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Original Research Article

Response of Germination Rate to Variable Drying Conditions and Moisture Contents for Storage of Dehisced Korean Ginseng Seeds

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Abstract - We compared the germination rate of dehisced ginseng (*Panax ginseng*) seeds that were dried under two different conditions, slowly at 15℃ [relative humidity (RH) 10-12%] and rapidly under a laminar airflow cabinet at 25℃ (RH 22-25%). The measurements showed that drying rate and seed moisture content (SMC) play important roles in storage ability and vigor. The seeds that were dried rapidly at 25℃ showed high GR compared with the seeds that were dried at 15℃ after 6 and 12 months of storage at -80℃ irrespective of MC. Seeds dried slowly at 15℃ with MC higher than 7.0% showed high GR maintenance after storage at -18℃ and at 4℃ in comparison with rapidly dried seeds. However, the GR of the slowly desiccated seeds decreased as mean SMC was reduced to less than 5.0%, whereas the rapidly dried seeds were distinguished by significantly high GR irrespective of the storage conditions. The ginseng seeds desiccated under different conditions showed differences in storage performance. Seeds with 7-9% MC that were dried slowly at 15℃ for 5-7 days showed high GR after 4℃ and -18℃ storage; however, longer periods of desiccation decreased the germination level remarkably compared with that of rapidly dried seeds.

Key words - Seed, Desiccation, Germination, Storage

Introduction

Developing a long-term storage methodology for various crop seeds and especially for ginseng seeds is considered crucial in scientific programs (Choi and Jeong, 2002; Lee *et al*., 2004; Yoon *et al*., 2005; Kim *et al*., 2008, Lee *et al*., 2016). The main factors associated with short seed longevity are seed water content (Vertucci, 1989; Pence, 1995; Pritchard *et al*., 1995), desiccation tolerance (Berjak *et al*., 1993; Pammenter *et al*., 1999; Wesley-Smith *et al*., 2001), storage conditions (Toole, 1960; Pritchard *et al*., 1995; Lee *et al*., 2004), and physio-biochemical state of the seeds (Koornneef *et al*., 2002: Kim *et al*., 2014), among others.

Many studies have cited protocols and developed gene bank guidelines for contained seed storage with international standards. Moreover, researchers have concentrated on finding the optimal moisture content that will facilitate longevity of seeds in storage and on developing protocols for long-term storage by using different approaches (Pritchard and Prendergast, 1986; Pence, 1992, 1995; Wesley-Smith *et al*., 1992; Chandel *et al*., 1995; Kim *et al*., 2008). However, excessive dehydration results in embryo death (Berjak *et al*., 1989; Vertucci *et al*., 1991; Pritchard *et al*., 1995), and studies have demonstrated that drying seeds beyond a certain critical seed moisture content provides little or no additional benefit to longevity (Dickie *et al*., 1990; Ellis and Hong, 2006; Nagel and Borner, 2009) and may even accelerate the rate of seed aging (Vertucci and Roos, 1990; Walters, 1998).

An efficient medium- and long-term storage technique has been reported for Korean red ginseng seeds (Lee *et al*., 2004; Yoon *et al*., 2005; Kim *et al*., 2008; Yi *et al*., 2015) and artificial seeds (Choi *et al*., 1999a; Choi and Jeong, 2002). Preliminary experiments performed with seeds or artificial seeds showed that they are capable of withstanding freezing after partial desiccation. However, after one year of cold storage, the dehiscence percentage of ginseng seeds was

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decreased to 38.7% and undehisced seeds failed to germinate (Lee *et al*., 1995). Additionally, a report by Lee *et al*. (2004) demonstrated that the germination percentage of ginseng seeds stored at 5℃ and 30% RH decreased after six years and decreased significantly to 2.5% after nine years. Kim *et al*. (2008) reported that determining the appropriate storage conditions for Korean ginseng seeds is difficult and noted that the lowest safe moisture content before loss of viability in the dehisced seeds was around 7.2% and that the high moisture freezing limit (HMFL) for cryopreservation was around 12.5%. These results suggest that it is necessary to continue research to identify the optimal conditions for the long-term storage of seed germplasm.

