Biosynthesis and Accumulation of Flavonoids in Fagopyrum spp.

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ABSTRACT

Buckwheat is a multipurpose crop used for both grains and greens and known to have several medicinal and nutritional properties. Buckwheat contains flavonoids such as rutin, anthocyanins, catechins, chlorogenic acid, 4-hydroxy-3-methoxy benzoic acid, caffeic acid, epicatechins, p-coumaric acid, ferulic acid etc. Fagopyrum esculentum and Fagopyrum tataricum are the major source of flavonoid called rutin. Seeds of F. tataricum contain higher rutin content in comparison to F. esculentum. This review discusses the physiological and molecular basis of flavonoid biosynthesis and accumulation in plants in general and rutin and anthocyanin content in Fagopyrum species, its correlation with the expression of flavonoid pathway genes and the effect of different environmental factors on flavonoid biosynthesis. The understanding of rutin biosynthesis in buckwheat is expected to supplement for genetic improvement of buckwheat for higher nutritional value.

Keywords: biosynthetic pathway, comparative genomics, gene, rutin
Abbreviations: 4CL, 4 Coumarate CoA ligase; CHI, Chalcone isomerase; CHS, Chalcone synthase; C4H, Cinnamate 4-Hydroxylase; DAS, day after seeding; DW, dry weight; F3H, Flavonol 3-Hydroxylase; F3’H, Flavonol 3’-Hydroxylase; FLS, Flavone synthase; PAL, Phenylalanine ammonia lyase

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INTRODUCTION

Flavonoids constitute a relatively diverse family of aromatic molecules that are derived from Phenylalanine and malonyl-coenzyme A (CoA; via the fatty acid pathway). These compounds include six major subgroups that are found in most of the plants: the chalcones, flavones, flavonols, flavandiols, anthocyanins, and condensed tannins (or proanthocyanidins); a seventh group, the aurones, is widespread, but not ubiquitous (Winkel-Shirley 2001).

Buckwheat belongs to the family Polygonaceae and refers to two cultivated species: common buckwheat (Fagopyrum esculentum) and tartary buckwheat (F. tataricum). Buckwheat (Fagopyrum spp.), a pseudocereal, is a multipurpose food crop used both for grains and greens and has seve-ral medicinal and nutritional properties (Campbell 1997; la Casa et al. 2000; Landberg et al. 2011). Buckwheat contains several kinds of flavonoids in the seeds, leaves and stems, and rutin is one of these compounds (Couch et al. 1946). It is an antioxidant that has many useful pharmacological effects (Yildizoglu-Ari et al. 1991). Rutin is also present in substantial amounts in various other plants, such as apple, citrus, Capris, tomato, etc. (McGregor and McKilligan 1952; Attansssova and Bagdassarian 2009), and buckwheat is considered to be a major dietary source of rutin. Rutin is a flavonol with antioxidative and anti-inflammatory activities such as it has hypotensive effect, positive inotropic effect (Matsubara et al. 1985), strengthens the capillary blood vessels (Campbell 1997). Rutin can also be used as a natural coloring agent, an oxidation inhibitor, sunburn preventative in cosmetics (rutin absorbs ultra violet rays) and as an ingredient in functional food applications (WIPO 2004). The demand for rutin and other flavonoids derived from buckwheat is growing in the food, pharmaceutical and cosmetic industries due to its nutritional and medicinal value (Table 1).

The high level of rutin in buckwheat leaves and stems is accompanied by another member of the flavonoid family –
anthocyanins, one of the final products of flavonoid bio-
synthetic pathways, which are water-soluble pigments in
the leaves, stems, flowers, and roots. Anthocyanins play an
important role in attracting insects or other animals for pol-
lination and seed dispersal (Horbowicz et al. 2008). They
also play a role as anti-oxidants and in protecting DNA and
the photosynthetic apparatus from high radiation fluxes
(Gould 2004). Anthocyanins have been associated with
enhanced tolerance to chilling and freezing temperatures
(Christie et al. 1994; McKown 1996; Hale et al. 2001;
Nozzolillo et al. 2002; Leng and Qi 2003), to heavy metals
(Krupa et al. 1996; Marrs and Walbot 1997; Hale et al. 2002)
and to water stress (Sherwin and Farrant 1998; Far-
rant 2000; Farrant et al. 2003). The flavonoids, particularly
proanthocyanidins in the seed coat, contribute to the main-
tenance of seed dormancy as well as increasing seed lon-
gevity in storage (Winkel-Shirley 1998; Debeaujon 2000;
Debeaujon et al. 2003).

Flavonoids are also of significant interest as antioxi-
dants and anticancer agents in the human diet (Rice-Evans
2001; Havsteen 2002; Stevens and Page 2004). Citrus fla-
vonoids have many medicinal properties such as anticar-
cinogenic, anti-inflammatory and antioxidant (Stavric 1993;
Elangovan et al. 1994) this also, resulted in reduction of
coronary heart disease (Hertog et al. 1993; Di Majo et al.
2005). The interest in these classes of compounds is due to
their pharmacological activity as radical scavengers (Cotelle
et al. 1996). Park et al. (2000) demonstrated that buckwheat
flowers have the potential of being used as a healthy food or
as a medicine by detecting rutin in boiled water from the
flowers. Clinical observations carried out on 75 diabetic
patients treated with tartary buckwheat biscuits showed a
decrease in the blood sugar level (Wang et al. 1992). Recent
evidence suggests that certain flavonoids reduce dental
caries and cariogenic bacteria incidence and are used as a
promising natural agent for non-invasive root caries therapy
(Wood 2007; Wu 2009). Flavonoids lower Alzheimer's dis-
case (AD) by lowering amyloid β production, which plays
an important role in AD (Paris et al. 2011). Flavonoids
clearly have the potential to directly affect signaling and
gene transcription through interaction with cytoplasmic and
nuclear proteins. It has been suggested that flavonoids func-
tion in gene regulation in plants, for example, by inhibiting
protein kinases that regulate the activity of transcription
factors required for the synthesis of auxin transport proteins
(Debeaujon et al. 2002; Buer and Muday 2004).

