ANALYSIS OF MOLECULAR DIVERSITY IN ACCESSIONS OF BUCKWHEAT (*FAGOPYRUM* SPP.) FROM HIMALAYAN RANGE

By

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The North-Eastern Hill University

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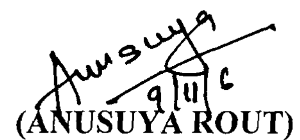
DECLARATION

I, Anusuya Rout, hereby declare that the subject matter of this thesis entitled "Analysis of molecular diversity in accessions of buckwheat (*Fagopyrum* spp.) from Himalayan range " is the record of work done by me, that the contents of this thesis did not form basis of the award of any previous degree to me or to the best of my knowledge to anybody else, and that the thesis has not been submitted by me for any research degree in any other University/Institute.

This is being submitted to the North-Eastern Hill University for the award of the degree of Doctor of Philosophy in Botany.



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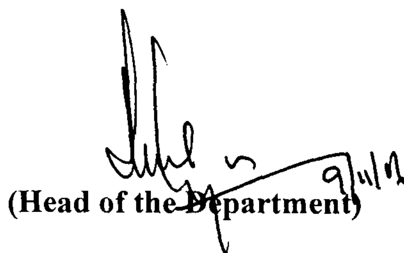

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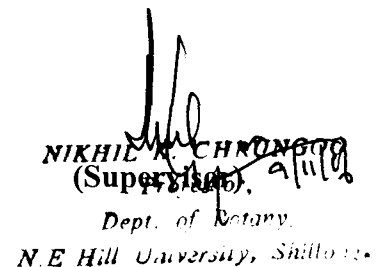

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Dedicated to my parents

ABBREVIATIONS

bp	: base pair
cpDNA	: chloroplast DNA
CTAB	: Hexadecetyltrimethyl - ammonium bromide
dATP	: 2'-Deoxyadenosine 5'-triphosphate.
dCTP	: 2'-Deoxycytosine 5'-triphosphate.
dGTP	: 2'-Deoxyguanosine 5'-triphosphate.
dTTP	: 2'-Deoxythymidine 5'-triphosphate.
DNA	: Deoxyribonucleic acid
EDTA	: Ethyl disodium tetra acetate
EtBr	: Ethidium Bromide
IPGRI	: International Plant Genetic Resource Institute
kb	: kilo base
kDa	: kilo Dalton
ME	: β- Mercaptoethanol
MQ	: Milli Q
PAGE	: Polyacrylamide gel electrophoresis
PCA	: Principal coordinate analysis
PCR	: Polymerase chain reaction
PIC	: Polymorphism information content
PMSF	: Phenyl-methyl sulphonyl fluoride
RAPD	: Random Amplification of Polymorphic DNA
R_f	: Relative front
*rpo*B	: RNA polymerase beta subunit
SDS	: Sodium dodecyl sulphate
TBE	: Tris- Borate EDTA
TEMED	: N, N, N, N-Tetramethylethylenediamine
Tris	: Tris (hydroxymethy) aminomethane
*trn*C	: tRNA- Cys (GCA)
UV	: Ultra Violet
UPGMA	: Unweighted Pair Group Method with Arithmetic Mean

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CHAPTER 1

INTRODUCTION

INTRODUCTION

Plant genetic resources are considered as one of the most important gifts of nature to mankind. They represent the sum total of diversity accumulated through years of cultivation under domestication and natural selection. Many of these genetic resources are also important sources of high nutritive value foods for human consumption. While the importance of conservation and use of genetic resources for the benefit to mankind can not be understated, the key to successful utilization of the existing genetic resources and the variability available in the broad gene pool requires a systematic evaluation of different agronomic traits in the available germplasm.

Out of the total crop genetic diversity available mankind has depended on a very limited number of crops to meet the needs of staple diets and on a very limited number of major non-food crops to meet associated needs. The narrowing of the number of crops upon which global food security and economic growth depend has placed the

future supply of food and rural incomes at risk. The shrinking portfolio of species used in agriculture reduces the ability of farmers and ecosystems to adapt to new environments, needs and opportunities. So far out of the estimated 75,000 species of edible plant only about 150 have been widely used. Even out of these, only about 30 species provide 90% of the world's food. Considering the ever-increasing demand for food materials, it is not only necessary to use the available rich diversity and wide genetic resources and to improve the existing conventional cultivars but also to look for non-conventional lesser known and underutilized food crops.

The Himalayan ranges of India are extremely rich in floristic wealth and are home to a large variety of traditional crops that could form an important component of human diet in times to come. Although Himalayan region is well established as a mega diversity region in the entire Indian sub-continent, the severe population pressure coupled with changes in the socio-economic life style of the peoples pose a serious threat to the unique biodiversity of the region. The region is home to a rich diversity of several plant species many of which are underutilized. These underutilized crops have a good potential for use as food or for industrial purposes. This rich genetic estate, extant in diverse ecosystem, nurtured by ingenious communities, provides ample opportunities for further development of agriculture in the region at a comparative advantage in terms of sustainability and diversification of farming systems. These crops could also constitute an important genetic base to look for suitable heterologous proteins and their genes, which could be used as tools in crop improvement programmes. Amongst the existing known plant resources, the International Plant Genetic Resources Institute (IPGRI) and Consultative Group on International Agriculture (CGIAR) have identified common buckwheat, grain amaranth and *Chenopodium* as important but underutilized

nutraceutical crops which could be used as the genetic base for identification and isolation of suitable heterologous genes coding for biomolecules of potential economic importance.

Traditionally, diversity is assessed by measuring variation in phenotypic traits such as flower colour, growth habit or quantitative agronomic traits like yield potential, stress tolerance, etc., which are of direct interest to users. Generally early distinctness, uniformity and stability of any cultivar have relied on morphological methods. This approach has certain limitations: genetic information provided by morphological characters is often limited and expression of quantitative traits is subjected to strong environmental influence and thus do not reflect the true genetic diversity of the collection (Green, 1971; Wikramaratne, 1981; Banerjee, 1992). Thus the morphological markers were not quite enough to expose the genetic diversity between the morphological overlap cultivars and the morphological identical accessions. The need, therefore, for new tool was desperate. Molecular tools such as isozyme patterns, seed storage protein polymorphism, Amplified Fragment Length Polymorphism (AFLP), Restriction Fragment Length Polymorphism (RFLP), Random Amplification of Polymorphic DNA (RAPD) provides virtually unlimited source of information on interspecific as well as intraspecific variations. These molecular tools also provide information about the genetic makeup of the plants which could be used as a tool in accessioning of the germplasm. Such analysis can also be an important tool tagging different agronomic traits to molecular markers for use in crop improvement programmes. The AFLP technique combines the RFLP reliability with the power of PCR to amplify simultaneously many restriction fragments (Vos *et al.*, 1995). This technique was used successfully to evaluate genetic diversity and genetic relationships

in wheat (Salamini *et al.*, 1997; Barrett and Kidwell, 1998), bean (*Phaseolus vulgaris* L.) (Tohme *et al.*, 1996), rice (Mackill *et al.*, 1996; Virk *et al.*, 2000), tea (*Camellia sinensis* Kuntze) (Paul *et al.*, 1997), barley (*Hordeum vulgare* L.) (Qi and Lindhout, 1997) and soybean (Maughan *et al.*, 1996).

The genus *Fagopyrum* consists of about 19 species, some of which have been recently discovered (Ohnishi, 1998; Ohsako and Ohnishi, 1998). Of the two cultivated species, *Fagopyrum esculentum* Moench and *Fagopyrum tataricum* Gaertn, cultivation of *Fagopyrum esculentum* extends from temperate Europe to Japan through the Indo-Mayanmar region. Cultivation of *Fagopyrum tataricum* is restricted mainly to the Himalayan region and China. *Fagopyrum cymosum*, the wild species of buckwheat occurs mostly in Himalayan foothills (Fig.1). The genus *Fagopyrum* has been divided into two phylogenetic groups *viz.* the cymosum group comprising of two cultivated species *F. esculentum* and *F. tataricum* and two wild species *F. cymosum* and *F. homotropicum* and the urophyllum group comprising *F. urophyllum* and other wild species (Ohnishi and Matsuoka, 1996; Yasui and Ohnishi, 1998a, b; Ohsako and Ohnishi, 2000). Baniya *et al.* (1992) have observed significant variation in days to maturity, plant height, number of branches and leaves, clusters and seeds per cyme, seed weight, grain yield, seed colour/ shape/ surface in different landraces of buckwheat. Evaluation of genotypes for their consistency of performance under different environments is important in plant breeding programmes. The occurrence of large genotype-environment (GE) interactions possesses a major problem of relating phenotypic performance to genetic constitution and makes the selection of genotypes difficult. Registration of buckwheat cultivars in gene banks is mainly based on morphologic and physiologic characteristics. Even though these descriptors are useful,

Fig 1: Plants of different accessions/cultivars of *Fagopyrum esculentum*, *"Fagopyrum himalianum"*, *Fagopyrum tataricum* and *Fagopyrum cymosum* collected from different areas of Himachal Pradesh, Uttaranchal and Arunachal Pradesh and Meghalaya growing in experimental beds in the botanical garden of the Department of Botany, NEHU.

gopyrum cymosum

they are limited in number and may be affected by environmental factors. Molecular markers are a useful complement to morphological and physiological characterization of cultivars because they are plentiful, independent of tissue or environmental effects, and allow cultivar identification early in plant development. RAPD and SDS PAGE profiles have been successfully used for analysis of diversity in many crops including buckwheat (Javonik & Kump, 1993; Tsuji & Ohnishi, 1998; Ohnishi & Asano,1999), cowpea (Mignouna *et al.*,1998), soybean (Thompson *et al.*, 1998), Brassica Juncea (Rabbani *et al.*, 1998), bean (Duarte *et al.*, 1999), *Vicia sativa* (Potokina *et al.*, 2000). Ohnishi (1998),, Ohsako and Ohnishi (1998) and Ohsako and Ohnishi (2000) have worked on phylogenetic relationships between different species of the genus *Fagopyrum*. However, not much information is available on interspecific variations in molecular fingerprints in the genus.

The objective of the proposed investigation is to elucidate the variation in different accessions of buckwheat at the molecular level and to develop suitable protein and RAPD based markers for the identification of various accessions of buckwheat.

CHAPTER II

REVIEW OF LITTERATURE

REVIEW OF LITERATURE

Plant genetic resources are considered as one of the most important gifts of nature to mankind. They represent the sum total of diversity accumulated through years of cultivation under domestication and natural selection. Since the dawn of agriculture, domestication of plant species has helped in the evolution of useful plant species and these resources have been exploited to our advantage. The unique process of domestication had the inherent objectives of cultivating plants with better traits to serve as basic source of food and raw materials for industry. So far out of the estimated 75,000 species of edible plant only about 150 have been widely used (Gautam and Singh, 1998). Of these, about 30 species provide 90% of the world's food. This tiny part of the existing biodiversity in species, however, has an extraordinary importance and involves huge inter- as well as intra-specific genetic diversity.

De Candolle (1886) presented the first documented account of origin and diversity of species occurring in the Indian Gene Centre. The document was primarily based on the Flora of British India (1876). The Indian Gene Centre focused prominently in this concept. The Indian Gene Centre is also recognized for its native wealth of plant genetic resources with over 1200 species having medicinal and aromatic importance.

The genetic diversity of agricultural crops is represented by bred cultivars, landraces and other genetic stock as well as by wild relatives of cultivated plants. All these materials constitute a gene pool which can be used for improvement/ value addition, broadening of a genetic base of cultivars and also as a source of new diversity for agriculture. One of the main justifications for the conservation of genetic resources is their utilization for crop improvement. However, the key to successful use of variability from broad gene pool requires the knowledge of desirable traits available in the germplasm and this requires a systematic evaluation of different agronomic traits in the available germplasm. Both characterization and evaluation results in recording of a number of agronomic traits and in the identity of accession with desirable traits for use in crop improvement. Assessment of genetic variation in a species is important to initiate an effective improvement programme for crop improvement because it provides the basis for tailoring the desirable genotypes. Information on genetic diversity is also valued for the management of germplasm and for evolving conservation strategies.

Morphological descriptions of plant cultivars often present problems in clear-cut identification because the phenotypic differences within species are too minute to discriminate between species. The use of genetic polymorphism assessed by various biochemical and molecular markers has been applied in many crops to determine genetic relationships for purposes of phylogenetic studies, varietal identification as well

as for QTL mapping. Molecular markers such as isozyme patterns, seed storage protein polymorphism, RFLP, RAPD can provide a virtually unlimited source of information on interspecific as well as intraspecific variations (Godshalk *et al.,* 1990; Dudley *et al.,* 1991; Boppenmaier *et al.,* 1993; Zhang *et al.,* 1995, 1996). Genome analysis based on molecular markers has generated a vast amount of information and a number of databases are now available. All molecular marker based assays generate a characteristic banding pattern for each individual/ population which can be assigned a marker genotype. The differences in allele frequencies at the marker loci can be used to calculate the genetic distance (GD) between individuals of a population or between populations of a species. When the banding pattern is complex and the genotype cannot be determined directly, the GD is usually estimated based on the uncommon bands between the individuals being studied (Nei and Li 1979).

A variety of molecular markers have been used to study the extent of genetic variation among the diverse group of important crops. More recently, DNA-based techniques have been used successfully in developing DNA fingerprints of several plant species such as sunflower (Hongtrakul *et al.*, 1997) and grapes (Cervera *et al.*, 1998) and in genetic diversity studies (Jain *et al.,* 1994; Sonnate *et al.*, 1997; Menkir *et al.*, 1997; Barrett and Kidwell, 1998; Sivolap *et al.*, 1998; Zhu *et al.*, 1998; Chowdari *et al.*, 1998; Gupta *et al.*, 1999; Sivolap *et al.*, 1999; De-Bustos *et al.*, 1999; Strelchenko *et al.*, 1999).

Seed protein polymorphism:

Traditional cultivar identification based on plant morphological characters is very time consuming and may be unreliable, since some of these characteristics are strongly affected by the environment. Morphological descriptions of plant cultivars also

present problems in clear-cut identification because in most cases differences at the phenotypic level may be too minute. Ideally the differences between cultivars should be based on the differences at the genetic level. Miege (1982) has suggested that intercultivar differences could be measured by comparing the differences in the products of gene activity indicating thereby the use of proteins as genotype markers. In recent years, seed protein electrophoresis has found wide application in resolving systematic relationships and in characterising cultivated varieties in crop plants including cereals and legumes and largely cross-fertilized pasture grasses (Ladizinsky and Hymowitz 1979; Ferguson & Grabe, 1986; Nevo and Payne, 1987; Chen *et al.*, 1987; Levy and Feldman, 1988; Gardiner & Forde, 1988; Clark *et al.*, 1989; Singh *et al.*, 1991; Yupsanis *et al.*, 1992; Jaradat 1991; Bernardo *et al.*, 1997; Przybylska *et al.*, 1998; De Bustos *et al.*, 1999a; Gonzalez-Castano, 1992). Ladizinski and Hymowitz (1979) have emphasized the importance of seed protein electrophoresis in resolving specific taxonomic and evolutionary problems.

Seed protein electrophoresis has been utilized as a powerful tool in solving taxonomic problems and explaining the origin and evolution of a number of cultivated plants including *Cicer* (Ahmad and Slinkard, 1992), *Lens* (Ahmad *et. al.*, 1997; Ladizinsky, 1979; Hussain *et al*, 1989; Sammour, 1994), *Capsicum anum* (Vladova *et al.*, 2000)(and)*Vigna mungo* (Ghafoor *et al.*, 2001),(*Chenopodium* (Bhargava *et al.*, 2005). Ahmad and Slinkard (1992) have analyzed the SDS PAGE profiles of seed storage proteins of one cultivated species and eight wild annual species of *Cicer*. They have reported that the seed protein profile was a conservative and species specific trait. Based on the differences in the seed storage protein profiles Ahmad and Slinkard (1992) have confirmed that *Cicer judaicum* and *Cicer pinnatifidum* were indeed two separate

species. They were also able to establish the relative genetic distances between different species of *Cicer*. On the basis of SDS PAGE profiles of different species of lentils, Sammour (1994) concluded that *L. culinaris* spp. *Orientalis* and *L. odemensis* were the wild progenitors of cultivated lentils. Ghafoor *et al* (2001) studied thirty seven diverse genotypes of *Vigna mungo* and three of *V. radiata* resembling to *V. mungo* for seed characters to determine the extent of genetic variation. Based on SDS PAGE, specific bands were suggested to be used for identifying *Vigna radiata* from mixed germplasm with *Vigna mungo*. They have suggested that while the two species showed a low level of inter-specific genetic diversity for agronomic characters and geographical origin, SDS PAGE profile of seed proteins could differentiate *Vigna radiata* and *Vigna mungo*. Bhargava *et al.* (2005) analyzed the seed storage protein SDS PAGE data for different accessions of Chenopodium to determine inter as well as intraspecific relationships between different accessions of Chenopodium. They observed that the seed protein data was congruent with the taxanomic position, crossability relationships and other biochemical characters for the various accessions of Chenopodium. SDS PAGE has also been used to discriminate high yielding soybean plants from the low yielding ones (Rashed *et al.*, 1997) as well as disease resistant plants from the susceptible ones. These results indicate the significance of use of seed storage protein SDS PAGE profiles as a tool for species identification. Variation of seed storage proteins has also been analyzed to estimate the center of genetic diversity and possible dissemination pathway in common bean (Gepts *et al.*, 1988).

Magdalena *et al* (2002) have assessed 59 Spanish landraces of common wheat belonging to different agrotypes for 11 seed protein loci to determine the level of agreement between the agrotype classification and the morphological and biochemical

variation of the wheat. At least 22 new gliadin, which were not catalogued before were found in the Spanish landraces studied. Multivariate and cluster analysis revealed that there was a clear relationship between seed protein composition and wheat classification for different agrotypes. Similar studies carried out by Aktad *et al* (2002) on *Triticum aestivum* and *Triticum durum*, have revealed the evolutionary history of the species. The SDS PAGE profiles obtained by these workers clearly distinguished species of *T. aestivum* from *T. durum*.

Rahman and Hirata (2004a, b) analyzed eighty five different cultivars of *Brassica rapa*, *Brassica juncea*, *Brassica napus*, *Brassica carinata*, *B. oleracea* and the hexaploid *Brassica* collected from Bangladesh, Japan, China and Denmark for seed and leaf protein variations by SDS PAGE to identify polymorphic genetic markers. While 10 polymorphic markers were identified from seed proteins no identifiable polymorphic band was detected from leaf proteins. The polymorphic markers developed by these workers clearly distinguished the yellow sarson self-compatible cultivars from the brown seeded, self-incompatible cultivars. Similar observations had been reported by Das *et al.* (2000) using RAPD and AFLP analysis. Rahman and Hirata (2004a, b) also reported that the hexaploid *Brasica* had the indices for % polymorphic bands, degree of phenotypic diversity, diversity value for genetic markers and the sum of effective number of alleles. The cluster analysis using data generated from SDS PAGE clearly separated the different seeded cultivars from different geographical areas of the Asia Pacific region. De Wet e*t al*., (1979) and Afzal *et al.* (1996) have analysed the intraspecific variation of foxtail millet, an important crop which was domesticated in the early stages of agriculture, *vis-à-vis* geographical distribution through protein polymorphism. Based on a comparison of the variation in seed storage protein profiles

the landraces of foxtail millet were classified into six different types. Despite the limited number of landraces from Thailand, Myanmar and Indonesia a geographical cline of SDS PAGE electrophoretogram was observed. Based on a comparison of seed protein electrophoretic profiles between wild and cultivated species of *Setaria* from Western Europe, Afghanistan, and East Central China, De Wet *et al.* (1979) have even suggested a mechanism for domestication through germplasm exchange for the species. Signor *et al.*, (2005) studied diversity in seed protein composition in fifty lines of *Medicago truncatula* and reported 26 polymorphic protein bands out of 46 major seed polypeptides. The polymorphism for the major seed protein classes allowed the clustering of the genotypes into four groups. There was no evidence of clustering according to geographical origin of the lines. However, all lines not belonging to either *M. truncatula* spp. *truncatula* or spp. *longispina* were clustered in a single group, demonstrating the value of seed protein profiles in delimiting species boundaries. Within-accession variation was investigated for one dimensional seed profiles, with additional lines obtained from the same ecotypes. Lines contrasting for qualitative traits and seed protein content were identified to allow for the genetic determination of these characters. Similar work carried out by Shawky *et al.*, 2005 on twenty-one varieties and sixteen landraces of faba bean (*Vicia faba*) showed a total of 30 distinguishable protein bands out of which 11 were polymorphic. Cluster analysis of the data resolved the populations into three major groups and seven subgroups. Even though much work has been done on analysis of seed protein polymorphism as a marker of genetic diversity in several crop plants not much information is available on buckwheat grain protein polymorphism (Zeller 2001). Rout and Chrungoo (2006) have determined the species relationship in Himalayan buckwheat by SDS PAGE of endosperm proteins. Their

results have indicated that the current practice of accessioning based on IPGRI defined descriptors could lead to erroneous cataloguing. They have emphasized the use of molecular markers like endosperm protein SDS PAGE profiles/ RAPD/ RFLP maps in determining species relationships in buckwheat. Rogl and Javornik (1996) and Yan *et al.*, (2003) have emphasized the usefulness of storage protein polymorphism as genetic markers for cultivar identification, crop origin and evolutionary studies.

The question of protein composition and its quantification is, however, still problematic. Webster (1986) has reported that the protein fractions obtained with Osborne method of protein separation and the homogenous polypeptides detected by SDS PAGE were hardly comparable. Application of modifications to the original Osborne method (Volker, 1975; Wieser *et al.*, 1980) could complicate the uniform protein characteristics and other possibilities of their use as markers. Electrophoretic studies of multilocus-enzymes (MLEE) and whole-cell protein (SDS PAGE) carried out by Rosa *et al.* (2000) to evaluate the parity between different methods for the characterization of five *Candida* species have revealed that SDS PAGE was more efficient in grouping strains in their respective species while MLEE had a much limited resolution in organizing all strains in their respective species-species clusters.

The analysis of genetic diversity based on endosperm proteins has previously been undertaken with species of several genera such a *Triticum* spp. (Nevo and Payne, 1987; Levy and Feldman, 1988), *Hordeum* spp. (Jaradat 1991; Bernardo *et al.*, 1997; De Bustos *et al.*, 1999), *Aegilops* spp. (Gonzalez-Castano, 1992) and *Thinopyrum* spp. (Moustakas *et al.*, 1986, 1988; Nieto *et al.*, 2003). The results obtained by these investigations have revealed population specific patterns of SDS PAGE profiles of endosperm proteins.

Isozymes as a tool for analysis of genetic diversity:

Electrophoretic separation and visualization of allozyme polymorphism is another technique to study the plant genetic diversity in several plant genera. Isozymes offer the advantage of environmental stability and co-dominant expression of isozymic genes. Cerezo *et al.*, (1989) have emphasized the rapidity and ease of reproducibility of the technique. Since the discovery of allozyme techniques by Hunter and Markert in 1957, the principle of isozyme analysis has been successfully applied to study population genetics (Gottlieb, 1977; Crawford, 1990) and germplasm management (Bvretting and Widrlechner, 1995). Although the limited number of isozymes makes it difficult to use these as markers for providing complete genome coverage, the use of isozymes remain as a quick, cheap and easy method for a preliminary survey based on a few markers (Ghereyaize *et al.*, 1995). Hamrick and Godt (1997) has highlighted that the Isozymes loci for a given enzyme are highly conserved in a given taxa. The number of loci which are polymorphic (% polymorphic loci) and the mean number of alleles per polymorphic loci are two of the genetic diversity parameters which gives an estimate of the extent of diversity maintained by plant species.

Electrophoretic separation and visualization of allozyme polymorphism has also been the most commonly used procedure to estimate plant genetic diversity in several plant genera such as *Phaseolus* (Acquaah *et al.*, 1994; Paredes and Gepts, 1995); *Glycine* (Griffin and Palmer, 1995); *Vigna* (Sonnante *et al.*, 1997) and *Cicer* (Labdi *et al.*, 1996) maize (Goodman *et al.*, 1980), wheat (Jaaska, 1983), tomato (Rick, 1983), or common bean (Vallejos *et al.*, 1992) etc. Isozymes have been used to identify cultivars, rootstocks and wild species in apples (Vinterhalter and James, 1983; Menendez *et al.*, 1986; Manganaris and Alston, 1992), cultivars in peach (Arulsekar *et al.*, 1986),

cultivars and rootstocks in grape (Walker and Lin, 1995), apricot (Byrne, 1989), *Citrus* (Torres *et al.*, 1978), in strawberry (Bringhurst *et al.*, 1981).

Tous *et al.*, (1992) studied variability of isozymes in 9 enzyme systems of 25 cultivars of carob (*Ceratonia siliqua* L.). Five enzymes *viz.* PGI, AAT, MDH, Aconitase and PGM were polymorphic, making it possible for the 25 cultivars to be classified into 8 phenotype categories. Similar investigations were carried out by Dudnikov (1998) on 24 Transcaucasian populations of *Aegilops squarrosa* and *A. strangulata*. Dudnikov (1998) reported the involvement of *Acph*1, *Est*2, *Est*5, *Got*1 and *Got*2 loci in the adaptive process of *Ae. Squarrosa* subspecies divergence. Investigations on genetic diversity through analysis of allozyme variations in Aegilops sp. was also studied by Kawahara (2000) who analyzed genetic variation at 21 loci in 73 accessions of *Aegilops comosa* and *A. uniaristata*, two species belonging to section Comopyrum of *Aegilops*. He concluded that the two species were genetically distant from each other, supporting the previously assigned different genome symbols M and N. Isozyme divergence has also been used as a tool to measure the level of diversity between two groups of rice breeding lines or cultivars which were screened as potential maintainers in hybrid rice breeding programmes (Devanand *et al.*, 1999). Their observations revealed that the majority of potential maintainers exhibited *japonica* type alleles whereas most of the potential restorers exhibited *indica* type alleles.

In order to clarify the genetic diversity of wild species as compared with its cultivated species it is important to use the wild species potential as a genetic resource. Some of the investigations in this direction were carried out by Ohnishi and Asano (1999) who assessed the genetic diversity of 19 populations of *Fagopyrum homotropicum*, a self fertilizing close relative of common buckwheat. Based on the

variation at 16 loci of 11 enzymes they could identify the tetraploid populations of the species. Yamane and Ohnishi (2001) have studied the allozyme variation among natural populations of perennial buckwheat for phylogenetic relationships. They reported that the phylogenetic tree constructed by the neighbour-joining method based on allozyme variation clarified two distinct groups of diploid populations of *F. cymosum* complex.

DNA based markers:

Molecular markers, which detect variation at the DNA level, provide a way to characterize germplasm accurately at a faster rate. An array of molecular marker techniques has been developed. RAPD markers remain popular because of their simplicity and low development cost. RAPD markers are generated by PCR amplification of random genomic segment with a single primer of arbitrary sequence (Williams *et al.*, 1990).

Compared with other methods of detecting DNA polymorphism, randomly amplified polymorphic DNA (RAPD) technique has potential advantage for the investigation of the rare plants. It is relatively inexpensive and technically straightforward for conducting experiment (Rosseto *et al.*, 1995). The amplified fragments provide a large number of polymorphic loci, which are especially useful for studies of species with low genetic variation (Dawson *et al.*, 1993).

The large number of accessions in gene banks usually demands a reliable, rapid and not complicated method, which is sufficiently discriminative to distinguish most or all genotypes held in a collection. The RAPD technique in general has been proven to be very successful in addressing different issues in the management of plant genetic resources, such as evaluation of germplasm (Novy *et al.*, 1994; Thorman *et al.*, 1994; S˘us˘tar-Vozlie and Javornik, 1999) identification of duplicate accessions (Waycott and

Fort, 1994; Virk *et al.*, 1995), detection of misclassifications (Margale *et al.*, 1995), and genetic improvement. The RAPD technique is an effective tool for identifying variation and estimating diversity in different biological system (Tingey and Tufo, 1993). Because of its simplicity, rapidity and reliability, the random amplified polymorphic DNA (RAPD) has been successfully used for diversity studies in many crops *viz.* buckwheat (Javonik & Kump, 1993; Tsuji & Ohnishi, 1998; Ohnishi & Asano, 1999), cowpea (Mignouna *et al.*, 1998), soybean (Thompson *et al.*, 1998), *Brassica Juncea* (Rabbani *et al.*, 1998), bean (Duarte *et al.*, 1999), *Vicia sativa* (Potokina *et al.*, 2000).

Many researchers have reported the use of RFLP and RAPD profiles for generating information on variability within plant germplasm (Beckmann & Soller, 1983; Tanksley *et al.*, 1989; Williams *et al.*, 1990; Skroch & Nienhuis, 1995; Ashburner *et al.*, 1997). The RFLP technique requires a large amount of relatively pure DNA which is time consuming and is technically demanding. RAPD methodology overcomes these limitations since a large number of polymorphic bands can be generated with relative ease from less DNA. Metais *et al.*, (2001) have assessed common bean diversity using RAPD and AFLP markers. They reported a higher level of polymorphism with RAPD than with AFLP analysis. However, both analyses led to the same type of clustering. RAPD markers have been successfully used in cultivar analysis in some cross (Hu and Quiros 1991, Yang and Quiros 1993, Koller *et al.*, 1993). Genetic analysis using RFLP has already been employed in genotyping Japanese tea cultivars (Matsumoto *et al.*, 1994), but variation was limited due to the low number of loci investigated.

During the last decade, the most widely utilized tool for phylogeny reconstruction in plants has been the analysis of chloroplast (cp) DNA, which is

haploid, multicopy and nonrecombining. A low evolutionary rate of cpDNA genomes generally makes this type of analysis unsuitable for the study of phylogenetic relationships among closely related taxa. However several informative studies on intraspecific variation of cpDNA sequences have focused on rapidly evolving regions in the chloroplast genome (Jordan *et al.*, 1996; Fujii *et al.*, 1997, 1999; Yasui and Ohnishi, 1998a; Ohsako and Ohnishi, 2000, 2001). In the genus *Fagopyrum*, 3' end of *rbc*L, *acc*D, and associated intergeneric spacer region of cpDNA (Yasui and Ohnishi, 1998a), the *trn*C-*rpo*B spacer, and the *trn*K/ *mat*K region (Ohsako and Ohnishi, 2000, 2001) has been used to study genetic relationships at lower taxonomic levels such as within a species.

Clegg *et al.*, 1991 has emphasized that the chloroplast genome evolves slowly in general making it an ideal system to assess phylogenetic relationships among genera. Such studies have been carried out in the family Onagraceae (Conti *et al.*, 1993), Apiaceae (Downie *et al.*, 1996), Asteridae (Olmstead *et al.*, 1992) including monocots (Davis, 1995) other seed plants (Chase *et al.*, 1993) and angiosperms (Nickrent and Soltis, 1995). Nucleotide variation of noncoding regions in cpDNA is being used for phylogenetic analyses at the intraspecific level because of their considerably higher evolutionary rate than gene-encoding regions (Dumolin-Lape′gue *et al.*, 1997; Fujii *et al.*, 1997).

The spacer region is useful for both phylogenetic assessment at species as well as population levels (Golenberg *et al.*, 1993; Manen *et al.*, 1994; Natali *et al.*, 1995). Several universal primers for amplifying noncoding spacers of the chloroplast genome have been reported (Taberlet *et al.*, 1991; Demesure *et al.*, 1995; Dumolin-Lapegue *et al.*, 1997). Most of the primers were designed for amplifying spacers between tRNA

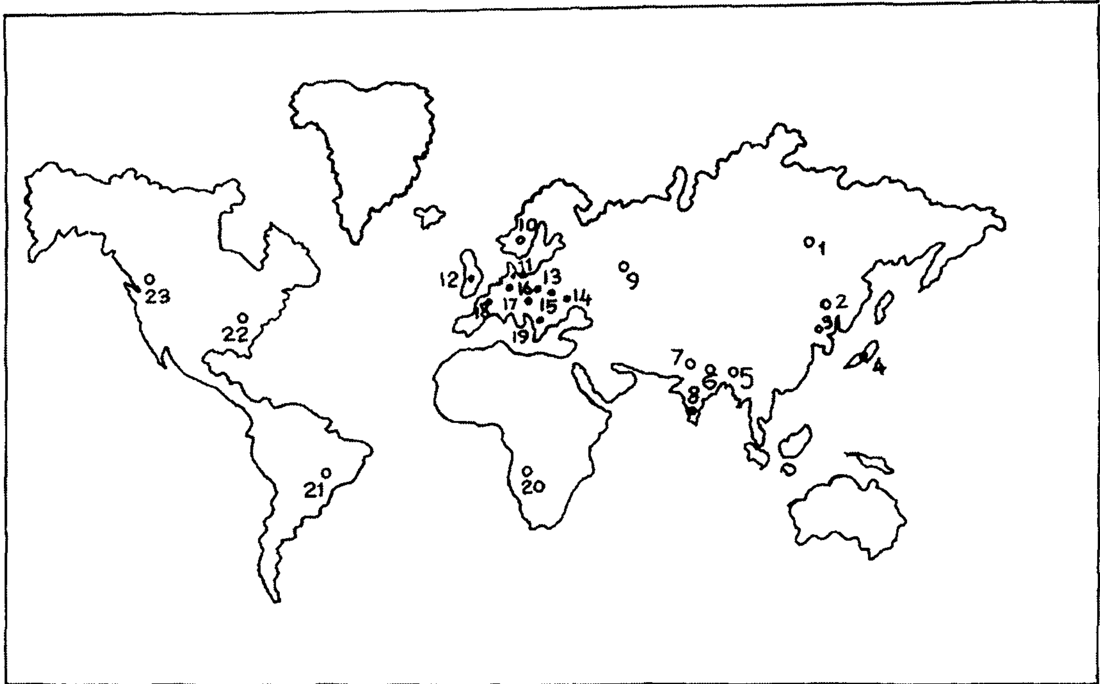
genes, which have been proved variable among species or populations (Demesure *et al.*, 1996). The sequences of the *rbc*L (the large subunit of ribulose-1, 5- bisphosphate carbolylase/ oxidase) gene have been used widely to construct plant phylogenies (e.g., Ritland and Clegg, 1987: Doebly *et al.*, 1990).

Analyses of the DNA sequences of *rbc*L gene and internal transcribed spacer (ITS) region of nuclear ribosomal RNA have been successfully used in classifying major groups of *Fagopyrum* (Yasui and Ohnishi, 1996; Yasui and Ohnishi, 1998a, b). Based on molecular data, the genus *Fagopyrum* has been divided into two phylogenetic groups *viz.* the *cymosum* group comprising of two cultivated species *F. esculentum* (Moench) and *F. tataricum* (L.) Gaertn and two wild species *F. cymosum* (Meissn), and *F. homotropicum* Ohnishi and the *urophyllum* group comprising *F. urophyllum* (Bur. et Franchet) H. Gross. and other wild species (Ohnishi and Matsuoka, 1996; Yasui and Ohnishi, 1998a, b; Ohsako and Ohnishi, 2000).

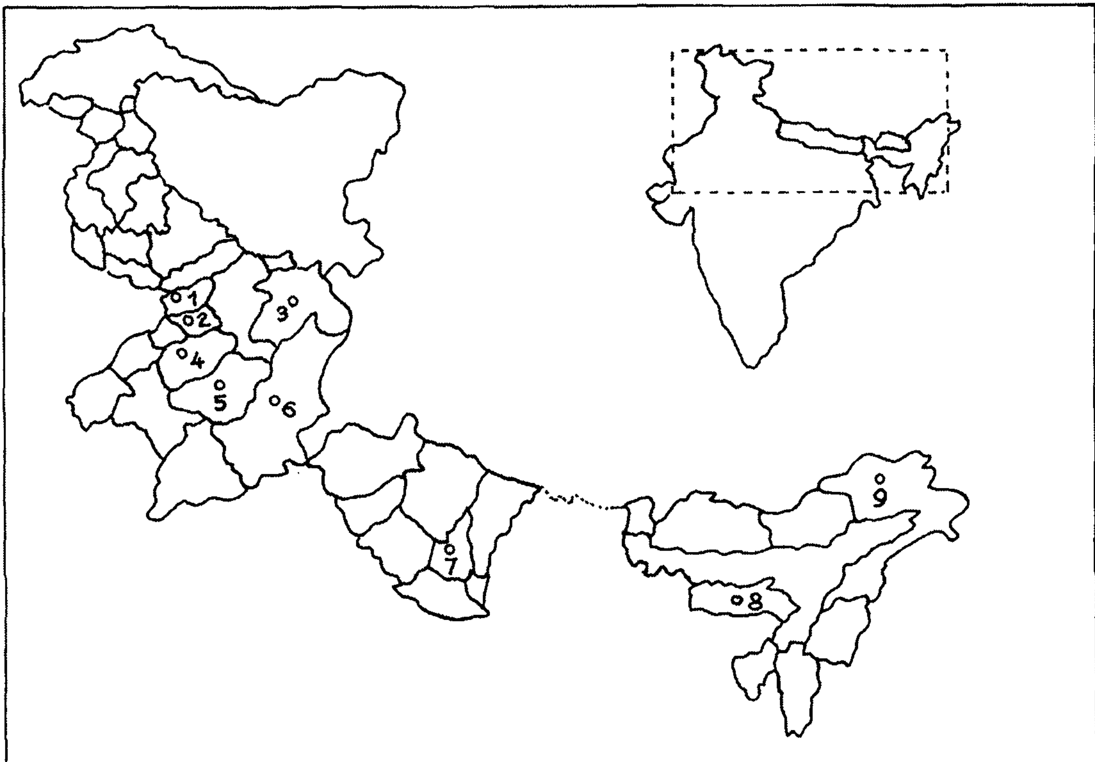
Common buckwheat (*Fagopyrum esculentum* Moench) has been a crop of secondary importance in many countries and yet it has persisted through centuries of civilization and enters into the agriculture of nearly every country where cereals are cultivated. The main regions of buckwheat cultivation across the globe include China, Russia, Ukraine, Kazakhstan, parts of Eastern Europe, Canada, Japan, Korea, Nepal and India. Buckwheat is grown throughout a large area of Asia and Southeast Asia as a crop that fits the farming system on marginal and fairly unproductive land. It is used as a subsistence crop in many of the more mountainous areas where it is often grown with barley at the higher altitudes. The crop grows extensively in the Himalayan foothills and is used by people living in the region as a staple diet (Fig.2). The plant is a pseudocereal of high economic importance because of short growth span, capacity to grow on poor

Fig 2 **a:** Areas of cultivation and distribution of buckwheat (*Fagopyrum* spp.) in the world. 1. China, 2: Manchuria, 3: Korean penusula, 4: Japan, 5: Myanmar, 6 & 7: Indian Himalayas, 8: Nilgiri Hills in Southern Indian penunsula, 9: USSR, 10: Sweden, 11: Denmark, 12: United Kingdom, 13: Poland, 14: Bulgaria, 15: Romania, 16: Austria, 17: Germany, 18: France, 19: Italy, 20: South Africa, 21: Brazil, 22: USA and 23: Canada

b: Areas of collection of accessions/cultivars of buckwheat for the present study.1: Chamba, 2: Kangra, 3: Kinnaur, 4: Mandi, 5: Kulu, 6: Shimla, 7:Almora, 8: Meghalaya and 9: Arunachal Pradesh.



a



b

soils and the high protein content of its grains. The plant is a rich source of trace elements (Ikeda *et al.*, 2000; Skrabanja *et al.*, 2004), rutin, vitamins B1,B2 and E (Watanabe *et al.*, 1995, Watanabe *et al.*, 1997) and dietary proteins for gluten-sensitive individuals (Skerritt, 1986).

Joshi and Paroda (1991) have evaluated 408 accessions of Buckwheat from the Himalayan region for 31 descriptor parameters including plant height, number of branches and leaves, clusters per cyme, seeds per cyme, days to maturity, seed weight, grain yield, seed colour/ shape/ surface. They have considered accession no. IC-13145 on the level of a species as "*Fagopyrum himalianum*". The accession, housed in the Regional Station, NBPGR, Phagli, Shimla (India), has been listed as "*F. tataricum* var. *himalianum*" by IPGRI (www.ipgri.cgiar.org/publication). Campbell (1997) has, however, considered it as a subspecies of *F. esculentum.*

Assessment of genetic variation in a species is important for initiation of effective breeding programmes because it provides the basis for tailoring desirable genotypes. The preparation of a list of Buckwheat Descriptors (IPGRI, 1994) was a major step forward in the coordination of passport data and descriptors on this crop. The National Bureau of Plant Genetic Resources has a large collection of indigenous buckwheat accessions. The bureau has also developed descriptor parameters based on morphological characteristics for these accessions. However, the evaluation protocol based on descriptors developed by NBPGR is a laborious process. In many cases the accessioning of germplasm based on morphological parameters only has led to incorrect/ duplicate accessioning. While there are some reports on marker based characterization of buckwheat accessions of China, Tibet and Japan (Onishi, 1998a, b; Tsuji *et al.*, 1999; Tsuji and Onishi, 2000), there has been no report on protein and DNA

based markers for the different indigenous accessions of this important crop. In contrast to the work done on the analysis of phylogenetic relationships between different species of the genus *Fagopyrum* (Javornic and Kump, 1993; Ohnishi, 1998; Ohsako and Ohnishi, 1998; Ohsako and Ohinishi, 2000), not much information is available on interspecific variations in molecular fingerprints in the genus, especially those cultivated in the Indian Himalayan region. With a vast resource of genetic diversity of minor cereals, legumes and other underutilized crops in mind, the present investigation was undertaken to study the different accessions/cultivars of buckwheat at morphological and molecular level and to elucidate the inter as well as intra specific variations in different accessions of buckwheat. Such a study would provide leads for identification of protein and DNA based markers for the identification of various accessions/cultivars of buckwheat.

CHAPTER III

MATERIALS AND METHODS

I.MATERIALS:

Plant Material: Grains of various accessions/cultivars of buckwheat (*Fagopyrum* spp.) used for the present investigation were procured from the National Bureau of Plant Genetic Resources, New Delhi, Vivekananda Laboratory of Hill Agriculture (Indian Council of Agricultural Research), Almora, East Khasi Hills in Meghalaya (India) and the Tawang District of Arunachal Pradesh (India). The accessions used for the present study are listed in Table 3.1.

Reagents: All the chemicals used in the present investigation were of analytical/ Molecular Biology grade and were procured from Sigma chemical co., St. Louis, (USA), Bangalore Genie, Bangalore, HiMedia, EMerk, Darmstadt (Germany) and BDH, England. The oligonucleotide primers used in the present study were procured from Microsynth GmbH, Switzerland, Operon Technology Inc., Alameda, Calif. USA and University of British Columbia, BC., Canada. The SDS-PAGE molecular weight markers were purchased from Roche Molecular Biological Ltd and Bangalore Genei, India.

Table 3.1: List of accessions/cultivars of different species of buckwheat studied in the present investigation

No	Accessions	Species	Source	Origin	
1	IC-188669	*F. esculentum*	NBPGR*	Himachal Pradesh	(Western Himalaya)
2	IC-18751	*F. esculentum*	NBPGR	Himachal Pradesh	(„)
3	IC-13376	*F. esculentum*	NBPGR	Himachal Pradesh	(„)
4	IC-13145	*F. himalianum*	NBPGR	Himachal Pradesh	(„)
5	IC-13141	*F. esculentum*	NBPGR	Himachal Pradesh	(„)
6	IC-13417	*F. esculentum*	NBPGR	Himachal Pradesh	(„)
7	Local	*F. esculentum*	VPKAS**	Uttaranchal	(Central Himalaya)
8	Kamroo Local	*F. esculentum*	VPKAS	Uttaranchal	(„)
9	OC-2	*F. esculentum*	VPKAS	Uttaranchal	(„)
10	VL-7	*F. esculentum*	VPKAS	Uttaranchal	(„)
11	IC-319588	*F. esculentum*	NBPGR	Arunanchal Pradesh	(East Himalaya)
12	IC-324313	*F. esculentum*	NBPGR	Arunanchal Pradesh	(East Himalaya)
13	IC-352992	*F. esculentum*	NBPGR	Arunanchal Pradesh	(East Himalaya)
14	IC-319595	*F. esculentum*	NBPGR	Arunanchal Pradesh	(East Himalaya)
15	IC-324244	*F. esculentum*	NBPGR	Arunanchal Pradesh	(East Himalaya)
16	SanglaB-1	*F. tataricum*	VPKAS	Himachal Pradesh	(Western Himalaya)
17	SanglaB-2	*F. tataricum*	VPKAS	Himachal Pradesh	(„)
18	SanglaB-3	*F. tataricum*	VPKAS	Himachal Pradesh	(„)
19	Sangla B-5	*F. tataricum*	VPKAS	Himachal Pradesh	(„)
20	Sangla B-6	*F. tataricum*	VPKAS	Himachal Pradesh	(„)
21	Sangla B-7	*F. tataricum*	VPKAS	Himachal Pradesh	(„)
22	KBB-3	*F. tataricum*	VPKAS	Uttaranchal	(Central Himalaya)
23	Himpriya	*F. tataricum*	VPKAS	Uttaranchal	(„)
24	Kuppa Local	*F. tataricum*	VPKAS	Uttaranchal	(„)
25	Shimla B-1	*F. tataricum*	VPKAS	Himachal Pradesh	(Western Himalaya)
26	IC-412744	*F. tataricum*	NBPGR	Arunanchal Pradesh	(East Himalaya
27	IC-412863	*F. tataricum*	NBPGR	Arunanchal Pradesh	(„)
28	IC-421598	*F. tataricum*	NBPGR	Arunachal Pradesh	(East Himalaya)
29	IC-412722	*F. tataricum*	VPKAS	Arunachal Pradesh	(„)
30	IC-421597	*F. tataricum*	VPKAS	Arunachal Pradesh	(„)
31	EC-323729	*F. esculentum*	NBPGR	Japan	
32	*F. cymosum*	*F. cymosum*	NBPGR	Meghalaya	(East Himalaya)

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**Vivekandada Parvatteeya Krishi Anusandhan Sanshan, Almora, India.

II.METHODS:

The grains were observed under a magnifying glass for morphological features like grain shape, colour, presence or absence of striations and wings etc. The parameters assessed and the score assigned to each qualitative parameter are listed in table 3.2.

The collection of germplasm was separated into different groups on the basis of their morphological features. The accessions/cultivars were grown in the experimental garden to study the qualitative as well as quantitative features during their growth span. The grains were sown in two different seasons, *viz*. as a summer crop during April and as a winter crop during late August. Sowing was done in rows with three rows for each accession. The crop was raised for three consecutive years for studying both qualitative as well as quantitative morphological parameters of the accessions.

For determination of grain weight, 25 grains from each accession/cultivars were weighed on Sartorius BP160P balance. The weight of each grain was calculated by dividing the gross weight by the number of grains. For determination of hull/ groat ratio, the grains were dehulled manually and weighed on a balance. The weight of hull was calculated as the difference between total grain weight and the weight of groat. The hull/ groat ratio was calculated by dividing the weight of the hull by the groat weight. The qualitative characters assessed for the present study are listed in table 3.3. Height of the plants was measured at 50 % flowering by measuring the distance from the base to the tip of the plant. The total leaf surface area of each leaf was first measured using leaf area meter. The surface area of all the individual leaves of a plant were added together to get total leaf area per plant. The parameters were recorded individually for 15 plants from each accession/cultivars. The mean of individual readings was used for final calculations.

Table 3.2: Qualitative morphological characters analyzed for determination of variation between the different accessions/cultivars of buckwheat (*Fagopyrum* spp.) studied in the present investigation.

Sl. No.	Traits	Variable*	
1.	Seed Shape 1	Winged (1);	Non-Winged (0)
2.	Seed Shape 2	Non-conical (0);	Conical (1)
3.	Seed colour	Brown (1);	Dark brown (0)
4.	Seed coat striations	Present (1);	Absent (0)
5.	Seed coat texture	Smooth (1);	Rough (0)
5.	Colour of the stem	Red (1);	Green (0)
6.	Blade shape 1	Cordate (1);	Sagittate (0)
7.	Blade shape 2	Non Hastate (0);	Hastate (1)
8.	Leaf margine colour	Red (1);	Green (0)
9.	Flower colour 1	White (1);	Green (0)
10.	Flower colour 2	Pink (1);	Non - Pink (0)

*Figures in parenthesis depict the score for the character

Table 3.3: Quantitative morphological characters analyzed for determination of variations between the different accessions/cultivars of buckwheat (*Fagopyrum* spp.) studied in the present investigation.

Sl. No.	Trait	Units of measurement
1.	Plant Height	Centimeter
2.	Number of Branches	Number
3.	Number Internode length	Number
4.	Leaf Area	Centimeter^2
5.	Days to Flower	Number
6.	Days to maturity	Number
7.	Total no of seeds/ plant	Number

Extraction of soluble proteins from grains and SDS PAGE profiling:

Mature and healthy grains from each group, representing accession/cultivars, were used for extraction of soluble proteins. The hull of each of the grains was removed with forceps to take out the groat fraction which was used directly for extraction of total grain proteins. For analyzing the SDS PAGE profile of endosperm proteins the dehulled grains were dissected with the help of a pointed forceps to remove the embryo so as obtain the endosperm portion for extraction of proteins. The groat fraction as well as the endosperm tissue was defatted by washing with cold acetone for 4-6 hours. Acetone was removed by filtration and the defatted meal dried under a continuous stream of dry air to remove the acetone completely. The dried defatted meal was powdered in liquid nitrogen and homogenized in a pre-chilled mortar and pestle in 50mM Tris-Cl buffer (pH 6.8) containing 100mM NaCl, 10Mm EDTA, 100mM glycine, 10% SDS and 1mM PMSF. The homogenate was allowed to stand for 45 min at 4^oC in a cold room .The homogenate was subsequently centrifuged at 10,000 rpm for 15 min at 4^oC. The extracted proteins were recovered as clear supernatant. Protein concentration in each sample was determined according to Bradford (1976). 5μl protein sample from each accession/cultivars was taken in separate eppendorf tubes and the volume was made to 100μl with distilled water. To the above sample, 1ml Bradford reagent was put and mixed thoroughly by gentle vortexing. Prior to taking the reading of protein sample a blank reading was taken with only Bradford reagent taken in cuvet. After taking blank reading, the prepared sample was transferred to cuvet and spectrophotometer (Beckman DU®530) reading was taken at A595 for all the accessions individually. Using the standard curve the equivalent protein concentration for the protein sample was recorded.

SDS PAGE of the extracted protein pool was carried out on 12% polyacrylamide gel following the method of Laemmli (1970). Suitable aliquot of the extracts, representing 50 µg protein from each sample, was mixed with 2X Laemmli buffer in the ratio of 1:1. The mixture was heated for 5 minutes in a boiling water bath followed by a brief centrifugation to sediment the debris. The supernatant was loaded into the wells of a 1.5 mm thick 12% acrylamide gel. An aliquot of a mixture of standard molecular weight markers (Bangalore Genei/ Roche) also was denatured similarly and loaded a lane of the same gel to serve as reference for determination of molecular mass of the resolved bands. Electrophoresis was carried out at a constant voltage of 100 V for 6 hours. After electrophoresis the gel was removed and washed briefly with distilled water. Proteins in the gel were fixed by immersing the gel for 30min in in a solution containing methanol: glacial acetic acid: water (4:1:5). The gel was subsequently stained for 3 hours in 0.25% (w/v) Coomassie Brilliant blue R-250 prepared in methanol: glacial acetic acid: water (4:1:5). Destaining was carried out in a destaining solution I composed of methanol: water: glacial acetic acid (4:5:1). The gels were further destained in destaining solution II composed of methanol: water: glacial acetic acid (4:5.3:0.7) and stored in the same solution till required. Protein bands were visualized on KODAK gel documentation system under white light. Electrophoretic mobility (R_f) of the bands was calculated as the ratio of the distance traveled by the band to the distance traveled by the dye front. For deriving a relationship between the molecular mass of the protein and its mobility on the polyacrylamide gel, the R_f value of the standard molecular weight marker was plotted against log molecular weight of the protein to obtain a standard curve. The molecular mass of the resolved proteins was determined by comparing their electrophoretic mobility with that of the standard molecular weight markers and by reference to the

standard curve drawn for the R_f value of the marker protein against log molecular weight. Consistency of result was ensured by studying a minimum of three seed samples from each groups of the accession and only the consistent bands were taken into account.

