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Possible Roles of Rutin in Buckwheat Plant

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ABSTRACT

Buckwheat contains rutin, a kind of flavonol, not only in the seeds but also in the cotyledon, leaf, stem and flower. To date, several reports have been published on the physiological roles of rutin. In this review, mainly based on our recent results, we summarize the main aspects of rutin related to its possible physiological roles. The enzymes, which catalyzed a part of rutin synthesis (quercetin glycosyl transferase) and decomposition (rutinosidase), have lower K_m especially for quercetin/UDP-glucose and rutin respectively compared to other similar compounds. This indicates that buckwheat developed glycosyl transferase and rutinosidase suit for rutin metabolism. The time course studies for rutin accumulation at seed ripening and leaf/cotyledon expansion show that rutin accumulation pattern is different for each organ. In seeds, rutin content per seed increases along with seed development, and mature seed contains the largest rutin concentration. In leaves, rutin content also increased along with development whereas senescent leaves contain little amount of rutin. In the cotyledon and mature leaves, rutin is distributed in the epidermis, and large amount of rutinosidase activity is present on surface of cotyledon during cotyledon expansion. In addition, rutin concentration and rutinosidase activity was increased by some stresses (UV-B radiation, cold and moisture stress) in leaves. Quercetin, the hydrolyzed moiety of rutin, possesses high antioxidant activity and the ability to be a precursor of a kind of anti fungal agents. Therefore, rutin and rutinosidase are suggested to be related to the enhancement of the defense system against stress conditions in buckwheat.

Keywords: glycosidase, quercetin, rutinose, rutinosidase, stress, Tartary buckwheat, UV
Abbreviations: 3GT, flavonoid 3-O-glucosyltransferases; **DAG**, days after germination; **f3g**, flavonol 3-glycosidase; **DAP**, days after pollination; **HPLC**, high performance liquid chromatography; **RDE**, rutin-degrading enzyme; **UDP**, uridine-5'-diphosphate; **UDP-Glc**, uridine-5'-diphospho-glucose

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INTRODUCTION

Buckwheat (*Fagopyrum esculentum* Moench) is a famous crop grown in some regions of the world, and is a useful and unique crop (Ikeda 2002; Kreft *et al*. 2003). In Japan, the focus on buckwheat is not only for its use as a traditional food, but also as a healthy food, because it contains useful compounds such as rutin. Rutin, a kind of flavonoid, exhibits beneficial effects on fragile human capillaries (Griffith *et al*. 1944; Shanno *et al*. 1946), and also shows antioxidative (Afanas'ev *et al.* 1989; Afanas'ev *et al.* 2001; Jiang *et al.* 2007; Awatsuhara *et al.* 2010), antihypertensive (Matsubara *et al.* 1985), anti-inflammatory activities (Afanas'ev *et al.* 2001) and α-glucosidase inhibitory activity (Li *et al.* 2009). Rutin is widely distributed in the plant kingdom (Sando and Lloyd 1924; Couch *et al.* 1946; Haley and Basin 1951; Bandyuko and Sergeeva 1974; Fabjan *et al.* 2003). Among them, buckwheat is the only known cereal to contain rutin in its seeds. Therefore, buckwheat has been utilized as a rutin-rich material for food (Kreft *et al.* 2006). Buckwheat contains rutin not only in the seed, but also in the cotyledon, leaves, stem and flower (Kalinova and Dadakova 2006). Rutin concentrations are surprisingly high, sometimes several percent on a dry weight bases. To date, the physiological functions of UV-B screening, antioxidant activity and disease resistance have been attributed to plant flavonoids (Harborne and Williams 2000). Similarly to the role of flavonoids in many plants, some papers have shown

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possible roles of rutin in buckwheat. To understand the physiological roles of rutin in buckwheat, it is important to investigate the accumulation patterns and tissue specific distribution of rutin during plant development, particularly at seed ripening, germination and vegetative growth. In addition, the enzymes involved in rutin metabolism are also important. In buckwheat, there are two major cultivated species, viz. common buckwheat (*Fagopyrum esculentum* Moench), and Tartary buckwheat (*Fagopyrum tataricum* Gaertn.). Both cultivated species contain large amount of rutin in the cotyledon and the leaves. However, seeds of Tartary buckwheat contain about 100 times larger amount of rutin than common buckwheat. Tartary buckwheat seed also presents higher level of rutinosidase activity than common buckwheat (Yasuda *et al.* 1992; Yasuda and Nakagawa 1994). These characteristics are suitable to elucidate physiological roles of rutin in buckwheat seed.