Buckwheat has been recognized as a healthy food
because its seed is rich in vitamin B1 and B2, its protein has
high biological value (Sure 1955), proteins are particularly
rich in lysine (6.1%), and contained less glutamic acid and
proline and more arginine and aspartic acid than cereal pro-
teins. Chemical analyses of buckwheat hydrolysates indi-
cated that the amino acid composition was nutritionally
superior to that of cereal grains (Pomeranz and Robbins
1972)

**Sources of Flavonoids**

The flavonoids in different vegetables are as (mg/100 g of
dry weight): broccoli 197, cauliflower 219, cabbage 147.5,
red chili 829, lemon grass 178, black tea 1491, garlic 957,
French bean 172.5, French peas 361 (Miean and Mohamed
2001). Of the Allium species, shallots and red onions rep-
resent the richest potential source of quercetin containing
95 and 64 mg/100 g, respectively. Quercetin 4′-glucoside
and quercetin 3,4′-diglucoside are, in most cases, reported
as the main flavonols in onions (Allium cepa L.) (Rune et al.
2007). The total flavonoid content of onion leaves (1497.5
mg/100 g quercetin, 391.0 mg/100 g luteolin, and 832.0
mg/100 g kaempferol) was followed by Semambu leaves
(2041.0 mg/100 g), bird chili (1663.0 mg/100 g), black tea
(1491.0 mg/100 g), papaya shoots (1264.0 mg/100 g), and
guava (1128.5 mg/100 g) (Miean and Mohamed 2001).
Green chilli pepper is one of the few vegetables that contain
both flavonols (quercetin, 11.39 mg/100 g) and flavones
(luteolin, 2.7 mg/100 g) at detectable levels. Celery and
sweet ball peppers are the main food sources of flavones
independent of flavonoids (Janet and Garry 2006). Among
the beverages, apple juice is one of the richest juice sources
of catechins (containing 6.3 mg (-)-epicatechin/100 ml and
0.8 mg (+)-catechin/100 ml) whereas cranberry juice con-
contains the richest potential source of flavonoids (F .
tatari-
num, 72.5 mg/100 g) (Miean and Mohamed 2001). The
European Journal of Plant Science and Biotechnology
6 (Special Issue 2), 17-25 ©2012 Global Science Books

**Flavonoid Biosynthesis**

Phenylpropanoids are a group of plant secondary metabo-
lites derived from phenylalanine which are important for

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### Table 1 Nutraceutical value of buckwheat.

<table>
<thead>
<tr>
<th>Product/compound</th>
<th>Effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gluten free proteins</td>
<td>Diet for celiac patients as an alternative to wheat</td>
<td>Dreziewski et al. 2003</td>
</tr>
<tr>
<td>Rutin</td>
<td>Strengthens capillaries and helps in arteriosclerosis or high blood pressure</td>
<td>Campbell 1997</td>
</tr>
<tr>
<td>Rutin</td>
<td>Controls cholesterol</td>
<td>Kayashita et al. 1997</td>
</tr>
<tr>
<td>Rutin</td>
<td>Protection against gastric lesions</td>
<td>La Casa et al. 2000</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Anti-depressants, prevents signs of aging such as wrinkles and skin damage</td>
<td>Watanabe and Ayugase 2008</td>
</tr>
<tr>
<td>Buckwheat polyphenols</td>
<td>Ameliorate spatial memory impairment</td>
<td>Pu et al. 2004</td>
</tr>
<tr>
<td>Buckwheat protein</td>
<td>Suppresses gallstone formation and cholesterol level by enhancing bile acid synthesis</td>
<td>Tomotake et al. 2000</td>
</tr>
<tr>
<td>Phenolic antioxidants</td>
<td>Protects humans from oxidative stress</td>
<td>Schramm et al. 2003</td>
</tr>
<tr>
<td>Fagopyritol B1</td>
<td>Treatment of diabetes, polycystic ovary</td>
<td>Sattanathan et al. 2011</td>
</tr>
<tr>
<td>Buckwheat protein extract</td>
<td>Retard memory carcinogenesis by lowering serum estradiol, causes muscle hypertrophy</td>
<td>Kayashita et al. 1999</td>
</tr>
<tr>
<td>Buckwheat polyphenols</td>
<td>Treatment of polycystic ovary syndrome</td>
<td>Campbell 1997</td>
</tr>
<tr>
<td><strong>Industrial value</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flowers</td>
<td>Produces dark flavored honey having high antioxidant property</td>
<td>Saeger and Dyck 2001</td>
</tr>
<tr>
<td>Flour</td>
<td>Used in desserts, ice cream cones, diabetic foods, pancake mixes, canned meat products, canned vegetable products, and dried breakfast cereals</td>
<td>Bonafaccia et al. 2003</td>
</tr>
<tr>
<td>Flour</td>
<td>Making soba noodles as staple food in Japan</td>
<td>Taylor and Belton 2002</td>
</tr>
<tr>
<td>Grain</td>
<td>Baby food and in energy drinks</td>
<td>Fabjan et al. 2003</td>
</tr>
<tr>
<td>Tartary buckwheat raw material</td>
<td>Bitter buckwheat tea</td>
<td>Fabjan et al. 2003</td>
</tr>
<tr>
<td>Hull</td>
<td>Making pillows for relief of neck and back pain, muscle tension</td>
<td>Campbell 1997</td>
</tr>
</tbody>
</table>
many aspects of plant growth and development, such as pigment production, photoprotection, and disease resistance. The biosynthesis and accumulation of rutin and other flavonoids is controlled at the molecular level by structural and regulatory genes in different plant species (Winkel-Shirley 2001; Buer et al. 2010) and the pathway genes are conserved among different plant species and have been characterized at the genetic, biochemical and enzymatic levels in plants of different groups, and that it has a cysteine rich residue at amino acid 169 that is thought to be part of the 4-coumarate-CoA binding site and which is required for enzyme activity. Afterward, chalcone synthase (CHS) catalyzes the production of a tetracyclic chalcone that is the precursor for all flavonoids. This step is the first dedicated reaction of the flavonoid biosynthesis pathway in higher plants. Lanz et al. (2003; Goodman et al. 2004).

