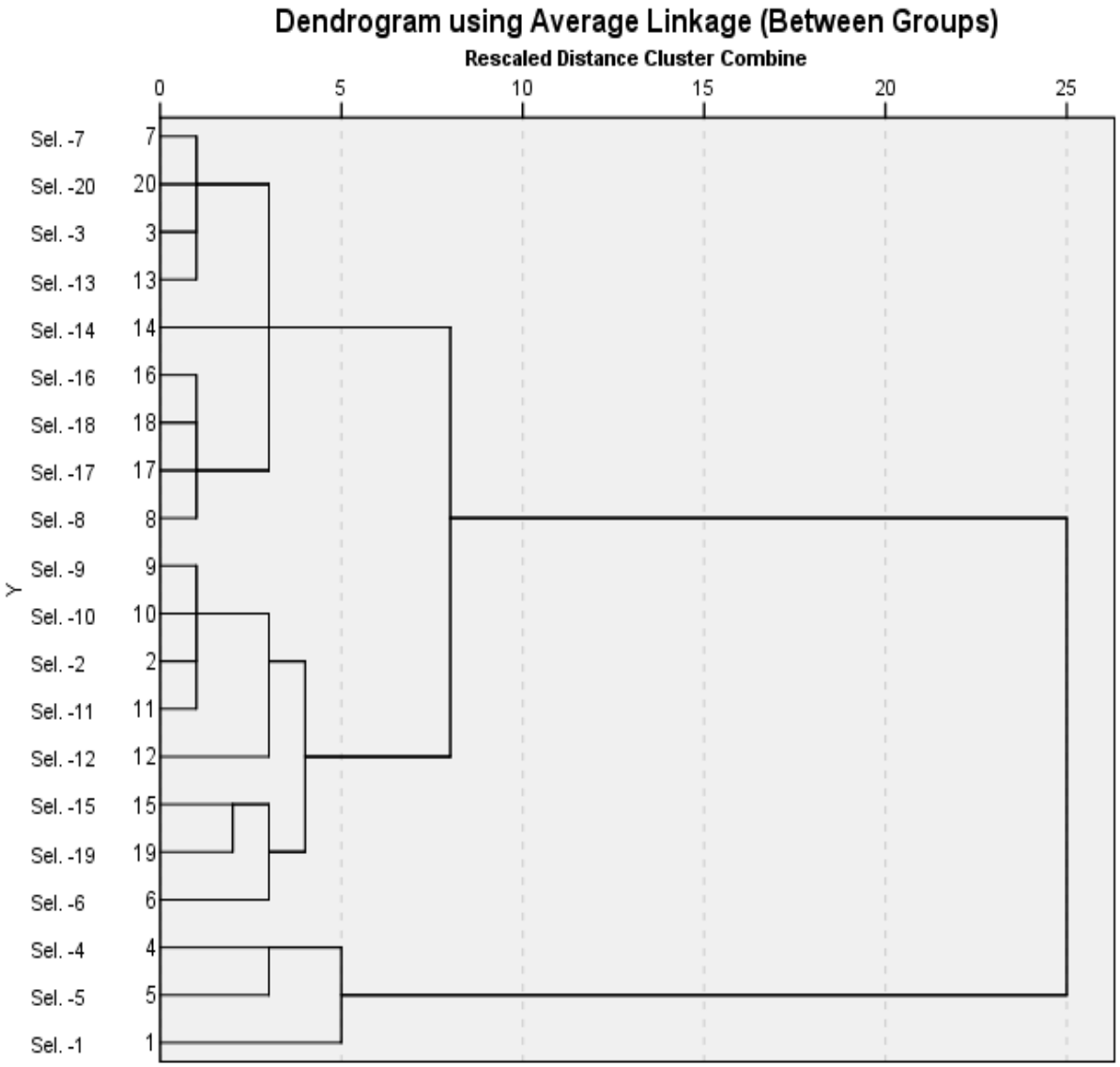

Plate 4.7 UPGMA dendrogram of 20 germplasm of water chestnut (*Trapa natans* var. *bispinosa* Roxb.) based on stomatal data

4.2.2 Morphological characters:

Three plants were selected from each pond and data were recorded for analysis of inter-varietal variability study through plant and leaf characters viz., number of rosette per plant), rosette spread (north-south) (cm), rosette spread (east-west) (cm), number of leaves per plant, length of leaves (cm), length of lamina (cm) widths of lamina (cm), petiole length (cm), pulvinus length (cm), pulvinus diameter (mm) and pulvinus diameter (mm) etc.

Number of rosette per plant

Observation on number of rosette per plant revealed significant difference among the morphotypes and number of rosette per plant was ranged from 3.67-12.17 with a grand mean of 6.70 (Table 4.11). The data on number of rosette are presented in Table 4.8 and Fig. 4.3A. The maximum number of rosette per plant was counted in T_{19} (12.17) and T_{11} (11.67) followed by T_{18} (9.67) while, the minimum number of rosette per plant was found in T_{13} (3.67).

Number of leaves per plant

The analysis of variance was carried out from the data obtained on number of leaves per plant which revealed significant difference among the morphotypes. The number of leaves was ranged from 21.67 to 29.67 with a grand mean of 18.79 (Table 4.11). The maximum number of leaves (29.67) were higher for T_6 followed by T_3 (26.65) and T_{14} (27.67), both of which were at par with each other (Table 4.8 and Fig. 4.3A). The minimum number of leaves were found in T_{10} (21.67) which was significantly lower from all other morphotypes under the study.

Rosette spread (north-south) (cm)

The rosette spread (north-south) (cm) ranged between 22.10-32.83cm and with a general mean of 27.63 cm. The data of rosette spread (north-south) and east west (cm) presented in Table 4.8 and Fig. 4.3B.The maximum rosette spread (north-south) had measured in T_1 (32.83cm) and T_9 (32.17cm) which were significantly higher to others, whereas Selection-4 had measured minimum in (22.10cm).

Rosette spread (east-west) (cm)

Rosette spread (east-west) (cm) under study showed wide variation 20.72 to 32.18cm having general mean of 27.27cm (Table 4.11). T_1 showed the significantly maximum (32.18 cm) followed by T_6 (31.88 cm) and T_7 (31.64cm). The minimum data was recorded for this trait for T_4 (20.72 cm) presented in Table 4.8 and Fig. 4.3B.

Table 4.8 Inter varietal variability in water chestnut (*Trapa natans* var. *bispinosa* Roxb.) collected from various blocks of district Lucknow on the basis of vegetative parameters

Germplasm	Number of rosette per plant			Number of leaves per plant			Rosette spread N-S (cm)			Rosette spread E-W (cm)		
	2016	2017	Pooled	2016	2017	Pooled	2016	2017	Pooled	2016	2017	Pooled
T_1	4.67	8.33	6.50	21.33	23.67	22.50	30.50	35.17	32.83	26.50	37.87	32.18
T_2	4.00	4.67	4.33	23.00	25.67	24.34	31.50	25.83	28.67	27.17	23.83	25.50
T_3	4.67	9.67	7.17	32.00	27.30	29.65	31.63	28.03	29.83	23.90	32.77	28.33
T_4	4.33	6.67	5.50	29.33	25.00	27.17	26.07	18.13	22.10	21.33	20.10	20.72
T_5	4.00	6.67	5.33	21.00	26.00	23.50	20.67	32.00	26.33	21.83	29.53	25.68
T_6	2.33	10.00	6.17	28.33	31.00	29.67	22.33	38.50	30.42	23.00	40.77	31.88
T_7	4.00	8.33	6.17	27.67	23.00	25.34	26.73	34.67	30.70	25.60	37.67	31.64
T_8	4.67	8.67	6.67	25.00	24.67	24.84	26.67	28.47	27.57	25.17	29.17	27.17
T_9	4.67	9.00	6.83	22.67	29.33	26.00	29.50	34.83	32.17	28.00	32.50	30.25
T_{10}	3.33	7.33	5.33	15.67	27.67	21.67	16.50	33.33	24.92	19.33	32.33	25.83
T_{11}	3.67	7.00	5.33	13.67	30.33	22.00	18.00	28.97	23.49	18.60	26.37	22.48
T_{12}	3.00	10.67	6.83	12.67	32.67	22.67	17.73	38.83	28.28	12.20	37.83	25.02
T_{13}	3.33	4.00	3.67	14.00	35.00	24.50	20.50	28.47	24.48	21.67	30.23	25.95
T_{14}	3.33	9.33	6.33	18.33	37.00	27.67	21.00	34.10	27.55	24.67	32.97	28.82
T_{15}	2.33	10.33	6.33	20.67	33.67	27.17	24.17	30.93	27.55	23.50	29.27	26.38
T_{16}	3.67	4.00	3.83	22.67	30.67	26.67	20.73	26.43	23.58	19.77	24.30	22.04
T_{17}	5.00	11.33	8.17	21.67	27.33	24.50	29.47	29.40	29.44	26.97	29.17	28.07
T_{18}	5.00	14.33	9.67	23.00	24.67	23.84	26.33	27.47	26.90	25.00	33.10	29.05
T_{19}	7.67	16.67	12.17	24.67	22.00	23.34	27.67	26.20	26.93	29.00	29.03	29.02
T_{20}	5.00	18.33	11.67	24.33	23.00	23.67	30.57	27.53	29.05	30.00	29.10	29.55
SE(m)±	0.71	0.81	0.54	1.82	2.29	1.46	1.45	1.33	0.98	1.13	1.25	0.84
CD (p=0.05)	**1.27**	**1.45**	**2.37**	**3.25**	**4.10**	**6.40**	**2.60**	**2.38**	**4.31**	**2.03**	**2.24**	**3.70**

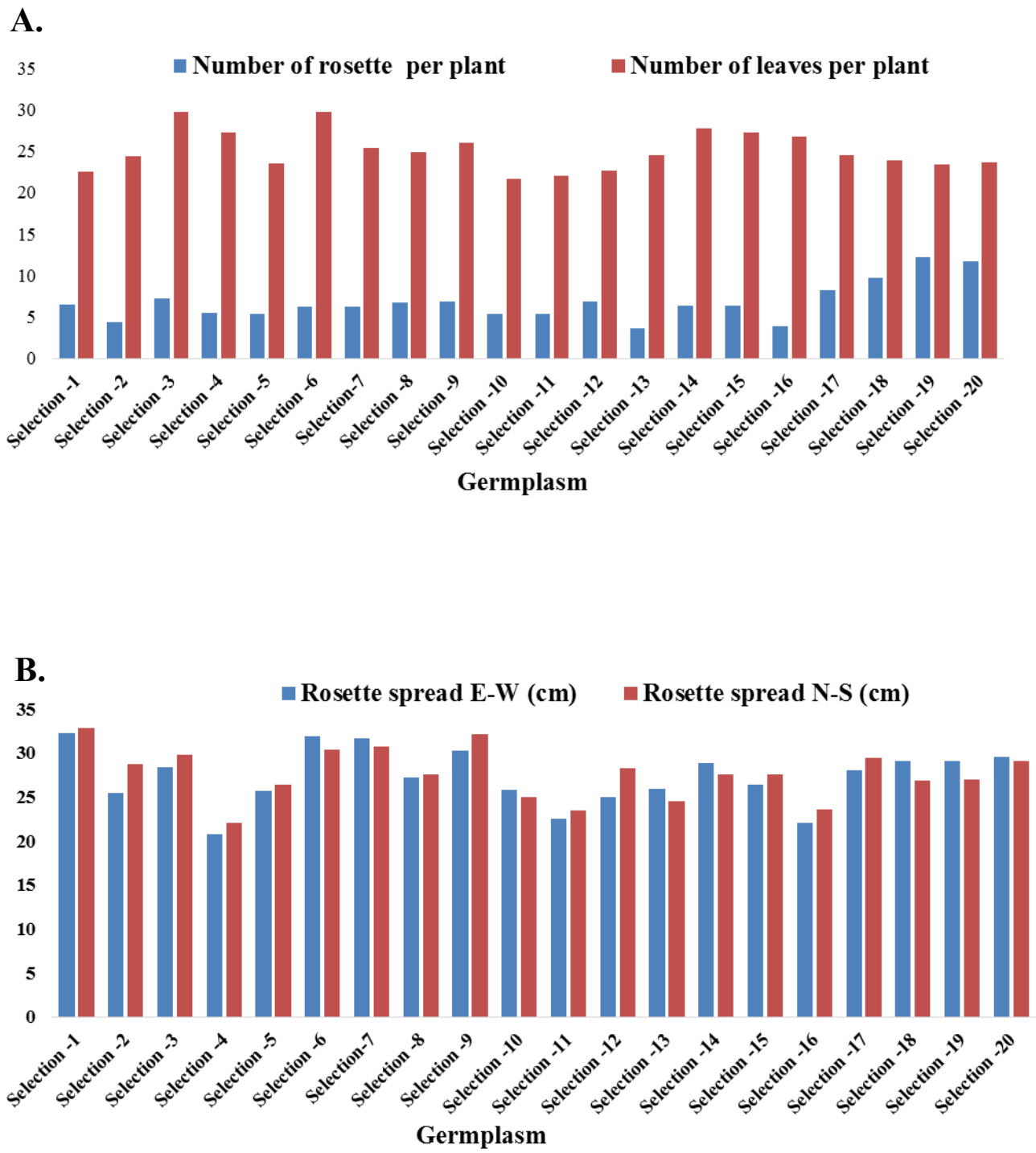

Fig. 4.3 Inter varietal variability in water chestnut (*Trapa natans* var. *bispinosa* Roxb.) collected from various blocks of district Lucknow on the basis of (A) number of rosette per plant) and number of leaves per plant); (B) rosette spread E-W (cm) and rosette spread N-S (cm)

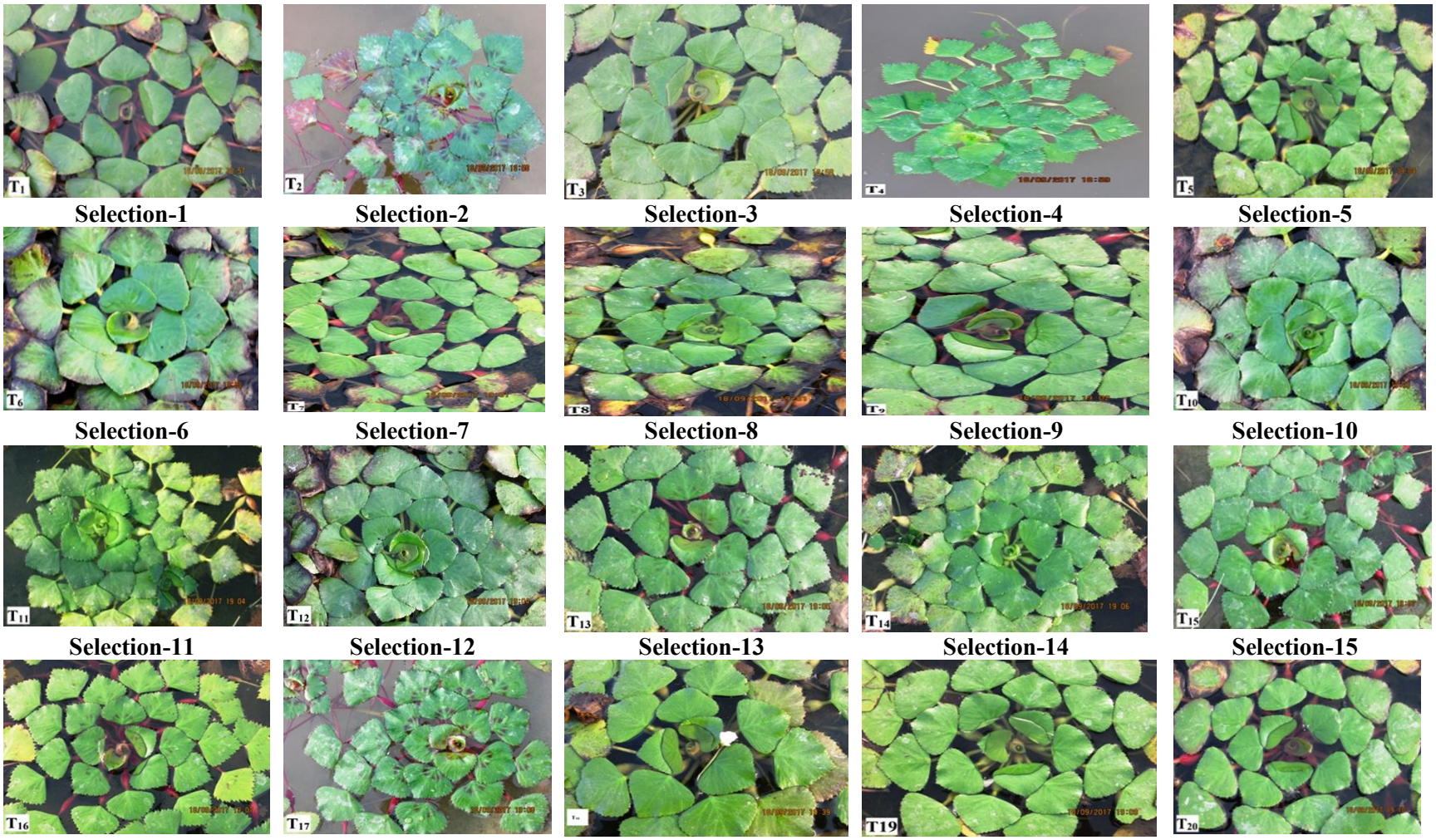

Plate 4.8 Inter varietal variability in rosette spread of twenty germplasm of water chestnut (*Trapa natans* var. *bispinosa* Roxb.) collected from various blocks of district Lucknow

Length of leaves (cm)

Germplasm statistically differed to each other for length of leaves. In the present investigation the length of leaves of germplasm varied between 11.93 to 19.78 cm and its general mean was recorded 15.62cm (Table 4.11). The germplasm T_6 produced maximum leaf length (19.78 cm) and minimum were recorded in T_{16} (11.93 cm). The length of leaves produced by germplasm T_7 (18.44 cm) and T_{10} (17.30 cm) were also significant higher after T_6 than other germplasm (Table 4.9 and Fig. 4.4A).

Length of lamina (cm)

The length of lamina (cm) was recorded from 4.20 to 6.44 cm with a general mean of 4.92 cm. The highest length of lamina was observed for Selection T_6 (6.44 cm) fallowed by T_1 (5.62 cm), T_9 (5.55cm), T_{12} (5.53 cm), T_{10} (5.29) and T_{11} (5.15), respectively. While the minimum value was recorded in T_4 (4.20) fallowed by T_{13} (4.22) (Table 4.9 and Fig. 4.4A).

Width of lamina (cm)

In the present investigation the range was found of different germplasm varied from 4.22 to 8.78 cm and with general mean of 6.20 cm (Table 4.11)**.** Selection T_6 had maximum (8.78 cm) widths of lamina followed by T_{12} (8.53 cm) and T_{10} (7.69 cm) which were significantly higher to others, whereas Selection T_4 had minimum (4.22 cm) widths of lamina as presented in Table 4.9 and Fig. 4.4B.

Petiole length (cm)

Petiole length (cm) revealed significant differences between various germplasm. The range of petiole length was recorded between 7.02-13.34 cm and its general mean of 10.78cm is shown in Table 4.11. The maximum petiole length (cm) was recorded in Selection T_6 (13.34) fallowed by T_7, T_8 and T_{12}, respectively. While the minimum petiole length (cm) was recorded in Selection T_4 (7.02) as data given in Table 4.9 and Fig. 4.4B.

