PROTEOMIC ANALYSIS OF *ACONITUM AUSTROKOREENSE* KOIDZ. SEEDS DURING DORMANCY BREAKING

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Abstract. This study examined the dormancy-breaking mechanism of *Aconitum austrokoreense* seeds and analyzed seed proteins in germination phase II. Dried seeds had undeveloped embryos and absorbed moisture within 72 h. However, seeds were not germinated at an appropriate temperature; hence, they entered morphophysiological dormancy. Seeds treated with low 6-benzylaminopurine (BAP) concentrations did not germinate; however, seed dormancy was significantly broken under high BAP concentrations (0.5, 1.0, and 5.0 mg/L; $F = 23.208$, $p < 0.01$). Protein accumulation was higher in phase II than in dry seeds, indicating protein activation to prepare for germination phase II. The presence of a 30S ribosomal protein subunit, cytochrome b_6-f complex subunit, and photosystem II protein D1 was confirmed in BAP-treated seeds in germination phase II. The increased 30S ribosomal subunit accumulation indicated that physiological changes prepared seeds for germination by dormancy breaking. The presence of photosystem II protein D1 and cytochrome b_6-f complex subunit indicated that the cotyledon prepared for photosynthesis immediately after germination. Unfortunately, the factor directly influencing dormancy breakage was not observed in germination phase II; however, differences in protein accumulation during germination were observed. These results provide a foundation for understanding seed physiological changes during germination phase II.
Keywords: *seed proteomics, germination phase II, 6-benzylaminopurine, cytochrome b_6-f complex subunit, photosystem II protein D*

Introduction

Dormancy is a specific mechanism in seeds that prevents seed germination under unfavorable environmental conditions and sustains strength until a suitable time for seedling growth (Bewley et al., 2006; Vleeshouwers et al., 1995). Water absorption by the seed signals the end of dormancy and is generally followed by the initiation of germination. However, not all water uptake leads to seedling growth (Marcus and Feeley, 1964).

When seeds are exposed to water, they undergo three phases of water uptake (Weitbrecht et al., 2011). In phase I, there is a rapid water influx into dry seeds, and the seeds swell in size, changing shape (imbibition state) (Woodstock, 1988; Robert et al., 2008). After sufficient water absorption, the moisture content of the seeds is maintained, and no visible changes in the seeds occur during phase II of water uptake (Lutts et al.,

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2016). In phase III, water absorption increases again, causing the radicle to protrude, indicating seed germination (Weitbrecht et al., 2011; Wolny et al., 2018).

Although phase II appears to be a stagnant state, various cellular and biochemical processes that prepare the seed for germination are active (Wolny et al., 2018). Among these processes, the levels of abscisic acid (ABA), a plant growth regulator related to dormancy induction, decrease along with a change in the accumulation of major regulators of ABA biosynthesis and degradation (Chiwocha et al., 2005; Seo et al., 2006). The transcription and translation of certain genes are upregulated alongside large metabolic changes involving enzymes in the energy production pathway (Müller et al., 2010; Kimura and Nambara, 2010). Furthermore, many major enzymes associated with the tricarboxylic acid cycle accumulate in phase II for the rapid production of adenosine triphosphate (Weitbrecht et al., 2011; Obroucheva and Antipova, 1997). Successful seed germination depends on the life-cycle transition events that occur during water uptake in phase II (Cernac et al., 2006).

Biochemical events that occur during the water absorption stages can be confirmed through accumulation proteomics, which identifies key proteins in specific stages based on differences in protein accumulation between physiological states (Gallardo et al., 2001). Two-dimensional (2D) gel-based proteomics confirms the activity of proteins involved in an event and determines how cell cycle-related genes function at specific stages of plant development (Rabilloud et al., 2010). This may uncover the cellular and structural events related to metabolic activity in quiescent embryos before seed germination (Cernac et al., 2006; Rabilloud et al., 2010).

The genus *Aconitum* is one of the largest genera of the family Ranunculaceae and has more than 300 species that are distributed in Asia, Europe, North Africa, and North America (Tamura, 1966). They have a common set of features that distinguish them from other taxa of the same family; they have large stature and erect stems, dark green leaves without stipules and are palmate or deeply palmately lobed, have numerous stamens and a rounded helmet-shaped sepal called “galea” (Tamura, 1966; Kosuge and Tamura, 1988).

Aconitum austrokoreense Koidz. is perennial herb, 30~80 cm tall, and the stem grows straight and does not branch. The leaves are triangular or pentagonal with serrated edges and alternate. The flowers are sky-blue helmet-shaped and bloom from August to September, and the flowers grow in racemes from the leaf axils (*Fig. 1*). The fruits ripen in October to November.

Figure 1. *Flowers of Aconitum austrokoreense Koidz.*

A. austrokoreense is found only in Korea, primarily in southern regions such as Gyeongsang-do and Jeollanam-do, where is naturally in cool places of mountain forests

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dominated by around valleys (Park et al., 2016). This species is also recorded as being critically endangered according to the International Union for Conservation of Nature Red List, indicating that protection of this species is urgently needed. Other than studies related to the distribution of *A. austrokoreense*, no studies have been conducted on its seed germination or reproduction.

