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# Reference proteome map of buckwheat (*Fagopyrum esculentum* and *Fagopyrum tataricum*) leaf and stem cultured under light or dark

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**Abstract**

Buckwheat sprouts are a vegetable to provide health benefit with their nutritionally important substances. Despite several reports on describing buckwheat as preventive medicine, little has been studied on the developmental physiology of buckwheat in protein level. Thus, we attempted for the first time to examine the proteomic profiles of leaf and stem from 7-day-old etiolated sprouts of common (*Fagopyrum esculentum* Moench.) and tatary buckwheat (*Fagopyrum tataricum* Gaertn.) seedlings under light and dark. By the gel-based proteomic approach, 166 unique proteins were identified and six proteins were commonly found from both buckwheat species in which 79 and 81 proteins were exclusively belonged to common and tatary buckwheat, respectively. The most abundant proteins were assigned to metabolism and cytoplasmic proteins were dominated by the classification of molecular function and intracellular localization. The sprouting leaf of 7-day-old etiolated common buckwheat seedlings showed light yellow under dark, suggesting the inhibition of light-dependent protochlorophyllide reductase leading to the reduction of chlorophyll biosynthesis. Light-inhibited storage protein, 13S globulin 3, was commonly found in the leaves of both buckwheat species under dark. In particular, the unknown pentatricopeptide repeat-containing proteins were identified in light-induced leaf of common and tatary buckwheat and also identified in dark-induced stem of tatary buckwheat. Thus, it suggests that the different and specific regulators are involved in the sterility and fertility from common and tatary buckwheat in a specific light-dependent manner. The reference proteomic map of buckwheat will give insight for understanding buckwheat physiology and application to buckwheat industry.

**Keywords:** Buckwheat sprouts, MALDI-TOF mass spectrometry, Proteomics, Two-dimensional electrophoresis

## Introduction

Buckwheat as a traditional pseudocereal crop has been widely considered as a nutritious and medicinal food source since buckwheat has gained its fame due to the different sorts of flavonoids to various health benefits, i.e. cholesterol reduction (Kayashita, 1997), tumor inhibition (Chan, 2003), hypertension regulation (Ma *et al.* 2006), control of inflammation and carcinogenesis (Ishii *et al.* 2008), and diabetes (Kawa, 2003). Buckwheat are consumed in many countries in different food forms such as noodles, pancakes, buckwheat corn muffins in China, Japan, Korea, Nepal, and some European and Western countries. Tatary buckwheat has attracted attention due to their compounds that are beneficial to health, and used as oriental medicine in China. Tatary buckwheat sprouting has a bitter component than that of common buckwheat. The higher amount of rutin contain in stem and leaf of common and tatary buckwheat, which is promoting the health (Ghimeray *et al.* 2010). The health promoting function of buckwheat is inherently related to the radical scavenging activity during the process of protein digestion. Thus, it is hypothesized that the hydrolysis of buckwheat protein can release the peptide fragments capable of stabilizing radical oxygen species and inhibiting lipid oxidation (Ma *et al.* 2010). The specific peptides or peptide fragments responsible for the antioxidant function were elucidated from *in vitro* digests of buckwheat proteins; however, *in vivo* proteins remain unknown. Recently, Buckwheat sprouts are a vegetable which is considered to be better than other cereals since buckwheat sprouts contain nutritionally important substances with high quantity of useful metabolites such as phenolics, quercetin, ascorbic acid, rutin, and γ-aminobutyric acid (Lin *et al.* 2008). Recently, buckwheat is evaluated as nutrition-improving gluten-free bread, where its nutritional values in terms of protein, fat, fiber and minerals were significantly higher than those of gluten-containing bread (Alvarez *et al.* 2009). Buckwheat is a common allergenic plant similar to allergies caused by soybean and peanuts (Satoh *et al.* 2010). Recently, a great variety of sprouts can be found in market such as broccoli

(*Brassica oleracea* L. var italic Plenck), common buckwheat (*F. esculentum* L. Monech), kale (*B. oleracea* L. var. encephala), mung bean (*Phaseolus aureus* Rob.), red cabbage (*B. oleracea* L. var. capitata f. rubra), and sobeay (*Glycin max* L. Marr.) (Suzuki *et al*. 2009). Tartary buckwheat seed contain high amount of rutin than that of common buckwheat (Suzuki *et al*. 2002). In our experiment, we conducted to cultivation of sprouts under light and dark condition, and try to screening, which is better condition for sprouts cultivation of buckwheat as a functional vegetable. Finally, we used buckwheat sprouts for proteomic analysis to identify the sprouts development responsive proteins, which regulated and responses under light and dark conditions of stem and leaf. Compared to common buckwheat, tatary buckwheat seed contains a very high amount of a rutin-degrading enzyme (Yasuda *et al.* 1994), rutin glucosidase, called flavonol 3-glucosidase (f3g) (Suzuki *et al.* 2002). The f3g catalyzes the hydrolysis of rutin to quercetin. Rutin and f3g in tatary buckwheat seed have physiological roles in seed ripening by screening ultraviolet light and/or by producing an anti-fungal agent (Suzuki *et al.* 2002). Recently, the buckwheat sprout has been reported to possess radical-scavenging activity (Watanabe and Shimizu, 2004). In particular, tatary buckwheat sprout contains higher content of functional molecules such as anthocyanin, rutin and flavonol glycoside compared to common buckwheat, resulting in a lowered heart disease (Kim *et al.* 2007). The biosynthesis of anthocyanin as a prevalent pigment of buckwheat is regulated by light and other internal factors such as hormone, other metabolites and nutrients. However, the investigation of protein activity in common and tatary buckwheat during seedling has not been executed so far. Therefore, we aimed to investigate the proteome profiles of buckwheat sprout under light and dark by gel-based proteomics. Two-dimensional gel electrophoresis (2-DE) is a platform technique of conventional proteomics, in which more than 100 proteins can be differentially visualized on 2-D gel when compared between species and culture conditions (Choi *et al.* 2008; Kamal *et al.* 2009a). In particular, the proteomic technology centered with matrix-assisted laser desorption/ionization mass spectrometry (MALDI-TOF-MS) has rapidly developed and becomes a powerful tool to identify proteins. MALDI-TOF-MS appears to be much more accurate and sensitive than any other MS technique; minimal quantity of less than 1 pmol of protein is sufficient to identify (Kamal *et al.* 2009b). The present study will present the overall sprout proteomic information in common and tatary buckwheat cultured under light and dark. The proteomics-based knowledge of buckwheat will give insights into the understanding of buckwheat sprouting development mechanism in protein level.