In this review, mainly based on our recent results, we summarize the main aspects of rutin related to its possible physiological roles. The review is divided into two major parts. The first part covers purification of rutin and characterization of rutin synthesis and decomposition in common and Tartary buckwheat. The second part covers concentration of rutin and related compounds in particular organs (cotyledons, leaves and seed) at different developmental stages of common and Tartary buckwheat. In addition, this part discusses changes in leaf rutin concentration and rutinosidase activity when the leaf was subjected to stress.

ENZYMES OF RUTIN DECOMPOSITION AND SYNTHESIS

Decomposition

To investigate physiological role of rutin, it is important to characterize enzymes, which catalyze rutin decomposition. Rutinosidase is a kind of flavonol 3-glycosidase (f3g), which catalyzes the hydrolysis of the 3-glycoside of flavonols such as rutin or isoquercitrin, speculated to be the precursor of rutin (Barber 1963; Barber and Behrman 1991) (**Fig. 1**). Rutinosidase activity has been found in a number of plants (Suzuki 1962; Yasuda and Nakagawa 1994; Suzuki *et al.* 2002; Baumgertel *et al.* 2003) and microorganisms (Hendson *et al.* 1992; Narikawa *et al.* 2000).

To date, several reports have been published on the methods to detect rutinosidase activity in buckwheat (Yasuda and Nakagawa 1994; Morishita *et al.* 1998; Suzuki *et al.* 2002; Baumgertel *et al.* 2003; Yang and Ren 2008; Chen and Gu 2011). Among them, HPLC method has been used in many reports. In addition, several methods have been reported about rutin evaluation (Brejcha and Horak 1958; Kreft *et al.* 1999; Danila *et al.* 2007; Stockova *et al.* 2009; Dadakova and Kalinova 2010), and HPLC methods have been also employed in many reports. A rutin-degrading enzyme, (RDE) has been purified from Tartary buckwheat seeds as the first report on buckwheat rutinosidase (Yasuda and Nakagawa 1994). In 2002, as the second report, f3g was also purified from Tartary buckwheat seeds (Suzuki *et al.* 2002). These enzymes have very similar characteristics except for molecular weight and kinetic properties. Both RDEs and f3g consisted of at least two major isozymes. Purification procedure and main characteristics of f3g are as follows. Two distinct enzymes of the f3g were purified to homogeneity from Tartary buckwheat seeds using ammonium-sulfate precipitation, ion-exchange chromatography and gel-filtration chromatography. Molecular weight of the isozymes was 58,200 (f3gI) and 57,400 (f3gII) on SDS-PAGE, and 89,000 for both isozymes on gel filtration. For both isozymes, rutin 3-glycosidase activity and isoquercitrin 3-glycosidase activities were optimal at pH 5.0, and 40°C. The kinetic constants, and V_{max}, with rutin and isoquercitrin as substrates were also similar in both isozymes. The optimal pH, optimum temperature and number of isozymes were very similar to that of RDEs (Yasuda and Nakagawa 1994). However, the molecular weight (RDEs:

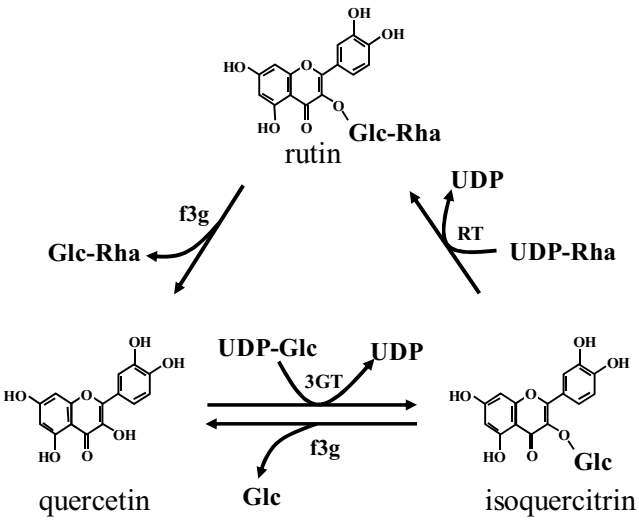

Fig. 1 Catalysis of rutin biosynthesis and decomposition. f3g: flavonol 3-glycosidase, 3GT: flavonol 3-*O*-glycosyltransferase, RT: rhamnosyltransferase.

