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Genetic and Genome Resources in Buckwheat – Present Status and Future Perspectives

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ABSTRACT

Buckwheat is an important crop because of its potential value to humans and livestock. The genus *Fagopyrum* has 15 known species, two of them namely, *Fagopyrum esculentum* (common buckwheat) and *F. tataricum* (tartary buckwheat) are cultivated while others occur as wild or as escapes in and around cultivated fields in the highlands of Euro-Asia. Germplasm comprising ~10,000 accessions has been preserved in genebanks across the world. A wide range of variation occurs in buckwheat germplasm accessions for agronomic and quality characters, including rutin content. Tartary buckwheat contains approximately 40 times more rutin than common buckwheat, and is therefore more important from an industrial perspective. Rutin biosynthesis involves 9 genes, phenylalanine ammonia lyase, cinnamate 4 hydroxylase, 4-coumarate CoA ligase, chalcone synthase, chalcone isomerase, flavonol synthase, flavanone-3-hydroxylase, flavanone-3'-hydroxylase, and glucosyl/rhamnosyl transferase. We are investigating comparative genomics of rutin biosynthesis genes in buckwheat by utilizing sequences from well characterized related species. Collecting germplasm from diversity-rich areas, identifying trait-specific genes including candidate genes involved in the biosynthesis of rutin and other secondary metabolites of economic importance would be useful for enhancing utilization of buckwheat genetic resources. Unfortunately, buckwheat genome resources are very limited, imposing a challenge to genetic improvement of the species. Use of genomic information from well characterized related taxa has been advocated for genetic improvement of buckwheat.

Keywords: biosynthetic pathway, genes, genome resources, rutin

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INTRODUCTION

Buckwheat (*Fagopyrum* sp.) is a multipurpose crop used for both grains and greens and known to have several medicinal and nutritional properties. The genus *Fagopyrum* belongs to the family *Polygonaceae* and has 15 known species which mainly occur in the highlands of Euro-Asia (Arora and Engels 1992; Ohnishi 1995). Tartary buckwheat (*Fagopyrum tataracum* Gaertn.) is cultivated at much higher elevations (~3500 m) than common buckwheat (*F. esculentum* L. Moench.), which grows at an elevation of ~2500 m and is considered to be more important in the Himalayan region (Rana 2004). Buckwheat genetic resources have been collected, evaluated and preserved at various gene banks worldwide. Buckwheat has high flavonoid content, especially rutin and quercetin. In addition to its ability to reduce hemorrhage in people with high blood pressure, rutin has several other medicinal properties including pharmacological, vasconstrictive, spasmolitic and positive inotropic effects (Campbell 1997; La Casa *et al.* 2000; Schramm *et al*. 2003; Tomotake *et al.* 2000; Wang *et al*. 2009). The seeds of tartary buckwheat contain higher amounts of rutin (about 0.8-1.7% d.w.) than those of common buckwheat (0.01% d.w.) (Fabjan *et al.* 2003). The demand for rutin and other flavonoids derived from buckwheat is growing in the food, pharmaceutical and cosmetic industries due to their desirable physiological activities, such as anti-oxidation, anti-inflammation and anti-hypertension (**Table 1**).

Taxonomically, buckwheat shares similarities with families like *Aizoaceae*, *Amaranthaceae*, *Plumbaginaceae*, and *Tamaricaceae* which have 27191, 26807, 6387, and 21709 expressed sequence tags (ESTs), respectively. We screened these EST sequences available at http://compbio.dfci.harvard.edu/tgi/tgipage.html from related families of buckwheat for identification of simple sequence repeats (SSRs) and isolated 141 SSRs. Of these, only 13 were able to amplify the buckwheat genome, indicating low cross-genera transferability of SSRs in buckwheat. The dip-

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Invited Review

Table 1 Potential medicinal and nutritional uses of buckwheat.

Product/compound	Effect	Reference
Rutin	Strengthens capillaries and so helps in arteriosclerosis or high blood pressure	Campbell 1997
Rutin, quercetin and quercitrin	UV-B radiations absorbing compounds	Kreft *et al*. 2002
Rutin	Protection against gastric lesions	La Casa *et al*. 2000
Leaves	Improve sight and hearing	Campbell 1997
Fagopyritol B1 (major soluble carbohydrate)	Seed desiccation tolerance	Horbowicz *et al*. 1998
Buckwheat protein product	Lowers plasma cholestrol and raises fecal neutral sterol	Kayashita *et al*. 1997
Buckwheat polyphenols	Ameliorate spatial memory impairment	Pu *et al.* 2004
Dietary rutin, quercetin	Nutritional value	Fabjan *et al.* 2003
Antimicrobial peptides Fa-AMP1 and Fa-AMP2	Toxic to plant pathogenic fungi, gram-positive and -negative bacteria	Fujimura *et al.* 2003
Phenolic antioxidants in buckwheat honey	Protects humans from oxidative stress	Schramm *et al*. 2003
Tartary buckwheat flavonoid (TBF)	Chemopreventive activity and may have therapeutic role for human leukemias	Ren *et al.* 2001
Buckwheat flour	Diabetes, obesity, hypertension and constipation	Li *et al.* 2001
Buckwheat protein	Suppresses gallstone formation and cholestrol level by enhancing bile acid synthesis	Tomotake *et al*. 2000
Buckwheat protein extract	Retard memory carcinogenesis by lowering serum estradiol	Kayashita *et al*. 1999
Buckwheat protein extract	Causes muscle hypertrophy, elevates carcass protein and reduces body fat	Kayashita *et al.* 1999
Roots and leaves	Aluminum tolerance in buckwheat roots	Ma *et al.* 1998
Buckwheat flour	Free radical scavenging activity	Qian *et al.* 1999
Buckwheat concentrate	Diabetes	Kawa *et al.* 2003
Buckwheat protein product	Colon carcinogenesis	Liu *et al.* 2001
Buckwheat extract	Ameliorates renal injury	Yokozawa *et al.* 2001
Bran extract	Reduction of serum trigycerides and total cholesterol level	Wang *et al*. 2009

loid nature (2n = 16), short life cycle (~70 days) and small genome size (~450 Mbp) makes it an ideal species for genetic investigation of the biosynthesis and accumulation of flavonoids. Rutin biosynthesis involves 9 genes, phenylalanine ammonia lyase, cinnamate 4 hydroxylase, 4-coumaryl CoA ligase, chalcone synthase, chalcone isomerase, flavanone synthase, flavanol-3-hydroxylase, flavanol-3′-hydroxylase, and glucosyl/rhamnosyl transferase. However, there are unidentified regulatory gene(s) controlling the expression of pathway genes in buckwheat. Towards this end, we are using genome information from related plant species through a comparative genomics approach to identify and characterize the structural and regulatory genes involved in rutin biosynthesis.

Buckwheat genetic diversity the world over has become enormously depleted during the past few decades due to several factors particularly resulting from changing cropping patterns and food habits. Identification of candidate genes for various traits of economic importance, including those involved in the biosynthesis of rutin and other secondary metabolites, would be useful for enhancing medicinal and nutritional properties of buckwheat both by conventional and non-conventional plant breeding methods. Unfortunately, the availability of genome resources in buckwheat is extremely limited, which impose challenges to the genetic improvement of crop and its industrial use. The paper reviews the present status of progress made in the area of genetic and genome resources, tissue culture and genetic transformation in buckwheat and the future directions needed to genetically improve the species in agricultural and industrial perspective.