Isozyme analysis:

For determination of isozyme profiles, 10-15 seeds from each accession/cultivars were germinated on germination paper in growth chamber under dark condition at 25°C. Cotyledons from 7 day old seedling or tissue extracts of young leaves (2 weeks old plant) were prepared in 0.1M Tris-Cl buffer (pH 8.0) containing 0.1% 2-Mercaptoetanol, 0.001M EDTA, 0.01M KCl, 0.01M $MgCl_2$, and 4% polyvinyl pyrrolidone (PVP).

The enzymes for which the isozyme profiles were determined included Malate dehydrogenase (MDH, E.C. no. 1.1.1.37), Phosphoglucomutase (PGM, E.C. no. 5.4.2.2), Peroxidase (PER, E.C. no. 1.11.1.7) and Esterase (EST, E.C. no. 3.1.1.1). Electrophoresis of the tissue extracts for determination of isozyme profiles was carried out on 12% polyacrylamide gels under native conditions in a cold room at a constant voltage of 80 volts according to Davis (1964). The gels were separately stained for detection of isozyme profiles of each enzyme. Each set of experiments was carried out twice to confirm the reproducibility of bands.

Staining procedures:

Malate dehydrogenase: Staining of gels for detection of isozymes of malate dehydrogenase was carried out as per the method described by Nichols and Ruddle (1973). The gel was incubated for 20-25 minutes in dark at 37°C in staining solution composed 0.1M Tris buffer (pH 7.5) containing 0.01M DL- Malate (pH 7.5) , 0.02 % NAD^+, 0.02% NBT and 0.005% PMS. After staining, the isozymes of malate dehydrogenase appeared as violet bands against a light brown background.

Peroxidase: Staining of gels for detection of peroxidase isozymes was carried out as per the method described by Shaw and Prasad (1970). The gel was incubated for 10-15 minutes in dark at 37°C in staining solution composed 0.1M acetate buffer (pH 5.6) containing 0.05% 3-amino, 9-ethyl carbazol N, N^+ dimethyl fomamide, 4mM $CaCl_2$ and 0.06% H_2O_2. Peroxidase isozymes appeared as dark blue bands against a light background.

Phosphoglucomutase: Staining of gel for detection of phosphoglucomutase isozyme activity in the gels was carried out according to the method described by Murphy *et al* (1990). The gel was incubated in dark at 37°C in a staining solution consisting of 0.1M Tris-Cl buffer (pH 8.0), 0.1M $MgCl_2$, 0.05 α-D-G-1-P disodium salt, 1 unit glucose-6-phosphate dehydrogenase, 0.008 % β-NADP, 0.008 % MTT and 0.004% PMS till distinct bands representing PGM activity appeared on the gels.

Esterase: Staining of gel for detection of esterase isozyme activity was carried out as per the method described by Scandalios (1974). The gel was incubated for 30 minutes in dark at room temperature in 0.1M phosphate buffer (pH. 6.0) containing 0.1% Fast blue RR salt and 0.02% α-naphthyl acetate prepared in 50 % acetone. Isozymes of esterase appeared as dark brown bands against a light pink background.

Post staining treatment and observations: The gels were fixed in 7% acetic acid after the staining and visualized under a transilluminator under visible light. The R_m value of each band was calculated on the basis of ratio of the distance traveled by the band to the distance traveled by the dye front. For each individual enzyme, the bands were sequentially designated as 'a', 'b', 'c' ..... 'n' for purposes of identification. The data matrix for the isozyme profile of each enzyme for different accessions was prepared as presence (1) or absence (0) of the band.

Isolation of Genomic DNA:

Total genomic DNA was isolated from 7 day old etiolate seedling by a modified CATB extraction protocol (Murray and Thompson 1980). Healthy grains from different accessions/cultivars of common buckwheat were surface sterilized by immersion in 0.01% $HgCl_2$ solution for 5 minutes followed by thorough rinsing with sterile distilled water. The grains were allowed to germinate on clean germination paper in an incubator at 27°C in dark. Etiolated seedlings were harvested 7 days after germination and used for isolation of genomic DNA. Approximately 1gm of leaf tissue from the etiolated seedlings was washed with cold distilled water and then crushed to fine power in a prechilled pestle and mortar under liquid nitrogen. Approximately 500 mg of the powdered tissue was lysed with 500µl of 2X CTAB buffer in an eppendorf tube followed by incubation at 65°C for 45 min. The incubation was followed by addition of chloroform: isoamyl alcohol mixture (24:1) in the ratio of 1:1. The mixture was mixed by gentle inversion. The solution was centrifuged at 13000 rpm for 5 min in a cooling centrifuge (Biofuge) at room temperature to separate the aqueous phase from the organic phase. After centrifugation, the aqueous phase was transferred to a fresh tube for further processing. DNA was precipitated from the aqueous phase by addition of 2/3 volumes of isopropanol. Complete precipitation of DNA was allowed by overnight incubation of the solution at -20°C. The precipitated DNA was pelletted by centrifugation for 15 minutes at 13000 rpm at room temperature in a microfuge. The pelletted DNA was washed thrice with 70% ethanol, vacuum dried and dissolved in 200 µl of water. The DNA was incubated with RNase A (20µg ml^{-1}) for 30 minute at 37°C to degrade the RNA present in the solution. The incubation was followed by addition of phenol: chroform: isoamyl alcohol (25:24:1) in the ratio of 1:1. The solution was mixed by gentle inversion and

centrifuged for 15 minutes at 13000 rpm at room temperature in a microfuge to separate the aquous and organic phases. The aqueous phase was transferred to a fresh tube and extracted once with equal volume of chloroform: isoamyl alcohol (24:1). DNA was precipitated from the aqueous phase by addition of 0.1 volume of 3M sodium acetate (pH 5.2) and 2 volumes of absolute alcohol. Complete precipitation was allowed by keeping the solution overnight at -20°C. The precipitated DNA was pelletted by centrifugation for 10 minutes at 13000 rpm at 4°C. The DNA pellet was washed twice with 70% alcohol, air dried and dissolved in nuclease free ultra pure water.

The isolated DNA was electrophoressed on 0.8% agarose gel at 50V for 3 hours in 1X TBE buffer (pH 7.5). After the electrophoresis was over, the gel was stained with ethidium bromide (0.5 μg ml^{-1}) for 20 minutes followed by destaining in water till the background fluorescence disappeared. DNA was visualized under UV light in a KODAK Gel Logic200 Imaging System.

Quantification of DNA: DNA was directly quantified by the Image analysis software of the Gel documentation system by comparison of the fluorescence of the ethidium bromide stained DNA bands with florescence of known amount of λDNA electrophoressed along with isolated DNA sample (Sambrook *et al.*, 1989). Quantification of isolated DNA sample also was carried out spectrophotometrically by measuring absorbance of the sample at 260 nm in a UV-Vis spectrophotometer with $A_{260}=1$ representing 50 μg/ml of the double stranded DNA. The purity of DNA preparation was checked by measuring the absorbance of the sample at 260 and 280 nm in a Beckman DU®530 spectrophotometer and determination of A_{260} / A_{280} ratio of the sample. The sample was considered as sufficiently pure if A_{260} / A_{280} was >1.8.

Table 3.4: Nucleotide sequences of random decamer oligonucleotide primers used for amplification of genomic DNA from accessions/cultivars of *Fagopyrum* spp.

Primer name/ number	Sequences	% GC content
1 OPD-6	5'ACCTGAAGC G3'	60
2 OPC-13	5'AAGCCTCGT C3'	60
3 OPD-14	5'AGCATGGCT C3'	60
4 OPO-16	5'TCGGCGGTT C3'	70
5 UBC-185	5'GTGTCTTCA C3'	50

Table 3.5: Primer pair used for amplification of the intergenic spacer between *trn*C and *rpo*B spacer.

Region	Primer sequences	Reference
Intergenic spacer region between *trn*C- *rpo*B	5'TGCCTTACCACTCGGCCAT3' 5'GTAGATATTCCCTCATTTCC3'	Oshako & Ohnishi (2000)

Random Amplification of Polymorphic DNA analysis:

RAPD profiles of the total genomic DNA isolated from different accessions/cultivars were determined by amplifying polymorphic DNA from the collected accessions with single decamer primers according to Williams *et al.*, (1990). List of decamer primers used in the study is listed in table 3.4. A typical 25µl reaction volume of the amplification mixture contained 15.2µl of sterile water, 200µM (2.5µl) dNTP mix., 1.5 mM (1.5µl) of $MgCl_2$, 2.5µl of 10X reaction buffer, 100 pmol (1.0µl) of decamer primer, 1 unit (0.3µl) of *Taq* DNA polymerase and 100ng (2.0µl) of genomic DNA as template. The reaction was performed in a thermal cycler (Techne, UK) programmed to one cycle of "hot start" (94°C, 5min); 35 cycles of denaturation (94°C, 1.0 min), annealing (37°C, 1min.), and polymerization/ primer extension (72°C, 1min.) and one cycle of chain elongation (72°C, 10min). Each set of amplification reaction mixtures had a positive (reaction mixture without the primer) and negative (reaction mixture without the template) control.

After the amplification cycles were complete, the mixture was electrophoressed on 1.2% agarose gel at 50V for 5 hours in 1X TBE buffer (pH 7.5). After the electrophoresis was over, the gel was stained with ethidium bromide (0.5 µg ml^{-1}) for 20 minutes followed by destaining in water till the background fluorescence disappeared. The amplified DNA was detected as fluorescent bands under UV light on a KODAK Gel Logic200 gel documentation system. The molecular mass of the resolved bands was determined by comparing their electrophoretic mobility with that of the standard molecular weight markers electrophoressed on the same gel.

Amplification was carried out under varying concentrations of DNA and $MgCl_2$, six initial reactions were set up with DNA concentration of 50 and 100 ng 25μl^{-1} of reaction volume and three different concentrations viz. 1.5mM, 2.0mM and 2.5mM of

$MgCl_2$. Each set of reactions was carried out thrice to check the reproducibility of the results. The bands were sequentially designated as 'a', 'b', 'c' ..... 'n' for purposes of identification, ignoring smeared and faint bands. The data matrix for the RAPD profile generated by each primer for different accessions was prepared as presence (1) or absence (0) of the band.

Amplification of intergenic spacer region between *trn*C and *rpo*B:

A typical 25µl reaction volume for amplification of the *trn*C and *rpo*B intergenic spacer region contained 14.2µl of sterile distilled water, 2.5µl of 10 mM dNTP mix, 25 mM (1.5µl) of $MgCl_2$, 1.0µl each (200 pmols) of the forward and reverse primers, 2.5µl of 10X reaction buffer, 0.3µl of *Taq* DNA polymerase (5units/µl) and 100 ng (2.0µl) of genomic DNA template. Primer pair used for amplification of the intergenic spacer between *trn*C and *rpo*B spacer is listed in table 3.5.

The reaction was performed in a thermal cycler (Techne, UK) programmed to one cycle of "hot start" (94°C, 5min); 35 cycles of denaturation (94°C, 1.0 min), annealing (57°C, 1 min), and polymerization/ primer extension (72°C, 1 min) and one cycle of chain elongation (72°C, 10min). Each set of amplification reaction mixtures had a positive (reaction mixture without the primer) and negative (reaction mixture without the template) control.

After the amplification, the mixtures were electrophoressed on 1.0 % agarose gel at 50V for 5 hours in 1X TBE buffer (pH 7.5). After the electrophoresis was over, the gel was stained with ethidium bromide (0.5 $\mu g\ ml^{-1}$) for 20 minutes followed by destaining in water till the background fluorescence disappeared. The amplified DNA was detected as fluorescent bands under UV light on a KODAK Gel Logic200 gel documentation system. The molecular mass of the resolved bands was determined by comparing their

electrophoretic mobility with that of the standard molecular weight markers electrophoreses on the same gel.

The amplified bands were eluted from the gel as per the protocol given by Byrnes *et al.*, (1995). The agarose slice containing the band of interest was chopped into tiny slices which were then transferred to a 1.5ml micro-centrifuge tube. The agarose slices were immersed in cold buffer saturated phenol and the tubes left overnight at 4°C. The tubes were subsequently incubated at 68°C for 20-30 minutes or till the agarose melted completely and then centrifuged at 12,000 rpm for 10 minutes in a microfuge at room temperature. The aqueous phase was transferred to a fresh microcentrifuge tube followed by addition of equal volume of chloroform: isoamyl alcohol (24:1). The solution was centrifuged at 12000 rpm for 10 minutes to separate the aqueous and organic phases. The aqueous phase was then transferred to a fresh tube. DNA precipitated from the aqueous phase with 0.5 volumes of 7.5mM ammonium acetate and two volumes of ice cold ethanol followed by incubation at -20°C overnight. The precipitated DNA was pelletted by centrifugation at 12000 rpm for 5 minutes, washed twice with cold 70% ethanol and dried under vacuum. After drying the pellet was dissolved in a suitable volume of ultra pure nuclease free water. The amplified DNA samples were digested with *Eco*R1 for 4 hours and then electrophoressed on 1.2% agarose gel at 50V for 5 hours in 1X TBE buffer (pH 7.5). The gel was subsequently stained with ethidium bromide and the banding pattern of digested DNA checked under a UV transilluminator.

Data analysis:

Mean, Standard deviation and standard error of mean have been calculated for the quantitative data on morphological characters using standard procedures. The data on qualitative morphological characters, SDS PAGE protein profiles, isozyme profiles and

RAPD was scored for the presence or absence of morphological character or protein/ isozyme/ DNA bands and converted into a data matrix indicating presence (1) or absence (0) of the character/ protein/ isozyme/ RAPD. Evaluation of variation in the total seed storage protein, endosperm protein, RAPD and isozyme profiles were performed by calculating the individual band frequency for each accession/cultivar. The binary data was used for determining polymorphism by dividing the number of polymorphic bands by the total number of bands. Polymorphism information content (PIC) was determined as per Botstein (1980) with the formula PIC= 2 Pi (1-Pi)

Where, Pi designates frequency of occurrence of polymorphic bands in different primers. The genetic relationships among the accessions were computed using Jaccard's coefficient of similarity (Jaccard, 1908) according to the formula:

$J = (N_{ab}) / (N_a + N_b - N_{ab})$

where

N_{ab}=Number of bands common in both accessions

N_a= Total number of bands present in accession 1

N_b= Total number of bands present in accession 2

Cluster analysis: Cluster analysis of the similarity matrices has been carried out by the UPGMA method to show the phenetic representation of genetic relationship among the accessions/cultivars. All computations were performed with NTSYS-PC Version 2.1 (Rohlf, 1993). Principal coordinate analysis (PCA) of the similarity matrix data has been performed following the method described by Sneath and Sokal (1973) using the NTSYS-pc Version 2.1 statistical Software package (Rohlf, 1993).

CHAPTER IV

RESULTS

EXPERIMENTAL:

Accessions of common buckwheat used in the present study were procured from the National Bureau of Plant Genetic Resources, New Delhi, Vivekananda Laboratory of Hill Agriculture (Indian Council of Agricultural Research), Almora, East Khasi Hills in Meghalaya (India) and the Tawang District of Arunachal Pradesh (India). The accessions/cultivars used for the present study are listed in Table 3.1. These accessions/cultivars belong to four different species of the genus *Fagopyrum* namely *Fagopyrum esculentum* (Moench), *Fagopyrum tataricum* (Gaertn.), "*Fagopyrum himalianum*" and *Fagopyrum cymosum* (Meissn). The collection included 12 accessions and 4 cultivars of *F. esculentum* and 5 accessions and 10 cultivars of *F. tataricum*. IC-13145, which has been identified as *F. himalianum* by NBPGR, was considered as one of the accession of this species. *F. cymosum* was represented by plants collected from wild with no specified accessions or cultivars within it. The accessions/cultivars were grown in the experimental garden to study the qualitative as well as quantitative features

during their growth span. The grains were sown in two different seasons i.e April as a summer crop and August as a winter crop. The grains were collected at harvest and used for studying the SDS PAGE profiles of total as well as endosperm proteins. Grains from each of the accessions/cultivars were germinated under sterile conditions in a seed germinator. Genomic DNA was isolated from 7 day old etiolated seedlings and used for RAPD analysis and for amplification of *trn*C and *rpo*B spacer region.

RESULTS:

Morphology:

The variations among the accessions/cultivars of different species of *Fagopyrum* were assessed using 17 diagnostic morphological traits; including 10 qualitative and 7 quantitative characters. The qualitative traits assessed were Grain type (Winged/Non winged), Grain shape (Conical/Non conical), Grain coat colour (Brownish/Greyish black), Striations on grain coat (Present/Absent), Leaf margin colour (Red/Green), Colour of the stem (Green/Red), Blade shape (Cordate/Non cordate), Blade shape (Hastate/Non hastate), Flower colour (White/Yellowish Green), Flower colour (Pink/Non-pink). The parameters assessed and the score assigned to each qualitative parameter are listed. The traits were scored as "1" for presence and "0" for absence (Table 4.1). The variations in grain morphology were observed within and across the accessions/cultivars of different species. Similarity matrix value for the scored parameters was calculated on the basis of the 1 or 0 values for different characters (Table 4.2) and a dendrogram was developed using NTSYS-PC version-2.1 (Rohlf, 1993). The quantitative traits studied includes Plant Height, Number of Branches on main stem, 2nd Internode length, Total Leaf surface area, Number of days to flower, Total number of grains per plant and Number of days to maturity.

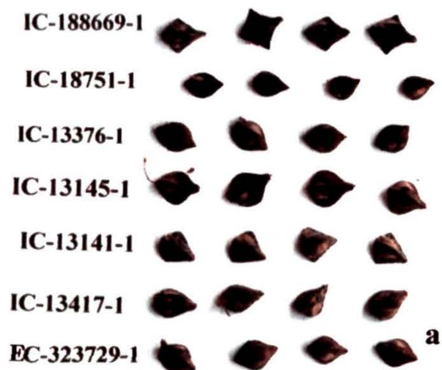
Table 4.1: Variations in grain shape, grain coat colour, presence or absence of striations on the coat of grains, stem colour, shape of leaf blade, colour of leaf margin and flower colour in different accessions/ cultivars of buckwheat of buckwheat (*Fagopyrum* spp.) studied in the present investigation.

Accessions	Grain shape (W/NW)	Grain coat colour (B/DB)	Striations (Y/N)	Colour of stem (R/G)	Blade shape (C/S)	Blade Shape (H/NH)	Flower colour (W/NW)	Flower colour (NP/P)	Grain shape (NC/C)	Leaf margin colour (R/G)
IC-188669-1	1	0	1	1	1	0	1	0	0	1
IC-188669-2	0	0	0	0	1	0	1	0	0	1
IC-188669-3	0	0	0	0	1	0	1	0	0	1
IC-188669-4	0	1	1	0	0	0	1	0	0	1
IC-188669-5	1	0	0	0	0	0	1	0	0	1
IC-188669-6	0	0	0	1	0	0	1	0	0	1
IC-188669-7	0	1	1	1	1	0	1	0	0	1
IC-18751-1	0	0	0	1	1	0	1	0	0	1
IC-18751-2	0	0	0	1	1	0	1	0	0	1
IC-18751-3	1	0	1	1	1	0	1	0	0	1
IC-18751-4	0	0	0	1	1	0	1	0	0	1
IC-18751-5	0	0	1	1	1	0	1	0	0	1
IC-13376-1	0	0	0	1	0	0	1	0	0	1
IC-13376-2	1	1	0	1	1	0	1	0	0	1
IC-13376-3	0	1	1	0	1	0	1	0	0	1
IC-13376-4	1	1	1	0	1	0	1	0	0	1
IC-13376-5	0	1	0	1	0	0	1	0	0	1
IC-13145-1	0	1	1	1	1	0	1	0	0	1
IC-13145-2	1	0	1	0	1	0	1	0	0	1
IC-13145-3	0	1	0	1	1	0	1	0	0	1
IC-13145-4	0	0	0	1	0	0	1	0	0	1
IC-13145-5	0	0	0	1	1	0	1	0	0	1
IC-13141-1	1	1	1	0	1	0	1	0	0	1
IC-13141-2	1	1	1	1	1	0	1	0	0	1
IC-13141-3	1	0	1	0	1	0	1	0	0	1
IC-13141-4	0	0	0	0	1	0	1	0	0	1
IC-13141-5	0	1	0	0	1	0	1	0	0	1
IC-13141-6	0	0	0	0	1	0	1	0	0	1
IC-13417-1	0	0	1	1	1	0	1	0	0	1
IC-13417-2	0	1	1	1	1	0	1	0	0	1
IC-13417-3	1	1	1	0	1	0	1	0	0	1
IC-13417-4	1	0	1	0	1	0	1	0	0	1
IC-13417-5	1	0	0	0	1	0	1	0	0	1
EC-323729-1	0	0	0	1	1	0	1	0	0	1
EC-323729-2	0	0	1	0	1	0	1	0	0	1
EC-323729-3	0	0	0	1	1	0	1	0	0	1
EC-323729-4	0	1	0	1	1	0	1	0	0	1
EC-323729-5	0	1	0	0	1	0	1	0	0	1
Local	0	0	0	0	1	0	0	1	0	1
Kamroo local	0	0	1	0	1	0	0	1	0	1
OC-2	0	0	1	0	1	0	0	1	0	1
VL-7	1	0	1	1	1	0	1	0	0	1
IC-319595	0	1	1	0	1	0	0	0	0	1
IC-319588	0	0	1	0	1	0	0	0	0	1
IC-324244	0	1	0	0	1	0	0	0	0	1
IC-324313	0	1	0	0	1	0	0	0	0	1
IC-352992	0	1	0	0	1	0	0	0	0	1
KBB-3	0	1	0	1	0	1	0	0	1	0
Himpriya	0	1	0	1	0	1	0	0	1	0
Kuppa Local	0	1	0	1	0	1	0	0	0	0
Shimla B-1	0	1	0	0	0	1	0	0	0	0
Sangla B-1	0	1	0	0	0	1	0	0	1	0
Sangla B-2	0	1	0	0	0	1	0	0	1	0
Sangla B-3	0	1	0	0	0	1	0	0	1	0
Sangla B-4	0	1	0	0	0	1	0	0	1	0
Sangla B-5	0	1	0	0	0	1	0	0	1	0
Sangla B-6	0	1	0	0	0	1	0	0	1	0
IC-412744	0	1	0	0	0	1	0	0	1	0
IC-421598	0	1	0	0	0	1	0	0	1	0
IC-412722	0	1	0	0	0	1	0	0	1	0
IC-412863	0	1	0	0	0	1	0	0	1	0
IC-421597	0	1	0	0	0	1	0	0	1	0
F. cymosum	0	1	0	0	0	0	1	0	0	1

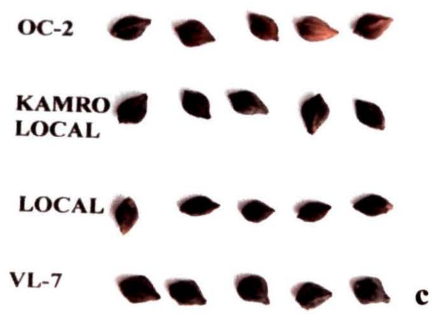
The grains of different accessions/cultivars of buckwheat showed a wide variation in shape (Fig. 4.1). While the grains of different accessions/cultivars of *F. esculentum* and *F. cymosum* were triangular in shape, those of *F. tataricum* were either conical or globous in shape. All the accessions/cultivars of *F. tataricum*, except Shimla B-1 and Kuppa Local, showed conical type of grain shape. The grains of Shimla B-1 and Kuppa Local were globous. One of the prominent features of grains of Shimla B-1 was the presence of deep furrows with prominent three lobes. The furrows were visible in Kuppa Local also but were not as deep as in Shimla B-1. The grains belonging to *F. cymosum* were triangular in shape. All the accessions/cultivars of *F. esculentum* had a smooth grain coat. Differences were, however, observed in the grain coat striations within different accessions of *F. esculentum* (Fig. 4.2a). Intra accession variation for presence or absence of striation was observed in the collections of *esculentum* from Himachal Pradesh. While there was no prominent striations on the grain coat of accessions IC-324313, IC-324244, IC-352992 and cultivar Local. The grain coat of Kamroo Local, OC-2 and VL-7 had prominent striations, which extended longitudinally across the surface of the coat. All the accessions/cultivars of *tataricum* and *cymosum* showed rough grain coat without any striations. The accessions/cultivars of *Fagopyrum* showed broadly two types of grains i.e. winged and non winged (Fig.4.2a). All winged type of grains were triangular in shape, each having a pair of winged type protrusion. The non-winged grains were triangular, conical or globous type. The triangular grains were either elongated or oblong in shape.

Variations were also observed in shape of the leaf blade, leaf margin colour and grain colour. While *F. esculentum* and "*F. himalianum*" showed both cordate and sagittate leaf blade morphology, *F. cymosum* had exclusively sagittate blades. The leaf

Fig 4.1: Variations in grain morphology in different accessions of buckwheat (*Fagopyrum* spp.) **a-c:** accessions/cultivars belonging to of *F. esculentum* and *F. himalianum*, **d-e:** accessions/cultivars belonging to of *F. tataricum* and **f:** accession of *F. cymosum.* EC-323729 is an exotic collection from Japan. All other accessions/cultivars are collected from Indian Himalayas.


IC-188669-1
IC-18751-1
IC-13376-1
IC-13145-1
IC-13141-1
IC-13417-1
EC-323729-1
a

IC-319588
IC-319595
IC-324313
IC-324244
IC-352992
b


OC-2
KAMRO LOCAL
LOCAL
VL-7
c

KBB-3
HIMPRIYA
KUPPA LOCAL
SHIMLA B-1
SANGLA-B-1
d

IC-412744
IC-421598
IC-412722
IC-412863
IC-421597
e

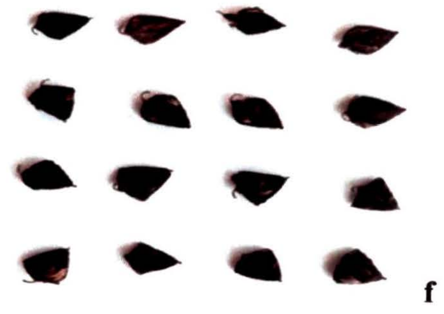
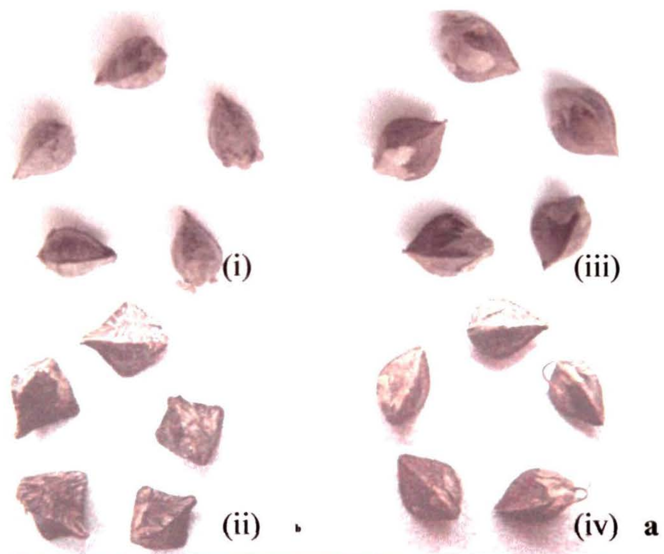
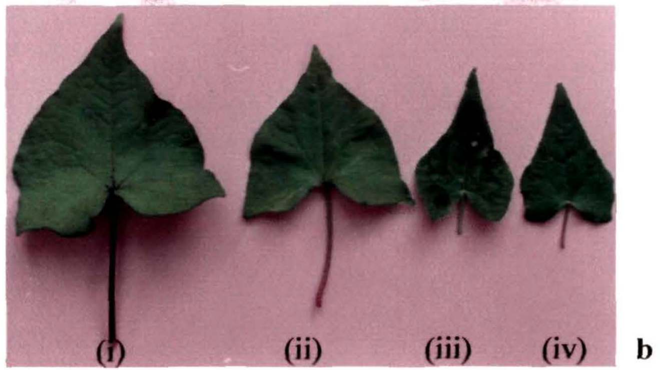

f

Fig 4.2 **a:** Variations in grain morphology in different accessions/cultivars of buckwheat (*Fagopyrum* spp.) i: grains without wings showing striations on the surface, ii: grains with wings and striations on the surface, iii & iv: grains without wings and any striations on the surface.

b: Variations in the shape of leaf blade in different species of buckwheat (*Fagopyrum* spp.) i. leaf of *F. cymosum* showing sagittate leaf blade, ii. leaf of *F. tataricum* showing hastate leaf blade, iii. leaf of *F. esculentum* showing cordate leaf blade and iv. leaf of *F. himalianum* showing cordate leaf blade.


(i)
(iii)
(ii)
(iv)
a


(i)
(ii)
(iii)
(iv)
b

blade shape in *F. tataricum* was hastate (Fig. 4.2b). All the accessions/cultivars of *F. esculentum, "F. himalianum" and F. cymosum* had leaves with red margin. The leaf margin of plants of *F. tataricum* was, however, green in colour. All the accessions/ cultivars of *F. esculentum and "F. himalianum"* showed either brownish or dark brown seed coat colour, while seeds of *F. tataricum* and *F. cymosum* were dark brown in colour.

While *F. esculentum, F. cymosum* and "*F. himalianum*" had white flowers, *F. tataricum* produced yellowish green flowers. Some cultivars of *F. esculentum* even produced pink flowers (Fig. 4.3). While the accessions IC-188669, IC-18751, IC-13376, IC-13141, IC-13417 and IC-323729 had white flowers and produced both winged as well as non-winged grains, the accession IC-319588, IC-319595, IC-324313, IC-324244, and IC-352992 had only white flowers which produced non-winged grains only. The grains of cultivars Local, Kamroo Local, OC-2 and VL-7 were exclusively non winged. Cultivars Local, Kamroo Local, OC-2 had pink flowers while those of VL-7 had white flowers. On the other hand accessions/cultivars belonging to *F. tataricum* showed yellowish green coloured flowers and produced only non-winged grains.

The pattern of variation observed for morphological traits among genotypes displayed statistical significant difference for quantitative traits. Considerable intraspecific diversity was observed between *F. esculentum* accessions/cultivars for grain shape, grain colour, striations, flower colour, shape of leaf blade and colour of the stem. Amongst the accessions/cultivars of *tataricum* intraspecific variation was observed only for grain shape and colour of the stem. *F. cymosum,* however, showed no intraspecific variation for any of the qualitative morphological characters studied in the present investigation. The similarity matrix based on ten morphological traits among

Fig 4.3: Photographs showing the variation in colour of sepals in flowers of buckwheat (*Fagopyrum* spp.) **a & b:** flowers of *Fagopyrum esculentum*, **c:** flowers of *F. himalianum*, **d:** flowers of *F. cymosum*, **e:** flowers of *F. tataricum* and **f:** a close up view of flower of *F. esculentum*.

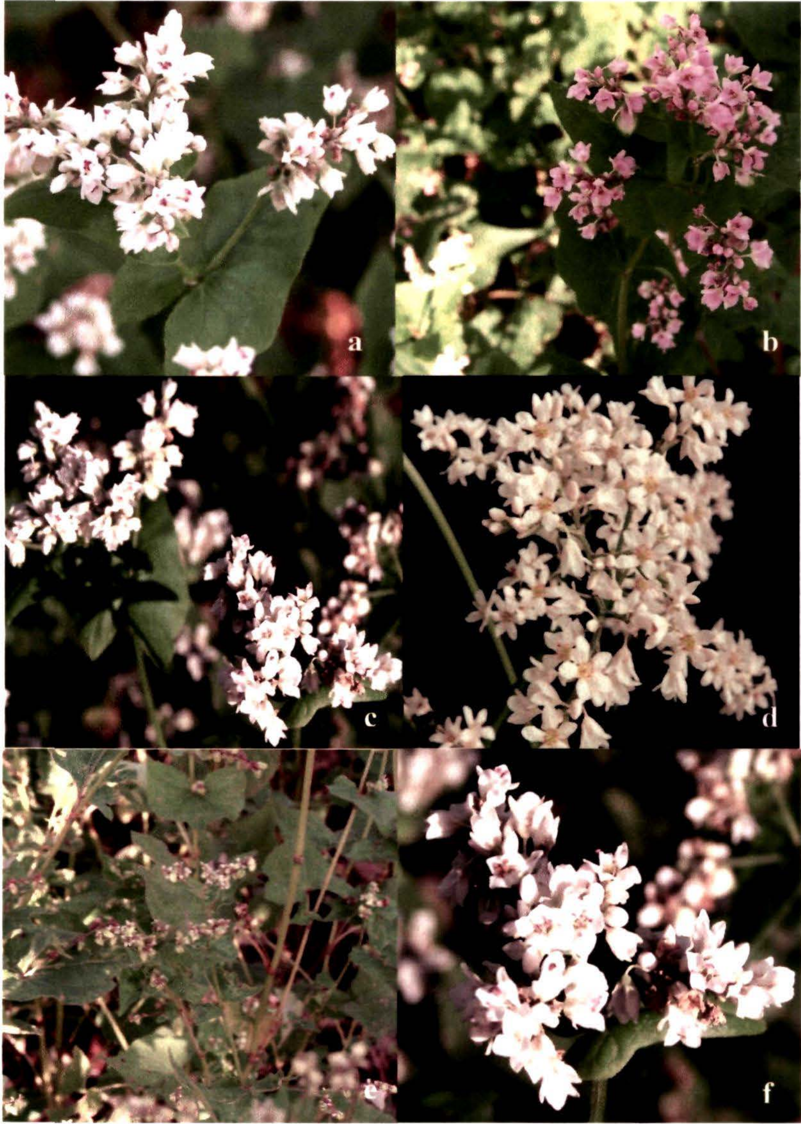

a
b
c
d
e
f

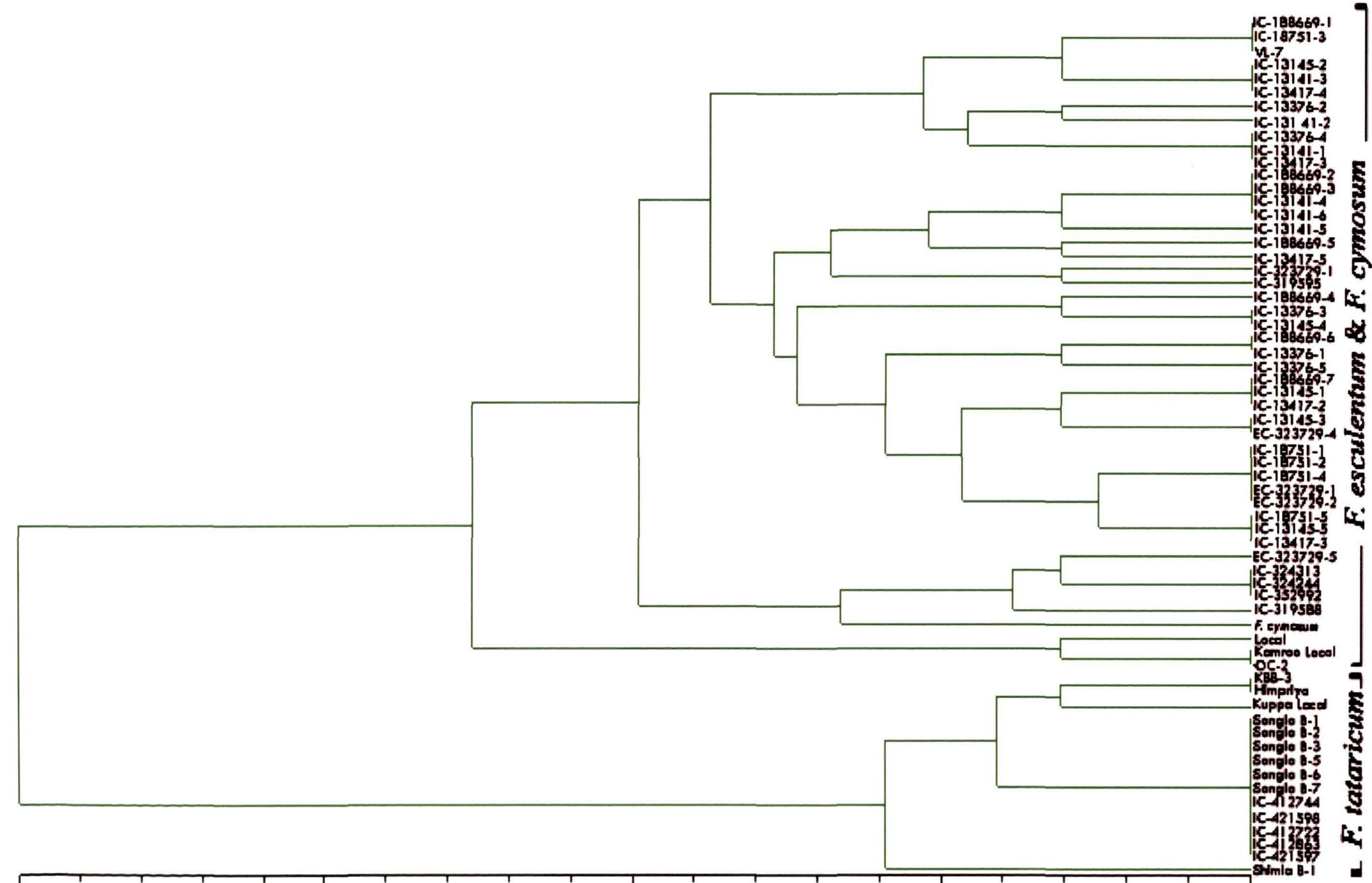
Table 4.2: Similarity matrix, based on Jaccard's similarity coefficient, of the qualitative morphological characters analyzed for determination of variation between different accessions of buckwheat (*Fagopyrum* spp.) studied in the present investigation.

Accessions	IC-188669-1	IC-188669-2	IC-188669-3	IC-188669-4	IC-188669-5	IC-188669-6	IC-188669-7	IC-18751-1	IC-18751-2	IC-18751-3	IC-18751-4	IC-18751-5	IC-13376-1	IC-13376-2	IC-13376-3	IC-13376-4	IC-13376-5	IC-13145-1	IC-13145-2	IC-13145-3	IC-13145-4	IC-13145-5	IC-13141-1	IC-13141-2	IC-13141-3	IC-13141-4	IC-13141-5	IC-13141-6	IC-13417-1	IC-13417-2	IC-13417-3	IC-13417-4	IC-13417-5	EC-323729-1	EC-323729-2	EC-323729-3	EC-323729-4	EC-323729-5	Local	Kamroo local	OC-2	VL-7	IC-319588	IC-324313	IC-352992	IC-319595	IC-324244	KBB-3	Himpriya	Kuppa local	Shimla B-1	Sangla B-1	Sangla B-2	Sangla B-3	Sangla B-5	Sangla B-6	Sangla B-7	IC-412744	IC-412863	IC-421598	IC-412722	IC-421597	F. cymosum
IC-188669-1	1.00
IC-188669-2	0.70	1.00
IC-188669-3	0.70	1.00	1.00
IC-188669-4	0.60	0.70	0.70	1.00
IC-188669-5	0.70	0.80	0.80	0.70	1.00
IC-188669-6	0.80	0.80	0.80	0.70	0.80	1.00
IC-188669-7	0.80	0.70	0.70	0.80	0.50	0.70	1.00
IC-18751-1	0.80	0.90	0.90	0.60	0.70	0.90	0.80	1.00
IC-18751-2	0.80	0.90	0.90	0.60	0.70	0.90	0.80	1.00	1.00
IC-18751-3	1.00	0.70	0.70	0.60	0.70	0.70	0.80	0.80	0.80	1.00
IC-18751-4	0.80	0.90	0.90	0.60	0.70	0.90	0.80	1.00	1.00	0.80	1.00
IC-18751-5	0.90	0.80	0.80	0.70	0.60	0.80	0.90	0.90	0.90	0.90	0.90	1.00
IC-13376-1	0.70	0.80	0.80	0.70	0.80	1.00	0.70	0.90	0.90	0.70	0.90	0.80	1.00
IC-13376-2	0.80	0.70	0.70	0.60	0.70	0.70	0.80	0.80	0.80	0.80	0.80	0.70	0.70	1.00
IC-13376-3	0.70	0.80	0.80	0.90	0.60	0.80	0.90	0.70	0.70	0.70	0.70	0.80	0.80	0.70	1.00
IC-13376-4	0.80	0.70	0.70	0.80	0.70	0.50	0.80	0.60	0.60	0.80	0.60	0.80	0.50	0.80	0.70	1.00
IC-13376-5	0.60	0.70	0.70	0.80	0.70	0.90	0.80	0.80	0.80	0.60	0.80	0.70	0.90	0.80	0.90	0.60	1.00
IC-13145-1	0.80	0.70	0.70	0.80	0.50	0.70	1.00	0.80	0.80	0.80	0.80	0.90	0.70	0.70	0.90	0.80	0.80	1.00
IC-13145-2	0.90	0.80	0.80	0.70	0.80	0.60	0.70	0.60	0.60	0.90	0.60	0.80	0.60	0.80	0.60	0.90	0.50	0.70	1.00
IC-13145-3	0.70	0.80	0.80	0.70	0.60	0.80	0.90	0.90	0.90	0.70	0.90	0.80	0.80	0.70	0.80	0.70	0.90	0.90	0.60	1.00
IC-13145-4	0.70	0.80	0.80	0.90	0.60	0.80	0.90	0.70	0.70	0.70	0.70	0.80	0.80	0.70	1.00	0.90	0.90	0.90	0.60	0.80	1.00
IC-13145-5	0.70	0.80	0.80	0.70	0.60	0.80	0.90	0.90	0.90	0.70	0.90	1.00	0.80	0.80	0.80	0.80	0.70	0.90	0.80	0.80	0.80	1.00
IC-13141-1	0.80	0.70	0.70	0.80	0.70	0.50	0.80	0.60	0.60	0.80	0.60	0.80	0.50	0.80	0.70	1.00	0.60	0.80	0.90	0.70	0.70	0.80	1.00
IC-13141-2	0.90	0.60	0.60	0.70	0.60	0.80	0.90	0.70	0.70	0.90	0.70	0.80	0.60	0.90	0.80	0.90	0.70	0.90	0.80	0.80	0.80	0.80	0.90	1.00
IC-13141-3	0.90	0.80	0.80	0.70	0.80	0.60	0.70	0.60	0.60	0.90	0.60	0.80	0.60	0.80	0.60	0.90	0.50	0.70	1.00	0.60	0.60	0.80	0.90	0.80	1.00
IC-13141-4	0.70	1.00	1.00	0.70	0.80	0.80	0.70	0.90	0.90	0.70	0.90	0.80	0.80	0.70	0.50	0.70	0.70	0.70	0.80	0.60	0.50	0.80	0.70	0.60	0.80	1.00
IC-13141-5	0.60	0.90	0.90	0.80	0.70	0.70	0.80	0.80	0.80	0.60	0.80	0.70	0.70	0.70	0.70	0.80	0.80	0.80	0.70	0.70	0.70	0.70	0.80	0.70	0.70	0.90	1.00
IC-13141-6	0.70	1.00	1.00	0.70	0.80	0.80	0.70	0.90	0.90	0.70	0.90	0.80	0.80	0.70	0.80	0.70	0.70	0.70	0.80	0.80	0.80	0.80	0.70	0.60	0.80	1.00	0.90	1.00
IC-13417-1	0.70	0.80	0.80	0.70	0.60	0.80	0.90	0.90	0.90	0.70	0.90	1.00	0.80	0.70	0.80	0.80	0.70	0.90	0.80	0.80	0.80	1.00	0.80	0.80	0.80	0.80	0.70	0.80	1.00
IC-13417-2	0.80	0.70	0.70	0.80	0.50	0.70	1.00	0.80	0.80	0.80	0.80	0.90	0.70	0.70	0.90	0.80	0.80	1.00	0.80	0.90	0.90	0.90	0.80	0.90	0.80	0.70	0.80	0.70	0.90	1.00
IC-13417-3	0.80	0.70	0.70	0.80	0.70	0.50	0.80	0.60	0.60	0.80	0.60	0.80	0.50	0.80	0.70	1.00	0.60	0.80	0.70	0.70	0.70	0.80	1.00	0.90	0.70	0.70	0.70	0.70	0.80	0.80	1.00
IC-13417-4	0.90	0.80	0.80	0.70	0.80	0.60	0.70	0.60	0.60	0.90	0.60	0.80	0.60	0.80	0.60	0.90	0.50	0.70	1.00	0.60	0.60	0.80	0.90	0.80	1.00	0.80	0.80	0.80	0.80	0.70	0.90	1.00
IC-13417-5	0.80	0.90	0.90	0.60	0.90	0.70	0.60	0.80	0.80	0.80	0.80	0.70	0.70	0.80	0.70	0.80	0.60	0.60	0.90	0.70	0.70	0.70	0.80	0.70	0.90	0.90	0.80	0.90	0.70	0.60	0.80	0.90	1.00
EC-323729-1	0.80	0.90	0.90	0.60	0.70	0.90	0.80	1.00	1.00	0.80	1.00	0.90	0.90	0.80	0.70	0.60	0.80	0.80	0.60	0.90	0.70	0.90	0.60	0.70	0.60	0.90	0.90	0.90	0.90	0.80	0.60	0.60	0.80	1.00
EC-323729-2	0.90	0.80	0.80	0.60	0.80	0.70	0.80	0.90	0.90	0.90	0.90	0.80	0.70	0.90	0.70	0.80	0.70	0.80	0.80	0.80	0.70	0.80	0.80	0.70	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.90	1.00
EC-323729-3	0.80	0.90	0.90	0.60	0.70	0.90	0.80	1.00	1.00	0.80	1.00	1.00	0.90	0.80	0.70	0.60	0.80	0.80	0.60	0.90	0.70	1.00	0.60	0.70	0.60	0.90	0.80	0.90	1.00	0.80	0.60	0.60	0.80	1.00	0.80	1.00
EC-323729-4	0.60	0.80	0.80	0.70	0.60	0.80	0.90	0.90	0.90	0.60	0.90	0.80	0.80	0.70	0.80	0.70	0.90	0.90	0.60	1.00	0.80	0.80	0.70	0.80	0.60	0.80	0.90	0.80	0.80	0.90	0.70	0.60	0.70	0.90	0.70	0.90	1.00
EC-323729-5	0.60	0.90	0.90	0.80	0.70	0.70	0.80	0.80	0.80	0.60	0.80	0.70	0.70	0.80	0.70	0.80	0.80	0.80	0.60	0.90	0.70	0.70	0.80	0.70	0.60	0.90	0.70	0.90	0.70	0.80	0.80	0.60	0.80	0.80	0.70	0.80	0.90	1.00
Local	0.50	0.80	0.80	0.50	0.60	0.60	0.50	0.70	0.70	0.50	0.70	0.60	0.60	0.50	0.40	0.50	0.50	0.50	0.70	0.50	0.40	0.60	0.50	0.40	0.70	0.80	0.70	0.80	0.60	0.50	0.50	0.70	0.70	0.70	0.60	0.70	0.50	0.70	1.00
Kamroo local	0.60	0.70	0.70	0.60	0.50	0.50	0.60	0.60	0.60	0.60	0.60	0.70	0.50	0.40	0.40	0.60	0.40	0.60	0.70	0.50	0.40	0.70	0.60	0.50	0.70	0.70	0.60	0.70	0.70	0.60	0.60	0.70	0.60	0.60	0.80	0.60	0.50	0.90	0.90	1.00
OC-2	0.60	0.70	0.70	0.60	0.50	0.50	0.60	0.60	0.60	0.60	0.60	0.70	0.50	0.40	0.50	0.50	0.40	0.60	0.70	0.50	0.50	0.70	0.50	0.50	0.70	0.70	0.60	0.70	0.70	0.60	0.50	0.70	0.60	0.60	0.80	0.60	0.50	0.60	0.90	1.00	1.00
VL-7	1.00	0.70	0.70	0.60	0.70	0.70	0.80	0.80	0.80	1.00	0.80	0.90	0.70	0.80	0.70	0.80	0.60	0.80	0.90	0.70	0.70	0.90	0.80	0.90	0.90	0.70	0.60	0.70	0.90	0.80	0.80	0.90	0.80	0.80	0.80	0.80	0.70	0.50	0.50	0.60	0.60	1.00
IC-319588	0.20	0.30	0.30	0.40	0.30	0.50	0.40	0.40	0.40	0.20	0.40	0.30	0.50	0.40	0.30	0.20	0.60	0.40	0.10	0.50	0.30	0.30	0.20	0.30	0.10	0.30	0.40	0.30	0.30	0.40	0.20	0.10	0.20	0.40	0.20	0.40	0.50	0.40	0.30	0.20	0.20	0.20	1.00
IC-324313	0.60	0.70	0.70	0.80	0.50	0.50	0.80	0.60	0.60	0.60	0.60	0.70	0.50	0.50	0.90	0.80	0.60	0.80	0.70	0.70	0.50	0.70	0.80	0.70	0.70	0.70	0.80	0.70	0.70	0.80	0.80	0.70	0.60	0.60	0.80	0.60	0.70	0.80	0.50	0.60	0.60	0.60	0.50	1.00
IC-352992	0.70	0.80	0.80	0.70	0.70	0.60	0.70	0.70	0.70	0.70	0.70	0.80	0.60	0.50	0.80	0.70	0.50	0.70	0.80	0.70	0.60	0.80	0.70	0.60	0.80	0.80	0.70	0.80	0.80	0.70	0.70	0.80	0.70	0.70	0.90	0.70	0.60	0.80	0.60	0.70	0.70	0.70	0.30	0.90	1.00
IC-319595	0.50	0.80	0.80	0.70	0.60	0.60	0.70	0.70	0.70	0.50	0.70	0.70	0.60	0.70	0.80	0.70	0.70	0.70	0.60	0.80	0.60	0.70	0.70	0.60	0.60	0.80	0.90	0.80	0.70	0.70	0.70	0.60	0.70	0.70	0.70	0.70	0.80	0.90	0.60	0.50	0.50	0.50	0.50	0.90	0.80	1.00
IC-324244	0.50	0.80	0.80	0.70	0.60	0.60	0.70	0.70	0.70	0.50	0.70	0.70	0.60	0.70	0.80	0.70	0.70	0.70	0.60	0.80	0.60	0.70	0.70	0.60	0.60	0.80	0.90	0.80	0.70	0.70	0.70	0.60	0.70	0.70	0.70	0.70	0.80	0.90	0.60	0.50	0.50	0.50	0.50	0.90	0.80	1.00	1.00
KBB-3	0.50	0.80	0.80	0.70	0.60	0.60	0.70	0.70	0.70	0.50	0.70	0.70	0.60	0.70	0.80	0.70	0.70	0.70	0.60	0.80	0.60	0.70	0.70	0.60	0.60	0.80	0.90	0.80	0.70	0.70	0.70	0.60	0.70	0.70	0.70	0.70	0.80	0.90	0.60	0.50	0.50	0.50	0.50	0.90	0.80	1.00	1.00	1.00
Himpriya	0.20	0.30	0.30	0.40	0.30	0.50	0.40	0.40	0.40	0.20	0.40	0.30	0.50	0.40	0.30	0.20	0.60	0.40	0.10	0.50	0.30	0.30	0.20	0.30	0.10	0.30	0.40	0.30	0.30	0.40	0.20	0.10	0.20	0.40	0.20	0.40	0.50	0.40	0.30	0.20	0.20	0.20	1.00	0.50	0.30	0.50	0.50	0.50	1.00
Kuppa local	0.30	0.40	0.40	0.50	0.40	0.60	0.50	0.50	0.50	0.30	0.50	0.40	0.60	0.50	0.50	0.30	0.60	0.50	0.20	0.70	0.50	0.40	0.30	0.40	0.20	0.40	0.50	0.40	0.40	0.50	0.30	0.20	0.30	0.50	0.30	0.50	0.70	0.50	0.40	0.30	0.30	0.30	0.90	0.50	0.40	0.60	0.60	0.60	0.90	1.00
Shimla B-1	0.20	0.20	0.20	0.60	0.50	0.50	0.40	0.40	0.40	0.20	0.40	0.30	0.50	0.40	0.60	0.40	0.60	0.40	0.30	0.50	0.60	0.30	0.40	0.30	0.30	0.20	0.60	0.20	0.30	0.40	0.40	0.30	0.40	0.40	0.40	0.40	0.50	0.60	0.50	0.30	0.30	0.20	0.80	0.60	0.50	0.70	0.70	0.70	0.80	0.90	1.00
Sangla B-1	0.10	0.10	1.00	0.50	0.40	0.40	0.30	0.30	0.30	0.10	0.30	0.20	0.40	0.30	0.50	0.30	0.50	0.30	0.20	0.30	0.50	0.20	0.30	0.20	0.20	1.00	0.50	1.00	0.20	0.30	0.30	0.20	0.30	0.30	0.20	0.30	0.30	0.40	0.40	0.30	0.30	0.10	0.90	0.50	0.30	0.50	0.50	0.50	0.90	0.80	0.80	1.00
Sangla B-2	0.10	0.10	1.00	0.50	0.40	0.40	0.30	0.30	0.30	0.10	0.30	0.20	0.40	0.30	0.50	0.30	0.50	0.30	0.20	0.30	0.50	0.20	0.30	0.20	0.20	1.00	0.50	1.00	0.20	0.30	0.30	0.20	0.30	0.30	0.20	0.30	0.30	0.40	0.40	0.30	0.30	0.10	0.90	0.50	0.30	0.50	0.50	0.50	0.90	0.80	0.80	1.00	1.00
Sangla B-3	0.10	0.10	1.00	0.50	0.40	0.40	0.30	0.30	0.30	0.10	0.30	0.20	0.40	0.30	0.50	0.30	0.50	0.30	0.20	0.30	0.50	0.20	0.30	0.20	0.20	1.00	0.50	1.00	0.20	0.30	0.30	0.20	0.30	0.30	0.20	0.30	0.30	0.40	0.40	0.30	0.30	0.10	0.90	0.50	0.30	0.50	0.50	0.50	0.90	0.80	0.80	1.00	1.00	1.00
Sangla B-5	0.10	0.10	1.00	0.50	0.40	0.40	0.30	0.30	0.30	0.10	0.30	0.20	0.40	0.30	0.50	0.30	0.50	0.30	0.20	0.30	0.50	0.20	0.30	0.20	0.20	1.00	0.50	1.00	0.20	0.30	0.30	0.20	0.30	0.30	0.20	0.30	0.30	0.40	0.40	0.30	0.30	0.10	0.90	0.50	0.30	0.50	0.50	0.50	0.90	0.80	0.80	1.00	1.00	1.00	1.00
Sangla B-6	0.10	0.10	1.00	0.50	0.40	0.40	0.30	0.30	0.30	0.10	0.30	0.20	0.40	0.30	0.50	0.30	0.50	0.30	0.20	0.30	0.50	0.20	0.30	0.20	0.20	1.00	0.50	1.00	0.20	0.30	0.30	0.20	0.30	0.30	0.20	0.30	0.30	0.40	0.40	0.30	0.30	0.10	0.90	0.50	0.30	0.50	0.50	0.50	0.90	0.80	0.80	1.00	1.00	1.00	1.00	1.00
Sangla B-7	0.10	0.10	1.00	0.50	0.40	0.40	0.30	0.30	0.30	0.10	0.30	0.20	0.40	0.30	0.50	0.30	0.50	0.30	0.20	0.30	0.50	0.20	0.30	0.20	0.20	1.00	0.50	1.00	0.20	0.30	0.30	0.20	0.30	0.30	0.20	0.30	0.30	0.40	0.40	0.30	0.30	0.10	0.90	0.50	0.30	0.50	0.50	0.50	0.90	0.80	0.80	1.00	1.00	1.00	1.00	1.00	1.00
IC-412744	0.10	0.10	0.10	0.50	0.40	0.40	0.30	0.30	0.30	0.10	0.30	0.20	0.40	0.30	0.50	0.30	0.50	0.30	0.20	0.30	0.50	0.20	0.30	0.20	0.20	0.10	0.50	0.10	0.20	0.30	0.30	0.20	0.30	0.30	0.20	0.30	0.30	0.40	0.40	0.30	0.30	0.10	0.90	0.50	0.30	0.50	0.50	0.50	0.90	0.80	0.80	1.00	1.00	1.00	1.00	1.00	1.00	1.00
IC-412863	0.10	0.10	0.10	0.50	0.40	0.40	0.30	0.30	0.30	0.10	0.30	0.20	0.40	0.30	0.50	0.30	0.50	0.30	0.20	0.30	0.50	0.20	0.30	0.20	0.20	0.10	0.50	0.10	0.20	0.30	0.30	0.20	0.30	0.30	0.20	0.30	0.30	0.40	0.40	0.30	0.30	0.10	0.90	0.50	0.30	0.50	0.50	0.50	0.90	0.80	0.80	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
IC-421598	0.10	0.10	0.10	0.50	0.40	0.40	0.30	0.30	0.30	0.10	0.30	0.20	0.40	0.30	0.50	0.30	0.50	0.30	0.20	0.30	0.50	0.20	0.30	0.20	0.20	0.10	0.50	0.10	0.20	0.30	0.30	0.20	0.30	0.30	0.20	0.30	0.30	0.40	0.40	0.30	0.30	0.10	0.90	0.50	0.30	0.50	0.50	0.50	0.90	0.80	0.80	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
IC-412722	0.10	0.10	0.10	0.50	0.40	0.40	0.30	0.30	0.30	0.10	0.30	0.20	0.40	0.30	0.50	0.30	0.50	0.30	0.20	0.30	0.50	0.20	0.30	0.20	0.20	0.10	0.50	0.10	0.20	0.30	0.30	0.20	0.30	0.30	0.20	0.30	0.30	0.40	0.40	0.30	0.30	0.10	0.90	0.50	0.30	0.50	0.50	0.50	0.90	0.80	0.80	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
IC-421597	0.10	0.10	0.10	0.50	0.40	0.40	0.30	0.30	0.30	0.10	0.30	0.20	0.40	0.30	0.50	0.30	0.50	0.30	0.20	0.30	0.50	0.20	0.30	0.20	0.20	0.10	0.50	0.10	0.20	0.30	0.30	0.20	0.30	0.30	0.20	0.30	0.30	0.40	0.40	0.30	0.30	0.10	0.90	0.50	0.30	0.50	0.50	0.50	0.90	0.80	0.80	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
F. cymosum	0.33	0.67	0.67	0.67	0.67	0.67	0.53	0.53	0.53	0.33	0.53	0.43	0.67	0.53	0.67	0.53	0.82	0.53	0.43	0.67	0.67	0.43	0.53	0.43	0.43	0.67	0.82	0.67	0.43	0.53	0.53	0.43	0.53	0.53	0.53	0.53	0.67	0.82	0.43	0.33	0.33	0.33	0.33	0.70	0.60	0.80	0.80	0.80	0.33	0.43	0.53	0.33	0.33	0.33	0.33	0.33	0.33	0.60	0.60	0.60	0.60	0.60	1.00

