Chapter - 1

INTRODUCTION

Fagopyrum tataricum

Fagopyrum esculentum

Quercetin

Quercetrin

Rutin

Major Secondary metabolites (flavonoids) present in Fagopyrum species
Nutraceuticals have off late received considerable interest because of their potential nutritional and therapeutic effects. Nutraceuticals (foods with nutritional and medicinal value), are being projected as functional foods due to desirable therapeutic outcomes with reduced side effects as compared to other therapeutic agents. Some popular nutraceuticals include buckwheat, oat, barley, flaxseed, psyllium, brown rice, soy and products, omega-3 eggs, calcium-enriched orange juice, green tea, etc. (Saikia and Deka 2011). The use of nutraceuticals, as an attempt to accomplish desirable therapeutic outcomes with reduced side effects, as compared with other therapeutic agents has met with great success (Rajasekaran et al., 2008). The major active nutraceutical ingredients in plants are flavonoids which represent a large family of low molecular weight polyphenolic secondary metabolites that are widespread throughout the plant kingdom, ranging from mosses to angiosperms (Winkel-Shirley 2001; Tapas et al., 2008). Over 6,000 flavonoids have been identified, and categorized, according to chemical structure, into flavonols, flavones, flavanones, isoflavones, catechins, anthocyanidins, dihydroflavonol and chalcones (Lehne and Saliba 2008; Sandhar et al., 2011). Flavonoids promote physiological survival of plant by protecting it from pathogenic microorganisms and UV radiations. In addition, flavonoids are involved in pigmentation for flowers, fruits and seeds to attract pollinators and seed dispersers, morphogenesis, sex-determination and in plant-microbe interactions (Winkel-Shirley 2001; Grotewold 2006; Sandhar et al., 2011; Zhou et al., 2011a). Flavonoids also contribute to nutraceutical qualities of fruits and vegetables and have long been recognized to possess anti-oxidant, anti-inflammatory, anti-allergic, hepato-protective, anti-thrombotic, antiviral, and anti-carcinogenic activities (Grotewold 2006; Tapas et al., 2008). The health-promoting effects of flavonoids may relate to interactions with key enzymes, signaling cascades involving cytokines and transcription factors, or antioxidant systems (Buer et al., 2010).
One of the bioactive flavonoid, rutin, is present in substantial amounts in various plant species such as *Viola tricolor* (3.36%), *Capris spinosa* (0.28%), Apple (0.17%), *Lycopersicon esculentum* (0.002%-0.009%) and many more (McGregor and McIllician 1952; Attanassova and Bagdassarian 2009; Sofic et al., 2010). The use of rutin as a medicinal agent for the treatment of vascular disorders characterized by abnormally fragile or permeable capillaries has stimulated interest in this compound (Campbell 1997). In addition to its ability to reduce hemorrhage in people with high blood pressure, rutin has several other medicinal properties, including pharmacological, vasconstrictive, spasmodic and positive inotropic effects (Campbell 1997; La Casa et al., 2000; Schramm et al., 2003; Wang et al., 2009a). Therefore, due to various medicinal and nutritional properties of rutin and other flavonoids, their demand is growing in the food, pharmaceutical and cosmetic industries (Wang et al., 2009a; Kuntić et al., 2011; Sandhar et al., 2011).

Buckwheat (*Fagopyrum* spp.), is a pseudo-cereal having many medicinal and nutraceutical properties, is the only food crop known as the richest source of rutin (McGregor and McIllician 1952). Buckwheat is a multipurpose crop used for both grains and greens (Campbell 1997). The genus *Fagopyrum* belongs to the family *Polygonaceae* and has 20 known species which mainly occur in the highlands of Euro-Asia (Arora and Engels 1992; Ohnishi 1995; Shao et al., 2011). Of these, two cultivated species, *F. esculentum* (Common buckwheat) and *F. tataricum* (Tartary buckwheat) are of high economic importance due to multiple uses such as a substitute for cereals in human consumption, as a vegetable crop, honey crop, and of ethno-botanical importance (Li and Zhang 2001; Schramm et al., 2003). Owing to the high lysine content, buckwheat proteins have a higher biological value than the cereal proteins, such as those of wheat, barley, rye and corn. The main protein solubility fraction in buckwheat is globulin
(Eggum et al., 1981). It is gluten free, thus making it a valuable nutrient in the diets of people who are sensitive to gluten (Drzewiecki et al., 2003; Sedej et al., 2011).

The difference in morphology of seeds of *F. esculentum* and *F. tataricum* reveal that the fruits of common buckwheat are triangular, large 3 edged achene in comparison to tartary buckwheat where fruits are small, ovoid, and conical with dull irregular faces on each side (Figure 1.1; Léder 2002). The nutritional composition of buckwheat seeds has been well studied (Bonafaccia et al., 2003). Buckwheat accounts for 70% of total carbohydrates with starch as the major component which is 73% in common buckwheat seeds and 70% in tartary buckwheat seeds (Campbell 1997). In tartary buckwheat, fagopyritols (mono-, di- and trigalactosyl derivatives of D-chiro-inositol) account for 40% of total soluble carbohydrates compared to 21% in common buckwheat thus, helps in the treatment of diabetes (Steadman et al., 2000). Total flavonoids are relatively higher in tartary buckwheat (40mg/g) compared to common buckwheat (10mg/g) of which rutin is the major component (Li and Zhang 2001). Tartary buckwheat seeds contain more rutin (about 0.8 to 1.7% DW) compared to common buckwheat seeds (0.01% DW) (Fabjan et al., 2003). Total B vitamin content is 2 times higher in tartary buckwheat than in common buckwheat (Bonafaccia et al., 2003). Hence, tartary buckwheat is considered an excellent food material with a potential for preventive nutrition due to the presence of proteins with high biological value (90%) and balanced amino acid composition, relatively higher crude fiber, and Vitamin B compared to common buckwheat. But, the seeds of tartary buckwheat have a tightly adhering hull that makes it difficult to de-hull and contains a bitter component that affects its palatability (Campbell 1997). Rice-tartary buckwheat is a type of tartary buckwheat with a non-adhering hull property that can be a potential nutraceutical food source (Figure 1.1; Wang and Campbell 2007). It has also been reported that this type of tartary buckwheat was
cultivated and used as a rice replacement in the staple diet in Nepal, Bhutan and southern China (Campbell 2003).