The purpose of this study was to investigate the dormancy type of *A. austrokoreense* seeds and their dormancy-breaking mechanism. In addition, the role of proteins in the different stages of seed germination, particularly in phase II, based on dormancy breaking of *A. austrokoreense* seeds was evaluated. These findings provide a seed protein reference map of protein accumulation in seeds during germination.

Materials and methods

Seed collection and characterization (plant material)

Mature *A. austrokoreense* seeds were collected on October 17, 2019, from Bonghwa, a native Korean habitat. Before use, all seeds were dried and stored at 15°C in the drying room of Baekdudaegan National Arboretum, Bonghwa, Korea. To evaluate seed shape and color, 10 seeds were randomly selected and photographed under a microscope (DVM6; Leica Microsystems GmbH, Wetzlar, Germany). The lengths and widths of the seeds were averaged by measuring the dimensions of 100 seeds. Thousand weights were obtained by measuring the mass of 100 seeds five times and multiplying the average value by 10 (Lovas-kiss et al., 2015). To determine the condition of the embryo, healthy and dried seeds were selected and cut along the long axis. Cross-sections of the seeds were observed and photographed using a microscope (DVM6, Leica Microsystems GmbH, Wetzlar, Germany). The E:S was calculated for dried or activated seeds (Vandelook et al., 2007).

Water uptake analysis

This experiment was conducted to determine the natural water absorption potential of the seeds. Three replicates of 20 seeds (selected randomly) were placed separately on two sheets of filter paper (Whatman no. 1, Bucks, UK) in 90 × 15 mm Petri dishes (cat. no. 11010; SPL Life Sciences, Pochon, South Korea). Seeds were sufficiently moist and kept at room temperature (20–25°C). The seeds were weighed for four days at 24 h intervals. The water uptake (% W*r*, the increase in seed mass) was calculated as follows:

$$\%\, Wr = [(Wf - Wi)/Wi] \times 100$$

where W*i* is the initial weight of the dry seed mass, and W*f* is the seed mass after a given interval of imbibition (Zhang et al., 2019).

Germination of seeds

Four replicates containing 25 seeds were separately placed on 0.8% plant agar medium containing 0.1, 0.5, 1.0, and 5.0 mg/L of BAP and cultivated at 15/6°C and 25/15°C (14/10 h, day/night). As a control, untreated seeds were simultaneously placed in an incubator. Seeds were defined as germinating when the root grew to > 1 mm. The number of germinating seeds was counted daily, and the germination (%) was

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calculated by summing the number of all germinated seeds after 30 days. After germination, the E:S ratios of all ungerminated seeds were measured.

Protein sampling and extraction

Seeds with roots (germinated by BAP treatment), seeds in phase II of the BAP treatment (before germination, with dormancy breaking in progress), and seeds that were not sufficiently activated for germination (only water was supplied, and BAP treatment was not provided) were prepared for protein analysis. The seeds were frozen in liquid nitrogen, ground to a fine powder using a mortar and pestle and used for protein extraction. Total soluble proteins were extracted from the seed powders using a motor-driven homogenizer (PowerGen125, ThermoFisher Scientific, Waltham, MA, USA) in sample lysis solution composed of 7 M urea, 2 M thiourea containing 4% (w/v) 3-[(3-cholamidopropyl) dimethyammonio]-1-propanesulfonate, 1% (w/v) dithiothreitol, 2% (v/v) pharmalyte, and 1 mM benzamidine. Protein concentrations were determined using the Bradford method (Bradford, 1976) and 600 μg of each protein was loaded onto a 3–10 pH immobilized pH gradient strip (Amersham, USA), and 2D-separations were performed using sodium dodecyl-sulfate polyacrylamide gel electrophoresis (20 × 24 cm, 10–16%). The gels were fixed, stained with Coomassie Brilliant Blue G-250 solution, and de-stained with acetic acid.

Protein analysis

Two-dimensional pattern matching with protein spots was performed using PDQuest software (version 7.0, Bio-Rad). The quantity of each spot was normalized to the intensity of the total number of valid spots, and protein spots showing a significant accumulation change of more than double that of the control group (dried seeds) were selected. Protein spots were enzymatically digested in-gel using modified porcine trypsin (Shevchenko et al., 1996), followed by analysis and identification using the Bruker Autoflex maX with LIFT™ ion optics (Bruker, Bremen, Germany). Both mass spectrometry (MS) and tandem mass spectrometry (MS/MS) data were acquired using the default calibration of the instrument without applying internal or external calibration. MS/MS ion searches were performed using the Mascot license for in-house use. All procedures for obtaining images, including protein extraction and 2D electrophoresis, were performed using Genomine (Pohang, Korea).

For protein identification, the acquired MS and MS/MS data were compared with data from the NCBI-nr and SwissProt databases.

Data analysis

Two-way analysis of variance was conducted, and the means were compared using Tukey's honestly significant difference mean separation test at a probability level of 0.05.