GENETIC RESOURCES

The genus *Fagopyrum* has around 15 species occurring in the temperate areas of Euro-Asia (Ye and Guo 1992). Of these, cultivated species *F. esculentum* and *F. tataricum* have wider distribution, while others such as *F. homotropicum*, *F. caudatum*, *F. sagittatum*, *F. cymosum*, *F. megaearpum*, *F. gracilipes*, *F. urophyllum*, *F. leptopodum*, *F. lineare*, *F. callianthum*, *F. pleioramosum*, *F. capillatum* and *F. statice* occur mainly in the highlands of Euro-Asia (Farooq and Tahir 1987; Anonymous 1988; Baniya 1994; Ohnishi 1995; Rana 2004). Furthermore, *F. tataricum* ssp. *annum* occurs in the Eastern Himalayas, *F. tataricum* ssp. *potanini* in Tibet, Kashmir Himalayas and northern Pakistan (Ohnishi 1989, 1991, 1992), while *F. tataricum* ssp. *himalianum*, and *F. tataricum* ssp. *emarginatum* are distributed in the cold arid regions of Western Himalayas of India (Rana 2004). Munshi (1982) described *F. kashmirianum* as a separate taxon but morphologically akin to *F. tataricum*, hence treated as same species. The wild types occupy open forests, wastelands, marginal lands, disturbed habitats and the weedy types occur more often in and around cultivated buckwheat fields (Anonymous 1988). Due to ancient trade links, there is the possibility of diffusion of buckwheat species from Tibet to Nepal and also *en route* to Bhutan and India and *vice versa.*

Buckwheat germplasm collection missions have been undertaken at national, regional and international levels. The current status of buckwheat genetic diversity collected and maintained in east and south Asia has been documented by the International Plant Genetic Resources Institute (now known as Biodiversity International) Regional Office for Asia, the Pacific and Oceania (IPGRl-APO 1999). Approximately 5,000 accessions of buckwheat have been collected in east and south Asia, which consist of about 52% of the world's buckwheat collections. Nearly 90% of the world's tartary buckwheat accessions are native to Asia. China has the largest collection of buckwheat accessions (2146) followed by India (954), Japan (746), DPR Korea (413), Nepal (327), North Korea (95) and Mongolia (30) (IPGRl-APO 1999; Zou and Zhang 1995; Zhang *et al.* 2004). Unfortunately, the diversity of wild species is not well represented in most of these collections. Accessions of nearly 50 wild species are maintained by China, Japan and India (Zhang *et al.* 2004). These accessions however, may have duplicates because of exchange of germplasm by various organizations within and between countries.

Characterization and evaluation of collected and introduced germplasm increases its utilization for various purposes. The germplasm collections have been characterized worldwide for agronomic characters, using descriptors developed by Biodiversity International. A wide range of variability has been noticed in the germplasm for many yield and yield-contributing characters across countries (Ujihara and Matano 1977; Ujihara 1983; Choi *et al.* 1992; Baniya *et al*. 1995; Joshi and Rana 1995; Rana and Sharma 2000; Rana 2004). The accessions varied with respect to days to 50% flowering (30-65), days to maturity (60-140), plant height (50-225 cm), number of internodes (9-28 cm), petiole length (1.4-8.9 cm), primary branches (1-20), leaf length (2.5-13.5 cm), leaf width (2.2-12.95 cm), cyme length (1.45-11.55 cm), seed yield per plant (1.75-124.15 g), and 1000-seed weight (3-35 g). Some of the promising accessions identified in the germplasm evaluated in India are

Table 2A Promising accessions identified for different traits from the germplasm evaluated in India.

Characters	Germplasm accessions	Accessions have value > or <
Days to flowering	IC329568, IC381130, IC13412, IC16558, IC42411, IC313301, IC313300, IC106836, IC326998	<35 days
Leaf length	IC341081, IC274444, IC310104, IC278957, IC274439, IC018870, EC018864, EC218764, EC323723, EC216630	>11 cm
Leaf width	IC341681, IC311004, IC310046, IC318859, IC310047, IC109458, IC202262, EC323730, EC216635, EC323726	>10 cm
No. of internodes	IC258244, IC258230, IC341680, IC313468, IC381077, IC109726, IC109757, EC125397	>20
No. of primary branches	IC274423, IC318859, IC329194, IC318859, EC216635, EC323723, EC018864, EC188664	>11
Cyme length	IC360826, IC547549, IC547346, IC547396, IC361635, IC341631, IC318859, EC125357, EC58322	>7 cm
Days to maturity	IC310104, IC341671, IC329568, IC381130, IC13412, IC16558, IC42411, IC24301, EC323731, EC323729	<80 days
1000-seed weight	IC381077, IC381098, IC381049, IC58322, EC323724, IC360829, IC360846, IC361359, EC216685, EC213682	>25 g
Seed yield/plant	IC18869, IC18889, IC318859, IC329401, IC329404 IC467923, IC447689, IC540858, EC218740	>100 g

Source: Rana 2004; IC: indigenous accessions; EC: exotic accessions

Table 2B Germplasm accessions marked for desirable traits.

Trait	Accessions/Breeding lines
Early type	VL 7, EC 323724, EC 323729, EC 323731
Dwarf type	VHC 26, EC 323729, EC 323724
No. of primary branches	Sangla local, PRB 9001, IC 18869
No. of leaves	Himpriya, IC 18889, VL 7
Leaf area	IC 18869, EC 323731
No. of internodes	KBB3, EC 323731, EC 323724, EC 323729
1000-Seed weight	EC 323729, EC 323731

Source: Rana 2004; IC: indigenous accessions; EC: exotic accessions

given in **Tables 2A** and **2B**.

Indian buckwheat germplasm has been subjected to genetic divergence (Rana 1998; Rana and Sharma 2000) and stability analysis (Joshi and Rana 1995). Germplasm has also been evaluated for quality characters such as amino acid, vitamins E and P contents (Keli and Dabiao 1992; Suzuki *et al*. 2005; Lin *et al.* 2008). The variation for protein content (8.20 to 15.10%), total phenols (1.4-1.70%), free phenols (0.27-0.94%) and available lysine (3.89-5.60%) was also noticed in 60 accessions evaluated under All India Coordinated Research Project on Under-Utilized Crops (Anonymous 2008). Accessions EC125938 and IC108499 (for protein content), IC341674, IC108500 and EC018282 (for lysine) and IC266947 and IC547385 (for total phenols) were found promising. The average amino acid content in *F. esculentum* germplasm ranged from 7.18-16.51%, whereas in *F. tataricum* accessions it varied from 7.04-15.83% (Zhou 1992).

We also evaluated rutin contents in mature seeds of 200 *F. tataricum* accessions, which showed relatively large variation, ranging from 0.6 to 2.0% (d.w) compared to only 0.07% (d.w) in selected accessions of *F. esculentum* (**Fig. 1**). These results are in complete agreement with previous reports on variation in rutin content in cultivated buckwheat (Bonafaccia and Fabjan 2003). Park *et al.* (2004) compared the variation in rutin content in different plant parts of *Fagopyrum* spp. (*F. tataricum*, *F. cymosum* and *F. esculentum*); highest content was in flowers, lowest in roots. There are reports of variation in rutin content in different *Fagopyrum* sp. coupled with antioxidant activity, which decreased in the order: *F. tataricum* > *F. homotropicum* > *F. esculentum* (Jiang *et al.* 2006). Morishita *et al.* (2007) reported 3-4 times higher antioxidant activity in tartary than common buckwheat grains. They further showed that rutin contributed 2% of the total antioxidant activity in common buckwheat while 11-13% was contributed by epichatechin in contrast to tartary buckwheat where rutin appeared to be the major antioxidant (85-90%) (Morishita *et al.* 2007). Research involving accessions with high and low rutin content (**Tables 3, 4**) is in progress in our laboratory. The variation in rutin content has been estimated from ≤ 10 µg/mg to ≥ 16 µg/mg through RP-HPLC.