Figure 1.1 Seed morphology of tartary (A), common (B) and rice-tartary buckwheat (C). Non-adhering hull of rice tartary buckwheat removed (D).

Contrary to most of the medicinal and aromatic plants prevalent in the Himalayas, the *F. tataricum* has an added advantage for investigating the molecular genetics of secondary metabolite production due to its annual growth habit with short life cycle, diploid nucleus, self fertility and higher seed set (Chauhan et al., 2010). Variation for rutin content or other flavonoids is highly desirable not only for identifying high content accessions but also to explore for novel genes/alleles contributing to higher flavonoids content. Variation for rutin content has been studied on a limited scale among *F. tataricum* genotypes. Yan et al. (2004) showed significant variation in rutin content among 14 genotypes of tartary buckwheat (0.87-1.4% rutin) and common buckwheat (0.4-0.5% rutin) grown in different ecological conditions. Rutin content variation was also observed among 50 strains of tartary buckwheat on the basis of differences in
seed shape and color (Park et al., 2004). However, in those studies rutin content was characterized by testing mature seeds of different buckwheat genotypes collected from diverse ecological regions, which can be largely influenced by the environmental conditions (altitude, light intensity, temperature, etc.) rather than only the genotype of seed samples. Hence, we report rutin content variation among *F. tataricum* accessions need to be studied by growing at the same environmental conditions so as to reflect genotypic variation.

Various molecular markers (AFLP, RAPD, RFLP and SSRs) have been successfully used in the diversity analysis and utilization of plant genetic resources (Botstein et al., 1980; William et al., 1990; Vos et al., 1995; Zietkiewicz et al., 1994). The genetic relationship was studied among natural populations and cultivated landraces of tartary buckwheat through RAPD and AFLP markers (Kump and Jovornik 2002; Sharma and Jana 2002b; Senthilkumaran et al., 2007). Microsatellite markers were also used for diversity analysis in tartary buckwheat (Li et al., 2007). However, no efforts have been made till date to correlate genetic relationship with the high vs. low rutin content in accessions of buckwheat which can provide helpful information for evaluation, breeding and conservation of germplasm. Therefore, characterization of tartary buckwheat for rutin content variation vis-à-vis DNA fingerprinting was undertaken among a collection of *F. tataricum* accessions from the Himalayan regions of India.

The flavonoid content in a particular tissue and developmental stage is largely influenced by different classes of regulatory genes, transporters, modifiers etc in addition to structural genes of the flavonoid biosynthetic pathway (Grotewold 2006; Bovy et al., 2007; Hichiri et al., 2011). Understanding the molecular biology of high rutin content (40-50x) and nutritional superiority in seeds of tartary buckwheat compared to common buckwheat would provide information on what genes/alleles in *F. tataricum* are contributing to high rutin content help in planning. The lack of
basic genome resources such as genetic map, seed ESTs, genome libraries, molecular markers, etc. further complicates molecular dissection of flavonoid machinery in buckwheat. The biosynthesis and accumulation of flavonoids is controlled by the structural and regulatory genes, which are studied in different plant species such as Zea mays, snapdragon, Petunia, Arabidopsis (Winkel-Shirley 2001; Grotewold 2006; Hichiri et al., 2011). The cloning and differential expression of anthocyanin biosynthesis pathway genes in different tissues (leaves, stem and flowers) of Fagopyrum species was studied without looking at rutin content variation vis-à-vis genes controlling or regulating rutin biosynthesis (Li et al., 2010; Park et al., 2011a). Therefore, physiological and molecular dissection of higher rutin content in F. tataricum compared to F. esculentum and its correlation with the expression of flavonoid pathway genes or uncovering novel genes would provide avenues for genetic improvement of buckwheat for enhanced flavonoid (rutin) content.

The lack of whole genome sequence and non-availability of ESTs from developing seeds of Fagopyrum spp. prompted us to utilize differential transcript profiling through cDNA-AFLP to decipher what genetic factors, in addition to flavonoid structural genes, contribute to high rutin content of rice-tartary compared to common buckwheat. The cDNA-AFLP has been suggested as an efficient method for isolation and identification of differentially expressed genes (Bachem et al., 1996). The cDNA-AFLP has an added advantage of not only capturing differences in the abundance and types of transcripts but can also detect differences in the DNA sequences of transcript fragments in two Fagopyrum species.
Keeping in view the economic importance of buckwheat, lack of information on rutin content variation in genotypes/landraces prevalent in North-Western Himalayas of India, virtually no information on molecular basis of high rutin biosynthesis in tartary buckwheat, the current study was undertaken with the following objectives:

**OBJECTIVES**

1. Rutin content profiling and AFLP fingerprinting of *F. tataricum* accessions from North-Western Himalayas of India

2. Cloning flavonoid pathway genes through comparative genomics and their expression vis-à-vis rutin content variation in different growth stages of *F. tataricum*

3. Differential transcript profiling to capture genes regulating biosynthesis, modification, transport and accumulation of flavonoids in *Fagopyrum* spp.