Results

General characteristics of A. austrokoreense seeds

The *A. austrokoreense* seeds were brown-black in color and had an obovoid shape. The length and width of the seeds were 2.64–3.96 mm and 1.50–2.63 mm, respectively,

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and their 1000-seed weight was 2.71 ± 0.0024 g. A morphologically undeveloped embryo was observed in the cross section of *A. austrokoreense* seeds, and the ratio of embryo length to seed length (E:S ratio) at this time was 0.166 ± 0.047 (*Fig. 2*).

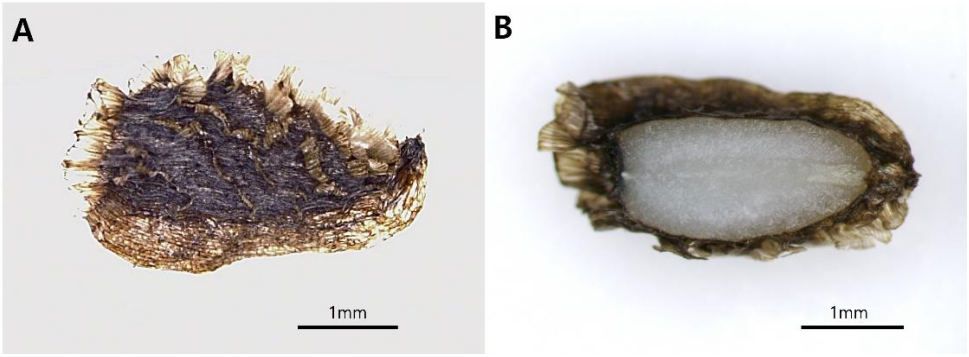

Figure 2. *Seed morphology of Aconitum austrokoreense. (A) Morphological characteristics of seeds. (B) Cross section of seed with undeveloped embryo*

This indicated that the embryos required morphological growth during germination. All *A. austrokoreense* seeds were able to absorb water immediately, and the seed weight increased by more than 50% after 24 h. Additionally, after 48 h, the absorption rate was 137.73 ± 0.002%. After 72 h, water absorption did not increase any further (*Fig. 3*).

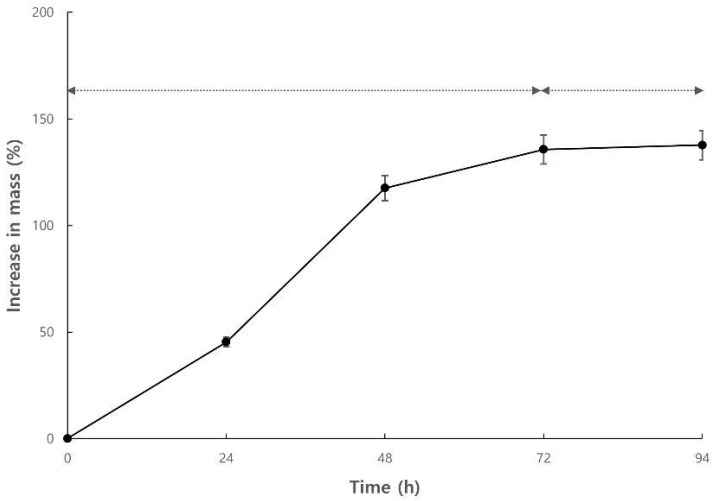

Figure 3. *Water absorption of Aconitum austrokoreense seeds*

Effect of 6-benzylaminopurine (BAP) on seed germination

Untreated seeds did not germinate at 15/6°C or 25/15°C (light/dark). Under BAP treatment, germination did not occur at 25/15°C, but occurred at 15/6°C (light/dark). The final germination of the seeds treated with 0.5, 1.0, and 5.0 mg/L BAP at 15/6°C was 9.0 ± 2.2%, 50.0 ± 2.6%, and 30.0 ± 1.3%, respectively, with a mean germination time (MGT) of 24.5 ± 0.90, 23.58 ± 1.55, and 25.84 ± 1.43 days, respectively. Seeds did not germinate under the 0.1 mg/L BAP treatment. These results demonstrate that BAP treatment affects the increase in germination and MGT (*Fig. 4*).

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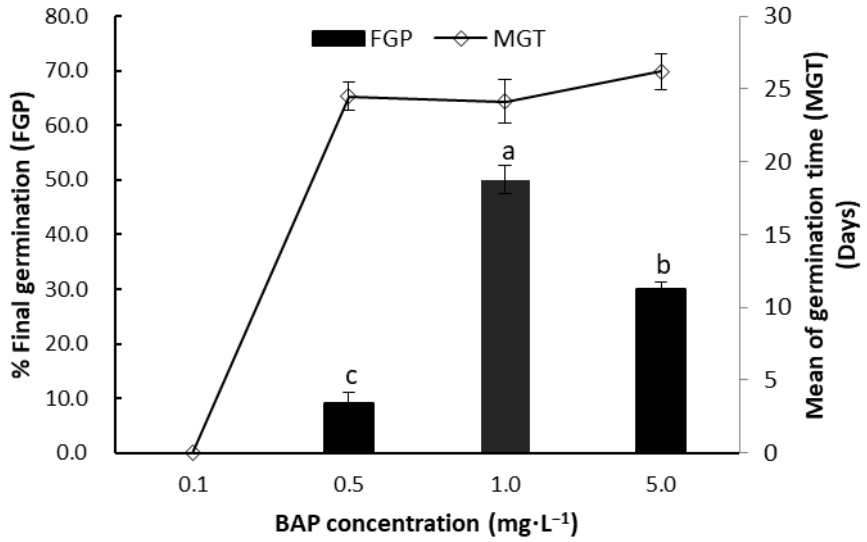