Table 4.9 Inter varietal variability in water chestnut (*Trapa natans* var. *bispinosa* Roxb.) collected from various blocks of district Lucknow on the basis of leaf parameters

Germplasm	Length of leaf (cm)			Length of lamina (cm)			Width of lamina (cm)			Petiole length (cm)		
	2016	2017	Pooled	2016	2017	Pooled	2016	2017	Pooled	2016	2017	Pooled
T_1	16.07	16.73	16.40	5.67	5.57	5.62	7.87	7.13	7.50	10.73	12.47	11.60
T_2	12.20	13.53	12.87	4.70	4.40	4.55	5.07	5.10	5.09	7.50	7.80	7.65
T_3	12.40	14.58	13.49	4.57	4.52	4.54	5.97	4.93	5.45	7.83	11.10	9.47
T_4	11.07	13.93	12.50	4.03	4.37	4.20	4.17	4.27	4.22	7.03	7.00	7.02
T_5	15.93	17.87	16.90	4.37	5.10	4.73	5.87	5.77	5.82	11.57	13.00	12.29
T_6	19.49	20.07	19.78	7.20	5.67	6.43	9.03	8.53	8.78	13.47	13.20	13.34
T_7	18.87	18.00	18.44	5.40	4.87	5.14	7.10	6.63	6.87	12.13	14.23	13.18
T_8	16.37	17.70	17.04	4.93	5.30	5.12	6.53	7.07	6.80	11.43	14.47	12.95
T_9	15.73	18.70	17.22	5.07	6.03	5.55	6.13	5.17	5.65	11.17	13.53	12.35
T_{10}	16.73	17.87	17.30	5.57	5.00	5.29	7.67	7.70	7.69	11.83	13.50	12.67
T_{11}	16.67	15.73	16.20	5.23	5.07	5.15	6.07	5.47	5.77	11.20	9.37	10.28
T_{12}	15.80	17.77	16.79	5.73	5.33	5.53	8.80	8.27	8.53	11.43	13.63	12.53
T_{13}	12.83	13.77	13.30	4.33	4.10	4.22	5.20	5.30	5.25	8.97	9.60	9.29
T_{14}	14.07	13.50	13.79	4.90	5.07	4.99	5.73	5.60	5.67	8.50	9.97	9.23
T_{15}	14.00	12.27	13.14	4.57	4.80	4.69	5.40	6.27	5.83	9.73	8.97	9.35
T_{16}	12.03	11.83	11.93	4.03	4.57	4.30	5.60	5.27	5.43	8.27	6.67	7.47
T_{17}	13.83	15.90	14.87	4.57	4.53	4.55	5.83	5.07	5.45	8.60	10.07	9.33
T_{18}	14.67	17.67	16.17	4.67	4.73	4.70	6.30	5.33	5.82	9.93	11.40	10.67
T_{19}	15.20	18.97	17.09	4.82	4.73	4.78	6.40	6.37	6.38	10.03	14.17	12.10
T_{20}	16.63	18.17	17.40	4.73	4.30	4.52	6.40	5.80	6.10	11.53	14.17	12.85
SE(m) ±	0.98	1.86	1.05	0.43	0.50	0.333	0.47	0.50	0.34	0.69	0.62	0.46
CD (p=0.05)	**1.76**	**3.3**	**4.60**	**0.78**	**0.90**	**1.45**	**0.83**	**0.89**	**1.49**	**1.23**	**1.11**	**2.03**

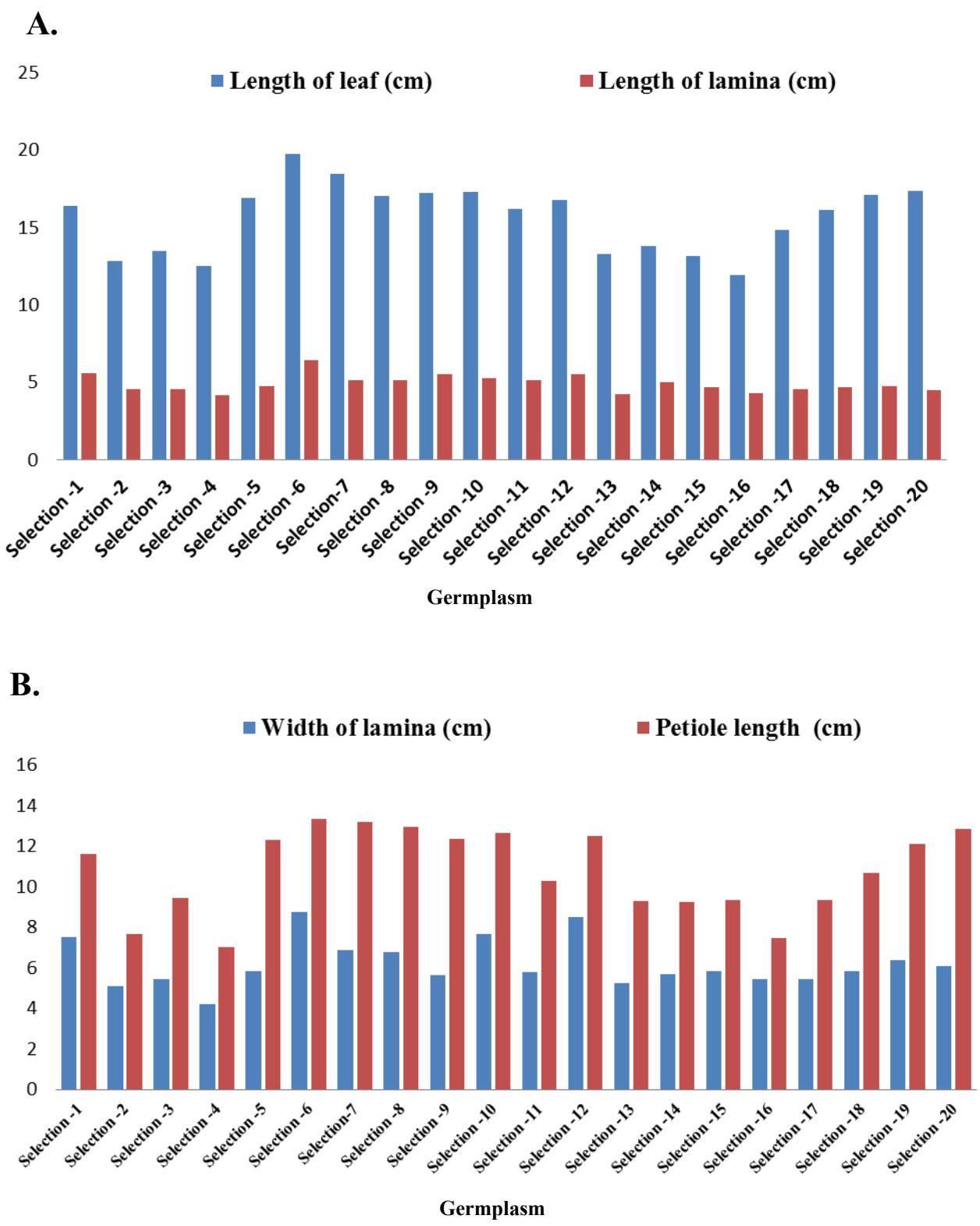

Fig. 4.4 Inter varietal variability in water chestnut (*Trapa natans* var. *bispinosa* Roxb.) collected from various blocks of district Lucknow on the basis of (A) length of leaf (cm) and length of lamina (cm); (B) width of leaf (cm) and petiole length (cm)

Pulvinus length (cm)

Pulvinus length (cm) showed wide variation under study and varied between 1.60 to 3.17 cm and its grand mean was recorded 2.45 for this trait (Table 4.11). The maximum pulvinus length (3.84 cm) was observed for Selection T_{10} (3.17 cm) followed by T_7 (3.05 cm) and T_8 (2.98 cm), both of which were at par with each other. The minimum pulvinus length was found in Selection T_4 (1.60 cm) which was significantly lower from all other morphotypes under the study (Table 4.10 and Fig. 4.5A).

Pulvinus diameter (mm)

The pulvinus diameter significantly varied between 3.84-15.94 mm and its general mean was recorded 8.51 mm (Table 4.11). The highest pulvinus diameter was showed in Selection T_{12} (15.94mm) fallowed by T_9 (11.13 mm), T_{11} (10.97 mm). The minimum pulvinus diameter showed in T_4 (Table 4.10 and Fig. 4.5A).

Pulvinus: Petiole ratio

The significantly range were found from 0.19-0.29 and its general mean 0.23 for character pulvinus: petiole ratio. The highest pulvinus: petiole ratio was observed in Selection T_{14} (0.29) fallowed by T_{15} (0.28), T_{12} and T_{13} (0.27), T_3 (0.26) and T_1 and T_2 (0.25). The minimum pulvinus: petiole ratio was recorded in T_5 (0.19) Table 4.10 and Fig. 4.5B.

Table 4.10 Inter varietal variability in water chestnut (*Trapa natans* var. *bispinosa* Roxb.) collected from various blocks of district Lucknow on the basis of leaf parameters

Germplasm	Pulvinus length (cm)			Pulvinus diameter (mm)			Pulvinus : Petiole ratio		
	2017	2017	Pooled	2016	2017	Pooled	2016	2017	Pooled
T_1	2.73	2.80	2.77	2.97	10.20	6.58	0.27	0.23	0.25
T_2	1.70	1.90	1.80	5.87	4.80	5.34	0.24	0.25	0.25
T_3	2.57	2.00	2.29	4.76	9.91	7.34	0.33	0.19	0.26
T_4	1.67	1.53	1.60	3.66	4.02	3.84	0.25	0.22	0.24
T_5	2.40	2.23	2.32	8.54	8.06	8.30	0.21	0.17	0.19
T_6	2.73	3.03	2.88	3.33	12.26	7.80	0.22	0.22	0.22
T_7	3.10	3.00	3.05	2.93	12.78	7.86	0.23	0.23	0.23
T_8	2.83	3.10	2.97	2.93	11.85	7.39	0.25	0.20	0.23
T_9	2.80	2.67	2.74	9.35	12.91	11.13	0.26	0.21	0.24
T_{10}	3.13	3.20	3.17	3.17	13.81	8.49	0.30	0.24	0.27
T_{11}	2.57	2.27	2.42	9.65	12.29	10.97	0.24	0.24	0.24
T_{12}	3.00	2.83	2.92	16.46	15.41	15.94	0.33	0.21	0.27
T_{13}	2.20	2.20	2.20	9.25	9.29	9.27	0.25	0.23	0.24
T_{14}	2.53	2.53	2.53	10.59	10.57	10.58	0.31	0.26	0.29
T_{15}	2.50	2.30	2.40	8.19	10.17	9.18	0.28	0.28	0.28
T_{16}	1.73	1.63	1.68	5.23	5.43	5.33	0.21	0.25	0.23
T_{17}	1.97	2.17	2.07	7.54	8.30	7.92	0.23	0.21	0.22
T_{18}	2.70	2.03	2.37	7.91	9.60	8.75	0.27	0.18	0.23
T_{19}	2.73	2.33	2.53	8.58	11.02	9.80	0.27	0.16	0.22
T_{20}	2.60	2.10	2.35	9.41	7.54	8.47	0.23	0.16	0.20
SE(m) ±	0.18	0.22	0.14	0.71	0.98	0.18	0.02	0.02	0.01
CD (p=0.05)	**0.32**	**0.39**	**0.62**	**1.27**	**1.76**	**0.32**	**0.09**	**0.03**	**0.06**

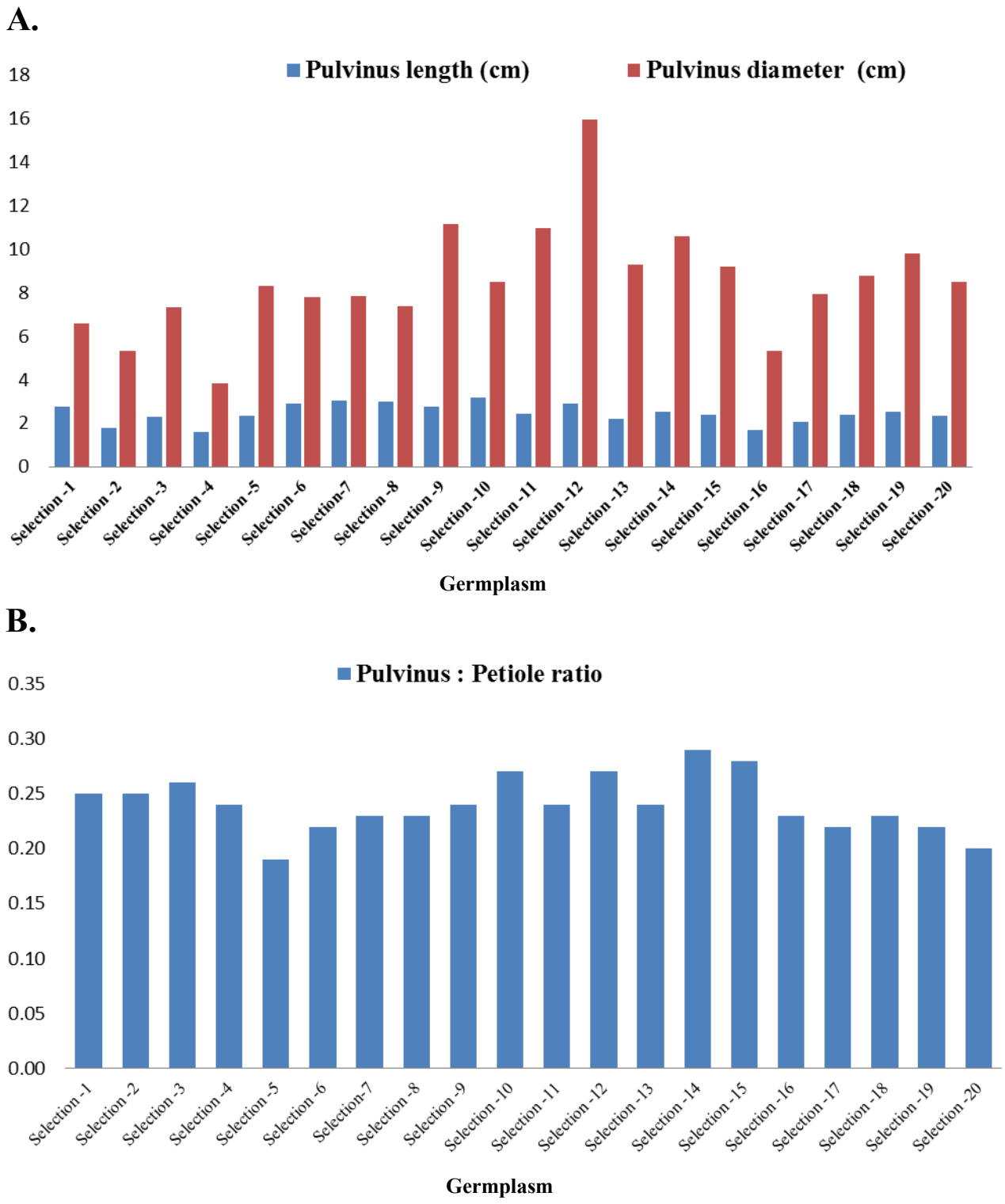

Fig. 4.5 Inter varietal variability in water chestnut (*Trapa natans* var. *bispinosa* Roxb.) collected from various blocks of district Lucknow on the basis of (A) pulvinus length (cm) and pulvinus diameter (cm); (B) pulvinus:petiole ratio

Biometrical techniques for assessment of inter-varietal variability for vegetative parameters:

The morphological data recorded was subjected to biometrical techniques for assessment which was computed through simple measures of variability *viz.,* range, grand mean and coefficient of variation (CV), genetic variability (PCV % and GCV %), heritability (h^2%), genetic advance (GA) and genetic advance as percent of mean (GAM%) for further elucidation of the data recorded. The highest CV (13.96), PCV (26.75%), GCV (22.82%), heritability (72.80%), genetic advance (5.54%) and genetic advance as percent of mean (82.70%) were recorded for number of rosette ($plant^{-1}$) (Table 4.11). The traits number of leaves per Plant, rosette spread north-south and east-west (cm) showed very negligible value for these heritability, genetic advance and genetic advance as percent of mean which indicates environmental effect. Regards in leaf parameters the maximum CV (11.70) was recorded for length of lamina. Among the leaf parameters the highest PCV (21.02%) and GCV (18.64%) were found for pulvinus diameter (mm). However, the wide heritability (84.80%) and genetic advance (7.44%) were observed for petiole length (cm) while, the maximum genetic advance as percent of mean (158.64%) was recorded for pulvinus length (cm) (Table 4.11).

Table 4.11 Range (minimum and maximum), grand mean, coefficient of variance (CV), phenotypic coefficient of variance (PCV%), genotypic coefficient of variance (GCV%), heritability h^2 (%), genetic advance (GA) and genetic advance as percent of mean (GAM%) for 11 plant and leaf morphology characters of 20 germplasm of water chestnut (*Trapa natans* var. *bispinosa* Roxb.) collected from various blocks of district Lucknow

Sr. No.	Characters	Grand mean	Range		CV	PCV (%)	GCV (%)	h^2 (%)	GA	GAM%
			Minimum	Maximum						
1	Number of rosette per plant	6.70	3.67	12.17	13.96	26.75	22.82	72.80	5.54	82.70
2	Number of leaves per plant	25.03	21.67	29.67	10.11	10.11	0.13	0.00*	0.00*	0.00*
3	Rosette spread N-S (cm)	27.63	22.10	32.83	6.16	6.16	0.11	0.00*	0.00*	0.00*
4	Rosette spread E-W (cm)	27.27	20.72	32.18	5.36	5.37	0.12	0.00*	0.00*	0.00*
5	Length of leaves (cm)	15.62	11.93	19.78	11.63	17.70	13.34	56.80	6.67	42.70
6	Length of lamina (cm)	4.92	4.20	6.43	11.70	15.37	9.96	42.00	1.34	8.56
7	Width of lamina (cm)	6.20	4.22	8.78	9.57	20.70	18.35	78.60	4.28	69.06
8	Petiole length (cm)	10.78	7.02	13.34	7.46	19.17	17.65	84.80	7.44	68.98
9	Pulvinus length (cm)	2.45	1.60	3.17	10.13	20.01	17.26	74.40	1.55	158.64
10	Pulvinus diameter (mm)	8.51	3.84	15.94	9.73	21.02	18.64	78.60	5.97	70.17
11	Pulvinus: Petiole ratio	0.23	0.19	0.29	10.14	10.65	3.22	9.10	0.00*	0.00*

Whereas, CV: Coefficient of variance, PCV: Phenotypic coefficient of variance, GCV: Genotypic coefficient of variance, h^2: Heritability, GA: Genetic advance and GAM: Genetic advance as percent of mean

Experiment III

4.3 To establish inter-varietal variability in water chestnut on the basis of physico-chemical characteristics of the fruits

4.3.1 Fruit morphology

Number of fruits per rosette

Observation of number of fruits revealed significant difference among the morphotypes and ranged from 11.67-25.50 with a general mean of 17.85 (Table 4.19). The data of number of fruits per rosette are presented in Table 4.12 and Fig. 4.6A. The maximum number of fruits per rosette was observed for T_1 and T_{12} (25.50) fallowed by T_{19} (25.33) and T_{20} (24.83), respectively. Whereas, the minimum number of fruits per plant was obtained for T_3 (11.67).

Skin colour of fruits

The colour of water chestnut fruit was observed by visual observation. The observed colour of all fruits of germplasm collected from various blocks of district Lucknow. It is lucid from the table that among the twenty germplasm Selection-1 recorded light red colour of the fruit. However, the dark red colour were found in T_2, T_7, T_9 and T_{16}. While reddish green colour was observed in T_3, T_5, T_6, T_{12} and T_{19}. Similarly, dark green colour was recorded for T_4, T_{10} and T_{17}. Whereas the pale green colour was showed for Selection-8, 11, 14 and 18. Greenish red colour were found for T_{13}, T_{15} and T_{20} are given in Table 4.4 and Plate 4.2.

Fruits weight (g)

The wide range of fruits weight varied from 5.43 to 8.90 (g) and with grand mean of 6.83 (g) as presented Table 4.19. The higher fruits weight (g) were found in T_{20} (8.90g) followed by T_1 (8.83g), Selection-17 (7.93g), respectively. Whereas the lowest fruits weight (g) was found in T_{11} (5.43 g) under the study the data are shown in Table 4.12 and Fig. 4.6A.

Fruit yield (g/plant)

The range ware found of the fruit yield from 76.67 to 225.07 g with a general mean of 133.01 g (Table 4.19). Observation of fruit yield revealed significant difference among the germplasm. The maximum fruit yield was observed for T_{12} (255.07g) and T_{20} (246.60g) fallowed by T_1 (201.60g), T_{19} (191.43g) and T_{18} (145.22g), respectively. Whereas, the minimum fruit yield was obtained for T_3 (76.67g) followed by T_{13} (80.67g). The data of number of fruit yield (g/Plant) are presented in Table 4.12 and Fig. 4.6B.

Volume of fruits (ml)

The average range of fruits volume (ml) was observed from 5.17 to 8.93 and with grand mean of 6.64 in (Table 4.19). The highest volume of fruits (ml) were recorded for T_{20} (8.93) fallowed by T_1 (8.85), T_{19} (4.43), respectively. The minimum value were recorded T_{11} (5.17) as presented data in Table 4.12 and Fig. 4.6B.