68,000 on SDS-PAGE and 70,000 by gel filtration) and kinetic constants (RDEs: K_m for rutin were 130 mM and 120 mM) were quite different. The amino acid sequences of the amino terminus of both f3g isozymes were identical within the range of residues analyzed (15 resides for f3gI and 10 for f3g II). These sequences shared identity with other glycosidases such as cyanogenic-beta-glucosidase (*Trifolium repens* L.), thioglucosidase [*Arabidopsis thaliana* (L.) Heynh.]. The f3g can catalyze not only rutin (K_m for rutin is about 0.12mM, and V_{max} is about 620 nkat mg protein^{-1}), but also isoquercitrin 3-glycosidase activity (K_m for isoquercitrin is about 1.1 mM, and V_{max} is about 67 nkat mg protein^{-1}), though the V_{max} value for isoquercitrin is one-tenth of that for rutin. Isoquercitrin was speculated to be the precursor of rutin (Suzuki 1962; Barber and Behrman 1991). The result that the f3g catalyzes hydrolysis of both rutin and isoquercitrin may relate to catalytic control of rutin levels in the buckwheat plant. It has been shown that a rutin complex with transition metals scavenges free radicals *in vitro* with great efficiency (Afanas'ev *et al.* 2001). The fact that f3g activity was strongly inhibited by copper ions is consistent with the objective of preserving the efficacy of the rutin-copper complex. In Tartary buckwheat seeds, rutin levels of about 15% (w/w) were detected in the portion of the embryo that will develop into the future cotyledons (Suzuki *et al.* 2002). Therefore, it may become a source of carbohydrates during germination. The stability of rutin against oxidative degradation was much greater than that of its aglycone quercetin (Afanas'ev *et al.* 1989). Therefore, f3g may play a role in controlling the amount of carbohydrate source, which, during germination, generates quercetin and the hydrolyzed moiety rutinose from rutin. Another possible role of rutin is the anti-fungal activity of its aglycone quercetin. It was reported that an anti-fungal agent, 3,4-dihydroxybenzoic acid, is formed by peroxidase-dependent oxidation of quercetin on browning onion scales (Takahama and Hirota 2000). Buckwheat also contained peroxidase activity (Kondo *et al.* 1982; Suzuki *et al.* 2005c, 2009). Therefore, in buckwheat seeds, the f3g might catalyze the first step in the generation of an anti-fungal agent during germination.

Synthesis

Barber (1963, 1991) proposed that rutin was synthesized via the 3-*O*-glycosylation of quercetin followed by the rhamnosylation of isoquercitrin (**Fig. 1**). The glucosylation of a flavonoid is catalyzed by flavonoid glucosyltransferases. Among these enzymes, UDP-Glc: flavonoid 3-*O*-glucosyltransferases (3GT) have been studied in maize (*Zea mays*

L.) (Futtek *et al.* 1988), snapdragon (*Antirrhinum majus* L.) (Fukuchi-Mizutani *et al.* 2003), barley (*Hordeum vulgare* L.) (Wise *et al.* 1990), grape (*Vitis vinifera* L.) (Ford *et al.* 1998) and gentian (*Gentiana triflora* Pall.) (Tanaka *et al.* 1996). Characterization of buckwheat 3GT is important because it catalyzes the glycosylation of quercetin as the first step in rutin biosynthesis using UDP-glucose as a glucose donor (**Fig. 1**). Buckwheat 3GT was purified and characterized only in cotyledon. It was purified to homogeneity from cotyledons of buckwheat seedlings, resulting in a 171-fold purification and final specific activity of 1.46 pkat per mg protein (Suzuki *et al.* 2005a). The molecular weight of 3GT was 58,600 on SDS-PAGE and 56,000 by gel filtration, suggesting that buckwheat 3GT is a monomeric enzyme similar to the other 3GTs. The optimal pH of the buckwheat 3GT was around pH 7.0, which was very close to that of other 3GTs, such as grape (Ford *et al.* 1998). Substrate specificity about sugar acceptor of the 3GT varied among flavonoids. The lowest K_m was detected for quercetin about 27 μM. Buckwheat had an at least 6-fold greater specificity for quercetin than kaempferol, apigenin, luteolin, or naringenin. This is a unique result in as much as the 3GT of grape (Ford *et al.* 1998) had a high affinity not only for quercetin but also for other flavonols such as kaempferol. For sugar donors, the 3GT had a lower K_m for UDP-Glc (1.04 mM) than other sugar donors. These K_m values were very close to those of grape 3GT (15 μM for quercetin and 1.04 mM for UDP-Glc; (Ford *et al.* 1998). Barber (1963) reported that TDP-Glc as well as UDP-Glc were good sugar donors in mung bean (*Phaseolus aureus* Roxb.) when quercetin was used as a sugar acceptor. In this study, however, TDP-Glc certainly played a role as a sugar donor, but the K_m value was much higher than that of UDP-Glc.