The goal of the present work was to investigate the efficiency of several long-term strategies with Korean ginseng seeds, namely, medium- and long-term storage of dehisced seeds, using two different dehydration methods with different seed moisture content (SMC) values, followed by precooling and rewarming treatments for seed storage in a deep-freezing chamber.

Materials and methods

Plant materials

The experiment was carried out at the National Agrobiodiversity Center, NAS, RDA (Republic of Korea). The seeds (*Panax ginseng* C.A. Meyer) used in this experiment were harvested and dehisced in 2013, and treated with a cold temperature (stratification) for 90 days. Investigation was begun in late February 2014. We used randomly selected seeds coated with endocarp in this study because seeds with different sizes have not shown significant differences in germination rate (Kim *et al*., 2014). The initial moisture content (IMC) and initial germination rate of the seeds (93.6% endocarp opened) were 55.9% and 84.0%, respectively. It should be noted that all experiments were started at the same time and that all seeds used were from the same harvest.

Measurement of moisture content and desiccation

Seed moisture content (SMC) was determined by drying the seeds in an oven at 105℃ for 24 h, followed by weighing the seeds (as presented below). To determine whether the seeds had reached the desired moisture content (DMC, %), seed moisture content was estimated by weighing the seeds before (initial seed weight, g) and after drying using the following formula:

Weight of seed (g) at DMC% = (100 - initial MC%) × initial seed weight (g) (100 − DMC%)

The rate of seed drying would depend on the amount of seed (particularly the depth of the layer of seeds), the circulation of dry air within the drying cabinet, and the species used. In this study, desiccation of seeds was carried out using two different methods. In the first (common) method, desiccation was carried out in a controlled drying room at 15℃ with relative humidity (RH) of 10-12% (Hong and Ellis, 1996). The second method was carried out by using a controllable growth airflow cabinet set at 25℃ with RH of about 22-23%. However, the MC of the seeds generally did not decrease below 4-5% after a certain period under the drying conditions. In this case, seeds were dried for 1 h in a drying oven at 40℃ until the desired level of MC was reached, followed by packing the seeds with laminated aluminum foil. For each treatment, 50 seeds and 5 replicates were used. For ginseng seeds, the high moisture freezing limit (HMFL), where freezing injury (ice crystallization peak) occurs, is around 12-13% (Kim *et al*., 2008); therefore, in our work, we used seeds with MC below 10%.

Medium- and long-term storage, precooling, rewarming, and germination of seeds

After desiccation for the desired MC (from 2% to 12%), the seeds were hermetically sealed in aluminum foil and divided into three groups. The first group of seeds was placed directly into a medium-term storage room (4℃) with no precooling, the second group of seeds was precooled at 2℃ for 24 h and then transferred into a long-term storage room (-18℃), and the third group of seeds was precooled and placed into a deep freezer (-80 ± 4.0℃) as follows. Before seed storage in the deep-freezing chamber, the seeds were hardened (precooled) slowly by decreasing the chamber temperature to 0 ± 0.3℃ in 1 h and then held for 24 h. Then, the temperature was reduced to -1.0℃ and the seeds were held for 3 h. Finally, the temperature was reduced further to -24.0℃ with 1 h intervals lowered in declines of 3℃. When the temperature reached the desired value (-24.0℃), the packed seeds were stored for 14 h and then transferred to the deep-freezer chamber.

Seed rewarming (recovery) from the deep storage was performed by rewarming the seeds with a water bath heater at 40℃ for 2 min.