Table 4.12 Inter varietal variability in water chestnut (*Trapa natans* var. *bispinosa* Roxb.) collected from various blocks of district Lucknow on the basis of fruit morphology

Germplasm	Number of fruits per plant			Fruit weight (g)			Fruit yield (g/plant)			Fruit volume (ml)		
	2016	2017	Pooled	2016	2017	Pooled	2016	2017	Pooled	2016	2017	Pooled
T_1	15.33	35.67	25.50	6.07	11.60	8.83	93.07	310.13	201.60	6.67	10.33	8.50
T_2	15.00	13.67	14.33	5.80	8.13	6.97	89.27	113.6	101.44	6.40	7.40	6.90
T_3	9.33	14.00	11.67	3.60	8.33	5.97	33.6	119.73	76.67	3.73	8.60	6.17
T_4	14.67	14.00	14.33	4.13	9.60	6.87	60.13	135.2	97.67	3.73	9.20	6.47
T_5	14.67	20.67	17.67	4.33	8.47	6.40	65.47	173.6	119.54	4.27	7.87	6.07
T_6	13.00	22.67	17.83	6.13	8.67	7.40	81.53	198	139.77	5.60	8.13	6.87
T_7	11.67	18.00	14.83	4.60	8.73	6.67	55.2	156.4	105.80	4.13	8.07	6.10
T_8	14.67	18.67	16.67	5.00	6.73	5.87	81.67	125.67	103.67	5.13	6.40	5.77
T_9	15.67	28.67	22.17	6.67	8.00	7.33	105.6	172.4	139.00	6.67	7.53	7.10
T_{10}	12.33	26.67	19.50	3.87	7.87	5.87	47.87	210.2	129.04	3.07	7.60	5.33
T_{11}	10.00	24.33	17.17	3.80	7.07	5.43	36.93	171.8	104.37	3.60	6.73	5.17
T_{12}	8.33	42.67	25.50	3.93	11.13	7.53	32.2	477.93	255.07	3.60	10.33	6.97
T_{13}	7.00	16.67	11.83	5.13	6.80	5.97	45.2	116.13	80.67	5.00	6.93	5.97
T_{14}	7.67	22.67	15.17	5.20	8.27	6.73	39.07	187.73	113.40	5.20	8.20	6.70
T_{15}	8.33	24.33	16.33	4.00	9.53	6.77	34.07	233.53	133.80	3.73	9.47	6.60
T_{16}	11.00	14.33	12.67	5.20	7.87	6.53	52.67	112.53	82.60	5.40	7.47	6.43
T_{17}	8.33	17.00	12.67	7.80	8.07	7.93	48.27	137.8	93.04	5.60	7.93	6.77
T_{18}	12.67	30.33	21.50	6.00	6.93	6.47	75.9	214.53	145.22	4.40	7.27	5.83
T_{19}	11.00	39.67	25.33	4.13	8.47	6.30	45.73	337.13	191.43	6.53	8.33	7.43
T_{20}	14.33	35.33	24.83	6.53	11.27	8.90	93.2	400	246.60	6.53	11.33	8.93
SE(m) ±	0.88	1.62	0.92	0.49	0.55	0.37	3.88	9.776	5.26	0.59	0.55	0.40
CD (p=0.05)	**1.58**	**2.90**	**4.04**	**0.88**	**0.99**	**1.62**	**6.95**	**17.5**	**23.03**	**1.05**	**0.98**	**1.75**

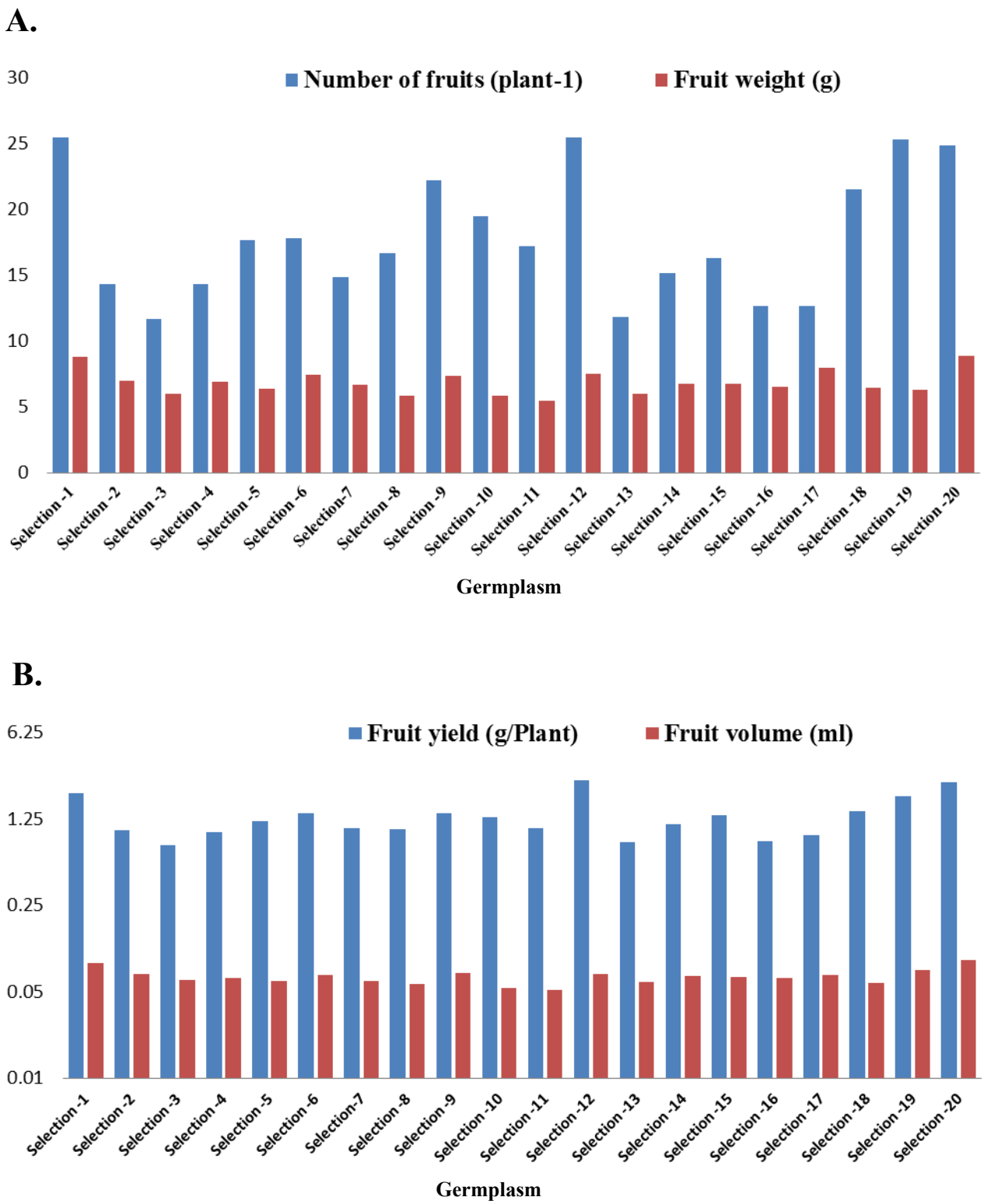

Fig. 4.6 Inter varietal variability in water chestnut (*Trapa natans* var. *bispinosa* Roxb.) collected from various blocks of district Lucknow on the basis of A. Number of fruits per plant and fruit weight (g); B. fruit yield (g/plant)

Specific gravity of the fruit (g/cc)

The range of specific gravity of fruit were recorded from 0.92 to 1.17g/cc with the general mean 1.01 g/cc (Table 4.19). The maximum specific gravity of fruit (1.17g/cc) was recorded for fruits from T_{12} followed by T_6, T_2, T_4 and T_5, respectively. The lowest specific gravity of fruit i.e. 1.01g/cc were observed for T_{13} the data are shown in Table 4.13 and Fig. 4.7A.

Fruits pedicel length (cm)

Under the investigation the study significant variance revealed between range 4.10 to 5.24 cm for the pedicel length of fruits and the grand mean of 4.61cm was noted for this trait (Table 4.19). The significantly result was highest showed for pedicel length of fruits for T_3 (5.24 cm) other than germplasm T_{20} (5.17 cm) and Selection- 18 (5.16 cm). The lowest data was recorded for this trait in T_{14} (4.10 cm) as presented in Table 4.13 and Fig. 4.7A

Length of fruit (mm)

Data pertaining to length of fruit (mm) of different morphotypes of water chestnut revealed significant inter-varietal variation among germplasm. T_{20} showed highest length of fruit (37.45mm) followed by T_{17} (37.07mm), T_9 (37.03mm) and T_1 (33.87mm), while minimum length of fruit (27.69 mm) was observed from T_3 (27.51mm) followed by T_{11} (27.69 mm) which were at par with each other and as shown in Table 4.13 and Fig. 4.7B.

Width of fruit (mm)

The significant inter-varietal variation was also observed for this character. The width of fruit was observed ranged from 27.65 to 36.62mm with grand mean 31.12mm (Table 4.19). The maximum width of fruit (36.62mm) was observed for T_5 followed by T_2 (36.31mm) and T_{20} (35.70 mm), T_{17} (34.37) and minimum width of fruit (27.65 mm) was recorded for T_{10} showing statistically similarity with each other and (Table 4.13 and Fig. 4.7B).

Table 4.13 Inter varietal variability in water chestnut (*Trapa natans* var. *bispinosa* Roxb.) collected from various blocks of district Lucknow on the basis of fruit morphology

Germplasm	Specific gravity of fruit (g/cc)			Length of fruit pedicel (cm)			Length of fruit (mm)			Width of fruit (mm)		
	2016	2017	Pooled	2016	2017	Pooled	2016	2017	Pooled	2016	2017	Pooled
T_1	0.90	1.14	1.02	4.83	5.01	4.92	30.67	37.07	33.87	32.82	33.99	33.41
T_2	1.03	1.05	1.04	4.23	4.64	4.43	31.24	33.23	32.23	41.33	31.29	36.31
T_3	0.98	0.97	0.98	5.28	5.19	5.24	21.51	33.51	27.51	24.10	33.56	28.83
T_4	1.06	0.99	1.03	4.85	4.63	4.74	25.42	33.37	29.39	24.54	30.81	27.68
T_5	1.02	1.03	1.03	4.54	4.39	4.46	23.32	35.06	29.19	42.01	31.22	36.62
T_6	1.25	1.00	1.12	4.26	4.38	4.32	30.48	37.20	33.84	31.48	32.26	31.87
T_7	0.89	1.08	0.98	4.53	4.91	4.72	27.00	33.85	30.43	26.42	32.56	29.49
T_8	0.99	1.03	1.01	4.81	4.27	4.54	30.74	31.21	30.97	29.73	26.87	28.30
T_9	1.00	1.01	1.00	4.32	4.39	4.36	39.98	34.07	37.03	39.26	29.22	34.24
T_{10}	1.00	0.97	0.99	4.40	3.96	4.18	24.42	34.52	29.47	24.99	30.32	27.65
T_{11}	1.05	1.00	1.03	4.11	4.31	4.21	19.41	35.96	27.69	26.86	31.73	29.30
T_{12}	1.28	1.06	1.17	4.25	4.68	4.47	19.86	37.77	28.82	28.12	29.15	28.63
T_{13}	0.87	0.98	0.92	4.37	4.13	4.25	25.81	30.11	27.96	26.75	29.37	28.06
T_{14}	1.00	1.01	1.00	4.15	4.06	4.10	29.62	35.05	32.33	29.74	29.04	29.39
T_{15}	1.02	1.00	1.01	4.70	4.81	4.76	25.28	32.67	28.97	22.60	33.02	27.81
T_{16}	0.97	1.05	1.01	4.49	4.43	4.46	30.57	34.63	32.60	29.82	32.89	31.35
T_{17}	0.97	1.01	0.99	4.36	5.04	4.70	43.29	30.85	37.07	37.69	31.05	34.37
T_{18}	1.04	1.01	1.03	5.56	4.76	5.16	33.93	33.62	33.78	34.28	30.25	32.27
T_{19}	0.93	0.98	0.96	4.72	5.53	5.13	28.42	34.44	31.43	29.02	33.65	31.33
T_{20}	1.00	1.00	1.00	5.27	5.08	5.17	36.85	38.04	37.45	35.86	35.54	35.70
SE(m) ±	0.11	0.07	0.07	0.27	0.28	0.20	1.64	1.21	1.02	1.29	1.05	0.83
CD (p=0.05)	**0.20**	**0.12**	**0.29**	**0.49**	**0.50**	**0.85**	**2.94**	**2.16**	**4.42**	**2.30**	**1.88**	**3.63**

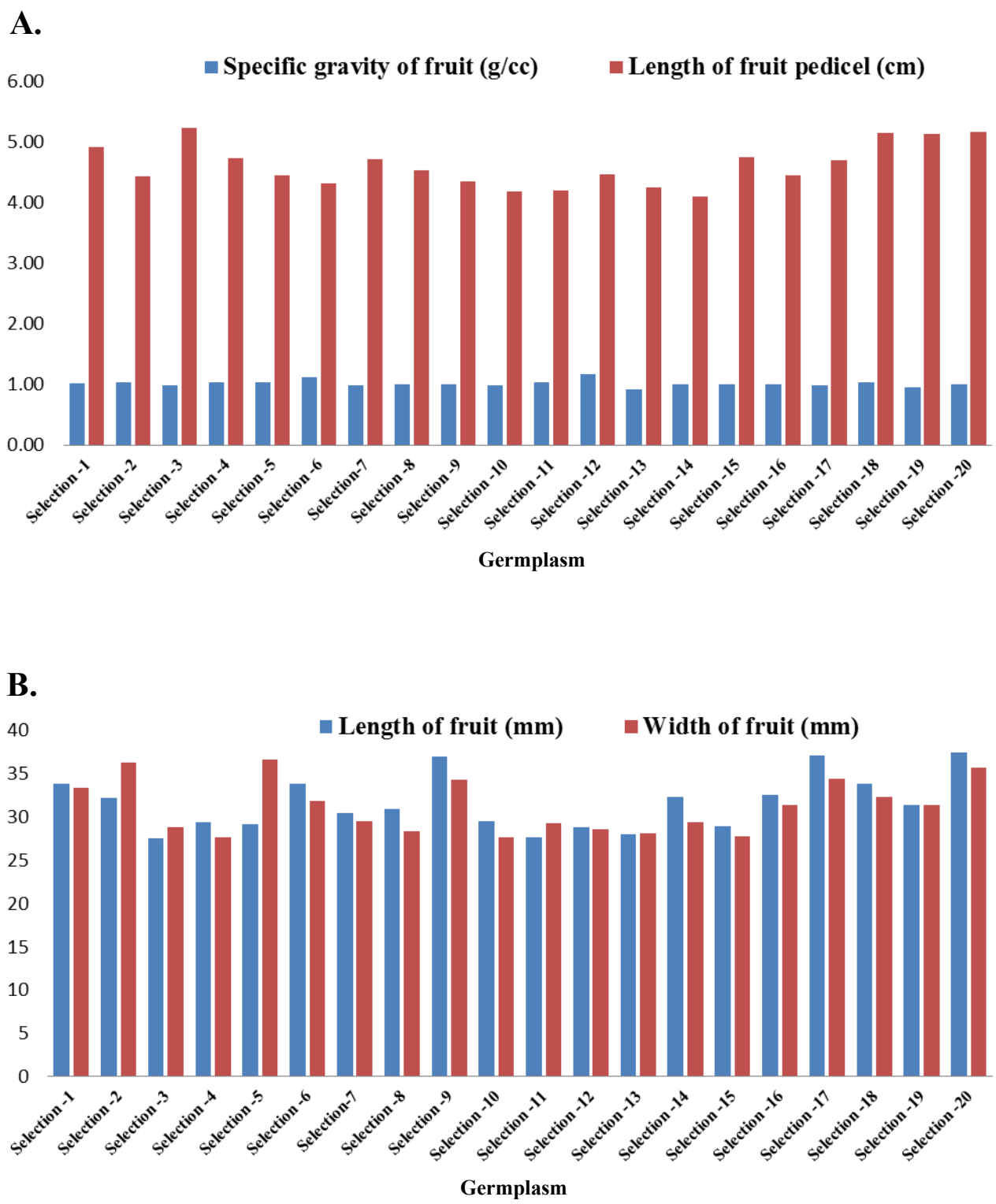

Fig. 4.7 Inter varietal variability in water chestnut (*Trapa natans* var. *bispinosa* Roxb.) collected from various blocks of district Lucknow on the basis of A. specific gravity of fruit (g/cc) and length of fruit pedicel (cm); B. length of fruit (cm) and width of fruit (cm)

Thickness of peel (mm)

The significantly value were recorded from ranged 1.76 to 2.46 with a grand mean 2.08 as presented in Table 4.19. Thickness of peel (mm) the maximum value were recorded in T_1 (2.46 mm) fallowed by T_3 and T_4 (2.44 mm), T_5 (2.41mm) and T_{14} (2.26) respectively. The minimum value of thickness of peel of fruit was obtained in T_{13} (1.76) fallowed by T_2 (1.81). Which are presented in Table 4.13 and Fig. 4.8A.

Peel weight (g)

Under the study significant variance of revealed between range 1.78 to 3.40 for the peel weight of fruits (g) and the grand mean of 2.57 was recorded for this trait. The significantly result were found for peel weight of fruits (g) for T_1 (3.40g) other than T_{20} (3.23), T_{17} (3.07) and T_{15} (3.03) while the minimum value were found for this trait in T_8 (1.78) (Table 4.14 and Fig. 4.8A).

Cheek diameter of fruit (mm)

The cheek diameter of fruit showed significant variation between various morphotypes and cheek diameter of fruit varied from 28.02 to 38.44 with a grand mean 32.61. (Table 4.19). The highest cheek diameter of fruit (37.04) was recorded for T_{20} followed by T_9 (36.87) and T_2 (36.62) and minimum cheek diameter of fruit (28.02) was recorded for Selection-7 as presented in Table 4.14 and Fig.4.8B.

Table 4.14 Inter varietal variability in water chestnut (*Trapa natans* var. *bispinosa* Roxb.) collected from various blocks of district Lucknow on the basis of kernel morphology

Germplasm	Thickness of peel (mm)			Peel weight (g)			Fruits cheek diameter (mm)		
	2016	2017	Pooled	2016	2017	Pooled	2016	2017	Pooled
T_1	2.50	2.42	2.46	1.67	5.13	3.40	33.60	34.77	34.18
T_2	1.58	2.04	1.81	2.60	3.00	2.80	40.93	32.31	36.62
T_3	2.61	2.27	2.44	1.47	3.13	2.30	25.53	35.02	30.28
T_4	2.58	2.30	2.44	1.47	4.33	2.90	25.38	31.68	28.53
T_5	2.42	2.40	2.41	1.87	4.07	2.97	42.33	34.55	38.44
T_6	2.14	2.10	2.12	1.40	4.13	2.77	32.79	33.96	33.38
T_7	2.00	1.99	2.00	1.33	4.00	2.67	25.46	30.57	28.02
T_8	1.72	1.92	1.82	1.73	1.83	1.78	30.92	30.61	30.77
T_9	2.10	2.03	2.06	1.73	3.40	2.57	42.18	31.56	36.87
T_{10}	2.27	2.10	2.18	1.13	3.33	2.23	25.70	32.37	29.03
T_{11}	2.06	1.89	1.97	1.53	3.00	2.27	30.57	34.27	32.42
T_{12}	2.25	1.93	2.09	1.00	3.27	2.13	32.66	32.76	32.71
T_{13}	1.61	1.90	1.76	1.67	2.00	1.83	29.00	31.54	30.27
T_{14}	2.09	2.43	2.26	1.67	3.27	2.47	29.92	32.26	31.09
T_{15}	1.96	2.10	2.03	1.40	4.67	3.03	26.51	35.05	30.78
T_{16}	1.82	2.14	1.98	1.60	3.20	2.40	31.23	32.13	31.68
T_{17}	1.95	1.96	1.95	2.13	4.00	3.07	35.71	31.29	33.50
T_{18}	1.98	2.09	2.03	2.33	1.93	2.13	35.16	32.49	33.83
T_{19}	1.77	1.87	1.82	1.73	3.47	2.60	30.97	34.69	32.83
T_{20}	2.20	1.87	2.03	2.20	4.27	3.23	37.23	36.85	37.04
SE(m) ±	0.27	0.13	0.15	0.11	0.27	0.14	0.91	1.35	0.91
CD (p=0.05)	**1.18**	**0.23**	**0.65**	**0.20**	**0.47**	**0.63**	**1.62**	**2.42**	**3.57**

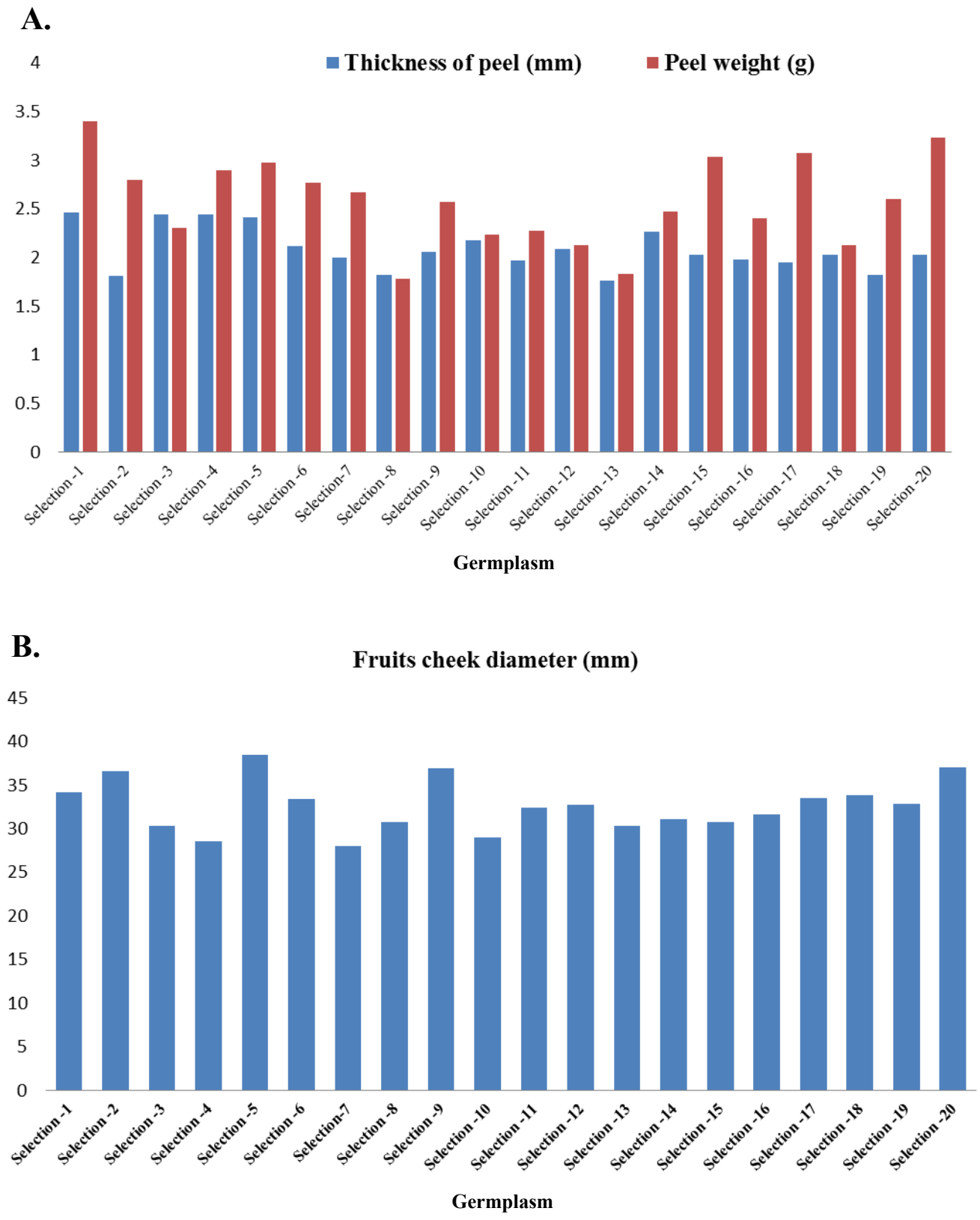

Fig. 4.8 Inter varietal variability in water chestnut (*Trapa natans* var. *bispinosa* Roxb.) collected from various blocks of district Lucknow on the basis of A. thicknesses of peel (mm) and peel weight (g); B. fruits cheek diameter (mm)

4.3.2 Kernel morphology

Weight of kernel (g)

The weight of kernel ranged from 3.17 to 5.67g with a grand mean of 4.21 g (Table 4.19). However, the maximum weight of kernel (5.67 g) was recorded for T_1 followed by (5.60 g) T_{20} and (4.93 g) T_{12} and minimum weight of kernel (3.17 g) was observed for T_{11}. Thus, weight of kernel of various germplasm showed wide variation between various germplasm and presented in Table 4.15 and Fig. 4.9A.