Buckwheat genetic resources are under a major threat of erosion worldwide. The authors, during exploration and collection missions during the last 15 years, have witnessed an alarming loss of diversity of buckwheat in the Indo-Tibet Himalayan region – an important region of buckwheat genetic resources. The area under buckwheat cultivation has declined substantially (60-92%) in the Western Himalayan region of India (Rana *et al.* 2000; Rao and Pant 2001). Similarly, in Japan reduction in area ranging from over 200,000 ha in 1800 AD to 25,000 ha by 1970 has been reported (Ujihara 1983). The reports on buckwheat acreage from other countries also depict similar trends in Nepal (Baniya *et al.* 1995), China (Zhou 1992) and Europe (Michalová 2001). Several factors are responsible for genetic erosion of genetic diversity in buckwheat and important among them are changing cropping patterns, low productivity, changing food habits and life styles, less alternative uses and products, and lack of awareness about its food value (Rana *et al*. 2010).

Table 3 List of 10 *Fagopyrum tataricum* accessions with high rutin content. EC- Exotic and IC- Indigenous accessions Source: unpublished data of Sunil K Sharma PhD.

Accession No.
IC26756
IC42421
IC107962
IC310045
IC14889
IC313136
EC18282
IC14253
IC274331
IC49667

Table 4 List of five *Fagopyrum tataricum* accessions with low rutin content. EC and IC accessions Source: unpublished data of Sunil K Sharma PhD.

Accession No.
IC49676
IC310046
EC99945
IC107583
IC18664

CURRENT STATUS OF GENOME RESEARCH

A significant amount of research has been conducted on the functionalities and properties of buckwheat proteins, flavonoids, flavones, phytosteroles, thiamin-binding proteins,

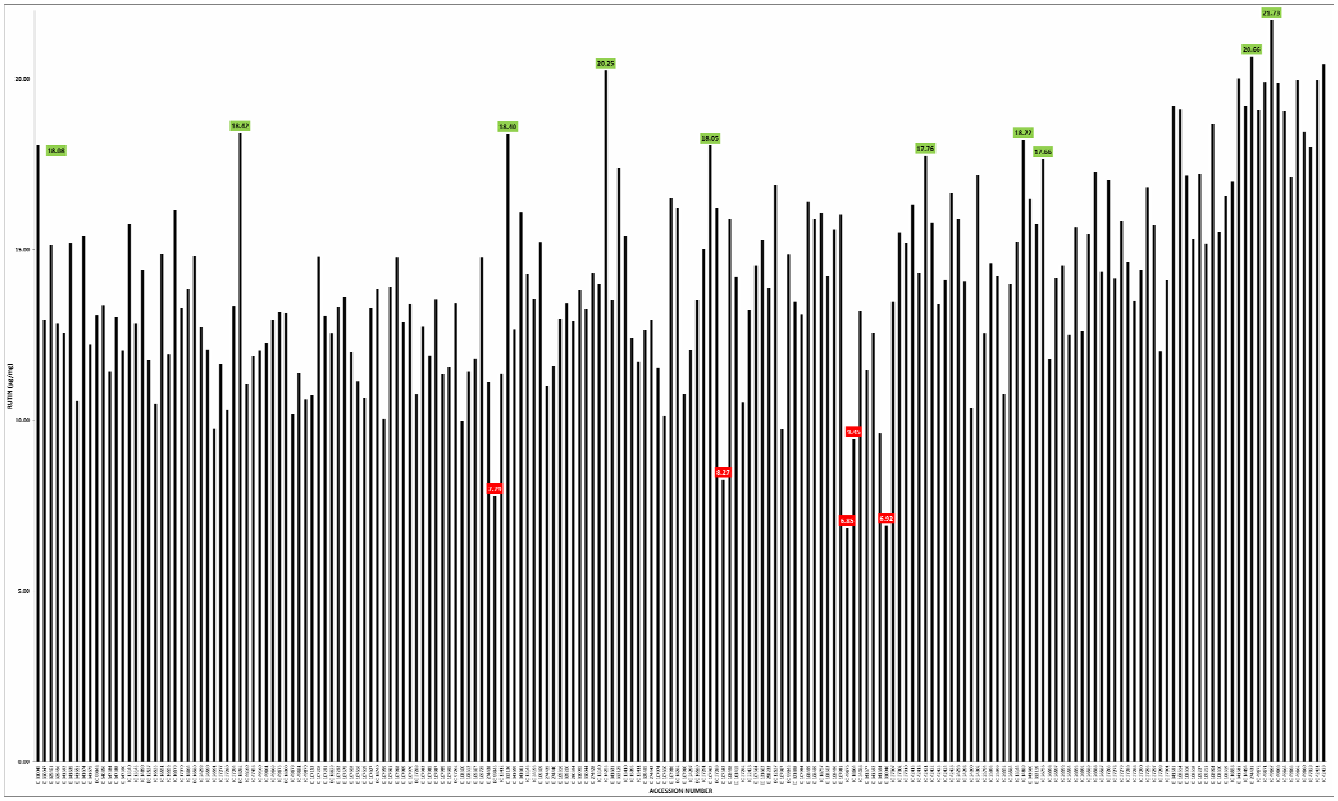

Fig. 1 Variation in rutin content among different accessions of *F. tataricum*. Source: unpublished data of Sunil K Sharma PhD.

and other rare compounds (Li *et al*. 2001; Tomotake *et al*. 2002; Kreft *et al*. 2006; Zielinski *et al*. 2009). However, little effort has been invested in the development of molecular markers and genome resources. The development and use of molecular markers for the detection and exploitation of DNA polymorphism is one of the most significant developments in the field of molecular genetics (Cullis 2002). A genetic marker can be defined in one of the following ways: (a) a chromosomal landmark or allele that allows for tracing a specific region of DNA; (b) a specific piece of DNA with a known position on the genome or (c) a gene whose phenotypic expression is easily distinguished, used to identify chromosomes, or locus. Since the markers and the genes they mark are located closely on the same chromosome, they tend to remain together in successive generations of propagation, thus allowing the development of genetic linkage maps. These genetic maps are used for detailed analysis of association between genes or quantitative trait loci (QTLs) and economically important traits and, thereby, aiding introgression of desirable genes or QTLs through marker-assisted selection (MAS). Use of several molecular markers such as random amplified polymorphic DNA (RAPD) (Aii *et al*. 1998), amplified fragment length polymorphism (AFLP), (Nagano *et al.* 2001; Matsui *et al.* 2004) and simple sequence repeats (SSRs) (Konishi *et al.* 2006) has been reported in buckwheat (details in **Table 5**). Much of the progress in research in molecular genetics and plant breeding has been achieved using common buckwheat, and only fragmentary research efforts have been made in tartary buckwheat.

DNA markers provide an efficient means of plant improvement through genome mapping and MAS. For this purpose, PCR-based methodology is more convenient than restriction fragment length polymorphism (RFLP) analysis because of the relative ease of detection and smaller amount of DNA required. Many fingerprinting techniques based on PCR, such as SSRs or microsatellites, RAPDs and AFLPs have been developed over the past several years. Sequence characterized amplified regions (SCARs) and sequence tagged sites (STSs) are also types of PCR markers, but they differ from the former methods by having single or two bands in agarose gels. RAPD provides a simple, inexpensive and efficient method of generating molecular data. Sharma and Jana (2002a) studied species relationships in *Fagopyrum* using RAPD markers. RAPD markers have also been used to elucidate genetic diversity in Indian and Chinese tartary buckwheat accessions (Sharma and Jana 2002b). The details of their findings have been reported in **Table 5**.