The 3GT from buckwheat cotyledons could use UDP-Glc as a sugar donor. An HPLC chromatogram of buckwheat cotyledons showed several unidentified peaks of polyphenolic compounds (Watanabe and Ito 2002; Kim *et al.* 2004), therefore, it is necessary to investigate not only galactosyltransferase activity but also its products. Watanabe and Ito (2002) reported that buckwheat sprouts contained apigenin-8-C-glucoside, apigenin-6-C-glucoside, luteolin-8-C-glucoside and luteolin-6-C-glucoside. These compounds were synthesized just after germination, and then the amounts gradually decreased. This is in contrast to the increase in the rutin concentration. It will be necessary to further investigate the mechanisms of rutin synthesis, including a determination of the characteristics of rhamnosyltransferase (RT) activity, which catalyzes the final step in rutin synthesis. An understanding of the relationship between the expression of 3GT and RT activity in controlling rutin biosynthesis is also needed.

ENZYMES IN PLANT ORGANS

To further investigate the physiological role of rutin in buckwheat, it was necessary to investigate rutin accumulation patterns and tissue specific distribution of rutin during buckwheat development. In addition, information about enzymes which relate to rutin synthesis and decomposition is also important. Some papers have investigated these questions at the developmental stages of germination (cotyledon), leaf expansion and seed ripening in common and Tartary buckwheat.

Cotyledon

The initial focus was on buckwheat seedlings. Changes in rutin concentration during the growth of common buckwheat seedlings have been the subject of previous investigations (Troyer 1955; Watanabe and Ito 2002; Kim *et al.* 2004; Kim *et al.* 2006, 2007; Krahl *et al.* 2008). During these studies, flavonoids other than rutin, such as orientine and isovitexine, were identified. After that, Suzuki *et al.* (2007) assessed changes in the concentrations of rutin and related compounds along with f3g and 3GT activity during seedling growth of common and Tartary buckwheat. In common buckwheat, the rutin concentration increased from 1 to 5 DAG (days after germination). From 3 to 5 DAG, the rutin concentration of cotyledons grown with light was greater than that of those grown without light. The rutin concentration of the mature cotyledon (5 DAG) was very high, roughly 4% of the dry weight. The highest rutin concentration for cotyledons grown with light was observed at 3 DAG, but without light it peaked at 4 DAG, and the peak was retained until 7 DAG with or without light. The 3GT activity increased prior to the increase of rutin and chlorophyll concentrations. It began to increase just after germination, reached a maximum value at 4 DAG and then rapidly decreased to 7 DAG. Quercetin (a sugar acceptor of 3GT) and isoquercitrin (the product of 3GT) concentrations were too low (around the detection limit) to perform a quantitative analysis. More than 50% of the rutin was distributed in the upper epidermis of the cotyledons. However, the 3GT activity was shown to have similar values between the upper epidermis, mesophyll and lower epidermis.