Kernel: Peel ratio

Kernel: Peel ratio also showed significant inter-varietal variation between different germplasm under the investigation and varied from 1.40 to 2.62 with general mean of 1.87 (Table 4.19). The maximum kernel: Peel (2.62) ratio was observed for T_8 followed by T_6 (2.38), T_{12} (2.31) and T_{13} and T_{18} (2.24) minimum kernel: Peel (1.40) was recorded for T_{15} which were at par with each other and as shown in Table 4.15 and Fig. 4.9A.

Volume kernel (ml)

The data pertaining to kernel volume of various morphotypes (Table 4.15 and Fig. 4.9B) showed significant variation between the germplasm. The ranged from 3.33-5.17 were found and with a general mean of this trait 4.24 (Table 4.19). The T_1 and T_{20} showed the highest kernel volume (5.17 ml) followed by T_9 and T_7 (4.90 ml) and T_6 (4.57ml). However, T_{11} (3.33) had minimum fruit volume followed by T_{10} (3.43 ml) which were at par.

Table 4.15 Inter varietal variability in water chestnut (*Trapa natans* var. *bispinosa* Roxb.) collected from various blocks of district Lucknow on the basis of kernel morphology

Germplasm	Kernel weight (g)			Kernel :peel ratio			Kernel volume (ml)		
	2016	2017	Pooled	2016	2017	Pooled	2016	2017	Pooled
T_1	4.40	6.80	5.67	2.62	1.45	2.04	4.27	6.07	5.17
T_2	3.20	5.13	4.17	1.46	1.94	1.70	3.40	5.13	4.27
T_3	2.13	5.20	3.67	1.47	1.75	1.61	2.33	4.73	3.53
T_4	2.67	5.27	3.97	1.82	1.26	1.54	2.80	5.07	3.93
T_5	3.47	4.40	3.93	1.82	1.10	1.46	3.67	4.67	4.17
T_6	4.73	4.53	4.63	3.61	1.16	2.38	4.67	4.47	4.57
T_7	3.27	4.80	4.03	2.39	1.31	1.85	3.67	5.00	4.33
T_8	3.27	5.00	4.13	1.97	3.26	2.62	3.53	5.13	4.33
T_9	4.93	4.60	4.77	2.91	1.38	2.14	5.07	4.73	4.90
T_{10}	2.33	4.47	3.40	2.14	1.35	1.75	2.60	4.27	3.43
T_{11}	2.27	4.07	3.17	1.48	1.54	1.51	2.40	4.27	3.33
T_{12}	2.67	7.20	4.93	2.69	1.93	2.31	2.87	6.40	4.64
T_{13}	3.47	4.80	4.13	2.04	2.44	2.24	3.87	5.00	4.43
T_{14}	3.47	5.00	4.24	2.16	1.54	1.85	3.80	4.87	4.33
T_{15}	2.53	4.53	3.53	1.78	1.01	1.40	2.73	4.20	3.47
T_{16}	3.60	3.87	3.73	2.30	1.21	1.75	3.93	3.80	3.87
T_{17}	5.67	4.07	4.87	2.66	1.03	1.85	5.93	3.87	4.90
T_{18}	3.67	5.00	4.33	1.58	2.89	2.24	3.93	5.00	4.47
T_{19}	2.40	4.27	3.33	1.46	1.40	1.43	2.47	4.93	3.70
T_{20}	4.33	7.00	5.60	2.04	1.64	1.84	4.40	5.93	5.17
SE(m) ±	0.24	0.45	0.26	0.28	0.19	0.17	0.28	0.36	0.23
CD (p=0.05)	**0.43**	**0.81**	**1.12**	**0.50**	**0.34**	**0.74**	**0.45**	**0.65**	**1.00**

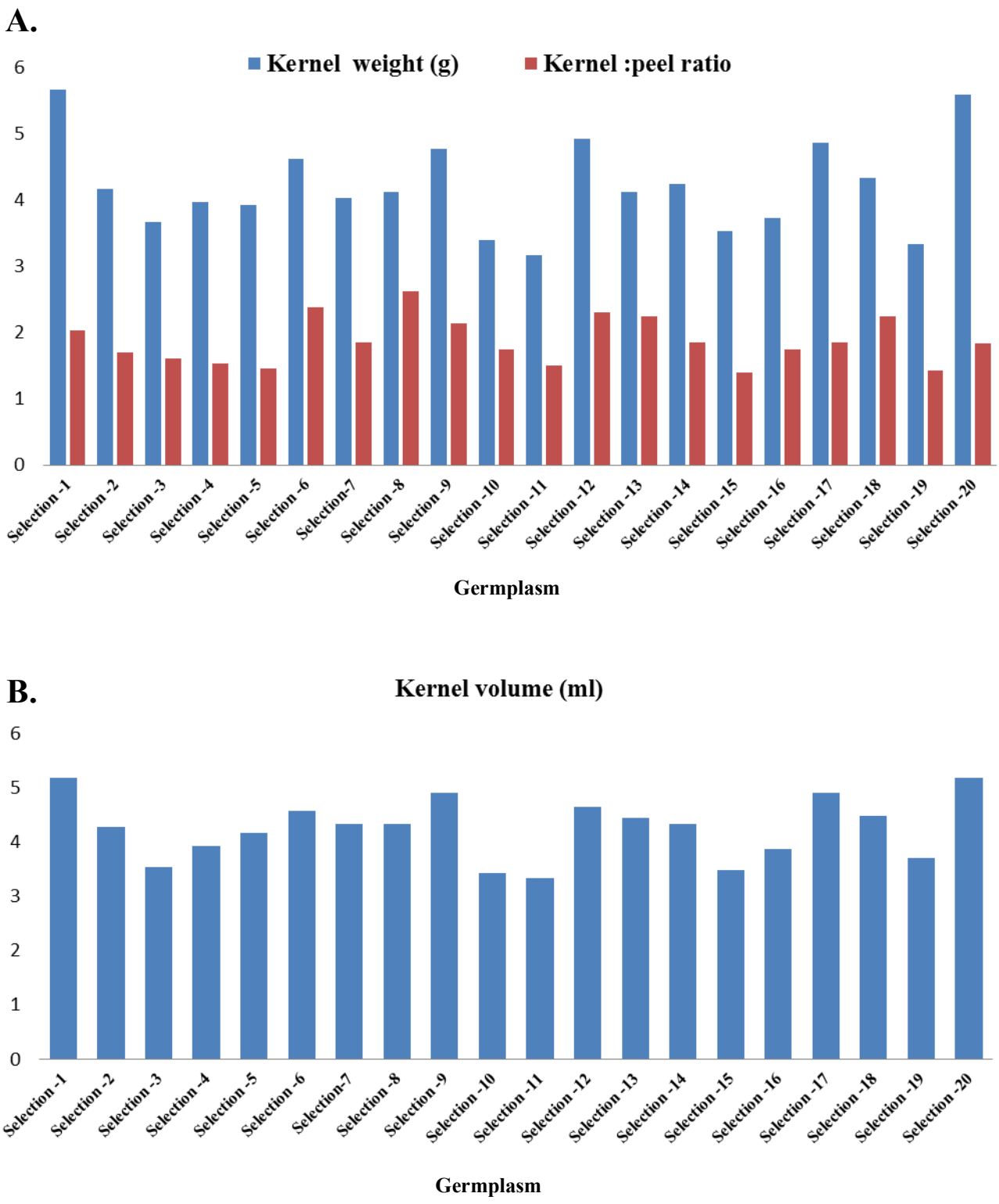

Fig. 4.9 Inter varietal variability in water chestnut (*Trapa natans* var. *bispinosa* Roxb.) collected from various blocks of district Lucknow on the basis of A. kernel weight (g) and kernel: peel ratio; B. kernel volume (ml)

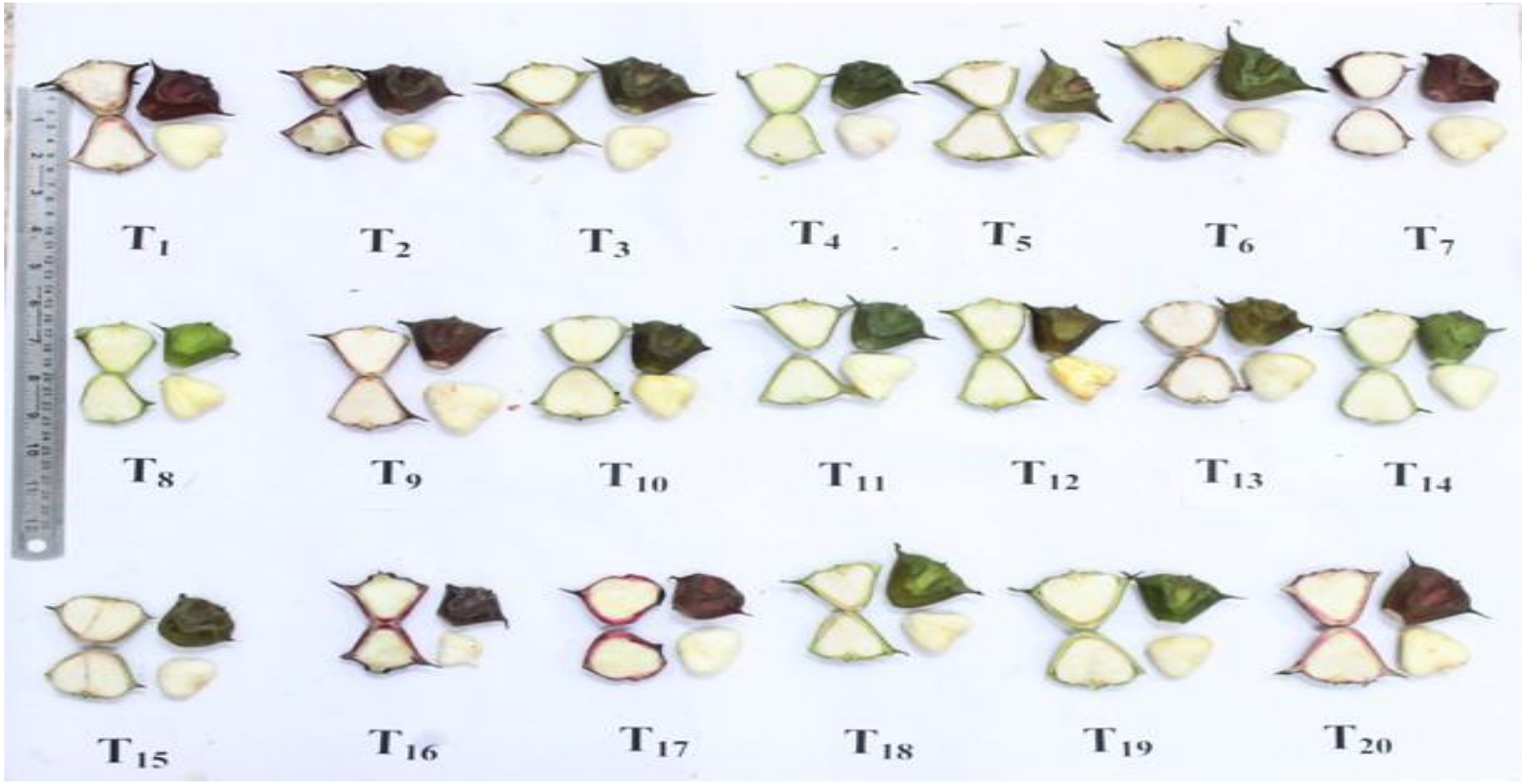

Plate 4.9 Inter varietal variability in kernels of water chestnut (*Trapa natans* var. *bispinosa* Roxb.) fruits of twenty germplasm collected from various blocks of district Lucknow

Specific gravity of kernel (g/cc)

The average range were found of specific gravity of kernel from 0.92 to 1.99 and grand mean 0.96 (Table 4.19). The maximum specific gravity of kernel was recorded for T_7 (1.99) fallowed by T_1 and T_3 (1.01), T_{11} (1.00) and T_6 (0.99) while the minimum specific gravity of kernel was observed for T_{10} and T_{13} (0.92) (Table 4.16 and Fig. 4.10A).

Length of kernel (mm)

The data pertaining to length of kernel of different germplasm revealed significant inter-varietal variation among the germplasm and length of kernel varied between 24.17-30.92 (mm) and grand mean of 26.79 (mm) (Table 4.19). However, the highest length of kernel (30.92 mm) was observed for T_6 followed by T_{10} (29.92 mm) and T_{11} (28.59 mm) respectively. The shortest length of kernel (24.17 mm) was observed for T_{13} which were at par with each other (Table 4.16 and Fig. 4.10A).

Width of kernel (mm)

A significant inter-varietal variation among different germplasm were recorded for width of kernel which ranged from 24.15 to 28.64 (mm) with a grand mean of 26.93 (mm) (Table 4.19). However, the maximum width of kernel (28.64 mm) was recorded in T_{10} followed by T_1 (28.38 mm), T_{16} (28.13 mm) and T_{20} (28.09 mm) which was significantly higher in comparison to other germplasm under study (Table 4.16 and Fig. 4.10B). Selection T_8 showed significant lower width of kernel (24.15 mm).

Kernel cheek diameter (mm)

The kernel cheek diameter showed variation among various germplasmvaried from 26.52 to 30.19 mm with a grand mean 28.28 mm (Table 4.19). However, the maximum cheek diameter (30.19 mm), (30.18mm), (29.91mm) (29.45mm) and (28.97mm) were observed from germplasm T_{20}, T_1, T_6, T_7 and T_{19}, respectively, and the minimum kernel cheek diameter (26.02mm) was observed from Selection T_{13} (Table 4.16and Fig.4.10B).

Colour of kernel

The colour of kernel kernel of water chestnut was observed visually in all twenty germplasm collected from the various blocks viz., of Mohanlalganj, Gosainganj, Sarojini nagar, Baksi Ka Talab and Mlihabad of district Lucknow and has been recorded and presented in Table 4.4 and Plate 4.2.

It is evident from the Plate 4.2 that Selection T_1, T_3, T_5, T_6, T_7, T_9, T_{17}, T_{18}, T_{19} and T_{20} were showed creamy white whereas, Selection T_4, T_{11}, T_{13}, T_{14} and T_{15}, T_{16} were found for colour of kernel. The Selection T_2, T_8, T_{10} and T_{12} were observed white.

Table 4.16 Inter varietal variability in water chestnut (*Trapa natans* var. *bispinosa* Roxb.) collected from various blocks of district Lucknow on the basis of kernel morphology

Germplasm	Specific gravity of kernel (g/cc)			Length of kernel (mm)			Widths of kernel (mm)			Kernel cheek diameter (mm)		
	2016	2017	Pooled	2016	2017	Pooled	2016	2017	Pooled	2016	2017	Pooled
T_1	1.02	1.00	1.01	24.88	30.18	27.53	27.70	29.05	28.38	30.48	29.87	30.18
T_2	0.92	0.98	0.95	26.17	27.06	26.61	26.76	27.53	27.14	27.37	27.83	27.60
T_3	0.92	1.10	1.01	25.58	27.17	26.38	26.93	29.14	28.04	27.89	30.22	29.06
T_4	0.94	0.98	0.96	24.23	26.02	25.13	24.80	24.87	24.84	27.01	26.50	26.75
T_5	0.93	0.95	0.94	24.98	28.00	26.49	25.83	27.82	26.83	29.59	27.91	28.75
T_6	1.00	0.98	0.99	25.81	36.03	30.92	26.09	29.32	27.71	30.00	29.82	29.91
T_7	1.03	2.96	1.99	24.61	29.35	26.98	25.68	29.70	27.69	28.72	30.18	29.45
T_8	0.92	1.01	0.97	22.38	27.73	25.06	23.36	24.95	24.15	28.57	26.37	27.47
T_9	0.98	0.97	0.97	25.91	27.86	26.89	27.12	26.26	26.69	28.94	27.46	28.20
T_{10}	0.89	0.95	0.92	30.18	29.65	29.92	30.61	26.67	28.64	29.03	28.33	28.68
T_{11}	1.05	0.95	1.00	27.47	29.72	28.59	28.01	26.70	27.35	26.84	29.54	28.19
T_{12}	0.88	1.02	0.95	25.92	29.63	27.77	27.48	24.97	26.22	28.29	26.63	27.46
T_{13}	0.89	0.94	0.92	23.45	24.88	24.17	24.02	26.56	25.29	24.86	27.18	26.02
T_{14}	0.93	1.03	0.98	25.48	29.07	27.27	26.52	27.81	27.17	28.58	27.55	28.07
T_{15}	0.92	1.00	0.96	24.76	27.24	26.00	25.94	29.64	27.79	27.43	29.63	28.53
T_{16}	0.91	0.96	0.94	24.14	28.34	26.24	26.52	29.73	28.13	27.83	29.83	28.83
T_{17}	0.95	0.95	0.95	22.80	26.05	24.43	23.67	27.71	25.69	25.95	27.09	26.52
T_{18}	0.93	1.00	0.96	23.80	25.80	24.80	24.61	25.78	25.20	27.28	26.35	26.81
T_{19}	0.96	0.96	0.96	25.74	27.59	26.67	26.13	29.46	27.80	28.80	29.13	28.97
T_{20}	0.99	1.01	1.00	25.28	31.02	28.15	25.73	30.45	28.09	30.07	30.32	30.19
SE(m)±	0.06	0.06	0.04	0.75	0.78	0.54	0.87	0.87	0.62	0.70	0.75	0.51
CD (p=0.05)	**0.11**	**0.10**	**0.18**	**1.34**	**1.40**	**2.36**	**1.56**	**1.56**	**2.69**	**1.25**	**1.35**	**2.25**