Figure 4. *Effect of 6-benzylaminopurine (BAP) concentration on Aconitum austrokoreense seed germination. Vertical bars represent mean ± standard deviation. Different letters indicate significant differences using Tukey' honestly significant difference test ($p < 0.05$)*

Moreover, embryonic development was observed after BAP treatment (*Fig. 5*). These results confirm that germination temperature and BAP treatment are important factors in *A. austrokoreense* seed germination.

Figure 5. *Embryonic development after 6-benzylaminopurine (BAP) treatment*

Protein changes in the germinating stage (phase II)

The days and BAP-treatments were confirmed to be significant in germination. Considering that BAP-treated seeds germinated from day 18, the period before that was considered as germination phase II. Therefore, proteins of BAP-treated seeds corresponding to day 17 were extracted as protein samples for germination phase II (*Fig. 6*).

The total number of proteins counted were those with an intensity value greater than 1000; 549, 519, 512, and 513 protein spots were observed in dried seeds, water-treated seeds, BAP-treated seeds (germination phase II), and germinated seeds, respectively. The change in spot intensity of each sample was investigated for increase, decrease, appearance, and disappearance and was compared with that of dry seeds (*Table 1; Fig. 7*).

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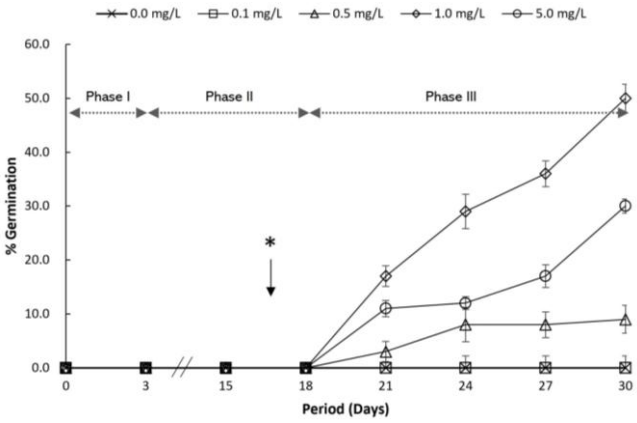

Figure 6. *Germination of Aconitum austrokoreense seeds cultivated on media containing different 6-benzylaminopurine (BAP) concentrations. *Proteins were analyzed on day 17*

Table 1. *Changes in the number of Aconitum austrokoreense seed proteins under different seed conditions*

Seed condition	Appeared	Disappeared	Increased	Decreased	Total
Dried (control)	–				549
BAP-treated (germination phase II)	20	7	220	89	519
Water-treated	21	46	219	88	512
Germinated	16	120	155	62	513

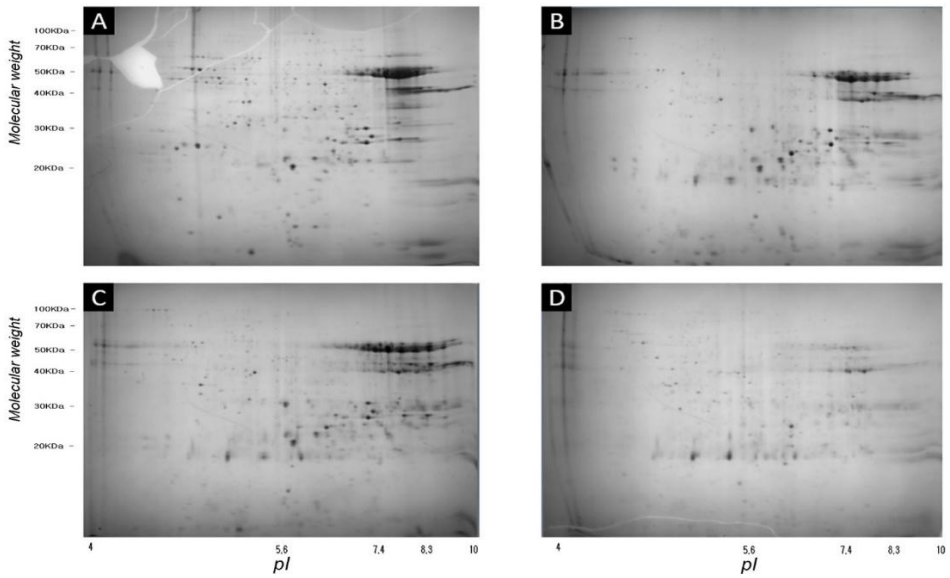

Figure 7. *Changes in spot intensity of each sample compared with that of dry seeds. Total protein patterns of (A) dried seeds, (B) BAP-treated seeds, (C) water-treated seeds, (D) germinated seeds*

Proteins in BAP-treated seeds were overexpressed compared with those under water-treatment and lowered (or disappeared) after germination. Accumulation of 40 proteins

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increased compared with that in dried seeds in germination phase II under BAP treatment (*Fig. 8A*). Moreover, 37 proteins in germination phase II under BAP treatment decreased compared with that in dried seeds and water-treated seeds (*Fig. 8B*).