In Tartary buckwheat, the rutin concentration gradually increased from 0 to 12 DAG (Suzuki *et al.* 2007). The mature seed contains a large amount of rutin (about 5% of the dry weight). This indicates that Tartary buckwheat cotyledons contain about 2.5% rutinose in the form of rutinoside, which may be a source of carbohydrate nutrition during germination and subsequent seedling growth. However, rutin concentration in the Tartary buckwheat cotyledon increased on both a dry weight and cotyledonary basis, indicating that it was unlikely that the rutin in the Tartary buckwheat cotyledon was consumed as a source of carbohydrates for nutrition during germination and cotyledon growth. Rutin was shown to be a deterrent of larval feeding of some kinds of insects (Simmonds 2003). Rutin concentrations in mature cotyledons were very high, about 4% of the dry weight. Therefore, the rutin in cotyledons may have a role in preventing damage from insects.

Several studies have shown that rutin functions as a UV screen under light (Margna *et al.* 1990; Suzuki *et al.* 2002, 2005b). However, in this study the rutin concentration as well as 3GT activity was high even in buckwheat cotyledons that were grown in darkness and thus were not exposed to UV light. This suggests that rutin in buckwheat cotyledons may have other roles in addition to UV screening, such as the enhancement of the defense system against cold or desiccation stress in Tartary buckwheat leaves (Suzuki *et al.* 2005b) as described in the next section.

The f3g activity, whose most part is localized in testa, began to decrease immediately after germination whereas 4DAG testa contained 80% of the f3g activity as compared to 0 DAG. From 0 DAG to 4 DAG, the testa adhered to the cotyledon and husk. From 5 DAG, testa (as well as husk) and cotyledon were easily separated. In 5DAG, the f3g activity was present at a high value (about 25% of that of the testa) on the surface of the cotyledon. The f3g on the surface of the cotyledon should have been exported from the testa because the cotyledon did not contain much f3g compared with the surface of the cotyledon. On the other hand, rutin was distributed mainly in the epidermis of the cotyledon, suggesting that rutin in the epidermis of the cotyledon can be hydrolyzed to quercetin by f3g at the surface of the cotyledon if the cotyledon is injured. In the browning of onion skin, the anti-fungal agent 3,4-dihydroxybenzoic acid is formed by peroxidase-dependent oxidation of quercetin (Takahama and Hirota 2000). The f3g activity on the surface of the cotyledon may play a similar role. From these results, rutin and f3g activity in Tartary buckwheat during cotyledon growth should have different physiological roles from those in common buckwheat.

Leaf

Buckwheat leaves contain rutin at a level similar to that obtained in the cotyledon. In other plants, flavonoids were mainly located in the epidermis of the leaves (Harborne and

Williams 2000). Some papers have described rutin accumulation in common and Tartary buckwheat leaf (Zhanaeva 1996; Kitabayashi *et al.* 1995a, 1995b). After that, Suzuki *et al.* (2005b) reported on rutin concentration, f3g activity and 3GT activity in Tartary buckwheat leaves at different leaf positions. Rutin concentration per leaf reached a maximum in the L7 leaf (L1 = cotyledon, L2 = senescent leaf, L3-L6 = mature leaf, L7 and L8 = young leaf), and then decreased with decreasing leaf number, until it was finally almost zero in the senescent leaf. The rutin concentration on a dry weight basis was highest in the L8 leaf (>20% of the dry weight), which is the youngest leaf, and then gradually decreased with a decrease in leaf number. These results show good agreement with the report of Zhanaeva (1996) on common buckwheat leaves. The f3g activity on a dry weight basis was higher in young leaves, reached a maximum in the L6 leaf, and then gradually decreased. The 3GT activity showed a pattern similar to that of rutin concentration, suggesting that 3GT plays a role in rutin synthesis. Rutin was mainly located in the epidermis of Tartary buckwheat leaves. This concurs in reports of Zhanaeva (1996) on common buckwheat leaves and reports of Harborne and Williams (2000) on leaves of other plant species. More than half of rutin in the leaf was located in the upper epidermis, which reinforces the idea that rutin in Tartary buckwheat leaf plays a role in UV screening. On the other hand, most f3g activity was located in the lower epidermis.

As described above, rutin is synthesized in the young leaves. Rutin could play important roles in young leaves, including UV screening. Long-term exposure to UV-B radiation has a significant negative effect on buckwheat growth (Gaberscik *et al.* 2002). Therefore, UV screening is very important for buckwheat plants. Lignin and wax can also absorb UV radiation. However, rutin should be a suitable UV absorbing compound especially in the young, expanding leaf. Leaves of Tartary buckwheat expand very quickly, generally within a few days, and the shape of the leaves changes dramatically. In such leaves, lignin or wax would physically prevent the leaf from expanding whereas rutin would not. After full expansion, the Tartary buckwheat leaf becomes hard and glossy, which indicates production of lignin and wax, which reinforces this idea.

