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

Dong-Hoon Shin1, Abu Hena Mostafa Kamal2, Tatsuro Suzuki3, Young-Ho Yun3, Moon-Soon Lee4, Keun-Yook Chung5, Heon-Sang Jeong6, Cheol-Ho Park7, Jong-Soon Choi8,9 *, Sun-Hee Woo1 *

1Dept. of Crop Science, Chungbuk National University, Cheong-ju 361-763, Korea
2National Agriculture and Food Research Organization for Hokkaido Region, Hokkaido 082-0071, Japan
3Highland Agriculture Research Center, RDA, Pyeongchang 232-955, Korea
4Dept. of Industrial Plant and Technology, Chungbuk National University, Cheong-ju 361-763, Korea
5Dept. of Agricultural Chemistry, Chungbuk National University, Cheong-ju 361-763, Korea
6Dept of Food Science and Technology, Chungbuk National University, Cheong-ju 361-763, Korea
7Dept of Plant Biotechnology, Kangwon National University, Chuncheon-si, 200-701, Korea
8Graduate School of Analytical Science and Technology, Chungnam National University, Daejeon 305-764, Korea
9Authors are equally contributed

*Corresponding authors: Sun-Hee Woo (shwoo@chungbuk.ac.kr); Jong-Soon Choi (jschoi@kbsi.re.kr)

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. Tatar buckwheat has attracted attention due to their compounds that are beneficial to health, and used as oriental medicine in China. Tatar buckwheat sprouting has a bitter component than that of common buckwheat. The higher amount of rutin contain in stem and leaf of common and tatar 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 allergen 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. Monch), 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, tartary 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 tartary 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, tartary 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 tartary 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 tartary 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 tartary 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 (PVTDF 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; Pombileva 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 tartary 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 genomic 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 tartary buckwheat is the reproduction style: common buckwheat can reproduce by cross-fertiliza
Table 1 Buckwheat sprouting tissue-specific proteins induced or inhibited by light

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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 seeding 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 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 pl-affecting post-translational modification and Mr-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 isoelectric point (pI) and/or mass range (Mr) 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 pl and/or Mr 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 Mr and pl value. While the theoretical Mr is often slightly higher or lower than the calculated Mr 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 Mr. As a result, an investigation into the causes of the difference in the theoretical Mr and the calculated Mr 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 tataray buckwheat; e, stem under light and f, stem under dark from common buckwheat; g, stem under light and h, stem under dark from tataray 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 tataray 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 tataray 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 tataray buckwheat were 79 and 81, respectively in Venn diagram (Fig. 3a). Commonly identified 6 proteins included 135 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 tataray 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 tataray 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 tataray buckwheat. Proteins belonging to cytoplasm dominated 40% (common buckwheat) and 35% (tataray 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 tataray 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 tataray 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 tataray buckwheat under light and dark**

The optimized protocol was used to investigate the profiles of leaf proteins from common and tataray 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 tatarum buckwheat are RNA-binding protein 1 homolog, At15g21990, 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 tatarum 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 tatarum buckwheat is probably caused by the different regulation of this unknown regulator. Identification of nitric oxide synthase in tatarum 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-protoclorophyllide 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 protoclorophyllide 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 tatarum 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 tatarum 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 tatarum buckwheat.

**Comparison between stem proteomes of common and tatarum 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 tatarum 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 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; Blanchin and Staiger, 2010). Peroxisomal (5)-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 photosrespiration 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 were identified as Rubisco small chain 4, auxin-responsive protein IAA18, formin-like protein 3, protein TIFY 11B, disease resistance protein RDL6/RF9, 13S globulin, scarcecrow-like protein 9, xylol glucan glycosyltransferase (XET) 2, and retinoblastoma-related (RB) 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 serve 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 (Izhaki 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 (Swure 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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