thirty two accessions/cultivars of *Fagopyrum* revealed a range of coefficient of similarity from 0.10 to 1.0 (Table 4.2). This indicated the diverse nature of collected germplasm. The dendrogram generated on the basis of Jaccard's similarity coefficient for the qualitative morphological traits showed the clustering of accessions/cultivars into two broad groups (Fig. 4.4). Cluster 1 comprised of all the accessions/cultivars of *Fagopyrum esculentum* and *F. cymosum.* While the accessions IC-324313, IC-324244 and IC-352992, which are collections from Arunachal Pradesh, showed a similarity coefficient of 1.0, IC-13145 showed 100% similarity with IC-13141-3 and IC-13417-4 of *F. esculentum*. IC-13145 has been designated by NBPGR as "*F. himalianum*". The cultivars Local, Kamroo Local and OC-2 of Uttaranchal clustered in same subgroup, with Kamroo Local and OC-2 showing 100% similarity and the variety Local showing 90% similarity with Kamroo Local and OC-2. Cluster 2 comprised of all the accessions/cultivars of *tataricum*. This cluster had three sub-clusters. The cultivars KBB-3 and Himpriya showed 100% similarity and emerged separately from rest of the accessions/cultivars. Kuppa Local and Shimla B-1 emerged out as a separate subgroup within this sub-cluster. While KBB-3 and Himpriya showed a similarity coefficient of 0.9 with Kuppa Local, these cultivars recorded a similarity coefficient of 0.8 with Shimla B-1. All the accessions of *tataricum* from Arunachal Pradesh showed 100% similarity amongst themselves. Similarly the six cultivars *viz.* Sangla B-1, B-2, B-3, B-5, B-6 and B-7 from Himachal showed a similarity coefficient of 1.0.

The crop was raised in two different seasons. The summer crop was grown during April to July while the winter crop was grown during August to November. For measurement of quantitative traits, readings were recorded individually for 15 plants

Fig 4.4: Dendrogram based on the UPGMA analysis generated from the similarity matrix of the qualitative morphological characters of accessions/cultivars of *Fagopyrum* spp. investigated in the present study.


IC-188669-1
IC-18751-3
VL-7
IC-13145-2
IC-13141-3
IC-13417-4
IC-13376-2
IC-131 41-2
IC-13376-4
IC-13141-1
IC-13417-3
IC-188669-2
IC-188669-3
IC-13141-4
IC-13141-6
IC-13141-5
IC-188669-5
IC-13417-5
IC-323729-1
IC-319595
IC-188669-4
IC-13376-3
IC-13145-4
IC-188669-6
IC-13376-1
IC-13376-5
IC-188669-7
IC-13145-1
IC-13417-2
IC-13145-3
EC-323729-4
IC-18751-1
IC-18751-2
IC-18751-4
EC-323729-1
EC-323729-2
IC-18751-5
IC-13145-5
IC-13417-3
EC-323729-5
IC-324313
IC-324244
IC-352992
IC-319588
F. cymosum
Local
Kamroo Local
OC-2
KBB-3
Himpriya
Kuppa Local
Sangla B-1
Sangla B-2
Sangla B-3
Sangla B-5
Sangla B-6
Sangla B-7
IC-412744
IC-421598
IC-412722
IC-412863
IC-421597
Shimla B-1
F. esculentum & F. cymosum
F. tataricum
Cluster 1
Cluster 2
0.35
0.51
0.67
0.84
1.0
Similarity coefficient

from each accessions/cultivar and average measurements were calculated. The crop was raised consecutively for three years during both the seasons.

F. cymosum showed significantly higher values for all the quantitative parameters studied. Amongst the accessions/cultivars of *F. esculentum*, plant height ranged from 55 cm in the cultivar "Local" to 88 cm in the accession IC-13141. Except IC-188669, which is a collection from Himachal Pradesh, the height of plants at 50% flowering in all the accessions of *esculentum* ranged between 76 to 88 cm. IC-188669 showed a height of 65 cm at 50% flowering. The cultivars collected from Uttaranchal, however, showed a wide range of variation in plant height which ranged from 55 cm in Local to 74 cm in OC-2. On the other hand, the accessions collected from Arunachal Pradesh showed a narrow range of variation in plant height. The height of plants at 50 % flowering in the accessions from Arunachal Pradesh ranged from 60 cm in IC-319588 to 70 cm in IC-352992. Amongst accessions/cultivars of *F. tataricum* the height of plants at 50 % flowering ranged between 59 cm for the cultivar Kuppa local to 95 cm for cultivar Shimla B-1. *F. cymosum* showed an average plant height of 156 cm (Table 4.3; Fig. 4.5). The accessions/cultivars belonging to *F. esculentum* and *F. tataricum* did not show marked variations in height during the winter season of growth. The height of plants at 50 % flowering during the winter season ranged from 34.6 cm for VL-7 to 48.2 cm for Shimla B-1. On the other hand *F. cymosum* showed an average plant height of 120 cm (Table 4.4; Fig. 4.5). There was no significant intraspecific variation in the number of branches per stem. While the number of branches recorded per stem was 4.0 in accessions/cultivars of *F. esculentum,* it ranged from 5.0 to 7.0 in accessions/cultivars of *F. tataricum*. On the other hand *F. cymosum* showed an average number of 15 branches per axis. The number of branches per axis was 3.0 in IC-13145, which has

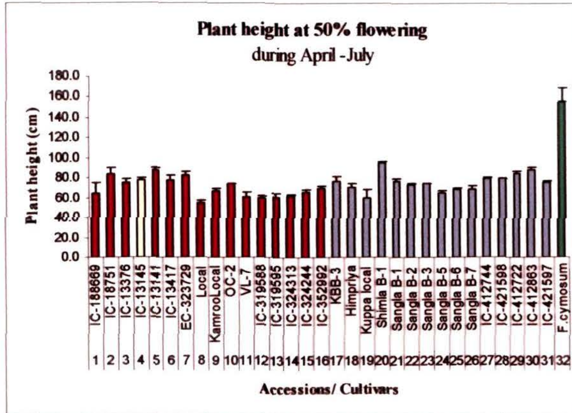
Table 4.3: Variation in Plant height, number of branches on main stem, number of internodes per plant, number of grains per plant, total leaf area, days to attain 50% flowering and days to maturity in different accessions/cultivars of buckwheat (*Fagopyrum* spp.) studied in the present investigation during summer season of growth.

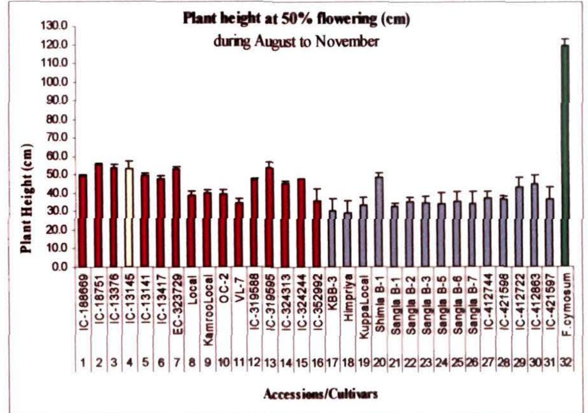
Sr. No.	Accessions	Species	Plant height (cm)	No. of branches	No. of internodes /plant	Number of grains/ plant	Total Leaf surface area (sq cm)	Days to 50% flowering	Days to maturity
1	IC-188669	*F. esculentum*	64.6 ± 11.3	4.0 ± 0.2	10.0 ± 0.5	60 ± 0.7	489.4 ± 43.9	39 ± 1.5	89 ± 11.0
2	IC-18751	*F. esculentum*	83.8 ± 6.6	4.0 ± 0.2	9.0 ± 1.4	67 ± 0.2	606.1 ± 72.8	39 ± 1.5	89 ± 11.0
3	IC-13376	*F. esculentum*	75.9 ± 2.7	4.0 ± 0.1	9.0 ± 0.9	55 ± 4.5	521.3 ± 136.0	39 ± 1.5	89 ± 11.0
4	IC-13145	*F. himalianum*	77.4 ± 2.7	4.0 ± 0.1	9.0 ± 0.8	60 ± 4.3	407.3 ± 38.2	39 ± 1.5	89 ± 11.0
5	IC-13141	*F. esculentum*	87.6 ± 2.0	4.0 ± 0.3	9.0 ± 1.0	56 ± 4.7	387.2 ± 111.0	39 ± 1.5	89 ± 11.0
6	IC-13417	*F. esculentum*	77.3 ± 5.0	4.0 ± 0.1	8.0 ± 1.3	61 ± 2.0	441.7 ± 56.4	39 ± 1.5	89 ± 11.0
7	EC-323729	*F. esculentum*	82.6 ± 3.3	4.0 ± 0.2	9.0 ± 1.3	61 ± 1.9	501.3 ± 55.7	39 ± 1.5	89 ± 11.0
8	Local	*F. esculentum*	55.0 ± 2.0	3.0 ± 0.3	7.0 ± 1.0	40 ± 2.2	300.9 ± 21.9	36 ± 2.0	104 ± 6.5
9	Kamroo Local	*F. esculentum*	67.2 ± 1.7	4.0 ± 0.2	10.0 ± 2.2	45 ± 4.8	366.6 ± 10.6	38 ± 0.5	101 ± 9.5
10	OC-2	*F. esculentum*	73.8 ± 0.6	4.0 ± 0.4	13.0 ± 2.5	41 ± 0.3	399.0 ± 4.0	39 ± 0.5	112 ± 1.5
11	VL-7	*F. esculentum*	61.2 ± 4.3	4.0 ± 0.9	8.0 ± 0.3	82 ± 6.8	346.1 ± 20.1	31 ± 1.5	76 ± 2.5
12	IC-319588	*F. esculentum*	60.0 ± 2.7	4.0 ± 0.2	8.0 ± 0.9	51 ± 4.6	420.0 ± 25.0	38 ± 2.0	83 ± 7.5
13	IC-319595	*F. esculentum*	60.6 ± 3.8	4.0 ± 0.1	8.0 ± 1.0	57 ± 7.4	450.5 ± 7.5	38 ± 2.0	83 ± 7.5
14	IC-324313	*F. esculentum*	61.5 ± 2.0	4.0 ± 0.1	8.0 ± 0.9	59 ± 3.8	506.5 ± 16.5	38 ± 2.0	83 ± 7.5
15	IC-324244	*F. esculentum*	65.2 ± 3.5	4.0 ± 0.2	9.0 ± 1.1	61 ± 2.7	503.5 ± 16.5	38 ± 2.0	83 ± 7.5
16	IC-352992	*F. esculentum*	69.9 ± 2.5	4.0 ± 0.4	9.0 ± 1.2	64 ± 2.0	505.0 ± 7.0	38 ± 2.0	83 ± 7.5
17	KBB-3	*F. tataricum*	76.7 ± 4.4	6.0 ± 1.0	17.0 ± 2.6	86 ± 14.1	548.6 ± 72.0	47 ± 1.5	94 ± 3.5
18	Himpriya	*F. tataricum*	71.1 ± 3.2	5.0 ± 0.4	17.0 ± 0.9	49 ± 0.7	409.6 ± 20.6	53 ± 5.5	111 ± 6.0
19	Kuppa local	*F. tataricum*	59.0 ± 9.1	5.0 ± 0.4	14.0 ± 1.4	52 ± 3.4	317.3 ± 15.7	53 ± 5.5	111± 6.0
20	Shimla B-1	*F. tataricum*	94.6 ± 1.3	5.0 ± 0.6	14.0 ± 1.1	100 ± 9.2	392.1 ± 6.4	39 ± 1.5	81 ± 2.5
21	Sangla B-1	*F. tataricum*	77.0 ± 2.0	5.0 ± 0.3	18.0 ± 2.4	54 ± 19.5	367.4 ± 22.1	53 ± 5.5	108 ± 2.0
22	Sangla B-2	*F. tataricum*	73.4 ± 0.9	5.0 ± 0.7	18.0 ± 2.9	53 ± 15.1	333.3 ± 12.0	53 ± 5.5	108 ± 2.0
23	Sangla B-3	*F. tataricum*	73.9 ± 0.2	4.0 ± 0.3	17.0 ± 2.9	22 ± 0.7	404.1 ± 6.5	53 ± 5.5	108 ± 2.0
24	Sangla B-5	*F. tataricum*	64.3 ± 2.9	4.0 ± 0.1	17.0 ± 1.0	50 ± 14.9	405.6 ± 14.6	53 ± 5.5	108 ± 2.0
25	Sangla B-6	*F. tataricum*	68.3 ± 1.4	4.0 ± 0.2	16.0 ± 0.7	53 ± 13.8	404.8 ± 25.4	53 ± 5.5	108 ± 2.0
26	Sangla B-7	*F. tataricum*	67.6 ± 3.6	5.0 ± 0.6	17.0 ± 1.9	48 ± 12.6	399.8 ± 10.8	53 ± 5.5	108 ± 2.0
27	IC-412744	*F. tataricum*	79.4 ± 1.0	6.0 ± 0.1	17.0 ± 2.8	77 ± 11.8	257.7 ± 2.3	48 ± 2.5	98 ± 2.5
28	IC-421598	*F. tataricum*	78.8 ± 0.4	6.0 ± 0.5	17.0 ± 2.8	76 ± 15.7	249.5 ± 6.5	48 ± 2.5	98 ± 2.5
29	IC-412722	*F. tataricum*	84.3 ± 2.4	6.0 ± 0.2	16.0 ± 1.3	79 ± 12.8	276.0 ± 44.0	48 ± 2.5	98 ± 2.5
30	IC-412863	*F. tataricum*	87.6 ± 2.3	7.0 ± 0.1	17.0 ± 1.3	79 ± 11.5	306.5 ± 28.5	48 ± 2.5	98 ± 2.5
31	IC-421597	*F. tataricum*	75.3 ± 1.2	5.0 ± 0.2	16.0 ± 1.5	77 ± 12.3	261.0 ± 10.0	50 ± 5.0	98 ± 2.5
32	*F.cymosum*	*F. cymosum*	156.0 ± 12.9	15.0 ± 1.1	26.0 ± 0.7	199 ± 8.4	1133.3 ± 50.7	125 ± 5.0	190 ± 10

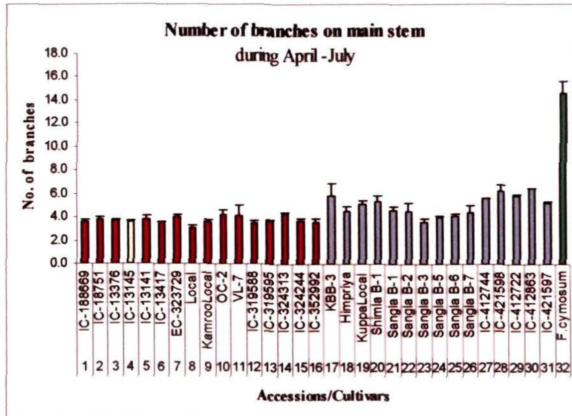
Table 4.4: Variation in Plant height, number of branches on main stem, number of internodes per plant, number of grains per plant, total leaf area, days to attain 50% flowering and days to maturity in different accessions/cultivars of buckwheat (*Fagopyrum* spp.) studied in the present investigation during winter season of growth.

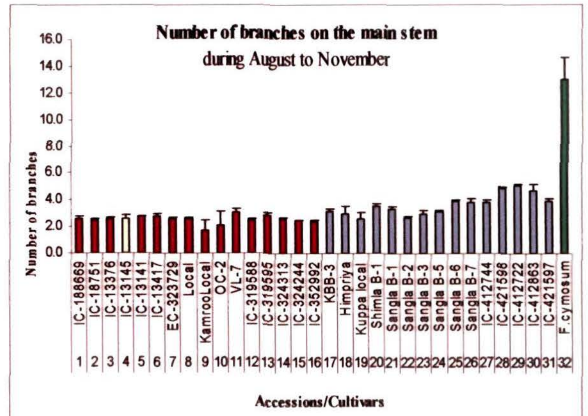
Sr. No.	Accessions	Species	Plant height (cm)	No. of branches	No. of internodes /plant	Number of grains/ plant	Total Leaf surface area (sq cm)	Days to 50% flowering	Days to maturity
1	IC-188669	*F. esculentum*	49.4 ± 0.5	3.0 ± 0.2	8.0 ± 0.7	28.0 ± 2.3	430.0 ± 30.7	34.0 ± 2.0	68.0 ± 2.0
2	IC-18751	*F. esculentum*	55.5 ± 0.6	3.0 ± 0.1	8.0 ± 0.6	35.0 ± 1.1	423.6 ± 8.6	34.0 ± 2.0	68.0 ± 2.0
3	IC-13376	*F. esculentum*	54.0 ± 1.7	3.0 ± 0.2	7.0 ± 0.5	41.0 ± 2.3	400.8 ± 19.6	34.0 ± 2.0	69.0 ± 2.0
4	IC-13145	*F. himalianum*	53.4 ± 3.9	3.0 ± 0.4	7.0 ± 0.3	31.0 ± 3.5	397.9 ± 24.4	34.0 ± 2.0	69.0 ± 2.0
5	IC-13141	*F. esculentum*	49.7 ± 1.1	3.0 ± 0.1	7.0 ± 0.9	32.0 ± 0.6	386.2 ± 26.9	34.0 ± 22	69.0 ± 2.0
6	IC-13417	*F. esculentum*	47.9 ± 1.9	3.0 ± 0.2	7.0 ± 1.0	33.0 ± 2.5	328.0 ± 15.6	34.0 ± 22	69.0 ± 2.0
7	EC-323729	*F. esculentum*	53.0 ± 1.4	3.0 ± 0.2	8.0 ± 1.2	36.0 ± 2.2	374.5 ± 11.6	34.0 ± 2.0	73.0 ± 2.0
8	Local	*F. esculentum*	38.7 ± 2.2	3.0 ± 0.2	7.0 ± 1.3	25.0 ± 5.2	271.2 ± 9.4	42.5 ± 12.5	67.5 ± 7.5
9	Kamroo Local	*F. esculentum*	39.6 ± 2.0	2.0 ± 0.8	8.0 ± 1.0	28.0 ± 5.1	202.5 ± 4.1	44.0 ± 11.0	68.5 ± 8.5
10	OC-2	*F. esculentum*	39.5 ± 2.0	2.0 ± 1.1	7.0 ± 0.3	25.0 ± 4.0	297.0 ± 43.0	48.0 ± 7.0	77.0 ± 7.0
11	VL-7	*F. esculentum*	34.6 ± 2.3	3.0 ± 0.4	6.0 ± 0.4	35.0 ± 4.2	305.3 ± 18.9	26.5 ± 1.5	56.5 ± 1.5
12	IC-319588	*F. esculentum*	47.3 ± 0.6	3.0 ± 0.1	9.0 ± 0.5	40.0 ± 6.6	390.0 ± 9.0	34.0 ± 2.0	70.0 ± 3.0
13	IC-319595	*F. esculentum*	53.9 ± 3.0	3.0 ± 0.3	8.0 ± 0.3	38.0 ± 6.8	358.5 ± 14.5	34.0 ± 2.0	70.0 ± 3.0
14	IC-324313	*F. esculentum*	45.4 ± 1.4	3.0 ± 0.2	8.0 ± 0.7	38.0 ± 6.8	372.5 ± 13.5	34.0 ± 2.0	70.0 ± 3.0
15	IC-324244	*F. esculentum*	47.5 ± 0.6	3.0 ± 0.1	8.0 ± 1.0	38.0 ± 7.7	416.0 ± 16.0	34.0 ± 2.0	70.0 ± 3.0
16	IC-352992	*F. esculentum*	35.7 ± 6.7	2.0 ± 0.1	7.0 ± 0.5	42.0 ± 4.7	353.5 ± 9.5	34.0 ± 2.0	70.0 ± 3.0
17	KBB-3	*F. tataricum*	30.1 ± 6.9	3.0 ± 0.3	10.0 ± 0.8	13.0 ± 0.1	358.1 ± 2.9	39.0 ± 6.0	75.0 ± 6.0
18	Himpriya	*F. tataricum*	29.3 ± 6.3	3.0 ± 0.5	10.0 ± 0.1	25.0 ± 0.5	356.7 ± 6.5	36.5 ± 6.5	75.5 ± 6.5
19	Kuppa local	*F. tataricum*	33.1 ± 4.5	3.0 ± 0.6	11.0 ± 0.2	22.0 ± 6.2	322.3 ± 13.4	40.5 ± 6.5	72.5 ± 6.5
20	Shimla B-1	*F. tataricum*	48.2 ± 2.8	4.0 ± 0.2	10.0 ± 0.3	45.0 ± 2.8	335.6 ± 9.3	32.5 ± 5.5	65.5 ± 5.5
21	Sangla B-1	*F. tataricum*	32.7 ± 2.0	3.0 ± 0.1	10.0 ± 0.2	22.0 ± 1.8	313.9 ± 16.6	45.0 ± 11.0	87.0 ± 11.0
22	Sangla B-2	*F. tataricum*	34.8 ± 2.5	3.0 ± 0.1	11.0 ± 0.2	28.0 ± 4.2	302.8 ± 12.9	45.0 ± 11.0	87.0 ± 11.0
23	Sangla B-3	*F. tataricum*	34.6 ± 3.4	3.0 ± 0.4	11.0 ± 0.0	12.0 ± 0.6	367.8 ± 22.1	45.0 ± 11.0	87.0 ± 11.0
24	Sangla B-5	*F. tataricum*	34.2 ± 5.9	3.0 ± 0.1	11.0 ± 0.6	29.0 ± 5.4	345.7 ± 30.1	45.0 ± 11.0	87.0 ± 11.0
25	Sangla B-6	*F. tataricum*	35.2 ± 5.3	4.0 ± 0.1	11.0 ± 0.5	32.0 ± 6.0	367.9 ± 22.1	45.0 ± 11.0	87.0 ± 11.0
26	Sangla B-7	*F. tataricum*	34.0 ± 6.8	4.0 ± 0.3	13.0 ± 0.0	30.0 ± 4.6	320.2 ± 30.3	45.0 ± 11.0	89.0 ± 11.0
27	IC-412744	*F. tataricum*	36.8 ± 3.5	4.0 ± 0.2	10.0 ± 0.1	38.0 ± 4.2	260.7 ± 28.3	42.0 ± 8.0	82.5 ± 7.5
28	IC-421598	*F. tataricum*	36.5 ± 1.4	5.0 ± 0.1	11.0 ± 0.2	40.0 ± 2.0	213.7 ± 27.7	42.0 ± 8.0	82.5 ± 7.5
29	IC-412722	*F. tataricum*	43.0 ± 5.1	5.0 ± 0.1	11.0 ± 0.4	40.0 ± 2.1	263.5 ± 13.9	42.0 ± 8.0	82.5 ± 7.5
30	IC-412863	*F. tataricum*	44.7 ± 4.9	5.0 ± 0.5	11.0 ± 0.4	41.0 ± 0.4	272.2 ± 19.7	42.0 ± 8.0	82.5 ± 7.5
31	IC-421597	*F. tataricum*	36.4 ± 6.2	4.0 ± 0.2	12.0 ± 0.2	38.0 ± 4.3	246.3 ± 3.1	42.0 ± 8.0	82.5 ± 7.5
32	*F. cymosum*	*F. cymosum*	120.0 ± 3.5	13.0 ± 1.6	22.0 ± 0.8	118.0 ± 6.4	1044.0 ± 66.0	113.5 ± 3.5	165.0 ± 5.0

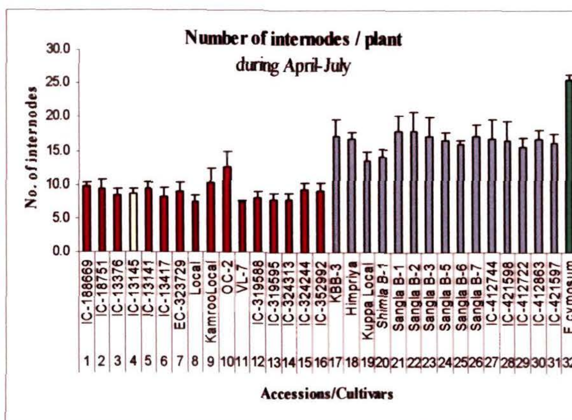
4.5: Graphical representation of plant height at 50% flowering, number of branches on the main stem and number of internodes per plant in plants of different accessions/cultivars of buckwheat (*Fagopyrum* spp.) grown as summer crop during April to July and as winter crop during August to November in the experimental fields of the Botanical garden at NEHU campus, Shillong. Columns in red signify accessions/cultivars of *F. esculentum*, yellow for *F. himalianum*, blue for *F. tataricum* and green for *F. cymosum*.

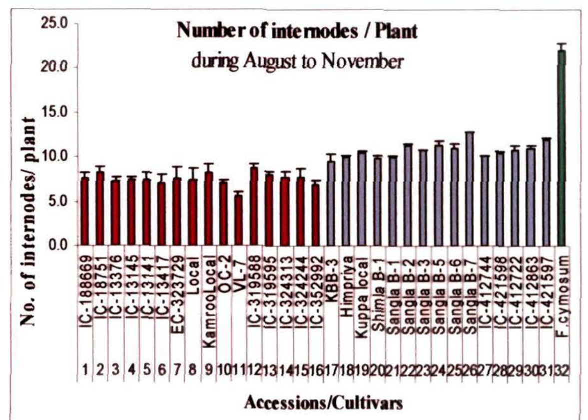

Plant height at 50% flowering
during April - July
Plant height (cm)
Accessions/ Cultivars


Plant height at 50% flowering (cm)
during August to November
Plant Height (cm)
Accessions/Cultivars


Number of branches on main stem
during April - July
No. of branches
Accessions/Cultivars


Number of branches on the main stem
during August to November
Number of branches
Accessions/Cultivars


Number of internodes / plant
during April-July
No. of internodes
Accessions/Cultivars


Number of internodes / Plant
during August to November
No. of internodes/ plant
Accessions/Cultivars

been identified as "*F. himalianum*" (Table 4.3; Fig. 4.5). During winter season *F. esculentum* and IC-13145 "*F. himalianum*" showed 2.0 to 3.0 branches while *F. tataricum* recorded 3.0 to 5.0 branches. *F. cymosum* showed an average number of branches of 13.0 (Table 4.4; Fig. 4.5).

A wide variation in the number of internodes on main axis was observed in plants from different accessions/cultivars during the summer season of growth. The number of internodes ranged from 7 in cultivar Local of *F. esculentum* to 26 in *F. cymosum*. Within the accessions/cultivars of *F. esculentum* the minimum number of internodes ranged from 7 in Local to 13 in OC-2. The magnitude of variation in the number of internodes per plant was, however, much less within accessions/cultivars of *F. tataricum*. In this species the number of internodes ranged between 14 to 18 (Table 4.3; Fig. 4.5). During winter season of growth there was no marked variation in the number of internodes per plant within a species. However, accessions/cultivars of *F. tataricum* showed a larger number of internodes on the main axis than the accessions/cultivars belonging to *F. esculentum*. Plants belonging to *F. cymosum* recorded the highest number of internodes on the main axis during winter season also (Table 4.4; Fig. 4.5).

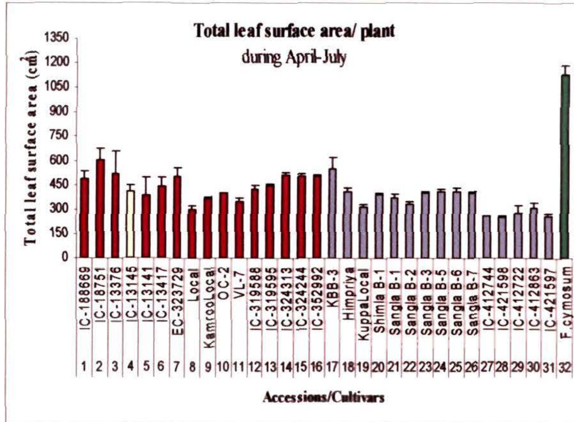
Irrespective of the season the total leaf surface area of plants belonging to different accessions/cultivars of *Fagopyrum* studied in the present investigation recorded a wide variation. During the summer season of growth the total leaf surface area per plant ranged from 249.5 cm^2 in IC-421598 to 1133.3 cm^2 in *F. cymosum*. Amongst the accessions/cultivars of *F. esculentum* the total leaf area per plant ranged from 301.1 cm^2 in the variety Local to 606.1 cm^2 in IC-18751. Similarly the total leaf surface area per plant in different accessions cultivars of *F. tataricum* ranged from 249.5

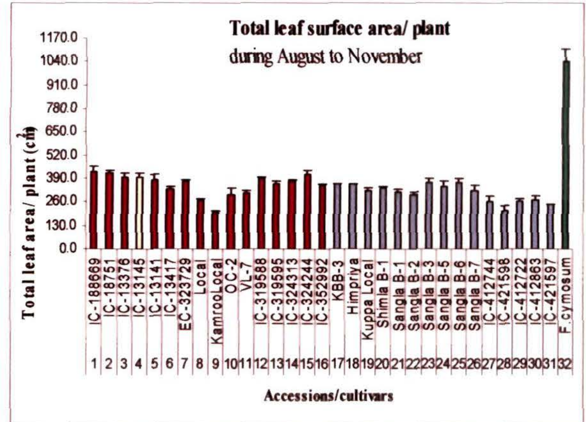
cm^2 in IC-421598 to 549.0 cm^2 in KBB-3 (Table 4.3; Fig. 4.6). During winter season, the range of variation in the total leaf surface area between different accessions/cultivars was much less compared to that observed in the crop grown during summer. *F. cymosum* recorded the highest leaf surface area per plant during this season also (Table 4.4; Fig. 4.6)

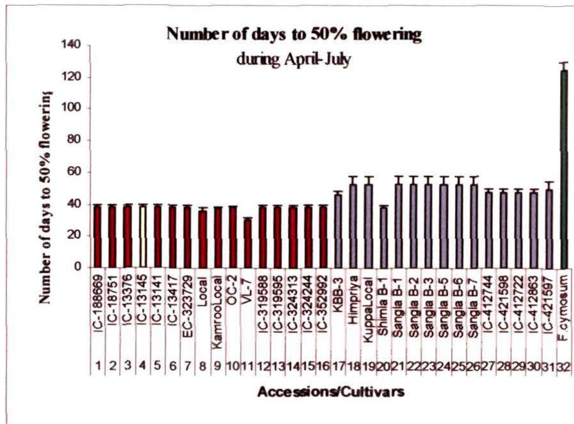
During summer season of growth the number of days to attain 50% flowering ranged from 31-39 days in accessions/cultivars of *F. esculentum* and 39 to 53 days in accessions/cultivars of *F. tataricum.* Within these two species VL-7 took the least number of days to attain 50% flowering. This cultivar attained 50% flowering in 31 days after sowing. On the other hand, Sangla B-6 took 53 days after sowing to attain 50% flowering. *F. cymosum*, however, attained 50% flowering in 125 days after sowing (Table. 4.3; Fig. 4.6). During winter season, the number of days to attain 50% flowering ranged from 26-48 days in accessions/cultivars of *F. esculentum* and 32 to 45 days in accessions/cultivars of *F. tataricum.* During this season VL-7 attained 50% flowering in 27 days. On the other hand, the variety OC-2 took 48 days to attain the same percentage of flowering. *F. cymosum* recorded 50% flowering in 104 days after sowing (Table. 4.4; Fig. 4.6).

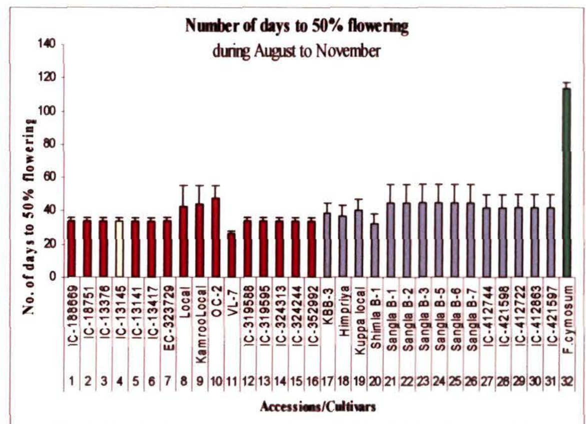
The crop raised during summer season showed a wide range of variation for number of grains per plant. Within the accessions/cultivars of *F. esculentum* the highest number of grains was recorded for VL-7 and the lowest number of grains yield was recorded in cultivar Local. Similar variation in the number of grains per plant was observed in accessions/cultivars of *F. tataricum.* Within this species Shimla B-1 produced the highest number of grains and Himpriya the lowest number of grains per

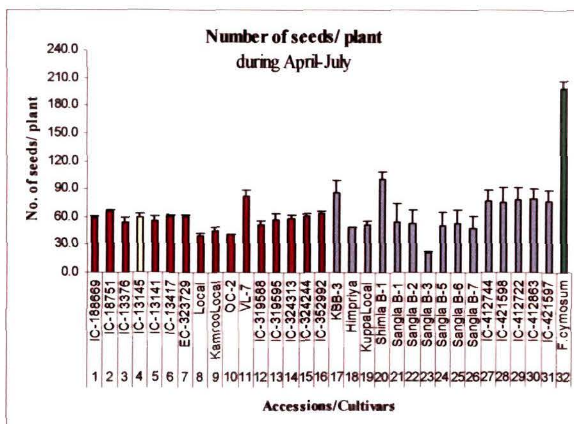
4.6: Graphical representation of total leaf surface area per plant, days to 50% flowering and grain yield per plant in plants of different accessions/cultivars of buckwheat (*Fagopyrum* spp.) grown as summer crop during April to July and as winter crop during August to November in the experimental fields of the Botanical garden at NEHU campus, Shillong.

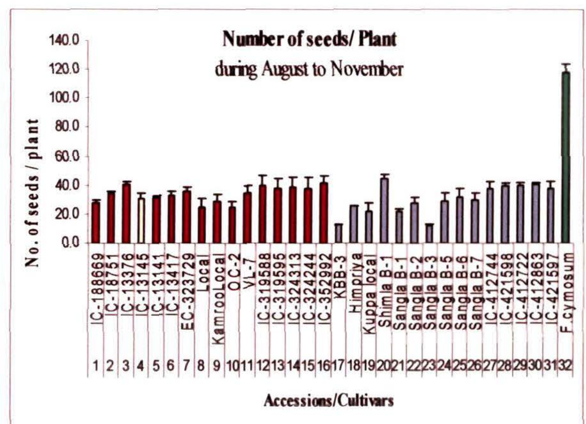

Total leaf surface area/ plant
during April-July
Total leaf surface area (cm²)
Accessions/Cultivars


Total leaf surface area/ plant
during August to November
Total leaf area/ plant (cm²)
Accessions/cultivars


Number of days to 50% flowering
during April-July
Number of days to 50% flowering
Accessions/Cultivars


Number of days to 50% flowering
during August to November
No. of days to 50% flowering
Accessions/Cultivars


Number of seeds/ plant
during April-July
No. of seeds/ plant
Accessions/Cultivars

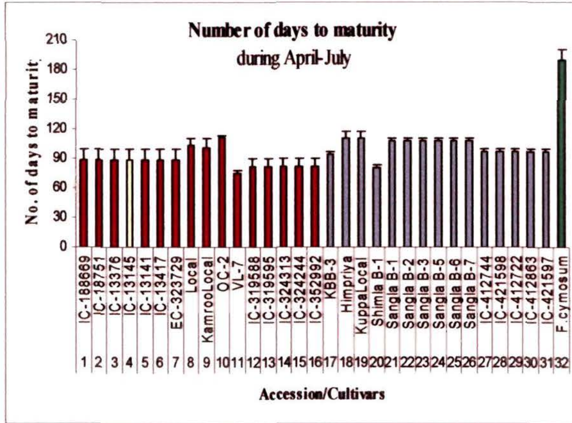

Number of seeds/ Plant
during August to November
No. of seeds / plant
Accessions/Cultivars

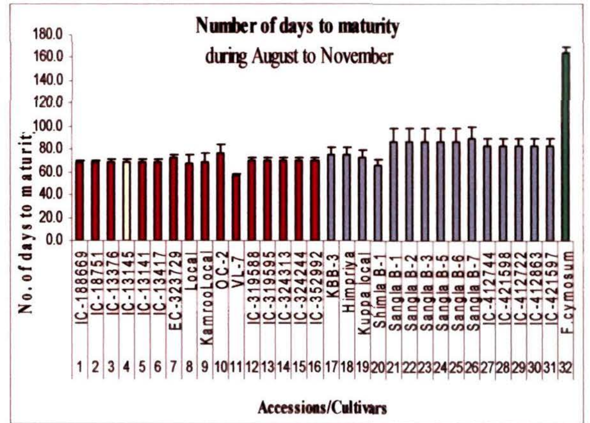
plant (Table. 4.3; Fig. 4.6). Similar variation in the number of grains produced per plant was also observed in the crop grown during the winter season (Table. 4.4; Fig. 4.6).

The number of days to attaining maturity has been calculated as the number of days taken from the day of sowing to 50% seed set. Amongst the accessions/cultivars of *F. esculentum* the number of days to attain maturity ranged from 76 in cultivar VL-7 to 112 in cultivar OC-2. IC-13145 attained the same level of maturity in 89 days. In accessions/cultivars of *F. tataricum* the number of days to attain maturity ranged from 81 in cultivar Shimla B-1 to 108 days in Sangla B-1 and Sangla B-6. *F. cymosum* took 190 days to attain the same level of maturity (Table. 4.3; Fig. 4.7). During the winter season of growth accessions/cultivars of *F. esculentum* matured between 57 to 77 days. While VL-7 took 57 days to attain maturity OC-2 attained the same level of maturity in 77 days. Amongst the accessions/cultivars of *F. tataricum* the number of days to attain maturity ranged from 66 in Shimla B-1 to 89 on Sangla B-7. *F. cymosum* took 165 days to attain the same level of maturity (Table. 4.4; Fig. 4.7).

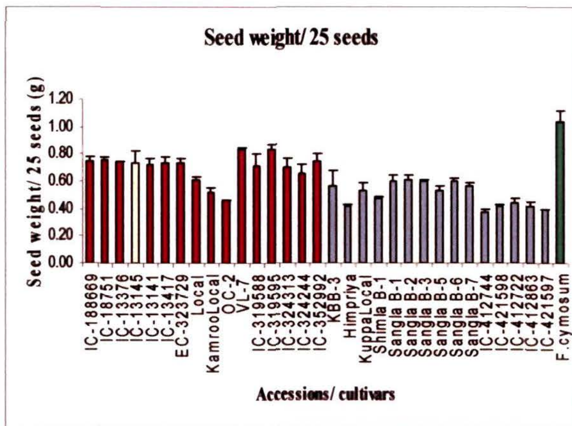
The grain weight per 25 grains ranged from a minimum weight of 0.46g in OC-2 to a maximum of 0.84g in cultivar VL-7 and in accession IC-319595 of *F. esculentum*. The accessions collected from Himachal Pradesh and Arunachal Pradesh showed the grain weight between 0.66-0.84g. The cultivars of *esculentum* collected from Uttaranchal showed the grain weight between 0.46g for OC-2 and 0.84g for VL-7. Amongst the *tataricum* group the minimum grain weight of 0.37g was shown by accession IC-412744 and maximum grain weight of 0.61g in Sangla B-2. The accessions of *tataricum* collected from Arunachal Pradesh showed comparatively lower grain weight (0.37-0.44g) than the cultivars collected from Uttaranchal (0.42-0.61g). *F. cymosum* showed grains weight of 1.0g, which was highest of all the species. IC-13145

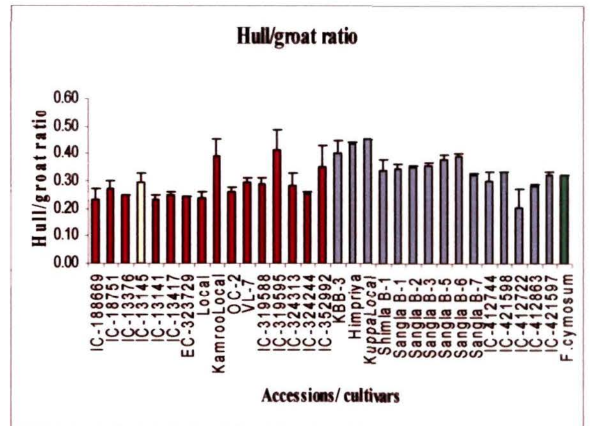
4.7: Graphical representation of **a:** number of days to attain maturity in plants of different accessions/cultivars of buckwheat (*Fagopyrum* spp.) grown as summer crop during April to July and as winter crop during August to November in the experimental fields of the Botanical garden at NEHU campus, Shillong and **b**: grain weight and hull groat ratio of different accessions of buckwheat (*Fagopyrum* spp.).





a





b

Table 4.5: Variations in grain weight 25 grains^{-1} and hull/ groat ratio in different accessions/cultivars of buckwheat (*Fagopyrum* spp.) studied in the present investigation.

Sr. No.	Accessions/Cultivars	Grain weight per 25 grains		Hull/ groat Ratio	
		Mean	Standard error	Mean	Standard error
1	IC-188669	0.75	0.03	0.23	0.03
2	IC-18751	0.76	0.02	0.27	0.03
3	IC-13376	0.74	0.01	0.25	0.00
4	IC-13145	0.74	0.09	0.29	0.04
5	IC-13141	0.72	0.05	0.23	0.02
6	IC-13417	0.74	0.04	0.25	0.01
7	EC-323729	0.74	0.03	0.24	0.00
8	IC-319588	0.71	0.09	0.29	0.02
9	IC-319595	0.84	0.04	0.41	0.07
10	IC-324313	0.70	0.07	0.28	0.05
11	IC-324244	0.66	0.07	0.26	0.00
12	IC-352992	0.74	0.06	0.35	0.08
13	Local	0.62	0.02	0.24	0.02
14	Kamroo local	0.52	0.03	0.39	0.06
15	OC-2	0.84	0.02	0.30	0.01
16	VL-7	0.46	0.00	0.26	0.02
17	KBB-3	0.57	0.11	0.40	0.04
18	Himpriya	0.42	0.01	0.44	0.01
19	Kuppa local	0.53	0.06	0.45	0.00
20	Shimla B-1	0.48	0.02	0.34	0.04
21	Sangla B-1	0.60	0.05	0.35	0.02
22	Sangla B-2	0.61	0.03	0.35	0.01
23	Sangla B-3	0.60	0.01	0.36	0.01
24	Sangla B-5	0.53	0.04	0.38	0.02
25	Sangla B-6	0.60	0.02	0.39	0.01
26	Sangla B-7	0.57	0.03	0.32	0.00
27	IC-412744	0.37	0.03	0.30	0.03
28	IC-421598	0.41	0.01	0.33	0.00
29	IC-412722	0.44	0.04	0.21	0.06
30	IC-412863	0.42	0.03	0.28	0.00
31	IC-421597	0.40	0.00	0.32	0.01
32	*F. cymosum*	1.05	0.08	0.32	0.00

"*F. himalianum*" showed grain weight of 0.74g which was similar to the weight of grains shown by many accessions of *esculentum* (Table 4.5, Fig. 4.7).

The hull/ groat ratio ranged from a minimum of 0.23 in IC-13141 to a maximum of 0.41 in IC-319595 of *F. esculentum.* The cultivars of *esculentum* collected from Uttaranchal showed hull/ groat ratio between 0.24g in Local and 0.39 in Kamroo local. OC-2 showed a ratio of 0.26 while VL-7 showed a ratio of 0.30. Amongst the *tataricum* group the minimum hull/ groat ratio of 0.21 was shown by IC-412722 0.21 and maximum of 0.45 in Kuppa local. The accessions of *tataricum* collected from Arunachal Pradesh showed comparative lower hull/ groat ration (0.21-0.33) than the cultivars collected from Uttaranchal (0.32-0.45). *F. cymosum* grains showed hull/ groat ration of 0.32. IC-13145 "*F. himalianum*" showed hull/ groat ration of 0.29 (Table 4.5, Fig. 4.7b).

Grain protein SDS PAGE analysis:

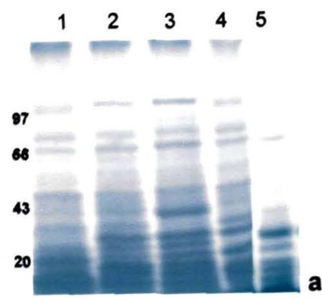
For determination of the SDS PAGE profile of total grain proteins, mature and healthy grains from each group, representing accession/cultivars, were used for extraction of soluble proteins. The hull fraction of each of the grain was removed and the dehulled grains representing the groat fraction was defatted. The defatted groat fraction was dried under a continuous stream of dry air and powdered in liquid nitrogen. The dried defatted meal was homogenized in 50mM Tris-Cl buffer (pH 6.8) containing 100mM NaCl, 10mM EDTA, 100mM glycine, 10% SDS and 1mM PMSF. The homogenate was allowed to stand for 45 min at 4°C in a cold room. The homogenate was subsequently centrifuged at 10,000 rpm for 15 min at 4°C and the supernatant used for SDS PAGE analysis. The SDS PAGE profile of each accession/cultivars was recorded as presence (1) or absence (0) of a band of a particular molecular weight. The

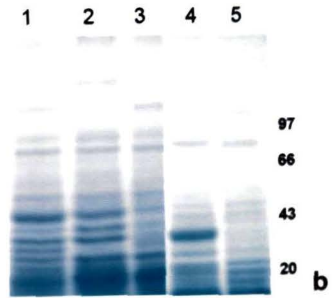
Jaccard's similarity coefficient between different accessions/cultivars was derived from the binary data showing the pair wise similarity between the accessions/cultivars.