1. Effects of stress on rutin and related enzymes in the leaf

Several studies have investigated the physiological roles of rutin in buckwheat (Kreft *et al.* 2002; Suzuki *et al.* 2002, 2005b; Yao *et al.* 2006; Suzuki *et al.* 2007). An increased level of ambient UV radiation sometimes results in negative effects on buckwheat growth (Yao *et al.* 2006; Mateja and Barbara 2007; Ozbolt *et al.* 2008) whereas sometimes results in an increase of leaf rutin concentration. Kreft *et al.* (2002) showed that after long-term UV-B radiation, a reduced UV-B radiation level produced a lower rutin concentration in common buckwheat leaves and flowers than did an ambient UV-B level. Yao *et al.* (2006) showed that leaf rutin concentration was increased by supplemental UV-B radiation in the field test. These facts, similarly to results described in previous sections, reinforce the idea that rutin serves as a UV screen.

Suzuki *et al.* (2005b) suggest another possible role of rutin as UV screen. They performed stress treatments on Tartary buckwheat leaves. They followed a special experimental protocol (**Fig. 2**) to decrease experimental error due to differences of growing stage or individual plant characteristics. At 28 DAG, an L7 leaf of field-grown Tartary buckwheat was harvested. By using the same leaf for stress treatment and for the corresponding control, they minimized variability. Rutin concentration and f3g activity did not change immediately after stress treatments had been applied. The rutin concentration was significantly increased by UV-B radiation or desiccation treatment compared with the untreated control. On the other hand, the rutin concentration was not significantly altered by cold treatment. There are many reports that flavonoids function as a UV

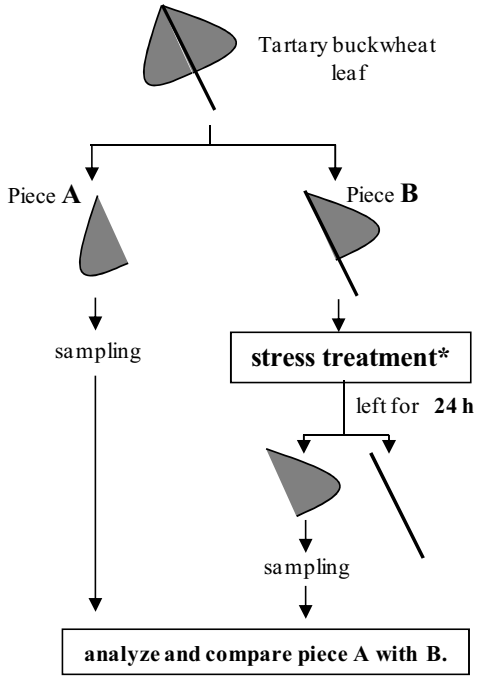

Fig. 2 Flowchart of environmental stress treatments. UV-B radiation: The leaf was treated with UV-B light (radiation peak, λ = 302 nm; duration, 30 min; intensity, 12.6 mW m^{-2}; total energy supplied 3.4 K Joules per m^2. Desiccation: The leaf was left at 22°C for 30 min without a water supply. Exposure to cold temperature: The leaf was exposed to –5°C for 5 min, then immediately returned to room temperature. As a control, an untreated leaf was used. Then, the treated leaf was left for 24 h at 22°C with adequate water. The rutin concentration and f3g activity were measured after removal of the vein, and the results of piece A and B were compared (value of piece A of each sample = 100).