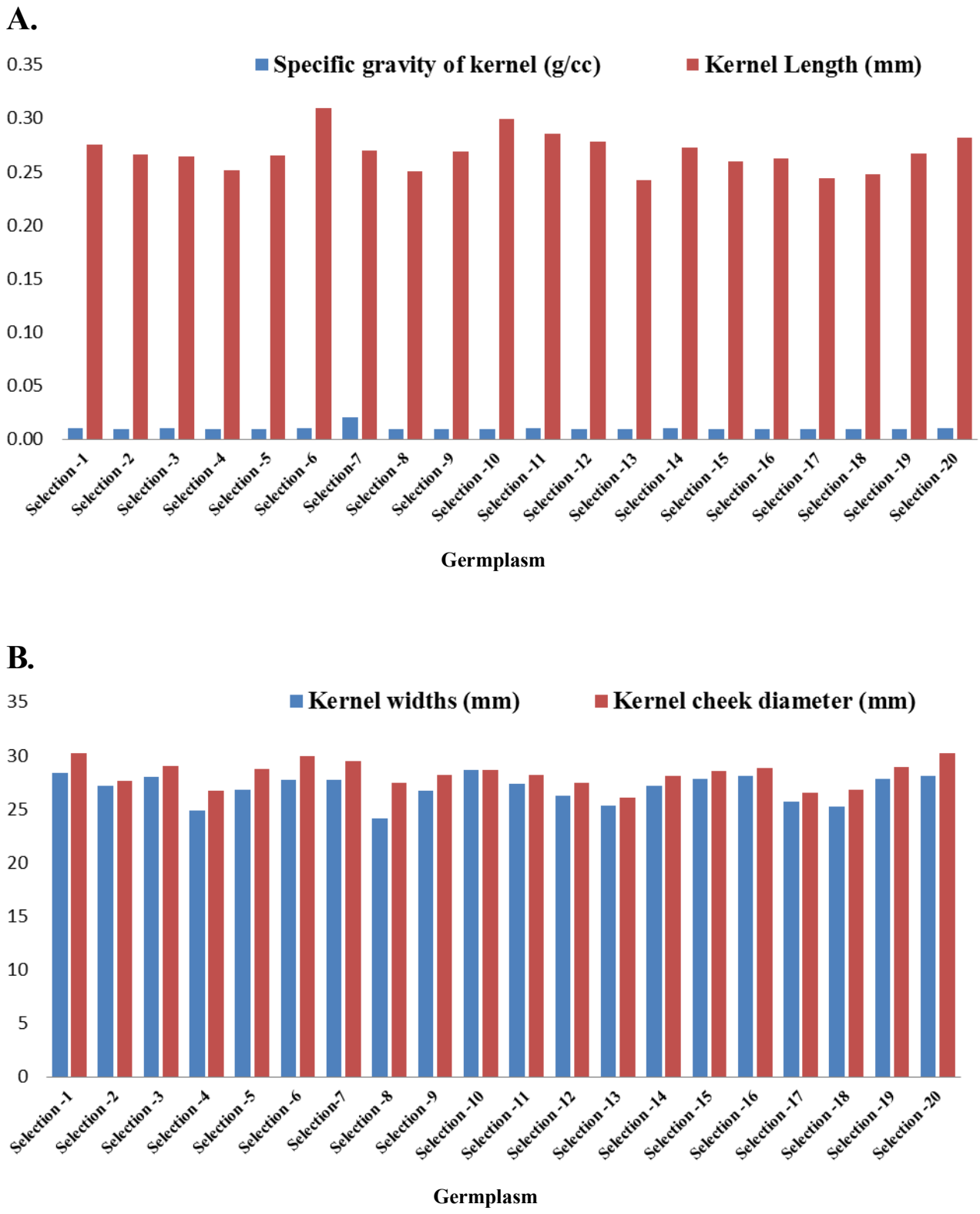

Fig. 4.10 Inter varietal variability in water chestnut (*Trapa natans* var. *bispinosa* Roxb.) collected from various blocks of district Lucknow on the basis of A. specific gravity of kernel (g/cc) and kernel length (mm); B. kernel width (cm) and kernel cheek diameter (mm)

4.3.3 Bio-chemical parameters of fruit:

Total soluble solid [TSS (ºBrix)]

The significant variation was observed for total soluble solids content in fruit juice among various germplasm, which ranged from 4.17 to 6.77 ºBrix with grand mean 5.41 ºBrix (Table 4.19). Therefore, the highest TSS (6.77 ºBrix) and (6.53 ºBrix) was observed from T_{19} and T_{12}, respectively followed by T_{17} (6.42ºBrix) which was at par with T_3 (6.15 ºBrix). Selection T_6 showed lowest TSS (4.17ºBrix) followed by T_{13} (4.27 ºBrix) as shown in Table 4.17 and Fig. 4.11A.

pH of the fruit pulp

pH of fruit pulp varied from 4.99 to 7.07 with grand mean of 5.97 (Table 4.19), showing maximum pH (7.07) in Selection T_{11} closely followed by T_7 (6.94) and T_{18} (6.65) which were at par with T_6 and T_{12} (6.34). The minimum pH of fruit pulp (4.99) was observed for T_{15} followed by T_1 and 8 (5.09) as showed in Table 4.17 and Fig. 4.11A.

Titratable acidity (%)

Titrable acidity in term of citric acid was determined for various germplasm and it varied from 0.04 to 0.10 % with grand mean of 0.06% (Table 4.19). However, the maximum titrable acidity (0.10 %) was observed from T_1 and T_{10} closely followed by T_9 and T_{16} (0.09%), T_2, T_{13}, T_{17} and T_{18} (0.08%) respectively while, the minimum titrable acidity (0.04%) was observed for Selection T_4, T_7 and T_{14} (0.04%) followed by T_5, T_{11} and T_{12} (0.05 %) (Table 4.17 and Fig. 4.11B).

Ascorbic acid (mg/100g)

Ascorbic acid in term of vitamin C was determined for various morphotypes and range were found from 7.80-8.83 % with grand mean of 8.26 2% (Table 4.19). However, the maximum Ascorbic acid (8.83 %) was observed from T_9 followed by T_{20} (8.69), T_5 (8.55), T_7 (5.50) and T_{18} (8.46), respectively while, the minimum Ascorbic acid (7.80%) was observed for T_{16}, followed by T_{17} (7.84 %) (Table 4.17 and Fig. 4.11B).

Table 4.17 Inter varietal variability in water chestnut (*Trapa natans* var. *bispinosa* Roxb.) collected from various blocks of district Lucknow on the basis of kernel morphology

Germplasm	TSS (^{0}Bricks)			pH of the fruit juice			Acidity (%)			Ascorbic acid (mg/100g)		
	2016	2017	Pooled	2016	2017	Pooled	2016	2017	Pooled	2016	2017	Pooled
T_1	6.13	6.07	6.10	4.89	5.28	5.09	0.09	0.10	0.10	7.51	8.34	7.93
T_2	5.73	5.53	5.63	5.47	5.43	5.45	0.07	0.10	0.08	8.31	8.40	8.36
T_3	6.47	5.83	6.15	5.13	5.48	5.31	0.07	0.04	0.06	7.13	9.22	8.18
T_4	7.27	4.63	5.95	5.93	6.32	6.13	0.05	0.04	0.04	8.31	7.91	8.11
T_5	4.03	5.50	4.77	5.06	5.36	5.21	0.07	0.03	0.05	8.48	8.62	8.55
T_6	3.87	4.47	4.17	6.07	6.61	6.34	0.02	0.11	0.07	7.50	9.12	8.31
T_7	4.10	5.47	4.78	6.77	7.11	6.94	0.07	0.01	0.04	8.83	8.17	8.50
T_8	5.97	6.20	6.08	5.00	5.18	5.09	0.06	0.05	0.06	8.29	8.57	8.43
T_9	4.27	5.20	4.73	6.47	6.49	6.48	0.07	0.12	0.09	9.07	8.58	8.83
T_{10}	3.60	6.07	4.83	6.53	6.37	6.45	0.08	0.11	0.10	7.48	8.67	8.07
T_{11}	4.53	5.03	4.78	7.12	7.01	7.07	0.05	0.04	0.05	7.60	9.23	8.42
T_{12}	7.50	5.57	6.53	6.52	6.16	6.34	0.07	0.03	0.05	7.89	8.39	8.14
T_{13}	3.63	4.90	4.27	6.03	5.41	5.72	0.08	0.08	0.08	8.01	8.06	8.04
T_{14}	6.03	4.90	5.47	6.38	6.08	6.23	0.04	0.05	0.04	7.85	8.47	8.16
T_{15}	5.20	4.83	5.02	4.77	5.20	4.99	0.08	0.09	0.09	8.36	8.01	8.19
T_{16}	5.70	6.37	6.03	5.97	6.02	6.00	0.08	0.11	0.09	7.24	8.35	7.80
T_{17}	5.93	6.90	6.42	5.98	6.26	6.12	0.11	0.04	0.08	8.10	7.58	7.84
T_{18}	4.60	5.50	5.05	6.30	7.00	6.65	0.10	0.06	0.08	8.32	8.61	8.46
T_{19}	7.13	6.40	6.77	5.77	6.07	5.92	0.07	0.04	0.06	7.70	8.81	8.25
T_{20}	4.63	4.70	4.67	5.37	6.72	6.05	0.07	0.07	0.07	9.06	8.31	8.69
SE(m)±	0.41	0.31	0.26	0.41	0.36	0.27	0.02	0.01	0.01	0.47	0.54	0.36
CD (p=0.05)	**0.74**	**0.55**	**1.13**	**0.74**	**0.64**	**1.20**	**0.03**	**0.02**	**0.15**	**0.84**	**0.97**	**1.58**

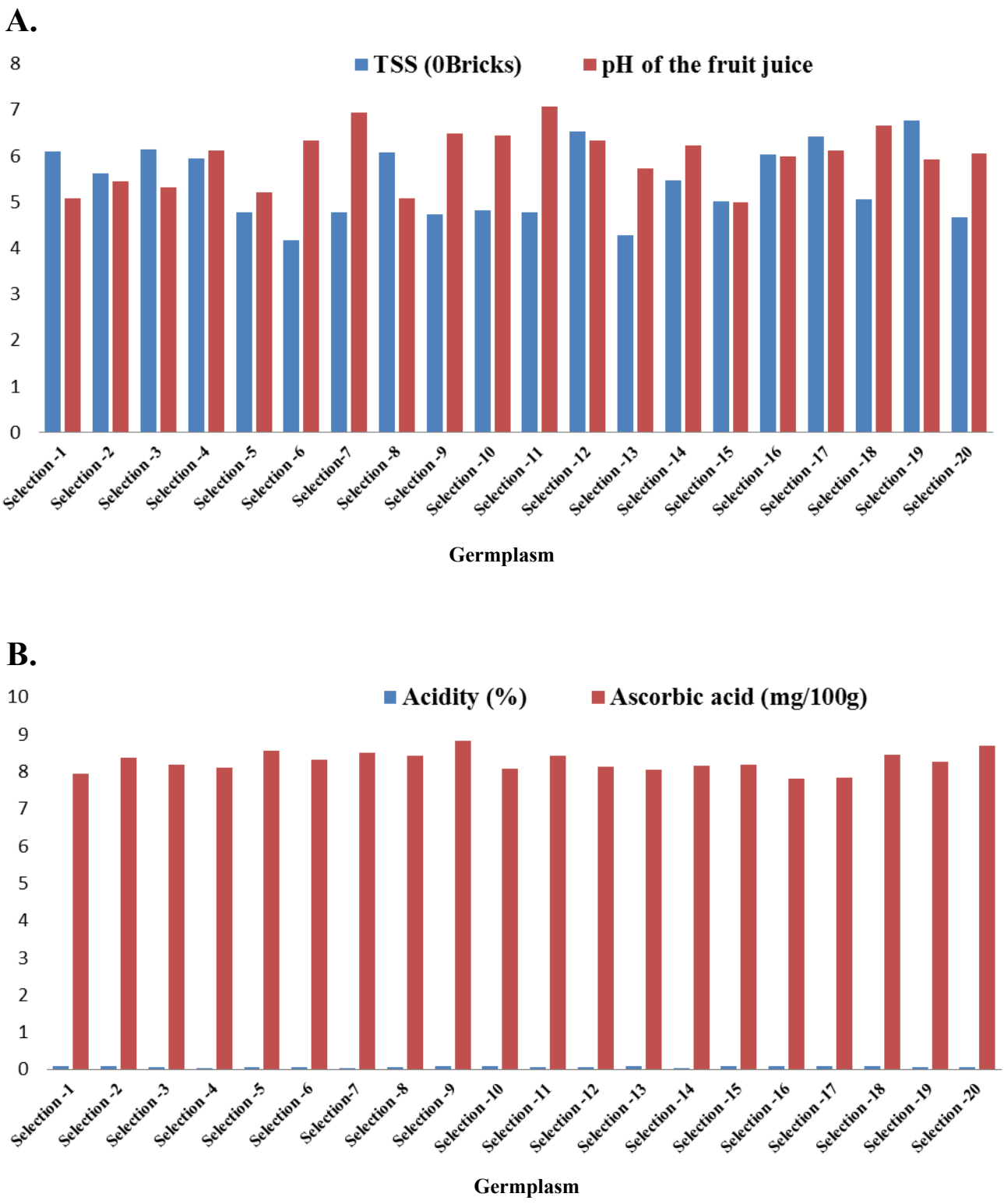

Fig. 4.11 Inter varietal variability in water chestnut (*Trapa natans* var. *bispinosa* Roxb.) collected from various blocks of district Lucknow on the basis of A. total soluble solids (°Brix) and pH of the fruit juice; B. acidity (%) and ascorbic acid (mg/100g)

Reducing sugars (%)

The data pertaining to reducing sugar of different water chestnut germplasm revealed that it varied from 1.65 to 1.87 % with a grand mean 1.77 % (Table 4.19). The maximum reducing sugar (1.87 %) was observed from Selection T_{15} followed by T_5, T_{12} and T_{15} (1.82 %), respectively. The minimum reducing sugar (1.65 %) was observed in T_6 followed by T_9 (1.68 %) which were at par with each other (Table 4.18and Fig. 4.12A).

Non-reducing sugar (%)

Non-reducing sugar ranged from 2.09 to 3.03% with a grand mean of 2.50 % (Table 4.19). The Selection T_6 (3.03%), showed significantly higher level of non-reducing sugar in comparison to other morphotypes under study. Lowest non-reducing sugar of 2.09 % was registered in T_{20} (Table 4.18 and Fig. 4.12A).

Total sugars (%)

The total sugars varied between 3.92 to 4.67% and its general mean was recorded 2.50 % (Table 4.19). The highest level of total sugars (4.67%) was recorded for Selection T_6 fallowed by T_{10} (4.57%), T_{12} (4.54%) and T_{18} (4.52%), respectively. Contained least total sugars as shown in T_{13} (9.92%) are given in Table 4.18 and Fig. 4.12B.

Table 4.18 Inter varietal variability in water chestnut (*Trapa natans* var. *bispinosa* Roxb.) collected from various blocks of district Lucknow on the basis of kernel morphology

Germplasm	Reducing sugar (%)			Non reducing sugar (%)			Total sugars (%)		
	2016	2017	Pooled	2016	2017	Pooled	2016	2017	Pooled
T_1	1.76	1.85	1.80	2.09	2.37	2.23	4.05	4.22	4.14
T_2	1.74	1.87	1.81	2.29	2.35	2.32	4.06	4.25	4.15
T_3	1.84	1.77	1.80	2.77	2.47	2.62	4.56	4.24	4.40
T_4	1.80	1.68	1.74	2.49	2.61	2.55	4.26	4.30	4.28
T_5	1.78	1.86	1.82	2.49	2.37	2.43	4.30	4.22	4.26
T_6	1.70	1.61	1.65	2.75	3.30	3.03	4.43	4.91	4.67
T_7	1.67	1.78	1.73	2.20	2.58	2.39	3.87	4.36	4.12
T_8	1.79	1.74	1.76	2.50	2.99	2.75	4.22	4.73	4.47
T_9	1.58	1.78	1.68	2.01	2.31	2.16	3.73	4.12	3.93
T_{10}	1.88	1.63	1.75	2.44	3.19	2.82	4.33	4.82	4.57
T_{11}	1.81	1.79	1.80	2.55	2.71	2.63	4.25	4.58	4.42
T_{12}	1.75	1.89	1.82	2.80	2.67	2.74	4.52	4.56	4.54
T_{13}	1.76	1.82	1.79	2.00	2.40	2.20	3.63	4.21	3.92
T_{14}	1.92	1.52	1.72	2.15	3.12	2.64	4.03	4.64	4.34
T_{15}	1.91	1.83	1.87	1.80	2.48	2.14	3.82	4.31	4.07
T_{16}	1.66	1.88	1.77	2.36	2.27	2.32	4.00	4.15	4.08
T_{17}	1.70	1.86	1.78	2.73	2.63	2.68	4.39	4.49	4.44
T_{18}	1.79	1.86	1.82	2.53	2.83	2.68	4.35	4.68	4.52
T_{19}	1.55	2.00	1.78	2.73	2.61	2.67	4.23	4.64	4.44
T_{20}	1.62	1.97	1.80	2.05	2.12	2.09	3.80	4.08	3.94
SE(m)±	0.10	0.08	0.06	0.12	0.18	0.11	0.12	0.15	0.10
CD (p=0.05)	**0.17**	**0.14**	**0.28**	**0.21**	**0.31**	**0.46**	**0.21**	**0.26**	**0.41**

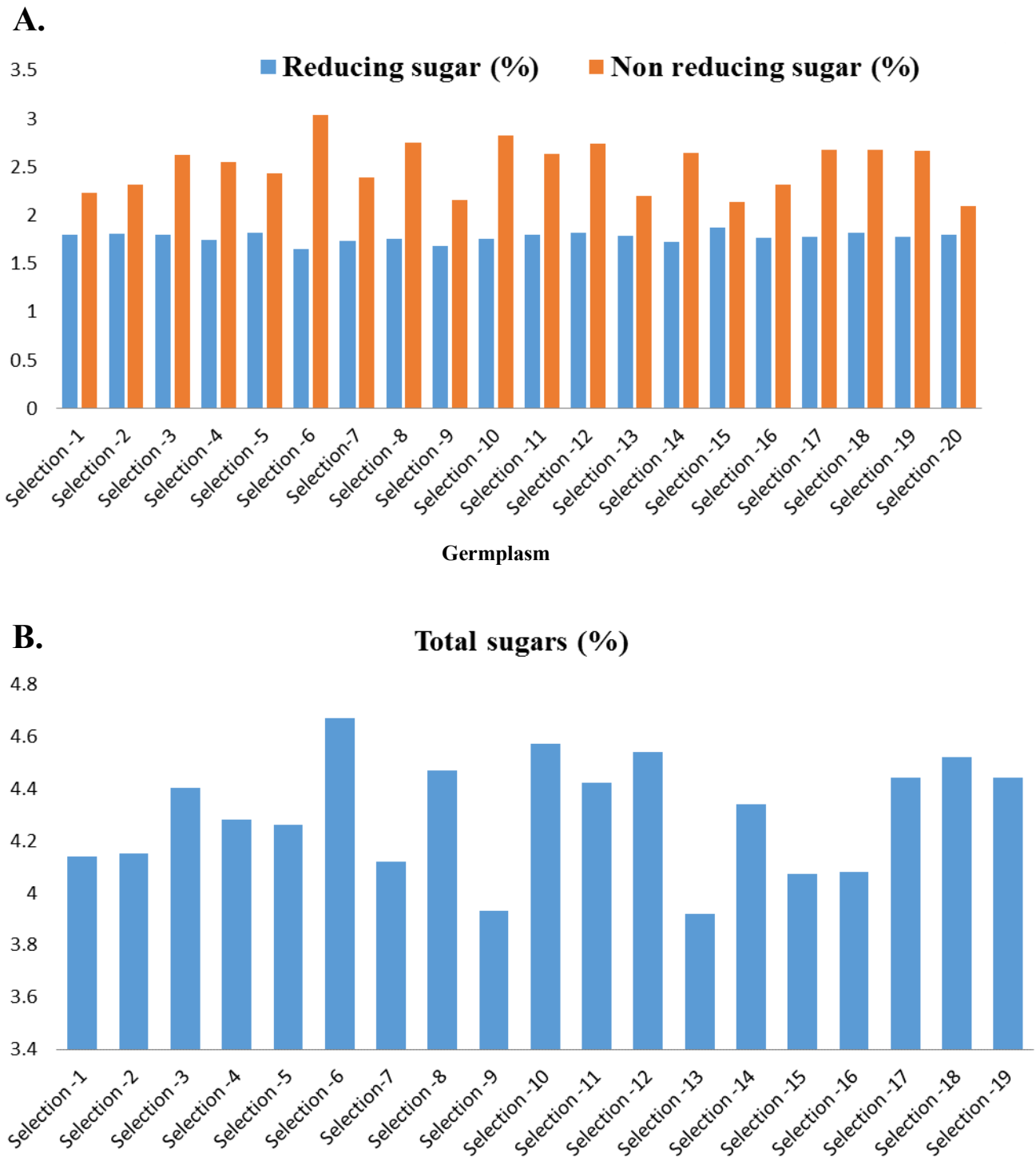

Fig. 4.12 Average performance of A. reducing sugar (%) and non-reducing sugar (%); B. total sugar (%) of 20 water chestnut (*Trapa natans* var. *bispinosa* Roxb.) germplasm collected from various blocks of district Lucknow

Biometrical techniques for assessment of inter-varietal variability for fruit physico-chemical traits of twenty germplasm of water chestnut:

The morphological data recorded was subjected to biometrical techniques for assessment which was computed through simple measures of variability viz., range, grand mean and coefficient of variation (CV), genetic variability (PCV % and GCV %), heritability, genetic advance and genetic advance as percent of mean (%) for further elucidation of the data recorded. Coefficient of variation, phenotypic coefficient of variation, genotypic coefficient of variation, heritability, genetic advance and genetic advance as percent of mean for fruit morphological characters showed more environmental influence (Table 4.19). However, the highest coefficient of variation (15.67), PCV (15.76%), GCV (8.93%) and genetic advance as percent of mean (21.63%) were recorded for thickness of peel (mm). While the maximum heritability (43.20%) was observed for pedicel length of fruits (cm).Whereas, higher genetic advance (3.52%) was found in number of fruits ($Plant^{-1}$). Among the fruit morphology most of the characters showed very negligible value for these CV, PCV, GCV, heritability, genetic advance and genetic advance as percent of mean which indicates environmental effect. CV, PCV, GCV, heritability, genetic advance and genetic advance as percent of mean were analyzed for kernel morphology which showed wide variation between PCV and GCV (Table 4.15). The highest coefficient of variation (15.67) and PCV (15.76%) was recorded for kernel: peel ratio whereas, the maximum GCV (4.82%), heritability (65.60%), genetic advance (4.44%) and genetic advance as percent of mean (16.57%) were observed for length of kernel (mm) (Table 4.19). However, the kernel morphology showed narrow difference between PCV and GCV.