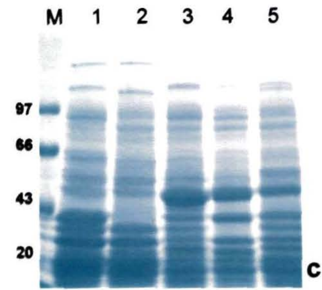
The SDS PAGE profile of grain proteins of different accessions/cultivars of *F. esculentum, "F. himalianum", F. tataricum* and *F. cymosum* is presented in Fig. 4.8 and Fig. 4.9. The number of bands resolved on the gels ranged from a minimum of 24 in the accession IC-13376-5, IC-13141-4 and IC-13417-5 of *F. esculentum* to a maximum of 33 in the accessions/cultivars of *F. tataricum*. The size of bands ranged between 17-121 kDa. Total 47 bands were observed out of which 14 were monomorphic to all the accessions/cultivars of different species and 33 bands were polymorphic in nature. Distinct qualitative variations were observed in the SDS PAGE profile of total grain proteins of *F. esculentum, F. tataricum* and *F. cymosum.* Variation amongst the accessions/cultivars was observed for both number and band sizes of polypeptides. The variation in band sizes of total grain protein was mainly seen in the molecular weight range of 100-121 kDa and 39-54 kDa.

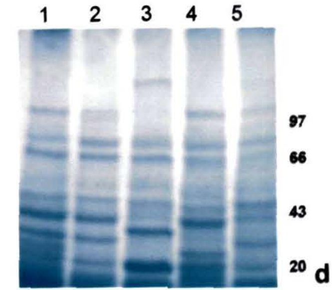
The SDS PAGE profile of total grain proteins of different accessions/cultivars of *F. esculentum* revealed a total of 36 polypeptides out of which 22 polypeptides showing molecular weights of 104, 98, 93, 87, 83, 76, 74, 72, 66, 62, 57, 35, 33, 31, 29, 26, 25, 24, 22, 21, 20, 17 kDa were monomorphic to all the accessions/cultivars. 14 polypeptides of molecular weights of 121, 100, 56, 55, 54, 51, 50, 49, 47, 44, 43, 41, 40, 39 kDa were polymorphic to the accessions/cultivars studied. One of the significant features of the profiles was the presence of a 47kDa band in IC-18751, IC-13145 and EC-323729 all of which belong to *F. esculentum*. This band could not be detected in any other accession/cultivars of *F. esculentum*. SDS PAGE profiles of grain proteins from IC-18751 and EC-323729 also revealed the presence of a 44 kDa and 47 kDa duplex.

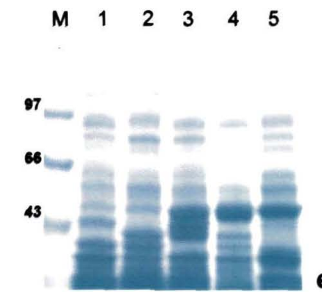
Fig 4.8: SDS PAGE profile of total grain proteins extracted from single grain of different accessions/cultivars of buckwheat (*Fagopyrum* spp.) investigated in the present study. **a:** Lanes1-5: IC-188669-1 - IC-188669-5; **b**: Lanes1-5: IC-18751-1- IC-18751-5; **c**: Lanes1-6: IC-13141-1- IC-13141-5; **d**: Lanes1-5: IC-13417-1 - IC-13417-5; **e**: Lanes1-5: IC-13376-1- IC-13376-5; **f**: Lanes1-5: EC-323729-1- EC-323729-5, **M**- Protein Molecular Weight Marker.


1 2 3 4 5
97
66
43
20
a


1 2 3 4 5
97
66
43
20
b


M 1 2 3 4 5
97
66
43
20
c


1 2 3 4 5
97
66
43
20
d


M 1 2 3 4 5
97
66
43
e

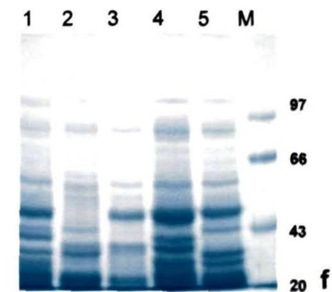
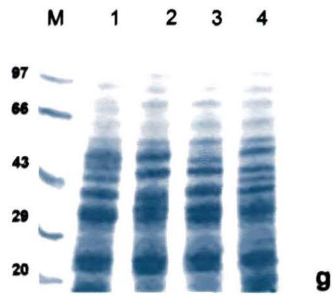
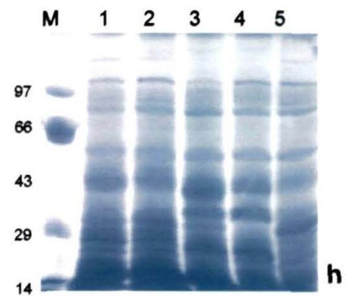

1 2 3 4 5 M
97
66
43
20
f

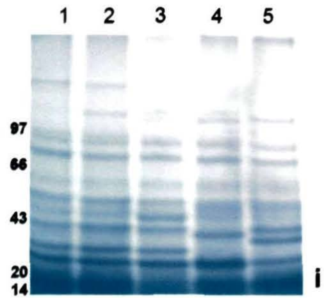
Fig 4.9: SDS PAGE profile of total grain proteins extracted from single grain of different accessions/cultivars of buckwheat (*Fagopyrum* spp.) investigated in the present study. **a**: Lane 1: OC-2, Lane 2: Local, Lane 3: Kamroo Local, Lane 4:VL-7; **b**: Lane 1: IC-319588, Lane 2: IC-324313, Lane 3: IC-352992, Lane 4: IC-319595, Lane 5: IC-324244; **c**: Lanes1-5: IC-13145 (*F. himalianum*); **d**: *F. cymosum,* **e**: Lane 11: IC-412744, Lane 12: IC-421598, Lane 13: IC-412722, Lane 14: IC-412863, Lane 15: IC-421597; **f**: Lane 1: KBB-3, Lane 2: Himpriya, Lane 3: Kuppa Local, Lane 4: Shimla B-1, Lane 5: Sangla B-1, Lane 6: Sangla B-2, Lane 7: Sangla B-3, Lane 8: Sangla B-5, Lane 9: Sangla B-6, Lane10: Sangla B-7, **M**- Protein Molecular Weight Marker.



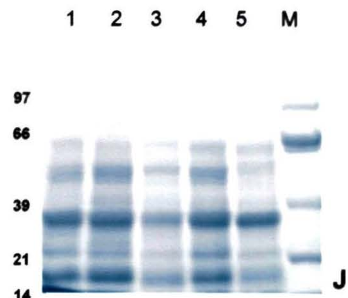
M 1 2 3 4
97
66
43
29
20
g



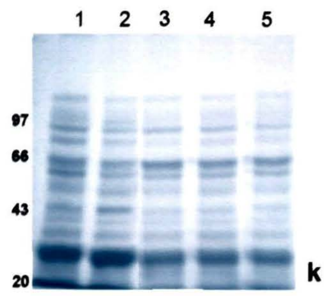
M 1 2 3 4 5
97
66
43
29
14
h



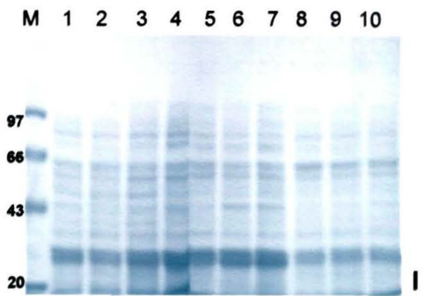
1 2 3 4 5
97
66
43
20
14
i



1 2 3 4 5 M
97
66
39
21
14
J



1 2 3 4 5
97
66
43
20
k



M 1 2 3 4 5 6 7 8 9 10
97
66
43
20
l

This duplex was not present in any other accession/cultivars of the species. The presence of 40 kDa band was observed exclusively in EC-323729, an exotic accession procured from Japan. A significantly high level of inter- as well as intra-accession/cultivars variation was observed in the SDS PAGE profiles of grain proteins isolated from different accessions/cultivars of *F. esculentum.* On the other hand there was no significant variation in the SDS PAGE profiles of grain proteins isolated from different accessions of *F. tataricum.* The profile revealed a total of 33 polypeptide bands ranging in molecular weight from 17-104kDa. There was no variation in the number as well as molecular mass of polypeptides in different accessions/cultivars belonging to the species. The polypeptides detected in accessions/cultivars of *tataricum* had molecular masses of 104, 102, 98, 97, 93, 87, 85, 83, 80, 74, 72, 70, 66, 62, 57, 56, 52, 49, 48, 41, 40, 39, 35, 33, 31,29, 26, 25, 24, 22, 21, 20, 17 kDa. When compared with that of *F. esculentum* the SDS PAGE profile revealed the presence 6 bands showing molecular weights of 102, 97, 85, 80, 70 and 48 kDa exclusive to *F. tataricum* only. These bands could be considered as marker band for the identification of this species. Protein bands which were common to accessions/cultivars of *F. esculentum* as well as *F. tataricum* included polypeptides showing molecular masses of 104, 98, 93, 87, 83, 74, 72, 66, 62, 57, 56, 49, 43, 41, 39, 35, 33, 31, 29, 26, 25, 24, 22, 21, 20, 17 kDa. Band size of 52 kDa was observed in the accessions/cultivars of *tataricum* and *cymosum* and not observed in any of the accessions/cultivars of *esculentum* group.

When subjected to SDS PAGE the total pool of proteins from grains of *F. cymosum* resolved into 25 bands ranging in molecular weight from 17 to 104 kDa. The SDS PAGE profile revealed no intra specific variation either in the number or molecular mass of different polypeptides in the species. The polypeptides detected in *F. cymosum*

had molecular masses of 104, 93, 90, 83, 79, 76, 65, 62, 59, 55, 52, 49, 47, 44, 38, 35, 33, 29, 26, 25, 24, 22, 21, 20, 17 kDa. The profile, however, revealed difference from the profiles of accessions/cultivars of *F. esculentum* and *F. tataricum*. Out of all the bands detected in the accessions/cultivars of *F. esculentum*, *F. tataricum* and *F. cymosum* protein bands showing apparent molecular weights of 90, 79, 65, 59, 55, 38 kDa were detected only in *F. cymosum* whereas protein bands showing molecular weights of 93, 83, 62, 49, 35, 33, 29, 26, 25, 24, 22, 21, 20, 17 kDa were universal to all the accessions/cultivars studied.

Analysis of pair wise similarity showed a range of coefficient of similarity from 0.63 to 1.0 between accessions/cultivars belonging to *F. esculentum* and 0.34 to 0.67 between accessions/cultivars of *F. esculentum* and *F. tataricum*. The Jaccard's similarity coefficient between IC-13145, which has been identified as "*F. himalianum*" by NBPGR, and different accessions/cultivars of *F. esculentum* ranged between 0.72- 1.00. IC-13145 showed a similarity coefficient of 0.53 with accessions/cultivars of *F. tataricum* and 0.41 with *F. cymosum*. The similarity values grouped *F. himalianum* along with the accessions/cultivars of *esculentum* (Table 4.6).

The dendrogram generated on the basis of SDS PAGE profiles of proteins, isolated from single grain, showed the clustering of the accessions/cultivars into 3 broad groups (Fig. 4.11a). Cluster 1 included all accessions/cultivars of *F. esculentum*. The coefficient of similarity within accessions/cultivars in this cluster ranged from 0.63 to 1.0. Within cluster 1, containing accessions/cultivars of *esculentum,* numerous sub groups were formed with majority of single grain showing a similarity between 90-100%. The accessions of *esculentum* from same geographic location showed no prominent difference in single seed grain protein SDS PAGE profile from accessions

Table 4.6: Similarity matrix based on Jaccard's coefficient of the SDS PAGE profile of total grain proteins of different accessions/cultivars of buckwheat (*Fagopyrum* spp.) studied in the present investigation.

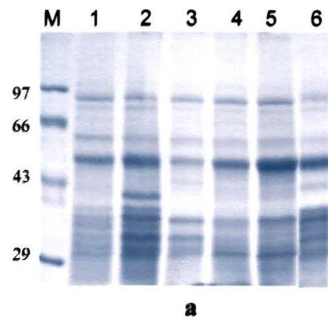
Accessions	IC-188669-1	IC-188669-2	IC-188669-3	IC-188669-4	IC-188669-5	IC-18761-1	IC-18761-2	IC-18761-3	IC-18761-4	IC-18761-5	IC-13376-1	IC-13376-2	IC-13376-3	IC-13376-4	IC-13376-5	IC-13145-1	IC-13145-2	IC-13145-3	IC-13145-4	IC-13145-5	IC-13141-1	IC-13141-2	IC-13141-3	IC-13141-4	IC-13141-5	IC-13417-1	IC-13417-2	IC-13417-3	IC-13417-4	IC-13417-5	EC-323729-1	EC-323729-2	EC-323729-3	EC-323729-4	EC-323729-5	IC-319588	IC-324313	IC-352982	IC-319595	IC-324244	OC-2	Kamroo local	Local	VL-7	KBB-3	Himpriya	Kuppa local	Shimla B-1	Sangla B-1	Sangla B-2	Sangla B-3	Sangla B-5	Sangla B-6	Sangla B-7	IC-412744	IC-412863	IC-421698	IC-412722	IC-421697	F. cymosum
IC-188669-1	1.00
IC-188669-2	0.83	1.00
IC-188669-3	0.91	0.91	1.00
IC-188669-4	0.83	0.76	0.91	1.00
IC-188669-5	0.91	0.88	0.96	0.88	1.00
IC-18761-1	0.88	0.88	0.96	0.88	0.92	1.00
IC-18761-2	0.80	0.88	0.88	0.88	0.92	0.92	1.00
IC-18761-3	0.91	0.83	0.91	0.83	0.88	0.96	0.88	1.00
IC-18761-4	0.96	0.88	0.88	0.88	0.84	0.92	0.84	0.96	1.00
IC-18761-5	0.80	0.83	0.83	0.76	0.96	0.88	0.88	0.91	0.88	1.00
IC-13376-1	0.91	0.91	1.00	0.91	0.96	0.96	0.88	0.91	0.88	0.83	1.00
IC-13376-2	0.95	0.79	0.87	0.95	0.83	0.83	0.83	0.87	0.91	0.79	0.87	1.00
IC-13376-3	0.68	0.78	0.78	0.78	0.75	0.83	0.83	0.87	0.83	0.86	0.78	0.82	1.00
IC-13376-4	0.83	0.83	0.91	0.91	0.87	0.87	0.87	0.83	0.79	0.83	0.91	0.86	0.86	1.00
IC-13376-5	0.95	0.95	0.95	0.87	0.91	0.91	0.63	0.95	0.91	0.87	0.95	0.87	0.82	0.86	1.00
IC-13145-1	0.87	0.87	0.87	0.87	0.83	0.91	0.91	0.95	0.91	0.87	0.87	0.87	0.90	0.86	0.91	1.00
IC-13145-2	0.91	0.75	0.83	0.91	0.87	0.79	0.79	0.83	0.87	0.83	0.83	0.87	0.86	0.90	0.86	0.86	1.00
IC-13145-3	0.91	0.83	0.83	0.84	0.88	0.88	0.80	0.91	0.96	0.91	0.83	0.83	0.86	0.83	0.87	0.87	0.91	1.00
IC-13145-4	0.83	0.83	0.83	0.76	0.88	0.88	0.80	0.91	0.88	1.00	0.83	0.76	0.86	0.83	0.87	0.87	0.83	0.91	1.00
IC-13145-5	0.79	0.79	0.83	0.72	0.83	0.83	0.83	0.83	0.83	0.95	0.83	0.72	0.90	0.78	0.83	0.83	0.78	0.87	0.95	1.00
IC-13141-1	0.80	0.88	0.96	0.96	0.92	0.92	0.92	0.88	0.84	0.96	0.96	0.73	0.83	0.87	0.83	0.83	0.79	0.67	0.96	0.91	1.00
IC-13141-2	0.87	0.87	0.87	0.79	0.91	0.83	0.83	0.87	0.83	0.91	0.87	0.79	0.82	0.86	0.91	0.86	0.86	0.65	0.91	0.91	0.91	1.00
IC-13141-3	0.91	0.83	0.91	0.91	0.96	0.88	0.80	0.83	0.80	0.76	0.91	0.83	0.71	0.83	0.87	0.83	0.86	0.83	0.76	0.79	0.80	0.79	1.00
IC-13141-4	0.78	0.78	0.86	0.86	0.83	0.83	0.83	0.78	0.75	0.78	0.86	0.78	0.81	0.95	0.82	0.82	0.86	0.78	0.78	0.74	0.83	0.82	0.86	1.00
IC-13141-5	0.91	0.83	0.91	0.91	0.88	0.88	0.80	0.91	0.88	0.83	0.91	0.83	0.78	0.83	0.87	0.79	0.91	0.91	0.83	0.79	0.88	0.87	0.91	0.86	1.00
IC-13417-1	0.91	0.87	0.95	0.87	0.91	0.91	0.63	0.95	0.91	0.87	0.95	0.87	0.82	0.91	1.00	0.91	0.86	0.65	0.87	0.83	0.83	0.91	0.95	0.82	0.87	1.00
IC-13417-2	0.87	0.87	0.91	0.91	0.88	0.88	0.80	0.91	0.88	0.83	0.91	0.83	0.78	0.91	0.87	0.79	0.91	0.91	0.83	0.79	0.88	0.87	0.91	0.86	1.00	0.87	1.00
IC-13417-3	0.83	0.83	0.91	0.91	0.87	0.87	0.87	0.83	0.79	0.75	0.91	0.83	0.77	0.90	0.86	0.86	0.82	0.75	0.75	0.71	0.79	0.78	0.91	0.95	0.83	0.86	0.83	1.00
IC-13417-4	0.87	0.79	0.87	0.79	0.91	0.83	0.76	0.79	0.83	0.79	0.87	0.79	0.74	0.86	0.83	0.75	0.86	0.87	0.79	0.75	0.88	0.83	0.95	0.90	0.95	0.83	0.95	0.86	1.00
IC-13417-5	0.86	0.71	0.78	0.86	0.83	0.75	0.75	0.78	0.83	0.78	0.78	0.86	0.81	0.86	0.82	0.82	0.95	0.86	0.78	0.74	0.75	0.82	0.86	0.82	0.86	0.82	0.86	0.86	0.90	1.00
EC-323729-1	0.83	0.91	0.83	0.91	0.88	0.88	0.88	0.83	0.80	0.83	0.83	0.83	0.78	0.83	0.79	0.87	0.83	0.83	0.83	0.72	0.80	0.72	0.83	0.78	0.83	0.79	0.83	0.83	0.79	0.78	1.00
EC-323729-2	0.83	0.73	0.80	0.80	0.84	0.77	0.77	0.80	0.77	0.73	0.80	0.80	0.75	0.79	0.83	0.83	0.79	0.88	0.73	0.69	0.70	0.76	0.88	0.75	0.73	0.83	0.73	0.79	0.76	0.75	0.73	1.00
EC-323729-3	0.88	0.88	0.80	0.80	0.84	0.84	0.77	0.88	0.84	0.80	0.80	0.80	0.75	0.72	0.83	0.83	0.79	0.76	0.80	0.76	0.77	0.76	0.80	0.68	0.73	0.83	0.73	0.72	0.83	0.75	0.88	0.77	1.00
EC-323729-4	0.76	0.91	0.83	0.83	0.80	0.88	0.83	0.83	0.73	0.76	0.83	0.76	0.78	0.83	0.79	0.87	0.75	0.76	0.76	0.72	0.80	0.72	0.83	0.78	0.76	0.79	0.76	0.75	0.83	0.71	0.91	0.80	0.88	1.00
EC-323729-5	0.79	0.87	0.79	0.79	0.76	0.83	0.83	0.87	0.83	0.79	0.79	0.79	0.82	0.78	0.83	0.91	0.78	0.79	0.79	0.75	0.76	0.75	0.79	0.74	0.72	0.83	0.72	0.78	0.75	0.74	0.87	0.83	0.91	0.87	1.00
IC-319588	0.80	0.88	0.96	0.96	0.92	0.92	0.92	0.88	0.84	0.96	0.96	0.73	0.83	0.87	0.83	0.83	0.79	0.67	0.96	0.91	1.00	0.91	0.80	0.83	0.88	0.83	0.88	0.79	0.88	0.75	0.80	0.70	0.77	0.80	0.76	1.00
IC-324313	0.80	0.88	0.96	0.96	0.92	0.92	0.92	0.88	0.84	0.96	0.96	0.73	0.83	0.87	0.83	0.83	0.79	0.67	0.96	0.91	1.00	0.91	0.80	0.83	0.88	0.83	0.88	0.79	0.88	0.75	0.80	0.70	0.77	0.80	0.76	1.00	1.00
IC-352982	0.91	0.83	0.91	0.91	0.96	0.88	0.80	0.83	0.80	0.76	0.91	0.83	0.71	0.83	0.87	0.83	0.86	0.83	0.76	0.79	0.80	0.79	1.00	0.86	0.91	0.95	0.91	0.91	0.95	0.86	0.83	0.88	0.80	0.83	0.79	0.80	0.80	1.00
IC-319595	0.78	0.78	0.86	0.86	0.83	0.83	0.83	0.78	0.75	0.78	0.86	0.78	0.81	0.95	0.82	0.82	0.86	0.78	0.78	0.74	0.83	0.82	0.86	1.00	0.86	0.82	0.86	0.95	0.90	0.82	0.78	0.75	0.68	0.78	0.74	0.83	0.83	0.86	1.00
IC-324244	0.87	0.79	0.87	0.79	0.91	0.83	0.76	0.79	0.83	0.79	0.87	0.79	0.74	0.86	0.83	0.75	0.86	0.87	0.79	0.75	0.88	0.83	0.95	0.90	0.95	0.83	0.95	0.86	1.00	0.90	0.79	0.76	0.83	0.83	0.75	0.88	0.88	0.95	0.90	1.00
OC-2	0.80	0.73	0.80	0.80	0.84	0.77	0.70	0.73	0.70	0.67	0.80	0.76	0.62	0.72	0.76	0.69	0.72	0.73	0.67	0.69	0.84	0.69	0.88	0.75	0.80	0.83	0.80	0.79	0.83	0.83	0.73	0.84	0.77	0.80	0.69	0.84	0.84	0.96	0.75	0.83	1.00
Kamroo local	0.83	0.76	0.83	0.83	0.88	0.80	0.73	0.76	0.73	0.69	0.83	0.79	0.64	0.75	0.79	0.72	0.75	0.76	0.69	0.72	0.88	0.72	0.91	0.78	0.83	0.87	0.83	0.83	0.87	0.78	0.76	0.80	0.73	0.76	0.72	0.88	0.88	0.91	0.78	0.87	0.88	1.00
Local	0.83	0.76	0.83	0.83	0.88	0.80	0.73	0.76	0.73	0.69	0.83	0.79	0.64	0.75	0.79	0.72	0.75	0.76	0.69	0.72	0.88	0.72	0.91	0.78	0.83	0.87	0.83	0.83	0.87	0.78	0.76	0.80	0.73	0.76	0.72	0.88	0.88	0.91	0.78	0.87	0.80	0.91	1.00
VL-7	0.88	0.80	0.88	0.88	0.92	0.84	0.77	0.80	0.77	0.73	0.88	0.83	0.68	0.79	0.83	0.76	0.79	0.80	0.73	0.76	0.92	0.76	0.96	0.83	0.88	0.91	0.88	0.87	0.91	0.83	0.80	0.84	0.77	0.80	0.76	0.92	0.92	0.96	0.83	0.91	0.88	0.88	0.77	1.00
KBB-3	0.56	0.56	0.61	0.56	0.59	0.59	0.55	0.56	0.55	0.56	0.61	0.34	0.52	0.60	0.48	0.53	0.55	0.56	0.56	0.53	0.59	0.47	0.61	0.57	0.38	0.48	0.38	0.55	0.63	0.57	0.53	0.50	0.50	0.52	0.48	0.59	0.59	0.61	0.57	0.63	0.59	0.67	0.61	0.55	1.00
Himpriya	0.56	0.56	0.61	0.56	0.59	0.59	0.55	0.56	0.55	0.56	0.61	0.34	0.52	0.60	0.48	0.53	0.55	0.56	0.56	0.53	0.59	0.47	0.61	0.57	0.38	0.48	0.38	0.55	0.63	0.57	0.53	0.50	0.50	0.52	0.48	0.59	0.59	0.61	0.57	0.63	0.59	0.67	0.61	0.55	1.00	1.00
Kuppa local	0.56	0.56	0.61	0.56	0.59	0.59	0.55	0.56	0.55	0.56	0.61	0.34	0.52	0.60	0.48	0.53	0.55	0.56	0.56	0.53	0.59	0.47	0.61	0.57	0.38	0.48	0.38	0.55	0.63	0.57	0.53	0.50	0.50	0.52	0.48	0.59	0.59	0.61	0.57	0.63	0.59	0.67	0.61	0.55	1.00	1.00	1.00
Shimla B-1	0.56	0.56	0.61	0.56	0.59	0.59	0.55	0.56	0.55	0.56	0.61	0.34	0.52	0.60	0.48	0.53	0.55	0.56	0.56	0.53	0.59	0.47	0.61	0.57	0.38	0.48	0.38	0.55	0.63	0.57	0.53	0.50	0.50	0.52	0.48	0.59	0.59	0.61	0.57	0.63	0.59	0.67	0.61	0.55	1.00	1.00	1.00	1.00
Sangla B-1	0.56	0.56	0.61	0.56	0.59	0.59	0.55	0.56	0.55	0.56	0.61	0.34	0.52	0.60	0.48	0.53	0.55	0.56	0.56	0.53	0.59	0.47	0.61	0.57	0.38	0.48	0.38	0.55	0.63	0.57	0.53	0.50	0.50	0.52	0.48	0.59	0.59	0.61	0.57	0.63	0.59	0.67	0.61	0.55	1.00	1.00	1.00	1.00	1.00
Sangla B-2	0.56	0.56	0.61	0.56	0.59	0.59	0.55	0.56	0.55	0.56	0.61	0.34	0.52	0.60	0.48	0.53	0.55	0.56	0.56	0.53	0.59	0.47	0.61	0.57	0.38	0.48	0.38	0.55	0.63	0.57	0.53	0.50	0.50	0.52	0.48	0.59	0.59	0.61	0.57	0.63	0.59	0.67	0.61	0.55	1.00	1.00	1.00	1.00	1.00	1.00
Sangla B-3	0.56	0.56	0.61	0.56	0.59	0.59	0.55	0.56	0.55	0.56	0.61	0.34	0.52	0.60	0.48	0.53	0.55	0.56	0.56	0.53	0.59	0.47	0.61	0.57	0.38	0.48	0.38	0.55	0.63	0.57	0.53	0.50	0.50	0.52	0.48	0.59	0.59	0.61	0.57	0.63	0.59	0.67	0.61	0.55	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Sangla B-5	0.56	0.56	0.61	0.56	0.59	0.59	0.55	0.56	0.55	0.56	0.61	0.34	0.52	0.60	0.48	0.53	0.55	0.56	0.56	0.53	0.59	0.47	0.61	0.57	0.38	0.48	0.38	0.55	0.63	0.57	0.53	0.50	0.50	0.52	0.48	0.59	0.59	0.61	0.57	0.63	0.59	0.67	0.61	0.55	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Sangla B-6	0.56	0.56	0.61	0.56	0.59	0.59	0.55	0.56	0.55	0.56	0.61	0.34	0.52	0.60	0.48	0.53	0.55	0.56	0.56	0.53	0.59	0.47	0.61	0.57	0.38	0.48	0.38	0.55	0.63	0.57	0.53	0.50	0.50	0.52	0.48	0.59	0.59	0.61	0.57	0.63	0.59	0.67	0.61	0.55	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Sangla B-7	0.56	0.56	0.61	0.56	0.59	0.59	0.55	0.56	0.55	0.56	0.61	0.34	0.52	0.60	0.48	0.53	0.55	0.56	0.56	0.53	0.59	0.47	0.61	0.57	0.38	0.48	0.38	0.55	0.63	0.57	0.53	0.50	0.50	0.52	0.48	0.59	0.59	0.61	0.57	0.63	0.59	0.67	0.61	0.55	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
IC-412744	0.56	0.56	0.61	0.56	0.59	0.59	0.55	0.56	0.55	0.56	0.61	0.34	0.52	0.60	0.48	0.53	0.55	0.56	0.56	0.53	0.59	0.47	0.61	0.57	0.38	0.48	0.38	0.55	0.63	0.57	0.53	0.50	0.50	0.52	0.48	0.59	0.59	0.61	0.57	0.63	0.59	0.67	0.61	0.55	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
IC-412863	0.56	0.56	0.61	0.56	0.59	0.59	0.55	0.56	0.55	0.56	0.61	0.34	0.52	0.60	0.48	0.53	0.55	0.56	0.56	0.53	0.59	0.47	0.61	0.57	0.38	0.48	0.38	0.55	0.63	0.57	0.53	0.50	0.50	0.52	0.48	0.59	0.59	0.61	0.57	0.63	0.59	0.67	0.61	0.55	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
IC-421698	0.56	0.56	0.61	0.56	0.59	0.59	0.55	0.56	0.55	0.56	0.61	0.34	0.52	0.60	0.48	0.53	0.55	0.56	0.56	0.53	0.59	0.47	0.61	0.57	0.38	0.48	0.38	0.55	0.63	0.57	0.53	0.50	0.50	0.52	0.48	0.59	0.59	0.61	0.57	0.63	0.59	0.67	0.61	0.55	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
IC-412722	0.56	0.56	0.61	0.56	0.59	0.59	0.55	0.56	0.55	0.56	0.61	0.34	0.52	0.60	0.48	0.53	0.55	0.56	0.56	0.53	0.59	0.47	0.61	0.57	0.38	0.48	0.38	0.55	0.63	0.57	0.53	0.50	0.50	0.52	0.48	0.59	0.59	0.61	0.57	0.63	0.59	0.67	0.61	0.55	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
IC-421697	0.56	0.56	0.61	0.56	0.59	0.59	0.55	0.56	0.55	0.56	0.61	0.34	0.52	0.60	0.48	0.53	0.55	0.56	0.56	0.53	0.59	0.47	0.61	0.57	0.38	0.48	0.38	0.55	0.63	0.57	0.53	0.50	0.50	0.52	0.48	0.59	0.59	0.61	0.57	0.63	0.59	0.67	0.61	0.55	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
F. cymosum	0.40	0.40	0.35	0.40	0.52	0.43	0.39	0.45	0.43	0.35	0.35	0.37	0.35	0.38	0.41	0.46	0.38	0.40	0.35	0.37	0.34	0.28	0.31	0.30	0.35	0.41	0.35	0.43	0.48	0.34	0.35	0.34	0.34	0.35	0.37	0.34	0.34	0.31	0.30	0.48	0.43	0.40	0.35	0.43	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.26	1.00

from other geographic location which could sub group accession on the basis of location of collection. Except VL-7 all the cultivars of *esculentum* collected from Uttaranchal (Local, Kamroo Local, and OC-2) clustered together. On the other hand, VL-7, which is a high yielding and early maturing cultivar of *F. esculentum* from Uttaranchal, showed more closeness with IC-13141-3 and IC-319595 which are not high grain yielding or early maturing accessions of *F. esculentum* as VL-7. The cultivar showed a Jaccard's coefficient of 0.96 with both the accessions IC-13141-3 and IC-319595. The only difference between the grain protein SDS PAGE profile of VL-7 and IC-13141-3 and IC-319595 was in the presence of a 55 kDa band in VL-7. This band could be used as marker band for the high yielding and early maturing genotype.

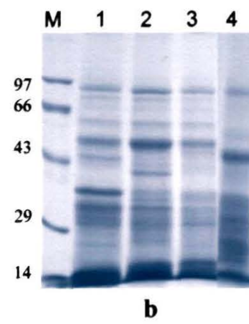
Cluster 2 includes all the accessions/cultivars of *F. tataricum.* There was no polymorphism observed between the accessions/cultivars of *tataricum. F. cymosum* emerged out separately both from *F. esculentum* and *F. tataricum* and formed cluster 3. There was no intraspecific variation in single seed profile of *cymosum. F. cymosum* showed least similarity coefficient of 0.26 with VL-7 and a maximum value of 0.52 with IC-188669-5. This indicates that *F. cymosum* was relatively closer to IC-188669-5 and least close to VL-7. A comparison of the SDS PAGE profiles of total proteins from grains of *F. cymosum* and *F. tataricum* revealed a Jaccard's coefficient of 0.30 indicating the low level of genetic closeness between the two species.

The SDS PAGE profile of endosperm grain proteins of different accessions/ cultivars of *F. esculentum, "F. himalianum", F. tataricum* and *F. cymosum* is presented in Fig. 4.10. The number of polypeptide bands observed in the accessions/cultivars ranged from a minimum of 17 in IC-13145 to a maximum of 25 in accessions/cultivars of *F. tataricum.* The size of resolved polypeptides ranged between 19 and 104 kDa. A

Fig 4.10: SDS PAGE profile of endosperm proteins extracted from single grain of different accessions/cultivars of buckwheat (*Fagopyrum* spp.) investigated in the present study **a**: Lane 1: IC-188669-1, Lane 2: IC- 188669-2, Lane 3: IC-18751-2, Lane 4: IC-18751-3, Lane 5: IC-13376-1, Lane 6: IC-13376-4; **b**: Lane 1: IC-13145-4, 2: IC- 13141-1, Lane 3: IC- 13141-5, Lane 4: IC-13145-2; **c**: Lane 1: IC-13417-2, Lane 2: IC-13417-5, Lane 3: EC-323729-1, Lane 4: EC-323729-5; **d**: Lane 1: VL-7, Lane 2: Local, Lane 3: Kamroo Local, Lane 4: OC-2; **e**: Lane 1: IC-319588, Lane 2: IC-324313, Lane 3: IC-352992, Lane 4: IC-319595, Lane 5: IC-324244; **f**: Lane 1: IC-412744, Lane 2: IC-421598, Lane 3: IC-412722, Lane 4: IC-412863, Lane 5: IC-421597; **g**: Lanel: Sangla B-1, Lane 2: Sangla B-2, Lane 3: Sangla B-3, Lane 4: Sangla B-5, 5: Sangla B-6; **h**: Lanel: KBB-3 Lane 2: Himpriya, Lane 3: Kuppa Local, Lane 4: Sangla B-7; **i**: Lanel: Shimla B-1, Lane 2: *F. cymosum*; **M**- Protein Molecular Weight Marker.



a



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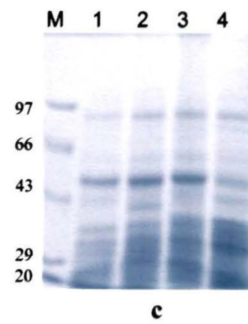

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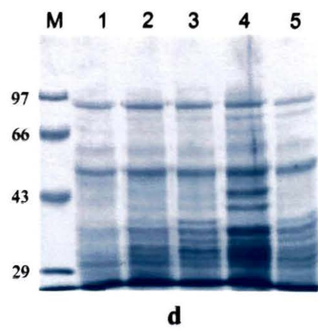
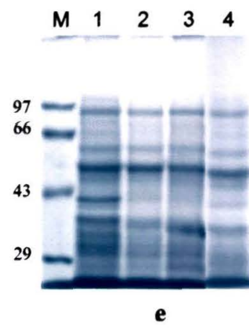
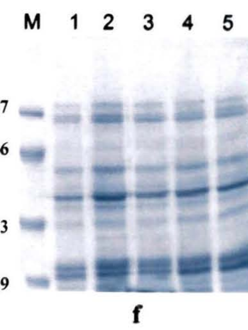


d



e



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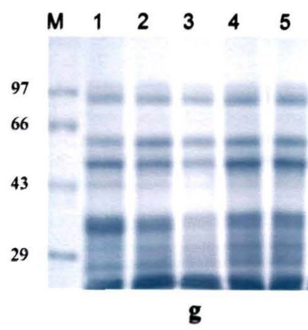
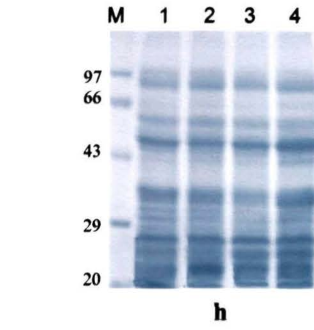
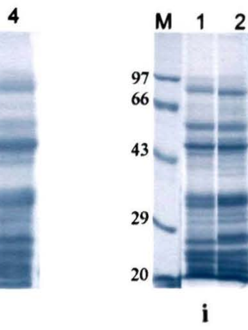


g



h



i

total 43 polypeptides were observed out of which 6 were monomorphic to all the accessions/cultivars and 37 were polymorphic.

The endosperm proteins extracted from single grain of different accessions/ cultivars of *F. esculentum* resolved into 39 bands ranging in size from 19 to 104 kDa. Out of these 10 polypeptides showing apparent molecular mass of 91, 87, 78, 76, 58, 56, 32, 28, 23, 19 kDa were monomorphic and 29 polypeptides showing apparent molecular mass of 104, 102, 74, 69, 65, 62, 60, 53, 52, 50, 49, 47, 42, 41, 39, 37, 36, 35, 33, 31, 30, 29, 28, 27, 25, 24.56, 23.84, 23, 21, 20.55 kDa were polymorphic to accessions of this species. Protein bands showing molecular mass of 104, 102 and 53 kDa were detected only in Local, Kamroo local, VL-7 and OC-2. These cultivars were procured from VPKAS, Almora, Uttaranchal. Similarly protein bands showing apparent molecular mass of 34, 41 and 52 kDa were detected only in accessions of *F. esculentum* collected from Arunachal Pradesh. One of the most important features of the SDS PAGE profiles of endosperm proteins was the presence of a 42 kDa band in accessions of *F. esculentum* having winged grains and a 31 kDa band in accessions with grains having prominent striations on the surface. Further a duplex band of size 41 and 39 kDa could be detected only in VL-7. This variety is high yielding and early maturing, suitable for cultivation in the middle hills. The variation in band sizes of endosperm grain protein were mainly seen in the molecular weight range of 102-104 kDa, 60-74 kDa, 33-56 kDa and 24-31 kDa. Analysis of pair wise similarity showed a range of coefficient of similarity from 0.46 to 1.0 between accessions/cultivars belonging to *F. esculentum* (Table 4.7).

SDS PAGE analysis of endosperm protein revealed the presence of 24 bands ranging in size from 19 to 104 kDa. in different accessions of *F. tataricum* and 22

Table 4.7: Similarity matrix, based on Jaccard's similarity coefficient, of the SDS PAGE profile of endosperm proteins of grains of different accessions/cultivars of buckwheat (*Fagopyrum* spp.) studied in the present investigation.

Accessions	IC-188669-1	IC-188669-2	IC-18751-2	IC-18751-3	IC-13376-1	IC-13376-4	IC-13145-2	IC-13145-4	IC-13141-1	IC-13141-5	IC-13417-2	IC-13417-5	EC-323729-1	EC-323729-3	IC-319588	IC-324313	IC-352992	IC-319595	IC-324244	VL-7	Local	Kamroo local	OC-2	KBB-3	Himpriya	Kuppa local	Shimla B-1	SanglaB-1	SanglaB-2	SangalB-3	SanglaB-5	SanglaB-6	SanglaB-7	IC-412744	IC-412863	IC-421598	IC-412722	IC-421597	*F. cymosum*
IC-188669-1	1.00																																						
IC-188669-2	0.93	1.00																																					
IC-18751-2	0.58	0.63	1.00																																				
IC-18751-3	0.61	0.59	0.71	1.00																																			
IC-13376-1	0.50	0.59	0.71	1.00	1.00																																		
IC-13376-4	0.62	0.66	0.67	0.96	0.88	1.00																																	
IC-13145-2	0.61	0.69	0.70	0.71	0.74	0.67	1.00																																
IC-13145-4	0.85	0.85	0.68	0.69	0.74	0.76	0.64	1.00																															
IC-13141-1	0.92	0.92	0.70	0.63	0.66	0.70	0.64	0.96	1.00																														
IC-13141-5	0.50	0.59	0.71	1.00	1.00	0.68	0.74	0.72	0.67	1.00																													
IC-13417-2	0.70	0.70	0.60	0.61	0.63	0.63	0.88	0.66	0.79	0.61	1.00																												
IC-13417-5	0.93	1.00	0.63	0.59	0.61	0.71	0.60	0.83	0.96	0.59	0.59	1.00																											
EC-323729-1	0.93	0.96	0.60	0.61	0.63	0.66	0.62	0.82	0.93	0.61	0.59	1.00	1.00																										
EC-323729-3	0.86	0.93	0.58	0.59	0.63	0.61	0.66	0.82	0.88	0.59	0.65	1.00	0.93	1.00																									
IC-319588	0.69	0.72	0.81	0.58	0.60	0.65	0.57	0.92	0.78	0.60	0.63	0.72	0.72	0.67	1.00																								
IC-324313	0.61	0.65	0.78	0.56	0.58	0.58	0.58	0.75	0.69	0.58	0.61	0.65	0.65	0.65	0.96	1.00																							
IC-352992	0.65	0.63	0.75	0.65	0.61	0.66	0.63	0.85	0.72	0.67	0.65	0.63	0.63	0.63	0.85	0.89	1.00																						
IC-319595	0.70	0.68	0.81	0.65	0.61	0.66	0.51	0.85	0.79	0.61	0.72	0.68	0.68	0.68	0.92	0.96	0.93	1.00																					
IC-324244	0.65	0.63	0.75	0.65	0.61	0.66	0.63	0.85	0.72	0.67	0.65	0.63	0.63	0.63	0.85	0.89	1.00	0.93	1.00																				
VL-7	0.63	0.51	0.52	0.53	0.53	0.51	0.58	0.46	0.49	0.53	0.56	0.46	0.53	0.51	0.59	0.58	0.66	0.66	0.66	1.00																			
Local	0.52	0.55	0.50	0.56	0.56	0.55	0.56	0.48	0.48	0.56	0.58	0.55	0.56	0.55	0.63	0.61	0.61	0.70	0.61	0.93	1.00																		
Kamroo local	0.52	0.55	0.50	0.56	0.56	0.55	0.56	0.48	0.48	0.56	0.58	0.55	0.56	0.55	0.63	0.61	0.61	0.70	0.61	0.93	1.00	1.00																	
OC-2	0.52	0.55	0.50	0.56	0.56	0.55	0.56	0.48	0.48	0.56	0.58	0.55	0.56	0.55	0.63	0.61	0.61	0.70	0.61	0.93	1.00	1.00	1.00																
KBB-3	0.38	0.41	0.48	0.46	0.47	0.56	0.46	0.46	0.42	0.46	0.46	0.41	0.41	0.45	0.47	0.50	0.55	0.49	0.55	0.47	0.57	0.57	0.57	1.00															
Himpriya	0.38	0.41	0.48	0.46	0.47	0.56	0.46	0.46	0.39	0.46	0.46	0.41	0.41	0.45	0.47	0.50	0.55	0.49	0.55	0.47	0.57	0.57	0.57	1.00	1.00														
Kuppa local	0.38	0.41	0.48	0.46	0.47	0.56	0.46	0.46	0.39	0.46	0.46	0.41	0.41	0.45	0.47	0.50	0.55	0.49	0.55	0.47	0.57	0.57	0.57	1.00	1.00	1.00													
Shimla B-1	0.37	0.41	0.52	0.44	0.46	0.58	0.48	0.58	0.47	0.49	0.49	0.43	0.43	0.47	0.52	0.50	0.41	0.49	0.41	0.47	0.50	0.47	0.47	0.90	0.90	0.90	1.00												
SanglaB-1	0.38	0.41	0.48	0.46	0.47	0.56	0.46	0.46	0.39	0.46	0.46	0.41	0.41	0.45	0.47	0.50	0.55	0.49	0.55	0.47	0.57	0.57	0.57	1.00	1.00	1.00	0.90	1.00											
SanglaB-2	0.38	0.41	0.48	0.46	0.47	0.56	0.46	0.46	0.39	0.46	0.46	0.41	0.41	0.45	0.47	0.50	0.55	0.49	0.55	0.47	0.57	0.57	0.57	1.00	1.00	1.00	0.90	1.00	1.00										
SangalB-3	0.38	0.41	0.48	0.46	0.47	0.56	0.46	0.46	0.39	0.46	0.46	0.41	0.41	0.45	0.47	0.50	0.55	0.49	0.55	0.47	0.57	0.57	0.57	1.00	1.00	1.00	0.90	1.00	1.00	1.00									
SanglaB-5	0.38	0.41	0.48	0.46	0.47	0.56	0.46	0.46	0.39	0.46	0.46	0.41	0.41	0.45	0.47	0.50	0.55	0.49	0.55	0.47	0.57	0.57	0.57	1.00	1.00	1.00	0.90	1.00	1.00	1.00	1.00								
SanglaB-6	0.38	0.41	0.48	0.46	0.47	0.56	0.46	0.46	0.39	0.46	0.46	0.41	0.41	0.45	0.47	0.50	0.55	0.49	0.55	0.47	0.57	0.57	0.57	1.00	1.00	1.00	0.90	1.00	1.00	1.00	1.00	1.00							
SanglaB-7	0.38	0.41	0.48	0.46	0.47	0.56	0.46	0.46	0.39	0.46	0.46	0.41	0.41	0.45	0.47	0.50	0.55	0.49	0.55	0.47	0.57	0.57	0.57	1.00	1.00	1.00	0.90	1.00	1.00	1.00	1.00	1.00	1.00						
IC-412744	0.38	0.41	0.48	0.46	0.47	0.56	0.46	0.46	0.39	0.46	0.46	0.41	0.41	0.45	0.47	0.50	0.55	0.49	0.55	0.47	0.57	0.57	0.57	1.00	1.00	1.00	0.90	1.00	1.00	1.00	1.00	1.00	1.00	1.00					
IC-412863	0.38	0.41	0.48	0.46	0.47	0.56	0.46	0.46	0.39	0.46	0.46	0.41	0.41	0.45	0.47	0.50	0.55	0.49	0.55	0.47	0.57	0.57	0.57	1.00	1.00	1.00	0.90	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00				
IC-421598	0.38	0.41	0.48	0.46	0.47	0.56	0.46	0.46	0.39	0.46	0.46	0.41	0.41	0.45	0.47	0.50	0.55	0.49	0.55	0.47	0.57	0.57	0.57	1.00	1.00	1.00	0.90	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00			
IC-412722	0.38	0.41	0.48	0.46	0.47	0.56	0.46	0.46	0.39	0.46	0.46	0.41	0.41	0.45	0.47	0.50	0.55	0.49	0.55	0.47	0.57	0.57	0.57	1.00	1.00	1.00	0.90	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00		
IC-421597	0.38	0.41	0.48	0.46	0.47	0.56	0.46	0.46	0.39	0.46	0.46	0.41	0.41	0.45	0.47	0.50	0.55	0.49	0.55	0.47	0.57	0.57	0.57	1.00	1.00	1.00	0.90	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	
F. cymosum	0.48	0.54	0.47	0.41	0.42	0.58	0.63	0.41	0.56	0.41	0.46	0.54	0.39	0.43	0.50	0.49	0.43	0.47	0.43	0.42	0.41	0.41	0.41	0.75	0.75	0.75	0.83	0.75	0.75	0.75	0.75	0.75	0.75	0.90	0.90	0.90	0.90	0.90	1.00

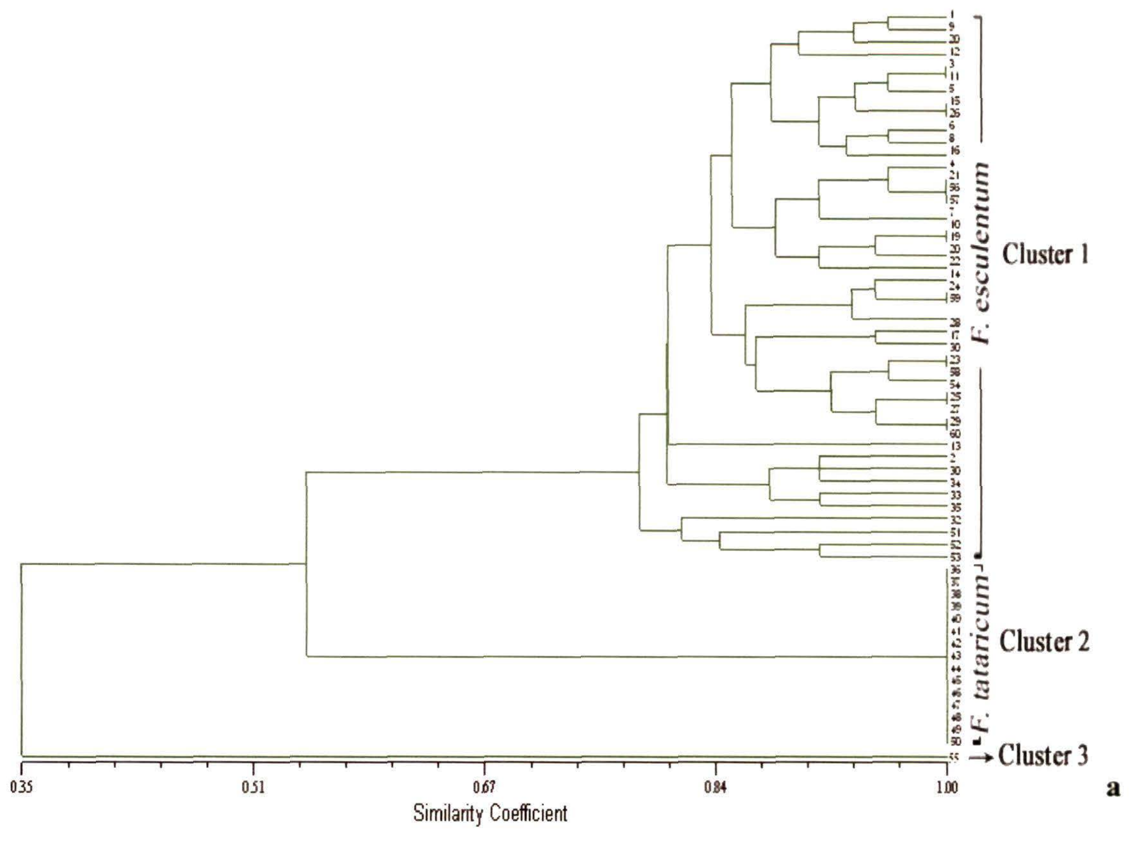
bands ranging from 19 to 104 kDa in *F. cymosum*. Except for Shimla B-1 there was no intraspecific variation in accessions/cultivars of *F. tataricum*. Analysis of pair wise similarity revealed a Jaccard's similarity coefficient between 0.9 to 1.0 amongst the accessions/cultivars of *F. tataricum* and 0.75 with accessions of *F. cymosum*.

The SDS PAGE profile of endosperm proteins of "*F. himalianum*" showed much similarity with that of *F. esculentum*. The Jaccard's similarity coefficient between IC-13145, which has been identified as "*F. himalianum*" by NBPGR, and different accessions/cultivars of *F. esculentum*, however, ranged between 0.46 to 0.96. The variation in the SDS PAGE profile between the two species was mainly observed in the molecular weight range of 27-50 kDa.