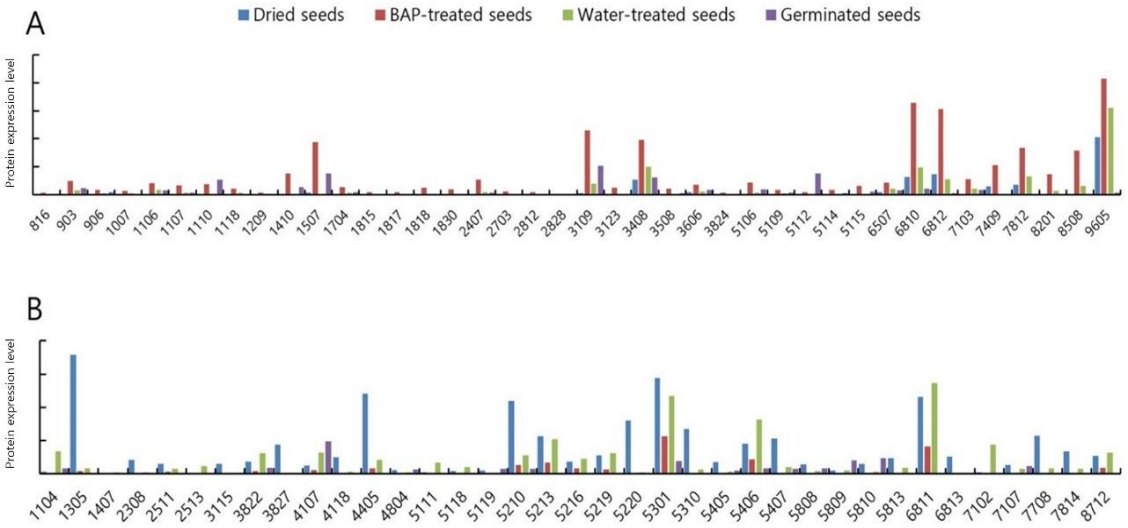

Figure 8. *Aconitum austrokoreense seed proteins with large degrees of change in their accumulation levels. (A) Spots with increased accumulation (40). (B) Spots with decreased accumulation (37) compared with those in dried seeds. BAP, 6-benzylaminopurine*

In particular, as a result of selecting points where the intensity value of BAP-treated seeds increased/decreased more than twice that of dried seeds, 12 spots were identified among proteins with increased accumulation and 11 spots were identified for proteins with decreased accumulation. The spots with increased protein accumulation were 1410, 1570, 3109, 3408, 6810, 6812, 7103, 7409, 7812, 8201, 8508, and 9605 (*Fig. 9*) and the spots with decreased protein accumulation were 1305, 4203, 4405, 5210, 5213, 5220, 5301, 5310, 5470, 6811, and 7708 (*Fig. 10*).

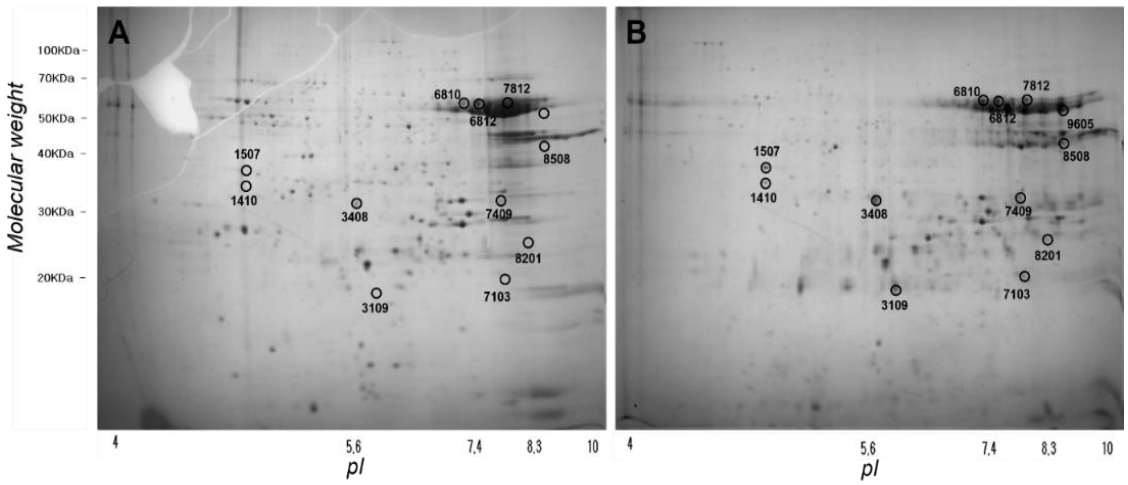

Figure 9. *Treatment with 6-benzylaminopurine (BAP) increases the accumulation of 12 proteins compared with that in dried seeds. (A) Dried seed. (B) BAP-treated seed. pI, isoelectric point*