To determine germination rate (GR), the dehisced seeds were sterilized with 1.0% sodium hypochlorite for 10 min followed by treatment with 70% ethanol for 1 min. Finally, the seeds were washed 5-6 times with distilled water. The sterilized seeds were dried in a laminar airflow cabinet at 25℃ for 3 h. Then, the dried seeds were placed in a moist paper towel that had been wetted with 200 mg/l^{-1} GA_3 solution; the moist paper towels were held within a large polythene box and placed into a controllable growth chamber at 10℃ (Yoon *et al*., 2005; Kim *et al*., 2008; Rajametov *et al*., 2014). The seed germination rate was tested by using 10-day to 70-day seed cultures with a 10-day interval, and the criterion for normal seed germination was the radicle reaching a length of more than 3 mm (Kim *et al*., 2008). Additionally, the vigor (growth rate of the radicle) of the seeds was determined. Data were developed after an interim analysis (30 days after bedding) and a final analysis (60-70 days after bedding).

Results

Desiccation of seeds under different drying conditions

Dehisced ginseng seeds were desiccated using two drying protocols, drying at 15℃ and using a controllable growth airflow cabinet set at 25℃. The MC of the seeds was reduced rapidly at 25℃ compared with that at 15℃. At 25℃, the MC reached the desired value (from 2% to 10%) within 1-5 days, whereas it took 5-40 days at 15℃ (Fig. 1). Thus, the desiccation of seeds at 25℃ reached the desired MC rapidly, whereas the desiccation at 15℃ reached it slowly. It should be noted that the SMCs obtained by drying at 25℃ were 3.2%, 4.4%, 6.3%, and 9.6%, and those for seeds dried at 15℃ were 2.2%, 4.4%, 7.2%, and 8.8%, respectively.

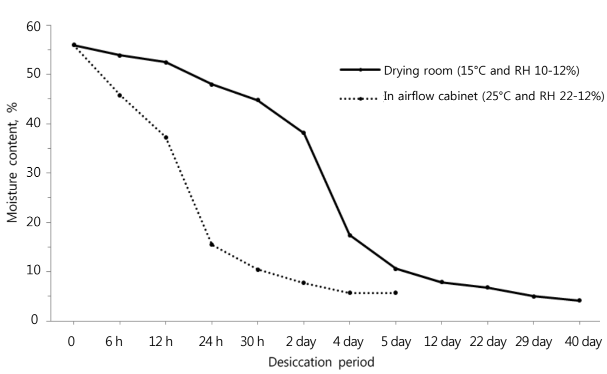

Fig. 1. Effect of the drying methods on seed desiccation rate.

Seed germination rate depends on desiccation and storage treatments

Response of the rapidly dried seeds (25℃) to storage conditions

The dehisced ginseng seeds that were dried at 25℃ and stored under the different conditions showed differences in vigor (Fig. 2). The seeds that were stored under the medium-term conditions (4℃) for 12 months, irrespective of MC, showed an increased germination rate compared with those of the 6-month storage period and reached a maximal value of 53.2% at 9.6% MC, compared with 40.8% for the 6-month storage. The minimal GRs of 30.8% and 38.0% for the 6- and 12-month storage treatments, respectively, were detected at 3.2% MC. However, for ginseng seeds with the storage period of 12 months, the vigor was decreased significantly at the interim analysis after 30 days of bedding for the seeds with 3.2% and 4.4% MC compared with the 6-month results, whereas for seeds with high MC, from 6.3% to 9.6%, the opposite trend, an increasing trend, occurred.

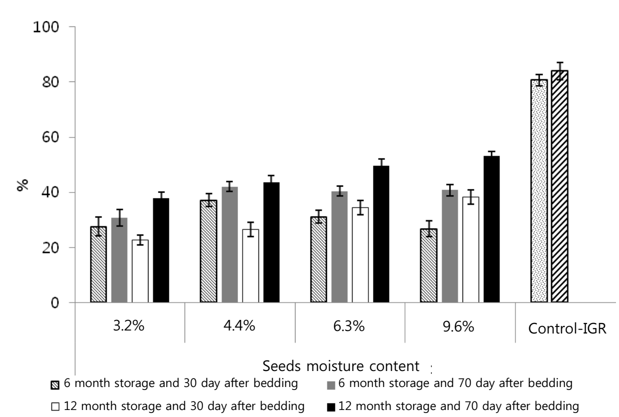

Fig. 2. Germination rate of ginseng seeds after drying at 25℃ and storage at +4℃. Data represented by mean ± SD (n = 5).