## Materials and Methods

### *Growth conditions and sample preparation*

Buckwheat (common buckwheat; *Fagopyrum esculentum* and tatary buckwheat; *Fagopyrum tataricum*) seeds were imbibed under dark for 24 hr and divided into two groups; growth under light and dark for 7 days. Experimental condition of light illumination was given with a normal light regime of 12-h light and 12-h dark at 28°C for 7 days. Light intensity was adjusted to 100 mmol photons/m2 sec. The sprouting leaves and stems were separately dried under freeze drier (PVTFD 30R, IIShin Lab, Korea) after milling with mortar. And then each sample was ground in a mortar with liquid nitrogen and collected to obtain flour (approximately 100 mg). The soluble proteins were obtained by trichloroacetic acid and acetone precipitation method as described previously (Damerval *et al.* 1986; Porubleva *et al.* 2001). The protein amount was determined using Bio-Rad protein assay kit (Bio-Rad, Seoul, Korea) using bovine serum albumin as a standard (Bradford, 1976).

### *Electrophoresis and mass spectrometry analysis*

Soluble proteins extracted from buckwheat sprouts were resolved on 2-D gel according to the previous protocol (O`Farrell 1975). Sample solution (50 μg) was loaded to the acidic side of the isoelectrofocusing (IEF) gel in the first dimension, and anodic and cathodic electrode solutions were filled in the upper and lower electrode chambers, respectively. The pH gradient of IEF was kept in the range of pH 3-10. SDS-PAGE in the second dimension was performed with 12% separation and 5% stacking polyacrylamide gels. Protein spots on 2-D gels were visualized by Coomassie Brilliant Blue (CBB) R-250 staining (Woo *et al.* 2002). Each sample was run three times and the best visualized gels were selected. According to the previous report (Choi *et al.* 2008; Kamal *et al.* 2009b) with some modifications, interest-of-gel spots were excised from the preparative gels and followed by reduction and alkylation of disulfide bonds of proteins. Subsequently, the prepared protein spot was overnight digested with trypsin (12.5 ng/μl, final volume of 20 μl) at 37°C. The resultant tryptic peptides were resuspended in 40 μl of 1% (v/v) trifluoroacetic acid (TFA) and 66% (v/v) acetonitrile and dried in vacuum dryer. After desalting with C18 zip tip (Millipore, Boston, MI), the peptides were loaded onto MALDI plates by α-cyano-4-hydroxy-cinnamic acid (CHCA) matrix solution (10 mg/ml CHCA in 0.5% TFA and 50% (v/v) acetonitrile, 1:1, v/v). With MALDI-TOF MS (AXIMA CFR+ Plus, Shimadzu, Japan), the protein identification was followed as described previously (Fukuda *et al.* 2003).

### *Bioinformatics analysis*

Buckwheat (common buckwheat and tatary buckwheat) sprout proteins were identified by searching NCBI non-redundant database using MASCOT version 2.2 (http://www.matrixscience.com/). Protein identification was conducted against whole plant databases due to the insufficient gnomic database of buckwheat. The search parameters were allowed for the modification of acetyl (K), carbamidomethyl (C), oxidation (M), propionamide (C) with peptide tolerance (±100 ppm). For MS/MS search, the fragmentation of a selected peptide molecular ion peak was used to identify with a probability of less than 5%. Thus, MS/MS spectra with MASCOT score higher than the significant score ($P$<0.05) were considered as valid. The proteins assigned with at least two peptides were finally chosen for further bioinformatics analysis. In the protein list, sequence lengths, gene names and possible protein functions were determined by searching Swiss-Prot/TrEMBL database using UniProt (http://www.uniprot.org).

## Results and Discussion

### *Morphological characteristics of light or dark-grown seedlings of buckwheat*

Morphological classification of buckwheat genus *Fagopyrum* was primarily based on the characters of embryo, cotyledons, and achenes containing perianths, the pubescence of ochreae and stems (Ohnishi, 1998). The most significant difference between common and tatary buckwheat is the reproduction style: common buckwheat can reproduce by cross-fertiliza

**Table 1** Buckwheat sprouting tissue-specific proteins induced or inhibited by light

| Spot # | Acc' # | Protein Identity | Spot # | Acc' # | Protein Identity |
|---|---|---|---|---|---|
| | | | A. Light-induced leaf proteins | | |
| Common Buckwheat | | | Tatary Buckwheat | | |
| A1 | Q0D831 | Os07g0187900 protein | C2 | P92985 | RNA-binding protein 1 homolog c |
| A2 | A2Z0C0 | Probable protein arginine N-methyltransferase 1 | C3 | B7ZWR6 | At5g21990 |
| A3 | Q39199 | DNA repair protein recA homolog 1, chloroplast | C4 | Q9M2C8 | Pentatricopeptide repeat-containing protein At3g61360 |
| A9 | NP_973524.1 | Nucleic acid binding / zinc ion binding | C6 | A6MZU0 | Tubulin alpha-1 chain |
| A10 | Q9LPC4 | Pentatricopeptide repeat-containing protein At1g01970 | C7 | Q9SFD8 | Nuclear transcription factor Y subunit B-9 |
| A11 | Q9C7Z8 | En/Spm-like transposon protein, 5' partial; 97242-96760 | C8 | P19124 | NAD(P)H-quinone oxidoreductase subunit J, chloroplast |
| | | | C9 | Q9XFI1 | Nitric oxide synthase |
| B. Light-inhibited leaf proteins | | | | | |
| Common Buckwheat | | | Tatary Buckwheat | | |
| B15 | P51431 | 40S ribosomal protein S27a | D8 | Q7XPL2 | Proporphyrinogen-III oxidase, chloroplast |
| B16 | Q9AVF | NADPH-protochlorophyllide oxidoreductase 1 | D9 | Q9XFI1 | Thioredoxin-like 3, chloroplast |
| B17 | 3 | mucin-like protein | D10 | Q8LMR4 | Putative peroxidase |
| B18 | Q5QL14 | Actin-depolymerizing factor 1 | D12 | B2XWP0 | DNA-directed RNA polymerase subunit beta' |
| B26 | P46251 | 13S globulin seed storage protein 3 | D19 | Q1W7A3 | Mitochondrial ATP synthesis coupled proton transport protein |
| B27 | Q9XFM4 | 13S globulin seed storage protein 3 | D21 | Q9LVK3 | Autophagy-related protein 12b |
| | Q9XFM4 | | D26 | Q8RWG8 | RNA-binding protein 1 homolog b |
| | | | D27 | Q9XFM4 | 13S globulin seed storage protein 3 |
| | | | D28 | Q9XFM4 | 13S globulin seed storage protein 3 |
| C. Light-induced stem proteins | | | | | |
| Common Buckwheat | | | Tatary Buckwheat | | |
| E1 | P31093 | Photosystem I reaction center subunit N, chloroplast | G2 | P12468 | Ribulose bisphosphate carboxylase small chain 4, chloroplast |
| E18 | P22177 | Proliferating cell nuclear antigen | G5 | O24408 | Auxin-responsive protein IAA18 |
| E19 | P12468 | Ribulose bisphosphate carboxylase small chain 4, chloroplast | G6 | O23373 | Formin-like protein 3 |
| E21 | O23373 | Formin-like protein 3 | G10 | Q9C9E3 | Protein TIFY 11B |
| E22 | Q08000 | Em protein H2 | G11 | Q94HW3 | Probable disease resistance protein RDL6/RF9 |
| E23 | Q9SN90 | Structural maintenance of chromosomes protein 2-2 | G12 | O23878 | 13S globulin seed storage protein 1 |
| E24 | B4FAF3 | UPF0737 protein 1 | G16 | O80933 | Scarecrow-like protein 9 |
| | | | G26 | Q7PC70 | Probable xyloglucan glycosyltransferase 2 |
| | | | G27 | A2YXJ7 | Retinoblastoma-related protein 1 |
| D. Light-inhibited stem proteins | | | | | |
| Common Buckwheat | | | Tatary Buckwheat | | |
| | | | H2 | Q9ZQR6 | Serpin-Z2 |
| | | | H3 | O82261 | Protease Do-like 2, chloroplast |
| | | | H4 | Q0JNL7 | Calmodulin-3 |
| | | None | H12 | Q8H6B1 | FACT complex subunit SPT16 |
| | | | H13 | Q9LTM4 | Cytochrome P450 71B19 |
| | | | H14 | Q5G1S8 | Pentatricopeptide repeat-containing protein At3g18110, chloroplast |