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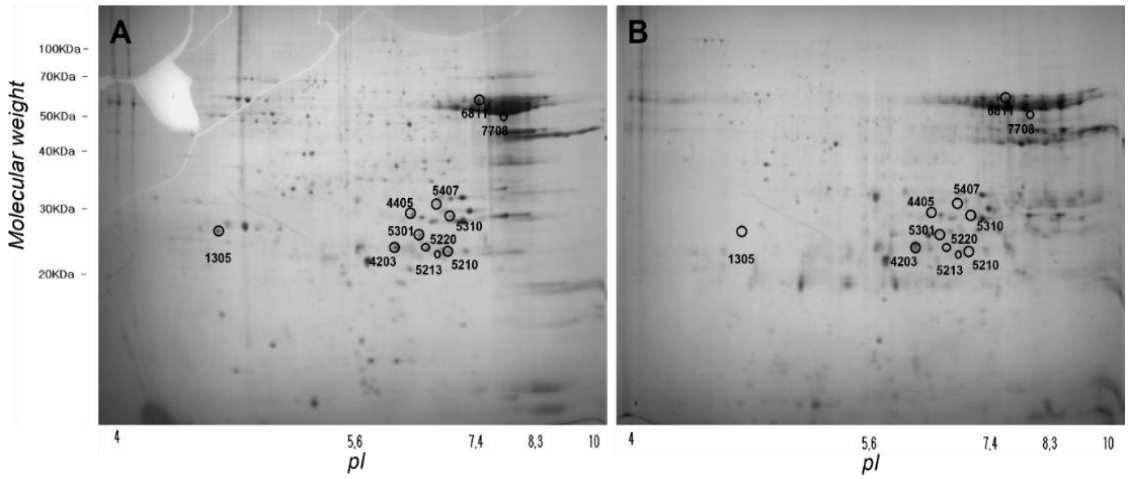

Figure 10. *Treatment with 6-benzylaminopurine (BAP) treatment decreases the accumulation of 11 proteins compared with that in dried seeds. (A) Dried seed. (B) BAP-treated seed. pI, isoelectric point*

Protein identification

The identity of the 11 proteins with decreased accumulation was not determined, while the 12 proteins with increased accumulation included several 30S ribosomal protein subunits, RNA polymerase subunits, and other proteins (*Table 2*). Moreover, spot 1410 and 7409 (cytochrome b_6-*f* complex subunit 8 and photosystem II protein D1, respectively) were confirmed to be specific germination phase II proteins that were not observed in water-treated seeds and disappeared or decreased after germination (*Fig. 11*).

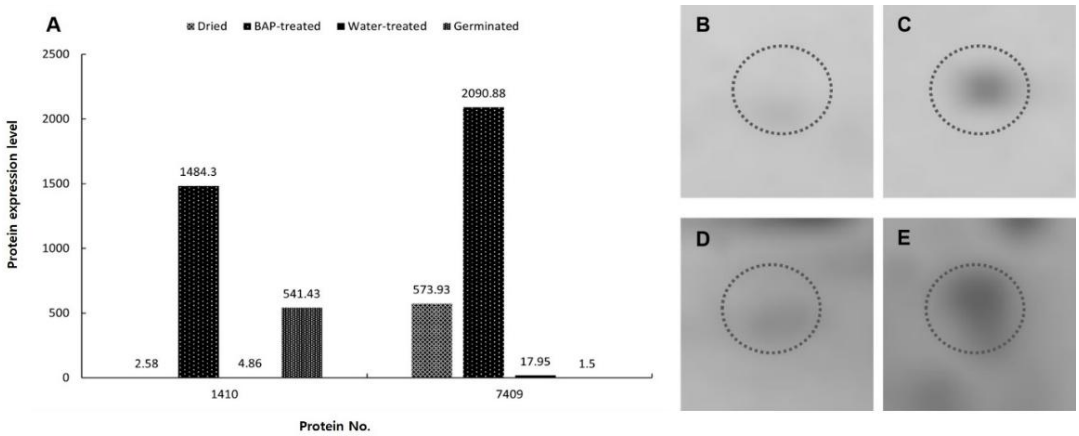

Figure 11. *The two proteins with increased accumulation levels after 6-benzylaminopurine (BAP) treatment. (A) Intensity of the 1410 and 7409 spots under each condition. (B) Spot 1410 of dried seeds. (C) Spot 1410 of BAP-treated seeds. (D) Spot 7409 of dried seeds. (E) Spot 7409 of BAP-treated seeds*

Discussion

Seed germination can be divided into phases I, II, and III according to the rate of change in water absorption (Weitbrecht et al., 2011). Phase II is the transformation stage before the emergence of the radicle, during which the seed prepares for

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germination or plant growth (Wolny et al., 2018). In particular, dormancy breaking caused by germination phase II is an essential step for promoting germination (Wolny et al., 2018; Cernac et al., 2006), and studies on the mechanism involved in breaking seed dormancy are limited, creating an urgency for in-depth research on dormancy-breaking mechanisms.