The second enzyme of the phenylpropanoid pathway, cinnamate 4-hydroxylase (C4H) is a member of the cytochrome P450 monooxygenase superfamily and it catalyzes the first oxygenation step during a phenylpropanoid metabolism, the hydroxylation of trans-cinnamate to p-coumarate (4-hydroxy trans-cinnamate). Next, 4-coumarate:CoA-ligase (4CL) converts p-coumarate to its coenzyme-A ester, which is a precursor for various phenylpropanoid biosynthetic derivatives, including lignins and flavonoids. Subsequently, chalcone synthase (CHS) catalyzes the production of a tetracyclic chalcone that is the precursor for all flavonoids. This step is the first dedicated reaction of the flavonoid biosynthesis pathway in higher plants. Lanze et al. (1991) have pointed out that this enzyme is well-conserved among plants of different groups, and that it has a cysteine residue at amino acid 169 that is thought to be part of the 4-coumaroyl-CoA binding site and which is required for enzyme activity. Afterward, chalcone isomerase (CHI) catalyzes the conversion of chalcone into flavone 3-hydroxylase (F3H) and flavonoid 3'-hydroxylase (F3'H), respectively. At this point, the pathway branches have two possible outcomes. In one branch, anthocyanidin synthase (ANS) catalyzes the conversion of leuco-anthocyanidin to anthocyanidin (Holton and Cornish 1995; Pelletier and Shirley 1996; Shirley 1996; Wisman et al. 1998; Chong et al. 2003; Groot et al. 2006). In the other branch, flavonol synthase (FLS) converts dihydroflavonols, such as dihydrokaempferol and dihydroquercetin, to flavonone 3-hydroxylase (F3'H) and flavonoid 3'-hydroxylase (F3'H), respectively. At this point, the structural and regulatory genes involved in rutin biosynthesis in tartary buckwheat. (Chauhan et al. 2010). More than one fragment was isolated for C4H, F3H and F3'H, but only the fragments showing maximum identity with the genes from other plant species were selected for designing primers for gene expression analysis. Primer pairs were designed from conserved regions of gene sequences retrieved from dicotyledon plants and amplified in Fagopyrum spp. (common, tartary and rice tartary buckwheat). Single band amplification was seen in CHS, 4CL and glucosyl/rhamnosyl transferases, whereas for F3H and C4H multiple copies of genes were amplified (Chauhan et al. 2010).

Isolation and sequence analysis of six anthocyanins biosynthetic genes was done in F. tataricum, which were cloned and characterized, namely, Fc4H, F4CL, FcCH, FcF3H, FcF3'H, and FcANS, which encodes C4H, 4CL, CHS, F3H, F3'H, and ANS, respectively (Park et al. 2011).

**SUBCELLULAR SITES / DISTRIBUTION OF FLAVONOIDs**

Flavonoids are found in most plant cell compartments, including the cytosol, vacuole, ER, chloroplast, nucleus and small vesicles, as well as the extracellular space. Anthocyanins are cytotoxic and unstable in the neutral pH of the cytoplasm. Therefore, sequestration of anthocyanins into the acidic vacuole is an important component of the pathway leading to anthocyanin accumulation. Quercetin and kaempferol glycosides have been detected in the chloroplasts, which are capable of flavonoid biosynthesis (Herández et al. 2009). In some tissues, such as the epidermis of leaves and flowers and endothelium of the developing seed coat, flavonoids are transported primarily to the vacuole by processes that appear to involve multidrug resistance-associated protein or multidrug and toxic compound extrusion proteins (Debeaujon et al. 2001; Mathews et al. 2003; Goodman et al. 2004).

Hrazdina and Wagner (1985) indicated that PAL, C4H, CHS, and UDP-glucose flavonoid glucosyltransferase (UGFT) function as part of one or more membrane-associated enzyme complexes in amaryllis, buckwheat, and red cabbage. Hrazdina (1992) stated that PAL, CHS, and UFGT were located in the cytosol, loosely associated with the cytoplasmic face of the endoplasmic reticulum (ER). An association of CHS with the cytoplasmic face of the rough ER (rER), but not with nuclei, plastids, mitochondria, Golgi, or tonoplasts, in buckwheat (F. esculentum) hypocotyls was also observed. In wild-type A. thaliana seedlings, flavonols accumulate in three main zones: the cotyledonary node, the hypocotyl–root transition zone and the root tip (Sheahan and Rechnitz 1993; Murphy et al. 2000; Peer et al. 2001; Saslowsky and Winkel-Shirley 2001). The wide distribution of flavonoids in plant cells and their probable biosynthetic sites on the cytosolic face of the ER imply that plants have efficient flavonoid transport systems with which to deliver these metabolites across various membrane-limited compartments. However, the mechanisms, including biosynthesis, trafficking of most primary and secondary metabolites are still poorly understood (Grotewold 2004). Two major hypothesis have been proposed for flavonoid transport: membrane vesicle-mediated transport and membrane transporter-mediated transport (Grotewold 2006). In vesicle-mediated flavonoid transport, anthocyanins were first assumed to be transport vesicles or sites of anthocyanin biosynthesis (Markham et al. 2000; Grotewold 2004; Braidot et al. 2008). Anthocyanic vacuolar inclusions (AVIs) are more likely to be storage complexes than to be involved in anthocyanin transport. Membrane mediated transportation is carried out by ABC and MATE family transporters.
Rutin content of tartary buckwheat is significantly higher than that of common buckwheat, with average values of 0.8 to 1.8% DW and 0.01% DW, respectively (Fabjan et al. 2003; Chauhan et al. 2010; Park et al. 2011). It is also higher compared to choline (81 g/100 g) of common buckwheat than in those of common buckwheat (Steadmam et al. 2001). In comparison, the amount of quercetin, another flavonoid present in buckwheat, is only 0.01-0.05% DW. Flowers are the richest source of rutin in buckwheat (6809 mg/100 g DW) with 1000 times more rutin in comparison to roots (6.25 mg/100 g DW) (Li et al. 2010) These results are consistent with previous studies that showed that the amount of rutin in buckwheat peaks at the full flowering stage (Dietrych-Szostak and Oleszek 1999; Gupta et al. 2011). There is more rutin in leaves positioned higher on the stem than in leaves at lower positions. Rutin concentration and rutin glucosidase activity by dry weight was high in young leaves (rutin content is more than 20% in unexpanded young leaves) and decreased along with the decrease of leaf position (Tatsuro et al. 2005). According to Kalinova and Dadakova (2006), the lowest amount of rutin was detected in buckwheat achenes and the plant parts, the richest in rutin were the leaves. However, amaranth inflorescences contained about half the amount of rutin compared to amaranth leaves (amaranth leaves contained up to 3% rutin per dry weight). According to Duke (1992), an amount of rutin similar to the level in amaranth (about 3%) is present in the leaves of parsley (Petroselinnum crispum).