Aii *et al.* (1998) developed SCAR markers that were closely associated with the *Sh* gene (homomorphic self-compatible having the middle-styled morph), and these proved useful in identification of heterozygosity (Aii *et al*. 1999). Using AFLP markers, Nagano *et al.* (2001) exploited the F_2 progeny of *F. esculentum* and *F. homotropicum* for fine mapping of the S^h allele (homostylar locus). Five AFLP markers linked to the *sht1* locus (genes linked to brittle pedicel in buckwheat) were identified (Matsui *et al.* 2004) and two of them were converted into STS markers, which were useful for MAS of non-brittle pedicel plants.

An interspecific linkage map using *F. esculentum* and *F. homotropicum* was developed (Yasui *et al.* 2004). The *F. esculentum* map has 8 linkage groups with 223 markers covering a total of 508.3 cM, whereas the *F. homotropicum* map consists of 211 markers covering 548.9 cM. One-to-one correspondence of the *F. esculentum* and *F. homotropicum* linkage groups exists. Morphological markers, distylous self-incompatibility and shattering habit were tightly linked to each other (1.3 cM) and located near the center of linkage group 1. Another marker, winged seed, was located on linkage group 4.

Microsatellite markers showed a higher level of polymorphism and higher expected heterozygosity than two other dominant (AFLP and RAPD) or codominant (RFLP) markers (Powell *et al.* 1996). In common buckwheat, only 5 microsatellite markers have been developed by sequencing 2785 clones from the libraries. 1483 clones contained microsatellites, which were enriched for $(CT)_n$ and $(GT)_n$ repeats. Primer pairs were designed for 237 of the microsatellite loci, of which 180 primer pairs were amplified. Of these, 54 primer pairs were highly variable. These primers were evaluated for their ability to detect variations in common buckwheat populations and utilized in 7 related *Fago-*

Table 5 Molecular markers reported in buckwheat.

Molecular marker	Studies done by authors	Results and findings	Reference
RAPD	Attempts were made to determine molecular markers linked with homostylar (H*o*) gene to determine basis of self compatibility.	F2 population was generated from an insterspecific hybrid between *Fagopyrum esculentum* and *F. homotropicum*. Three RAPD markers $OPB14_{1250}$, $OPP8_{1000}$ and $OPQ7_{800}$ were identified to be linked with the H*o* gene.	Aii *et al.* 1998
	RAPD markers were used to study the species relationship between 28 different accessions which belong to 14 different species.	They revealed that *F. tataricum* is closer to its wild ancestor *F. tataricum* ssp. *potanini* Batalin, closely followed by *F. giganteum*. Cultivated common buckwheat (*F. esculentum*) showed affinity with its putative wild ancestor *F. esculentum* ssp. *ancestrale* and the other closely related diploid species *F. homotropicum*. The results showed that RAPD can be utilized for analysis of species relationship in *F.* and construction of genetic maps.	Sharma and Jana 2002a
	The objectives of the study were to (i) determine the feasibility of using RAPD for diversity analysis in *F. tataricum*, (ii) group *F. tataricum* accessions collected from different ecoregions on the basis of their genetic diversity, and (iii) study the relatedness of wild ancestor *F. tataricum* ssp. *potanini* with cultivated tartary buckwheat germplasm.	The similarity between cultivated tartary buckwheat accessions ranged from 0.61 to 1.00. Four distinct clusters were formed which corresponded well with the geographic distribution of the tartary buckwheat. Nepalese accessions showed maximum diversity followed by Chinese accessions. The wild buckwheat accession did not group with any of the three cultivated tartary buckwheat groups, and formed its own single-entry group. The study demonstrated the usefulness of the RAPD technique for the characterization of plant genetic resources and assessment of diversity between species.	Sharma and Jana 2002b
AFLP	The objective was to find tightly linked markers in buckwheat homostylar locus, concerned with self-compatibility. Approximately 500 polymorphic loci were screened on the bulked segregant pools from F_2 progeny of the cross between *F. esculentum* (pin) and *F. homotropicum*.	Of the nine markers, two were confirmed to have been derived from a single region. Nucleotide sequence information from each flanking region of the two single locus markers was used to design region-specific primers for PCR amplification. These markers can be utilized for fine mapping of the *Sh* allele in buckwheat and for positional cloning of the gene.	Nagano *et al.* 2001
	Shattering habit in buckwheat is due to brittle pedicel produced by two complementary, dominant genes, *Sht1* and *Sht2*. To detect molecular makers linked to the *sht1* locus, AFLP analysis was used in combination with bulked segregant analysis of segregating progeny of a cross between non-brittle common buckwheat and a brittle self-compatible buckwheat line.	312 primer combinations were screened and linkage map was constructed around the *sht1* locus by using 102 F_2 plants. Five AFLP markers linked to the *sht1* locus (genes linked to brittle pedicle in buckwheat) were identified.	Matsui *et al.* 2004
	Linkage analysis of *F. esculentum* and its wild self-pollinated relative *F. homotropicum* has been studied.	An interspecific linkage map using *F. esculentum* and *F. homotropicum* was developed.	Yasui *et al.* 2004
Microsatellites	The purpose was to develop a larger number of microsatellite markers in common buckwheat. By sequencing 2785 clones from the libraries, 1483 clones contained microsatellites, of which 352 had unique sequences. Primer pairs were designed for 237 of the microsatellite loci, of which 180 primer pairs each amplified PCR products. Fifty-four primer pairs that each amplified a clear PCR product of the expected size were evaluated for their ability to detect variations in common buckwheat populations and to be utilized in seven related *Fagopyrum* spp.	Forty-eight (88.9%) out of the 54 microsatellite markers tested were found to be highly variable (the average number of alleles was 12.2 and the average polymorphism information content (PIC) was 0.79) in a population of cultivated buckwheat. A high rate of successful amplification of common buckwheat microsatellite markers was observed in closely related species The developed microsatellite markers will be useful in molecular breeding of common buckwheat.	Konishi *et al.* 2006
	136 new SSR markers developed in *F. esculentum* ssp. *esculentum* and their application to related species in the genus *Fagopyrum* has been investigated.	Forty-one of the 136 SSRs amplified sequences in other *Fagopyrum* spp., including *cymosum* and *urophyllum* groups. However, of the 136 SSRs, only 10 were polymorphic on 41 accessions of diverse species origin. The phylogenetic relationships revealed that the use of SSRs showed consistent results as compared to using other marker systems	Ma *et al.* 2009

AFLP: amplified fragment length polymorphism; RAPD: random amplified polymorphic DNA

pyrum species, including *F. tataricum* (Konishi *et al.* 2006). Efforts have also been made to develop microsatellite markers in tartary buckwheat (Li *et al.* 2007), which includes the construction of a genomic library enriched with $(gT)_n$ repeats by using 5′-anchored PCR for the development of microsatellite markers. Ma *et al.* (2009) reported 136 new SSR markers in *F. esculentum* and showed their application for diversity analysis in related species of the genus *Fagopyrum*. However, of the 136 SSRs, only 10 were polymorphic on 41 accessions of diverse species origin.

ESTs are a partial sequence of a gene and are mapped on the respective gene position on the genome either by PCR-based markers or RFLP probes. Because EST markers are derived from the gene-coding regions, they are more likely to be conserved across populations and species than markers derived from random regions of DNA. The applicability of 17 EST primers developed from common buckwheat was tested in other wild and cultivated *Fagopyrum* spp. (Joshi *et al.* 2006). The amplification products differed in band intensity. The results indicated that the transferability of common buckwheat EST markers decreased with an increase in genetic distance between species.