tion while tatary buckwheat by self-fertilization. Due to the traits of higher seed yields, self-pollination ability, and cold-resistance of tatary buckwheat, *F. tataricum* has been utilized as a parent in inter-specific crosses (Adachi *et al.* 1989). Recently, 16 compatible and incompatible hybridization patterns among *Fagopyrum* species were established according to the pistils-pollen interaction. Inter-specific cross between *F. tataricum* and *F. esculentum* pin type showed slightly compatible (Woo *et al.* 2008). The genetic distance between *F. esculentum* and *F. tataricum*, based on the allozyme variability, showed the relatively closer than other *Fagopyrum* species (Ohnishi and Matsuoka, 1996). Many buckwheat scientists stated that buckwheat genetic and polymorphism depend on origin of buckwheat ancestor, cultivars, and climate conditions. Buckwheat has highly diversity among Indian, China, and European countries ancestors (Senthilkumaran *et al.* 2008). High content of anthocyanin is accumulated in buckwheat hypocotyls and cotyledons after 3 days of seedling under light (Horbowicz *et al.* 2008). Likewise, the stem of light-grown buckwheat showed clearly reddish color, indicating the higher content of major pigment anthocyanin. Buckwheat seeds were cultured under dark and light from seed germination. Compared to the greening buckwheat, the sprouting leaves and stems of 7-day-old etiolated seedlings turned to light yellow and white, respectively (Fig. 1). Light-grown buckwheat seedlings underwent the reduced stem elongation compared to etiolated seedlings, opening of the sprouting leaves, and greening leaves by matured development of chloroplasts. This dramatic change can be caused by chloroplast development, pigment synthesis, and photosynthetic apparatus in thylakoids (Yang *et al.* 2007). Furthermore, buckwheat cotyledons were vertically straight grown under light whereas cotyledons under dark formed more curved. The stems of 7-day-old etiolated tatary buckwheat were shown as more branched and more arrow-shaped. The size of sprouting leaf from common buckwheat showed bigger than that of tatary buckwheat (Fig. 1). The morphology of dark-grown buckwheat looks similar, however, the curved stems of tatary buckwheat were much more observed than those of tatary buckwheat. This minute difference of phenotypes is caused by different genetic trait and its downstream proteomic profiles.

***Analysis of buckwheat reference proteome on 2-DE***

In order to compare the proteomic profiles of common and tatary buckwheat, we performed the gel-based qualitative proteomics with sprouting leaves and stems cultivated under light or dark. The proteome reference map of buckwheat was attempted for the first time to establish with dominantly expressed proteins in 7 days old sprouts of buckwheat seedlings. Proteomics of buckwheat sprouts represents proteins responsible for different physiological condition. The high-quality reference proteome maps of various buckwheat tissues (leaf and stem) were obtained after evaluating protein extraction and solubilization methods. Extraction of buckwheat proteins are difficult due to the presence of interfering materials such as nucleic acid, phenolics, salts, organic acids, pigments, terpenes and so on. Proteins were extracted from common and tatary buckwheat cultured under light or dark according to the methods of protein extraction and solubilization (Damerval *et al.* 1986; Porubleva *et al.* 2001). The separation of buckwheat proteins on 2-D gel was not satisfactorily visualized around the wide range of neutral pH (pH 4-7). Thus, we adopted the wide range of pH (pH 3-10) for one-dimensional separation. As shown in Fig. 2, predominant proteins of stem and leaf tissue


**Fig 1.** Morphological characteristics of buckwheat gown under light or dark for 7 days after germination. Under light, the sprouting leaf size of common buckwheat (a, b) was bigger than that of tatary buckwheat (c, d).

from common and tatary buckwheat were clearly separated in the range of pH 3-10 on 2-D gel. In general, the wide range of IEF around pH 3-10 is used to avoid the overlapping protein spots and to increase the resolution capacity. In previous proteomics studies, wheat grain proteins were successfully separated in the first dimension of IEF around pH 4-7 and pH 6-11 (Woo *et al.* 2002; Kamal *et al.* 2009a, 2009b). As a result of wide range of proteome separation, approximately 700 protein spots were displayed, in which a total of 166 unique proteins were identified from 194 spots chosen (Supplementary Table 1 and 2), suggesting the existence of multiple spots caused by possible post-translational modifications (Muller *et al.* 1999). For instance, spots of G3, G8, and G9 were analyzed by mass fingerprinting and Mascot search using non-redundant NCBI database and assigned to probable protein phosphatase 2C from stem of tatary buckwheat under light (Fig. 1c). Thus, this multi-spot identification may be caused by the unknown isoforms of proteins in buckwheat by p*I*-affecting post-translational modification and M*r*-affecting protein process (Choi *et al.* 2008). Two-dimensional gel electrophoresis for the separation of complex protein samples coupled with mass spectrometry for protein identification has been used to analyze protein expression patterns for many sample types. The use of this technique provides different kind of information about not only the full-length protein expression but also the modification of proteins. Any protein modification that leads to a change in overall of isoelectic point (p*I*) and/or mass range (M*r*) will generate a different spot on the 2D gel. Modification-specific staining can identify whether a specific post-translational modification (phosphorylation, glycosylation, methylation, acetylation and so on) is liable for the shift, and mass spectrometry can potentially identify the source of p*I* and/or M*r* differences (Hart *et al.* 2003; Schulenberg *et al.* 2004). Due to the lack of complete coverage for a protein's amino acid sequence using either MALDI-TOF-MS or high-performance liquid chromatography (HPLC) tandem mass spectrometry (LC-MS/MS), there has been limited success in using MS to identify exact M*r* and p*I* value. While the theoretical M*r* is often slightly higher or lower than the calculated M*r* of the fully processed protein due to cleavage of signal and propeptides, there can also be post-translational modifications that increase the protein's gel M*r*. As a result, an investigation into the causes of the difference in the theoretical M*r* and the calculated M*r* as seen in the gel can