Amaranthus hybrid and A. cruentus were good sources of rutin. These species can provide 10-20 kg of rutin per hectare (in case of biomass production of about 2 t/ha DW) in a 60 days period of growth which is less than data obtained for common buckwheat. Kalinova and Dadakova (2006) determined rutin production of about 90 kg/ha (DW) at the flowering stage of buckwheat (about 60 days after sowing). In amaranth, about 45-90 kg/ha of rutin was obtained at the end of the growing period, which is due to the high production of biomass (about 30-45 t/ha), similar to the amount produced by common buckwheat.

Variation in rutin content in different plant parts of Fagopyrum spp. (F. esculentum, F. tataricum, F. cyamoides) was reported as highest in the flowers and lowest in the roots (Park et al. 2004). Rutin content in inflorescences of tartary buckwheat was shown to be 9.5 times higher than in those of common buckwheat and 2.2 times higher than in those of F. esculentum. Rutin content in tartary buckwheat was 34.9 times higher than in those of common buckwheat. Kalinova and Dadakova (2006) determined rutin production of about 90 kg/ha (DW) at the flowering stage of buckwheat (about 60 days after sowing). In amaranth, about 45-90 kg/ha of rutin was obtained at the end of the growing period, which is due to the high production of biomass (about 30-45 t/ha), similar to the amount produced by common buckwheat.

Differences in rutin content in seeds and in other tissues and in different stages of two Fagopyrum species, vis-à-vis expression profiling of flavonoid pathway genes has been reported by Gupta et al. (2011). Biosynthesis and accumulation of rutin showed significant variation among different growth stages from S1 to S9 (seed germination to seed maturation) of the Fagopyrum species, namely F. tataricum (IC-14889, IC-329457) and F. esculentum (IC-540858). Acces- sions of F. tataricum showed more or less similar pattern of rutin biosynthesis and accumulation in different growth stages to that of F. esculentum. Rutin content was higher during seedling stages of F. tataricum (3.6- to 4.6-fold) compared to F. esculentum and then increased exponentially from stages S3 to S6 (different leaf maturing and flowering stage) of F. esculentum, whereas the biosynthesis and accumulation of rutin showed a zigzag pattern during stages S2 to S6 of both the accessions (IC-14889, IC-329457) of F. tataricum. The dynamics of rutin biosynthesis and accumulation in different growth stages of both the Fagopyrum species suggested that the higher amounts of rutin starts accumulating during post-flowering stages of F. tataricum as seed maturing stages of F. tataricum contains 40-50x higher rutin than F. esculentum.

ANTHOCYANINS IN BUCKWHEAT AND THEIR ACCUMULATION

The petals and sprouts of a common buckwheat cultivar (F. esculentum) contain four anthocyanins: cyanidin 3-O-gluco- side, cyanidin 3-O-rutinoside, cyanidin 3-O-galactoside and cyanidin 3-O-galactopyranosyl-rhamnoside (Kim et al. 2007). Total amount of anthocyanins increases with flower development and the concentration of anthocyanins in the petals of buckwheat determine the colour of the flowers. For example, the ‘Gan-Chao’ cultivar which has red flowers, contains 4.69 μg of anthocyanins/petal, while the cultivar ‘Kitawasesoba’ which has white flowers, contains 0.06 μg of anthocyanins/petal.

All the organs of ‘Hokkai T10’ contained 2.6-6 times more anthocyanins than those of ‘Hokkai T8’ (Park et al. 2011). Hence the total anthocyanin content was higher in ‘Hokkai T10’ than in ‘Hokkai T8’, which is consistent with previous studies (Suzuki et al. 2009) which showed that anthocyanin accumulation was cultivar specific. These cultivar specific differences may be due to differential gene expression in different plant organs (Park et al. 2011); however, naringenin chalcone, a flavonoid, was absent from ‘Hokkai T10’ seedlings based on fluorescence microscopy, hence the accumulation of flavonoids and anthocyanins are inversely related in ‘Hokkai T8’ and ‘Hokkai T10’. In terms of quantity, the major anthocyanin compound in buckwheat flowers is cyanidin 3-O-rutinoside (Watanabe 2007). Cyanidin 3-O-glucoside and cyanidin 3-O-rutinoside concentrations in 6-10 day seedling sprouts of ‘Hokkai T10’ ranged from 0.16 to 0.20 mg/g DW and from 5.55 to 6.57 mg/g DW, respectively. In addition, dark-grown sprouts of ‘Hokkai T10’ accumulated 0.091 and 2.77 mg/g DW of cyanidin 3-O-glucoside and cyanidin 3-O-rutinoside whereas other varieties/breeding lines accumulated trace amounts of anthocyanins (Kim et al. 2007).