The same desiccated seeds were observed after storage at -18℃. In this case, different GR and vigor were also identified (Fig. 3). The seeds stored for the 6- and 12-month periods with MC below 4% and above 9% showed significantly low rates of germination. SMC between 4% and 7% was best for the long-term storage and showed GRs of more than 55%, whereas rates were lower for the medium-term storage (4℃). However, there was a noted reduction of vigor at the interim analysis following 30 days of bedding for the 12-month storage treatment.

Keeping the dehisced ginseng seeds in the deep freezer at -80℃ for 12 months decreased their vigor (Fig. 4). In the first GR test, the seeds that were stored for 6 months with MC above 6% showed high values and the same tendency was preserved after 12 months of storage. Additionally, in this case, a decrease of the seed vigor ability was observed from 6 to 12 months of storage, and a substantial reduction was seen after 30 days of bedding and 12 months of storage. The maximal GR was observed for seeds with an MC of about 10%.

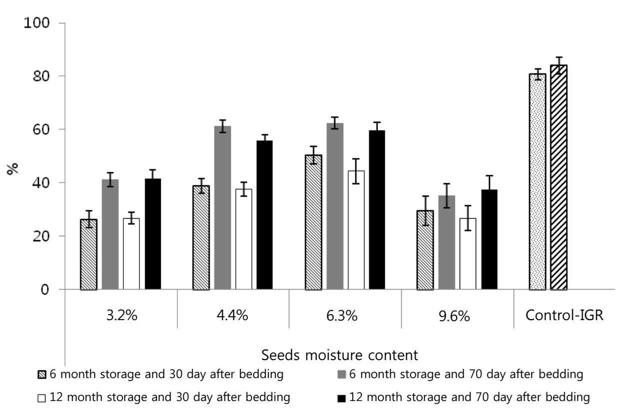

Fig. 3. Germination rate of ginseng seeds after drying at 25℃ and storage at -18℃. Data represented by mean ± SD (n = 5).

Response of the slowly dried seeds (15℃) to storage conditions

The seeds that were dried slowly showed a different response in storage ability compared with the rapidly dried seeds described above in the discussion of the rapid drying treatment. The dehisced ginseng seeds dried slowly at 15℃ with SMC below 5% showed low storage ability and GR; especially when the seed MC was 2.2%, the GR was almost equal to zero for the medium-term storage, the long-term storage, and the deep-freezing chamber (Fig. 5). For the medium-term storage (4℃), an increase of GR to more than 55% was revealed in seeds with MC between 7% and 9% in comparison with seeds that were dried rapidly, but the vigor of the seeds that were stored for 6 to 12 months was reduced negligibly at the interim and the final analysis of the seeds.

For the long-term storage conditions (-18℃), an unessential increase of 3-5% of the GR of seeds to more than 60% was observed for storage of 12 months compared with storage of 6 months, except for seeds with MC of 2.2% (Fig. 6). The same pattern was observed for the rapidly dried (at 25℃) seeds that were stored under the medium-term conditions. The highest level of GR of more than 60% was detected in seeds with MC from 7% to 9%.

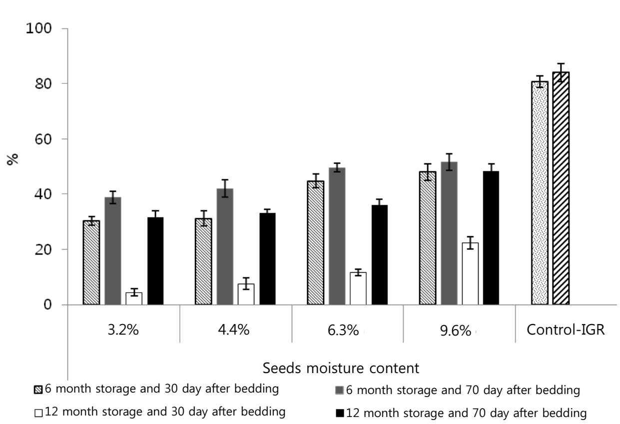

Fig. 4. Germination rate of ginseng seeds after drying at 25℃ and storage at -80℃. Data represented by mean ± SD (n = 5).

