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

By

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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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Dedicated to my parents

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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.


Fagopyrum 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.