The expression of flavonoid biosynthesis regulatory genes appears to be highly dependent on tissue type and/or response to internal or external signals which affect the signal transduction and gene expression involved in biosynthesis (Tsukaya et al. 1991; Dixon and Paiva 1995; Levya et al. 1995; Mol et al. 1996; Laura et al. 2007; Ferri et al. 2009). The type and amount of flavonoids in plants depends on genotype and developmental stage (Hahlbrock and Grisebach 1979). Expression of flavonoid pathway genes was compared in F. esculentum (IC-540858) and F. tataricum (IC-329457) and their correlation with rutin content was reported by Gupta et al. (2011). Rutin content vis-à-vis expression analysis was carried out in different growth stages of Fagopyrum species. Out of 9 genes, expression levels of C4H, 4CL, GT, F3’H and F3H genes were not significant, whereas four genes, namely PAL, CHS, CHI and FLS showed differential expression with relatively higher amounts of transcripts in rice tertiary buckwheat compared to common buckwheat during different growth stages. A correlation was observed between the expression of PAL, FLS, CHS and CHI genes and the rutin content, as there was a significant increase in these genes transcripts during the S6 (inflorescence) stage of F.tataricum compared to F. esculentum. PAL and CHS genes were highly expressed in the mature seeds of F. tataricum in S9, which results in higher rutin content than F. esculentum (F. tatar- icum contained 43-55x higher rutin compared to F. esculen- tum). CHI transcript level is higher in S7 (early seed develop- lopment) in F.tataricum as compared to F.esculentum.
According to Park et al. (2011), the gene transcripts for all of the enzymes of the flavonoid biosynthetic pathway were expressed in every organ of *F. esculentum*, the expression levels were the highest in the stems and roots. The expression level of FePAL in the stems and roots was higher than in the flowers and leaves. In contrast to C4H, which was strongly expressed in all organs (flower, stem, leaf and root), FeF3H, FeF3TH, FeDFR, FeFLS1, FeFLS2, and FeF3H were expressed at low levels in all these organs. In addition, FeFLS2 was expressed at very low levels in the roots, unlike FeFLS1.

**EXPRESSION OF ANTHOCYANIN BIOSYNTHETIC GENES IN DIFFERENT ORGANS OF *F. TATARICUM***

Gene expression was compared in different organs and developmental stages of tartary buckwheat cultivars ‘Hokkai T8’ and ‘Hokkai T10’ (Park et al. 2011). Anthocyanin content was directly correlated with the expression of flavonoid biosynthesis genes. During flowering and seed ripening the FtANS gene was more highly expressed than other genes. Among the various genes for the anthocyanin pathway, the highly expressed genes were FtPAL, FtC4H, FtF3H, FtF3HR, FtCH, FtCHI, FtF3H and FtANS in the flowers (for example the total anthocyanin content of the flowers of ‘Hokkai T10’ was 4.5 times that of ‘Hokkai T8’), FtPAL, FtCHS and FtANS in the leaves, FtPAL, Ft4CL, FtC4H, FtCHS and FtANS in the stem, and FtC4H, Ft4CL, FtCHS, FtF3H and FtANS in the roots. During flowering and seed ripening the FtANS gene was more highly expressed than the other genes.

Quantitative real-time PCR analysis showed that these biosynthetic genes are more highly expressed in the lower parts of the plant (i.e. stems and roots) than in the higher parts of the plant (i.e. flower and leaves). There is an inverse relationship between the expression of flavonoid biosynthetic genes and the accumulation of their products in *F. tataricum*, similarly to *F. esculentum*. This may be due to transport of flavonoids within *Fagopyrum* species (Li et al. 2010; Park et al. 2011). Similar results of transport and accumulation were found in other plants. In *Arabidopsis* roots flavonoids were also found in the stele, which did not contain detectable levels of CHS or CHI, suggesting that flavonoids may be transported between cells (Saslowsky and Winkel-Shirley 2001). The expression of AsPAL and AsC4H transcripts in *Allium sativum* was highest in the roots but surprisingly low in the bulbils, where phenylpropanoid compounds are most concentrated. These results suggest that the phenylpropanoids are synthesized in the roots and subsequently transported to the bulbils of *A. sativum* (Tuan et al. 2010).

**ENVIRONMENTAL FACTORS AFFECTING FLAVONOID BIOSYNTHESIS, TRANSPORT AND ACCUMULATION***

Cultivar and environmental factors, such as soil and climate, including temperature, UV radiation, sunlight, as well as cultural practices like sowing time and fertilizer also have an effect on the flavonoid content of plants. Individual biosynthetic genes may be regulated in response to a number of developmental and environmental signals. For example, in flowers the biosynthetic genes change their activities as a consequence of light and spatio-temporal developmental factors for the production of anthocyanins in the petal epidermal cells, coincidently with flower fertility (Braidot et al. 2008). *Ginkgo biloba* GbPAL was also observed to be induced by a variety of stresses including UV-B, wounding, cold and salicylic acid. PAL is a key enzyme in plant stress response. Its biosynthesis is stimulated on pathogenic attack, tissue wounding, UV irradiation, low temperature, or low levels of nitrogen, phosphate, or iron (Dixon and Paiva 1995). The enzyme is accumulated in the vicinity of the affected tissue (Mauch-Mani and Slusarenko 1996; Ehres et al. 1997).

A decrease of flavonoid biosynthesis has been observed when either endogenous (e.g. plant hormones), or exogenous factors (e.g. water and temperature stress, light, fertilizer, etc.) are limiting or excessive. In particular, plant hormones could affect flavonoid biosynthesis in a complex way. Abscisic acid, auxin and ethylene are responsible for an increase of flavonoids, while gibberellic acid and inhibitors of the ethylene receptors decrease their synthesis (Deikman and Hammer 1995; Dan and Lee 2004; Jeong et al. 2004). The gene expression is induced by sucrose, jasmonic acid and light irradiation, leading to an enhanced anthocyanin accumulation (Braidot et al. 2008). Methyl jasmonate inhibits anthocyanin biosynthesis and accumulation in hypocotyls of seedlings of common buckwheat (*F. esculentum* Moench) (Horbowicz 2008).