screen (Harborne and Williams 2000). The increased rutin concentration after UV-B radiation was consistent with these observations. In common buckwheat, long-term UV-B radiation results in an increase in UV-B absorbing compounds (Kreft *et al.* 2002). Rutin concentration is affected by humidity and irrigation, with the highest rutin content found in the driest conditions (Ghouzbdi *et al.* 2009) Therefore, rutin may play a role in the protection against desiccation. The f3g activity increased significantly in all stress treatments: 363% under UV-B radiation, 190% under cold treatment and 158% under desiccation, compared to the control (Suzuki *et al.* 2005b). After UV-B radiation, both rutin concentration and f3g activity increased concurrently. This is the first demonstration that rutinosidase activity is increased in stress conditions. The f3g activity results in an increase of quercetin and rutinose concentrations. One of the possible roles of f3g activity may be to supply quercetin as a substrate of peroxidase. In stress conditions, peroxidase plays an important role in the defense of plants against oxidative damage (Bradley *et al.* 1992; Kolattukudy *et al.* 1992). Because quercetin is a suitable substrate for guaiacol peroxidase (Amako *et al.* 1994), quercetin, which was produced from rutin, may be used as a substrate of guaiacol peroxidase in the defense of the Tartary buckwheat leaf. This reinforces the concept that the stability of rutin against oxidative degradation is much greater than its aglycone quercetin (Afanas'ev *et al.* 1989). Another possible role of f3g activity is to supply carbohydrate substrates for respiration. In stress conditions, Tartary buckwheat leaves may be deficient in sugar for respiration because of damage to the photosynthetic system or to the mechanism of translocation of carbohydrates from source organs. Such damage would be most serious in young leaves (L8, L7) because they have an undeveloped photosynthetic system. In such conditions, rutinose produced by f3g activity may be used as a substrate of respiration. This idea is reinforced by the fact that young

leaves (L8, L7) contain more than 15% w/w rutin. To clarify this hypothesis, it would be necessary to investigate changes in organ and tissue distributions of rutin concentration, quercetin concentration and f3g activity in stress-treated leaves.

Seed

Several reports have investigated rutin concentration and f3g activity in fully ripe seeds of common and Tartary buckwheat (Yasuda and Nakagawa 1994; Kitabayashi *et al.* 1995a, 1995b; Ohsawa and Tsutsumi 1995; Morishita and Tetsuka 2002; Suzuki *et al.* 2002; Végvári *et al.* 2008). Seed rutin concentration of Tartary buckwheat was ranging from 1,100 to 1,950 mg/100 g DW, whereas the level in common buckwheat was two orders magnitude less than that. Rutin concentrations in Tartary buckwheat seeds, as well as common buckwheat, increased at the early stage of seed ripening and reached a maximum at 30 DAP (days after pollination) (Ren and Tang 1992; Suzuki *et al.* 2002).

Jiang *et al*. (2007) investigated rutin contents of three buckwheat species such as common buckwheat, Tartary buckwheat, and *F. cymosum*, and concluded that seed rutin plays an important role in the antioxidant activity of buckwheat seed. The rutin 3-glycosidase activity also increased at the early stage of seed ripening, reaching a maximum at 23 DAP and decreasing only slightly at 30 DAP (the fully-ripe seed stage). During seed ripening, both rutin and isoquercitrin concentrations, and f3g and 3GT activities increased in the same seed. The f3g activity in Tartary buckwheat seeds was sufficient to hydrolyze rutin (about 1 to 2% w/w) in the flour within a few minutes after the addition of water. In both Tartary and common buckwheat, rutin was mainly detected in the embryo, and most of the rutin 3-glycosidase activity was located in the testa. Therefore, embryo rutin was separated from rutin 3-glycosidase activity by difference in organ distribution. This result is consistent with report on food utilization of Tartary buckwheat seed (Mukasa *et al.* 2009). In the early stage of seed-development, cells in the embryo divide actively. In such seed, damage to DNA from free radicals or UV light has a significant influence on plant. Rutin may be important in the seeds, especially at the early stage of ripening, as an antioxidant and/or a UV-absorbing compound.

CONCLUSION

Compared to the well-known functions of flavonoids in certain plant species, only limited-information was available from the literature on the physiological functions of rutin in buckwheat. To understand the function of rutin in buckwheat, biochemical and physiological approaches have been adopted using common buckwheat and Tartary buckwheat species in the current study. The kinetic analyses suggest that both f3g and 3GT are involved in the metabolism of rutin in this plant. Organ and tissue localization of rutin and enzyme activities corresponding to its synthesis and decomposition suggest that rutin could be involved in the defense mechanisms against UV radiation. In addition, stress-response in Tartary buckwheat leaves suggests that rutin and f3g are involved in the enhancement of the defense system to different kinds of stress.

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