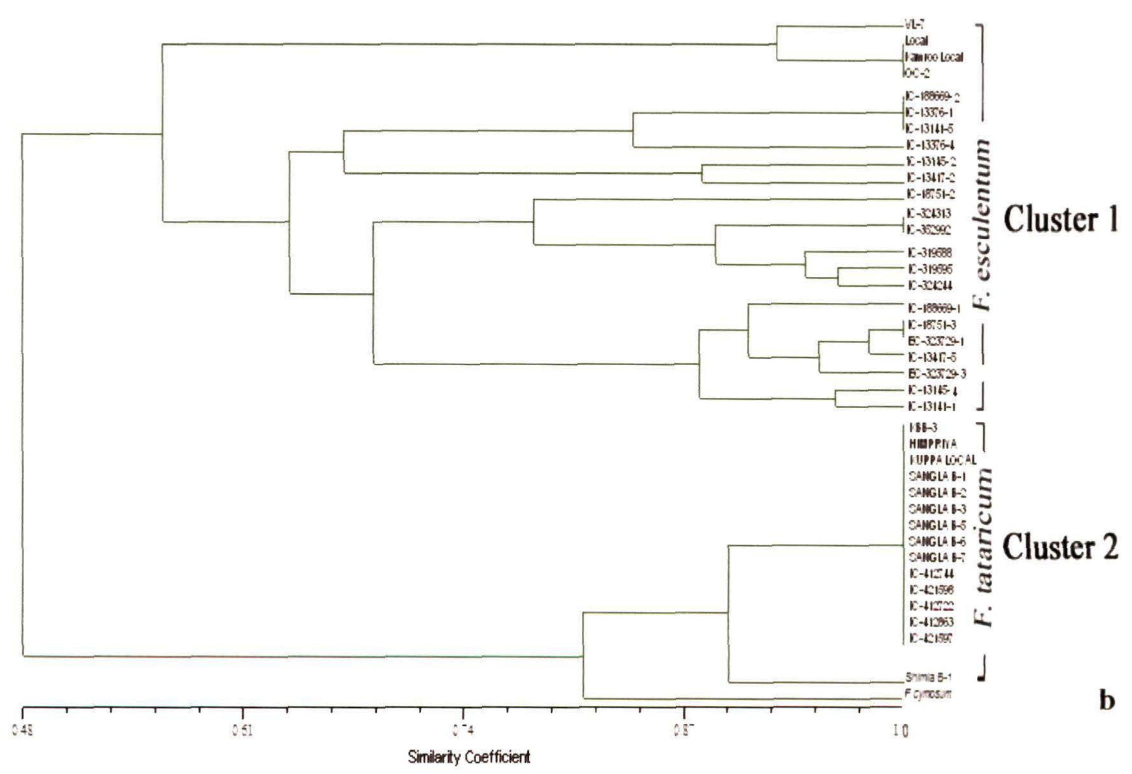
The dendrogram generated on the basis of SDS PAGE profile of endosperm grain proteins revealed the clustering of the accessions/cultivars into three broad clusters (Fig 4.11b). Cluster 1 included all the accessions/cultivars of *F. esculentum*. Within this cluster three different subgroups were noticed. Subgroup I included cultivars designated as Kamroo local, OC-2 and VL-7 which were collected from VPKAS, Almora. Even though Kamroo local and OC-2 have been identified as two different cultivars, they showed 100% identity in endosperm protein profile. Accessions having winged seeds and those having striations on the grain coat formed sub cluster II and III respectively. IC-13145, which had been identified as a separate species *viz.* "*F. himalianum*", showed >90% similarity with IC-13376 (*F. esculentum*). The two accessions clustered together even on the basis of their morphological descriptors. Our results indicate that "*F. himalianum*" belongs to the *esculentum* group rather than qualifying as a different species. Cluster 2 included all the accessions/cultivars of *F. tataricum*. All the accessions/cultivars of *F. tataricum*, except Shimla B-1, clustered into one group with

Fig 4.11 a: Dendrogram based on the UPGMA analysis generated from the similarity matrix developed from the SDS PAGE profiles of total seed proteins extracted from single seeds of different accessions/cultivars of *Fagopyrum* spp. investigated in the present study. **1:** IC-188669-1, **2**: IC-188669-2, **3**: IC-188669-3, **4**: IC-188669-4, **5**: IC-188669-5, **6**: IC-18751-1, **7**: IC-18751-2, **8**: IC-18751-3, **9**: IC-18751-4, **10**: IC-18751-5, **11**: IC-13376-1, **12**: IC-13376-2, **13**: IC-13376-3, **14**: IC-13376-4, **15**: IC-13376-5, **16**: IC-13145-1, **17**: IC-13145-2, **18**: IC-13145-3, **19**: IC-13145-4, **20**: IC-13145-5, **21**: IC-13141-1, **22**: IC-13141-2, **23**: IC-13141-3, **24**: IC-13141-4, **25**: IC-13141-5, **26**: IC-13417-1, **27**: IC-13417-2, **28**: IC-13417-3, **29**: IC-13417-4, **30**: IC-13417-5, **31**: IC-323729-1, **32**: IC-323729-2, **33**: IC-323729-3, **34**: IC-323729-4, **35**: IC-323729-5, **36**: KBB-3, **37**: Himpriya, **38**: Kuppa Local, **39**: Shimla B-1, **40**: Sangla B-1, **41**: Sangla B-2, **42**: Sangla B-3, **43**: Sangla B-5, **44**: Sangla B-6, **45**: Sangla B-7, **46**: 11:IC-412744, **47**: IC-421598, **48**: IC-412722, **49**: IC-412863, **50**: IC-421597, **51**: OC-2, **52**: Kamroo Local, **53**: Local, **54**: VL-7, **55**: *F. cymosum*, **56**: 319588, **57**: IC-324313, **58**: IC-352992, **59**: IC-319595 and **60**: IC-324244.

b: Dendrogram based on the UPGMA analysis generated from the similarity matrix developed from the SDS PAGE profiles of endosperm proteins extracted from single grains of different accessions/cultivars of *Fagopyrum* spp. investigated in the present study.



Cluster 1
Cluster 2
Cluster 3
F. esculentum
F. tataricum
0.35
0.51
0.67
0.84
1.00
Similarity Coefficient
a



VL-7
Local
OC-2
IC-188669-2
IC-13376-1
IC-13141-5
IC-13376-4
IC-13145-2
IC-13417-2
IC-18751-2
IC-324313
IC-319688
IC-319695
IC-324244
IC-188669-1
IC-18751-3
EC-323729-1
IC-13417-5
EC-323729-3
IC-13145-4
IC-13141-1
HIMPRIYA
RUPPA LOCAL
SANGLA B-1
SANGLA B-2
SANGLA B-3
SANGLA B-5
SANGLA B-6
SANGLA B-7
IC-412744
IC-421598
IC-412722
IC-412863
Shimla B-1
F. cymosum
Cluster 1
Cluster 2
F. esculentum
F. tataricum
1.0
Similarity Coefficient
b

100% similarity. Cluster 3 included *F. cymosum*, which clustered as a separate group distinct from both *esculentum* as well as *tataricum*. *F. cymosum* showed least similarity of 39.4% with *F. esculentum* and a maximum of 57% similarity with *F. tataricum*.

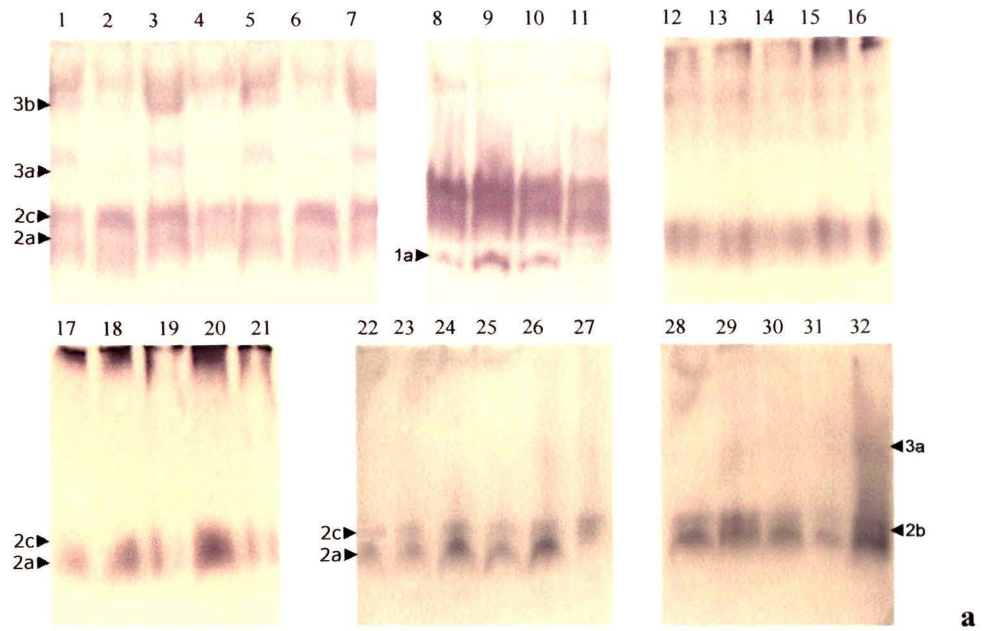
Isozymes:

Isozymes profiles of Malate dehydrogenase (MDH), Phosphoglucomutase (PGM), Peroxidase (PER) and Esterase (EST) were determined in extracts of cotyledonary tissues of 7 day old seedling or young leaves from 2 weeks old plant with 0.1M Tris-Cl buffer (pH 8.0) containing 0.05ml 0.1% 2-Mercaptoetanol, 0.001M EDTA, 0.01M KCL, 0.01M $MgCl_2$ hexahydrate, 1g 4% polyvinyl pyrrolidone (PVP). The extracts were centrifuged at 10000 rpm 15 min at 4^oC. The supernatant was collected and used for isozyme analysis.

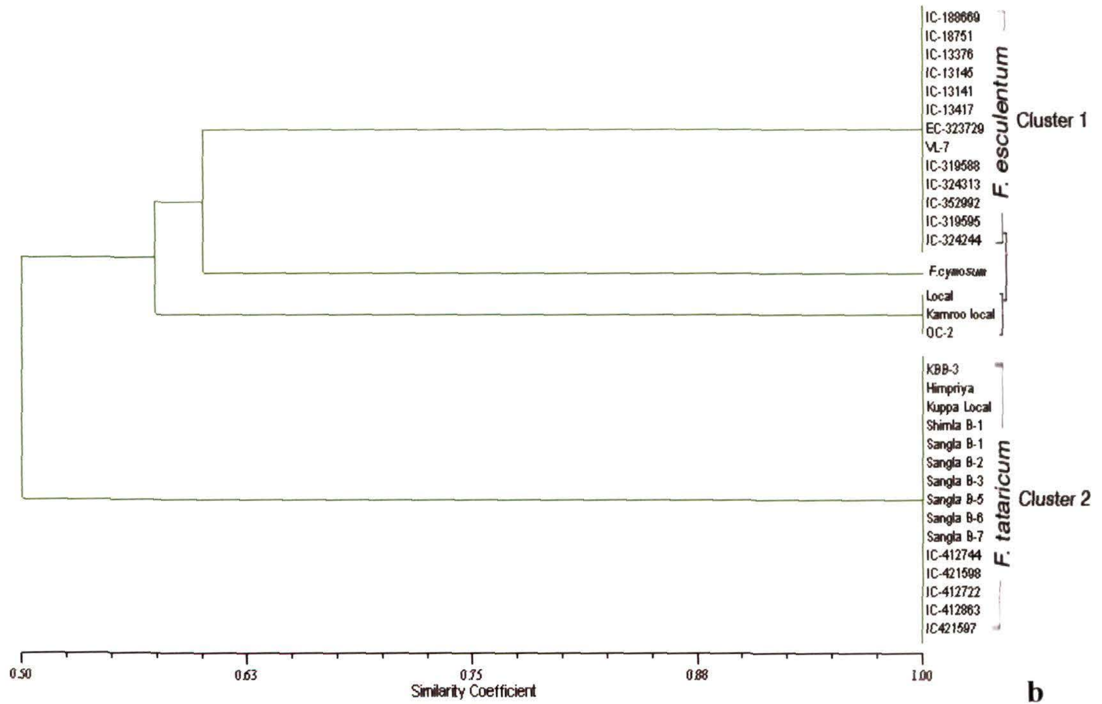
The isozymes profiles of MDH showed 3 zones/loci of activity with a total of 6 bands designated as 1a, 2a, 2b, 2c, 3a & 3b (Fig. 4.12a). Two bands of Rm values 0.55 & 0.68 (alleles 2c and 2a) were monomorphic to all the species. Other bands showing Rm values of 0.20, 0.38, 0.56 & 0.76 (alleles 3b, 3a, 2b and 1a) were polymorphic. The isozyme band of Rm 0.2 representing allele 3b was detected in all the accession/cultivars of *F. esculentum* and *F. himalianum* but not in accessions/cultivars of *F. tataricum* and *F. cymosum*. The isozyme band showing Rm value of 0.76 representing the allele 1a was observed only in three cultivars of *F. esculentum viz.* Local, Kamroo local and OC-2. This allele could not be detected in any other accession/cultivars. On the other hand the cathodal allele 3a having Rm value of 0.38 was observed in all accessions/cultivars of *F. esculentum* except Local, Kamroo local and OC-2, *F. himalianum* and *F. tataricum*. The accessions/cultivars of *F. tataricum* showed the presence of only two bands of Rm values 0.55 & 0.68. These represented

Fig 4.12 a: Isozymes profiles of malate dehydrogenase from cotyledonary tissues of different accessions/cultivars of buckwheat (*Fagopyrum* spp.) investigated in the present study. Lane 1:IC-188669, Lane 2:IC-18751, Lane 3:IC-13376, Lane 4:IC-13145, Lane 5:IC-13141, Lane 6:IC-13417, Lane 7:EC-323729, Lane 8:Local, Lane 9:Kamroo local, Lane 10:OC-2, Lane 11:VL-7, Lane 12:IC-319588, Lane 13:IC-324313, Lane 14:IC-352992, Lane 15:IC-319595, Lane 16:IC-324244: Lane 17:IC-412744, Lane 18:IC-421598, Lane 19:IC-412722, Lane 20:IC-412863, Lane 21:IC-421597, Lane 22:KBB-3, Lane 23:Himpriya, Lane 24:Kuppa local, Lane 25:Shimla B-1, Lane 26:Sangla B-1, Lane 27:Sangla B-2, Lane 28:Sangla B-3, Lane 29:Sangla B-5, Lane 30:Sangla B-6, Lane 31:Sangla B-7 and Lane 32:*F. cymosum.*

b: Dendrogram based on the UPGMA analysis generated from the similarity matrix developed from the isozymes profiles of malate dehydrogenase of different accessions/cultivars of buckwheat (*Fagopyrum* spp.) investigated in the present study.



a



b

Table 4.8: Similarity matrix, generated on the basis of Jaccard's coefficient, of MDH isozyme profiles for different accessions/cultivars of buckwheat (*Fagopyrum* spp.) studied in the present investigation.

Accessions	IC-188669	IC-18751	IC-13376	IC-13145	IC-13141	IC-13417	EC-323729	IC-319588	IC-324313	IC-352992	IC-319595	IC-324244	Local	Kamroo local	OC-2	VL-7	KBB-3	Himpriya	Kuppa local	Shimla B-1	Sangla B-1	Sangla B-2	Sangla B-3	Sangla B-5	Sangla B-6	Sangla B-7	IC-412744	IC-412863	IC-421598	IC-412722	IC-421597	*F. cymosum*
IC-188669	1.00																															
IC-18751	1.00	1.00																														
IC-13376	1.00	1.00	1.00																													
IC-13145	1.00	1.00	1.00	1.00																												
IC-13141	1.00	1.00	1.00	1.00	1.00																											
IC-13417	1.00	1.00	1.00	1.00	1.00	1.00																										
EC-323729	1.00	1.00	1.00	1.00	1.00	1.00	1.00																									
IC-319588	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00																								
IC-324313	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00																							
IC-352992	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00																						
IC-319595	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00																					
IC-324244	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00																				
Local	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	1.00																			
Kamroo local	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	1.00	1.00																		
OC-2	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	1.00	1.00	1.00																	
VL-7	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.50	0.50	0.50	1.00																
KBB-3	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	1.00															
Himpriya	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	1.00	1.00														
Kuppa local	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	1.00	1.00	1.00													
Shimla B-1	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	1.00	1.00	1.00	1.00												
Sangla B-1	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	1.00	1.00	1.00	1.00	1.00											
Sangla B-2	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	1.00	1.00	1.00	1.00	1.00	1.00										
Sangla B-3	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	1.00	1.00	1.00	1.00	1.00	1.00	1.00									
Sangla B-5	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00								
Sangla B-6	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00							
Sangla B-7	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00						
IC-412744	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00					
IC-412863	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00				
IC-421598	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00			
IC-412722	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00		
IC-421597	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	
F. cymosum	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.33	0.33	0.33	0.60	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	1.00

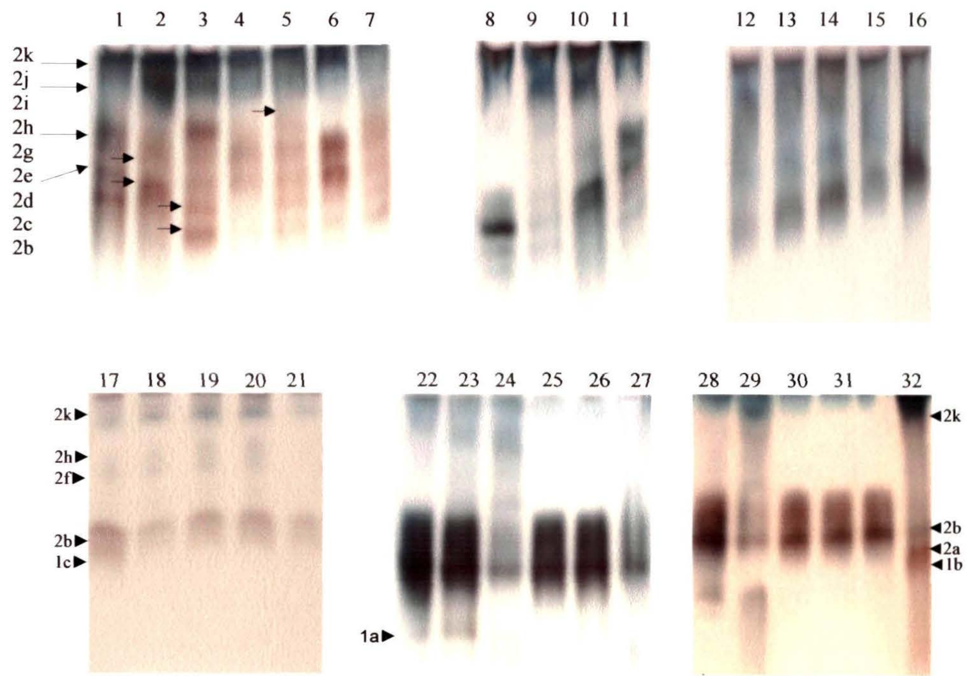
the alleles 2c and 2a. *F. cymosum* showed the presence of alleles 2a, 2b, 2c & 3a alleles. Analysis of pair wise similarity showed a range of coefficient of similarity from 0.33 between *F. cymosum* and the cultivars Local, Kamroo Local and OC-2 of *F. esculentum* to 1.0 within the accessions of *F. esculentum* except the cultivars Local, Kamroo Local and OC-2. Accessions/cultivars of *F. tataricum* also showed a similarity coefficient of 1.0 for MDH isozymes (Table 4.8). The dendrogram generated on the basis of similarity matrix for isozymes of MDH revealed clustering of the accessions/cultivars into two distinct clusters (Fig. 4.12b). Cluster 1 comprised of 15 accessions/cultivars of *F. esculentum*, IC-13145 "*F. himalianum*" and *F. cymosum.* Cluster 2 comprised of all the accessions/cultivars of *F. tataricum.* The dendrogram revealed a similarity coefficient of 0.5 between cluster 1 and cluster 2.

The electrophoretic banding pattern of isozymes of PER revealed a total of 14 bands. While the accessions/cultivars of *F. esculentum* showed 9 bands those of *F. tataricum* showed 6 bands for PER activity. *F. cymosum* showed 4 bands for activity of isozymes of peroxidase (Fig. 4.13a).

Analysis of pair wise similarity revealed a range of value for coefficient of similarity, from minimum of 0.11 between accessions of *F. esculentum* and *F. cymosum* to 1.0 within accessions/cultivars of *F. esculentum* and *F. tataricum* (Table 4.9). The dendrogram generated on the basis of similarity matrix for isozymes of PER revealed clustering of the accessions/cultivars into three distinct clusters (Fig. 4.13b). Cluster 1 comprised accessions/cultivars of *F. esculentum* and *F. himalianum.* While the accessions/cultivars belonging to *F. tataricum* comprised the 2nd cluster and *F. cymosum* emerged out as a separate cluster as cluster 3.

Fig 4.13 a: Isozymes profiles of peroxidase from leaf tissues of different accessions/cultivars of buckwheat (*Fagopyrum* spp.) investigated in the present study. Lane 1:IC-188669, Lane 2:IC-18751, Lane 3:IC-13376, Lane 4:IC-13145, Lane 5:IC-13141, Lane 6:IC-13417, Lane 7:EC-323729, Lane 8:Local, Lane 9:Kamroo local, Lane 10:OC-2, Lane 11:VL-7, Lane 12:IC-319588, Lane 13:IC-324313, Lane 14:IC-352992, Lane 15:IC-319595, Lane 16:IC-324244, Lane 17:IC-412744, Lane 18:IC-421598, Lane 19:IC-412722, Lane 20:IC-412863, Lane 21:IC-421597, Lane 22:KBB-3, Lane 23:Kuppa local, Lane 24:Himpriya, Lane 25: SanglaB-1, Lane 26:Sangla B-2, Lane 27:Sangla B-3, Lane 28:Sangla B-5, Lane 29:Shimla B-1, Lane 30: Sangla B-6, Lane 31:Sangla B-7 and Lane 23:*F. cymosum*.

b: Dendrogram based on the UPGMA analysis generated from similarity matrix developed from the isozymes profiles of peroxidase of different accessions/cultivars of *Fagopyrum* spp.



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16
2k
2j
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2g
2e
2d
2c
2b
17
18
19
20
21
22
23
24
25
26
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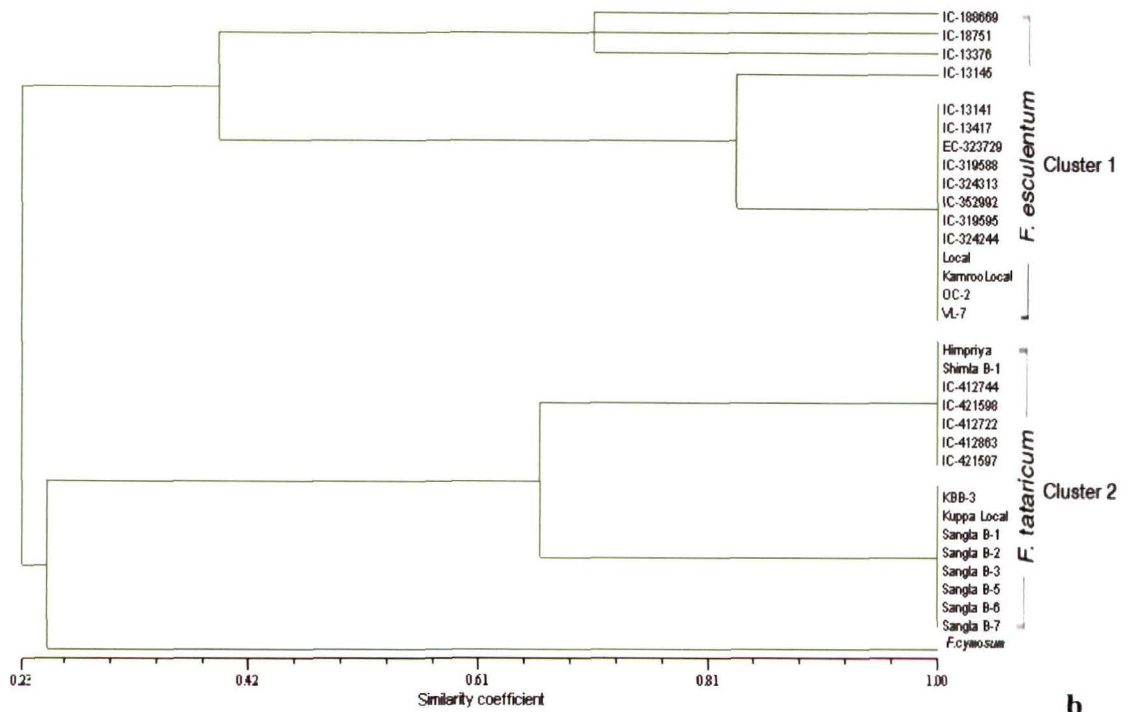


IC-188669
IC-18751
IC-13376
IC-13145
IC-13141
IC-13417
EC-323729
IC-319588
IC-324313
IC-352992
IC-319595
IC-324244
Local
Kamroo Local
OC-2
VL-7
Himpriya
Shimla B-1
IC-412744
IC-421598
IC-412722
IC-412863
IC-421597
KBB-3
Kuppa Local
Sangla B-1
Sangla B-2
Sangla B-3
Sangla B-5
Sangla B-6
Sangla B-7
F.cymosum
F. esculentum
Cluster 1
F. tataricum
Cluster 2
0.23
0.42
0.61
0.81
1.00
Similarity coefficient
b

Table 4.9: Similarity matrix, generated on the basis of Jaccard's coefficient, of PER isozyme profiles for different accessions/cultivars of buckwheat (*Fagopyrum* spp.) studied in the present investigation.

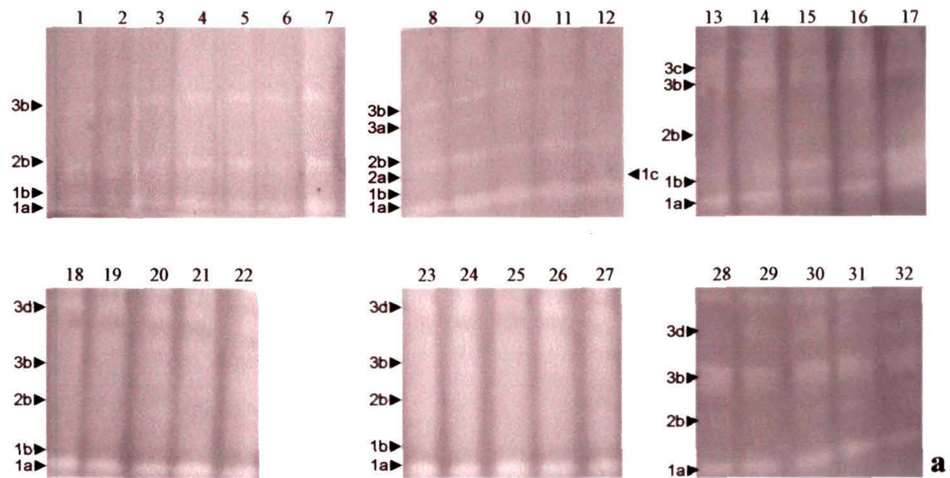
Accessions	IC-188669	IC-18751	IC-13376	IC-13145	IC-13141	IC-13417	EC-323729	IC-319588	IC-324313	IC-352992	IC-319595	IC-324244	Local	Kamroo local	OC-2	VL-7	KBB-3	Kuppa local	Himpriya	Shimla B-1	Sangla B-1	Sangla B-2	Sangla B-3	Sangla B-5	Sangla B-6	Sangla B-7	IC-412744	IC-412863	IC-421598	IC-412722	IC-421597	*F. cymosum*
IC-188669	1.00																															
IC-18751	0.71	1.00																														
IC-13376	0.71	0.71	1.00																													
IC-13145	0.38	0.38	0.57	1.00																												
IC-13141	0.33	0.50	0.50	0.83	1.00																											
IC-13417	0.33	0.50	0.33	0.83	1.00	1.00																										
EC-323729	0.33	0.50	0.33	0.83	1.00	1.00	1.00																									
IC-319588	0.33	0.50	0.33	0.83	1.00	1.00	1.00	1.00																								
IC-324313	0.33	0.50	0.33	0.83	1.00	1.00	1.00	1.00	1.00																							
IC-352992	0.33	0.50	0.33	0.83	1.00	1.00	1.00	1.00	1.00	1.00																						
IC-319595	0.33	0.50	0.33	0.83	1.00	1.00	1.00	1.00	1.00	1.00	1.00																					
IC-324244	0.33	0.50	0.33	0.83	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00																				
Local	0.33	0.50	0.33	0.83	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00																			
Kamroo local	0.33	0.50	0.33	0.83	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00																		
OC-2	0.33	0.50	0.33	0.83	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00																	
VL-7	0.33	0.50	0.33	0.83	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00																
KBB-3	0.33	0.20	0.18	0.38	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	1.00															
Kuppa local	0.33	0.20	0.18	0.38	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	1.00	1.00														
Himpriya	0.33	0.33	0.30	0.38	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.71	0.71	1.00													
Shimla B-1	0.33	0.33	0.33	0.38	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.71	0.71	1.00	1.00												
Sangla B-1	0.33	0.20	0.18	0.38	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	1.00	1.00	0.71	0.71	1.00											
Sangla B-2	0.33	0.20	0.18	0.38	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	1.00	1.00	0.71	0.71	1.00	1.00										
Sangla B-3	0.33	0.20	0.18	0.38	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	1.00	1.00	0.71	0.71	1.00	1.00	1.00									
Sangla B-5	0.33	0.20	0.18	0.38	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	1.00	1.00	0.71	0.71	1.00	1.00	1.00	1.00								
Sangla B-6	0.33	0.20	0.18	0.38	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	1.00	1.00	0.71	0.71	1.00	1.00	1.00	1.00	1.00							
Sangla B-7	0.33	0.20	0.18	0.38	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	1.00	1.00	0.71	0.71	1.00	1.00	1.00	1.00	1.00	1.00						
IC-412744	0.33	0.33	0.30	0.38	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.71	0.71	1.00	1.00	0.71	0.71	0.71	0.71	0.71	0.71	1.00					
IC-412863	0.33	0.33	0.30	0.38	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.71	0.71	1.00	1.00	0.71	0.71	0.71	0.71	0.71	0.71	1.00	1.00				
IC-421598	0.33	0.33	0.30	0.38	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.71	0.71	1.00	1.00	0.71	0.71	0.71	0.71	0.71	0.71	1.00	1.00	1.00			
IC-412722	0.33	0.33	0.30	0.38	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.71	0.71	1.00	1.00	0.71	0.71	0.71	0.71	0.71	0.71	1.00	1.00	1.00	1.00		
IC-421597	0.33	0.33	0.30	0.38	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.71	0.71	1.00	1.00	0.71	0.71	0.71	0.71	0.71	0.71	1.00	1.00	1.00	1.00	1.00	
F. cymosum	0.11	0.11	0.25	0.29	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	1.00

The electrophoretic banding pattern of PGM isozymes revealed a total of 9 bands (Fig. 4.14a). Out of these one band with Rm value of 0.76 and representing the allele 1b was monomorphic and 8 bands with Rm values of 0.086, 0.17, 0.21, 0.34, 0.58, 0.69, 0.72 & 0.86 were polymorphic. One of the significant features of the profile was the presence of band having Rm value of 0.17 representing allele 3c in accessions of *F. esculentum* collected from Arunachal Pradesh and Uttaranchal. While the band at Rm 0.08 representing the allele 3d was exclusively found in accessions of *F. tataricum,* Rm value of 0.72 representing the allele 1c was exclusive to *F. cymosum.*

Cluster analysis of the similarity matrix generated from the electrophoretic profile of isozymes of PGM revealed clustering of the accessions/cultivars into two distinct clusters (Fig. 4.14b). Accessions/cultivars of *F. esculentum*, *F. himalianum* and *F. cymosum* clustered together in one cluster. Cluster 1 revealed three sub clusters within it. The entire collection of accessions of *F. esculentum* from Himachal Pradesh showed a similarity coefficient of 1.0 amongst them and formed one subgroup. The cultivars Local, Kamroo Local, OC-2 and VL-7 from Uttaranchal showed 100% similarity amongst them and formed another subgroup. All other accessions of *F. esculentum* except IC-319588 Arunachal Pradesh showed 100% similarity within them and clustered together as the 3rd subcluster within cluster 1. Cluster 2 comprised of all the accessions/cultivars of *F. tataricum*. Two sub clusters were observed within this cluster, the cultivars from Uttaranchal clustered in one sub group while the accessions from Arunachal Pradesh formed another sub group. *F. cymosum* showed a mean closeness of 48% with accessions/cultivars of *F. esculentum*, while 38% with the accessions/cultivars of *F. tataricum* (Table 4.10) and clustered along with cluster 1.

Fig 4.14 a: Isozymes profiles of phosphoglucomutase from cotyledonary tissues of different accessions/cultivars of buckwheat (*Fagopyrum* spp.) investigated in the present study. Lane 1:IC-188669, Lane 2:IC-18751, Lane 3:IC-13376, Lane 4:IC-13145, Lane 5:IC-13141, Lane 6:IC-13417, Lane 7:EC-323729, Lane 8:Local, Lane 9:Kamroo local, Lane 10:OC-2, Lane 11:VL-7, Lane 12:*F. cymosum,* Lane 13:IC-319588, Lane 14:IC-324313, Lane 15:IC-352992, Lane 16:IC-319595, Lane 17:IC-324244, Lane 18:KBB-3, Lane 19:Himpriya, Lane 20:Kuppa, local, Lane 21:Shimla B-1, Lane 22:Sangla B-1, Lane 23:Sangla B-2, Lane 24:Sangla B-3, Lane 25:Sangla B-5, Lane 26:Sangla B-6, Lane 27:Sangla B-7, Lane 28:IC-412744, Lane 29:IC-421598, Lane 30:IC-412722, Lane 31:IC-412863 and Lane 32:IC-421597.

b: Dendrogram based on the UPGMA analysis generated from similarity matrix developed from the isozymes profiles of phosphoglucomutase of different accessions/cultivars of *Fagopyrum* spp.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3b
2b
1b
1a
8 9 10 11 12
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18 19 20 21 22
3d
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3d
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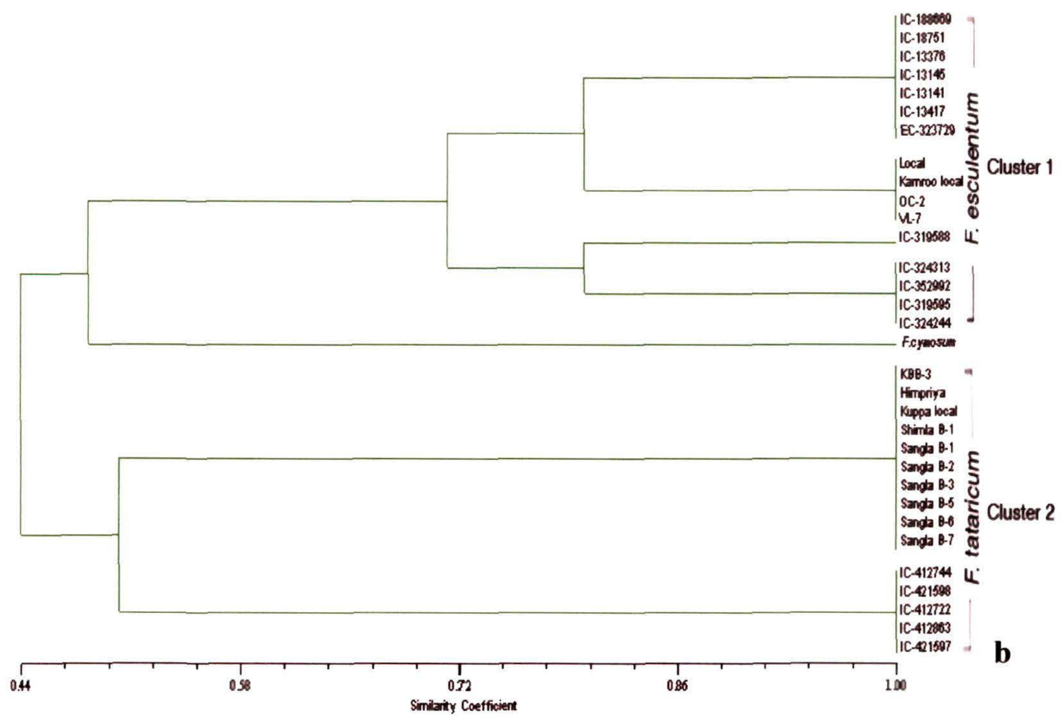

IC-188669
IC-18751
IC-13376
IC-13146
IC-13141
IC-13417
EC-323729
Local
Kamroo local
OC-2
VL-7
IC-319588
IC-324313
IC-352992
IC-319595
IC-324244
F.cymosum
KBB-3
Himpriya
Kuppa local
Shimla B-1
Sangla B-1
Sangla B-2
Sangla B-3
Sangla B-5
Sangla B-6
Sangla B-7
IC-412744
IC-421598
IC-412722
IC-412863
IC-421597
F. esculentum
Cluster 1
F. tataricum
Cluster 2
b
0.44
0.58
0.72
0.86
1.00
Similarity Coefficient

Table 4.10: Similarity matrix, generated on the basis of Jaccard's coefficient, of PGM isozyme profiles for different accessions/cultivars of buckwheat (*Fagopyrum* spp.) studied in the present investigation.

Accessions	IC-188669	IC-18751	IC-13376	IC-13145	IC-13141	IC-13417	EC-323729	IC-319588	IC-324313	IC-352992	IC-319595	IC-324244	Local	Kamroo local	OC-2	VL-7	KBB-3	Himpriya	Kuppa local	Shimla B-1	Sangla B-1	Sangla B-2	Sangla B-3	Sangla B-5	Sangla B-6	Sangla B-7	IC-412744	IC-412863	IC-421598	IC-412722	IC-421597	*F. cymosum*
IC-188669	1.00																															
IC-18751	1.00	1.00																														
IC-13376	1.00	1.00	1.00																													
IC-13145	1.00	1.00	1.00	1.00																												
IC-13141	1.00	1.00	1.00	1.00	1.00																											
IC-13417	1.00	1.00	1.00	1.00	1.00	1.00																										
EC-323729	1.00	1.00	1.00	1.00	1.00	1.00	1.00																									
IC-319588	0.60	0.60	0.60	0.60	0.60	0.60	0.60	1.00																								
IC-324313	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	1.00																							
IC-352992	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	1.00	1.00																						
IC-319595	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	1.00	1.00	1.00																					
IC-324244	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	1.00	1.00	1.00	1.00																				
Local	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.50	0.66	0.66	0.66	0.66	1.00																			
Kamroo local	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.50	0.66	0.66	0.66	0.66	1.00	1.00																		
OC-2	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.50	0.66	0.66	0.66	0.66	1.00	1.00	1.00																	
VL-7	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.50	0.66	0.66	0.66	0.66	1.00	1.00	1.00	1.00																
KBB-3	0.44	0.44	0.44	0.44	0.44	0.44	0.44	0.50	0.43	0.43	0.43	0.43	0.66	0.66	0.66	0.66	1.00															
Himpriya	0.44	0.44	0.44	0.44	0.44	0.44	0.44	0.50	0.43	0.43	0.43	0.43	0.66	0.66	0.66	0.66	1.00	1.00														
Kuppa local	0.44	0.44	0.44	0.44	0.44	0.44	0.44	0.50	0.43	0.43	0.43	0.43	0.66	0.66	0.66	0.66	1.00	1.00	1.00													
Shimla B-1	0.44	0.44	0.44	0.44	0.44	0.44	0.44	0.50	0.43	0.43	0.43	0.43	0.66	0.66	0.66	0.66	1.00	1.00	1.00	1.00												
Sangla B-1	0.44	0.44	0.44	0.44	0.44	0.44	0.44	0.50	0.43	0.43	0.43	0.43	0.66	0.66	0.66	0.66	1.00	1.00	1.00	1.00	1.00											
Sangla B-2	0.44	0.44	0.44	0.44	0.44	0.44	0.44	0.50	0.43	0.43	0.43	0.43	0.66	0.66	0.66	0.66	1.00	1.00	1.00	1.00	1.00	1.00										
Sangla B-3	0.44	0.44	0.44	0.44	0.44	0.44	0.44	0.50	0.43	0.43	0.43	0.43	0.66	0.66	0.66	0.66	1.00	1.00	1.00	1.00	1.00	1.00	1.00									
Sangla B-5	0.44	0.44	0.44	0.44	0.44	0.44	0.44	0.50	0.43	0.43	0.43	0.43	0.66	0.66	0.66	0.66	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00								
Sangla B-6	0.44	0.44	0.44	0.44	0.44	0.44	0.44	0.50	0.43	0.43	0.43	0.43	0.66	0.66	0.66	0.66	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00							
Sangla B-7	0.44	0.44	0.44	0.44	0.44	0.44	0.44	0.50	0.43	0.43	0.43	0.43	0.66	0.66	0.66	0.66	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00						
IC-412744	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.14	0.29	0.29	0.29	0.29	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	1.00					
IC-412863	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.14	0.29	0.29	0.29	0.29	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	1.00	1.00				
IC-421598	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.14	0.29	0.29	0.29	0.29	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	1.00	1.00	1.00			
IC-412722	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.14	0.29	0.29	0.29	0.29	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	1.00	1.00	1.00	1.00		
IC-421597	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.14	0.29	0.29	0.29	0.29	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	1.00	1.00	1.00	1.00	1.00	
F. cymosum	0.44	0.44	0.44	0.44	0.44	0.44	0.44	0.60	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.16	0.16	0.16	0.16	0.16	1.00

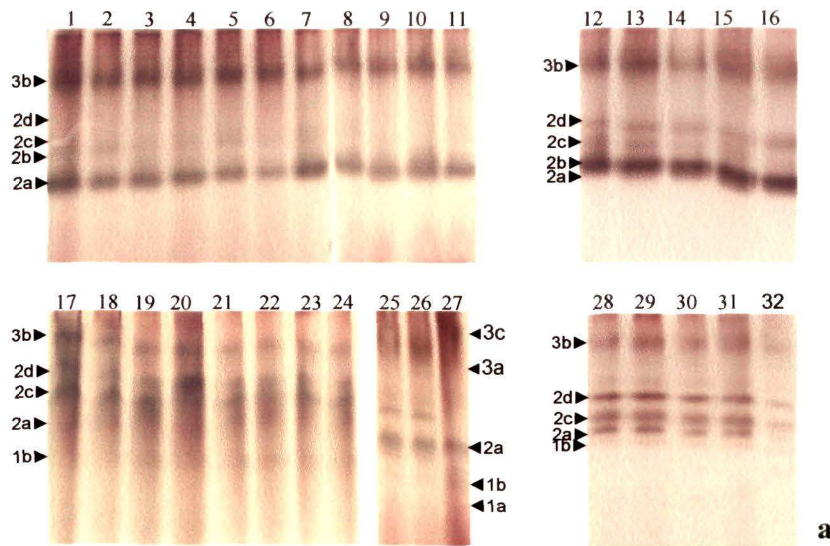
The electrophoretic profile for isozymes of EST revealed three loci of activity with a total of nine alleles. While the anodal alleles were designated as 1a and1b the cathodal alleles were designated as 3a, 3b and 3c. The alleles which were intermediate between the anodal and cathodal ends were designated as 2a, 2b, 2c and 2d (Fig. 4.15a). Five alleles were observed amongst the accessions/cultivars of *F. esculentum.* One of these was cathodal and four alleles were intermediate in position. The profile did not reveal the presence of any anodal allele in accessions/cultivars of *F. esculentum* and *F. himalianum.* Accessions/cultivars of *F. tataricum* had five alleles of EST. Out of these allele one was cathodal, three were intermediate in position and one was anodal. The isozymes profile of *F. cymosum* showed five alleles out of which two were cathodal, two were anodal and one was intermediate in position. No intraspecific variation was observed in any of the species studied. Pairwise genetic similarities observed between *esculentum* and *cymosum* was 0.25, between *esculentum* and *tataricum* was 0.67 and between *tataricum* and *cymosum* was 0.25. There was no intraspecific variation observed in any of the species studied (Table 4.11).

The dendrogram constructed with UPGMA clustering of the similarity matrix generated from the EST isozymes profiles revealed clustering of accessions/cultivars into three distinct clusters. Accessions/cultivars of *F. esculentum* and *F. himalianum* clustered together in one cluster. Cluster 2 comprised of all the accessions/cultivars of *F. tataricum*. *F. cymosum,* which was observed to be equidistant from *F. esculentum* as well as *F. tataricum*, formed the 3rd cluster (Fig. 4.15b).

The values for %age of polymorphism contributed by each isozymes and the average polymorphism of all the isozymes are presented in Table 4.12. It describes

Fig 4.15 a: Isozymes profiles of esterase from cotyledonary tissues of different accessions/cultivars of buckwheat (*Fagopyrum* spp.) investigated in the present study. Lane 1:IC-188669, Lane 2:IC-18751, Lane 3:IC-13376, Lane 4 IC:13145, Lane 5:IC-13141, Lane 6:IC-13417, Lane 7:EC-323729, Lane 8:VL-7, Lane 9:OC-2, Lane 10:Kamroo local, Lane 11:Local, Lane 12:IC-319588, Lane 13:IC-324313, Lane 14:IC-352992, Lane 15:IC-319595, Lane 16:IC-324244, Lane 17:KBB-3, Lane 18:Himpriya, Lane 19:Kuppa local, Lane 20:Shimla B-1, Lane 21:Sangla B-1, Lane 22:Sangla B-2, Lane 23:Sangla B-3, Lane 24:Sangla B-5, Lane 25:Sangla B-6, Lane 26:Sangla B-7, 27:*F .cymosum,* 28:IC-412744, 29:IC-421598, Lane 30:IC-412722, Lane 31:IC-412863 and Lane 32:IC-421597.

b: Dendrogram based on the UPGMA analysis generated from similarity matrix developed from the isozymes profiles of esterase of different accessions/cultivars of *Fagopyrum* spp.


1 2 3 4 5 6 7 8 9 10 11
12 13 14 15 16
3b
2d
2c
2b
2a
17 18 19 20 21 22 23 24 25 26 27
28 29 30 31 32
3c
3a
1b
1a
a

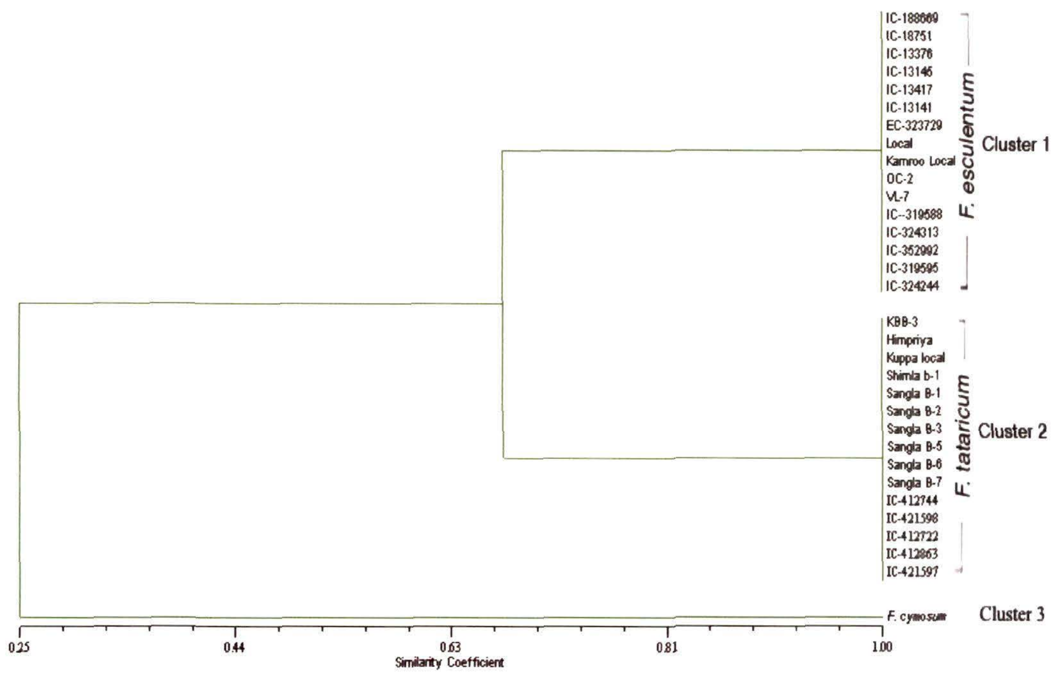

IC-188669
IC-18751
IC-13376
IC-13146
IC-13417
IC-13141
EC-323729
Local
Kamroo Local
OC-2
VL-7
IC--319588
IC-324313
IC-352992
IC-319595
IC-324244
KBB-3
Himpriya
Kuppa local
Shimla b-1
Sangla B-1
Sangla B-2
Sangla B-3
Sangla B-5
Sangla B-6
Sangla B-7
IC-412744
IC-421598
IC-412722
IC-412863
IC-421597
F. cymosum
F. esculentum
F. tataricum
Cluster 1
Cluster 2
Cluster 3
0.25
0.44
0.63
0.81
1.00
Similarity Coefficient
b

Table 4.11: Similarity matrix, generated on the basis of Jaccard's coefficient, of EST isozyme profiles for different accessions/cultivars of buckwheat (*Fagopyrum* spp.) studied in the present investigation.

Accessions	IC-188669	IC-18751	IC-13376	IC-13145	IC-13141	IC-13417	EC-323729	IC-319588	IC-324313	IC-352992	IC-319595	IC-324244	Local	Kamroo local	OC-2	VL-7	KBB-3	Himpriya	Kuppa local	Shimla B-1	Sangla B-1	Sangla B-2	Sangla B-3	Sangla B-5	Sangla B-6	Sangla B-7	IC-412744	IC-412863	IC-421598	IC-412722	IC-421597	*F. cymosum*
IC-188669	1.00																															
IC-18751	1.00	1.00																														
IC-13376	1.00	1.00	1.00																													
IC-13145	1.00	1.00	1.00	1.00																												
IC-13141	1.00	1.00	1.00	1.00	1.00																											
IC-13417	1.00	1.00	1.00	1.00	1.00	1.00																										
EC-323729	1.00	1.00	1.00	1.00	1.00	1.00	1.00																									
IC-319588	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00																								
IC-324313	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00																							
IC-352992	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00																						
IC-319595	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00																					
IC-324244	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00																				
Local	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00																			
Kamroo local	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00																		
OC-2	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00																	
VL-7	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00																
KBB-3	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	1.00															
Himpriya	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	1.00	1.00														
Kuppa local	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	1.00	1.00	1.00													
Shimla B-1	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	1.00	1.00	1.00	1.00												
Sangla B-1	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	1.00	1.00	1.00	1.00	1.00											
Sangla B-2	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	1.00	1.00	1.00	1.00	1.00	1.00										
Sangla B-3	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	1.00	1.00	1.00	1.00	1.00	1.00	1.00									
Sangla B-5	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00								
Sangla B-6	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00							
Sangla B-7	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00						
IC-412744	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00					
IC-412863	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00				
IC-421598	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00			
IC-412722	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00		
IC-421597	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	
F. cymosum	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	1.00

Table 4.12: Polymorphic percentage and polymorphism information content derived from analysis of isozyme profiles of malate dehydrogenase, phosphoglucomutase, peroxidase and esterase from leaf tissues of different accessions/cultivars of buckwheat (*Fagopyrum* spp.) studied in the present investigation.

Enzyme	Total number of bands	Number of polymorphic bands	% age polymorphism	Average frequency of polymorphic bands	PIC
MDH	6	4	66.66	0.51	0.49
PGM	9	8	88.88	0.52	0.50
PER	14	13	92.85	0.41	0.48
EST	9	8	88.88	0.56	0.49
Total	38	33	86.80	Average	0.49

average frequency of polymorphic bands by isozymes and the polymorphism information content. While isozymes profile of PER showed 92.85% polymorphism in the banding pattern isozymes profile of EST and PGM showed 88.88 percent polymorphism. The lowest level of polymorphism was recorded for isozymes profile of MDH. This enzyme showed 66.6% polymorphism in the banding pattern. The isozymes profiles of PGM and MDH showed the highest values for PIC (polymorphism information content) followed by isozymes profiles of EST and PER. As the polymorphism exhibited by all the isozymes were close to each other and very close to average PIC value, all the isozymes were suitable for characterization of *Fagopyrum* germplasm.