**Light/UV light***

Arabidopsis roots grown in complete darkness do not accumulate flavonoids since the expression of genes encoding enzymes of flavonoid biosynthesis are light-dependent (Charles et al. 2007). Different light exposures of fruits demonstrate that shading decreases significantly the flavonoid content of the organs. Long light hours could stimulate the increase of flavonoid content (Kim and Lee 2002), as flavonoid content of common buckwheat subjected to long sunlight hours was two times higher than that subjected to short sunlight hours. The total rutin content of buckwheat plant was highest under the natural light, intermediate under the blue-light, and lowest under red-light. The amount of rutin is also affected by the light and dark conditions (Suzuki et al. 1987; Campbell 1997), whereas other flavonoids, except gallic acid, are not significantly affected. In sprouts grown under light condition, rutin content (556.71 mg/100 g at 12 DAS) was 60% more than grown under dark conditions (343.25 mg/100 g at 12 DAS) (Park et al. 2011). The light-dependent biosynthesis would be limited only to flavonoids, a result that is consistent with the role that these molecules play in protecting tissues from UV light (Downey et al. 2004). Expression of genes of flavonoid synthesis, except PAL and SISy, is coordinately enhanced by light in grapes (*Vitis vinifera*) (Sparvoli et al. 1994). Blue-light significantly decreased stem length, the number of nodes and number of nodes in both common and tartary buckwheat cultivars.

Flavonoid biosynthetic genes are induced by a Blue/UV-B Light Receptor. UV-B radiations alter the enzyme activity or gene expression and hence affect the flavonoid content of the plant. Several studies, on the expression, have shown that the production of flavonoid and anthocyanin compounds in response to light is controlled, at least in part, at the level of transcription (Feinbaum and Ausubel 1988; Taylor and Briggs 1990; Feinbaum et al. 1991). For example, in parsley tissue culture cells CHS gene expression has been shown to be regulated by a UV-B light receptor, a blue light receptor, and phytochrome (Brun et al. 1986; Ohi et al. 1987). UV-B was able to induce the activity of diphenylethyl phthalocyanine and the synthesis of rutin, and was also capable of inducing the accumulation of rutin in the organs (Kreft et al. 2002; Suzuki et al. 2005a). Tartary buckwheat leaf treated with stress using UV-B radiation, cold and desiccation showed an increase in rutin concentration of 122% by UV-B radiation and 129% by desiccation, whereas rutin glucosidase activity was increased 363% by UV-B radiation, 100% by cold treatment and 158% by desiccation treatment over the control. Hence, it was proposed that rutin and rutin glucosidase activity may be related to enhancement of the defense system against stress conditions in tartary buckwheat leaf (Suzuki et al. 2005a). When common buckwheat was exposed to reduced, ambient, and enhanced UV-B radiation, the total amounts of UV-absorbing compounds (rutin, quercetin, quercitrin and other flavonoids) were lower in buckwheat grown under reduced UV-B, as compared to...
those under the ambient and enhanced radiation (Germ
2004). Modest amount of UV-B radiation may stimulate the
synthesis of rutin, however, buckwheat plants may be dam-
aged by higher doses (Gao et al. 2002; Kreft et al. 2002).

Temperature

The “accumulation of anthocyanin is more a function of
temperature than of light”. Furthermore, anthocyanin content
seems to be sensitive to diurnal differences in tem-
perature, being higher in the presence of colder nights with
respect to constant (high) temperature (Downey et al. 2006).
More rutin is synthesized at higher average or day-night
temperature (24.5°C daytime, and 18°C at night) in compar-
tion to lower temperature (18°C daytime, and 12°C at
night) (Schneider et al. 1996). Effect of temperature during the
growth of strawberry on antioxidant capacity in the plant
was studied by Wang and Zheng (2001), and it was found
that high temperature conditions significantly en-
hanced the content of p-coumaroylglucose, dihydroflavonol,
quercetin 3-glucoside, quercetin 3-glucuronide, kaempferol
3-glucoside, kaempferol 3-glucuronide, cyanidin 3-gluco-
side, pelargonidin 3-glucoside, pelargonidin 3-rutinoside,
cyani din 3-glucoside-succinate, and pelargonidin 3-gluco-
side-succinate in strawberry juice. Plants grown in the cool
day and cool night temperature (18/12°C) generally had the
lowest phenolic acid, flavonols, and anthocyanins. An in-
crease in night temperature from 12 to 22°C, with the day
temperature kept constant at 25°C, resulted in a significant
increase in phenolic acid, flavonols, and anthocyanins.
These conditions also resulted in a significant increase in the
antioxidant capacity. The effects of temperature on rutin content
of seedling, leaf, and seed of buckwheat were studied by Chen and Wen (2005) in a growth chamber. The
results showed that rutin content of the seedling was sig-
nificantly higher than that of the seed, and it decreased with the
increasing temperature. The rutin content of leaf and seed increased with the increasing temperature during the
grain-filling stage.

As an essential factor for plant growth and metabolism, sugars are not only energy sources and structural compo-
nents, but also are physiologic signals regulating the expres-
sion of a variety of genes involved both in primary and
secondary metabolism (Koch 1996). Higher level of sugars
 – the first product of photosynthesis (and light) – could be
the main reason for increased ANS in some plants exposed
to light (Weiss et al. 1995; Vinterhalter et al. 2007; Horbo-
wicz et al. 2008; Li et al. 2011). Boss et al. (1996) reported
that the expression of 7 genes involved in anthocyanin bio-
synthesis was enhanced during the development of berry
skins in V vinifera under the influence of sugars.

Influence of sucrose on rutin content and flavonoid bio-
synthetic gene expression in seedlings of common buck-
wheat was studied by Li et al. (2011) The growth of buck-
wheat was inhibited when the concentration of sucrose was
increased to 50 g/L in seedlings; however, the expression of
most flavonoid biosynthetic genes was increased after 1 or
2 days of treatment and rutin content showed a marked in-
crease when the concentration of sucrose was increased from
10 g/L to 50 g/L. Series of genes, namely, FeC4H, FeCHS,
FeF3H, FeF3L1, FeFLS2 and FeANS was up-
regulated during sucrose treatment (Li et al. 2011).

Fertilizers and water stress

Rutin content and the PAL activity were higher with in-
creasing amounts of nitrogen application in grapevine. The
highest values were detected at a nitrogen fertilizer level of
225 kg/ha. The rutin content and the PAL activity in the
NPK combined application treatment were higher than in
the NP, NK, PK. The changing trends in rutin contents were
rather similar to PAL activity, which indicates that the rutin
content was closely correlated with the PAL activity in
buckwheat (China papers 2010). Flavonoid accumulation
was induced by exposing plants for one week to nitrogen
depletion at 10°C, giving high levels of anthocyanins and
the 3-glucoside-7-rhamnoses, the 3,7-di-rhamnoses, and
the 3-rutinoside-7-rhamnoses of kaempferol and quercetin
in buckwheat (Horbowicz et al. 2008).