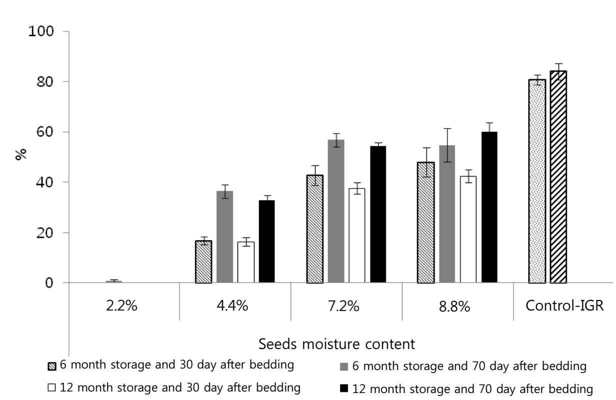

Fig. 5. Germination rate of ginseng seeds after drying at 15℃ and storage at 4℃. Data represented by mean ± SD (n = 5).

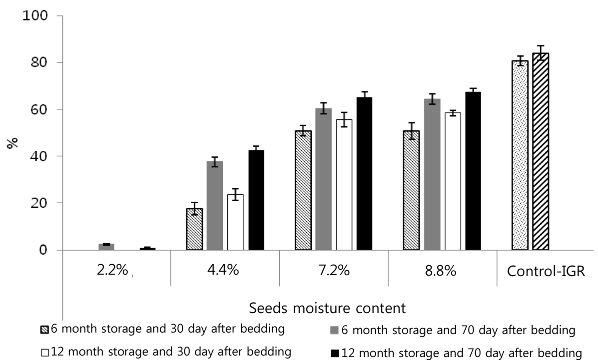

Fig. 6. Germination rate of ginseng seeds after drying at 15℃ and storage at -18℃. Data represented by mean ± SD (n = 5).

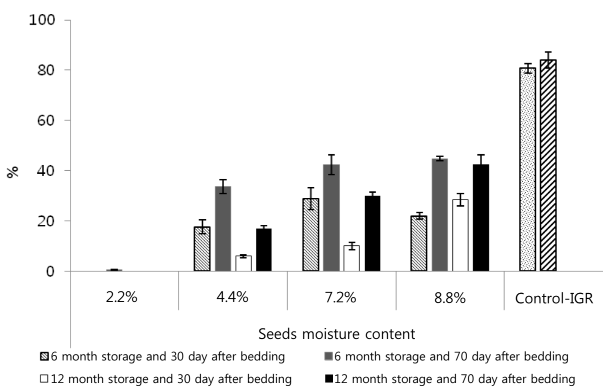

Fig. 7. Germination rate of ginseng seeds after drying at 15℃ and storage at -80℃. Data represented by mean ± SD (n = 5).

The storage behavior of the slowly dried ginseng seeds held in the deep-freezing chamber (-80℃) showed the same trend as revealed for the rapidly dried seeds, in which decreasing GR was observed for storage from 6 to 12 months and significantly reduced seed vigor ability was observed in the assay (Fig. 7). Irrespective of storage period, the maximal GR of more than 40% was detected in seeds with MC of around 9%. However, the slowly dehydrated seeds at MC lower than 4% showed no germination, whereas the rapidly dried seeds with MC lower than 4% showed a relatively high level of GR of more than 30%.

Discussion

This investigation compared the effect of the drying method and the sensitivity of seeds with different moisture contents to storage conditions; it identified different responses in rapidly dried and slowly dried seeds. Such differences depend on various factors, such as water content, physio-chemical state, rehydration, and ice crystallization peak (Ashworth, 1992; Sparks *et al*., 2001; Yoon *et al*., 2005).