RAPD profiling & amplification of *trn*C- *rpo*B spacer region:

Random amplified Polymorphic DNA (RAPD) profiles for different accessions/ cultivars of buckwheat used in the present study were generated by PCR amplification of genomic DNA isolated from different accessions/cultivars of buckwheat. PCR was carried out in a Techne thermal cycler programmed for 35 cycles with an initial strand separation at 94 °C for 5 min and 94 °C for 1 min., followed by annealing at 37 °C for 1 min and extension at 72° C for 1 min. After 35 cycles there was a final extension at 72°C for 10 min. The amplifications were carried out under optimized reaction conditions with amplification conditions being identical for all the reactions. Optimization of RAPD assay was done by titrating different concentration of DNA samples, dNTPs, primers and *Taq* polymerase enzyme. In the present study, six initial reactions were set up with DNA concentration of 50 and 100 ng $25\mu l^{-1}$ of reaction volume and three different concentrations *viz.* 1.5mM, 2.0mM and 2.5mM of $MgCl_2$. The PCR assay was optimized with 100 ng DNA, 200 µm dNTPs, 100 pM primers, 1.5

mM $MgCl_2$ and 1 unit of *Taq* DNA polymerase. Each set of reactions was repeated thrice and only the reproducible ones were included in the analysis. For the present study, fifteen decamer primers were used to analyze the RAPD variation in 32 accessions/cultivars of buckwheat from Indian Himalayas. Out of these, only five primers produced reproducible polymorphic amplified products. These primers produced a total of 150 bands ranging in size from 70-2152bp.

The CTAB extraction protocol used in the current investigation yielded good quality DNA from etiolated buckwheat seedlings. Analysis of the isolated DNA revealed that the preparation was free of RNA and had not undergone any shearing during isolation. The protocol gave a yield of about 20 μgm of DNA gm^{-1} tissue with a 260/280 absorption ratio of 1.9. Under UV light, the DNA was detected as a single high molecular band corresponding to a molecular mass of approximately 25 kb in all species taken for study (Fig. 4.16).

Amplification of genomic DNA with primer 1 (OPD-6) resulted in the amplification of fourteen polymorphic bands ranging in size from 250 bp to 2152 bp (Fig. 4.17a-c). The number of amplified products in different accessions ranged from 7 to 14, with an average of 11 bands per accession. A significant feature of the profiles obtained with primer 1 was the detection of a 851 bp RAPD in accessions of *F. esculentum* only. While all the accessions of *F. esculentum* collected from Himachal Pradesh and Arunachal Pradesh showed the presence of a 741 bp RAPD those from Arunachal Pradesh showed the presence of RAPDs having molecular mass of 600 and 1558bp. The 600 and 1558bp amplicons could not be detected in accessions/cultivars of *F. esculentum* collected from either Uttaranchal or Himachal Pradesh. The profiles did not reveal any prominent differences among accessions having winged or non-winged

Fig 4.16: Electrophoretic profile of total genomic DNA isolated from 7 day old etiolated seedlings of different accessions of buckwheat (*Fagopyrum* spp.).

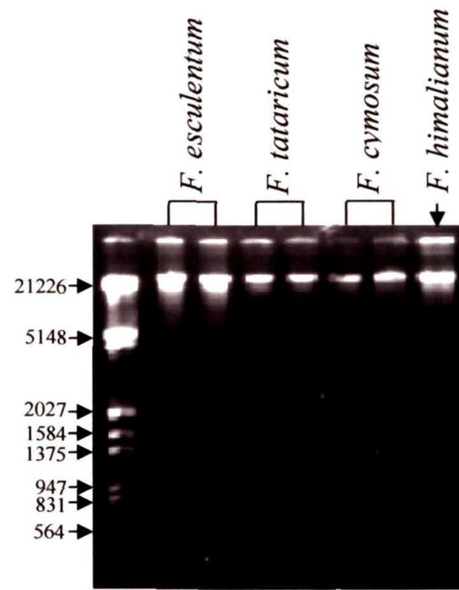
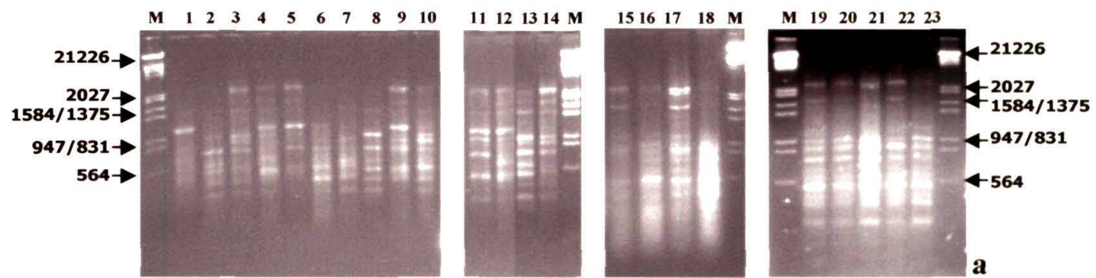

F. esculentum
F. tataricum
F. cymosum
F. himalianum
21226
5148
2027
1584
1375
947
831
564

Fig 4.17: RAPD profile of genomic DNA extracted from 7 day old etiolated seedlings of different accessions/cultivars of buckwheat (*Fagopyrum* spp.) studied in the present investigation using the primer OPD-6. **a:** Lane 1:IC-13417-2, Lane 2:IC-13376-1, Lane 3:IC-13376-4, Lane 4:IC-13141-5, Lane 5:IC-188669-1, Lane 6:IC-188669-2, Lane 7:IC-18751-2, Lane 8:IC-13145-4, Lane 9:IC-13145-2, Lane 10:IC-13141-1, Lane 11:IC-18751-3, Lane 12:IC-13417-5, Lane 13:EC-323729-1, Lane 14:EC-323729-3, Lane 15:OC-2, Lane 16:VL-7, Lane 17:Local, Lane 18:Kamroo Local, Lane 19:IC-319588, Lane 20:IC-324313, Lane 21:IC-352992, Lane 22:IC-319595, Lane 23:IC-324244; **b:** Lane 1:Sangla B-1, Lane 2:Sangla B -2, Lane 3:Sangla B-3, Lane 4:Sangla B-5, Lane 5:Sangla B-6, Lane 6:Sangla B-7, Lane 7:KBB-3, Lane 8:Himpriya, Lane 9:Kuppa Local, Lane 10:Shimla B-1; Lane 11:IC-412744, Lane 12:IC-421598, Lane 13:IC-412722, Lane 14:IC-412863, Lane 15:IC-421597; **c**: Lane 1: *F. cymosum.* M: λ DNA *Eco*R1/*Hind* III double digest; **d:** Dendrogram based on the UPGMA analysis generated from similarity matrix of the RAPD profiles of different accessions of buckwheat (*Fagopyrum* spp.) studied in the present investigation using Primer OPD-6.



M 1 2 3 4 5 6 7 8 9 10
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15 16 17 18 M
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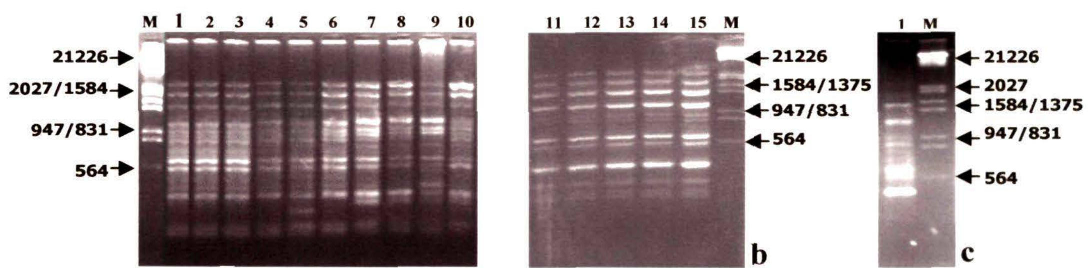
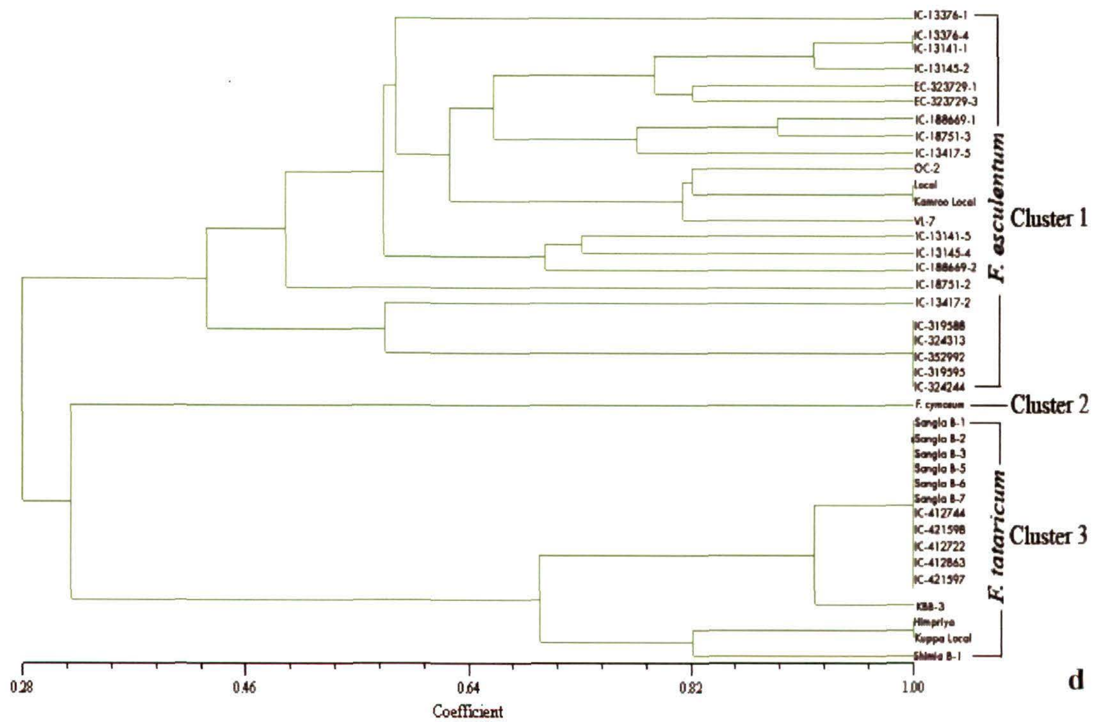
21226
2027
1584/1375
947/831
564
a



M 1 2 3 4 5 6 7 8 9 10
11 12 13 14 15 M
1 M
21226
2027/1584
947/831
564
1584/1375
2027
b
c



IC-13376-1
IC-13376-4
IC-13141-1
IC-13145-2
EC-323729-1
EC-323729-3
IC-188669-1
IC-18751-3
IC-13417-5
OC-2
Local
Kamroo Local
VL-7
IC-13141-5
IC-13145-4
IC-188669-2
IC-18751-2
IC-13417-2
IC-319588
IC-324313
IC-352992
IC-319595
IC-324244
F. cymosum
Sangla B-1
Sangla B-2
Sangla B-3
Sangla B-5
Sangla B-6
Sangla B-7
IC-412744
IC-421598
IC-412722
IC-412863
IC-421597
KBB-3
Himpriya
Kuppa Local
Shimla B-1
F. esculentum
F. tataricum
Cluster 1
Cluster 2
Cluster 3
0.28
0.46
0.64
0.82
1.00
Coefficient
d

grains. The primer amplified 314 and 1489bp RAPDs exclusively in accessions /cultivars of *F. tataricum*. While a 1154bp RAPD was detected in all cultivars /accessions of *F. tataricum*, it could not be detected in Shimla B-1. Shimla B-1 is an early maturing and high yielding cultivar. Out of all the bands amplified a 250 bp RAPD was detected only in varieties KBB-3, Himpriya, Kuppa Local and Shimla B-1 collected from Uttaranchal. The frequency of occurrence of polymorphic bands across all accessions ranged from 0.03 to 1.00 with an average frequency of 0.43 and percentage polymorphism of 96.0 (Tables 4.13; 4.14).

The similarity matrix based on RAPD score with OPD-6 is presented in table 4.15. The coefficient of similarity revealed least similarity with a coefficient of 0.11 between Kuppa local and IC-188669-1. On the other hand accessions/cultivars of *F. tataricum* showed the maximum level of similarity with a coefficient of 1.0. The dendrogram generated on the basis similarity matrix developed for the RAPD profiles of the accessions/cultivars revealed clustering into three distinct clusters (Fig. 4.17d). While Cluster 1 included all the accessions/cultivars of *F. esculentum*, clusters 2 and 3 included *F. cymosum* and accessions/cultivars *F. tataricum* respectively. Cluster 1 had three sub clusters within it with IC-13376-1, IC- 13376-4, IC-18751-2, IC-13145-4, EC-323729-1, EC-323729-3, IC-188669-1, IC-18751-3 and IC-13417-5 forming one subgroup. The cultivars Local, Kamroo Local, OC-2 and VL-7 of Uttaranchal formed another subgroup with similarity coefficient value ranging from 0.82 to 1.0. The accessions IC-319588, IC-324313, IC-352992, IC-319595 and IC-324244 from Arunachal Pradesh showed 100% similarity amongst them and emerged as separate sub cluster. Cluster 2 comprised the species *F. cymosum* which emerged out as a separate cluster. Cluster 3 included all the accessions/cultivars of *F. tataricum*. All the

Table 4.13: Number of bands amplified and frequency of occurrence of polymorphic bands in RAPD analysis.

Sr. No.	Number of RAPD bands produced				Frequency of occurrence of polymorphic bands	
	Primer	**Band sizes**	**Range**	**Average**	**Range**	**Average**
1	OPD-6	2152-250	7- 14	11.0	0.03- 1.00	0.43
2	OPC-13	1450-173	7- 12	10.0	0.05- 1.00	0.36
3	OPC-14	1488-347	5- 11	8.0	0.05- 1.00	0.46
4	OPO-16	2140-173	6-19	12.0	0.03- 0.77	0.22
5	UBC-185	1362- 70	7-13	10.0	0.03- 1.00	0.48

Table 4.14: Polymorphic percentages and polymorphism information content derived from the RAPD profiles of genomic DNA isolated from 7 day etiolated seedlings of different accessions/cultivars of buckwheat (*Fagopyrum* spp.) studied in the present investigation.

Sr. No.	Primers	Total number of bands	Number of polymorphic bands	%age of polymorphic bands	Average frequency of polymorphic bands	PIC
1	OPD-6	25	24	96	0.43	0.49
2	OPC-13	21	20	95	0.36	0.46
3	OPD-14	17	16	94	0.46	0.50
4	OPO-16	65	65	100	0.21	0.33
5	UBC-185	22	20	91	0.48	0.50

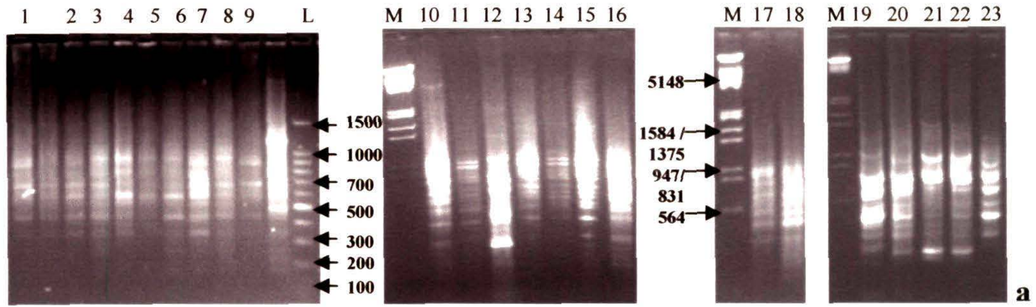
Table 4.15: Similarity matrix, based on Jaccard's coefficient, of the RAPD profile generated by primer OPD-6 of different accessions/cultivars of buckwheat (*Fagopyrum* spp.) studied in the present investigation.

Accessions	IC-188669-1	IC-188669-2	IC-18751-2	IC-18751-3	IC-13376-1	IC-13376-4	IC-13145-2	IC-13145-4	IC-13141-1	IC-13141-5	IC-13417-2	IC-13417-5	EC-323729-1	EC-323729-3	IC-319588	IC-324313	IC-352992	IC-319595	IC-324244	OC-2	VL-7	Local	Kamroo local	KBB-3	Himpriya	Kuppa local	Shimla B-1	Sangla B-1	Sangla B-2	Sangla B-3	Sangla B-5	Sangla B-6	Sangla B-7	IC-412744	IC-412863	IC-421598	IC-412722	IC-421597	*F. cymosum*
IC-188669-1	1.00																																					
IC-188669-2	0.54	1.00																																				
IC-18751-2	0.50	0.50	1.00																																			
IC-18751-3	0.58	0.89	0.33	1.00																																		
IC-13376-1	0.43	0.43	0.29	0.36	1.00																																	
IC-13376-4	0.57	0.57	0.67	0.62	0.69	1.00																																
IC-13145-2	0.71	0.60	0.57	0.62	0.60	0.73	1.00																															
IC-13145-4	0.62	0.62	0.73	0.53	0.62	0.92	0.67	1.00																														
IC-13141-1	0.69	0.38	0.43	0.62	0.47	0.60	0.73	0.53	1.00																													
IC-13141-5	0.57	0.57	0.67	0.62	0.90	1.00	0.63	0.92	0.60	1.00																												
IC-13417-2	0.21	0.55	0.36	0.38	0.55	0.46	0.40	0.38	0.36	0.46	1.00																											
IC-13417-5	0.50	0.73	0.38	0.82	0.62	0.77	0.77	0.56	0.64	0.77	0.54	1.00																										
EC-323729-1	0.54	0.64	0.50	0.73	0.54	0.83	0.69	0.71	0.47	0.83	0.36	0.75	1.00																									
EC-323729-3	0.54	0.64	0.50	0.73	0.54	0.83	0.69	0.71	0.47	0.83	0.36	0.75	1.00	1.00																								
IC-319588	0.29	0.47	0.33	0.36	0.38	0.50	0.44	0.44	0.26	0.50	0.57	0.40	0.53	0.47	1.00																							
IC-324313	0.29	0.47	0.33	0.36	0.38	0.50	0.44	0.44	0.26	0.50	0.57	0.40	0.53	0.47	1.00	1.00																						
IC-352992	0.29	0.47	0.33	0.36	0.38	0.50	0.44	0.44	0.26	0.50	0.57	0.40	0.53	0.47	1.00	1.00	1.00																					
IC-319595	0.29	0.47	0.33	0.36	0.38	0.50	0.44	0.44	0.26	0.50	0.57	0.40	0.53	0.47	1.00	1.00	1.00	1.00																				
IC-324244	0.29	0.47	0.33	0.36	0.38	0.50	0.44	0.44	0.26	0.50	0.57	0.40	0.53	0.47	1.00	1.00	1.00	1.00	1.00																			
OC-2	0.46	0.58	0.42	0.64	0.38	0.62	0.53	0.67	0.50	0.62	0.45	0.54	0.58	0.58	0.40	0.40	0.40	0.40	0.40	1.00																		
VL-7	0.46	0.58	0.42	0.54	0.58	0.62	0.44	0.73	0.50	0.62	0.60	0.57	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.80	1.00																	
Local	0.50	0.62	0.46	0.67	0.64	0.77	0.67	0.69	0.64	0.77	0.63	0.69	0.62	0.62	0.44	0.44	0.44	0.44	0.44	0.82	0.82	1.00																
Kamroo local	0.50	0.62	0.46	0.67	0.64	0.77	0.67	0.69	0.64	0.77	0.63	0.69	0.40	0.40	0.44	0.44	0.44	0.44	0.44	0.82	0.82	1.00	1.00															
KBB-3	0.16	0.26	0.18	0.33	0.22	0.26	0.24	0.28	0.20	0.20	0.27	0.29	0.31	0.31	0.50	0.50	0.50	0.50	0.50	0.25	0.24	0.44	0.44	1.00														
Himpriya	0.24	0.17	0.12	0.25	0.24	0.21	0.14	0.29	0.15	0.21	0.20	0.22	0.24	0.24	0.35	0.35	0.35	0.35	0.35	0.18	0.18	0.22	0.22	0.92	1.00													
Kuppa local	0.11	0.31	0.12	0.29	0.24	0.21	0.19	0.22	0.15	0.21	0.20	0.25	0.27	0.27	0.35	0.35	0.35	0.35	0.35	0.18	0.18	0.22	0.22	0.92	1.00	1.00												
Shimla B-1	0.12	0.27	0.13	0.29	0.19	0.24	0.15	0.25	0.17	0.26	0.23	0.25	0.27	0.27	0.40	0.40	0.40	0.40	0.40	0.20	0.20	0.25	0.25	0.75	0.82	0.82	1.00											
Sangla B-1	0.17	0.31	0.19	0.31	0.24	0.21	0.25	0.29	0.21	0.25	0.29	0.28	0.29	0.29	0.35	0.35	0.35	0.35	0.35	0.25	0.25	0.29	0.29	0.92	0.69	0.69	0.66	1.00										
Sangla B-2	0.17	0.31	0.19	0.31	0.24	0.21	0.25	0.29	0.21	0.25	0.29	0.28	0.29	0.29	0.35	0.35	0.35	0.35	0.35	0.25	0.25	0.29	0.29	0.92	0.69	0.69	0.66	1.00	1.00									
Sangla B-3	0.17	0.31	0.19	0.31	0.24	0.21	0.25	0.29	0.21	0.25	0.29	0.28	0.29	0.29	0.35	0.35	0.35	0.35	0.35	0.25	0.25	0.29	0.29	0.92	0.69	0.69	0.66	1.00	1.00	1.00								
Sangla B-5	0.17	0.31	0.19	0.31	0.24	0.21	0.25	0.29	0.21	0.25	0.29	0.28	0.29	0.29	0.35	0.35	0.35	0.35	0.35	0.25	0.25	0.29	0.29	0.92	0.69	0.69	0.66	1.00	1.00	1.00	1.00							
Sangla B-6	0.17	0.31	0.19	0.31	0.24	0.21	0.25	0.29	0.21	0.25	0.29	0.28	0.29	0.29	0.35	0.35	0.35	0.35	0.35	0.25	0.25	0.29	0.29	0.92	0.69	0.69	0.66	1.00	1.00	1.00	1.00	1.00						
Sangla B-7	0.17	0.31	0.19	0.31	0.24	0.21	0.25	0.29	0.21	0.25	0.29	0.28	0.29	0.29	0.35	0.35	0.35	0.35	0.35	0.25	0.25	0.29	0.29	0.92	0.69	0.69	0.66	1.00	1.00	1.00	1.00	1.00	1.00					
IC-412744	0.17	0.31	0.19	0.31	0.24	0.21	0.25	0.29	0.21	0.25	0.29	0.28	0.29	0.29	0.35	0.35	0.35	0.35	0.35	0.25	0.25	0.29	0.29	0.92	0.69	0.69	0.66	1.00	1.00	1.00	1.00	1.00	1.00	1.00				
IC-412863	0.17	0.31	0.19	0.31	0.24	0.21	0.25	0.29	0.21	0.25	0.29	0.28	0.29	0.29	0.35	0.35	0.35	0.35	0.35	0.25	0.25	0.29	0.29	0.92	0.69	0.69	0.66	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00			
IC-421598	0.17	0.31	0.19	0.31	0.24	0.21	0.25	0.29	0.21	0.25	0.29	0.28	0.29	0.29	0.35	0.35	0.35	0.35	0.35	0.25	0.25	0.29	0.29	0.92	0.69	0.69	0.66	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00		
IC-412722	0.17	0.31	0.19	0.31	0.24	0.21	0.25	0.29	0.21	0.25	0.29	0.28	0.29	0.29	0.35	0.35	0.35	0.35	0.35	0.25	0.25	0.29	0.29	0.92	0.69	0.69	0.66	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	
IC-421597	0.17	0.31	0.19	0.31	0.24	0.21	0.25	0.29	0.21	0.25	0.29	0.28	0.29	0.29	0.35	0.35	0.35	0.35	0.35	0.25	0.25	0.29	0.29	0.92	0.69	0.69	0.66	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	
F. cymosum	0.27	0.36	0.13	0.27	0.36	0.31	0.28	0.25	0.24	0.24	0.33	0.29	0.27	0.27	0.31	0.31	0.31	0.31	0.31	0.29	0.29	0.25	0.25	0.31	0.25	0.25	0.29	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	1.00

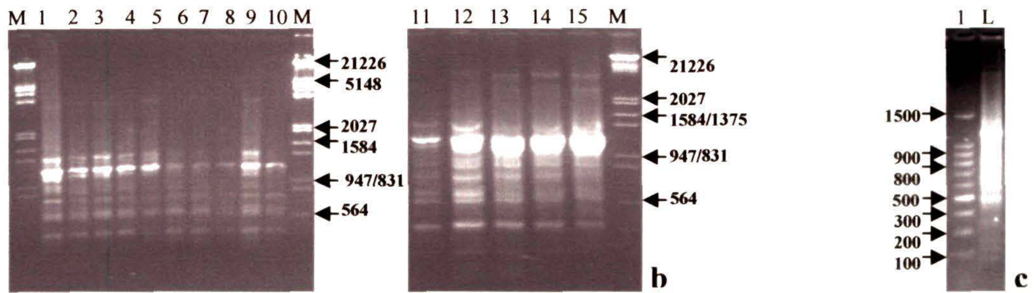
accessions/cultivars of *F. tataricum* except KBB-3, Kuppa Local, Himpriya and Shimla B-1, formed one subgroup with 100% similarity between them. The cultivars Himpriya and Kuppa Local, which showed a similarity coefficient of 1.0 amongst them, emerged out as another subgroup within this cluster.

Primer 2 (OPC-13) generated 21 polymorphic bands with sizes ranging from 173 bp to 1450bp (Fig. 4.18a-c). While the number of amplified products per accession ranged from 7 to 12 with an average of 10 bands per accession the frequency of polymorphic bands ranged from 0.05 to 1.00 with an average value of 0.36 (Table 4.13). The number of amplified bands ranged between 7 and 12 in accessions of *F. esculentum.* While IC-13141-1 and IC-13141-4 generated 7 bands VL-7 produced 12 bands. A 1033 bp amplicon was detected only in the variety VL-7. VL-7 is one of the high yielding and early maturing variety belonging to the species *F. esculentum.* The RAPD profiles of accessions belonging to *F. tataricum* revealed the presence of 10 bands in all the accessions. These bands ranged in size from 413 to 1450 bp. There was no variation in the RAPD profile of accessions/cultivars of *F. tataricum*. The primer amplified 14 polymorphic DNA bands ranging in size from 173 to 1450 bp from genomic DNA of *F. cymosum.* Out of these 5 bands were common to *F. tataricum* and 9 were common to *F. esculentum*. Two RAPD bands of 218 and 500 bp were, however, exclusive to *F. cymosum*. An amplicon of 447 bp obtained using this primer was monomorphic to all the accession/cultivars studied in the present investigation. Estimation of the polymorphism content revealed 95 percent polymorphism content and a similarity coefficient ranging from 0.11 to 1.0 (Table 4.13; 4.14). Cluster analysis of the genetic similarity estimates revealed clustering of the accessions/cultivars into two broad clusters (Fig. 4.18d). All the accessions/cultivars belonging to *F. esculentum*

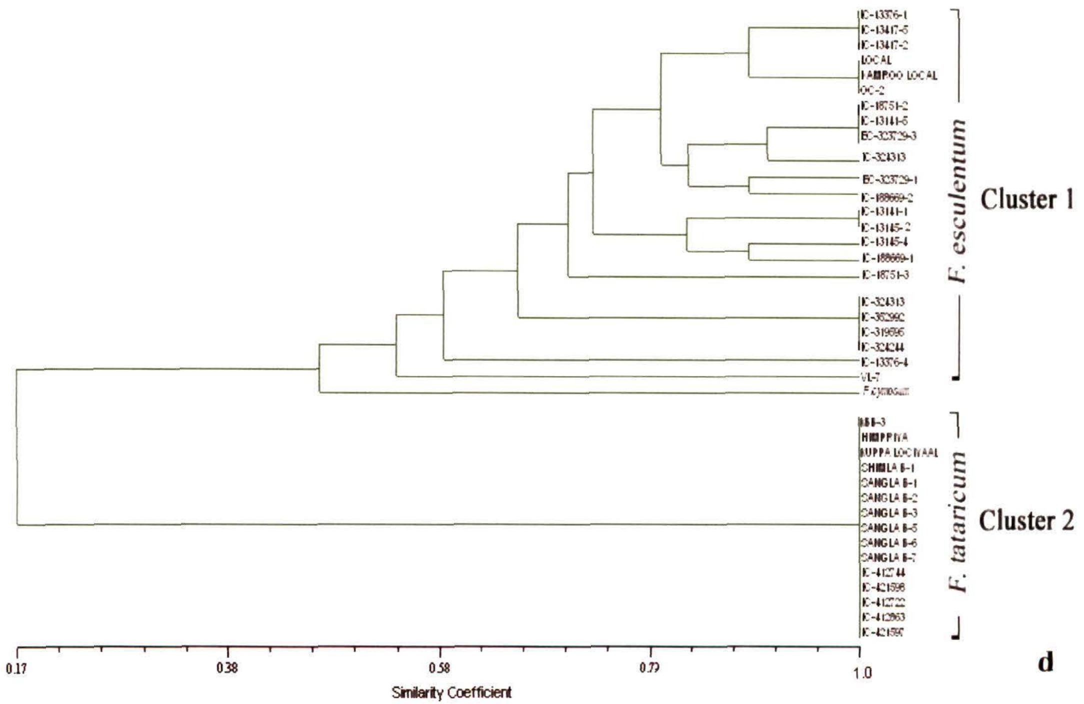
Fig 4.18: RAPD profile of genomic DNA extracted from 7 day old etiolated seedlings of different accessions/cultivars of buckwheat (*Fagopyrum* spp.) studied in the present investigation using the primer OPC-13. **a**:Lane1:IC-13376-1, Lane 2:IC-13417-5, Lane 3:IC-13417-2, Lane 4:IC-18751-2, Lane 5:Local, Lane 6:Kamroo Local, Lane 7:OC-2, Lane 8:VL-7, Lane 9:IC-18751-3, Lane 10:IC-13141-1, Lane 11:IC-13376-4, Lane 12:IC-13141-5, Lane 13:EC-323729-1, Lane 14:IC-188669-2; **c**: Lane 15:IC-13145-4, Lane 16:IC-188669-1, Lane 17:IC-13145-2, Lane 18: EC-323729-3, Lane 19:IC-319588, Lane 20:IC-324313, Lane 21:IC-352992, Lane 22:IC-319595, Lane 23:IC-324244; **b**: Lane1:KBB-3, Lane 2:Himpriya, Lane 3:Kuppa Local, Lane 4:Shimla B-1, Lane 5:Sangla B-1, Lane 6:Sangla B-2, Lane 7: Sangla B-3, Lane 8:Sangla B-5, Lane 9:Sangla B-6, Lane 10:Sangla B-7, Lane 11:IC-412744, Lane 12:IC-421598 Lane 13:IC-412722, Lane 14:IC-412863, Lane 15:IC-421597, **c**: Lane1: *F. cymosum*; M: λDNA *Eco*R1*Hind* III double digest; **d:** Dendrogram based on the UPGMA analysis generated from similarity matrix of the RAPD profiles of different accessions of buckwheat (*Fagopyrum* spp.) studied in the present investigation using Primer OPC-13.



1 2 3 4 5 6 7 8 9 L
M 10 11 12 13 14 15 16
M 17 18
M 19 20 21 22 23
1500
1000
700
500
300
200
100
5148
1584 /
1375
947/
831
564
a



M 1 2 3 4 5 6 7 8 9 10 M
11 12 13 14 15 M
1 L
21226
5148
2027
1584
947/831
564
21226
2027
1584/1375
947/831
564
b
1500
900
800
500
300
200
100
c



F. esculentum
Cluster 1
F. tataricum
Cluster 2
0.17
0.38
0.58
0.79
1.0
Similarity Coefficient
d

Table 4.16: Similarity matrix, based on Jaccard's coefficient, of the RAPD profile generated by primer OPD-13 of different accessions/cultivars of buckwheat (*Fagopyrum* spp.) studied in the present investigation.

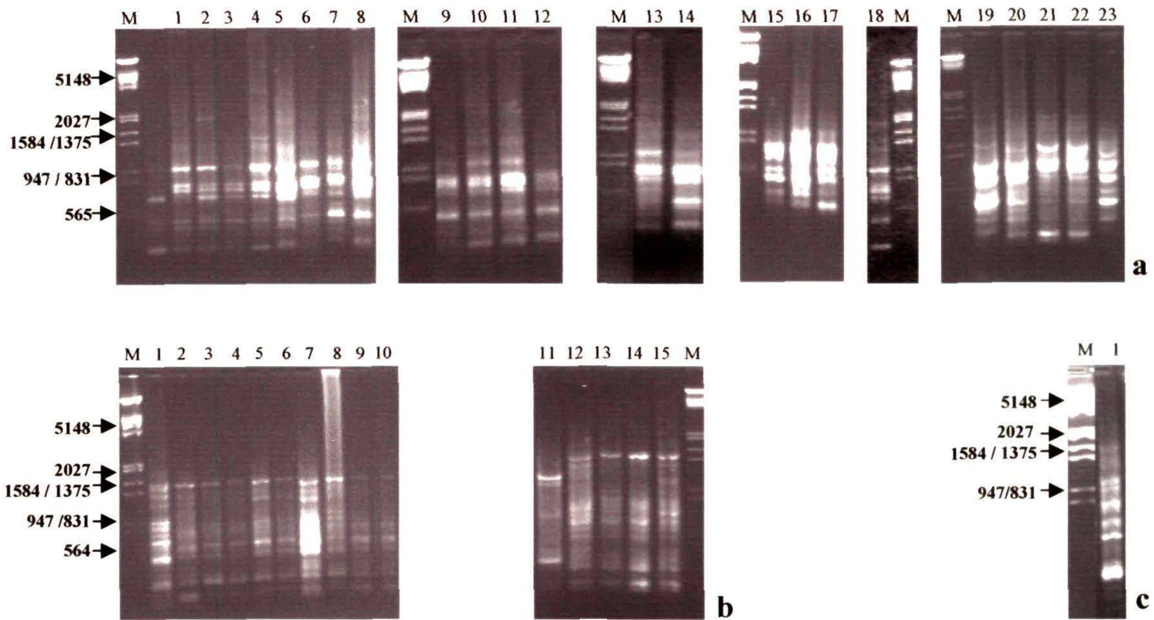
Accessions	IC-188669-1	IC-188669-2	IC-18751-2	IC-18751-3	IC-13376-1	IC-13376-4	IC-13145-4	IC-13145-2	IC-13141-1	IC-13141-5	IC-13417-5	IC-13417-2	EC-323729-1	EC-323729-3	IC-319588	IC-324313	IC-352992	IC-319595	IC-324244	Local	Kamroo Local	OC-2	VL-7	KBB-3	Himpriya	Kuppa Local	Shimla B-1	SanglaB-1	SanglaB-2	SanglaB-3	SanglaB-5	SanglaB-6	SanglaB-7	IC-412744	IC-412863	IC-421598	IC-412722	IC-421597	*F. cymosum*
IC-188669-1	1.00																																						
IC-188669-2	0.70	1.00																																					
IC-18751-2	0.90	0.80	1.00																																				
IC-18751-3	0.80	0.70	0.73	1.00																																			
IC-13376-1	0.80	0.89	0.90	0.80	1.00																																		
IC-13376-4	0.50	0.60	0.54	0.60	0.78	1.00																																	
IC-13145-4	0.89	0.78	0.64	0.70	0.89	0.60	1.00																																
IC-13145-2	0.78	0.67	0.70	0.60	0.78	0.56	0.88	1.00																															
IC-13141-1	0.78	0.67	0.70	0.60	0.78	0.56	0.88	1.00	1.00																														
IC-13141-5	0.90	0.80	1.00	0.73	0.90	0.54	0.64	0.70	0.70	1.00																													
IC-13417-5	0.80	0.89	0.90	0.80	1.00	0.78	0.89	0.78	0.78	0.90	1.00																												
IC-13417-2	0.80	0.89	0.90	0.80	1.00	0.78	0.89	0.78	0.78	0.90	1.00	1.00																											
EC-323729-1	0.64	0.89	0.90	0.64	0.80	0.50	0.70	0.60	0.60	0.90	0.80	0.80	1.00																										
EC-323729-3	0.90	0.60	1.00	0.73	0.90	0.54	0.64	0.70	0.70	1.00	0.90	0.90	0.90	1.00																									
IC-319588	0.82	0.73	0.91	0.67	0.82	0.47	0.73	0.64	0.64	0.91	0.82	0.82	0.82	0.91	1.00																								
IC-324313	0.64	0.55	0.73	0.64	0.64	0.50	0.55	0.60	0.60	0.73	0.64	0.64	0.64	0.73	0.82	1.00																							
IC-352992	0.64	0.55	0.73	0.64	0.64	0.50	0.55	0.60	0.60	0.73	0.64	0.64	0.64	0.73	0.82	1.00	1.00																						
IC-319595	0.64	0.55	0.73	0.64	0.64	0.50	0.55	0.60	0.60	0.73	0.64	0.64	0.64	0.73	0.82	1.00	1.00	1.00																					
IC-324244	0.64	0.55	0.73	0.64	0.64	0.50	0.55	0.60	0.60	0.73	0.64	0.64	0.64	0.73	0.82	1.00	1.00	1.00	1.00																				
Local	0.70	0.78	0.80	0.70	0.89	0.67	0.78	0.88	0.50	0.64	0.89	0.89	0.70	0.80	0.73	0.70	0.70	0.70	0.70	1.00																			
Kamroo local	0.70	0.78	0.80	0.70	0.89	0.67	0.78	0.88	0.50	0.64	0.89	0.89	0.70	0.80	0.73	0.70	0.70	0.70	0.70	1.00	1.00																		
OC-2	0.70	0.78	0.80	0.70	0.89	0.67	0.78	0.88	0.50	0.64	0.89	0.89	0.70	0.80	0.73	0.70	0.70	0.70	0.70	1.00	1.00	1.00																	
VL-7	0.50	0.54	0.57	0.50	0.62	0.58	0.54	0.46	0.46	0.57	0.62	0.62	0.50	0.57	0.64	0.50	0.50	0.50	0.50	0.54	0.54	0.54	1.00																
KBB-3	0.19	0.13	0.18	0.12	0.12	0.11	0.13	0.13	0.13	0.18	0.12	0.12	0.19	0.18	0.17	0.27	0.27	0.27	0.27	0.13	0.13	0.13	0.22	1.00															
Himpriya	0.19	0.13	0.18	0.12	0.12	0.11	0.13	0.13	0.13	0.18	0.12	0.12	0.19	0.18	0.17	0.27	0.27	0.27	0.27	0.13	0.13	0.13	0.22	1.00	1.00														
Kuppa local	0.19	0.13	0.18	0.12	0.12	0.11	0.13	0.13	0.13	0.18	0.12	0.12	0.19	0.18	0.17	0.27	0.27	0.27	0.27	0.13	0.13	0.13	0.22	1.00	1.00	1.00													
Shimla B-1	0.19	0.13	0.18	0.12	0.12	0.11	0.13	0.13	0.13	0.18	0.12	0.12	0.19	0.18	0.17	0.27	0.27	0.27	0.27	0.13	0.13	0.13	0.22	1.00	1.00	1.00	1.00												
SanglaB-1	0.19	0.13	0.18	0.12	0.12	0.11	0.13	0.13	0.13	0.18	0.12	0.12	0.19	0.18	0.17	0.27	0.27	0.27	0.27	0.13	0.13	0.13	0.22	1.00	1.00	1.00	1.00	1.00											
SanglaB-2	0.19	0.13	0.18	0.12	0.12	0.11	0.13	0.13	0.13	0.18	0.12	0.12	0.19	0.18	0.17	0.27	0.27	0.27	0.27	0.13	0.13	0.13	0.22	1.00	1.00	1.00	1.00	1.00	1.00										
SanglaB-3	0.19	0.13	0.18	0.12	0.12	0.11	0.13	0.13	0.13	0.18	0.12	0.12	0.19	0.18	0.17	0.27	0.27	0.27	0.27	0.13	0.13	0.13	0.22	1.00	1.00	1.00	1.00	1.00	1.00	1.00									
SanglaB-5	0.19	0.13	0.18	0.12	0.12	0.11	0.13	0.13	0.13	0.18	0.12	0.12	0.19	0.18	0.17	0.27	0.27	0.27	0.27	0.13	0.13	0.13	0.22	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00								
SanglaB-6	0.19	0.13	0.18	0.12	0.12	0.11	0.13	0.13	0.13	0.18	0.12	0.12	0.19	0.18	0.17	0.27	0.27	0.27	0.27	0.13	0.13	0.13	0.22	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00							
SanglaB-7	0.19	0.13	0.18	0.12	0.12	0.11	0.13	0.13	0.13	0.18	0.12	0.12	0.19	0.18	0.17	0.27	0.27	0.27	0.27	0.13	0.13	0.13	0.22	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00						
IC-412744	0.19	0.13	0.18	0.12	0.12	0.11	0.13	0.13	0.13	0.18	0.12	0.12	0.19	0.18	0.17	0.27	0.27	0.27	0.27	0.13	0.13	0.13	0.22	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00					
IC-412863	0.19	0.13	0.18	0.12	0.12	0.11	0.13	0.13	0.13	0.18	0.12	0.12	0.19	0.18	0.17	0.27	0.27	0.27	0.27	0.13	0.13	0.13	0.22	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00				
IC-421598	0.19	0.13	0.18	0.12	0.12	0.11	0.13	0.13	0.13	0.18	0.12	0.12	0.19	0.18	0.17	0.27	0.27	0.27	0.27	0.13	0.13	0.13	0.22	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00			
IC-412722	0.19	0.13	0.18	0.12	0.12	0.11	0.13	0.13	0.13	0.18	0.12	0.12	0.19	0.18	0.17	0.27	0.27	0.27	0.27	0.13	0.13	0.13	0.22	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00		
IC-421597	0.19	0.13	0.18	0.12	0.12	0.11	0.13	0.13	0.13	0.18	0.12	0.12	0.19	0.18	0.17	0.27	0.27	0.27	0.27	0.13	0.13	0.13	0.22	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	
F. cymosum	0.44	0.47	0.50	0.44	0.53	0.40	0.47	0.40	0.40	0.50	0.53	0.53	0.44	0.50	0.56	0.44	0.44	0.44	0.44	0.47	0.47	0.47	0.44	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	1.00

clustered together as cluster 1. *F. cymosum* emerged separately from the accessions /cultivars of both *F. esculentum and F. tataricum,* but showed more closeness to *esculentum* group than *tataricum* group. Amongst all the genotypes belonging to *F. esculentum,* VL-7 clustered separately with an average similarity matrix value of 0.54 with other genotypes of this species. Cluster 2 clubbed together all the accessions/ cultivars of *F. tataricum.* The accessions/cultivars of *tataricum* showed a similarity coefficient of 1.0 (Table 4.16).

Primer 3 (OPD-14) amplified seventeen polymorphic bands in *Fagopyrum* species with sizes ranging from 347 to 1488 bp (Fig. 4.19a-c). The RAPD profiles revealed a polymorphism percentage of 94.0 and frequency of occurrence of polymorphic bands across all genotypes ranging from 0.05 –1.00 (Table-4.13; 4.14). There was variation in the RAPD profile of different accessions/cultivars *F. esculentum.* The primer amplified 10-11 bands in accessions/cultivars *F. tataricum* and 10 bands *in F. cymosum.* All the accessions/cultivars of *F. tataricum,* except KBB-3 and IC-412744, showed the same RAPD profiles. These two accessions showed a 1176 bp amplicon which could not be detected in other accessions/cultivars. Out of the 10 amplified bands observed in *F. cymosum,* 9 bands were common with the accessions /cultivars of *F. tataricum* 6 bands were common to both the accessions/cultivars of *F. esculentum* and *F. himalianum.* Analysis of the profiles revealed the lowest similarity coefficient of 0.29 between IC-18751-2 and *F. cymosum.* On the other hand the highest similarity coefficient of 1.0 was recorded amongst many accessions/cultivars of *F. esculentum* and *F. tataricum* (Table 4.17).

Within the accessions of *F. esculentum* the similarity matrix ranged from 0.38 between IC-324244 and IC- 13376-1 to 1.0 between EC-323729-5, IC-188669-1, EC-

Fig 4.19: RAPD profile of genomic DNA extracted from 7 day old etiolated seedlings of different accessions/cultivars of buckwheat (*Fagopyrum* spp.) studied in the present investigation using the primer OPD-14. **a:** Lane1:IC-188669-2, Lane 2:IC-188669-1, Lane 3:IC-18751-2, Lane 4:EC-323729-1, Lane 5:VL-7, Lane 6: Local, Lane 7:Kamroo Local, Lane 8:OC-2; B: Lane 9:IC-13376-1, Lane 10:IC-13145-4, Lane 11:IC-13417-5, Lane 12:IC-13376-4, Lane 13:IC-13417-2, Lane 14:EC-323729-3, Lane 15:IC-18751-3, Lane 16:IC-13141-5, Lane 17:IC-13145-2; Lane 18:IC-13141-1, Lane 19:IC-319588, Lane 20:IC-324313, Lane 21:IC-352992, Lane 22:IC-319595, Lane 23:IC-324244; **b:** Lane 1:KBB-3, Lane 2:Himpriya, Lane 3:Kuppa Local, Lane 4:Shimla B-1, Lane 5:Sangla B-1, Lane 6: Sangla B-2, Lane 7: Sangla B-3, Lane 8: Sangla B-5, Lane 9: Sangla B-6, Lane 10: Sangla B-7; **c:** Lane 11:IC-412744, Lane 12:IC-421598 Lane 13:IC-412722 Lane 14:IC-412863 Lane 15:IC-421597; **c:** Lane1: *F. cymosum*; M: λDNA *Eco*R1 + *Hind* III double digest; **d:** Dendrogram based on the UPGMA analysis generated from similarity matrix of the RAPD profiles of different accessions of buckwheat (*Fagopyrum* spp.) studied in the present investigation using Primer OPC-14.


M 1 2 3 4 5 6 7 8
M 9 10 11 12
M 13 14
M 15 16 17
18 M
M 19 20 21 22 23
5148
2027
1584 /1375
947 / 831
565
a
M 1 2 3 4 5 6 7 8 9 10
11 12 13 14 15 M
5148
2027
1584 / 1375
947 /831
564
b
M 1
5148
2027
1584 / 1375
947/831
c

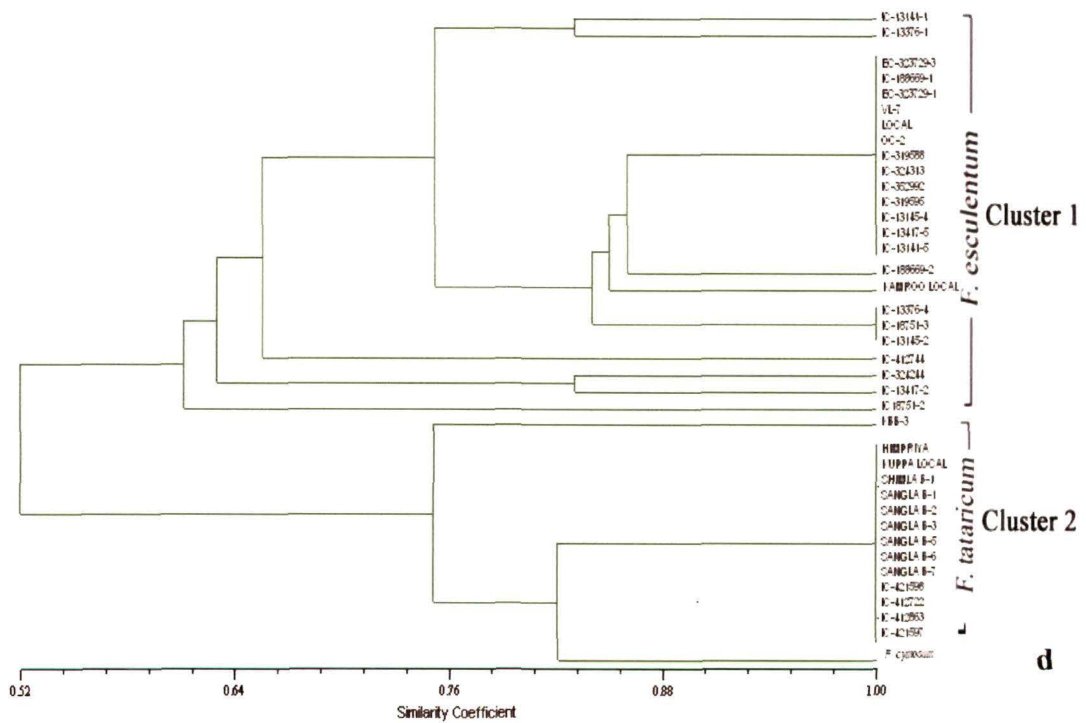

IC-13141-1
IC-13376-1
EC-323729-3
IC-189669-1
EC-323729-1
VL-7
LOCAL
OC-2
IC-319588
IC-324313
IC-352990
IC-319596
IC-13145-4
IC-13417-5
IC-13141-5
IC-189669-2
IC-13376-4
IC-18751-3
IC-13145-2
IC-412744
IC-324244
IC-13417-2
IC 18751-2
PUPPA LOCAL
SANGLA B-1
SANGLA B-2
SANGLA B-3
SANGLA B-5
SANGLA B-6
SANGLA B-7
IC-421596
IC-412722
IC-412863
IC-421597
F. esculentum
Cluster 1
F. tataricum
Cluster 2
d
0.52
0.64
0.76
0.88
1.00
Similarity Coefficient

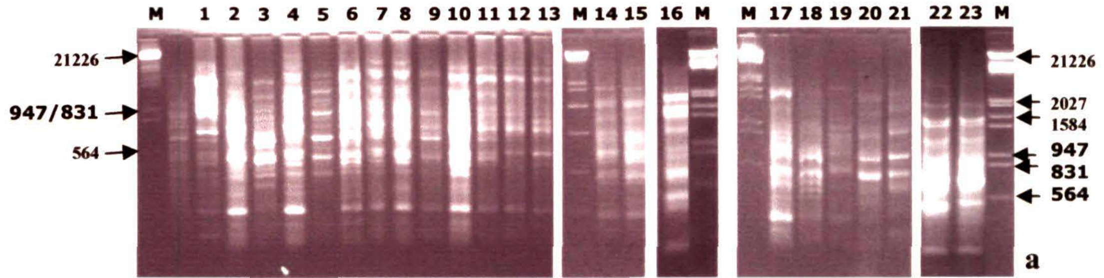
Table 4.17: Similarity matrix, based on Jaccard's coefficient, of the RAPD profile generated by primer OPD-14 of different accessions/cultivars of buckwheat (*Fagopyrum* spp.) studied in the present investigation.