Recently, genome analysis has taken a basic position in genetics and plant breeding and several genome libraries have been constructed for major crops (Nakamura *et al.* 1997; http://www.hsls.pitt.edu/guides/genetics/obrc/plant/general, http://www.tigr.org/db.shtml). The presence of a genome library is useful for achieving advances in genomics and breeding of buckwheat through MAS. For example, several interesting and economically important genes

can be isolated by positional cloning, such as the heterostylous self compatibility gene (Aii *et al.* 1998) or various stress resistance genes. A BAC library has been constructed from wild buckwheat, *F. homotropicum* (Nagano *et al*. 2001). This library contains 24,096 clones. A random sample of 250 BACs from this library indicated an average insert size of 97 Kb. Based on the genome size of 1,080 Mbp, the library coverage is 1.9 haploid genome equivalents. The library provides useful resource for isolation of genes, e.g., the *S*-locus of buckwheat. Another BAC library for *F. esculentum* has been constructed, which includes 142,005 clones with an average insert size of ~76 kb (Yasui *et al.* 2008).

Chloroplasts often show uniparental inheritance over generations, which provide valuable information on interspecies relationships. The complete chloroplast genome sequence of the wild ancestor of cultivated buckwheat, *F. esculentum* ssp. *ancestrale* has been made available by Logacheva *et al.* (2008). The gene content and chloroplast genome order in buckwheat are similar to that of *Spinacia oleracea*. However, it has some unique structural features distinct from previously reported complete chloroplast genome sequences. Phylogenetic analysis of the dataset, including the new sequence from non-core *Caryophyllales*, supports the sister relationship between *Caryophyllales* and asterids.

TISSUE CULTURE AND GENETIC TRANSFORMATION

Plant tissue culture is used widely in plant science; it also has a number of commercial applications. For buckwheat, which has immense nutraceutical importance, tissue culture assumes greater significance for *in vitro* production of important plant metabolites. Large-scale growth of plant cells in liquid culture inside bioreactors as a source of secondary products, like recombinant proteins can be used as biopharmaceuticals. At present, information about tissue culture in buckwheat is limited and is mainly restricted to micropropagation. *Fagopyrum* spp. are diploid, 2n =16; but tetraploid varieties either occur spontaneously or can be induced. Buckwheat has for centuries remained a crop with low seed set due to certain characteristics which prevent the application of conventional breeding methods (Kreft 1983). The main obstacles in buckwheat breeding include its very strong self/cross-incompatibility and its indeterminate type of growth and flowering. Modern biotechnology may provide means to address these problems in a novel way (Nešković *et al.* 1995). *In vitro* regeneration of buckwheat has been reported from various explants (**Table 6**) such as cotyledons and hypocotyls of seedling of buckwheat by addition of 2, 4-dichlorophenoxyacetic acid (2,4-D) with concentration of 5-10 mg/l to White's basal media (Yamane 1974). Another report shows regeneration of calli from protoplasts in *F. tataricum* which is one of the prerequisites for the successful use of somatic hybridization. As it has

Table 6 An account of tissue culture studies in buckwheat.

Tissue culture studies	Medium used	Plant growth regulators (conc.)	Light, temperature conditions	References
Induction of diploid restored plants from callus of buckwheat	White's basal medium pH-5.6	2,4-D (10 mg/l)	Dark (transferred to fresh media after every 14-21 days), 23^0 C	Yamne 1974
Plant regeneration from Immature Inflorescence	B5 media pH-5.8	NAA (0.2 mg/l), BA (0-2 mg/l), IBA (1 mg/l)	16-h photoperiod, 25°C	Takahata 1988
Plant regeneration from protoplast of Common Buckwheat	MS medium	Gibberellic acid (0.1 mg/l)	16-h photoperiod, 25°C	Adachi *et al.* 1989
Callus regeneration from hypococtyl protoplast of Tartay Buckwheat	MS medium Sucrose 3%, Mannitol 0.5M, 5mM $CaCl_2$ pH-5.8	BA (1 mg/l), NAA (2 mg/l)	Dark for 4 weeks followed by 16-h photoperiod, 25°C	Lachmann and Adachi 1990
Anther culture and androgenetic plant regeneration	Gellan-gum solidified MS medium, 90 g/l maltose	BA (2.5 mg/l), IAA (0.5 mg/l)	16-h photoperiod, 25°C	Bohanec *et al*. 1993
Regeneration of plants from cotyledon tissue of Common Buckwheat	MS medium Sucrose 3% pH-5.7	2,4-D (1-3 mg/l), Kinetin (0.2 mg/l), NAA (0.1-0.5 mg/l) Optimum medium (0.2 mg/l KIN, 2.0 mg/l BAP, 3% sucrose)	16-h photoperiod, 25°C	Woo *et al.* 2000
Somatic embryogenesis in common buckwheat by use of explants from hypocotyls of young seedlings.	Distilled water solidified by agar (0.8%) Sub cultured on liquid and solid (with 3% phytogel) media, based on the mineral salts B5, supplemented with *myo*-inositol (100 mg/l), thiamin-HCl (1 mg/l), pyridoxine-HCI (1 mg/l), nicotinic acid (1 mg/l), casein hydrolysate (2 g/l), sucrose (30 or 100 g/l); pH 5.5-5.6.	2,4-D (1.0-15.0 mg/1) Regeneration medium contains IAA (0.175 mg/l) and 6-BAP (2.23 mg/1)	16-h photoperiod, 25°C	Gumerova *et al.* 2001
Shoot organogenesis from leaf callus for common buckwheat.	MS medium Sucrose 3% pH-5.7	2, 4-D (2 mg/l), Kinetin (0.2 mg/l), NAA (0.1-0.5 mg/l). For sub culturing (0.2 mg/l KIN, 2.0 mg/l BAP, 3% sucrose)	16-h photoperiod, 25°C	Woo *et al.* 2004
High frequency plant regeneration of common buckwheat.	MS medium pH-5.7 For sub culturing (1/2 MS medium containing 1.0 mg/l IBA,0.5 mg/l NAA, 3% sucrose)	2, 4-D (2 mg/l), 6-BA (1.0-2.0 mg/l)	16-h photoperiod, 25°C	Chen and Xu 2006
Shoot organogenesis and plant regeneration for lateral cotyledonary meristems of buckwheat.	MS medium pH-5.8	BAP (4.0 mg/l), $AgNO_3$ (7 mg/l)	16-h photoperiod, 25°C	Lee *et al*. 2009

2,4-D: 2,4-dichlorophenoxyacetic acid, BA: benzyl adenine, BAP: benzlaminopurine, IBA: indole-3-butyric acid, KIN: kinetin NAA: 1-napthalene acetic acid

been found that without a nurse callus, protoplasts start dividing 7-10 days after isolation independent of the culture conditions but, the development stops in two celled stage, hence cultures collapse. Therefore, *in vitro* planting efficiency can be raised to 25% by means of nurse cell culture technique (Lachmann and Adachi 1990). Callus and shoot regeneration has also been reported through cotyledons (Srejović and Nešković 1981; Mijuš-Đjukić *et al*. 1992; Chen and Xu 2006; details in **Table 6**). However, these explants could not be used for clonal propagation, therefore, a procedure for plant regeneration from the immature inflorescence culture (**Table 6**) was developed (Takahata 1988). Plant regeneration through cultured anthers of common buckwheat has been reported (Adachi *et al*. 1989; Bohanec *et al.* 1993). Somatic embryogenesis has been reported (**Table 6**) in cultures of immature embryos of common buckwheat (Rumyantseva *et al.* 1989) and of tartary buckwheat (Rumyantseva *et al.* 1989; Lachmann and Adachi 1990; Woo *et al.* 2000). This regeneration system has been suggested to be valuable for genetic transformation and cell line selection in common buckwheat.