Effects of water stress on flavonoids, rutin and querc-
etin content of seedlings of tartary buckwheat were studied by
Na et al. (2008). The results showed that the quercetin content
was very low in buckwheat seedlings. The rutin and
flavonoid content of the seedling increased under water
stress. Anthocyanin accumulation is also modified by the
water status of the plant. During water stress, the synthesis of
anthocyanins is paralleled by an increase of the expres-
sion of flavonoid transporter(s) (Braidot et al. 2008).

TRANSCRIPTIONAL REGULATION OF
FLAVONOID BIOSYNTHESIS

Several transcription factors involved in the regulation of
metabolic pathway genes have been isolated and studied.
There are indications that transcription factor activity itself
is regulated by internal or external signals leading to con-
trolled responses. Regulators belonging to different trans-
cription factor families, including WD40 (beta-transducin
repeat), WRKY, basic-leucine zipper (bZIP), MADS-box,
R2R3-MYB and basic helix-loop-helix (bHLH) factors, are
involved in the transcriptional control of flavonoid biosyn-
thesis genes (FBGs) and are reviewed in the work of Ram-
say and Glover (2005).

The transcriptional control of flavonoid biosynthesis has
been intensively studied (Broun 2005), and several
classes of transcriptional regulators have been identified.
The first group includes four MYB (myeloblastosis) pro-
teins, production of anthocyanin pigments PAP1, PAP2,
MYB113, and MYB114. Overexpression of any one of
these, results in an increase of anthocyanin accumulation
(Borevitz et al. 2000; Gonzalez et al. 2008). The second
group encodes three redundant basic helix-loop-helix
(bHLH) factors, transient testa-8 (TT8), glabrous-3
(GL3), and enhancer of glabra-3 (EGL3), with their simul-
taneous inactivation causing anthocyanin deficiency (Zhang
et al. 2003). MYB and bHLH proteins combine with the
WD40 repeat-containing protein transient testa glabra 1
(TTG1) to form a transcriptional complex that activates
anthocyanin biosynthetic genes, including anthocyanidin
synthase (ANS), DFR, F3’H, leucoanthocyanidin dioxy-
genase, UDP-glucosyl transferase 78d2 (UGT78D2), and
UDP-glucosyl transferase 75c1 (UGT75C1) (Gonzalez et al.
2008). Three other closely related MYB proteins, MYB11,
MYB12, and MYB111, regulate early steps of the flavonoid
pathway, including those catalyzed by the enzymes encoded
by CHS, CHI, F3’H, and FLS1 (Stracke et al. 2007). In
addition, MYB12, an R3-MYB–related protein, acts as a
represser by interfering with the formation of the MYB-
bHLH-WD40 complex (Dubos et al. 2008; Matsui et al.
2008). It has been reported that the transcription factor genes MYB75/PAP1and PAP2/MYB90 play an es-
sential role in the sucrose-induced anthocyanin biosynthesis path-
way (Lloyd and Zakhleniuk 2004; Tao et al. 2005). It is
possible that such regulatory genes have a close relationship
and contribute to the increase in rutin synthesis (Li et al.
2011).

The fine regulation of flavonoid biosynthesis is achieved by combinatorial action(s) of transcription factors,
expressed in a spatially and temporally controlled. MYB
factor PAP1, but not PAP2, strongly stimulates the expres-
sion of the anthocyanin structural gene encoding flavonol
reductase, but neither factor affected the expres-
sion of the early flavonoid biosynthesis gene encoding
chalcone synthase. All bHLH genes (TT8, EGL3 and
GL3) showed light induction, and in seedlings their expres-
sion preceded that of the late structural genes, suggesting
their possible role in light regulation of these structural
genes. The first functional characterization of a light-indu-
cible MYB transcription factor controlling flavonol synthe-
sis was studied in developing grape berries. Flavonol-spe-
Specific MYB transcription factor VvMYBF1 activates promoters of the flavonoid pathway genes VvCHI, VvCHS and VvFLS1 required for flavonol synthesis in fruits (grapevine) (Stefan et al. 2009). Arabidopsis HY5 encodes a bZIP factor that is a key positive regulator of light signaling during plant development, and that regulates numerous genes during photomorphogenesis, including AtCHS, AtFLS and AtMYB2 (Lee et al. 2007). ANAC078 protein is associated with the induction of genes related to flavonoid biosynthesis (the transcript levels of PAP, AtMYB4, CHI, F3H and EGL3 was higher), leading to the accumulation of anthocyanins, in response to HL stress. In Arabidopsis, members of the AP2/ERF (apetala2/ethylene response factor), bZIP, NAC, (homeodomain and leucine zipper) HD-ZIP, and MYB/MYC families, as well as several classes of zinc finger domain proteins, are induced by cytokinins in Arabidopsis seedlings (Yang et al. 2004). The Arabidopsis NFTY5 (Nuclear transcription factor Y subunit A-5) transcription factor is regulated transcriptionally and posttranscriptionally to promote drought resistance (Wen-Xue et al. 2008), and related transcription factor, NFTY1, was reported to confer drought tolerance not only in Arabidopsis but also in maize, when overexpressed. The utility of NFTY1 (Nuclear transcription factor-Y beta) overexpression is in stabilizing crop yield under drought conditions (Nelson et al. 2007).

CONCLUSION

The biosynthesis and accumulation of flavonoids in different growth stages of Fagopyrum species along with the expression of flavonoid biosynthetic genes, would help in understanding the physiological and molecular dissection of expression of flavonoid biosynthetic genes, which is a key positive regulator of light signaling during plant development, and that regulates numerous genes during photomorphogenesis, including AtCHS, AtFLS and AtMYB2 (Lee et al. 2007). The Arabidopsis NFTY5 (Nuclear transcription factor Y subunit A-5) transcription factor is regulated transcriptionally and posttranscriptionally to promote drought resistance (Wen-Xue et al. 2008), and related transcription factor, NFTY1, was reported to confer drought tolerance not only in Arabidopsis but also in maize, when overexpressed. The utility of NFTY1 (Nuclear transcription factor-Y beta) overexpression is in stabilizing crop yield under drought conditions (Nelson et al. 2007).