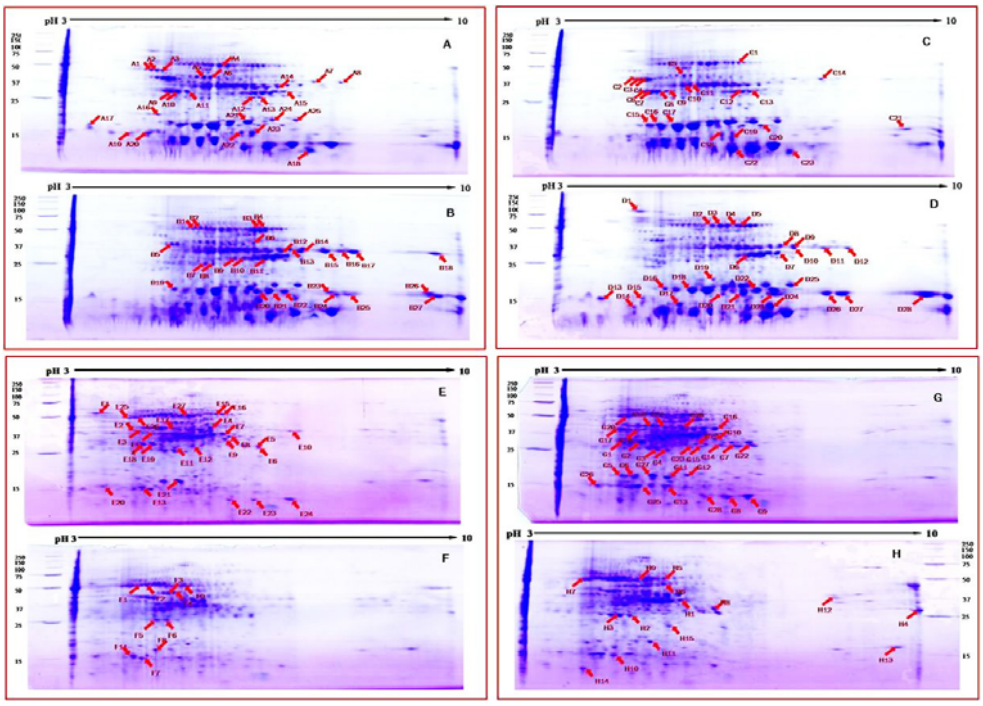

**Fig 2.** 2-D gel analyses of proteins extracted from mature buckwheat tissues (a, leaf under light and b, leaf under dark from common buckwheat; c, leaf under light and d, leaf under dark of tatary buckwheat; e, stem under light and f, stem under dark from common buckwheat; g, stem under light and h, stem under dark from tatary buckwheat). First-dimensional electrophoresis was performed on IEF with pH 3-10. In the second dimension, SDS-PAGE was used and proteins were visualized using CBB R-250.

yield information about the state of the protein (Person *et al.* 2006). Proteins identified as *Fagopyrum esculentum* were counted for 9 corresponding to 5.5% out of all the hit proteins. This low number is probably caused by the incomplete genome database of buckwheat. Top-ranked proteins were hit by *Arabidopsis thaliana* (77 hit, 47 %) followed by *Oryza sativa* (36 hit, 21.9%), *Zea mays* (11 hit, 6.7%), and *Glycine max* (7 hit, 4.1%).

***Differential protein classification of identified proteins of common and tatary buckwheat***

Based on 2D-gel and MALDI-TOF MS analysis, 88 protein spots of common buckwheat were successfully identified: leaf light (24 spots), leaf dark (26 spots), stem light (27 spots), and stem dark (11 spots). Similarly, 90 spots of tatary buckwheat were identified: leaf light (23 spots), leaf dark (23 spots), stem light (29 spots), and stem dark (15 spots). 166 uniquely proteins were identified in buckwheat. Interestingly, some detected spots were revealed same proteins (3 spots detected same proteins in both of buckwheat) (Supplementary Table 1 & 2). Exclusively identified proteins of common and tatatry buckwheat were 79 and 81, respectively in Venn diagram (Fig. 3a). Commonly identified 6 proteins included 13S globulin seed storage protein 3, metallothionin-like protein 3B, NAD(P)H-quinone oxidoreductase subunit 1, forming-like protein 3, Rubisco small chain, and naringenin, 2-oxoglutarate 3-dioxygenase from both buckwheat species. The identified buckwheat proteins were further classified on the basis of molecular function and intracellular localization according to annotations of Swiss-Prot database and UniProt program. A total of 172 proteins (85 proteins in common buckwheat and 87 proteins in tatary buckwheat) were classified into 16 molecular function groups and 11 intracellular compartment localization groups. As shown in Fig. 3b, the distribution of molecular function of common buckwheat proteins was displayed in sequence of metabolism (16.9%), unknown (11%), cellular process, signal transduction, transcription, transport (8%) and so on. In contrast to common buckwheat, the distribution of molecular function of tartatry buckwheat showed unexpectedly top group of transcription (22%) followed by metabolism (14%), transport (12%), defense and translation (6.9%). From the distribution of intracellular compartment localization of buckwheat proteins, the overall pattern was similar between common and tatary buckwheat. Proteins belonging to cytoplasm dominated 40% (common buckwheat) and 35% (tatary buckwheat) out of total proteins. Proteins belonging to plastid (21.7%) and nucleus (17%) showed next abundant ones in common buckwheat whereas nucleus (22%), plastid (17.7%) in tatary buckwheat (Fig. 3c). Interestingly, chloroplast ATP synthase complex (ATP synthase F1 beta, delta chain precursor, vacuolar type proton subunit d1) and NADPH-quinone oxidoreductase subunit H were found in plasma membrane of common and tatary buckwheat leaves under dark (Supplementary Table 1). Furthermore, NADPH-quinone oxidoreductase subunit J, formin-like protein 3, and calcineurin B-like proteins 3 belonged to plasma membrane were identified in buckwheat stem under light.