Kim *et al*. (2008) reported that seed MC needs to be strictly controlled for routine application of cryopreservation to Korean ginseng seeds due to the morphological and physiological heterogeneity of the seeds (seed length and thickness, dehiscence) and that the hydration window for cryopreservation of Korean ginseng seeds is narrow, namely 8-11%.

In the case of seed storage in a deep freezer, we used pre- and post-treatment techniques, as presented in the methodology section, and found that the high-moisture freezing limit for ginseng seeds is around 12-13% and that MC above 13.5% results in the occurrence of ice crystallization (Kim *et al*., 2008), which leads to cell wall injury by the formation of irregular and different shapes of ice in the extracellular and intracellular regions, causing an imbalance in the cell water balance (Leprince *et al*., 2000) and tension (Rajashekar and Burke, 1996).

It has been revealed that the onset temperature of crystallization during the cooling of ginseng seeds increased from -30 to -10.8℃ when seed MC was increased from 14% to 58.8% and that the ice melting peak for seeds is above the MC of 13.5% (Kim *et al*., 2008). Additionally, there is a lack of information on the response of seeds to the air deep freezing condition and the methods of storage at deep-freezing chamber temperature, which is as low as -80℃. It is known that the response of seeds to the slow air freezing (in the case of the deep freezer) and the rapid liquid nitrogen freezing states is different. To avoid ice crystallization in the deep freezer, we used precooling and rewarming treatments.

Kim *et al*. (2008) also noted that the three desiccation methods did not affect the germination percentage in desiccated ginseng seeds. The lowest safe moisture content (LSMC), where loss of viability occurs in dehisced seeds, was around 7.2%. The critical point of GR (34.9%) was detected for seeds with 5.3% MC during cryopreservation. According to Lee *et al*. (2004), in medium-term storage conditions at 5℃ with 30% RH and 12% MC, the GR of ginseng seeds was above 70% for seven years, but dropped significantly to 2.5% after nine years, and the storage period also affected the radicle and shoot growth, which was also observed in our present work (data not shown).

In our study, the drying methods showed different GRs and significantly affected seeds, which had essentially low GR values compared with those of control seeds (initial GR) with initial MC. It should be noted that we did not investigate seeds with MC of more than 10% to 20%, as noted above; seeds with MC of more 12% can be stored well under medium- and long-term storage conditions compared with those with low MC below 10%. This would be better for use in further research of medium- and long-term storage, especially in the deep-freezing chamber conditions, which in our case showed high GR in rapidly dried seeds that had about 10% MC.

Lowered MC reduces the likelihood of freezing of recalcitrant seed embryos at sub-freezing temperatures (Becwar *et al*., 1983; Vertucci *et al*., 1991), but excessive dehydration results in embryo death (Berjak *et al*., 1989; Pence, 1990; Pritchard *et al*., 1995). Slow drying with intact seeds reduced embryo growth ability and different crops have shown differential critical water contents (Pritchard *et al*. 1995). Contents below the critical point are lethal, as drying rate affects the distribution of water within axes, which could be relevant to the level of stress experienced by germinated cells (Wesley-Smith *et al*., 2001). According to our results for rapidly dried seeds, the LSMC is around 6-7% for medium-term storage in the deep freezer and above 4% for long-term storage. For medium- and long-term storage of slowly dried seeds in a deep freezer, LSMC must be 7%. These results suggest that the same seeds have different desiccation tolerance, storage ability, and vigor. The mechanisms by which rapid drying allows lower water contents to be tolerated in recalcitrant materials have not been fully elucidated, but evidence indicates that the duration of the benefit is short (Vertucci and Farrant, 1995; Walters *et al*., 2001b).