Accessions	IC-188669-1	IC-188669-2	IC-18751-2	IC-18751-3	IC-13376-1	IC-13376-4	IC-13145-2	IC-13145-4	IC-13141-1	IC-13141-5	IC-13417-2	IC-13417-5	EC-323729-1	EC-323729-3	IC-319588	IC-324313	IC-352992	IC-319595	IC-324244	VL-7	Local	Kamroo local	OC-2	KBB-3	Himpriya	Kuppa local	ShimlaB-1	SanglaB-1	SanglaB-2	SanglaB-3	SanglaB-5	SanglaB-6	SanglaB-7	IC-412744	IC-412863	IC-421598	IC-412722	IC-421597	*F.cymosum*
IC-188669-1	1.00																																						
IC-188669-2	0.86	1.00																																					
IC-18751-2	0.67	0.56	1.00																																				
IC-18751-3	0.86	0.71	0.56	1.00																																			
IC-13376-1	0.71	0.83	0.63	0.57	1.00																																		
IC-13376-4	0.86	0.71	0.56	1.00	0.57	1.00																																	
IC-13145-2	0.86	0.71	0.56	1.00	0.57	1.00	1.00																																
IC-13145-4	1.00	0.86	0.67	0.86	0.71	0.86	0.86	1.00																															
IC-13141-1	0.86	0.71	0.75	0.71	0.83	0.71	0.71	0.86	1.00																														
IC-13141-5	1.00	0.86	0.50	0.86	0.71	0.86	0.86	1.00	0.86	1.00																													
IC-13417-2	0.71	0.57	0.63	0.57	0.67	0.57	0.57	0.71	0.83	0.71	1.00																												
IC-13417-5	1.00	0.86	0.67	0.86	0.71	0.86	0.86	1.00	0.86	1.00	0.71	1.00																											
EC-323729-1	1.00	0.86	0.67	0.86	0.71	0.86	0.86	1.00	0.86	1.00	0.71	1.00	1.00																										
EC-323729-3	1.00	0.86	0.50	0.86	0.71	0.86	0.86	1.00	0.83	1.00	0.71	1.00	1.00	1.00																									
IC-319588	1.00	0.86	0.67	0.86	0.71	0.86	0.86	1.00	0.86	1.00	0.71	1.00	1.00	1.00	1.00																								
IC-324313	1.00	0.86	0.67	0.86	0.71	0.86	0.86	1.00	0.86	1.00	0.71	1.00	1.00	1.00	1.00	1.00																							
IC-352992	1.00	0.86	0.67	0.86	0.71	0.86	0.86	1.00	0.86	1.00	0.71	1.00	1.00	1.00	1.00	1.00	1.00																						
IC-319595	1.00	0.86	0.67	0.86	0.71	0.86	0.86	1.00	0.86	1.00	0.71	1.00	1.00	1.00	1.00	1.00	1.00	1.00																					
IC-324244	0.63	0.71	0.40	0.50	0.38	0.50	0.50	0.63	0.50	0.63	0.83	0.63	0.63	0.63	0.63	0.63	0.63	0.63	1.00																				
VL-7	1.00	0.86	0.67	0.86	0.71	0.86	0.86	1.00	0.86	1.00	0.71	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.63	1.00																			
Local	1.00	0.86	0.67	0.86	0.71	0.86	0.86	1.00	0.86	1.00	0.71	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.63	1.00	1.00																		
Kamroo local	0.86	0.71	0.56	0.71	0.57	0.71	0.71	0.86	0.71	0.86	0.57	0.86	0.86	0.86	0.86	0.86	0.86	0.86	0.71	0.86	0.86	1.00																	
OC-2	1.00	0.86	0.67	0.86	0.71	0.86	0.86	1.00	0.86	1.00	0.71	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.63	1.00	1.00	0.86	1.00																
KBB-3	0.64	0.42	0.36	0.55	0.33	0.55	0.55	0.64	0.55	0.64	0.33	0.64	0.64	0.64	0.64	0.64	0.64	0.64	0.42	0.64	0.64	0.42	0.64	1.00															
Himpriya	0.55	0.45	0.38	0.60	0.36	0.60	0.60	0.55	0.45	0.55	0.36	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.33	0.55	0.55	0.45	0.55	0.75	1.00														
Kuppa local	0.55	0.45	0.38	0.60	0.36	0.60	0.60	0.55	0.45	0.55	0.36	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.33	0.55	0.55	0.45	0.55	0.75	1.00	1.00													
Shimla B-1	0.55	0.45	0.38	0.60	0.36	0.60	0.60	0.55	0.45	0.55	0.36	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.33	0.55	0.55	0.45	0.55	0.75	1.00	1.00	1.00												
SanglaB-1	0.55	0.45	0.38	0.60	0.36	0.60	0.60	0.55	0.45	0.55	0.36	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.33	0.55	0.55	0.45	0.55	0.75	1.00	1.00	1.00	1.00											
SanglaB-2	0.55	0.45	0.38	0.60	0.36	0.60	0.60	0.55	0.45	0.55	0.36	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.33	0.55	0.55	0.45	0.55	0.75	1.00	1.00	1.00	1.00	1.00										
SangalB-3	0.55	0.45	0.38	0.60	0.36	0.60	0.60	0.55	0.45	0.55	0.36	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.33	0.55	0.55	0.45	0.55	0.75	1.00	1.00	1.00	1.00	1.00	1.00									
SanglaB-5	0.55	0.45	0.38	0.60	0.36	0.60	0.60	0.55	0.45	0.55	0.36	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.33	0.55	0.55	0.45	0.55	0.75	1.00	1.00	1.00	1.00	1.00	1.00	1.00								
SanglaB-6	0.55	0.45	0.38	0.60	0.36	0.60	0.60	0.55	0.45	0.55	0.36	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.33	0.55	0.55	0.45	0.55	0.75	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00							
SanglaB-7	0.55	0.45	0.38	0.60	0.36	0.60	0.60	0.55	0.45	0.55	0.36	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.33	0.55	0.55	0.45	0.55	0.75	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00						
IC-412744	0.67	0.56	0.45	0.75	0.44	0.75	0.75	0.67	0.56	0.67	0.44	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.40	0.67	0.67	0.56	0.67	0.73	0.64	0.64	0.64	0.64	0.64	0.64	0.64	0.64	0.64	1.00					
IC-412863	0.55	0.45	0.38	0.60	0.36	0.60	0.60	0.55	0.45	0.55	0.36	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.33	0.55	0.55	0.45	0.55	0.75	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.64	1.00				
IC-421598	0.55	0.45	0.38	0.60	0.36	0.60	0.60	0.55	0.45	0.55	0.36	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.33	0.55	0.55	0.45	0.55	0.75	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.64	1.00	1.00			
IC-412722	0.55	0.45	0.38	0.60	0.36	0.60	0.60	0.55	0.45	0.55	0.36	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.33	0.55	0.55	0.45	0.55	0.75	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.64	1.00	1.00	1.00		
IC-421597	0.55	0.45	0.38	0.60	0.36	0.60	0.60	0.55	0.45	0.55	0.36	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.33	0.55	0.55	0.45	0.55	0.75	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.64	1.00	1.00	1.00	1.00	
F.cymosum	0.55	0.45	0.29	0.60	0.36	0.60	0.60	0.55	0.45	0.55	0.36	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.33	0.55	0.55	0.45	0.55	0.75	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.82	0.50	0.82	0.82	0.82	0.82	1.00

3232729-1, VL-7, Local, OC-2, IC-319588, IC-324313, IC-352992, IC-319595, IC-13145-4, IC-13417-5, IC-13145-5. There was no prominent and distinct band found amongst accessions/cultivars of *F. esculentum* which could separate them on the basis of their collection from different geographic location. The exotic collection EC-323729 showed 100% similarity with accessions/cultivars of *F. esculentum* collected from different geographic location. IC-13145, identified as *F. himalianum,* showed 100% similarity with other accessions/cultivars of *F. esculentum*. Cluster analysis of the genetic similarity estimates revealed clustering of the accessions/cultivars into two broad clusters (Fig. 4.19d). While cluster 1 included all the accessions/cultivars of *F. esculentum* cluster 2 included all the accessions/cultivars of *F. tataricum* and *F. cymosum*.

Primer 4 (OPO-16) amplified seventeen polymorphic bands in *Fagopyrum* species with sizes ranging from 173 to 2140 bp (Fig. 4.20a-c). While frequency of occurrence of polymorphic bands across all accessions was 0.21, the profiles did not reveal the presence of any monomorphic band (tables 4.13, 4.14). One of the significant features of the profiles was the detection of 3 bands having molecular mass of 2140 bp, 1820bp and 1294 bp only in accessions of *F. esculentum* collected from Arunachal Pradesh. While a RAPD of 1350 bp was detected to be monomorphic to accessions of *F. tataricum*. All the cultivars of Sangla group showed the same amplification profile. The primer produced 8 amplicons with genomic DNA from *F. cymosum*. Out of these amplicons an amplicon of 1321 bp was found to be exclusive to this species. All other bands of *F. cymosum* was either common with accessions/cultivars of *esculentum* or *tataricum*. The values for Jaccard's coefficient of similarity based on the RAPD score with primer 4 (OPO-16) are presented in Table 4.18. Cluster analysis of the genetic

Fig 4.20: RAPD profile of genomic DNA extracted from 7 day old etiolated seedlings of different accessions/cultivars of buckwheat (*Fagopyrum* spp.) studied in the present investigation using the primer OPO-16.**a**: Lane1:IC-18751-3, Lane 2:IC-13376-1, Lane 3:IC-13145-2, Lane 4:IC-13141-1, Lane 5:13417-5, Lane 6:EC-323729-3, Lane 7:Local, Lane 8:Kamroo Local, Lane 9:VL-7, Lane 10:OC-2; Lane 11:IC-319588, Lane 12:IC-324313, Lane 13:IC-352992; Lane 14:IC-18751-2, Lane 15:IC-13376-4, Lane 16 IC-13141-5, Lane 17:IC-188669-1, Lane 18:13417-2, Lane 19:EC-323729-1, Lane 20:IC-13145-4, Lane 21:IC-188669-2, Lane 22:IC-319595, Lane 23:IC-324244; **b**: Lane1:KBB-3, Lane 2:Himpriya, Lane 3:Kuppa Local, Lane 4:Shimla B-1, Lane 5:Sangla B-1, Lane 6: Sangla B-2, Lane 7: Sangla B-3, Lane 8: Sangla B-5, Lane 9: Sangla B-6, Lane 10: Sangla B-7, Lane 11:IC-412744, Lane 12:IC-421598, Lane 13: IC-412722, Lane 14:IC-412863, Lane 15: IC-421597; **c**: Lane1:*F. cymosum*; M: λDNA *Eco*R1 + *Hind* III double digest; **d:** Dendrogram based on the UPGMA analysis generated from similarity matrix of the RAPD profiles of different accessions of buckwheat (*Fagopyrum* spp.) studied in the present investigation using Primer OPO-16.



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947/831
564
21226
2027
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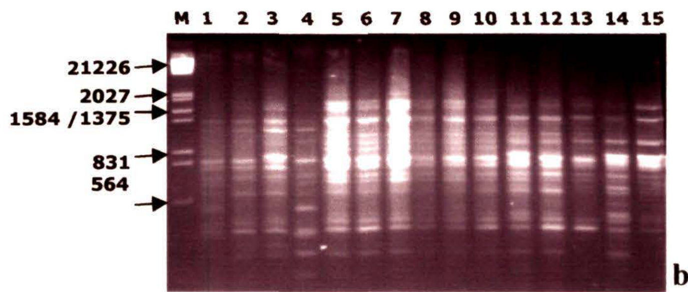
947
831
564
a



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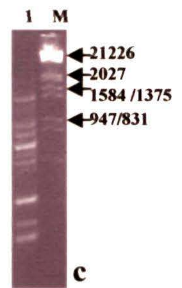
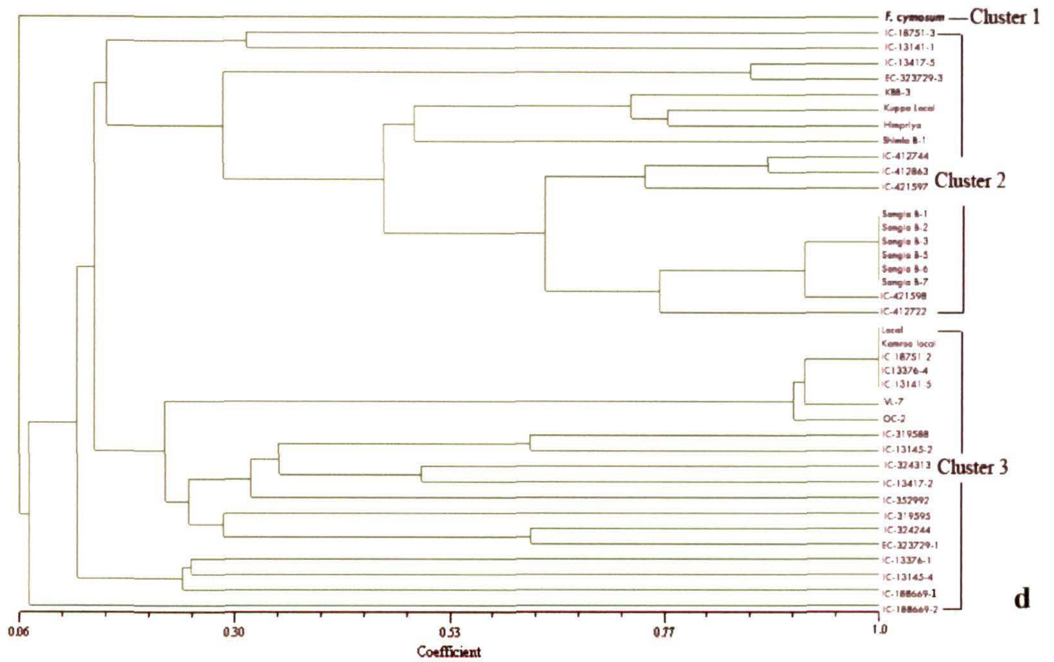
21226
2027
1584 /1375
831
564
b



1 M
21226
2027
1584 /1375
947/831
c



F. cymosum
Cluster 1
IC-18751-3
IC-13141-1
IC-13417-5
EC-323729-3
KBB-3
Kuppa Local
Himpriya
Shimla B-1
IC-412744
IC-412863
IC-421597
Cluster 2
Sangla B-1
Sangla B-2
Sangla B-3
Sangla B-5
Sangla B-6
Sangla B-7
IC-421598
IC-412722
Local
Kamroo local
IC 18751 2
IC13376-4
IC 13141 5
VL-7
OC-2
IC-319588
IC-13145-2
IC-324313
Cluster 3
IC-13417-2
IC-352992
IC-319595
IC-324244
EC-323729-1
IC-13376-1
IC-13145-4
IC-188669-1
IC-188669-2
d
0.06
0.30
0.53
0.77
1.0
Coefficient

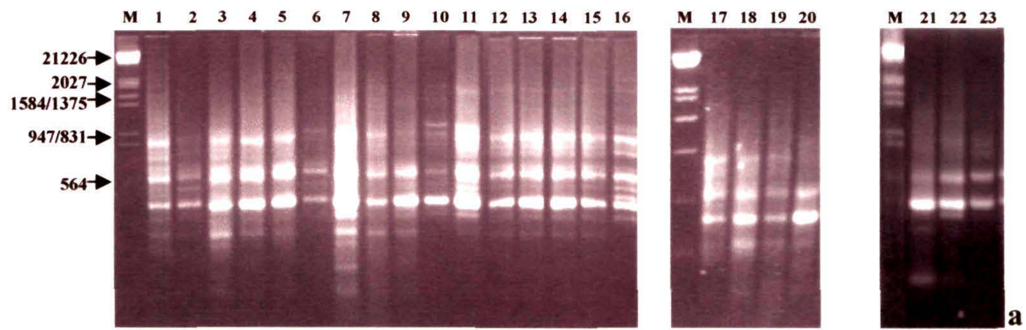
Table 4.18: Similarity matrix, based on Jaccard's coefficient, of the RAPD profile generated by primer OPO-16 of different accessions/cultivars of buckwheat (*Fagopyrum* spp.) studied in the present investigation.

Accessions	IC-188669-1	IC-188669-2	IC-18751-2	IC-18751-3	IC-13376-1	IC-13376-4	IC-13145-2	IC-13145-4	IC-13141-1	IC-13141-5	IC-13417-2	IC-13417-5	EC-323729-1	EC-323729-3	IC-319588	IC-324313	IC-352992	IC-319595	IC-324244	Local	Kamroo Local	VL-7	OC-2	KBB-3	Himpriya	Kuppa local	Shimla B-1	SanglaB-1	SanglaB-2	SanglaB-3	SanglaB-5	SanglaB-6	SanglaB-7	IC-412744	IC-412863	IC-421598	IC-412722	IC-421597	F. cymosum
IC-188669-1	1.00																																						
IC-188669-2	0.13	1.00																																					
IC-18751-2	0.15	0.12	1.00																																				
IC-18751-3	0.15	0.08	0.11	1.00																																			
IC-13376-1	0.24	0.05	0.08	0.21	1.00																																		
IC-13376-4	0.15	0.12	1.00	0.11	0.08	1.00																																	
IC-13145-2	0.24	0.16	0.17	0.13	0.25	0.17	1.00																																
IC-13145-4	0.13	0.00	0.13	0.14	0.17	0.13	0.31	1.00																															
IC-13141-1	0.13	0.00	0.13	0.31	0.20	0.13	0.07	0.05	1.00																														
IC-13141-5	0.15	0.12	1.00	0.11	0.08	1.00	0.17	0.13	0.13	1.00																													
IC-13417-2	0.18	0.23	0.17	0.12	0.09	0.17	0.26	0.15	0.09	0.17	1.00																												
IC-13417-5	0.30	0.30	0.18	0.19	0.08	0.18	0.22	0.08	0.22	0.18	0.85	1.00																											
EC-323729-1	0.05	0.07	0.11	0.04	0.14	0.11	0.00	0.07	0.09	0.11	0.06	0.08	1.00																										
EC-323729-3	0.08	0.05	0.13	0.13	0.20	0.13	0.03	0.11	0.11	0.13	0.09	0.86	0.33	1.00																									
IC-319588	0.00	0.16	0.13	0.10	0.03	0.13	0.07	0.62	0.11	0.13	0.50	0.18	0.50	0.07	1.00																								
IC-324313	0.00	0.16	0.13	0.17	0.07	0.13	0.07	0.62	0.11	0.13	0.50	0.18	0.50	0.07	0.11	1.00																							
IC-352992	0.00	0.16	0.13	0.10	0.07	0.13	0.07	0.62	0.11	0.13	0.50	0.18	0.50	0.07	0.07	0.07	1.00																						
IC-319595	0.00	0.16	0.13	0.10	0.07	0.13	0.07	0.62	0.11	0.13	0.50	0.18	0.50	0.07	0.03	0.03	0.03	1.00																					
IC-324244	0.50	0.13	0.13	0.17	0.07	0.13	0.07	0.16	0.11	0.13	0.50	0.18	0.62	0.07	0.07	0.07	0.07	0.07	1.00																				
Local	0.15	0.12	1.00	0.11	0.08	1.00	0.17	0.13	0.13	1.00	0.17	0.18	0.11	0.13	0.42	0.42	0.42	0.42	0.42	1.00																			
Kamroo local	0.15	0.12	1.00	0.11	0.08	1.00	0.17	0.13	0.13	1.00	0.17	0.18	0.11	0.13	0.42	0.42	0.42	0.42	0.42	1.00	1.00																		
VL-7	0.16	0.13	0.92	0.11	0.08	0.92	0.18	0.13	0.13	0.92	0.11	0.08	0.11	0.08	0.30	0.30	0.30	0.30	0.30	0.92	0.92	1.00																	
OC-2	0.14	0.11	0.92	0.10	0.12	0.92	0.12	0.12	0.17	0.92	0.16	0.18	0.10	0.12	0.40	0.40	0.40	0.40	0.40	0.92	0.92	0.85	1.00																
KBB-3	0.16	0.00	0.15	0.15	0.24	0.15	0.13	0.13	0.18	0.15	0.11	0.29	0.18	0.24	0.13	0.13	0.13	0.13	0.14	0.15	0.15	0.10	0.14	1.00															
Himpriya	0.17	0.00	0.16	0.12	0.25	0.16	0.14	0.14	0.14	0.16	0.12	0.18	0.19	0.25	0.14	0.14	0.14	0.14	0.12	0.16	0.16	0.11	0.15	0.75	1.00														
Kuppa local	0.14	0.00	0.14	0.19	0.22	0.14	0.12	0.12	0.17	0.14	0.10	0.18	0.29	0.27	0.12	0.12	0.12	0.12	0.12	0.19	0.19	0.14	0.18	0.71	0.77	1.00													
Shimla B-1	0.14	0.05	0.18	0.22	0.12	0.18	0.16	0.11	0.16	0.18	0.10	0.18	0.28	0.26	0.12	0.12	0.12	0.12	0.13	0.18	0.18	0.14	0.17	0.56	0.50	0.42	1.00												
SanglaB-1	0.10	0.00	0.20	0.15	0.13	0.20	0.04	0.06	0.17	0.20	0.11	0.43	0.31	0.29	0.13	0.13	0.13	0.13	0.13	0.14	0.14	0.10	0.14	0.53	0.47	0.67	0.37	1.00											
SanglaB-2	0.10	0.00	0.14	0.15	0.13	0.14	0.04	0.06	0.17	0.14	0.11	0.43	0.17	0.29	0.13	0.13	0.13	0.13	0.13	0.14	0.14	0.10	0.14	0.53	0.47	0.67	0.37	1.00	1.00										
SanglaB-3	0.10	0.00	0.14	0.15	0.13	0.14	0.04	0.06	0.17	0.14	0.11	0.43	0.24	0.29	0.13	0.13	0.13	0.13	0.13	0.14	0.14	0.10	0.14	0.53	0.47	0.67	0.37	1.00	1.00	1.00									
SanglaB-5	0.10	0.00	0.14	0.15	0.13	0.14	0.04	0.06	0.17	0.14	0.11	0.43	0.17	0.29	0.13	0.13	0.13	0.13	0.13	0.14	0.14	0.10	0.14	0.53	0.47	0.67	0.37	1.00	1.00	1.00	1.00								
SanglaB-6	0.10	0.00	0.14	0.15	0.13	0.14	0.04	0.06	0.17	0.14	0.11	0.43	0.17	0.29	0.13	0.13	0.13	0.13	0.13	0.14	0.14	0.10	0.14	0.53	0.47	0.67	0.37	1.00	1.00	1.00	1.00	1.00							
SanglaB-7	0.10	0.00	0.14	0.15	0.13	0.14	0.04	0.06	0.17	0.14	0.11	0.43	0.17	0.29	0.13	0.13	0.13	0.13	0.11	0.14	0.14	0.10	0.14	0.53	0.47	0.67	0.37	1.00	1.00	1.00	1.00	1.00	1.00						
IC-412744	0.18	0.00	0.13	0.13	0.20	0.13	0.15	0.05	0.15	0.13	0.04	0.29	0.14	0.20	0.11	0.11	0.11	0.11	0.12	0.23	0.23	0.18	0.22	0.37	0.32	0.56	0.38	0.69	0.69	0.69	0.69	0.69	0.69	1.00					
IC-412863	0.09	0.00	0.25	0.19	0.17	0.25	0.08	0.06	0.17	0.25	0.10	0.43	0.22	0.27	0.12	0.12	0.12	0.12	0.13	0.32	0.32	0.26	0.30	0.41	0.44	0.73	0.42	0.92	0.92	0.92	0.92	0.92	0.92	0.87	1.00				
IC-421598	0.10	0.00	0.35	0.15	0.13	0.35	0.04	0.06	0.18	0.35	0.05	0.29	0.18	0.18	0.13	0.13	0.13	0.13	0.11	0.21	0.21	0.16	0.20	0.38	0.24	0.60	0.25	0.77	0.77	0.77	0.77	0.77	0.77	0.63	0.71	1.00			
IC-412722	0.18	0.00	0.17	0.13	0.20	0.17	0.15	0.05	0.11	0.17	0.04	0.18	0.14	0.20	0.11	0.11	0.11	0.11	0.13	0.23	0.23	0.18	0.22	0.30	0.32	0.47	0.38	0.59	0.59	0.59	0.59	0.59	0.59	0.88	0.65	0.53	1.00		
IC-421597	0.15	0.00	0.26	0.15	0.17	0.26	0.13	0.06	0.13	0.26	0.05	0.18	0.17	0.17	0.13	0.13	0.13	0.13	0.13	0.20	0.20	0.15	0.19	0.28	0.29	0.47	0.37	0.60	0.60	0.60	0.60	0.60	0.60	0.69	0.67	0.64	0.80	1.00	
F.cymosum	0.12	0.00	0.05	0.08	0.10	0.05	0.00	0.08	0.05	0.05	0.00	0.11	0.13	0.15	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.06	0.17	0.12	0.13	0.11	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.00	0.05	0.00	1.00

similarity estimates revealed clustering of the accessions/cultivars into three broad clusters (Fig. 4.20d). A significant feature of the dendrogram was the emergence of *F. cymosum* as a separate group with a similarity coefficient of 0.06 with both *F. esculentum* and *F. tataricum*. The accessions/cultivars of *tataricum* clustered together in one group with similarity coefficient ranging from 0.42 to 1.0. IC-412744, IC-412863, IC-421597, which are collections from Arunachal Pradesh, showed their distinctness from each other as separate accessions.

Primer 5 (UBC-185) amplified seventeen polymorphic bands in *Fagopyrum* species (Fig. 4.21a-c). The average frequency of occurrence of polymorphic bands was 0.48 with 91.0% polymorphism (Table.4.13; 4.14). A significant feature of the profiles was the detection of a 692 bp RAPD in all the accessions/cultivars of *esculentum* and only in Shimla B-1 of *tataricum* species. Amongst the accessions/cultivars of *F. tataricum*, Shimla B-1 is an early maturing and high yielding variety. There was no clear variation in the number or size of amplicons on the basis of different geographic location of collection sites of the accessions/cultivars. The values for Jaccard's coefficient of similarity based on the RAPD score with primer 5 (UBC-185) are presented in Table 4.19. The values for similarity coefficient ranged between 0.62-1.0 for accessions/cultivars belonging to *F. esculentum*. Within this species the lowest value of 0.62 was recorded between IC-18751-3 and EC-323729 and the highest value of 1.0 was recorded between IC-13376-1, IC-13145-2, IC-13141-1, Local, Kamroo Local, OC-2, IC-319588, IC-324313, IC-352992, IC-319595, IC-324244, IC-13141-5, IC-13417-5, IC-188669-2, IC-18751-2, IC-13376-4. All the cultivars of Sangla group showed similarity coefficient of 1.0. Cluster analysis of the genetic similarity estimates revealed clustering of the accessions/cultivars into three broad clusters (Fig. 4.21d). While cluster

Fig 4.21: RAPD profile of genomic DNA extracted from 7 day old etiolated seedlings of different accessions/cultivars of buckwheat (*Fagopyrum* spp.) studied in the present investigation using the primer UBC-185.**a**: Lane1:IC-188669-1, Lane 2:IC-18751-3, Lane 3:IC-13376-1, Lane 4:IC-13145-2, Lane 5:IC-13141-1, Lane 6:IC-13417-2, Lane 7:EC-323729-1, Lane 8:Local, Lane 9:Kamroo Local, Lane 10:VL-7, Lane 11:OC-2, Lane 12:IC-319588, Lane 13:IC-324313, Lane 14:IC-352992, Lane 15:IC-319595, Lane 16:IC-324244, Lane 17:IC-13141-5, Lane 18:IC-13417-5, Lane 19:IC-188669-2, Lane 20:EC-323729-3, Lane 21:IC-18751-2, Lane 22: IC:13376-4, Lane 23:IC-13145-4; **b**: Lane1:KBB-3, Lane 2:Himpriya, Lane 3:Kuppa Local, Lane 4:Shimla B-1, Lane 5:Sangla B-1, Lane 6: Sangla B-2, Lane 7: Sangla B-3, Lane 8: Sangla B-5, Lane 9: Sangla B-6, Lane 10: Sangla B-7, Lane 11:IC-412744, Lane 12:IC-421598, Lane 13:IC-412722, Lane 14:IC-412863, Lane 15:IC-421597; **c**: Lane1:*F. cymosum*; M: λDNA *Eco*R1 + *Hind* III double digest; **d:** Dendrogram based on the UPGMA analysis generated from similarity matrix of the RAPD profiles of different accessions of buckwheat (*Fagopyrum* spp.) studied in the present investigation using primer UBC-185.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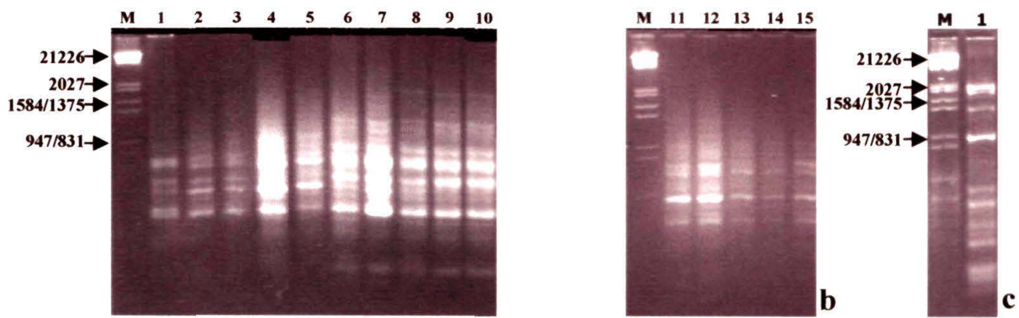
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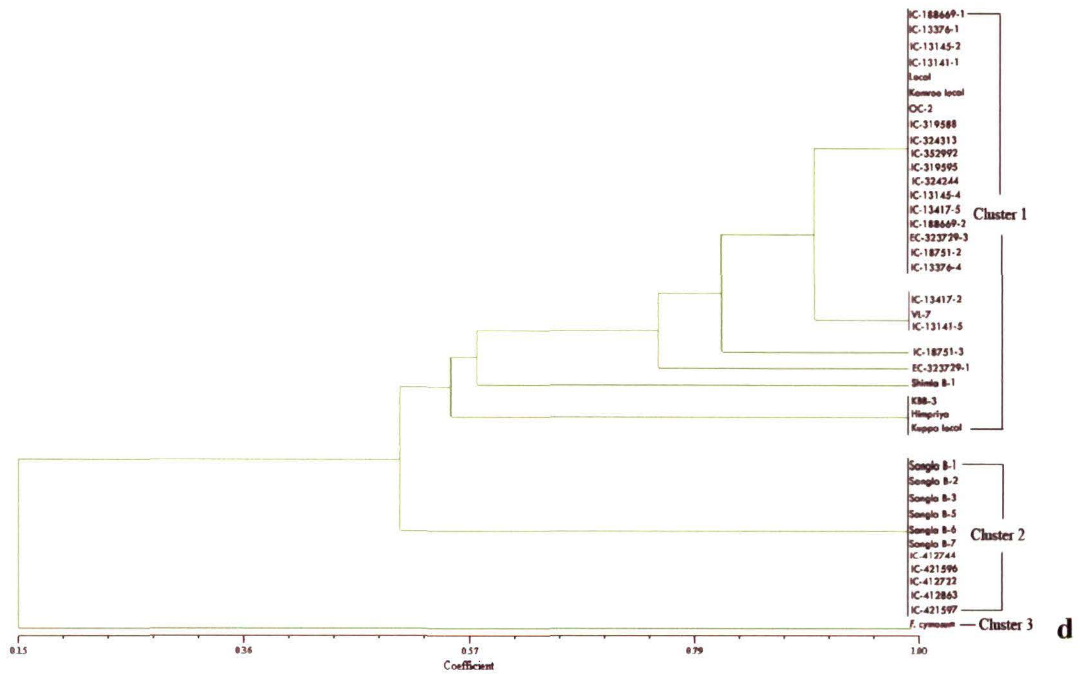

IC-188669-1
IC-13376-1
IC-13145-2
IC-13141-1
Local
Kamroo local
OC-2
IC-319588
IC-324313
IC-352992
IC-319595
IC-324244
IC-13145-4
IC-13417-5
IC-188669-2
EC-323729-3
IC-18751-2
IC-13376-4
IC-13417-2
VL-7
IC-13141-5
IC-18751-3
EC-323729-1
Shimla B-1
KBB-3
Himpriya
Kuppa local
Cluster 1
Sangla B-1
Sangla B-2
Sangla B-3
Sangla B-5
Sangla B-6
Sangla B-7
IC-412744
IC-421596
IC-412722
IC-412863
IC-421597
Cluster 2
F. cymosum
Cluster 3
0.15
0.36
0.57
0.79
1.00
Coefficient
d

Table 4.19: Similarity matrix, based on Jaccard's coefficient, of the RAPD profile generated by primer UBC-185 of different accessions/cultivars of buckwheat (*Fagopyrum* spp.) studied in the present investigation.

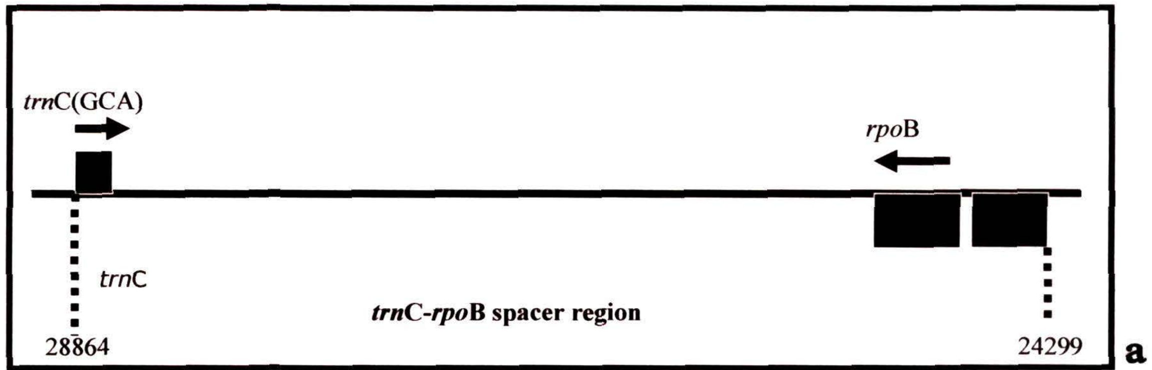
Accessions	IC-188669-1	IC-188669-2	IC-18751-2	IC-18751-3	IC-13376-1	IC-13376-4	IC-13145-2	IC-13145-4	IC-13141-1	IC-13141-5	IC-13417-2	IC-13417-5	EC-323729-1	EC-323729-3	IC-319588	IC-324313	IC-352992	IC-319595	IC-324244	Local	Kamroo local	VL-7	OC-2	KBB-3	Himpriya	Kuppa local	Shimla B-1	Sangla B-1	Sangla B-2	Sangla B-3	Sangla B-5	Sangla B-6	Sangla B-7	IC-412744	IC-412863	IC-421598	IC-412722	IC-421597	*F. cymosum*
IC-188669-1	1.00																																						
IC-188669-2	1.00	1.00																																					
IC-18751-2	1.00	1.00	1.00																																				
IC-18751-3	*0.80*	*0.80*	*0.80*	*1.00*																																			
IC-13376-1	1.00	1.00	1.00	0.80	1.00																																		
IC-13376-4	1.00	1.00	1.00	0.80	1.00	1.00																																	
IC-13145-2	1.00	1.00	1.00	0.80	1.00	1.00	1.00																																
IC-13145-4	0.90	0.90	0.90	0.89	0.90	0.90	0.90	1.00																															
IC-13141-1	1.00	1.00	1.00	0.80	1.00	1.00	1.00	0.90	1.00																														
IC-13141-5	1.00	1.00	1.00	0.80	1.00	1.00	1.00	0.90	1.00	1.00																													
IC-13417-2	0.90	0.90	0.90	0.89	0.90	0.90	0.90	1.00	0.90	0.90	1.00																												
IC-13417-5	1.00	1.00	1.00	0.80	1.00	1.00	1.00	0.90	1.00	1.00	0.90	1.00																											
EC-323729-1	0.77	0.77	0.77	0.62	0.77	0.77	0.77	0.69	0.77	0.77	0.69	0.77	1.00																										
EC-323729-3	1.00	1.00	1.00	0.80	1.00	1.00	1.00	0.90	1.00	1.00	0.90	1.00	0.77	1.00																									
IC-319588	1.00	1.00	1.00	0.80	1.00	1.00	1.00	0.90	1.00	1.00	0.90	1.00	0.77	1.00	1.00																								
IC-324313	1.00	1.00	1.00	0.80	1.00	1.00	1.00	0.90	1.00	1.00	0.90	1.00	0.77	1.00	1.00	1.00																							
IC-352992	1.00	1.00	1.00	0.80	1.00	1.00	1.00	0.90	1.00	1.00	0.90	1.00	0.77	1.00	1.00	1.00	1.00																						
IC-319595	1.00	1.00	1.00	0.80	1.00	1.00	1.00	0.90	1.00	1.00	0.90	1.00	0.77	1.00	1.00	1.00	1.00	1.00																					
IC-324244	1.00	1.00	1.00	0.80	1.00	1.00	1.00	0.90	1.00	1.00	0.90	1.00	0.77	1.00	1.00	1.00	1.00	1.00	1.00																				
Local	1.00	1.00	1.00	0.80	1.00	1.00	1.00	0.90	1.00	1.00	0.90	1.00	0.77	1.00	1.00	1.00	1.00	1.00	1.00	1.00																			
VL-7	1.00	1.00	1.00	0.80	1.00	1.00	1.00	0.90	1.00	1.00	0.90	1.00	0.77	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00																		
Kamroo local	0.90	0.90	0.90	0.89	0.90	0.90	0.90	1.00	0.90	0.90	1.00	0.90	0.69	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	1.00																	
OC-2	1.00	1.00	1.00	0.80	1.00	1.00	1.00	0.90	1.00	1.00	0.90	1.00	0.77	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.90	1.00																
KBB-3	0.55	0.55	0.55	0.66	0.55	0.55	0.55	0.60	0.55	0.55	0.60	0.55	0.43	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.60	0.55	1.00															
Himpriya	0.55	0.55	0.55	0.66	0.55	0.55	0.55	0.60	0.55	0.55	0.60	0.55	0.43	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.60	0.55	1.00	1.00														
Kuppa local	0.55	0.55	0.55	0.66	0.55	0.55	0.55	0.60	0.55	0.55	0.60	0.55	0.43	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.60	0.55	1.00	1.00	1.00													
Shimla B-1	0.55	0.55	0.55	0.66	0.55	0.55	0.55	0.78	0.55	0.55	0.78	0.55	0.43	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.78	0.55	0.56	0.56	0.56	1.00												
Sangla B-1	0.53	0.53	0.53	0.40	0.53	0.53	0.53	0.47	0.53	0.53	0.47	0.53	0.63	0.53	0.53	0.53	0.53	0.53	0.53	0.53	0.53	0.47	0.53	0.43	0.43	0.43	0.43	1.00											
Sangla B-2	0.53	0.53	0.53	0.40	0.53	0.53	0.53	0.47	0.53	0.53	0.47	0.53	0.63	0.53	0.53	0.53	0.53	0.53	0.53	0.53	0.53	0.47	0.53	0.43	0.43	0.43	0.43	1.00	1.00										
Sangla B-3	0.53	0.53	0.53	0.40	0.53	0.53	0.53	0.47	0.53	0.53	0.47	0.53	0.63	0.53	0.53	0.53	0.53	0.53	0.53	0.53	0.53	0.47	0.53	0.43	0.43	0.43	0.43	1.00	1.00	1.00									
Sangla B-5	0.53	0.53	0.53	0.40	0.53	0.53	0.53	0.47	0.53	0.53	0.47	0.53	0.63	0.53	0.53	0.53	0.53	0.53	0.53	0.53	0.53	0.47	0.53	0.43	0.43	0.43	0.43	1.00	1.00	1.00	1.00								
Sangla B-6	0.53	0.53	0.53	0.40	0.53	0.53	0.53	0.47	0.53	0.53	0.47	0.53	0.63	0.53	0.53	0.53	0.53	0.53	0.53	0.53	0.53	0.47	0.53	0.43	0.43	0.43	0.43	1.00	1.00	1.00	1.00	1.00							
Sangla B-7	0.53	0.53	0.53	0.40	0.53	0.53	0.53	0.47	0.53	0.53	0.47	0.53	0.63	0.53	0.53	0.53	0.53	0.53	0.53	0.53	0.53	0.47	0.53	0.43	0.43	0.43	0.43	1.00	1.00	1.00	1.00	1.00	1.00						
IC-412744	0.53	0.53	0.53	0.40	0.53	0.53	0.53	0.47	0.53	0.53	0.47	0.53	0.63	0.53	0.53	0.53	0.53	0.53	0.53	0.53	0.53	0.47	0.53	0.43	0.43	0.43	0.43	1.00	1.00	1.00	1.00	1.00	1.00	1.00					
IC-412863	0.53	0.53	0.53	0.40	0.53	0.53	0.53	0.47	0.53	0.53	0.47	0.53	0.63	0.53	0.53	0.53	0.53	0.53	0.53	0.53	0.53	0.47	0.53	0.43	0.43	0.43	0.43	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00				
IC-421598	0.53	0.53	0.53	0.40	0.53	0.53	0.53	0.47	0.53	0.53	0.47	0.53	0.63	0.53	0.53	0.53	0.53	0.53	0.53	0.53	0.53	0.47	0.53	0.43	0.43	0.43	0.43	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00			
IC-412722	0.53	0.53	0.53	0.40	0.53	0.53	0.53	0.47	0.53	0.53	0.47	0.53	0.63	0.53	0.53	0.53	0.53	0.53	0.53	0.53	0.53	0.47	0.53	0.43	0.43	0.43	0.43	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00		
IC-421597	0.53	0.53	0.53	0.40	0.53	0.53	0.53	0.47	0.53	0.53	0.47	0.53	0.63	0.53	0.53	0.53	0.53	0.53	0.53	0.53	0.53	0.47	0.53	0.43	0.43	0.43	0.43	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	
F. cymosum	0.13	0.13	0.13	0.25	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.17	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.15	0.15	0.15	0.15	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	1.00

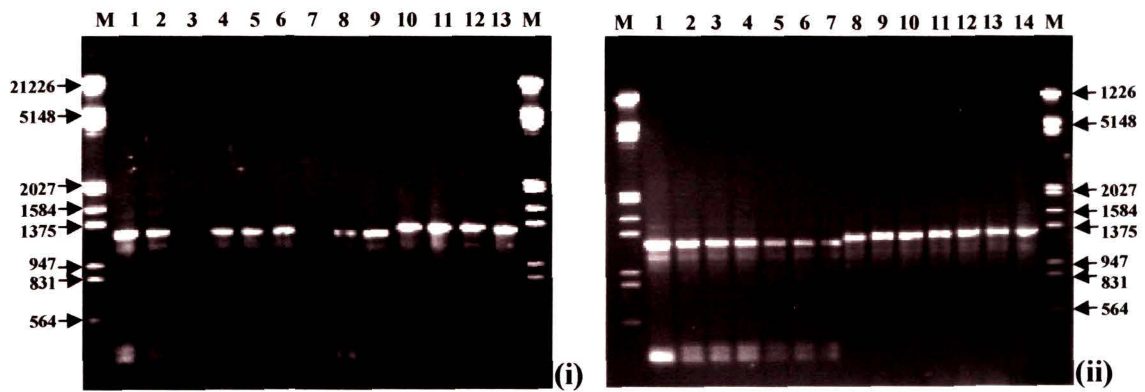
1 included all the accessions/cultivars of *F. esculentum* and four cultivars of *F. tataricum*, cluster 2 included the accessions/cultivars of *F. tataricum*. Cluster 1 had three sub clusters with majority of the accessions/cultivars *viz.* IC-188669-2, IC-18751-3, IC-13145-2, IC-13141-1, Local, Kamroo local, OC-2, IC-319588, IC-324313, IC-352992, IC-319595, IC-324244, IC-13141-5, IC-13417-5, IC-188669-2, EC-323729 -3, IC-18751-1, IC-13376-4 forming one sub cluster. While IC-13417-2, IC13145-5 and variety VL-7 formed the 2nd sub cluster. KBB-3, Himpriya and Kuppa local formed the third sub cluster. Accessions/cultivars within each sub cluster showed 100% similarity amongst themselves. Cluster 2 included Sangla B-1, Sangla B-2, Sangla B-3, Sangla B-5, Sangla B-6, Sangla B-7, IC-412744, IC-421596, IC-412722, IC-412863 and IC-421597 of *tataricum*. *F. cymosum* emerged out separately in Cluster 3.

PCR amplification of the intergenic spacer region between *trn*C and *rpo*B was carried out using primer listed in table 3.5. Diagrammatic representation of organization of *trn*C-*rpo*B intergeneric spacer region is presented in Fig. 4.22a. The amplification resulted in generation of a 1.36 kbp DNA fragment in accessions/cultivars of *F. esculentum*, a 1.42 kbp fragment in accessions/cultivars of *F. tataricum* and a 1.39 kbp fragment in *F. cymosum* (Fig. 4.22b). There was no intraspecific variation in the size of the amplicon in any of the species tested. However, the profile revealed distinct inter-specific variation in the size of the amplified product. The amplified DNA fragment from each accession/cultivars was eluted from the gels using a modified protocol of Byrnes *et al.*, (1995). The eluted DNA fragments were digested with *Eco*RI. The restriction digestion pattern did not reveal any intraspecific variation in the banding pattern of the digested DNA. However, differences were observed in the banding pattern between accessions/cultivars belonging to different species (Fig. 4.23).

Fig 4.22 a: Diagrammatic representation of organization of *trn*C-*rpo*B intergeneric spacer region.

b: Polymerase chain reaction (PCR) amplification of the intergenic spacer region between *trn*C and *rpo*B of different accessions/cultivars of buckwheat (*Fagopyrum* spp.) studied in the present investigation. **(i):** Lane 1:Local, Lane 2:Kamroo Local, Lane 3:IC-319588, Lane 4:IC-324313, Lane 5:IC-324244, Lane 6:IC-188669, Lane 7:Empty, Lane 8:IC-13376, Lane 9:EC-323729, Lane 10: Sangla B-1, Lane 11: Sangla B-2, Lane 12: IC-412744, Lane13: *F. cymosum*; **(ii):** Lane 1:IC-319588, Lane 2:VL-7, Lane 3:OC-2, Lane 4:IC-18751, Lane 5:IC-13141, Lane 6:IC-13417, Lane 7:EC-323729, Lane 8:KBB-3, Lane 9:Himpriya, Lane 10:Kuppa local, Lane 11:Shimla B-1, Lane 12:SanglaB-3 13:IC-421598, Lane 14:*F. cymosum*; **(iii):** Lane 1:IC-352992, Lane 2:IC: 319595, Lane 3:IC-324244, Lane 4:*F. cymosum*, Lane 5:IC-421598 Lane 6:IC-412722, Lane 7:IC-412863, Lane 8:IC-421597, Lane 9:Sangla B-5, Lane 10:Sangla B-6, Lane 11:Sangla B-7.


trnC(GCA)
rpoB
trnC
trnC-rpoB spacer region
28864
24299
a


M 1 2 3 4 5 6 7 8 9 10 11 12 13 M
21226
5148
2027
1584
1375
947
831
564
(i)
M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 M
1226
5148
2027
1584
1375
947
831
564
(ii)

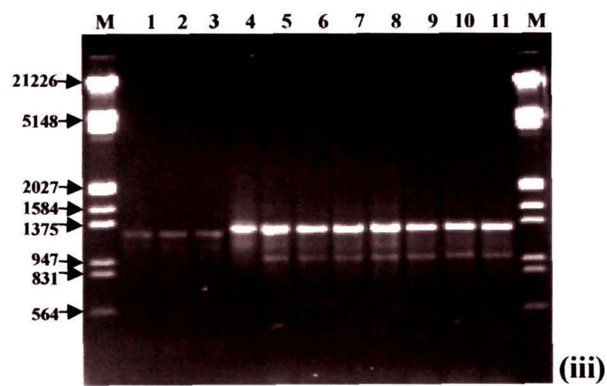
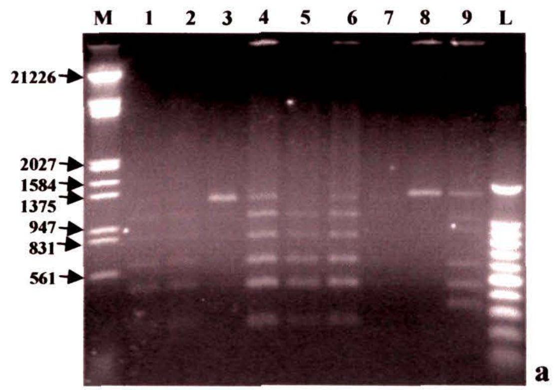

M 1 2 3 4 5 6 7 8 9 10 11 M
21226
5148
2027
1584
1375
947
831
564
(iii)
b

Fig 4.23: Restriction fragment length profile (RFLP) of amplicons of *trn*C-*rpo*B intergeneric spacer region of different accessions/cultivars of buckwheat (*Fagopyrum* spp.) studied in the present investigation. **a:** Lane 1:Local, Lane 2:Kamroo Local, Lane 3:Undigested DNA, Lane 4:IC-13376, Lane 5:EC-323729, Lane 6:IC-324313, Lane 7:IC-319595, Lane 8:Undigested DNA, Lane 9:*F. cymosum*; **b:** Lane 1:Undigested DNA, Lane 2:IC-13141, Lane 3:IC-13417, Lane 4:IC-319588, Lane 5:KBB-3, Lane 6:Himpriya, Lane 7:Kuppa Local, Lane 8:Shimla B-1, Lane 9:Sangla B-2, Lane 10:IC-412744, Lane 11: IC-421598, Lane 12: *F. cymosum.*


M
1
2
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4
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8
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L
21226
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1584
1375
947
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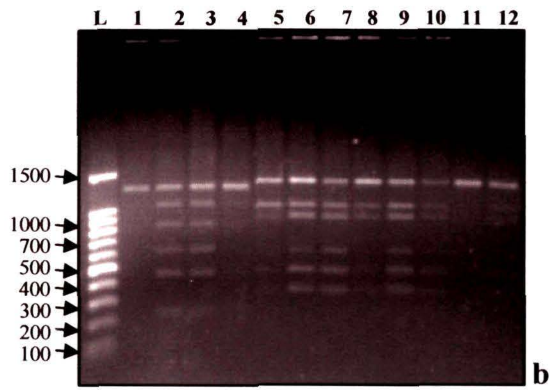
561
a


L
1
2
3
4
5
6
7
8
9
10
11
12
1500
1000
700
500
400
300
200
100
b

CHAPTER : V

DISCUSSION

DISCUSSION:

The genus *Fagopyrum* belongs to the family Polygonaceae. The Polygonaceae family has leaves that vary in size, arrangement and shape, but the leaf stalk is always surrounded by a membranous or chaffy sheath at the base. The flowers are often grouped in clusters that are showy owing to the colour of the sepals or bracts, for there are no petals. The fruit is a triangular nut, sometimes prominently winged. The common buckwheat plant is a broadleaved, erect annual with a single main stem and a branching habit. Common buckwheat is an indeterminate species in photoperiodic response. The flowers of *F. esculentum* are perfect but incomplete. They have no petals, but the calyx is composed of five petal-like sepals that are usually white, pink or dark pink. The flowers are showy and densely clustered in racemes at the ends of the branches or on short pedicels that arise from the axils of the leaves. This species is dimorphic, having plants bearing one of two flower types. The pin flowers have long pistils and short stamens

while the thrum flowers have short pistils and long stamens. Flowers with pistils and stamens of similar length (Esser 1953; Marshall 1969) and lines with only one floral type (Marshall 1969; Fesenko and Antonov 1973) have also been reported.

Despite the large number of collections there appears to be little agreement on important traits to be documented for characterization of different accessions of the crop. Ohnishi (1995) has given a key for identification of different species of *Fagopyrum* on the basis of morphological features. While the key holds well for identification upto the level of genus it does not hold well for identification upto the level of species. The key identifies a species of the genus *Fagopyrum* as *F. cymosum* if the achene has a smooth surface. However, the achene of *F. cymosum* has a rough surface. The preparation of a list of Buckwheat Descriptors (IPGRI 1994) has been the only major step forward in the coordination of passport data and descriptors on this crop. The descriptors used for development of passport data for most of the collections have included plant height, number of branches, number of internodes, petiole length, days to flowering and maturity, grain shape, grain size, grain yield etc. Since most of these traits can be influenced by environment, the evaluation data has not lead to development of reliable morphological markers for determination of diversity in buckwheat. This has lead to a greater focus on identification of qualitative traits for determining genetic diversity in the crop.