***Comparison between leaf proteomes of common and tatary buckwheat under light and dark***

The optimized protocol was used to investigate the profiles of leaf proteins from common and tatary buckwheat. More than 360 protein spots were reproducibly detected on CBB stained gels (Fig. 2a, 2b, 2c, and 2d). Among them, 102 spots of buckwheat leaves were successfully identified in which 23 to 28 spots were selected in each group. Based on the spot intensity, exclusively light-induced leaf proteins of common buckwheat are as follows; nodule-specific protein-like, putative arginine N-methyltransferase 1, chloroplast recA homolog, nucleic acid/Zn-binding protein, pentatricopeptide

repeat-containing protein, En/Spm-like transposon, and photosystem II (PSII)-reaction center protein M (Table 1). Arginine methyltransferase (AtPRTM5) in *Arabidopsis thaliana* is involved in promoting growth and flowering (Niu *et al.* 2007). As a nucleic acid/zinc-binding protein, bundle sheath defective 2 was known to regulate *rbcL* gene of maize by post-translational modification (Brunell *et al.* 1999). Likewise, the exclusively light-induced leaf proteins of tatary buckwheat are RNA-binding protein 1 homolog, At5g21990, tubulin alpha-1 chain, nuclear transcription factor Y subunit B9, nitric oxide synthase, and chloroplast NAD(P)H-quinone oxidoreductase subunit J. In addition, the induction of pentatricopeptide repeat-containing protein was similarly observed in tatary buckwheat. A pentatricopeptide repeat-containing protein is involved in cytoplasmic male sterility and restorer for the production of hybrid seed in plant (Bentolila *et al.* 2002). Thus, the difference of sterility and fertility from common and tatary buckwheat is probably caused by the different regulation of this unknown regulator. Identification of nitric oxide synthase in tatary buckwheat is notable that nitric oxide is an endogenous signal in plants and regulates the buckwheat greening by intrinsic hormones (Neil *et al.* 2008). Light-inhibited leaf proteins of common buckwheat are listed as ribosomal protein S27a, NADPH-protochlorophyllide oxidoreductase (POR) 1, mucin-like protein, actin depolymerizing factor 1, and 13S globulin seed storage protein 3. Interestingly, the inhibition of POR by light makes sense because the light-dependent reduction of protochlorophyllide to chlorophyllide is a key regulator of chlorophyll biosynthesis. The light-inhibited POR 1 is in agreement with the previous proteomic study of finding POR in dark-grown plant (Blomqvist *et al.* 2008). Similarly, proporphyrinogen-III oxidase was decreased in tatary buckwheat sprouting leaf under dark, in which the tetrapyrrole synthesis is involved in plant greening development (Kruse *et al.* 1995). Besides this, light-inhibited proteins in tatary buckwheat were thioredoxin-like 3, putative peroxidase, RNA polymerase beta', mitochondrial ATP synthesis-coupled proton transport protein, autophagy-related protein 12b, and RNA-binding protein 1 homolog b. Thioredoxin (Trx) in plant chloroplast has been extensively studied because the significance of Trx-mediated redox control connects to the cascade metabolic enzyme reaction such as Calvin cycle, starch synthesis, and chlorophyll biosynthesis (Balmer *et al.* 2003; Lindahl and Kieslbach, 2009; Hall *et al.* 2010). Photosynthetic light quality acclimation affects redox-controlled changes in plastid gene expression including plastid-encoded transcription, RNA-binding protein, and ribosomal subunits and chaperones (Steiner *et al.* 2009). Light-inhibited 13S globulin as the main storage protein of buckwheat seeds, in which this storage protein can cause allergies to subjects (Zhang *et al.* 2008), was commonly found in common and tatary buckwheat.

***Comparison between stem proteomes of common and tatary buckwheat under light and dark***

From the systemic separation by 2D-gel and identification with MALDI-TOF-MS, 82 spots of buckwheat stems were successfully identified in which 38 and 44 spots were belonged to common and tatary buckwheat, respectively. Throughout the 2D-gels of buckwheat stem, many stem proteins were prevalently displayed in acidic region compared to those of buckwheat leaf (Fig. 2e, 2f, 2g, and 2h). This 2D-gel pattern was similarly observed in the proteomic analysis of rice leaf and stem (Nozu *et al.* 2006). Buckwheat stem proteins such as formin-like protein 3 and ribulose bisphosphate carboxylase (Rubisco) small chain 4 were

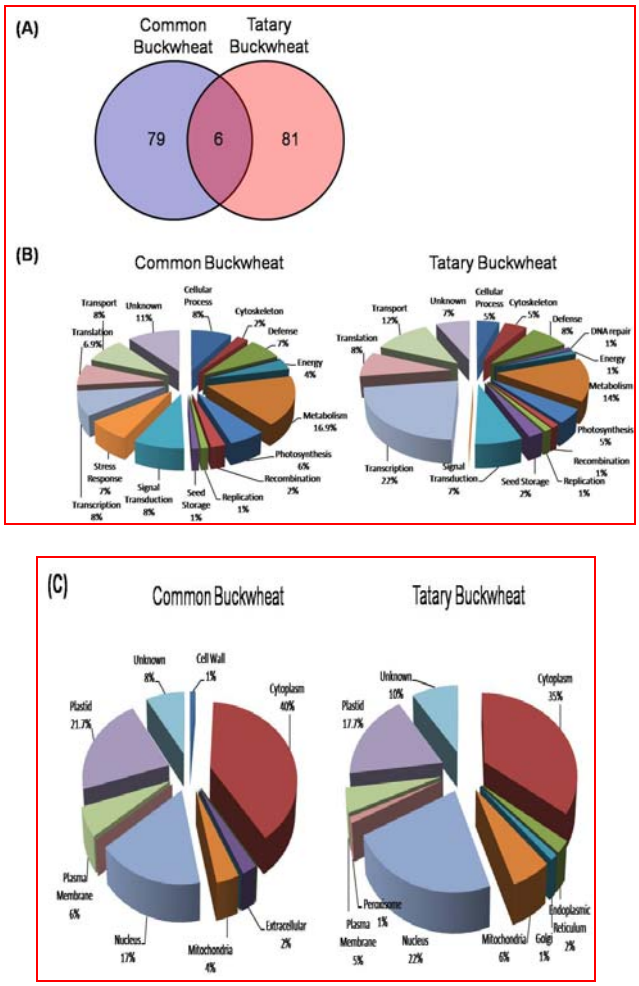


**Fig 3.** Functional classifications of identified proteins from common and tatary buckwheat leaf and stem under light or dark. (a) A Venn diagram of identified proteins from common and tatary buckwheat. Distribution of identified proteins was pie-charted according to (b) molecular function and (c) intracellular compartment localization.