Among the fruit biochemical parameters the highest CV (31.43) and GCV (10.31%) was observed for titrable acidity (%) (Table 4.19) similarly, the highest PCV (12.67%) was recorded for character TSS while, the highest heritability (60.20%), genetic advance (1.91%) and genetic advance as percent of means (31.99%) were observed for pH. The parameter titrable acidity (%) and ascorbic acid (%) showed very negligible. (Table 4.19).

Table 4.19 Range (minimum and maximum), grand mean, coefficient of variance (CV), phenotypic coefficient of variance (PCV%), genotypic coefficient of variance (GCV%), heritability h^2 (%), genetic advance (GA) and genetic advance as percent of mean (GAM%) for 25 fruit, kernel and biochemical characters of 20 germplasm of water chestnut (*Trapa natans* var. *bispinosa* Roxb.) collected from various blocks of district Lucknow:-

Sr. No.	Characters	Grand mean	Range		CV	PCV (%)	GCV (%)	h^2 (%)	GA	GAM (%)
			Minimum	Maximum						
1	Number of fruits (Plant^{-1})	17.85	11.67	25.50	8.95	11.57	7.33	40.10	3.52	19.73
2	Fruit weight (g)	6.83	5.43	8.90	9.36	10.02	3.54	12.50	0.37	5.41
3	Yield (Plant^{-1})	133.01	76.67	255.07	6.84	6.86	0.34	0.30	0.10	0.07
4	Pedicel length of fruits (cm)	4.61	4.10	5.24	7.32	9.72	6.39	43.20	0.82	17.87
5	Fruits Volume (ml)	6.64	5.17	8.93	10.42	11.13	3.90	12.30	0.39	5.87
6	Specific gravity of fruits (g/cc)	1.01	0.92	1.17	11.30	11.73	3.11	7.10	0.04	3.96
7	Fruits length (mm)	31.60	27.51	37.45	5.58	5.58	0.10	0.00*	0.00*	0.00*
8	Fruits width (mm)	31.12	27.65	36.62	4.61	4.62	0.10	0.00*	0.00*	0.00*
9	Cheek diameter of fruit (mm)	32.61	28.02	38.44	4.32	5.38	3.20	35.30	2.64	8.09
10	Thickness of peel (mm)	2.08	1.76	2.46	12.40	15.29	8.93	34.10	0.45	21.63
11	Pee weight (g)	2.57	1.78	3.40	9.63	9.72	1.23	1.60	0.02	0.77
12	Weight of kernel (g)	4.21	3.17	5.67	10.56	11.55	4.68	16.4	0.32	7.60
13	Volume of kernel (ml)	4.24	3.33	5.17	9.32	9.36	0.74	0.60	0.02	0.47
14	Specific gravity of Kernel (g/cc)	0.96	0.92	1.99	7.52	8.20	3.27	15.9	0.06	6.25
15	Length of kernel (mm)	26.79	24.17	30.92	3.48	5.95	4.82	65.60	4.44	16.57

16	Width of kernel (mm)	26.93	24.15	28.64	3.96	4.53	2.19	23.30	1.21	4.49
17	Cheek diameter of kernel (mm)	28.28	26.52	30.19	3.15	4.54	3.27	51.90	2.82	9.97
18	Kernel: peel ratio	1.87	1.40	2.62	15.67	15.76	1.69	1.10	0.02	1.06
19	Total soluble solids [TSS (ºBrix)]	5.41	4.17	6.77	8.34	12.67	9.53	56.6	1.64	30.31
20	pH of the fruit juice	5.97	4.99	7.07	7.96	12.62	9.79	60.20	1.91	31.99
21	Titrable Acidity (%)	0.06	0.04	0.10	31.43	33.08	10.31	9.70	0.00*	0.00*
22	Ascorbic acid (mg/100g)	8.26	7.80	8.83	7.57	7.59	0.38	0.30	0.00*	0.00*
23	Reducing sugar (%)	1.77	1.65	1.87	6.27	6.52	1.78	7.50	0.04	2.25
24	Non-reducing sugar (%)	2.50	2.09	3.03	7.43	10.98	8.08	54.10	0.63	25.52
25	Total sugars (%)	4.28	3.92	4.67	3.84	5.97	4.57	58.6	0.63	14.71

Whereas, CV: Coefficient of variance, PCV: Phenotypic coefficient of variance, GCV: Genotypic coefficient of variance, h^2: Heritability, GA: genetic advance and GAM: genetic advance as percent of mean

EXPERIMENT IV

4.4 Molecular characterization of intervarietal variability in water chestnut

Knowledge of genetic diversity and homozygosity in the germplasm is a prerequisite for any crop improvement programme. Fraction of proteins bands by Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) serve as an indispensable tool for assessing genetic diversity and species relationship. Protein profile of 16 germplasm of water chestnut was prepared using SDS-PAGE for estimation of their genetic diversity at molecular level. The profiles collected are presented in Plate 4.10 for 16 genotypes which survived finally from the germplasm.

The protein profiling showed distinct polymorphism in electrophoretic banding patterns and led to the detection of total of 25 bands. In the initial screening the molecular weight of the 25 bands obtained ranged from 322 kDa to 18 kDa. The highest 322 kDa value band recorded in T_{15} which was not present in other germplasm and minimum (18 kDa) value band was found in all germplasm. The maximum number of bands (25 bands) was observed for T_{15} followed by T_1 (22 bands), T_{16} (21 bands), T_{11} and T_{14} (20 bands), T_{13} (19 bands), T_3, T_4, T_6, T_9 and T_{12} (18 bands), respectively. The minimum number of bands (14 bands) was reported for T_5 followed by T_2 (15 bands) (Table 4.20).

Genetic relationships would be useful in utilization and management of the morphotypes during inter varietal breeding programs. The genetic relationships among the water chestnut germplasm were assessed by a cluster analysis of the similarity matrix. Jaccard's coefficient of similarity was used to evaluate the similarity between the germplasm based on the protein profiling. The value of similarity index (SI) is presented in Table 4.21. The similarity value showed the similarities between varieties ranged from 0.2 to 1.0. 0.7% similarity showed with T_{10} followed by T_3, T_6, T_8, T_9, T_{11}, T_{13} with T_{15} showed 0.6% similarity (Table 4.21).

Genetic distances among the different germplasm were analyzed with a UPGMA-derived dendrogram. The UPGMA dendrogram of the protein profile obtained using hierarchical genetic distance based clustering revealed two main clusters (Plate4.11). Cluster-I was further divided into three sub-clusters (IA, IB and IC). Sub cluster-IA comprises 7 germplasm (T_7, T_{10}, T_{11}, T_{13}, T_9 T_1 and T_6), sub cluster-IB comprises germplasm (T_4 T_2 and T_3) and sub cluster-IC comprises 2 germplasm (T_5 and T_8) presented in Table 4.22. Cluster-II contained 4 germplasm (T_{12}, T_{14}, T_{15} and T_{16}).

Table 4.20 Protein profiling for 16 water chestnut (*Trapa natans* var. *bispinosa Roxb.*) germplasm collected from various blocks of district Lucknow showing different bands and their corresponding molecular weight (kDa)

S.N.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	322	-
2	-	-	-	-	-	-	-	316	-	-	-	316	-	-	-	316
3	-	-	-	-	-	-	-	-	-	-	310	-	-	-	-	-
4	-	-	-	-	298	-	298	-	-	-	-	-	298	298	-	-
5	-	-	286	-	-	-	-	-	-	-	-	-	-	-	281	-
6	257	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	250
8	-	-	-	-	-	-	-	-	227						227	-
9	-	-	-	-	-	222	-	-	--	--	-	-	-	-	-	-
10	-	-	-	-	-	-	214	-	-	212	-	-	-	218	-	-
11	210	-	-	210	-	-	-	-	-	-	-	-	-	-	-	-
12	-	-	-	-	-	-	-	200	-	-	-	204	-	-	-	-
13	-	-	191	-	-	-	-	-	-	-	-	-	-	-	-	-
14	180	-	-	-	-	179	-	-	177	-	-	-	-	-	-	-
15	-	-	-	-	172	-	172	172	-	175	175	-	175	-	172	-
16	-	166	166	166	-	-	-	-	-	-	-	169	-	169	-	169
17	-	-	-	-	-	-	-	--	-	-	-	-	-	154	-	-
18														-	148	-
19	-	-	-	119	-	-	-	-	-	-	-	-	-	115	115	115
20	106					106	106	-	-	106	106	-	107	-	-	-
21	-	102	102	102	104	-	-	104	104	-	-	104	-	100	100	99
22	-	-	-	-	-	-	-	92	-	-	-	94	-	-	-	-
23	-	-	-	-	-	86	85	-	-	-	86	-	-	-	-	-
24	81	-	-	-	82	-	-	-	-	-	-	-	-	81	-	-
25	-	79	78	79	-	-	77	77	77	77	77	-	79	-	-	-
26	-	-	-	-	-	-	-	-	-	-	-	74	-	74	74	74
27	68	-	-	67	-	67	66	-	66	66	67	-	-	-	-	-
28	-	65	65	-	65	-	-	64	-	-	62	64	65	62	60	60
29	57	-	-	54	-	54	-	-	-	54	56	-	-	-	-	-

30	50	-	-	-	-	47	-	-	47	-	-	46	46	46	49	46
31	34	33	32	34	35	35	34	34	33	33	32	33	34	33	33	33
32	28	28	28	28	29	29	28	28	28	28	28	28	28	28	28	28
33	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
34	25	-	25	26	26	26	26	26	26	25	25	25	26	26	26	26
35	23	23	23	23	23	23	23	23	23	23	23	23	23	23	23	23
36	22	22	22	22	-	22	22	22	22	22	22	22	22	22	22	22
37	21	-	21	21	21	21	21	21	-	21	21	-	21	21	21	21
38	20	-	-	-	-	-	-	-	-	-	-	-	-	-	20	20
39	20	20	20	-	-	20	-	-	20	20	20	20	20	-	19	19
40	19	-	19	-	19	-	-	19	19	19	19	19	19	19	19	19
41	19	19	19	19	19	19	19	19	19	19	19	19	19	19	19	-
42	19	19	19	19	19	19	19	19	19	19	-	-	19	-	18	-
43	-	18	-	-	-	-	-	-	-	-	-	-	19	-	18	18
44	18	18	-	18	-	-	-	-	18	-	18	-	-	-	18	18
45	18	18	18	18	-	18	18	-	18	-	18	18	18	18	18	18
46	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18
TNB	**22**	**15**	**18**	**18**	**14**	**18**	**17**	**17**	**18**	**17**	**20**	**18**	**19**	**20**	**25**	**21**

TNB: Total number of bands, 1: T_1, 2: T2, 3: T_3, 4: T_4, 5: T_5, 6: T_6, 7: T_7, 8: T_8, 9: T_9, 10: T_{10}, 11: T_{11}, 12: T_{12}, 13: T_{13}, 14: T_{14}, 15: T_{15}, 16: T_{16}

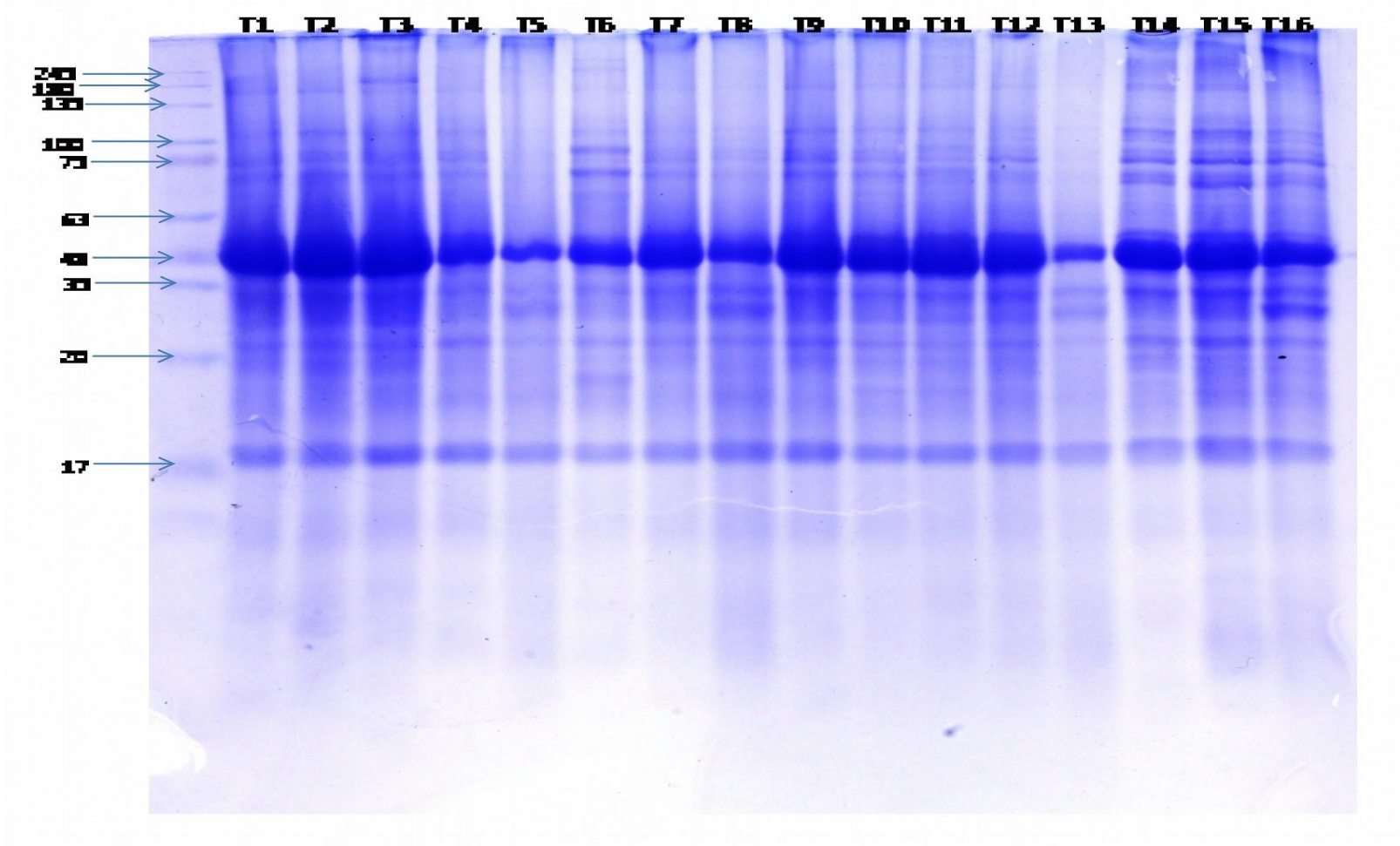

Plate 4.10 Protein profiling for 16 water chestnut (*Trapa natans* var. *bispinosa Roxb.*) germplasm showing different bands and their corresponding molecular weight (kDa)

Table 4.21 Jaccard's coefficient of similarity values between 16 germplasm of water chestnut (*Trapa natans* var. *bispinosa Roxb.*) collected from various blocks of district Lucknow

S. N.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	1.0	0.3	0.4	0.5	0.3	0.6	0.4	0.3	0.6	0.5	0.5	0.3	0.5	0.4	0.4	0.4
2		1.0	0.6	0.5	0.3	0.3	0.3	0.4	0.5	0.3	0.4	0.5	0.5	0.4	0.4	0.5
3			1.0	0.5	0.5	0.4	0.4	0.5	0.5	0.5	0.5	0.5	0.6	0.5	0.5	0.5
4				1.0	0.3	0.5	0.5	0.4	0.5	0.5	0.5	0.3	0.4	0.4	0.4	0.4
5					1.0	0.3	0.4	0.6	0.3	0.4	0.4	0.3	0.5	0.5	0.4	0.3
6						1.0	0.5	0.3	0.5	0.5	0.5	0.3	0.5	0.3	0.3	0.3
7							1.0	0.4	0.4	0.7	0.6	0.2	0.6	0.4	0.3	0.2
8								1.0	0.4	0.5	0.4	0.5	0.5	0.4	0.4	0.4
9									1.0	0.5	0.5	0.5	0.5	0.4	0.5	0.4
10										1.0	0.6	0.3	0.6	0.3	0.4	0.3
11											1.0	0.4	0.6	0.3	0.4	0.4
12												1.0	0.4	0.5	0.4	0.6
13													1.0	0.5	0.5	0.4
14														1.0	0.5	0.5
15															1.0	0.6
16																1.0

1: T_1, 2: T2, 3: T_3, 4: T_4, 5: T_5, 6: T_6, 7: T_7, 8: T_8, 9: T_9, 10: T_{10}, 11: T_{11}, 12: T_{12}, 13: T_{13}, 14: T_{14}, 15: T_{15}, 16: T_{16}

Table 4.22 Non-hierarchical Euclidean Clusters analysis in 20 germplasm of water chestnut (*Trapa natans* var. *bispinosa* Roxb.) based on molecular weight (kDa)

Clusters	Germplasm
Clusters I	T_1, T_2, T_3, T_4 , T_5, T_6, T_7, T_8, T_9, T_{10} and T_{11}
Clusters II	T_{12}, T_{14}, T_{15} and T_{16}

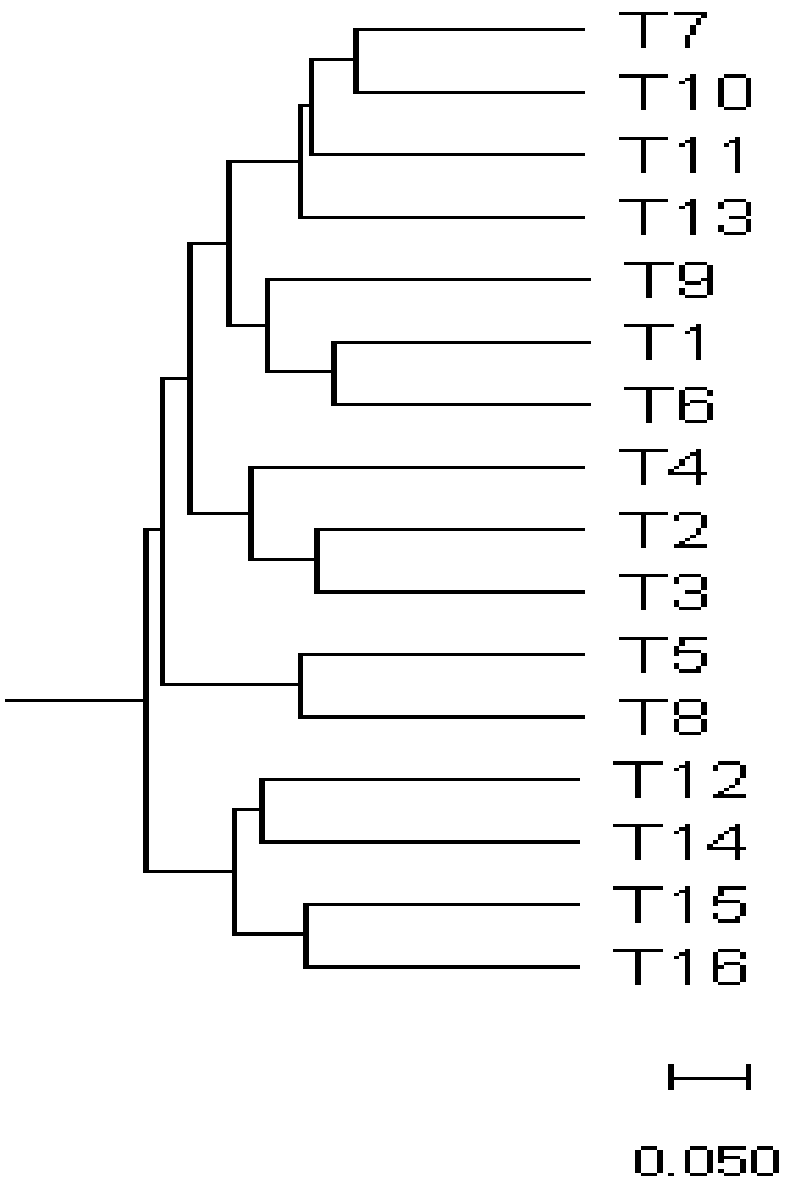

Plate 4.11 UPGMA dendrogram of 16 germplasm of water chestnut (*Trapa natans* var. *bispinosa* Roxb.) based on Protein profiling

Chapter V

DISCUSSIONS

In this chapter an attempt has been made to elucidate the findings of the present investigation entitled "**Morphological and Molecular Analysis of Intervarietal Variability in Water Chestnut (*Trapa natans* var. *bispinosa* Roxb.) in Central Uttar Pradesh"** has been discussed critically in the light of recent review of literature. The findings have been discussed a ccording to objective of the thesis.