REFERENCES


Bio synthesis and accumulation of flavonoids in Fagopyrum spp. Panwar et al.


Frisch H, Griesbach H (1975) Biosynthesis of cyanidin in cell cultures of Haplopappus gracilis. Phytochemistry 14, 2437-2442


Haplopappus gracillus. Agronomy Journal 94, 89-100


Miean KH, Mohamed S (2001) Flavonoid (myricetin, quercetin, kaempferol, luteolin, and apigenin) content of edible tropical plants. The European Journal of Plant Science and Biotechnology 6 (Special Issue 2), 17-25 ©2012 Global Science Books
transduction, and gene expression involved in anthocyanin biosynthesis. Critical Reviews in Plant Sciences 15, 525-557
Murphy A, Peer WA, Taiz L (2000) Regulation of auxin transport by amino
peptidases and endogenous flavonoids. Planta 211, 315-324
Na C, Rong D, Peng C (2008) Effects of water stress on flavonoid content of
tartary buckwheat seedlings. Acta Agriculturae Bororlai-Occidentalis Sinica
Nelson DE, Repetti PP, Adams TR, Warner DC, Ans-
noff R, Cardelli M, Caragianides PP, Vola CA, Cifuentes MG, Hinchee BS,
mechanism against excess light in the resurrection plants Craterostigma
wilmsii and Xerophyta viscosa. Plant Cell Environ 20, 241-248
of gene expression in the drought and cold stress responses. Current Opinion
in Plant Biology. 6, 410-417
molecular analysis of structural genes involved in flavonoids and stilbene
biosynthesis in grapes (Vitis Vinifera L.). Plant Molecular Biology 24, 743-
755
Stevic B (1993) Antimutagens and anticarcinogens in foods. Food and Chemic-
tal Toxicology 31, 79-90
Steadman KJ, Burgon MS, Lewis BA, Edsoson SE, Obendorf RL (2001)
Minerals, phytic acid, tannin and rutin in buckwheat seed milling
fractions. Journal of the Science of Food and Agriculture 81, 1091-1094
Stevens JF, Page JE (2004) Xanthohumol and related prenyllflavonoids from
hop and beer. To your good health. Phytochemistry 65, 1317-1330
Stracke R, Ishihara H, Hugd B, Arch R, Mehrtsens F, Niehaus K, Weis-
shaar B (2007) Differential regulation of closely related R2R3-MYB tran-
scription factors controls flavonol accumulation in different parts of the Arabi-
dopsis thaliana seedling. Plant Journal 50, 601-617
Sure B (1985) Nutrative value of proteins in buckwheat and their role as
supplements to proteins in cereal grains. Journal of Agriculture and Food
Chemistry 3, 793-795
Suzuki T, Honda Y, Mukasa Y (2005a) Effects of UV-B radiation, cold
and desiccation stress on rutin concentration and rutin glucosidase activity in
tartary buckwheat (Fagopyrum tataricum) leaves. Journal of Plant Science 168,
1303-1307
Taylor JRN, Belton PS (2002) Pseudoexcretes and Less Common Cereals,
Springer, 269 pp
Taylor LP, Briggs WR (1990) Genetic regulation and photocoction of antho-
cyanin accumulation in maize seedlings. Plant Cell 2, 115-127
Teng S, Keurentjes J, Beuissl AK, Koornneef M, Smeekens S (2005) Suc-
rrose-specificity of the anthocyanin biosynthesis in Arabidopsis requires the
MYB/B5/PAP1 gene. Plant Physiology 139, 1840-1852
Tomokane H, Shimaoka I, Kayashita J, Yokoyama F, Nakajoh M, Kato N
(2005) A buckwheat protein suppresses gallstone formation and plasma
colesterol more strongly than soy protein isolate in hamsters. Journal
of Nutrition 135, 1670-1674
ten expression of the CHS-A gene for chalcone synthase from petunia in
transgenic Arabidopsis. Plant Physiology 97, 1414-1421
Tuan PA, Park NI, Li X, Xu R, Park SU (2007) Carbohydrate and charac-
terization of phenylalanine, ammonia-lyase and cinnamate 4-
hydroxylase in the phenylpropanoid biosynthesis pathway in garlic (Allium
saizum). Journal of Agricultural and Food Chemistry 55 (20), 10911-10917
Vinterhalter B, Ninkovici S, Kozomara B, Vinterhalter D (2007) Carbohydr-
ate nutrition and anthocyanin accumulation in light grown and etiolated
shoots of carobs (Ceratonia siliqua L.). Archives of Biological Sciences
59, 51-56
Vom Endt D, Kijne JW, Memelink J (2002) Transcription factors controlling
plant secondary metabolism: What regulates the regulators? Phytochemis-
try 61, 107-114
Wang J, Liu Z, Fu X, Run M (1992) A clinical observation on the hypogly-
cemic effect of Xinjiang buckwheat in. In: Proceedings of 5th International Sym-
opsum on Buckwheat, Taiyuan, pp 465-467
capacity in strawberry. Journal of Agriculture and Food Chemistry 49 (10),
4977-4982
Watanabe M (2007) An anthocyanin compound in buckwheat sprouts and
its contribution to antioxidant capacity. Bioscience, Biotechnology and Bio-
chemistry 71, 579-582
Watanabe M, Ayagae J (2008) Anti-stress effects of flavonoids from buck-
wheat sprouts in mice subjected to restraint stress. Food Science and Tech-

Bioaccumulation and accumulation of flavonoids in Fagopyrum spp. Powaret et al.


Winkel-Shirley B (1998) Flavonoids in seeds and grains: Physiological function, agronomic importance and the genetics of biosynthesis. *Seed Science Research* 8, 415-422