There is an increasing interest in common buckwheat as a possible experimental material in plant molecular biology research. Transgenic techniques have the potential of modifying, decreasing or even removing allergenic substances in buckwheat. Attempts have been made to develop transgenic hypoallergic buckwheat genotypes. In 1987, a semi-dwarf buckwheat line was developed at the Agriculture Canada Research Station (Campbell 1987). Reduction in height is due to decrease in length of first six internodes of the plant. Kojima *et al.* (2000) demonstrated successful *Agrobacterium*-mediated transformation in *F. esculentum*. The transformation method includes inoculation of *Agrobacterium tumefaciens* cells on to the apical meristem of seedlings with height of 7-8 cm after 4-5 days of culturing. The transformation efficiency of plants was estimated by detection of β-glucuronidase (GUS) gene and southern blot analysis. There is a need to develop a transformation system that does not require sterile conditions and allow rapid analysis of gene functions. To this end, two different *in planta* transformation methods, vacuum infiltration and infiltration by syringe, have been optimized (Bratić *et al.* 2007). The transformation efficiency was measured by monitoring *A. tumefaciens* culture cell density, as well as pressure conditions and time elapsing between transformation event and GUS activity measurement. The vacuum infiltration method was found to be much more efficient as GUS activity was 57.3% (median value) higher than that obtained with infiltration with a syringe. The *Arabidopsis thaliana* tonoplast Na^+/H^+ antiporter gene, *AtNHX1*, was transferred into common buckwheat by *Agrobacterium* (Cheng *et al.* 2007). The transformants confirmed by PCR, Southern blotting, RT-PCR and Northern blotting analysis exhibited higher levels of salt tolerance to wild type plants. Moreover, the rutin content of the roots, stems and leaves of transgenic buckwheat also increased than those of the control plants. Transgenic common buckwheat plants over-expressing *AtNHX1*, a vacuolar Na (+)/H (+) antiporter gene from *A. thaliana*, were regenerated after transformation with *A. tumefaciens* (Chen *et al.* 2008). These plants were able to grow, flower and accumulate more rutin in the presence of 200 mmol/l NaCl. Moreover, the content of important nutrients in buckwheat was not affected by the high salinity of the soil. These results demonstrated the potential value of these transgenic plants for agricultural use in saline soil. Khadeeva *et al.* (2009) have demonstrated the possibility to use *Agrobacterium*-mediated transformation of leaf discs to produce resistance to bacterial infections in tobacco and potato plants by introduction of a single gene encoding the serine proteinase inhibitor BWI-1a (ISP) from buckwheat seeds.

DEVELOPMENT OF MOLECULAR MARKERS IN *F. TATARICUM*

The absence of a well developed linkage map and availability of only a limited number of molecular markers in buckwheat prompted us to look for *in silico* alternatives for rapid identification of additional molecular markers. We utilized ESTs available in other plant species belonging to a taxonomically common order of *Fagopyrum* spp. Buckwheat belongs to the family *Polygonaceae*, order *Caryophyllales*, therefore, we chose those plant species that fall under the same order for identification of molecular markers such as SSRs. All ESTs available in a particular plant species (**Table 7**) were downloaded from the TIGR database (http://compbio.dfci.harvard.edu/tgi/tgipage.html). The SSRs were identified in ESTs using PGG Bioinformatics at www.hornbill.cspp.latrobe.edu.au and primers were designed. Primer pairs were synthesized for 141 SSRs based on repeat length, out of which only 13 SSRs were successfully amplified on *F. tataricum* genotypes, indicating poor transferability of SSRs (**Table 8**). Fifty four SSRs, which were identified by Konishi *et al.* (2006) in *F. esculentum*, were also tested on selected accessions of *F. tataricum*, but no polymorphism was found.

Table 7 Status of ESTs in plant species related to *Fagopyrum*. Source: unpublished data of Nidhi Gupta PhD.

Family	Plant species	Number of ESTs
Aizoaceae (ice plant family)	*Mesembryanthemum crystallinum*	27,191
Amaranthaceae (goosefoot family)	*Beta vulgaris*	25,834
	Suaeda salsa	973
Plumbaginaceae (leadwort family)	*Limonium bicolor*	4,686
	Plumbago zeylanica	1,701
Tamaricaceae (tamarix family)	*Tamarix androssowii*	4,627
	Tamarix hispida	17,082

Table 8 SSR primers derived from ESTs and tested on *Fagopyrum tataricum*. Source: unpublished data of Nidhi Gupta PhD.

Forward primer sequence (5′-3′)	Reverse primer sequence (5′-3′)	SSR motif	Product size	Plant
GAACCAACAACATCAGTTTCAGC	GTTTCGAGGTTAGTAGCTGGGAT	(cag)17	300-400 bp	*Beta vulgaris*
AATTTCTGGATGGAGTAGTGCCT	ATGGACAATGATGGAGTGTAACC	(ga)28	300-400 bp	(Goosefoot family)
CCTCTTCTCTCTTCCCTTGCTAC	GTGAAACAAAGAGACTGAGCCAT	(ca)4(ac)3a(ac)17	100-200 bp	
GCTCTTAGGCAGTTGGGTCC	TCTCTCTCTCCCTTTCTCCTCTC	(tc)20	200 bp	
CAGCTCCAATTCCTTTCTTCTTC	GCTTCATATCTCTCTCCTCCCTC	(ct)17	500 bp	
CCTTCCTTACTCACCCTTTGTCT	CAATAAGTGAGAGTTGCGTCTCC	(tac)14	400 bp	*Mesembryanthemum*
CCTAGTCTAGACGATCGCGG	AGGAAATGAGGAGAGTGGTTAGG	(ga)55	200 bp	*crystallinum*
CTACCACCACTCCACCACTCTC	GACCTCGGAGAAGAAGACGTAG	(cca)15	200 bp	(Ice plant family)
AACCCTCTCAGTATCAGCAGTTG	AACTTCTGCAGTTGCTACCAAAG	(tc)21	300 bp	
CATATCCTCCGTGTCCATAGTGT	AGTATGTCAAGCAACCCAATCAC	(ag)38	200 bp	
CAAATTGAGAAAGACGGAGAGG	GAAGATCCGACTCAACTTCTTCA	(gaa)7	500 bp	*Plumbago zeylanica*
TCTGACGAAGAAAGAGAGATGCT	AAGTCTTAGCCAACTAGATCCCG	(gaaag)7	400 bp	(Leadwort family)
ACTCAGGATGTAACAGGGTTGAG	GATGTTAGGTGATCTTGTGGGAA	(caa)5	200 bp	