EXPERIMENT I

5.1 Survey and collection of germplasm of water chestnut from the various blocks of district Lucknow.

Survey of water chestnut growers (Table 3.1) in various blocks *viz*., Mohanlalganj, Gosainganj, Sarojini nagar, Bakshi Ka Talab and Malihabad of district Lucknow was conducted in June – July 2016 to explore the possibility of intervarietal variability in water chestnut. Intervarietal variability was observed on the basis of data collected during the survey. The survey was conducted on twenty germplasm of water chestnut which were identified on the basis of previous studies and an extensive survey of the water chestnut growing areas around district Lucknow. These were collected for further evaluation. The crop (*Trapa natans* var. *bispinosa*) contributes significantly to the food of poor rural masses of these districts and has the potential for nutritional security and can also improve the socio-economic condition of the local farmers with the increasing markets for the crop. However, availability of this underutilized crop is poor owing to lack of standard varieties, cultivation practices, postharvest management etc. These result are closely related to a report by Suriyagoda *et al.* (2007) and Deb *et al*. (2013) for water chestnut in world. Similarly, earlier exploration, collection and evaluation studies were done in water chestnut by Suriyagoda *et al.* (2007) and Dwivedi *et al.* (2011a) in order to explore the possibility of genetic discrepancy in water chestnut by Arima *et al.* (1999), who explored local lines of 7 species i.e. 5 small and 2 medium from Japan and 5 large fruit lines from China on the basis of morphological performance of plant, fruit and its yield through the survey and analysed vegetative and yield performance of water chestnuts.

Previously related survey was studied for collection of multi-crop germplasm for exploring diversity in the state of Mizoram in six districts, i.e. Kolasib, Aizawl, Serchhip, Lunglei, Lawngtlai and Saiha. During these exploration trips, 344 different agri-horticultural crop germplasm were collected by Rathi *et al.* (2013). In consonance to these finding Manivel *et al.* (2019) which reported in salparni and to explore the natural population and to conserve the variability in the gene bank and also reported distinct variability on the basis of morphological characters. Mukherjee (1983) also observed similar survey in mango growing belts in West Bengal for description and characterization of variability in several clones of important mango varieties through vegetative, floral and fruit character and tolerance to biotic and abiotic stress conditions. Similarly, survey was studied in mango on the basis of questionnaire and complemented by visits to orchards in 35 districts of Bihar and 8 of Jharkhand (Singh *et al*., 2019).

Exploration of intra varietal variability in fruits crops on the basis of survey has been reported by several researchers (De Souza and Lima, 2004; Singh *et al.,* 2009; Rocha *et al.,* 2012 and Begum *et al.,* 2014a) which were later supported with molecular analysis in 'Cherukurasam' cultivar of mango. Begum *et al.* (2014c) conducted eco-geographic survey, in Baneshan, the choicest table cultivar of mango (*Mangifera indica* L.) cultivated commercially for more than a century, covering the three regions of Andhra Pradesh to study intra cultivar heterogeneity based on morphological fruit traits and microsatellite markers. Mukherjee (1983) conducted a survey in the mango growing belts in West Bengal and described several clones of important mango varieties. Mal *et al.* (2010) also reported to exploration and collecting, characterization and evaluation, identification of promising/elite lines, documentation, conservation, training and capacity building, socioeconomic analysis, information dissemination, collaboration and networking, impacts and sustainability of efforts.

EXPERIMENT II

5.2 To evaluate the intervarietal variability in water chestnut on the basis of botanical descriptors i.e. root, leaf, stem, flower and fruit

5.2.1 Botanical descriptors

Intervarietal variability in water chestnut was evaluated by studying variability in the vegetative parameters on the basis of botanical descriptors i.e. root, leaf, stem, flower and fruit, through anatomical studies of the different plant parts and stomatal studies.

Similarly studies have reported Çakılcıoğlu *et al.* (2010) which analysed 41 medical plants species belonging to 17 families identified in Yazıkonak and Yurtbası Districts of Elazığ Province, Turkey. These were collected herbarium prepared for nomenclature of the specimens.

Therefore, botanical classification of plants has always provided better contribution to conserve existing plant names, for the benefit of researchers, botanists, taxonomists, pharmacognocists and other users. A concise botanical classification of water chestnut (*Trapa natans* var. *bispinosa* Roxb) is available in previous literature and the descriptors developed in the present study as described in the previous chapter 4 are in consonance with the earlier reports with minor variations in leaf shape and size, etc. Similarly previous results have been reported of many researchers (Sculthorpe, 1967; Groth *et al.* 1996; Prajapati *et al.,* 2003 and Singh *et al*., 2010) for this crop.

In these present study roots were found assimilatory which contain chlorophy ll. These are green, aerial and adventitious roots which prepare food materials by photosynthesis. Stems to be noted cylindrical, flexuous and ascending in nature and produce several branches, each terminating in a rosette. The leaves were free-floating, hydrophytic and spongy in nature, arranged in a rosette and were alternate and feather like and long up to the 11.93 to 19.78 cm recorded. The average petiole length was recorded from 7.02-13.34 cm. Leaf shape was mostly rhombic to rhombic ovate, rhombic deltoid and in some germplasm found fan shaped. Margin of lamina was serrate inciso and light serrate and abaxial surface of the leaves were mostly found pale green, dark red and dark green. Flowers were generally seen inflorescence inconspicuous, solitary axillary, pedicillate, white in colour and complete flowers. Four white petals and four green sepals, superior ovary, six androecium and monogynoecious ovary in a flower and were located in the centre of the rosette. Flowering begins in July and continues until the plants are killed by frost. The flowers convert into fruits in approximately one week. Fruits were of medium size, triangular in shape, having two spiny horns. Fruits colour ranging from green to greenish red and red were recorded.

It was observed in this present study, that roots also contain chlorophyll and appear like submerged leaves with segments comparable to the terrestrial roots. There is no primary root system, just the adventitious roots that extend from the hypocotyls. These result are closely related to Hummel and Kiviat (2004); Adkar *et al*. (2014) who demonstrated that *Trapa* is an aquatic floating plant with flexuous ascending stem. The upper stem swelling has a lacunate pith and four or five rings of air apace in the cortex whereas the

remaining pith is compact having only two rings of cortical lacunae in the lower stem (Naylor, 2003). Similarly result were found 24 cm long, upper leaves are slightly rhombic to rhombic ovate and are sharply dentate along the leaf margins (Groth *et al.*, 1996 and Naylor, 2003) reach up to 15 cm long. The petioles of the floating leaves are 0.6-1.8 cm long. Prajapati *et al.* (2003) reported to leaving simple alteration, crowded at the upper part of the stem in rosettes, rhomboidal, apex triangular, irregular inciso–serrate in the upper part a dark green above, reddish purple beneath, petioles dilated near the apex into a large spongy float. Karmakar, (2011) has reported that the reddish green leaves are villous on the dorsal side and about 5 to 8 cm long, have hairy petioles 10 to 15 cm in length (Sculthorpe 1967; Cozza *et al.* 1999 and Chandana *et al.* 2013). Similarly, earlier studies have been done by several researchers in water chestnut Kadono and Schneider (1986); Arima *et al*. (1999); Hummel and Kiviat (2004); Karmakar (2011) and Chandana *et al.* (2013) for flower morphology. O'Neill (2006) also reported on the basis of colour of the husk, it categorised into three types: completely green, completely red and green blended with red. Water chestnut have two forms, one is red (leaf, petiole and fruit) and other is green (leaf, petiole and fruit) each bearing fruit large in size having two dull spines (Faruk *et al.* 2012). Fruits ripen in above a month and can remain viable for up to about twelve years. Each seed can give rise to ten to fifteen rosettes and each rosette may produce as many as twenty seeds. When mature, the fruits fall from the plant and sink to the bottom of the body of water. Seed dormancy can be from four months to twelve years. The horns may act as anchors to limit movement of the seed, thus keeping them at suitable water depths **(**Naylor, 2003; O'Neill, 2006; Prajapati *et al.,* 2003 and Karmakar, 2011). Hoque *et al.* (2001) reported water chestnut plant is propagated mainly through seeds. A single seed can give rise to 10 to 15 plant rosettes.

5.2.2 Anatomical and stomatal studies:

Intervarietal variability studies on the basis of anatomical and stomatal studies were done through section cutting of plant part *viz*., root, stem, leaf and pulvinus and scanning electron microscopy (SEM). The lateral roots contained only one strand of xylem and phloem. The upper stem swelling had a lacunate pith and four or five rings of air apace in the cortex whereas, the remaining pith is compact having two rings of cortical lacunae in the lower stem, air chamber, spongy tissue pith was visible in section cutting (Plate 4.4). In case of leaves gases move through stomata in the upper epidermis of the leaves,

which was absent on the adaxial surface and vascular tissues were generally poorly developed in the leaves, these result were confirmed by studies of Sculthorpe (1967) and Naylor (2003). Leaves have little or no lignin and the vascular tissues are generally poorly developed in the leaves and buoyant tissues in the stem (Groth *et al.,* 1996 and Sculthorpe, 1967). The upper stem swelling has a lacunate pith and four or five rings of air spaces in the cortex whereas the remaining pith is compact having only two rings of cortical lacunae in the lower stem (Naylor, 2003). Similarly, previous by Bercu (2004) reported anatomical and histological features of the leaves of *Trapa natans* a free-floating hydrophyte, living in the Danube Delta channels and the special characteristics of both surfacing and submerged leaves of this plant which are similar to the present study were described and discussed.

Stomatal studies of stomatal traits have showed significant variation among the germplasm. Stomatal length varied from, 11.48-17.43 µm, width 4.68 to 8.05 µm, pore size length 6.64-13.52µm, width 1.40-5.16µm) and stomatal density 25-50 μm^{-2} (Table 4.5). Stomatal length has been also reported to correlate with genome size (Aasamaa *et al.,* 2006 and Xu and Zhou 2008). Therefore, the genetic and developmental basis for high stomatal density and conductance and its application in germplasm studies is exploited as a research priority in plant physiology, agriculture and paleo-biology (Roche, 2015 and Wang *et al.,* 2015). Larger stomata are usually distributed in low densities (Dillen *et al.,* 2008). Preliminary studies were done by several researchers Ferris *et al*. (2002) and Tognetti *et al.* (2004) who explained stomatal density and length as a characters for establishing the existence of the large clonal variability in germplasm. Marron (2005) postulated that stomatal traits could be used as early indicators of growth potential in poplar as well as a criteria for clonal discrimination in the genus and stomatal density is reported to differ significantly even among clones belonging to different parentages, between different canopy positions and on leaf surfaces besides varying within leaves, plants and individuals of a single species (Afas *et al.,* 2006).

Inter varietal variability existence among the germplasm was determined in terms of CV, PCV% and GCV%, h^2%, genetic advance and GAM%. The PCV was higher than GCV for all traits. The maximum PCV (35.44%), GCV (35.07%), h^2 (97.90%) and GAM (147.19%) were recorded for stomatal pore width (µm).Whereas, the highest genetic advance (26.01%) was observed for stomatal density (Table 4.6). Gailing *et al.* (2008) have observed that stomata appear to be genetically determined and are controlled by

additive genes since stomatal initiation is controlled by both environmental and genetic factors (Casson and Hetherington 2010).

These results are also in consonance with findings of Riaz and Chaudhary (2003) that high estimates of heritability for stomatal characters indicate that these character are transmitted to the offspring and were governed by additive gene. Usefulness of any character is related to its onward transmission to the progeny and characters with high heritable are easy to select for breeding purpose. Higher values of heritability of stomatal characters in the present study indicates that either these were simply inherited characters governed by a few major genes or additive gene effects even if, they were under polygenic control and therefore, selection of these characters would be more effective for improvement and can be exploited at an early stage of development of the plants. Thus, heritability and genetic gain (GA) aid in referring valuable conclusion for effective selection in a germplasm.

Considering the stability of stomatal characteristics at the genetic level as above, a dendrogram was prepared on the basis of stomatal characteristics of 20 germplasm of water chestnut in order to establish their relatedness to each other. In the present study, the samples under study were noted to be very closely related and grouped into two major clusters (cluster I and II) (Table 4.7 and Plate 4.7) with additional sub-clusters, differentiating the germplasm collected from different blocks of district Lucknow. Cluster-I consisted of 17germplasm which further divided into four sub-groups (cluster IA, IB, IC and ID) while cluster-II comprised three germplasm which was divided into three sub-groups (cluster IIA, IIB and IIIC). Similarly, preliminary studies conducted in the same crop and other fruit crops also classify existing germplasm in different plant species through dendrogram prepared a on the basis of RAPD and AFLP data, (Hoque *et al.,* 2005 and Anu *et al.,* 2015).

5.2.3 Morphological studies

Inter varietal variability existence among the germplasm based on morphological traits is presented in the previous chapter (Table 4.8). It was observed that number of rosettes per plant ranged from 3.67-12.17, number of leaves per plant (21.67 to 29.67), rosette spread in north-south direction (22.10 to 32.83 cm), rosette spread in east-west direction (20.72-32.18 cm), Leaf characters showed a variable range for length of expanded leaves (11.93 to 19.78 cm) which was recorded from the base of the petiole

till the tip of the leaf, length of lamina (4.20 to 6.44cm), width of lamina (4.22 to 8.78 cm), pulvinus length (1.60 to 3.17cm), petiole length (7.02 to 13.34 cm), pulvinus diameter (3.84 to 15.94 mm) and pulvinus: petiole ratio (0.19 to 0.29). Until recently, morphological methods have been used for the characterisation of intervarietal variability in fruit crops (Shukla *et al.,* 2012; Singh *et al*., 2009) and similar variability has been reported in previous studies conducted *in situ* in water chestnut at the farmers pond (Dwivedi *et al*., 2011a; Babu and Dwivedi, 2012b; Babu *et al.,* 2013) with respect of morphology of fruit and leaf as well as other characteristics, variability in fruit biochemical parameters and its capacity for bioaccumulation of heavy metals based on which the collection of the accessions has also been done. However, morphological characters may be controlled by epistatic and plieotropic gene effects which affects heritability and they may be prone to error due to environmental effects (Anu *et al.,* 2015) and hence, it becomes important that supportive techniques may be used for further elucidation of the variations present in the population. These leaf morphological characters helped to identify primarily the intervarietal variability of fruit crops as also described by other scientific workers who narrated morphological characterisation as one of the simple, rapid and inexpensive methods which was conventionally applied in mango (Begum *et al.,* 2014b; Kishor *et al.,* 2019), in banana (Kundu *et al.*, 2018) and in *Gingko biloba* (Klimko *et al.*, 2015) which even facilitated the identification and classification of fragments of *Gingko* fossil leaves. But, number of these traits is limited, unstable and unable to establish variations in closely related accessions.

The morphological data recorded was subjected to biometrical techniques for assessment which was computed through simple measures of variability *viz.,* range, grand mean and coefficient of variation (CV), genetic variability (PCV % and GCV %), heritability (h^2 %), genetic advance (GA) and genetic advance as percent of mean (GAM%) for further elucidation of the obtained data. The highest CV (13.96), PCV (26.75%), GCV (22.82%), heritability (72.80%), genetic advance (5.54%) and genetic advance as percent of mean (82.70%) were recorded for number of rosette per plant are given in Table 4.11. The characters rosette spread north-south and east-west (cm) showed very negligible value for these CV, PCV, GCV, heritability, genetic advance and genetic advance as percent of mean which indicates to effect of environment. Regards in leaf parameters the maximum CV (11.70) was recorded for length of lamina. Among the leaf parameters the highest PCV (21.02%) and GCV (18.64%) were found

for pulvinus diameter (mm). However, the wide heritability (84.80%) and genetic advance (7.44%) were observed for petiole length (cm) while, the maximum genetic advance as percent of mean (158.64%) was recorded for pulvinus length (cm) is given in Table 4.11.

These may however, be controlled by epistatic and pleiotropic gene effects and face heritability problems (Begum *et al.,* 2014a). Thus, prime advantages of genotyping on the basis of morphological traits are simplicity and rapid, inexpensive assays, even from herbarium specimens and other dead tissues (Begum *et al.*, 2014b). However, it is limited and lack decisiveness because environmental variations also affect expression of these characteristics. Thus, these morphological characters may not adequately represent the genetic variability among accessions of a single cultivar.

EXPERIMENT III

5.3 To establish intervarietal variability in water chestnut on the basis of physico-chemical characteristics of the fruits.

5.3.1 Variability Studies through fruit Morphology

Variability studied of water chestnut and another fruit crops genotypes from India and other countries has been characterized based on morphological traits and genetic markers (Arima *et al.,* 1999; Babu *et al.,* 2011; Dwivedi *et al.,* 2011; Hoque *et al.,* 2005; Shukla *et al.,* 2008; Singh *et al*., 2009; Singh *et al.,* 2010 and Rocha *et al*., 2012). Morphological characterization of the vegetative parts as well as the fruits, is traditionally the most common method used. Until recently, on the basis of fruits morphological methods have been used for the characterization of intervarietal variability in horticultural fruit crops (Pandey, 1998; Suriyagoda *et al.,* 2007; Ertan, 2007; Singh *et al.*, 2009 and Rumana *et al.,* 2016) observed significant variation with regard to fruit shape, size, color and quality of the fruits which was ascribed to bud mutations. Very little work has been done on physico- chemical parameters of water chestnut. However, germplasm studied as earlier in 1967 on aonla (Singh and Arora, 1967); on Guava (Ball and Khehra, 2005); on mango (Kishor *et al.*, 2019) indicates clearly that variability can be established on the basis of physical and chemical parameters. Available research findings reviewed earlier have been referred briefly in

the present chapter and the salient finding pertaining to the present investigation have been documented in a classified manner, so as to use it as reference literature.

The fruit morphological traits showed significant variation in all germplasm collected from different regions in Uttar Pradesh for the fruit and peel as well as kernel traits under study.

In this study, it is evident that there was significant variation in fruit and kernel morphology among the 20 germplasm of water chestnut under study. The number of fruit varied between (11.67 to 25.50), fruit weight (5.43 to 8.90 g), fruit yield (g/plant) (76.67 to 225.07), pedicel length of the fruit (4.10 to 5.24 cm), fruit volume (5.17 to 8.93 ml), specific gravity of fruit (0.92 to 1.17 g/cc), fruit length (27.51 to 37.45 mm) fruit width 27.65 to 36.62 mm), cheek diameter of fruit (28.02-37.04), peel thickness (1.76 to 2.46 mm), peel weight (1.78 to 3.40 g), kernel weight (3.17 to 5.67g), kernel volume (3.33 to 5.17 ml), specific gravity of kernel (0.92 to 1.99 g/cc), kernel length (27.17 to 30.92 mm), kernel width (24.15 to 28.64 mm), cheek diameter of kernel (26.52 to 30.19 mm), kernel: peel ratio (1.40 to 2.62). TSS (4.17 to 6.77), pH (4.99 to 6.94), Acidity (0.10 to 0.04), Ascorbic acid (7.80 to 8.83), reducing sugar (1.65 to 1.87), non-reducing sugar (2.09 to 3.03) and total sugars (3.93 to 4.67). These result are closely related to report by Singh *et al.* (2010) who demonstrated that physico-chemical properties of water chestnut (*Trapa natans* L. var. *bispinosa* Roxb.) contents of total soluble solids and titrable acidity determined was 7.2 and 0.142%, respectively. Similarly, Gani *et al*., (2010) also analysed of physio-chemical analysis of *Trapa natans* var. *bispinosa* with respect of weight and size and nuts and kernel. The variations observed in fruiting behaviours may be attributed to the genetic nature of varieties and weather parameter.

In the present study, fruits physico-chemical traits of all germplasm of water chestnut were found variable. These result are indirectly supported by a report of Singh *et al.* (2009) who also detected that prominent discrepancy in mango cultivar Banganapalli based on morphological analysis of 17 fruit characters. The present findings are also in agreement with those of Bally *et al.* (1996), which has also determined phenotypic variation in the type of fruit in 15 accessions of Kensington Pride, a polyembryonic cultivar of mango. Conventionally also, the intracultivar heterogeneity of mango has been characterized mostly at the morphological level of fruits by several researchers (Naik, 1948; Oppenheinmer, 1956; Gan *et al*., 1981; Pandey, 1998; Singh *et al*., 2009).

Variability estimates in terms of CV, GCV%, PCV%, h^2%, genetic advance and genetic advance as percent of mean indicates that the highest morphological characters showed more environmental influence (Table 4.19). However, the highest coefficient of variation (15.67), PCV (15.76%), GCV (8.93%) and genetic advance as percent of mean (21.63%) were recorded for thickness of peel (mm). While the maximum heritability (43.20%) was observed for pedicel length of fruits (cm), whereas, higher genetic advance (3.52%) was found in number of fruits per plant. Among the fruit morphology most of the characters showed very negligible value for these CV, PCV, GCV, heritability, genetic advance and genetic advance as percent of mean which indicates environmental effect. CV, PCV%, GCV%, h^2%, GA and GAM % were analyzed for kernel morphology which showed wide variation between PCV and GCV (Table 4.19). The highest coefficient of variation (15.67) and PCV (15.76%) was recorded for kernel: peel ratio whereas, the maximum GCV (4.82%), heritability (65.60%), genetic advance (4.44%) and genetic advance as percent of mean (16.57%) were observed for length of kernel (mm) (Table 4.19). However, the kernel morphology showed narrow difference between PCV and GCV indicating that the kernel parameters are more under the genotypic control rather than being affected by the environment as is recent reported in mango (Kishor *et al.,* 2019) and in maize (Ogunniyan and Olakojo, 2014).

Among the fruit biochemical parameters the highest coefficient of variation (31.43) and GCV (10.31%) was observed for titrable acidity (%) (Table 4.19). Similarly, the highest PCV (12.67%) was recorded for character TSS while, the highest heritability (60.20%), genetic advance (1.91%) and genetic advance as percent of means (31.99%) were observed for pH. The parameter titrable acidity (%) and ascorbic acid (%) showed very negligible values. (Table 4.19).