The grains of different accessions/cultivars of buckwheat studied in the present investigation showed a wide variation in shape. While the grains from plants belonging to different accessions/cultivars of *F. esculentum* and *F. cymosum* were triangular in shape those of *F. tataricum* were either conical or globous in shape. All the accessions/cultivars of *F. tataricum*, except Shimla B-1 and Kuppa Local, showed conical type of grain shape.

One of the prominent features of grains of Shimla B-1 was the presence of deep furrows with prominent three lobes. All the accessions/cultivars of *F. esculentum* had a smooth seed coat. Differences were, however, observed in the grain striations within different accessions/cultivars of *F. esculentum.* Intra accession variation for presence or absence of striation was observed in the collections of *esculentum* from Himachal Pradesh. While there was no prominent striation on the grain coat of IC-319588, IC-319595, IC-324313, IC-324244, IC-352992 and Local the seed coat surface of Kamroo Local, OC-2 and VL-7 had prominent striations which extended longitudinally across the surface of the coat. All the accessions/cultivars of *tataricum* and *cymosum* showed rough seed coat without any striations. The absence of any striations on the grain coat in the cultivar "Local" indicates that this cultivar is a distinct local cultivar of Uttaranchal. Significantly Kamroo local, OC-2 and VL-7, which were also the collections of Uttaranchal, did not show any striations on the grain coat. The accessions/cultivars of *Fagopyrum* showed broadly two types of grains i.e. winged and non-winged. These observations clearly indicate that grain morphology can be used as an important feature to differentiate accessions/cultivars of *F. esculentum* and *F. cymosum* from those of *F. tataricum.*

While the accessions IC-188669, IC-18751, IC-13376, IC-13141, IC-13417, and IC-323729 had white flowers and produced both winged as well as non-winged grains, the accessions IC-319588, IC-319595, IC-324313, IC-324244, and IC-352992 had only white flowers which produced non winged grains only. Cultivars Local, Kamroo Local, OC-2 and VL-7 were exclusively non winged. Cultivars Local, Kamroo Local and OC-2 had pink flowers while those of VL-7 white flowers. On the other hand accessions/cultivars belonging to *F. tataricum* showed yellowish green coloured flowers and produced only non-winged grains. These observations are in conformity with those

of Joshi and Paroda (1991) and Campbell (1997) who has described in detail the morphology of *F. esculentum*, *F. tataricum* and *F. cymosum.* Variations were also observed in shape of the leaf blade and leaf margin colour between different species of buckwheat. While *F. esculentum* and "*F. himalianum*" showed both cordate and sagittate leaf blade morphology, *F. cymosum* had exclusively sagittate blades. The leaf blade shape in *F. tataricum* was hastate. The presence of hastate leaf blade thus appears to be a feature exclusively associated with *F. tataricum.*

The qualitative parameters analyzed in the present investigation indicate that IC-13145, which has been identified by NBPGR as "*F. himalianum*", belongs to the *esculentum* group rather than as a separate species. Similarly IC-13141, which has been identified by NBPGR as an accession of *F. esculentum,* is described as *F. tataricum* by Joshi and Paroda (1991). Our results indicate that the accession belongs to *F. esculentum* and not *F. tataricum* as described by Joshi and Paroda (1991).

Accessions belonging to *F. esculentum* showed intra-accession variation in morphological features of the grains including shape, colour, and presence or absence of wings and striation on the grains, shape of the leaf blade and colour of the stem. On the other hand there was no intra-accession variation in flower colour and leaf margin colour. These observations indicate that while the morphological features of the grains and shape of the leaf blade and stem colour can not be used solely for identification of any of the accessions/cultivars of *F. esculentum,* features like flower colour and leaf margin colour could be used as pointers towards identification of accessions/cultivars in this species. Within *F. tataricum*, however, intra-accession variations were detected in the shape of the grains and colour of the stem. These observations point towards the presence of a high level of heterozygosity in accessions/cultivars of *F. esculentum* compared to that in

F. tataricum. This could be ascribed to the cross pollinating nature of accessions of *F. esculentum* and the self pollinating nature of accessions/cultivars belonging to *F. tataricum* species. Ohnishi (1998b) has indicated that the loss of variability in tartary buckwheat probably occurred during the process of domestication. These workers have suggested that tartary buckwheat could have acquired four variants through mutation. Each of the mutated lines was fixed in several local populations during diffusion of buckwheat cultivation to other parts of China and to Himalayas.

Cluster analysis of data on qualitative traits of different accessions/cultivars of buckwheat studied in the present investigation indicates a high level of genetic variability amongst them. However these features could not be correlated to the accession/cultivars collected from different geographical location *i.e.* presence of a particular character in accessions/cultivars from a particular geographic location.

The accessions of *F. esculentum* collected from Himachal Pradesh and cultivars from Uttaranchal showed a comparatively higher level of inter-accession variation in plant height and number of branches per stem, there was no significant variation in plant height and number of branches per stem in accessions of the species collected from Arunachal Pradesh. This could probably indicate that either the collections from Arunachal Pradesh belonged to a uniform gene pool or these accessions had a greater adaptive capability to the environmental conditions of Arunachal Pradesh. Amongst all the accession/cultivars of *esculentum* studied in the present investigation, VL-7 took least number of days to mature. This identifies VL-7 as an early maturing cultivar. The little variation in yield of VL-7 during the winter season could be due to better adaptation of VL-7 as a summer crop.

. The accessions of *esculentum* did not show significant variation in grain weight except IC-319595. While the cultivars collected from Uttaranchal showed variation, with a minimum grain weight of 0.46g in OC-2 and 0.84g in VL-7 for 25 grains. VL-7 was identified as a cultivar with highest grain weight. This cultivar proves to be very important from high yielding, early maturing and high grain weight point of view. The accessions/cultivars of *tataricum* showed low grain weight than *F. esculentum*. KBB-3 proves to be an important cultivar amongst the *tataricum* group as it showed high grain weight. *F. cymosum* showed highest grain yield of all the species, thus making it an important species to be considered for crop improvement.

The accessions/cultivars of *esculentum* did no show much variation in hull/ groat ratio except for Kamroo local, IC-319595 (0.41) and IC-352992 (0.35). Amongst the *tataricum* group the minimum hull/ groat ratio was shown by IC-412722 (0.21) and 0.45 in Kuppa local. For crop improvement programme accessions having high hull/ groat ratio are avoided as they have greater hull weight compared to other accessions of same species. In *F. cymosum* high grain weight and less hull/ groat ratio revealed higher grout weight.

Compared to *F. esculentum* and *F. tataricum, F. cymosum* showed the higher values for all the quantitative morphological features studied in the present investigation. The reason for *F. cymosum* showing higher quantitative value for all parameters studied could be due to its tetraploid nature (Yamne & Ohnishi, 2001). *F. cymosum* is heterostylous out breeding perennial shrub (Ohnishi and Matsuka, 1996). One of the greatest drawback with this species is the brittleness of the pedicel. Though the species produces the highest number of flowers and seeds per plant most seeds shed off before attaining complete ripening.

Principal coordinate analysis of the data matrix of ten morphological parameters for the accessions/cultivars of buckwheat studied in the present investigation revealed that the accessions/cultivars could be grouped into two groups (Fig.5a). While the accessions/cultivars belonging to *F. esculentum* and *F. cymosum* formed one group those belonging to *F. tataricum* formed the other group. The accessions/cultivars of *F. esculentum* and *F. cymosum* clustered together while those of *F. tataricum* emerged out as a separate group. These results indicate that on the basis of morphological features *F. cymosum* is closer to *F. esculentum* than to *F. tataricum.* Similar observations have been made by Campbell (1997). Our results indicate a relatively higher level of variability in the *esculentum* group. Within the *esculentum* group the cultivars "Local" and Kamroo local and OC-2 together emerged out separately from others. These cultivars have pink flowers, while in "Local" the grains do not have any striations on the surface other cultivars have. Within the *tataricum* group the cultivars Shimla B-1, KBB-3 and Himpriya together and Kuppa local could be identified as different cultivars on the basis of qualitative morphological characters.

Multivariate methods of analysis, such as the PC and cluster analyses have been extensively used to assess genetic diversity in several species including quinoa (Ortiz *et al.* 1998), blackgram (Ghafoor *et al.* 2001), sorghum (Grenier *et al.* 2001), sugarcane (Tai and Miller 2002), chickpea (Ghafoor *et al.* 2003) and cacti (Carmona and Casas 2005). Our results support the significance of use of Principal coordinates analysis for analysis of genetic diversity even in species which have a high level of heterozygosity.

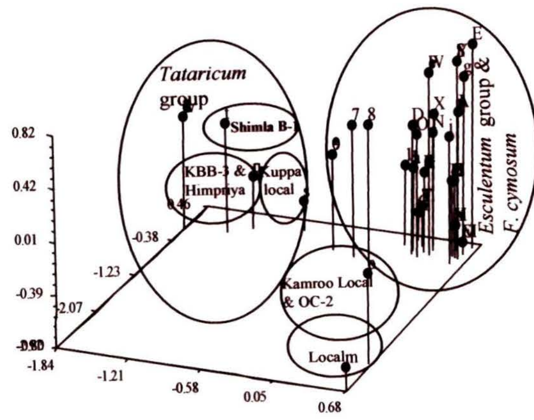
Joshi and Paroda (1991) reported on 408 collections of buckwheat grown in single row observation plots during the years 1985 and 1986 at the Regional Station of the NBPGR at Phagli, Shimla. A wide range of variation was found in characters like plant

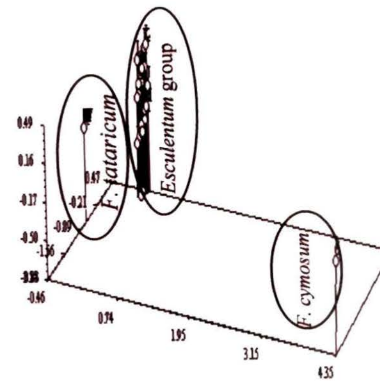
Fig 5a: Plot of principal coordinate analysis based on qualitative morphological characters of different accessions/cultivars of buckwheat (*Fagopyrum* spp.) investigated in the present study.

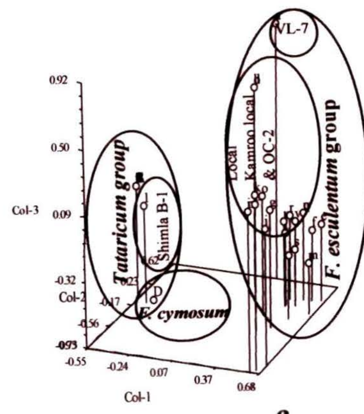
Fig 5b: Plot of principal coordinate analysis based on SDS PAGE profile of total seed proteins extracted from single grains of different accessions/cultivars of buckwheat (*Fagopyrum* spp.) investigated in the present study.

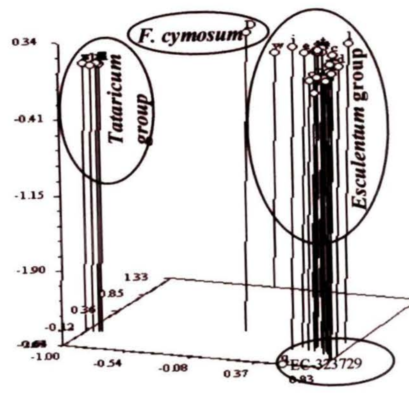
Fig 5c: Plot of principal coordinate analysis based on SDS PAGE profile of endosperm protein extracted from single grains of different accessions/cultivars of buckwheat (*Fagopyrum* spp.) investigated in the present study.

Fig 5d: Plot of principal coordinate analysis based on RAPD profile of genomic DNA extracted from 7 day old etiolated seedlings of different accessions/cultivars of buckwheat (*Fagopyrum* spp.) studied in the present investigation using Primer OPD-6.


Tataricum group
Shimla B-1
KBB-3 & Himpriya
Kuppa local
Esculentum group & F. cymosum
Kamroo Local & OC-2
Localm
0.82
0.42
0.01
-0.39
-1.84
-1.21
-0.58
0.05
0.68
a


F. tataricum
Esculentum group
F. cymosum
0.49
0.16
-0.17
-0.50
0.74
1.95
3.15
4.35
b


VL-7
Tataricum group
Shimla B-1
Local
Kamroo local & OC-2
F. esculentum group
F. cymosum
Col-3
Col-2
Col-1
0.92
0.50
0.09
-0.32
-0.55
-0.24
0.07
0.37
0.68
c


F. cymosum
Tataricum group
Esculentum group
EC-323729
0.34
-0.41
-1.15
-1.90
1.33
0.85
0.36
-0.12
-1.00
-0.54
-0.08
0.37
0.83
d

height, number of branches, number of internodes, days to flower, days to maturity. This is in conformity with the results obtained in the present investigation indicating the existence of a great deal of variability that could be utilized in the development of lines for different growing periods or for use in double-cropping systems. However, the range of values for different parameters reported by Joshi and Paroda (1991) differ from the values reported by us for the same parameters and the same accessions. This variation could be ascribed to differences in the environmental conditions between Phagli (Shimla) and NEHU (Shillong). These observations clearly indicate these morphological descriptors may not be reliable parameters accessioning of buckwheat germplasm. Since the quantifiable morphological parameters are susceptible to environmental influences their use for studying genetic relationships has been criticized by many workers (Gubbels, 1978; Kumar, 1999). Clements & Cowling, (1994) have suggested that morphological features along with molecular characterization for identification of genetic diversity could facilitate better germplasm management and assembling a core collection of crop genetic resources.

The accessions/cultivars of *F. esculentum, F. himalianum, F. tataricum* and *F. cymosum* were analyzed for variations in the profile of grain proteins by SDS PAGE to identify the polymorphic genetic markers for evaluation of genetic resources. SDS PAGE profile of total grain protein revealed a total of 47 bands out of which 14 bands were monomorphic and 33 polymorphic bands revealing high amount of polymorphism. Most of the variations in the profile detected in protein bands showing molecular weights between 121-100 kDa and 54-39 kDa. On the other hand, Rogl and Javornik (1995) reported that the variations in the profiles were restricted to protein bands having molecular weights between 30-54 kDa. Three distinct regions revealing 18 scorable

protein band were detected in this range. They have suggested that the polymorphism revealed by the pool of total proteins was actually the polymorphism of proteins stored in cotyledons. Significantly the SDS PAGE profile of total grain proteins of different accessions of *Fagopyrum* revealed a total of 47 bands out of which 14 were monomorphic to all the accessions and 33 were polymorphic. A significantly high level of inter- as well as intra- accession variation was observed in the SDS PAGE profiles of grain proteins isolated from different accessions/cultivars of *F. esculentum*. On the other hand there was no significant variation in the SDS PAGE profiles of grain proteins isolated from different accessions of *F. tataricum*. Compared with the SDS PAGE profiles of different accessions of *F. esculentum,* the profiles of accessions/cultivars belonging to *F. tataricum* revealed the presence of 6 bands having molecular weights of 102, 97, 85, 80, 70, and 48 kDa only *F. tataricum*. These bands could be considered as marker bands for of this species.

The dendrogram generated on the basis of similarity matrix for SDS PAGE profiles of total proteins, isolated from single grain of different accessions/cultivars of *Fagopyrum*, showed the clustering of the accessions/cultivars into 3 clusters. While cluster 1 included all accessions/cultivars of *F. esculentum* cluster 2 included all the accessions/cultivars belonging to *F. tataricum*. Cluster 2 includes all the accessions /cultivars of *F. tataricum*. There was no polymorphism observed amongst the accessions /cultivars of *tataricum*. A similarity coefficient of 1.0 for these accessions/cultivars indicates that these accessions/cultivars may be genetically similar. *F. cymosum* emerged out separately both from accessions/cultivars of *F. esculentum* and *F. tataricum* and formed cluster 3. A comparison of the SDS PAGE profiles of total proteins from grains

of *F. cymosum* and *F. tataricum* revealed a Jaccard's coefficient of 0.30 indicating the low level of genetic closeness between the two species.

SDS PAGE profile of endosperm proteins revealed a total of 43 bands out of which 6 were monomorphic to all the accessions/cultivars and 37 polymorphic. The variation in band sizes of endosperm seed protein were mainly seen in the molecular weight range of 102-104kDa, 60-74 kDa, 33-56 kDa and 24-31 kDa. Interestingly, most of the protein bands in the 26-54 kDa category belong to the legumin type family of seed proteins (Rout and Chrungoo, 1996; Bharali, 2002). The SDS PAGE profile of endosperm proteins of "*F. himalianum*" showed much similarity with that of *F. esculentum*. Accessions of *F. tataricum* and *F. cymosum*, on the other hand, did not show any significant intraspecific variations in the SDS PAGE profile of grain endosperm proteins. These results are consistent with the observations of Nishiyama *et al.* (1991) and Svetek (1994). One of the most important features of the profile was the presence of a 42 kDa band in accessions/cultivars of *F. esculentum* having winged grains and a 31 kDa band in grains having prominent strips. Further a duplex of 41 and 39 kDa could be detected only in VL-7. This cultivar is high yielding and early maturing, suitable for cultivation in the middle hills (Arora *et al.*, 1995). This protein band could be used as a marker for the identification of this cultivar. Another important feature of the endosperm protein profile was the presence of a 31 kDa band in grains having prominent striations on seed coat surface. The presence or absence of a protein band or a group of bands which are in association with particular character might indicate a relationship between the protein and the particular feature. Similar observations have been made by Rao and Pernolett (1981) who have demonstrated a relationship between wrinkled phenotype and the presence of a 60 kDa vicilin in *Pisum sativum.*

Significant intraspecific variations were detected in the endosperm protein profile of different accessions/cultivars of *F. esculentum*. This could be ascribed to a high degree of grain protein polymorphism in *F. esculentum*. Similar observations have been made by Marija *et al.* (2004) on the endosperm proteins of common buckwheat. Results of the present investigation revealed 40 % polymorphism in the grain proteins of *F. esculentum*. These observations are, however, contradictory to those obtained by Ikeda (1995) and Rogl & Javornik (1996) who have reported high polymorphism of cotyledon storage proteins but high endosperm protein homogeneity in accessions of common buckwheat. Rogl & Javornik (1996) have also demonstrated the relationship between frequencies of protein bands revealed by single seed analysis and their appearance on the bulk seed analyses. Our studies revealed a clear distinction in the electrophoretic profiles of endosperm proteins between accessions of *F. esculentum* and *F. tataricum*. While *F. esculentum* is self-incompatible *F. tataricum* is a self compatible species. Similar results have been reported by Ikeda (1995). These results clearly indicate that SDS PAGE of endosperm proteins can be used as a tool to differentiate accessions/cultivars of *F. esculentum* from *F. tataricum*. Thanh *et al.* (2003) and Rahman *et al.* (2004) have demonstrated the usefulness of SDS PAGE of grain proteins in differentiating the self compatible yellow sarson cultivars from the self-incompatible the brown sarson cultivars.

Principal coordinate analysis (PCA) of the results of SDS PAGE profiles of total grain proteins carried out in the present investigation supports the taxonomic treatment of the three species and the relationship within accessions within the genus *Fagopyrum* (Fig. 5b). Species *esculentum*, *tataricum* and *cymosum* emerged out separately in three distinct groups, with of *esculentum* showing intra specific variation while the species *tataricum* and *cymosum* showed no intra specific variation.

There were no differences in the SDS PAGE profiles of endosperm proteins of different accessions/cultivars of *F. tataricum* except Shimla B-1. A similarity coefficient of 1.0 for these accessions indicates that these accessions/cultivars may be genetically similar. The variation in electrophoretic profiles of endosperm proteins between Shimla B-1 and other accession/cultivars of *F. tataricum* was mainly observed due to the absence of 102, 38 and 34 kDa protein bands in Shimla B-1, while these bands were present in other accessions of *tataricum.* Shimla B-1 is an early maturing and high yielding cultivar of *F. tataricum*. This differentiation could not be made by SDS PAGE profile of total grain proteins. Endosperm proteins have the advantage of reflecting numerous variations since they belong to very polymorphic multigenic families (Doll and Brown, 1979).

F. tataricum is self compatible and is cultivated extensively in the Indian Himalayan foot hills. The loss of variability in *tatary* buckwheat could have occurred during the process of domestication. Ohnishi (1998b, 2004) has suggested that *tartary* buckwheat acquired four variants through mutations during the process of domestication. Each of the variants got fixed in the local populations during diffusion of buckwheat cultivation in the Indian Himalayas. The presence of common protein bands among all the accessions/cultivars of the species may be an evidence of evolutionary origins of the cultivars studied.

The dendrogram generated on the basis of SDS PAGE profile of endosperm proteins revealed the clustering of the accessions into three broad groups. Cluster 1 included all the accessions of *F. esculentum*. Within this cluster three different subgroups were noticed. Subgroup1 included cultivars designated as Kamroo local, OC-2 and VL-7 which were collected from VPKAS, Almora. Accessions having winged seeds and those having strips on the seed coat formed 2nd and 3rd subgroup respectively. IC-13145, which

had been identified as a separate species *viz.* "*F. himalianum*", clustered together with accessions of *F. esculentum* in cluster 1. Our results indicate that "*F. himalianum*" belongs to the *esculentum* group rather than qualifying as a different species. Cluster 2 included all the accessions belonging to *F. tataricum*. All the accessions/cultivars of *F. tataricum*, except Shimla B-1 clustered into one group with 100% similarity. Cluster 3 included the accessions of *F. cymosum*. *F. cymosum* showed least similarity of 39.4% with *F. esculentum* and a maximum of 57% similarity with *F. tataricum*. Our results on SDS PAGE indicate that *F. cymosum*, which to a greater extent resembles *F. esculentum* morphologically, is closer to *F. tataricum*. These observations are in conformity with earlier findings reported on species relationships in *Fagopyrum* using a different marker approach (Kishima *et al.* 1995; Sharma & Jana, 2002).

Principal coordinate analysis (PCA) of SDS PAGE profiles of endosperm grain proteins carried out in the present investigation supports the taxonomic treatment of the three species and the relationship within accessions/cultivars within the genus *Fagopyrum* (Fig. 5c). One of the significant features revealed by PCA was the emergence of VL-7 as cultivar distinct from other accessions/cultivars of the same species. It could also distinguish cultivars Local, Kamroo local and OC-2 as one entity separately from other accessions/cultivars of *esculentum*. VL-7 is a cultivar released by VPKAS, Almora. These results indicate that SDS PAGE profile of endosperm proteins can be used to determine intraspecific variability in *Fagopyrum* spp.

The four enzyme systems analyzed in the present investigation revealed 11 loci with 38 alleles. Out of 38 bands present 33 bands were polymorphic. Of all the isozymes phosphoglucomutase revealed highest polymorphism information content and revealed intra specific variations. Analysis of pair wise similarity between different accessions of

buckwheat on the basis of isozyme profiles revealed a range of values for Jaccard's coefficient of similarity from 0.1 between *esculentum* and *tataricum* groups. These results indicate a wide range of genetic distance between the two species. The values for Jaccard's coefficient of similarity ranged between 0.60-1.0 between different accessions/cultivars of *F. esculentum* and between 0.50-1.0 within the accessions/ cultivars of *F. tataricum.* This indicates a relatively higher level of intraspecific genetic similarity in the genus. This is, however, in contradiction with the observations on SDS PAGE profiles of endosperm proteins which have been able to establish a significantly high level of intraspecifc variation in *F. esculentum.* Based on the results obtained on isozyme profiles of different species of buckwheat Ohnishi (1993) and Chen (1999) have reported that while isozyme profiling could reveal differences between different species of the genus *Fagopyrum* they revealed little information about intraspecific variations in the genus. Results obtained in the present investigation partially supports this observation. While the isozyme profiles of MDH, PGM, EST did not reveal significant information on intraspecific variations in the genus the isozyme profiles of PER could reveal differences between some of the accessions of the same species in the genus. Out of 14 alleles for the enzyme peroxidase detected in the present investigation only one was observed to be monomorphic and the rest polymorphic. The profiles showed accessions specific alleles for IC-188669, IC-18751, IC-13376, IC-13145.

Our result revealed 5 alleles of PGM in *F. tataricum.* This is in conformity with the findings of Ohnishi (1998) on wild *tartary* buckwheat from Yunnan-Sichuan Tibet boarder area. Since *Tartary* buckwheat is self fertilizing, the level of genetic variability in a population is expected to be very limited. Of the 20 loci investigated Ohnishi (1998b) could detect allozyme variation at only three loci only. The allozyme polymorphism

detected in small populations of wild *tartary* buckwheat was ascribed either to partial out crossing or adaptation of different genotypes to different environments (Ohnishi, 2000).

In the present investigation, fifteen decamer primers were used to analyze the RAPD variation in 32 accessions/cultivars of buckwheat from Indian Himalayas. Out of these, only five primers produced reproducible polymorphic amplified products. These primers differed greatly in their efficiency for revealing polymorphism. Since the reaction conditions were kept uniform for all primers, differences in the amplification resolution and the clarity in the banding patterns were probably due to specific requirements of the primer. While the primer OPO-16 generated the 65 RAPD fragments the primer OPD-14 amplified only seventeen bands. A total of 150 bands were observed out of which 96.6% were polymorphic. While primer OPO-16 detected the highest level of polymorphism the primer UBC-185 detected least level of polymorphism in buckwheat genomic DNA. The primers also revealed unique banding patterns for most species, indicating the wide genetic base of the *Fagopyrum* species. The pattern of polymorphism generated by RAPD markers show different degrees of genetic relationships among the species involved in the study. Similar observations on variation in band number and RAPD profile in different species of buckwheat have been made by Sharma and Jana (2000). The presence of unique composite RAPD markers among various *Fagopyrum* species indicates the usefulness of the approach for fingerprinting purposes.

A significant feature of the profile was the amplification of a 851 bp amplicon only in accessions belonging to *F. esculentum* and 314 and 1489 bp amplicons exclusively in accessions/cultivars of *F. tataricum* with primer OPD-6. The primer also amplified a 600 bp and a 1558 bp amplicon in accessions of *F. esculentum* collected from Arunachal Pradesh. While a 1154 bp RAPD was detected in all accessions of *F.*

tataricum except Shimla B-1, a 250 bp RAPD was detected only in KBB-3, Himpriya, Kuppa Local and Shimla B-1. The 250 bp RAPD was not detected in Sangla group of cultivars. Presence of this band in KBB-3, Himpriya, Kuppa Local and Shimla B-1 and its absence from Sangla group of accessions reveals the difference between them. The grouping pattern of genotypes revealed that the composition of clusters were heterogeneous with regard to their geographical origin. Such specific fragments or bands can be used for species identification or accession/cultivars identification which is important. Relationship between the presence or absence of a RAPD band *vis a vis* geographic location of an accession has also been reported by Sharma and Jana (2000). Our results indicate that primer OPD-6 is very effective not only in elucidating genetic relationships between different species of the genus *Fagopyrum* studied in the present investigation. The primer could also differentiate between accessions from the same species individually, as well as on the basis of their location. The absence of 1154bp band from cultivar Shimla B-1, which is high yielding and early maturing cultivar of *F. tataricum*, could be used as a diagnostic of this cultivar.

No single primer or combination of two primers could discriminate between banding pattern for all 32 individuals. Out of many primers used, the UPGMA dendrogram generated using the primers OPD-6 and OPO-16 elucidated the inter-accession as well as inter-specific differences in accessions of buckwheat used in the current study. The RAPD data obtained by different primers supports the taxonomic treatment of different accessions/cultivars in the species. Our data clearly identifies IC-13145, which was identified as "*F. himalianum*" by NBPGR, as an accession of *F. esculentum*. Cluster analysis of the genetic similarity estimates using the primers OPD-6 grouped the cultivars Local, Kamroo local, OC-2 and VL-7 into one subgroup within the

cluster containing accessions/cultivars belonging to *F. esculentum*. Accessions collected from Himachal Pradesh and accessions from Arunachal constituted separate subgroup within the cluster. Even though Tsuji and Ohnishi, (1998, 2000a, b) could not discriminate between cultivated landraces of buckwheat collected from different geographical locations on the basis of RAPD profiles our results clearly indicate the effectiveness of the primers in differentiating between accessions/cultivars collected from different geographical locations.

In contrast to the observations based on morphological parameters and isozyme analysis, the similarity matrix developed on the basis of RAPD profiles could not separate the accessions of *F. tataricum* collected from Arunachal Pradesh from other accessions of the same species. Similarly Sangla group of cultivars could not be distinguished on the basis of RAPD profiles. Several factors may account for the discordance between morphologic and RAPD markers. Johns *et al.*, (1997) suggested that discordance might occur if morphological similarity was due to different combinations of alleles producing similar phenotypes. Discordance between morphologic and molecular markers could occur if a single or few genes controlled the expression of morphological traits, and were not detected by RAPDs (Steiner and Garcia de los Santo 2001). Discordance may also be due to difference in evolutionary rates between morphologic characters and characters originating from selectively neutral, non coding DNA, especially if morphological characters have adaptive value and molecular markers reflects functional neutrality (Johns et al. 1997; Linhart and Grant 1996). Morphological variation was strongly associated with environmental variation. Reasons for inconsistencies may include differences in geographic scale examined, type of breeding system in studied species and level of heterogeneity in environment examined.

The similarity matrix generated on RAPD by using primer OPD-6 revealed that *F. cymosum* showed greater similarity with *F. tataricum* than with *F. esculentum*. Similar results were observed with Primer OPD-14, OPO-16 and UBC-185. These results are in agreement with the observations of Kishima *et al.* (1995), Yasui and Ohnishi (1998a, b) and Sharma & Jana (2002) who have placed *F. cymosum* closer to *F. tataricum* than *F. esculentum*. While the observations of Kishima *et al.* (1995) are based on RFLP analysis of cpDNA those of Yasui and Ohnishi (1998a, b) are based by comparison of the nucleotide sequences of the *rbcL-accD* region of cpDNA and nuclear DNA sequences of different species of the genus *Fagopyrum*. The observations of Sharma & Jana (2002) are based on observations on RAPD profiles of different species of the genus *Fagopyrum*. Our results are in agreement with these observations.

Principal coordinate analysis (PCA) of the RAPD profiles (Fig. 5d) supports the taxonomic treatment of the three species and the relationship within accessions/cultivars within the genus *Fagopyrum*. One of the significant features revealed by PCA was the emergence of EC-323729 as an accession distinct from other accessions of the same species. While EC-323729 is an East European accession the other accessions investigated in the present investigation are from Indian Himalayas.

PCR amplification of the intergenic spacer region between *trn*C and *rpo*B and the restriction digestion profile of the amplicon revealed distinct inter-specific variation while no intraspecific variation. The *trnC-rpoB* spacer region can be used to study genetic relationships at lower taxonomic levels such as within a species.

CHAPTER : VI

GENERAL SUMMARY AND CONCLUSIONS

INTRODUCTION:

Plant genetic resources are considered as one of the most important gifts of nature to mankind. They represent the sum total of diversity accumulated through years of cultivation under domestication and natural selection. Many of these genetic resources are also important sources of high nutritive value foods for human consumption. While the importance of conservation and use of genetic resources for the benefit to mankind can not be understated, the key to successful utilization of the existing genetic resources and the variability available in the broad gene pool requires a systematic evaluation of different agronomic traits in the available germplasm.

Out of the total crop genetic diversity available mankind has depended on a very limited number of crops to meet the needs of staple diets and on a very limited number of major non-food crops to meet associated needs. The narrowing of the number of crops upon which global food security and economic growth depend has placed the future

supply of food and rural incomes at risk. The shrinking portfolio of species used in agriculture reduces the ability of farmers and ecosystems to adapt to new environments, needs and opportunities. So far out of the estimated 75,000 species of edible plant only about 150 have been widely used. Even out of these only about 30 species provide 90% of the world's food. Considering the ever-increasing demand for food materials, it is not only necessary to use the available rich diversity and wide genetic resources and to improve the existing conventional cultivars but also to look for non-conventional lesser known and underutilized food crops.

The Himalayan ranges of India are extremely rich in floristic wealth and are home to a large variety of traditional crops that could form an important component of human diet in times to come. Although Himalayan region is well established as a mega diversity region in the entire Indian sub-continent, the severe population pressure coupled with changes in the socio-economic life style of the peoples pose a serious threat to the unique biodiversity of the region. The region is home to a rich diversity of several plant species many of which are underutilized. These underutilized crops have a good potential for use as food or for industrial purposes. This rich genetic estate, extant in diverse ecosystem, nurtured by ingenious indigenous communities, provides ample opportunities for further development of agriculture in the region at a comparative advantage in terms of sustainability and diversification of farming systems. These crops could also constitute an important genetic base to look for suitable heterologous proteins and their genes, which could be used as tools in crop improvement programmes. Amongst the existing known plant resources, the International Plant Genetic Resources Institute (IPGRI) and Consultative Group on International Agriculture (CGIAR) have identified common buckwheat, grain amaranth and *Chenopodium* as important but underutilized

nutraceutical crops which could be used as the genetic base for identification and isolation of suitable heterologous genes coding for biomolecules of potential economic importance.

Buckwheat (*Fagopyrum spp.*) is an ancient crop, which has long been grown in East Asia and the Himalayan region. It is a major staple food crop in the high altitude zones including the Daliang Mountain in Southwest China (Jiang and Xing, 1992). It is the most important crop of mountain region above 1800m elevation both for grain and greens. Unlike common cereals which are deficient in lysine, the buckwheat has excellent protein quality in terms of essential amino acid composition. Despite the large number of collections there appears to be little agreement on important traits to be documented for characterization of different accessions of the crop. Ohnishi (1995) has given a key for identification of different species of *Fagopyrum* on the basis of morphological features. While the key holds well for identification upto the level of genus it does not hold well for identification upto the level of species. The key identifies a species of the genus *Fagopyrum* as *F. cymosum* if the achene has a smooth surface. However, the achene of *F. cymosum* has a rough surface. The preparation of a list of Buckwheat Descriptors (IPGRI 1994) has been the only major step forward in the coordination of passport data and descriptors on this crop. The descriptors used for development of passport data for most of the collections have included plant height, number of branches, number of internodes, petiole length, days to flowering and maturity, grain shape, grain size, grain yield etc. Since most of these traits can be influenced by environment, the evaluation data has not lead to development of reliable morphological markers for determination of diversity in buckwheat. This has lead to a greater focus on identification of qualitative traits for determining genetic diversity in the crop.

The genus *Fagopyrum* has been divided into two phylogenetic groups *viz.* the *cymosum* group comprising of two cultivated species *F. esculentum* (Moench) and *F. tataricum* (L.) Gaertn and two wild species *F. cymosum* (Meissn), and *F. homotropicum* Ohnishi and the *urophyllum* group comprising *F. urophyllum* and other wild species (Ohnishi and Matsuoka, 1996; Yasui and Ohnishi, 1998a, b; Ohsako and Ohnishi, 2000). Baniya *et al.* (1992) have observed significant variations in plant height, number of branches and leaves, clusters per cyme, seeds per cyme, days to maturity, seed weight, grain yield, seed colour/ shape/ surface in different landraces of buckwheat in Nepal. Joshi and Paroda (1991) have evaluated 408 accessions of Buckwheat from Himalayan region for 31 descriptor parameters including plant height, number of branches and leaves, clusters per cyme, seeds per cyme, days to maturity, seed weight, grain yield, seed colour/ shape/ surface. They have considered accession no. IC-13145 on the level of a species as "*Fagopyrum himalianum*". The accession, housed in the Regional Station, NBPGR, Phagli, Shimla (India), has been listed as "*F. tataricum* var. *himalianum*" by IPGRI (www.ipgri.cgiar.org/publication). Campbell (1997) has, however, considered it as a race of *F. esculentum.* Morphological descriptions of plant cultivars often present problems in clear-cut identification because the phenotypic differences within species are too minute to discriminate between them. The occurrence of large genotype-environment (GE) interactions possesses a major problem of relating phenotypic performance to genetic constitution and makes the selection of genotypes difficult. Molecular tools such as isozyme patterns, seed storage protein polymorphism, Amplified Fragment Length Polymorphism (AFLP), Restriction Fragment Length Polymorphism (RFLP), Random Amplification of Polymorphic DNA (RAPD) provides virtually unlimited source of information on interspecific as well as intraspecific variations. Even though some work

has been done on the analysis of phylogenetic relationships between different species of the genus *Fagopyrum* using isozyme profiling and RFLP variations in cpDNA (Ohnishi, 1998; Ohsako and Ohnishi, 1998; Ohsako and Ohinishi, 2000), not much information is available on inter- as well as intra-specific variations in molecular fingerprints in this genus. The objective of the proposed investigation is to elucidate the variation in different accessions of buckwheat at the molecular level and to develop suitable protein and RAPD based markers for the identification of various accessions of buckwheat.

Accessions of buckwheat used for the present investigation were procured from the National Bureau of Plant Genetic Resources, New Delhi, Vivekananda Laboratory of Hill Agriculture (Indian Council of Agricultural Research), Almora, East Khasi Hills in Meghalaya (India) and the Tawang District of Arunachal Pradesh (India). Germplasm collected from different places was separated into different groups based on their morphological characters. The accessions were grown in the experimental garden to study the qualitative as well as quantitative features during their growth span. The seeds were sown in two different seasons i.e April-July and August-November. Variation in the morphological characters was determined by visual checking of the features on the plants from each accession. Each character was scored either as (1) or (0). Evaluation of variation in the endosperm proteins and RAPD profile was performed by calculating the individual band frequency for each accession. Polymorphism was scored for the presence (1) or absence (0) of bands. Cluster analysis was performed on the similarity matrix based on Jaccard's coefficient by the UPGMA method. All computations were performed with NTSYS-PC version 2.1 (Rohlf, 1993).

RESULTS:

The grains of different accessions/cultivars of buckwheat studied in the present investigation showed a wide variation in shape. While the grains from plants belonging to different accessions/cultivars of *F. esculentum* and *F. cymosum* were triangular in shape those of *F. tataricum* were either conical or globous in shape. All the accessions/cultivars of *F. tataricum*, except Shimla B-1 and Kuppa Local, showed conical type of grain shape. One of the prominent features of grains of Shimla B-1 was the presence of deep furrows with prominent three lobes. All the accessions/cultivars of *F. esculentum* had a smooth grain coat surface. Differences were, however, observed in the grain coat surface striations within different accessions of *F. esculentum*. While there was no prominent striations on the grain coat surface of IC-319588, IC-319595, IC-324313, IC-324244, IC-352992 and Local, the grain coat surface of Kamroo Local, OC-2 and VL-7 had prominent striations which extended longitudinally across the surface of the coat. All the accessions/cultivars of *tataricum* and *cymosum* showed rough seed coat without any striations. The absence of any striations on the coat in the cultivar "Local" indicates that this cultivar is a distinct local cultivar of Uttaranchal. Significantly Kamroo local, OC-2 and VL-7, which are also grown in Uttaranchal, did not have any striations on the grain coat. The accessions of *Fagopyrum* showed broadly two types of grains i.e. winged and non-winged. While the accessions IC-188669, IC-18751, IC-13376, IC-13141, IC-13417, IC-323729 had white flowers and produced both winged as well as non-winged grains, the accessions IC-319588, IC-319595, IC-324313, IC-324244, IC-352992 had only white flowers which produced non winged grains only. The grains belonging to cultivars Local, Kamroo Local, OC-2 and VL-7 were exclusively non-winged. Cultivars Local, Kamroo Local and OC-2 had pink flowers while those of VL-7 white flowers. On the other hand

accessions/cultivars belonging to *F. tataricum* showed yellowish green coloured flowers and produced only non-winged grains. Variations were also observed in shape of the leaf blade and leaf margin colour between different species of buckwheat. While *F. esculentum* and "*F. himalianum*" showed both cordate and sagittate leaf blade morphology, *F. cymosum* had exclusively sagittate blades. The leaf blade shape in *F. tataricum* was hastate. The presence of hastate leaf blade thus appears to be a feature exclusively associated with *F. tataricum.*

The pattern of variation observed for morphological traits among genotypes displayed statistical significant. The similarity matrix based on ten morphological traits among thirty two accessions/cultivars of *Fagopyrum* revealed a range of coefficient of similarity from 0.10 to 1.0. This indicated the diverse nature of collected germplasm. The dendrogram generated on the basis of Jaccard's similarity coefficient for the qualitative morphological traits showed the clustering of accessions/cultivars into two broad groups. Cluster 1 comprised of all the accessions/cultivars of *Fagopyrum esculentum* and *F. cymosum.* IC-13145, which has been identified as *F. himalianum* by NBPGR, showed 100% similarity with accessions IC-13141-3 and IC-13417-4 of *F. esculentum*. Cluster 2 comprised of all the accessions/cultivars of *F. tataricum*. This cluster had three sub-clusters. All the accessions of *F. tataricum* from Arunachal Pradesh showed 100% similarity amongst themselves. The qualitative parameters analyzed in the present investigation indicate that IC-13145, which has been identified by NBPGR as "*F. himalianum*", belongs to the *esculentum* group rather than as a separate species. Similarly, IC-13141 which has been identified by NBPGR as an accession of *F. esculentum* and *F. tataricum* by Joshi and Paroda (1991) appears to belong to *F. esculentum.*

The accessions/cultivars of *F. esculentum, F. himalianum, F. tataricum* and *F. cymosum* were analyzed for variations in the profile of grain proteins by SDS PAGE to identify the polymorphic genetic markers for evaluation of genetic resources. SDS PAGE profile of total grain protein revealed a total of 47 bands out of which 14 bands were monomorphic and 33 polymorphic bands revealing high amount of polymorphism. Most of the variations in the band size in protein profile were detected between 121-100 kDa and 54-39 kDa. The size of resolved polypeptides ranged between 17-121 kDa. A significantly high level of inter- as well as intra- accession variation was observed in the SDS PAGE profiles of grain proteins isolated from different accessions/cultivars of *F. esculentum.* On the other hand there was no significant variation in the SDS PAGE profiles of grain proteins isolated from different accessions/cultivars of *F. tataricum.*

The dendrogram generated on the basis of similarity matrix for SDS PAGE profiles of total proteins, isolated from single grain of different accessions of *Fagopyrum*, showed the clustering of the accessions/cultivars into 3 clusters. While cluster 1 included all accessions/cultivars of *F. esculentum* cluster 2 included all the accessions belonging to *F. tataricum*. There was no polymorphism observed in the accessions/cultivars of *tataricum*. *F. cymosum* emerged out separately both from accessions/cultivars of *F. esculentum* and *F. tataricum* and formed cluster 3.

SDS PAGE profile of endosperm proteins revealed a total of 43 bands out of which 6 were monomorphic to all the accessions and 37 polymorphic. The variation in band sizes of endosperm grain protein was mainly seen in the molecular weight range of 104-102 kDa, 74-60 kDa, 56-33 kDa and 31-24 kDa. Interestingly, most of the protein bands were in the 26-54 kDa category belongs to the legumin type family of grain proteins (Rout and Chrungoo, 1996; Bharali, 2002). One of the most important features

of the profile was the presence of a 42 kDa band in accessions of *F. esculentum* having winged grains and a 31 kDa band in grains having prominent striations on grain coat surface. Further a duplex of 41 and 39 kDa could be detected only in VL-7. This cultivar is high yielding and early maturing, suitable for cultivation in the middle hills (Arora *et al.*, 1995). This protein band could be used as a marker for the identification of this accession.

Significant intraspecific variations were detected in the endosperm protein profile of different accessions of *F. esculentum*. Our results indicate 40% polymorphism in the grain proteins of *F. esculentum*. The dendrogram generated on the basis of SDS PAGE profile of endosperm proteins revealed the clustering of the accessions/cultivars into three broad groups. Cluster 1 included all the accessions/cultivars of *F. esculentum*. Within this cluster three different subgroups were noticed. Subgroup1 included cultivars designated as Kamroo local, OC-2 and VL-7 which were collected from VPKAS, Almora. Accessions having winged seeds and those having strips on the seed coat formed 2nd and 3rd subgroup respectively. IC-13145, which had been identified as a separate species *viz.* "*F. himalianum*", clustered together with accessions of *F. esculentum* in cluster 1. Our results indicate that "*F. himalianum*" belongs to the *esculentum* group rather than qualifying as a different species. Cluster 2 included all the accessions/cultivars belonging to *F. tataricum*. All the accessions/cultivars of *F. tataricum*, except Shimla B-1 clustered into one group with 100% similarity. Cluster 3 included the species *F. cymosum*. *F. cymosum* showed least similarity of 39.4% with *F. esculentum* and a maximum of 57% similarity with *F. tataricum*. Our results on SDS PAGE indicate that *F. cymosum*, which to a greater extent resembles *F. esculentum* morphologically, is closer to *F. tataricum*. These observations are in conformity with earlier findings reported on species

relationships in *Fagopyrum* using a different marker approach (Kishima *et al.* 1995; Sharma & Jana, 2002).

The four enzyme systems analyzed in the present investigation revealed 11 loci with 38 alleles. Out of 38 bands present 33 bands were polymorphic. Based on the results obtained on isozyme profiles of different species of buckwheat Ohnishi (1993) and Chen (1999) have reported that while isozyme profiling could reveal differences between different species of the genus *Fagopyrum* they revealed little information about intraspecific variations in the genus. Results obtained in the present investigation partially support this observation. While the isozyme profiles of MDH, PGM, EST did not reveal significant information on intraspecific variations in the genus the isozyme profiles of PER could reveal differences between accessions of the same species in the genus. Our result revealed 5 alleles of PGM in *F. tataricum.* This is in conformity with the findings of Ohnishi (1998) on wild tartary buckwheat from Yunnan-Sichuan Tibet boarder area. Since *Tartary* buckwheat is self-fertilizing, the level of genetic variability in a population is expected to be very limited.

In the present investigation, fifteen decamer primers were used to analyze the RAPD variation in 32 accessions/cultivars of buckwheat from Indian Himalayas. Out of these, only five primers produced reproducible polymorphic amplified products. These primers differed greatly in their efficiency for revealing polymorphism. While the primer OPO-16 generated 65 RAPD fragments the primer OPD-14 amplified only seventeen bands. While OPO-16 detected the highest level of polymorphism the primer UBC-185 detected least level of polymorphism in buckwheat genomic DNA. A significant feature of the profile was the amplification of 851 bp amplicon only in accessions belonging to *F. esculentum* and 314 and 1489 bp amplicons exclusively in accessions/cultivars of *F.*

tataricum with primer OPD-6. The primer also amplified a 600 bp and a 1558 bp amplicon in accessions of *F. esculentum* collected from Arunachal Pradesh. While an 1154 bp RAPD was detected in all accessions of *F. tataricum* except Shimla B-1, a 250 bp RAPD was detected only in KBB-3, Himpriya, Kuppa Local and Shimla B-1. The 250 bp RAPD was not detected in Sangla group of cultivars. Presence of this band in KBB-3, Himpriya, Kuppa Local and Shimla B-1 and its absence from Sangla group of accessions reveals the difference between them. The absence of 1154bp band from cultivar Shimla B-1, which is high yielding and early maturing variety of *F. tataricum,* could be used as a diagnostic of this cultivar. The grouping pattern of genotypes revealed that the composition of clusters were heterogeneous with regard to their geographical origin. A significant feature of the profiles obtained by UBC-185 was the detection of a 692 bp RAPD in all the accessions/cultivars of *esculentum* and only in Shimla B-1 of *tataricum* species. Amongst the accessions belonging to *F. tataricum,* Shimla B-1 is an early maturing and high yielding variety.

No single primer or combination of two primers could discriminate between banding pattern for all 32 individuals. Out of many primers used, the UPGMA dendrogram generated using the primers OPD-6 and OPO-16 elucidated the inter-accession as well as inter-specific differences in accessions of buckwheat used in the current study. The RAPD data obtained by different primers supports the taxonomic treatment of different accessions/cultivars in the species. Our data clearly identifies IC-13145, which was identified as "*F. himalianum*" by NBPGR, as an accession of *F. esculentum.* Cluster analysis of the genetic similarity estimates using the primers OPD-6 grouped the cultivars Local, Kamroo local, OC-2 and VL-7 into one subgroup within the cluster containing accessions/cultivars belonging to *F. esculentum.* Accessions collected

from Himachal Pradesh and accessions from Arunanchal constituted separate subgroup within the cluster. Even though Tsuji and Ohnishi, (1998, 2000a,b) could not discriminate between cultivated landraces of buckwheat collected from different geographical locations on the basis of RAPD profiles our results clearly indicate the effectiveness of the primers in differentiating between accessions/cultivars collected from different geographical locations.

The similarity matrix generated on RAPD by using primer OPD-6 revealed that *F. cymosum* showed greater similarity with *F. tataricum* than with *F. esculentum*. These results are in agreement with the observations of Kishima *et al.* (1995), Yasui and Ohnishi (1998a, b) and Sharma & Jana (2002) who have placed *F. cymosum* closer to *F. tataricum* than *F. esculentum*.

Principal coordinate analysis of the SDS PAGE profile of total grain protein, endosperm protein and RAPD profiles supports the taxonomic treatment of the three species and the relationship within accessions/cultivars within the genus *Fagopyrum*. One of the significant features revealed by PCA of SDS PAGE profile of endosperm protein by was the emergence of VL-7. PCA of RAPD using primer OPD-6 could identify EC-323729 as an accession distinct from other accessions of the same species. While VL-7 is a cultivar released by VPKAS, Almora EC-323729 is an East European accession.

PCR amplification of the intergenic spacer region between *trn*C and *rpo*B resulted in amplification of a 1.36 kbp DNA fragment in accessions/cultivars of *F. esculentum*, a 1.42 kbp fragment in accessions of *F. tataricum* and a 1.39 kbp fragment in *F. cymosum*. There was no intraspecification in the size of the amplicon. The restriction digestion pattern did not reveal any intraspecific variation in the banding pattern of the digested DNA.

CHAPTER : VII

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APPENDIX

Buffers:

The composition of the commonly used buffers and solutions are given below:

Bradford reagent:

Solution I

0.2% Coomassie Blue G250 in 95% ethanol.

Solution II

85% phosphoric acid

Solution III

Sol. 1 and sol. II added in the ratio of 1:2

The Bradford's reagent comprised of 15% solution III in distilled water.

Protein extraction buffer:

50 mM Tris-Cl (pH 6.8),100 mM NaCl,100 mM EDTA, glycine,10 mM EDTA, 2% SDS and 1 mM PMSF.

Coomassie Brilliant Blue Stain:

0.25% Coomassie Brilliant Blue R-250 in 40% Methanol, 10% Glacial acetic acid.

Fixing solution: 40% Methanol, 10% Glacial acetic acid

Destaining Solution I: 40% Methanol, 10% Glacial acetic acid

Destaining Solution II: 10% Methanol, 5% Glacial acetic acid

Tank buffer for SDS-PAGE: 0.025 M Tris, 0.192 M Glycine, 0.1% SDS, pH 8.3

Isozyme Extraction buffer:

0.1 M Tris-Cl buffer (pH 8.0) containing 0.1% 2-Mercaptoetanol, 0.001 M EDTA, 0.01 M KCL, 0.01 M Magnesium chloride ($MgCl_2$) hexahydrate, 1g 4% polyvinyl pyrrolidone (PVP).

Tank buffer for native gels: 0.025 M Tris, 0.192 M Glycine, pH 8.3

Agarose Gel Electrophoresis Buffer: Tris-Borate EDTA (TBE) buffer.

1X TBE is composed of 0.09 M Tris Borate, 0.002 M EDTA. For 5X concentration stock; 54 g Tris-Cl, 27.5 g boric acid, 20 ml 0.5 M EDTA pH 8.0 and the volume made upto 1 litre.

Gel loading buffer (5X):

5 ml Glycerol, 20 μl of 0.05 M EDTA, 250 μl of 50X TE and 20 mg of Bromophenol blue and the volume adjusted to 10 ml.

Buffer for plant genomic DNA isolation:

Composition of 2X CTAB Buffer: 100 mM Tris-Cl pH-8.0, 20 mM EDTA, 1.4 M NaCl, 2% CTAB, 0.2% Mercaptoethanol.