commonly found under light, irrespective of buckwheat species (Supplementary Table 2). Plant formin belongs to a new class of conserved actin nucleator, suggesting playing key roles on cell expansion (Yi *et al.* 2005; Blanchoin and Staiger, 2010). Peroxisomal (*S*)-2-hydroxy-acid oxidase 2 and naringenin/2-oxoglutarate 3-dioxygenase from stem tissue were commonly found, irrespective of light or dark-grown condition. Glycolate oxidase, an FMN-dependent peroxisomal oxidase, functions in photorespiration but the exact function is unclear (Vignaud *et al.* 2007). The family of 2-oxoglutarate and ferrous iron-dependent oxygenase contains anthocyanin synthase, flavonol synthase and flavonone 3 beta-hydroxylase, in which these enzymes suggested to be constitutively expressed for flavonoid biosynthesis in buckwheat stem (Turnbull *et al.* 2004). Light-induced stem proteins of common buckwheat are listed as photosystem I reaction center subunit N (PSI-N), proliferating cell nuclear antigen (PCNA), Rubisco small chain 4, formin-like protein 3, Em protein-H2, chromosomal protein 2-2, and UPF0737 protein1. PSI-N is restricted to higher plant, localizing in the thylakoid lumen, which are engaged in the energy transfer of photosystem I (Haldrup *et al.* 1999). The presence of PCNA in germinating seeds and its absence from mature plants were suggested to play a crucial role

during early stage of plant development (Strzalka and Ziemienowics, 2007). Likewise, light-induced stem proteins of tatary buckwheat are identified as Rubisco small chain 4, auxin-responsive protein IAA18, formin-like protein 3, protein TIFY 11B, disease resistance protein RDL6/RF9, 13S globulin, scarecrow-like protein 9, xyloglucan glycosyltransferase (XET) 2, and retinoblastoma-related (RBR) protein 1. According to previous study, auxin-responsive promoter was expressed in the epidermis and cortex of stems and petioles in transgenic tobacco and light treatment reverted to its activity (Li *et al.* 1991). Thus, auxin-responsive protein can be one of the light-responsive proteins in tatary buckwheat. The TIFY family is a novel plant-specific gene family, in which the function is involved in the regulation of development of phytohormones in *Arabidopsis* (Ye *et al.* 2009). Thus, light-induced TIFY 11B in tatary buckwheat can be signature of light-induced biological process. The presence of RDL6/RF9 from tatary buckwheat stem was also reported in the stem of potato EST sequencing (D'Agostino *et al.* 2009). The enzyme XET facilitates the cell wall extensibility and hypocotyl elongation of plant seedling (Wu *et al.* 2005). The root and shoot apical meristems are produced during embryogenesis and serves as a progenitor cells for plant growth. The small group of cells centered for the progenitor of root and stem inhibit the surrounding cells by accumulating auxin via auxin efflux carrier, Scracrow (Holmes *et al.* 2008). It is notable that the finding of RBR protein in tatary buckwheat stem was involved in plant root development (Dinney and Benfey, 2005; Wildwater *et al.* 2005). Light-inhibited stem proteins of common buckwheat are not observed. Instead, light-inhibited stem proteins of tatary buckwheat such as serpin Z2, protease Do-like 2, calmodulin 3, FACT complex subunit SPT16, cytochrome P450 71B19, and pentatricopeptide repeat-containing protein were identified as up-regulated protein under dark. Serpin protein family plays a critical role in the control of irreversible proteolysis (Roberts and Hejgaard, 2007). Protease Do is serine-type heat shock protease that can be found in the thylakoid inner membrane (Itzhaki *et al.* 1998). Calmodulin, heat-resistant and acidic polypeptide possessing calcium-binding characteristics, is localized prevalently in root tip and lesser amounts in xylem of stem (Lin *et al.* 1986). FACT complex as a conserved cofactor of RNA polymerase II elongation through nucleosome contributes to chromatin integrity by histone chaperone activity (Stuwe *et al.* 2008). Cytochrome P450 71B19 was up-regulated under the lithium exposure to *Brassica carinata* seedlings (Li *et al.* 2009). Interestingly, other type of pentatricopeptide repeat-containing protein was increased under dark from tatary buckwheat in contrast to the induction of this protein under light from common and tatary buckwheat.

## Conclusion

With the gel-based proteomic approach, we systematically examined the proteomic profiles of 7-day-old sprouting leaf and stem from common and tatary buckwheat under light or dark. A total 166 unique buckwheat proteins were successfully identified, in which 79 and 81 proteins were exclusively found in common and tatary buckwheat, respectively, whereas 6 proteins commonly found in both buckwheat species. By the classification of molecular function, proteins belonging to metabolism such as carbon, nitrogen, amino acid, pigment biosynthesis were dominated in common buckwheat whereas proteins belonged to transcription were predominant in tatary buckwheat. By the classification of intracellular compartment localization, most abundant proteins were assigned to cytoplasm followed by plastid and nucleus, in which the proteomic profiles contained several membrane proteins assigned to plasma membrane, mitochondria, and endoplasmic reticulum. The sprouting leaves of 7-day-old etiolated common buckwheat seedlings turned to light yellow, suggesting the inhibition of light-dependent protochlorophyllide reductase by proteomic analysis. Light-inhibited storage protein, 13S globulin 3, was commonly found in the leaves of both buckwheat species under dark. In particular, putative pentatricopeptide repeat-containing proteins were specifically identified in light-induced leaves in common and tatary buckwheat and also identified in light-inhibited stem of tatary buckwheat. It suggests that the different regulator isoforms are involved in the sterility and fertility from common and tatary buckwheat in a specific light-dependent manner. The proteomic attempt to buckwheat sprout will be helpful to understand the developmental physiology of unknown non-cereal crop buckwheat.


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## References


Adachi T, Yamaguchi A, Miike Y, Hiffman F (1989) Plant regeneration from protoplasts of common buckwheat (*Fagopyrum esculentum*). Plant Cell Rep. 8:247-250\

Alvarez JL, Arendt EK, Gallagher E (2009) Nutritive value and chemical composition of pseudocereals as gluten-free ingredients. Int J Food Sci Nutr. 4:240-257

Balmer Y, Vensel WH, Tanaka CK, Hurkman WJ, Gelhaye E, Rouhier N, Jacquot JP, Manieri W, Schürmann P, Droux M, Buchanan BB (2003) Proteomics gives insight into the regulatory function of chloroplast thioredoxins. Proc Natl Acad Sci USA. 100:370-375

Bentolila S, Alfonso AA, Hanson MR (2002) A pentapeptide repeat-containing gene restores fertility to cytoplasmic male-sterile plants. Proc Natl Acad Sci USA. 99:10887-10892

Blanchoin L, Staiger CJ (2010) Plant formins: diverse isoforms and unique molecular mechanism. Biochim Biophys Acta. 1803:201-206