The results are in agreement with those of Munshi and Behera (2000), Singh *et al.* (2005) and Gupta *et al.* (2009) in chilli. The narrow difference were recorded between PCV and GCV for kernel length, kernel cheek diameter which indicated that the phenotypic expression of characters is primarily due to the genotype, since environmental influence is negligible and expression of these characters could be controlled by some epistatic and pleiotropic gene effect useful for crop improvement through selection as in mango (Begum *et al*., 2014a, Kishor *et al*., 2019) and banana (Kundu *et al*., 2018). However, a wide variation between PCV and GCV were recorded

for other fruit morphological parameters (Table 4.19) which was influenced by environmental effect and thus, cannot be considered reliable for crop improvement through selection (Ranpise and Desai, 2003) of these parameters. Although the genotypic coefficient of variation and phenotypic coefficient of variation are the measures of genetic variability, the amount of genetic gain can be estimated from genotypic coefficient of variation and phenotypic coefficient of variation along with heritability (Ogunniyan and Olakojo, 2014). Estimates of GCV alone are not sufficient to quantify the amount of variation which is heritable and genotypic coefficient of variation effects, together with heritability estimates, furnish more reliable information.

Knowledge of heritability of a character is important as it indicates the possibility and extent to which improvement is possible through selection. Higher heritability does not always ensure an increased genetic advance. The character having high heritability with high genetic advance generally indicates that heritability is more due to the additive gene effect and advocated the use of high estimates of heritability along with high magnitude of genetic advance for genetic improvement in any trait through selection. Morphological traits are visually evaluated in most cases and are thereby subjective morphological characteristics that can improve characterizations for defining the potential use of any genotype. These traits have long been the means of studying variability among populations in fruit crops. In fruit tree species, quantitative and qualitative fruit traits have been found useful in identification and assessment of intervarietal heterogeneity and selection of elite forms for fruit production on a large scale as these traits help in developing the ideotypes. In mango, the oldest and most widely used markers were the morphological traits, which may still be optimal for certain cases, where the cultivars were identified based on leaf, panicle, fruit and other physical characteristics. In spite of it, actual identity of some cultivars is still in question, because similar cultivars grown in different areas often have various names (Lakshminarayana, 1980).

The prime advantages of morphological traits are simplicity and rapid, inexpensive assays, even from herbarium specimens and other dead tissues. Although morphological traits are very useful, they have several disadvantages. They are often limited in number. They suffer from lack of decisiveness. They face heritability problems as they may be controlled by epistatic and pleiotropic gene effects. Qualitative characteristics provide quite useful information particularly from nutritional and biochemical point of

view. The main factors responsible for variation in fruit composition are climatic and nutritional conditions and fruit load on plants.

Genetic diversity represents the heritable variation within and between populations of organisms. It is the basis of survival and adaptation and makes it possible and advance the adaptive process on which evolutionary success and to some extent the survival depends (Rao and Hodgkin, 2002). A better understanding of genetic diversity and distribution is essential for it conservation and use. It helps in determining what to conserve as well as where to conserve. This also improves our understanding of the taxonomy, origin and evolution of plant species of interest.

Morphological characterizations are error prone due to environmental variations affecting expression of these characteristics. In addition, these observations are time consuming and this mode of identification is slow because of long juvenile periods. Therefore, these morphological characters may not adequately represent the genetic heterogeneity among accessions of a cultivar. Hence, characterization of intravarietal heterogeneity based on morphological traits needs complementation with microscopic studies and protein profiling as they can contribute greatly to the utilization of inter varietal heterogeneity through descriptive information of structure of genotypes, analyses of relatedness, the study of identity and location diversity.

EXPERIMENT IV

5.3 Molecular characterization of intervarietal variability in water chestnut

Genetic diversity represents the heritable variation within and between populations of organisms. It is the basis of survival and adaptation and makes it possible to advance the adaptive process on which evolutionary success and to some extent, the survival of species depends (Rao and Hodgkin, 2002). A better understanding of genetic diversity and distribution is essential for it conservation and use. It helps in determining what to conserve as well as where to conserve.

Smith and Smith (1992) concluded that morphological differences cannot be interpreted to provide accurate estimates of genetic differences. Moreover, identification of cultivars using classical methods based on morphological and physiological characters has become increasingly difficult because of the large number of lines being released and convergence of these lines on a few of the most desirable characters. Time and resource requirements of grow out tests and their dependence on normal environmental conditions make such procedure impractical for routine screening (Weeden, 1984). Thus,

identification of crop cultivars through biochemical markers i.e. proteins, have been used to measure the genetic diversity for conspicuous species and specific and highly stable characteristics. Accessions among cultivated plants from different geographical areas and adapted to diverse ecological zone still possess the same basic profile (Sridhar *et al.,* 2018). Seed protein variants have been observed to be the most widely used biochemical markers during the last century. Its reliability depends on polymorphism of seed and seedlings proteins and the fact that these proteins represent primary gene products. Moreover these have advantage of being scrabble from inevitable organs or tissues and the electrophoretic protocol for bulk protein assay is generally simpler than of isozyme (Cooke, 1984). Mann *et al.* (2005) reported that the number of bands give an account of polypeptides present in a protein. As equal amount of proteins is loaded in all, thus the banding pattern is indicative of the range of the proteins present in the different varieties.

Therefore, knowledge of the genetic structure for the population is limited, and there are very few reports available on the molecular characterization of *T. natans.*

The protein profiling in the present sample showed distinct polymorphism in electrophoretic banding patterns and led to the detection of total of 25 bands. In the initial screening the molecular weight of the 25 bands obtained ranged from 322 kDa to 18 kDa. The highest 322 kDa value band recorded in T_{15} which was not present in other germplasm and minimum (18 kDa) value band was found in all germplasm. The maximum number of bands (25 bands) was observed for T_{15} followed by T_1 (22 bands), T_{16} (21bands), T_{11} and T_{14} (20), T_{13} (19), T_3, T_4, T_6, T_9 and T_{12} (18 bands), respectively. The minimum number of bands (14 bands) was reported for T_5 followed by T_2 (15 bands) (Table 4.20). These results were in conformity with those in pumpkin (Kumar *et al*., 2006) and other cucurbits like bitter gourd (Tewari, 1997), muskmelon (Singh *et al.*, 1999).

Genetic relationships would be useful in utilization and management of the germplasm during inter varietal breeding programs. The genetic relationships among the water chestnut germplasm were assessed by a cluster analysis of the similarity matrix. Jaccard's coefficient of similarity was used to evaluate the similarity between the germplasm based on the protein profiling. The value of similarity index (SI) is presented in Table 4.21. Similarity value showed the similarities between varieties ranged from 0.2 to 1.0. 0.7% similarity showed with T_{10} followed by T_3, T_6, T_8, T_9, T_{11}, T_{13} with T_{15} showed 0.6% similarity (Table 4.21).

Similarity matrix and UPGMA cluster analysis

The UPGMA dendrogram of the protein profile obtained using hierarchical genetic distance based clustering revealed two main clusters (Plate 4.10). Cluster-I was further divided into three sub-clusters (IA, IB and IC). Sub cluster-IA comprises 7 germplasm (T_7, T_{10}, T_{11}, T_{13}, T_9, T_1 and T_6), sub cluster-IB comprises germplasm (T_4, T_2, T_3) and sub cluster-IC comprises two germplasm (T_5 and T_8) presented in Table 4.22. Cluster-II contained four germplasm (T_{12}, T_{14}, T_{15} and T_{16}). In contrast to the present study, the previous report based on RAPD marker Hoque *et al.,* 2005 also examined in water chestnut that genetic distances among the different varieties were analyzed with a UPGMA-derived dendrogram and similarity matrix showed the similarities between varieties ranged from 0.25 to 1.0. The dendrogram results suggested that the European varieties were in the same cluster group and also similar result were detected in pumpkin (Kumar *et al*., 2006) and other cucurbits like bittergourd (Tewari, 1997), muskmelon (Singh *et al.*, 1999). Thus, it was evident that the genotypes with similar morphological traits were accordingly grouped together and could be easily distinguished through electrophoresis. This was in conformation with the study in bottle gourd (Padiyar, 2007). Discussed some of the traits which could lead to yield increase, with a focus on how natural genetic variation could be harnessed. Moreover, such studies provide insights for advancing understanding of the molecular aspects governing plant growth and yield, and propose future avenues for improvement of crop yield. It is also suggested that knowledge accumulated over the last decade in the field of molecular physiology should be integrated into development of new ideotypes.

Chapter-VI

Summary and Conclusion

The present investigation entitled **"Morphological and Molecular Analysis of Inter-varietal Variability in Water Chestnut (*Trapa natans* var. *bispinosa* Roxb.) in Central Uttar Pradesh"** was conducted during the year 2016-2018 in various blocks of district Lucknow and Ph. D Horticultural Laboratory, Department of Horticulture, School of Agricultural Sciences and Technology, Babasaheb Bhimrao Ambedkar University, Lucknow with the following objective

- Survey and collection of germplasm of water chestnut from the various block of district Lucknow.
- To evaluate the inter-varietal variability in water chestnut on the basis of botanical descriptors i.e. root, leaf, stem, flower and fruit.
- To establish inter-varietal variability in water chestnut on the basis of physico-chemical characteristics of the fruits.
- Molecular characterization of inter-varietal variability in water chestnut.

Data of 20 germplasm were analyzed for exploring intervarietal variability through botanical descriptors, stomatal characters, vegetative, physico-chemical as well as protein profiling. The experiment was laid out in Completely Randomized Design (CRD) with 20 treatments (germplasm) and from each, three replications (plant) were selected for the study. Standard methods and procedures were followed for recording various vegetative, stomatal attributes and physico-chemicals as well as protein profiling. The salient findings are summarized below.

6.1 Survey and collection of germplasm

A survey was conducted in various blocks *viz.,* Mohanlalganj, Gosainganj, Sarojini nagar, Bakshi Ka Talab and Mlihabad of district Lucknow. According to farmer feedback water chestnut is an annual aquatic plant forming dense mats on the surfaces of lakes, ponds and slow moving waters. As most of the cultivation is done by traditional methods, lack of scientific knowledge of cultivation is also considered as one of the major constraints in popularity of this crop. The traditional system of cultivation of water chestnut is very easy and provides the farmers opportunities to cultivate cereals

and other field crops in the same fields in a year. Information was collected through the feedback of water chestnut growing farmer during in June-July, 2016 to explore the possibility of existence of intervarietal variability in water chestnut.

6.2 Botanical descriptors

Roots were found submerged terrestrial assimilatory which contain chlorophyll. Stem was cylindrical and flexuous, ascending in nature and 1 to 5 m long, nodes of the stem have slender linear roots while the plant is anchored in the sediment by the lower roots that emerged from propagating seed hull. The leaves were free-floating, hydrophytic and spongy in nature, arranged in a rosette. The leaves were alternate and feather like and long (up to the 11.93 to 19.78 cm were recorded). The average petiole length was recorded from 7.02-13.34 cm. Generally, leaf shape were found rhombic to rhombic ovate, rhombic deltoid and in some morphotypes found fan shaped. Inflorescence has inconspicuous, solitary axillary, pedicillate, white in colour, complete flowers were found, formed in the axils of the surfacing leaves in July, continues until the plants are killed by frost and flowers were converted into fruits in approximately one week. Fruits were of medium size, triangular in shape, having two spiny horns. 11.67-25.50 fruits were observed per rosette.

Generally vascular tissues were poorly developed in the leaves. Spongy tissue, air chamber, pith were visible in root, stem and leaf section.

Stomatal parameters

The Selection T_{19} was found better for stomatal length (µm), stomatal pore length (µm) and stomatal pore width (µm) while, the Selection T_{13} resulted best for stomatal width (µm) and stomatal density (μm^{-2}). However, T_5 was superior for stomatal pore width (µm).

The stomatal morphological data recorded was subjected to biometrical techniques for assessment computed through simple measures of variability *viz.,* range, grand mean and coefficient of variation (CV), genetic variability (PCV % and GCV %), heritability, genetic advance and genetic as percent of mean (%) for further elucidation of the data recorded. Among the stomatal traits the maximum coefficient of variation showed for stomatal pore length (µm). However, the maximum PCV (%), GCV (%), heritability (%) and genetic advance as percent of mean (%) were recorded for stomatal pore width (µm) while, the highest genetic advance (%) was observed for stomatal density.

A UPGMA dendrogram was prepared on the basis of stomatal characteristics of 20 germplasm of water chestnut in order to establish their relatedness to each other. The result were examined very closely related and grouped into two major (Cluster-I consisted of 17 germplasm which further divided into four sub-groups (cluster IA, IB, IC and ID).

Vegetative parameters:

The maximum number of rosettes per plant was observed for Selection T_{19} and the minimum was observed in T_{13}, while rosette spread in north-south direction, rosette spread in east-west direction, number of fruits recorded for T_1 and the minimum was recorded for T_3 and T_4.

The maximum number of leaf, leaf length, lamina length, lamina width and petiole length were recorded for T_6, while the maximum pulvinus diameter was recorded for T_{12}. However, then minimum number of leaf and leaf length for T_{16} while lamina length, lamina width and petiole length observed in T_4 among the 20 germplasm.

The highest pulvinus length, pulvinus diameter and Pulvinus: petiole ware observed in T_{10}, T_{12} and T_{14} while minimum value was recorded for T_4 and T_5.

6.3 Fruit bio- chemical parameters

Among the fruits morphology the highest fruit weight (g), fruit volume (ml), fruit length (mm), fruit width (mm) and kernel check diameter were recorded from Selection T_{20} and the minimum data were recorded for T_3, T_{10}, T_{11} and T_{13} respectively. However, the maximum peel thickness, peel weight, kernel weight (g) and kernel volume (g/ml) observed from T_1 and the minimum was observed in Selection T_8, T_{11}, T_{13} and T_{19} respectively. Whereas, the maximum specific gravity of fruit and kernel, fruit check diameter (mm), kernel length, kernel widths and kernel: peel ratio were recorded for Selection T_{12}, T_7, T_5, T_6, T_{10} and T_8 respectively. While the minimum data were recorded from T_3, T_{10}, T_7, T_{13}, T_8 and T_{15} respectively.

The maximum TSS was observed for Selection T_{19} and minimum was recorded for T_6. However, the maximum pH, acidity and ascorbic acid were recorded for T_9. While the minimum were recorded for T_{14}.

The maximum reducing sugar was recorded for T_5 and minimum was recorded for T_6. However, the maximum non reducing sugar and Total sugar were observed for T_6 while the minimum were observed for T_{20} and T_{13}.

The highest coefficient of variation, PCV (%), GCV (%) and genetic advance as percent of mean (%) were recorded for thickness of peel (mm). While the maximum heritability (%) was observed for pedicel length of fruits (cm).Whereas, higher genetic advance (%) was found in number of fruits ($Plant^{-1}$). Among the fruit morphology most of the characters showed very negligible value for these CV, PCV, GCV, heritability, genetic advance and genetic advance as percent of mean which indicates environmental effect. CV, PCV, GCV, heritability, genetic advance and genetic advance as percent of mean were analyzed for kernel morphology which showed wide variation between PCV and GCV (Table 4.15). The highest coefficient of variation (CV) and PCV (15.76%) was recorded for kernel: peel ratio whereas, the maximum GCV (%), heritability (%), genetic advance (%) and genetic advance as percent of mean (%) were observed for length of kernel (mm). However, the kernel morphology showed narrow difference between PCV and GCV.

Among the fruit biochemical parameters the highest coefficient of variation and GCV (%) was observed for titrable acidity (%). Similarly, the highest PCV (%) was recorded for character TSS while, the highest heritability (%), genetic advance (%) and genetic advance as percent of means (%) were observed for pH. The parameter titrable acidity (%) and ascorbic acid (%) showed very negligible.

6.4 Protein profiling of different germplasm

The initial screening the molecular weight of the 25 bands obtained ranged from 322 kDa to 18 kDa. The highest 322 kDa value band recorded in Selection T_{15} which was not present in other germplasm and minimum (18 kDa) value band was found in all germplasm. The maximum number of bands (25 bands) was observed for T_{15} followed by T_1 (22 bands), T_{16} (21 bands), T_{11} and T_{14} (20 bands), T_{13} (19 bands), T_3, T_4, T_6, T_9 and T_{12} (18 bands), respectively. The minimum number of bands (14 bands) was reported for Selection T_5 followed by T_2 (15 bands).

Jaccard's coefficient of similarity was used to evaluate the similarity between the germplasm based on the protein profiling. The similarity value showed the similarities between varieties ranged from 0.2 to 1.0. 0.7% similarity showed with Selection T_{10} followed by T_3, T_6, T_8, T_9, T_{11}, T_{13} with T_{15} showed 0.6% similarity.

Genetic distances among the different germplasm were analyzed with a UPGMA-derived dendrogram. The UPGMA dendrogram of the protein profile obtained using hierarchical genetic distance based clustering revealed two main clusters (Cluster-I and Cluster-II).

Conclusion

Water chestnut (*Trapa natans* var. *bispinosa)* is an important crop with immense nutritional value for its edible fruit. It has the capacity to grow in aquatic waterlogged ecosystems and is thus, a potential alternative horticultural crop. Till date there are no standard cultivars of the crop and thus, in the present study native germplasm of water chestnut in the regions around district Lucknow has been surveyed, collected and evaluated for its variability.

Statistically significant variation has been recorded in the morphological parameters of the plant as well as fruit. Botanical descriptors show variations in leaf shape, fruit and kernel parameters. However, biometrical values for these characters show a higher PCV as compared to GCV values indicating an environmental effect on expression of the characters. These have been further elucidated with the help of the significant variability in the anatomical and stomatal characters of the samples studied which show higher GCV values and higher heritability and genetic advance. Hence, morphotype T_6 which has the highest GCV% and GAM% for stomatal characters i.e. stomatal pore width has shown corresponding high values for fruit quality parameters i.e. reducing sugar, non-reducing sugar, total sugars and also recorded the highest yield. Hence, it can be interpreted that based on the study of stomatal characters which have shown higher GCV % value, we can derive conclusion which could help for further improvement in the selection of morphotype for varieties, improvement of water chestnut.

Intervarietal variability thus established through different techniques was further substantiated through molecular studies. SDS- PAGE of the various samples under study has elucidated the variations in the banding patterns obtained for the different samples indicating expression of different proteins in different morphotypes which is deduced to be indicative of existing variability. This however, needs deeper and more focussed analysis. Cluster analysis of the various parameters has shown that the sample population forms two main clusters. One cluster further divided into three sub-clusters (IA, IB and IC).

In view of the importance of the crop for sustainable horticulture under the changing climate patterns the study gains significance and needs to be further extended to cover other parameters of study.

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WHETHER DATA OF LUCKNOW

Period	Mean Temperature ºC				Relative Humidity (%)				Total Rainfall (mm)		Raining Day	
Month	Maximum		Minimum		7 Hours		14 Hours					
	2016-2017	2017-2018	2016-2017	2017-2018	2016-2017	2017-2018	2016-2017	2017-2018	2016-2017	2017-2018	2016-2017	2017-2018
January	19.0-22.0	22.0-20.5	8.7-7.8	7.8-5.4	92-95	95-96	52-52	52-52	62.2-16.5	16.5-4.0	3-3	3-0
February	24.5-26.7	26.7-26.9	10.4-10.3	10.3-10.4	83-91	91-89	38-37	37-39	33.6-0.0	0.0-0.0	1-0	0-0
March	30.6-31.8	31.8-33.6	13.8-14.9	14.9-15.6	74-76	76-73	28-25	25-24	0.0-5.4	5.4-0.0	0-1	1-0
April	40.2-38.5	38.5-38.7	22.3-22.3	22.3-19.9	47-61	61-61	16-25	25-26	0.0-0.0	0.0-9.2	0-0	0-2
May	39.0-39.5	39.5-38.9	25.4-24.9	24.9-24.8	65-67	67-67	36-32	32-29	39.6-18.4	18.4-16.8	6-2	2-2
June	37.9-38.8	38.8-34.4	27.6-26.7	26.7-27.3	77-74	74-70	51-42	42-45	92.4-85.8	85.8-123.4	8-4	4-5
July	33.4-32.7	32.7-32.4	26.3-26.0	26.0-26.2	92-91	91-88	77-78	78-68	219.6-296.4	296.4-318.7	20-15	15-13
August	33.7-33.4	33.4-34.2	25.7-26.3	26.3-25.4	90-92	92-94	73-75	75-78	243.4-232.4	232.4-564.4	15-12	12-14
September	33.4-34.4	34.4-33.8	25.2-25.4	25.4-23.9	92-91	91-90	69-64	64-65	202.4-54.0	54.0-227.8	09-03	03-7
October	33.5-34.3	34.3-33.8	20.3-20.0	20.0-17.6	92-96	96-92	46-45	45-37	54.6-0.0	0.0-0.0	02-0	0-0
November	28.6-28.4	28.4-28.8	11.9-11.8	11.8-11.7	95-94	94-92	42-41	41-39	0.0-0.0	0.0-0.0	0-0	0-0
December	22.2-24.3	24.3-23.7	9.1-8.8	8.8-5.1	98-94	94-95	62-45	45-37	0.0-0.0	0.0-0.0	0-0	0-0