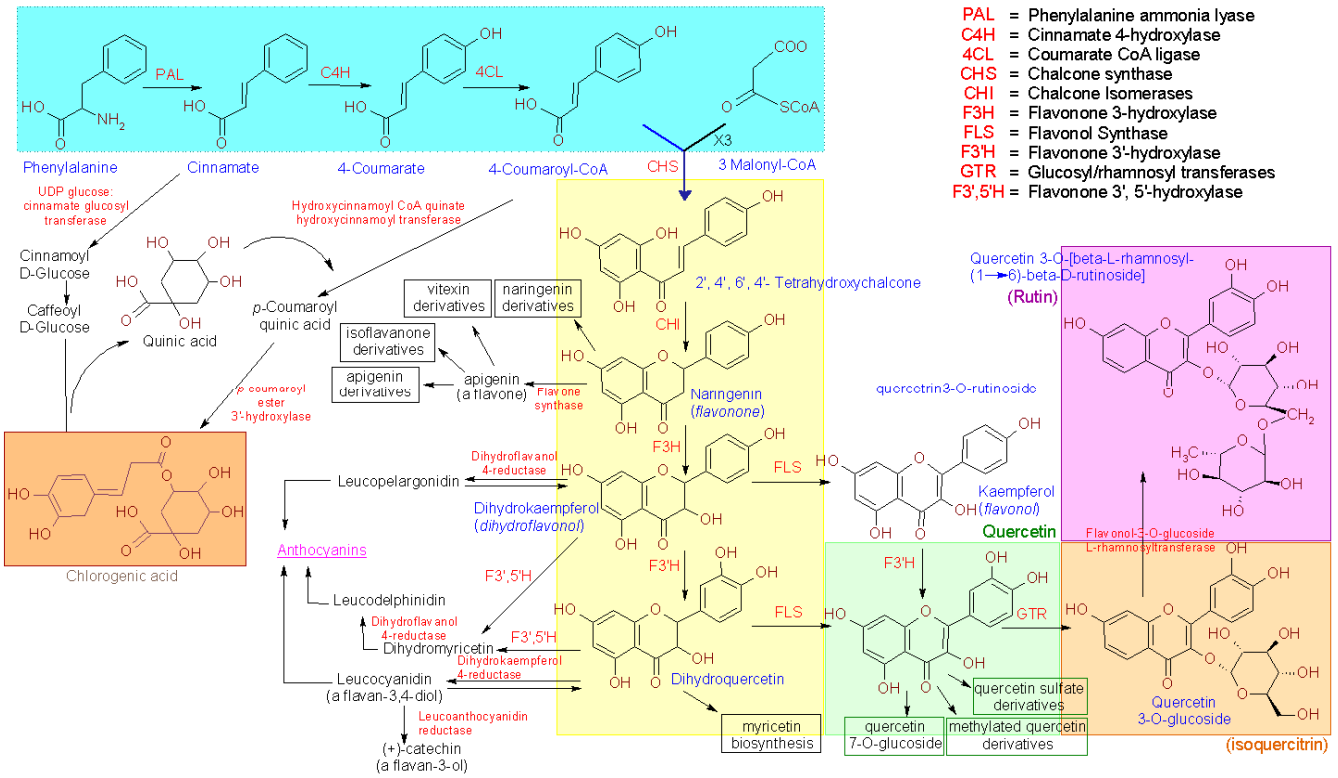

Fig. 2 Rutin biosynthetic pathway. Created by using information from KEGG (Kyoto Encyclopedia of Genes and Genomes) and Comino *et al.* (2007).

RUTIN BIOSYNTHETIC PATHWAY

The rutin biosynthetic pathway has been elucidated in various plant species. Nine genes are known to be involved in the pathway: phenylalanine ammonia-lyase (PAL), cinnamate-4-hydroxylase (C4H), 4-coumarate CoA ligase, Chalcone synthase (CHS), Chalcone isomerase (CHI), Flavonol synthase (FLS), Flavanone-3-hydroxylase (F3H), Flavanone-3'-hydroxylase (F3'H) and glucosyl/rhamnosyl transferase (**Fig. 2**).

PAL, EC 4.3.1.5 catalyzes the first step in the phenylpropanoid pathway and is considered an important regulation point between primary and secondary metabolism.

CHS, a key enzyme in flavonoid biosynthesis, catalyses sequential decarboxylative condensations of *p*-coumaroyl-CoA with three malonyl-CoA molecules. CHS biosynthesis is specific to the formation of flavonoid pigments and isoflavonoids. Chalcone synthase gene has been found to be critical in rutin biosynthesis because down regulation of CHS in tomato fruit by RNA interference (RNAi) resulted in suppression of CHS, and subsequently reduced accumulation of flavonoids (Schijlen *et al.* 2007)

CHI, an enzyme in the isoflavonoid pathway in plants, catalyzes the cyclization of chalcone into (*2S*)-naringenin. CHI gene activity appears to be critical as in tomato peel tissue, the expression of a sequence encoding the *P. hybrida* chalcone isomerase leads to a large increase in the level of quercetin-glycoside accumulation (Verhoeyen *et al.* 2002). CHI-suppression by RNA interference (RNAi) showed reduced pigmentation and change of flavonoid components in flower petals of transgenic *Nicotiana tabacum* (Nishihara *et al.* 2005). The plants also accumulated high levels of chalcone in pollen, showing a yellow coloration.

F3H catalyses the hydroxylation of flavanones at the 3 position of C ring leads to the formation of dihydroflavanols. The full-length cDNA (1071 bp) and genomic DNA sequences of F3H gene were isolated from *Ginkgo biloba* for the first time (Shen *et al.* 2006). The full-length cDNA encoding a 357-amino-acid protein with a calculated molecular weight of about 40 kDa and isoelectric point (pI) of 5.57. In addition, F3H gene expression appears to be vital in the regulation of the flavonoid pathway as it is coordinately regulated with CHS and CHI in petunia (Pelletier *et al.* 1997). The strong correlation between the concentrations of catechins in *Camellia sinensis* F3H expression indicates its critical role in catechin biosynthesis (Singh *et al.* 2008).

FLS catalyse the formation of kempferol and quercitin from dihydrokempferol and dihydroquercitin respectively. Knock-out alleles of genes involved in flavonoid biosynthesis were generated in *Arabidopsis*. One mutant line containing an *En-1*(maize transposable element) insertion in the FLS showed drastic reduced levels of kaempferol. Allelism tests with other lines containing *En-1* insertions in the F3H demonstrated that *transparent testa 6* (*tt6*) encodes F3H (Wisman *et al.* 1998).

Glucosyl/rhamnosyl transferases catalyse the glycosylation of quercetin following the rhamnosylation of isoquercitrin. Among these enzymes, uridine-diphosphoglucose (UDPGlc), flavonoid 3-*O*-glucosyltransferases (3GT) have been studied in different plants. In the present 3GT in buckwheat cotyledons has been purified and characterized (Suzuki *et al.* 2005). It has been studied that, the expression pattern of a UGT gene, *UGT89C1*, was found to be highly interrelated with known flavonoid biosynthetic genes which is identified as flavonol 7-*O*-rhamnosyltransferase. This was done by using transcriptome co-expression analysis accessible on the ATTED-II public database (Yonekura-Sakakibara *et al.* 2007).

COMPARATIVE GENOMICS OF GENES INVOLVED IN RUTIN BIOSYNTHESIS

Of the 9 genes involved in rutin biosynthesis, two genes CHS and glucosyl transferase have been identified in *F. esculentum* and *F. tataricum*, respectively (Hrazdina *et al.* 1986; Suzuki *et al.* 2005). We used comparative genomics to identify and clone the remaining rutin biosynthesis genes in tartary buckwheat. As most of the genes are present in multiple copies in the genomes of plants, we used the *Arabidopsis* genome information to identify the most significant copy of each gene (**Tables 9, 10**). The nucleotide and protein sequences of genes involved in rutin biosynthesis were retrieved from different plant species and the multiple sequence alignments (MSA) were done in order to find out

Table 9 Copy number of rutin biosynthesis genes in different plant species.