Blomqvist LA, Ryberg M, Sundqvist C (2008) Proteomic analysis of highly purified prolamellar bodies reveals their significance in chloroplast development. Photosyn Res. 96:37-50

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 72:248-254

Brutnell TP, Sawers RJ, Mant A, Langdale JA (1999) BUNDLE SHEATH DEFECTIVE2, a novel protein required for post-translational regulation of the rbcL gene of maize. Plant Cell. 11:849-864

Chan PK (2003) Inhibition of tumor growth in vitro by the extract of *Fagopyrum cymosum* (fago-c). Life Sci. 72:1851-1858

Choi JS, Cho SW, Kim TS, Cho K, Han SS, Kim HK, Woo SH, Chung KY (2008) Proteome analysis of greenhouse-cultured lettuce with the natural soil mineral conditioner liter. Soil Biol Biochem. 40:13701-378

D'Agostino N, Traini A, Frusciante L, Chiusano ML (2009) SolEST database: a "one-stop shop" approach to the study of Solanaceae transcriptomes. BMC Plant Biol. 9:142

Damerval C, de Vienne D, Zivy M, Thiellement H (1986) The technical improvements in two-dimensional electrophoresis increase the level of genetic variation detected in wheat-seedling proteins. Electrophoresis. 7:52-54

Dinneny JR, Benfey PN (2005) Stem cell research goes underground: the retinoblastoma-related gene in root development. Cell. 123:1180-1182

Fukuda M, Islam N, Woo SH, Yamagishi A, Takaoka M, Hirano H (2003) Assessing matrix assisted laser desorption/ ionization-time of flight-mass spectrometry as a means of rapid embryo protein identification in rice. Electrophoresis. 24:1319-1329

Ghimeray AK, Sharma P, Briatia X (2010) Phenolic compounds and free radical scavenging activity of seed, seedling and sprout of buckwheat. The proceeding of the Intl. Symp. On Buckwheat Sprouts. Bongpyoung, Korea, pp 41-45.

Haldrup A, Naver H, Scheller HV (1999) The interaction between plastocyanin and photosystem I is inefficient in transgenic Arabidopsis plants lacking the PSI-N subunit of photosystem I. Plant J. 17:689-698

Hall M, Mata-Cabana A, Akerlund HE, Florencio FJ, Schröder WP, Lindahl M, Kieselbach T (2010) Thioredoxin targets of the plant chloroplast lumen and their implications for plastid function. Proteomics. 10:987-1001

Hart C, Schulenberg B, Steinberg TH, Leung WY, Patton WF (2003) Detection of glycoproteins in polyacrylamide gels and on electroblots using Pro-Q Emerald 488 dye, a fluorescent periodate Schiff-base stain. Electrophoresis. 24:588-598

Holmes P, Goffard N, Weiller GF, Rolfe BG, Imin N (2008) Transcriptional profiling of *Medicago truncatula* meristematic root cells. BMC Plant Biol. 8:21

Horbowicz M, Grzesiuk A, Debski H, Koczkodaj D, Saniewski M (2008) Methyl jasmonate inhibits anthocyanin synthesis in seedlings of common buckwheat (*Fagopyrum esculentum* Moench.). Acta Biol Crac Ser Bot. 50: 71-78

Ishii S, Katsumura T, Shiozuka C, Ooyauchi K, Kawasaki K, Takigawa S, Fukushima T, Tokuji Y, Kinoshita M, Ohnishi M, Kawahara M, Ohba K (2008) Anti-inflammatory effect of buckwheat sprouts in lipopolysaccharide-activated human colon cancer cells and mice. Biosci Biotech Biochem. 72:3148-3157

Itzhaki H, Naveh L, Lindahl M, Cook M, Adam Z (1998) Identification and Characterization of DegP, a Serine Protease Associated with the Luminal Side of the Thylakoid Membrane. J Biol Chem. 273:7094-7098

Kamal AHM, Kim KH, Shin DH, Seo HS, Shin KH, Park CS, Heo HY, Woo SH (2009a) Proteomics profile of pre-harvest sprouting wheat by using MALDI-TOF Mass Spectrometry. Plant Omics J. 2:110-119

Kamal AHM, Kim KH, Shin KH, Seo HS, Tsujimoto H, Heo HY, Choi JS, Park CS, Woo SH (2009b) Diversity of novel glutenin subunits in bread wheat (*Triticum aestivum* L.). J Plant Biol. 52:533–542

Kawa JM, Taylor CG, Przybylski R (2003) Buckwheat concentrate reduces serum glucose in streptozotocin-diabetic rats. J Agric Food Chem. 51:7287-7291

Kayashita J, Shimaoka I, Nakajoh M, Yamazaki M, Norihisa K (1997) Consumption of buckwheat protein lowers plasma cholesterol and raises fecal neutral sterols in cholesterol-fed rats because of its low digestibility. J Nutr. 127:1395-1400

Kim SJ, Maeda T, Sarker MZI, Takigawa S, Matsuura-Endo C, Yamaguchi H, Mukasa Y, Saito K, Hashimoto N, Noda T, Saito T, Suzuki T (2007) Identification of anthocyanins in the sprouts of buckwheat. J Agric Food Chem. 55:6314-6318

Kruse E, Mock HP Grimm B (1995) Coproporphyrinogen III oxidase from barley and tobacco-sequence analysis and initial expression studies. Planta. 196:796-803

Li X, Gao P, Gjetvaj B, Westcott N, Gruber MY (2009) Analysis of the metabolome and transcriptome of *Brassica* carinata seedlings after lithium chloride exposure. Plant Sci. 177:68-80

Li Y, Hagen G, Guilfoyle TJ (1991) An auxin-responsive promoter is differentially induced by auxin gradients during tropisms. Plant Cell. 3:1167-1175

Lin CT, Sun D, Song SX, Wu JY (1986) Calmodulin: localization in plant tissues. J Histochem Cytochem. 34:561-567.