It is assumed that the normal functions of the cell may be perturbed at water contents intermediate between full hydration and the lower limit of survival, resulting in deleterious reactions due to unregulated metabolism (Berjak and Pammenter, 1997; Leprince *et al*., 2000; Walters *et al*., 2001b). In addition, the comparative decrease of GR in ginseng seeds with MC below 7%, dried at 15℃ in our case, can be related to the long period of desiccation, which may lead to seed aging (King and Roberts, 1980; Chandel *et al*., 1995; Walters *et al*., 2001b) or seed coat cracking (Hong *et al*., 2005), whereas the rapidly dried seeds (at 25℃) showed stable rates of GR irrespective of MC.

Wesley-Smith *et al*. (2001) found that the common cause of the desiccation damage, which became evident on rehydration, was reversible following rapid drying, but was more severe in axes that were dried slowly. The area occupied by vacuoles increased significantly only following rehydration, and this was similar in axes dried rapidly or slowly. Prolonged exposure to partial hydration may contribute greater sensitivity to vacuoles, causing damage during rehydration resulting in membrane breakdown in cells in either rapidly or slowly dried treatments.

However, it is commonly stated in the literature that the primary site of desiccation injury of the cell is the membrane, which causes dysfunctional metabolism (e.g., Senaratna and McKersie, 1986; Vertucci, 1993; Salmen Espindola *et al*., 1994; Leprince *et al*., 1999).

Walters *et al*. (2001b) proposed that the early damage caused by dehydration stress may be indirect and reversible; however, with sufficient time at water potentials (Leprince *et al*., 2000; Walters *et al*., 2001a), respiration rate (Leprince *et al*., 1999) can initiate events analogous to aging and lead to cell death. We assumed that cell functional ability was not significantly destroyed in seeds dried rapidly for a short period with MC from 6% to 10%, whereas in cells are continued physio-chemical synthesis, as in the common condition, without any membrane breakdown and did not feel extremal osmotic stress. In slowly dried seeds, owing to long dehydration, mainly when MC reaches values below 7%, which may require more than 3 weeks, cells experience metabolic stress and disorders, which contribute to a reduction of cell size, vacuoles, plasmalemma, and plastids, among others, as well as the acquisition of abnormal shapes and the depletion of cell sap concentration. It is well known that, during the dormancy stage (in December), ginseng seeds show significantly high embryo growth rates, total endogenous GA, lipids, proteins, and carbohydrates, as well as decreasing ABA contents (Kim *et al*., 2008; Kim *et al*., 2014). The maximal embryo growth rate is reached in early March, when the seeds are dehisced and we see the opposite tendency of decreasing GA, lipids, proteins, and carbohydrates, and increasing ABA. Eventually, ABA acts as a key regulator in seeds for water loss, desiccation, and maturation during late embryogenesis (Aalen, 1999; Black *et al*. 1999). Moreover, both GA and BA play an important role in seed dormancy and germination (Koornneef *et al*., 2002; Nambara *et al*., 2010; Kim *et al*., 2014). This tendency of seed chemical synthesis associated with different MC may affect cell physiological functions, especially cell sap metabolism during long-term drying and after storage rehydration and in rapid drying.

As we reported previously (Rajametov *et al*., 2014), pre-storage treatments are not the only main factor for long-term conservation (cryopreservation) of ginseng seeds, as post-storage treatments such as rewarming and germination medium are also important factors that can significantly affect the vigor and growth ability of the seeds. In particular, these should be applied to seeds that are stored at under freezing conditions.

In conclusion, ginseng seeds dried under different conditions have differences in responses to storage at low temperatures. Seeds with MC of about 7-9% that were dried slowly at 15℃ for 5-7 days showed high GR after medium-term (4℃) and long-term (-18℃) storage; however, the longer desiccation period greatly decreased the GR when compared with rapid drying. Moreover, in all treatment conditions, seeds were identified to have reduced vigor, such as of radicle and shoot, after the storage period.

Therefore, in future research to find the optimal storage conditions for undehisced and dehisced ginseng seeds, it will be very important to pay close attention to seed MC, dormancy period, stage of embryo growth ratio, respiration rate, and chemical conditions because all of these are strongly related to storage and the post-treatment growth metabolic process.

Acknowledgment

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