Wild plant seeds specifically display dormancy as a survival strategy (Fenner et al., 2005; Linkies et al., 2010; Silveira, 2013). Dormancy is classified into physical (PY), morphological (MD), physiological, morphophysiological (MPD), and combinational dormancy based on inhibitory factors (Baskin and Baskin, 1998; Kildisheva et al., 2020; Nikolaeva, 1977).

In general, seeds are considered impermeable to moisture when their initial seed weight increases by less than 20% after water absorption (Baskin and Baskin, 2003). This is a typical feature of physical dormancy, which suppresses water uptake using a thick seed coat (Baskin and Baskin, 1998). In this study, all seeds readily absorbed water; seed mass increased by more than 50% after 24 h and more than 100% after 48 h of steady water supply. Therefore, the *A. austrokoreense* seeds did not display PY or combinational dormancy.

Morphologically, a basal rudimentary embryo was observed in the brown-black oval-shaped seeds of *A. austrokoreense* (*Fig. 2*), and the E:S ratio of the seeds ranged from 0.166 to 0.047. Seeds with undeveloped embryos can be classified as MD or MPD and require additional time for embryo development (Baskin and Baskin, 1998, 2004; Campbell, 1946).

Most plants belonging to the genus *Aconitum* are native to mountainous regions and grow in shaded, cool, and moist environments (Srivastava, 2002; Park et al., 2016). Furthermore, the optimum germination temperature for wild plants in temperate regions is 24–30°C (Hartmann and Kester, 1975). Considering these two conditions, a general germination test was conducted at 25/15°C, and 15/6°C (day/night, 24/10 h). Germination did not occur, and the E:S ratio was calculated to be less than 0.320 (data not shown). MD and MPD are distinguished by the occurrence of general germination and embryonic development within 30 days under optimal environmental conditions (Baskin and Baskin, 2004). Therefore, the seed dormancy type of *A. austrokoreense* was MPD.

Seed dormancy is an evolutionary adaptation that prevents a seed from germinating under unfavorable conditions (Linkies et al., 2010). Therefore, understanding the mechanisms involved in dormancy breaking and inducing germination is important for the propagation of agricultural and horticultural plants (Zhang et al., 2021; Lee et al., 2015).

Plant hormones affect gene accumulation and transcription, cell division, and growth, even at low concentrations (Ku et al., 2018; Srivastava, 2002). BAP is a plant growth regulator that induces cell division along with plant growth and development (Sipes and Einset, 1983; Akhtar et al., 2020). BAP-induced germination in *Aconitum balfourii* and *Aconitum heterophyllum* has been reported (Rana and Sreenivasulu, 2013; Jabeen et al., 2006); similarly, *A. austrokoreense* seeds germinated after BAP treatment at 15/6°C (light/dark) (*Fig. 4*). Moreover, dormancy of *A. austrokoreense* seeds was broken after BAP treatment at concentrations of 0.5, 1.0, and 5.0 mg/L (*Fig. 6*), and the effect of different BAP concentrations on germination was statistically significant ($F = 23.208$, $p < 0.01$).

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Table 2. *Identification of the proteins with increased accumulation following BAP treatment*

Spot No.	Protein name	Organism	Expt. MW (KDa)	Expt. *pI*	No. of queries matched	Score	Seq coverage (%)	Sequence of the matched proteins	UniProt accession no.
1410	Cytochrome b_6-*f* complex subunit 8	*Gossypium barbadense*	35.13	4.95	4	24	86	M.DIVSLAWAALMVVFTFSLSLVVWGR.S	A0ZZ28
1507	30S ribosomal protein S15, chloroplastic	*Carica papaya*	37.68	4.94	1	11	7	R.KDYLSQR.G	B1A991
3109	RNA polymerase subunit beta	*Acorus americanus*	19.39	6.01	1	9	2	QDMPYLQDGTPVDMVFNPLGVPSR	A9LYH7
3408	30S ribosomal protein S7, chloroplastic	*Asarum canadense*	33.60	5.01	1	4	8	QAIRGVTPDIAVK	Q9GFN2
6810	30S ribosomal protein S19, chloroplastic	*Pisum sativum*	19.71	7.57	4	11	10	K.EHLPVYITDR.M	P31162
6812	30S ribosomal protein S7, chloroplastic	*Asarum canadense*	54.71	7.44	2	21	14	K.TETNPLSVLR.Q R.QAIRGVTPDIAVK.A	Q9GFN2
7103	30S ribosomal protein S7, chloroplastic	*Sciadopitys verticillate*	20.73	7.80	3	3	7	LVNMLVNRILK	Q6KGW1
7409	Photosystem II protein D1	*Illicium oligandrum*	32.15	7.77	2	1	3	VINTWADIINR	A6MMS4
7812	30S ribosomal protein S7, chloroplastic	*Asarum canadense*	55.17	7.85	3 7	21	14	K.TETNPLSVLR.Q R.QAIRGVTPDIAVK.A	Q9GFN2
8201	30S ribosomal protein S19, chloroplastic	*Pisum sativum*	25.93	8.07	3	13	10	K.EHLPVYITDR.M	P31162
8508	30S ribosomal protein S8, chloroplastic	*Nymphaea alba*	41.52	8.28	1	2	< 1	GIMTDREAR	Q6EW16
9605	30S ribosomal protein S8, chloroplastic	*Nymphaea alba*	50.00	8.22	1	1	< 1	GIMTDREAR	Q6EW16