Lin LY, Peng CC, Yang YL, Peng RY (2008) Optimization of bioactive compounds in buckwheat sprouts and their effect on blood cholesterol in hamsters. J Agric Food Chem. 56:1216-1223

Lindahl M, Kieselbach T (2009) Disulfide proteomes and interactions with thiordoxin on the track towards understanding redox regulation in chloroplasts and cyanobacteria. J Proteomics. 72:416-438

Ma MS, Bae IY, Lee HG, Yang CB (2006) Purification and identification of angiotensin I-converting enzyme inhibitory peptide from buckwheat (*Fagopyrum esculentum* Moench.). Food Chem. 96:36-42

Ma Y, Xiong YL, Zhai J, Zhu H, Sziubla T (2010) Fractionation and evaluation of radical-scavenging peptides from in vitro digests of buckwheat protein. Food Chem. 118:582-588

Muller DR, Schindler P, Coulot M, Voshol H, van Oostrum J (1999) Mass spectrometric characterization of stathmin isoforms separated by 2D PAGE. J Mass Spectrom. 34:336-345

Neil S, Barros R, Bright J, Desikan R, Hancock J, Harrison J, Morris P, Ribeiro D, Wilson I (2008) Nitric oxide, somatal closure, and abiotic stress. J Exp Bot. 59:165-176

Niu L, Lu F, Pei Y, Liu C, Cao X (2007) Regulation of flowering time by the protein arginine methyltransferase AtPRMT10. EMBO Rep. 8:1190-1195

Nozu Y, Tsugita A, Kamijo K (2006) Proteomic analysis of rice leaf, stem and root tissues during growth course. Proteomic. 6:3665-3670

O`Farrell PH (1975) High resolution two-dimensional electrophoresis of proteins. J Biol Chem. 250:4007-4021

Ohnishi O (1998) Search for the wild ancestor of buckwheat I. Description of new *Fagopyrum* (Polugonaceae) species and their distribution in China and the Himalayan hills. Fagopyrum. 15:18-28

Ohnishi O, Matsuoka Y (1996) Search for the wild ancestor of buckwheat II. Taxonomy of *Fagopyrum* (Polyhonaceae) species based on morphology, isozymes and cpDNA variability. Genes Genet Syst. 71:383-390

Person MD, Shen J, Traner A, Hensley SC, Lo HH, Abbruzzese JL, Li D (2006) Protein Fragment Domains Identified Using 2D Gel Electrophoresis/MALDI-TOF. J Biomol Tech. 17:145-156

Porubleva L, Velden KV, Kothari S, Oliver DJ, Chitnis PR (2001) The proteome of maize leaves: Use of gene sequences and expressed sequence tag data for identification of proteins with peptide mass fingerprints. Electrophoresis. 22:1724-1738

Roberts TH, Hejgaard J (2007) Serpins in plants and green algae. Funct Integr Genomic. 8:1-27

Satoh R, Koyano S, Takagi K, Nakamura R, Teshima R (2010) Identification of an IgE-binding epitope of a major buckwheat allergen, BWp16, by SPOTs assay and mimotope screening. Int Arch Allergy Immunol. 153:133-140

Schulenberg B, Goodman TN, Aggeler R, Capaldi RA, Patton WF (2004) Characterization of dynamic and steady-state protein phosphorylation using a fluorescent phosphoprotein gel stain and mass spectrometry. Electrophoresis. 25:2526-2532

Senthilkumaran R, Bisht IS, Bhat KV, Rana JC (2008) Diversity in buckwheat ( *Fagopyrum* spp.) landrace populations from north-western Indian Himalayas . Genet Resour Crop Evol. 55:287-302.

Steiner S, Dietzel L, Schroeter Y, Fey V, Wagner R, Pfannschmidt T (2009) The role of phosphorylatiopn in redox regulation of photosynthesis genes psaA and psbA during photosynthetic acclimation of mustard. Mol Plant. 2:416-429

Strzalka W, Ziemienowicz A (2007) Molecular cloning of Phaseolus vulgaris cDNA encoding proliferating cell nuclear antigen. J Plant Physiol. 164:209-213

Stuwe T, Hothorn M, Lejeune E, Rybin V, Bortfeld M, Scheffzek K, Ladurner A (2008) The FACT Spt16 "peptidase" domain is a histone H3-H4 binding module. Proc Natl Acad Sci USA. 105:8884-8889

Suzuki T, Honda Y, Funatsuki W, Nakatsuka K (2002) Purification and characterization of flavonol 3-gluosidase, and its activity during the ripening in tatary buckwheat seeds. Plant Sci. 163:417-423

Suzuki T, Mukasa Y, Yokota S, Takigawa S, Yamauchi H, Matsuura-Endo C, Hashimoto N, Noda T, Morishita T, Kim SJ (2009) Development of tatary buckwheat varieties for sprouts. The proceeding of the Intl. Sym.on Buckwheat Sprouts, Bongpyoung, Korea, pp-80-91

Turnbull JJ, Nakajima J, Welford RW, Yamazaki M, Saito K, Schofield CJ (2004) Mechanistic studies on three 2-oxoglutarate-dependent oxygenases of flavonoid biosynthesis: anthocyanidin synthase, flavonol synthase, and flavanone 3beta-hydroxylase. J Biol Chem. 279:1206-1216

Vignaud C, Pietrancosta N, Williams EL, Rumsby G, Lederer F (2007) Purification and characterization of recombinant human liver glycolate oxidase. Arch Biochem Biophys. 465:2410-416

Watanabe M, Shimizu H (2004) Composition of flavonoid compounds in seedlings of tatary buckwheat. Tohoku Agric Res (Japanses). 57:267-268

Wildwater M, Campilho A, Perez-Perez JM, Heidstra R, Blilou I, Korthout H, Chatterjee J, Mariconti L, Gruissem W, Scheres B (2005) The retinoblastoma-related gene regulates stem cell maintenance in Arabidopsis roots. Cell.123:1337-1349

Woo SH, Fukuda M, Islam N, Takaoka M, Kawasaki H, Hirano H (2002) Efficient peptide mapping and its application to identification of embryo proteins in the rice proteome analysis. Electrophoresis. 23:647-654

Woo SH, Kim SH, Tsai SK, Chung KY, Jong SK, Adachi T, Choi JS (2008) Pollen-tube behavior and embryo development in interspecific crosses among the genus *Fagopyrum*. J Plant Biol. 51:302-310

Wu Y, Jeong BR, Fry SC, Boyer JS (2005) Change in XET activities, cell wall extensibility and hypocotyl longation of soybean seedlings at low water potential. Planta. 220:593-601

Yang P, Chen H, Liang Y, Shen S (2007) Proteomic analysis of de-etiolated rice seedlings upon exposure to light. Proteomics. 7:2459-2468

Yasuda T, Nakagawa H (1994) Purification and characterization of rutin-degrading enzymes in tatary buckwheat seeds. Phytochem. 37:133-136

Ye H, Du H, Tang N, Li X, Xiong L (2009) Identification and expression profiling analysis of TIFY family genes involved in stress and phytohormone responses in rice. Plant Mol Biol. 71:291-305

Yi K, Guo C, Chen D, Zhao B, Yang B, Ren H (2005) Cloning and functional characterization of a forming-like protein (AtFH8) from *Arabidopsis*. Plant Physiol. 138:1071-1082

Zhang X, Yuan JM, Cui XD, Wang ZH (2008) Molecular cloning, recombinant expression, and immunological characterization of a novel allergen from tatary buckwheat. J Agric Food Chem. 56:10947-10953