Gene	Plant species/Gene copy number	References
CHS (Chalcone synthase)	*Citrus*/2(CHS 2 in embryogenesis)	Moriguchi *et al.* 1999
	Poplar (6)	Tsai *et al.* 2006
	Arabidopsis (1)	Feinbaum and Ausubel 1988
	Soyabean (7)	
	Walnut/2 (98% identity)	Claudot *et al.* 1999
	C. sinensis/2 (CHS 2 plays key role)	Moriguchi *et al.* 1999
	V. vinifera (4)	Harker *et al.* 1990
	Petunia/8 complete and 4 partial	Koes *et al*. 1987
PAL (Phenylalanine ammonia-lyase)	*V. vinifera*/13 (1, 2, 3 are major ones)	Saparvoli *et al.* 1994
	Poplar (5)	Subramaniam *et al.* 1993
	Arabidopsis/4 (1 and 2 are major)	Olsen *et al*. 2007
C4H (Cinnamate 4 hydroxylase)	*C. sinensis*/2 (C4H 2 plays major role)	Betz *et al*. 2001
	Arabidopsis (1)	Mizutani *et al.* 1997
	V. vinifera (1)	Saparvoli *et al.* 1994
	Poplar (2)	Tsai *et al.* 2006
	Ipomea purpurea/6	Durbin *et al*. 1995
4CL (4Coumarate CoA ligase)	*Arabidopsis*/14 genes, 4 are major (*At4cl1*, *At4cl2*, *At4cl3*, and *At4cl5*). At4CL3 plays role	Hamberger and Hahlbrock 2004
	Raspberry (4)	Kumar and Ellis 2004
	V. vinifera (1)	Sparvoli *et al*. 1994
	Poplar (5)	Ehlting *et al*. 2002
CHI (Chalcone isomerase)	*Camellia sinensis* (1)	Lucheta *et al.* 2007
	Petunia hybrida (2) and *Glycine max* (5)	Van Tunen *et al*. 1988; Ralston *et al*. 2005
	Arabidopsis (1)	Shirley *et al*. 1992
	Poplar (1)	Lucheta *et al.* 2007
	V. vinifera (1)	Lucheta *et al.* 2007
F3H (Flavanone 3-hydroxylase)	Poplar (3)	Tsai *et al.* 2006
	Arabidopsis (1)	Pelletier and Shirley 1996
	V. vinifera (1)	Saparvoli *et al.* 1994
FLS (Flavonol synthase)	Poplar (4)	Tsai *et al.* 2006
	Arabidopsis/6 (AtFLS 1 is major)	Owens *et al.* 2008
	V. vinifera (1)	Saparvoli *et al.* 1994
GTR (Glucosyl/Rhamnosyl transferase)	Poplar (1)	Lucheta *et al.* 2007
	Arabidopsis (1)	Shirley *et al.* 1996

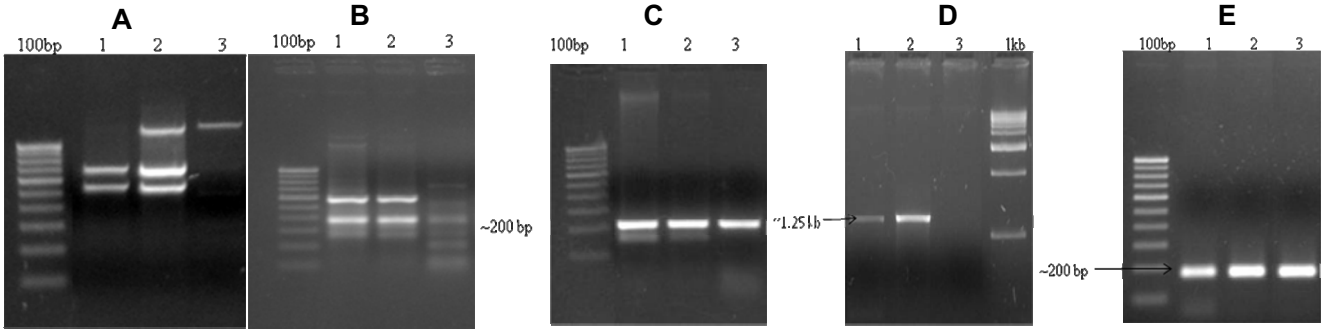

Fig. 3 Amplification of rutin biosynthetic pathway genes. (**A**) Flavanone-3 Hydroxylase, (**B**) Cinnamate-4 Hydroxylase, (**C**) Chalcone synthase, (**D**) 4-Coumarate CoA Ligase, (**E**) Glucosyl/ Rhamnosyl Transferase in *Fagopyrum* species. 1 = *F. tataricum* (tartary buckwheat), 2 = *F. tataricum* (rice-taratary buckwheat), 3 = *F. esculentum* (common buckwheat). Source: unpublished data of Nidhi Gupta PhD.

the extent of sequence similarity (**Table 11**). Primer pairs were designed from conserved regions of gene sequences retrieved from dicot plants and amplified in *Fagopyrum* spp. (common, tartary and rice-tartary buckwheat). We observed single band amplification in CHS, 4CL and glucosyl/rhamnosyl transferases, whereas for F3H and C4H, multiple copies of genes were amplified (**Fig. 3**).

CONCLUSION AND FUTURE RESEARCH NEEDS

Cultivated buckwheat, despite its consumption and use in many parts of the world lacks breeding research, primarily due to its distribution in resource-poor countries. Future collections are required to be made from the areas where genetic erosion is taking place, largely due to changed cropping patterns and the replacement of buckwheat with more remunerative crop species. There is pressing need for a regional as well as global database on the status of buckwheat *ex situ* collections with basic passport data and a minimum of characterization data. Systematic and concerted germplasm characterization efforts are required to identify the trait specific germplasm such as ultra early maturing types, synchronous maturity, high rutin content, resistance to shattering, easy-to de-hull, especially in tartary buckwheat, tolerance to lodging and frost, increased groat percentage, etc. Wild species diversity needs further taxonomic and biosystematic approach to classify and authenticate variations at species and subspecies level. More focus is required on the utilization of material for crop improvement, particularly wild gene pool for incorporating tolerance to biotic and abiotic factors. Genomic resources in buckwheat are limited. Efforts need to be directed towards developing genomic libraries, identification and validation of molecular markers and generating linkage maps with deeper coverage that can assist breeders in QTL dissection and marker-assisted breeding. Optimization of *in vitro* protocols for large-scale production of secondary metabolites of industrial importance like rutin can be very useful. Research on development of transgenics for various biotic and abiotic stresses is another area that needs attention.

Table 10 Rutin biosynthetic pathway genes in *Arabidopsis thaliana*. Source: http://www.ncbi.nlm.nih.gov/nucleotide/

Gene name	Gene id
PAL	AT3G53260
C4H	AT2G30490
4CL	AT5G67010, AT3G21240
CHS	AT5G13930
CHI	AT3G55120
F3H	AT3G51240
F3'H	AT5G07990
FLS	AT5G08640
GTR	AT2G22590

Table 11 Sequence Identity among rutin biosynthesis genes from different plant species with *Arabidopsis thaliana*. Source: unpublished data of Nidhi gupta PhD.

Gene name	Plant species	Percent identity
PAL	*Populus*	60
	Nicotiana tabacum	60
	Brassica rapa	63.3
	Brassica napus	79.9
	Jatropha curcas	58
C4H	*N. tabacum*	71.5
	Populus	67
	Camellia sinensis	68.9
	B. napus	83
	B. rapa	81.2
	Capsicum	68
	G. max	67.9
	Allium cepa	67.4
4CL	*B. napus*	63.1
	B. oleracea	68
	B. rapa	63.1
CHS	*Fagopyrum*	40
	Solanum lycopersicon	60
CHI	*Citrus sinensis*	70.1
	P. communis	60
	B. rapa	61.1
	Raphnus sativus	60.9
F3H	*B. napus*	62.8
	Glycine max	55.4
FLS	*N. tabacum*	60
	P. communis	60
	Fragaria	51.5
F3'H	*Glycine max*	64
	Populous	62.2
	Centaurea cyanus	78.9
	Brassica rapa	87

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