BAP, 6-benzylaminopurine; Expt, experimental; No., number; Seq, sequence; *pI*, isoelectric point

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BAP-treated seeds were selected on the 17th day during germination phase II, and protein analysis was conducted (*Fig. 6*). Protein accumulation was higher in other seeds than in dry seeds. Therefore, seed proteins were activated to prepare for germination in phase II and 79 proteins were preferentially selected (*Fig. 8*).

Considering that BAP treatment influences protein activation during germination, spots showing little or no protein accumulation in water-treated seeds and overexpressed proteins in BAP-treated seeds were selected. Proteins with reduced accumulation levels in germinated seeds were also considered, indicating that these proteins were specifically overexpressed in the germinating state. Finally, 23 proteins were identified based on their accumulation levels. Among them, 12 proteins from BAP-treated seeds, which were overexpressed compared to those in water-treated seeds and reduced in accumulation compared to those in germinated seeds, were evaluated using the National Center for Biotechnology Information non-redundant protein sequence (NCBI-nr) and SwissProt databases. Protein identification confirmed the presence of a subunit of the 30S ribosomal protein and two characteristic proteins, the cytochrome b_6-*f* complex subunit and photosystem II protein D1 (*Table 2*).

Ribosomes are organelles found in all organisms, which synthesize proteins by recognizing codons in mRNA according to genetic instructions (Savir and Tlusty, 2013; Frank and Agrawal, 2000). Ribosomes comprise RNA and protein complexes, and small (30S) and large (50S) subunits (Cech, 2000; Yusupov et al., 2001). The 30S ribosomal subunit ensures the accuracy of translating genetic information into proteins by identifying transfer RNA (tRNA) that do not match the codons of the mRNA, along with the 50S ribosomal subunit, contributing to the translocation of tRNA by exactly one codon (Carter et al., 2000; Wimberly et al., 2000). Synthesized proteins catalyze intracellular biochemical reactions and transmit signals to perform essential roles, such as metabolism (Ardejani et al., 2017; Branden and Tooze, 2012). Here, the increased accumulation of the 30S ribosomal subunit in the germinating state (phase II) is a result of the activity of proteins to induce physiological changes to germinate seeds by overcoming dormancy. Thus, several physiological changes occur in seeds, causing a switch from minimal to active metabolism.

The protein identification analysis revealed two spots related to photosynthesis. Photosynthesis is a process by which plants convert light energy into chemical energy and begins when proteins known as reaction centers absorb light (Whitmarsh, 1999; Singhal et al., 2012; Orf et al., 2018). Reaction centers are complexes of several proteins, pigments, and other cofactors that execute the primary energy conversion reactions of photosynthesis (Gisriel et al., 2017; Lancaster and Michel, 2011). Molecular excitation and transfer of excitation energy derived directly from light result in electron transfer reactions along pathways involving a series of protein-binding co-factors (Gisriel et al., 2017; Sadekar et al., 2006). Representative light-harvesting complexes in plants have two types of reaction centers that are part of a larger super complex, known as photosystems II and I (Barber et al., 1997; Caffarri et al., 2014). The energy harvested by light is converted into usable electrons, which are then transported by electron transporters (Bendall and Manasse, 1995). Many electron acceptors have been reported in the electron transport chains of green plants, including pheophytin, quinone, plastoquinone, cytochrome *bf*, and ferredoxin (Bendall and Manasse, 1995). Spot 7409 was identified as photosystem II protein D1. Photosystem II is the first protein complex in a light-dependent reaction and provides electrons for photosynthesis using electrons from a water-splitting reaction (Caffarri et al., 2014; Ambreen et al.,

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2021). The D1 protein is a core protein of photosystem II, a group of proteins that constitute the photosynthetic reaction center (Zouni et al., 2001; Komenda, 2000). Spot 1410 was identified as a cytochrome b_6-*f* complex subunit, which facilitates the transfer of electrons between photosynthetic reaction center complexes, transferring electrons from photosystem II to photosystem I (Hasan et al., 2013; Nelson and Neumann, 1972). The overaccumulation patterns of these two proteins were observed in germination phase II of the seeds after BAP treatment (*Fig. 11*). Hence, the cotyledon on which the embryo grew was ready for photosynthesis immediately after germination.

When the seed dormancy of *A. austrokoreense* was disrupted by BAP, physiological changes were confirmed through the activation of 30S during germination phase II. In particular, the accumulation of photosystem II protein D1 and the cytochrome b_6-*f* complex subunit enabled photosynthesis immediately after germination, confirming that dormancy was completely broken during germination phase II. Although a direct factor influencing the breakage of dormancy was not found in germination phase II, differences in protein accumulation during germination were confirmed, and these could be used as a basis to understand the physiological changes in seeds during germination phase II.

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