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Germination, osmotic adjustment, and antioxidant enzyme activities of gibberellin-pretreated *Picea asperata* seeds under water stress

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Abstract Germination of dragon spruce (*Picea asperata* Mast.) seeds pretreated with gibberellin (GA) in response to water stress and changes in the levels of osmotic adjustments as well as in activities of antioxidant enzymes were investigated. With decreasing water potential caused by increasing concentrations of PEG 6000, germination percentage and germination index decreased gradually; the decrease was especially prominent under the serious water stress from PEG −0.6 MPa. In contrast, osmotic regulation substances (free proline and soluble sugar contents), lipid peroxidation (MDA), and activities of antioxidant enzyme (ascorbate peroxidase, catalase, and peroxidase) increased markedly with decreased water potential. Similarly, the values in all parameters under −0.6 MPa PEG treatment were markedly higher than the control and −0.2 MPa PEG treatment. These results suggested that *P. asperata* seed germination was insensitive to water stress. In addition, seeds pretreated with GA had increased tolerance to water stress as measured by germination percentage and germination index, osmotic regulation substance, and antioxidant enzyme activities.

Keywords Antioxidant enzymes · Germination · Gibberellin · Osmotic adjustment · Water stress

Abbreviations
APX Ascorbate peroxidase
CAT Catalase

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EDTA	Ethylenediaminetetraacetic acid
GA	Gibberellin
MDA	Lipid peroxidation
PEG	Polyethylene glycol
POD	Peroxidase
PVP	Polyvinylpyrrolidone
TCA	Trichloro acetic acid

Introduction

Seed germination is affected by many factors, such as temperature, light, and soil water content. The availability of retrievable water in the soil is considered the most important factor for controlling seed germination (Sharma 1973; Sy et al. 2001). This might be due to the fact that water influences the progress and kinetics of most biologically significant reactions. As a solvent, water provides a fluid medium through which substrates diffuse to active sites and enzymes undergo the conformational changes necessary for catalytic activity. Research has shown that water stress strongly inhibited germination at extremely high or extremely low temperatures (Zheng et al. 2004), and it may also reduce the probability of seedling establishment because of the effect of low soil water emergence, content on seedling survival, and growth of surviving seedlings (Shao et al. 2008a; Villalobos and Peláez 2001).

The development of a plant from the seed comprises the germination phase, seedling survival, and subsequent growth of the plant. For woody plants with potentially long lifespan and low post-establishment mortality, seedling recruitment is a critical life-history stage (Scholes and Archer 1997). The physiological responses of plants to water deficits change in relation to their sensitivity to stress (Carvalho et al. 2005; Shao et al. 2008b).

Gibberellin (GA) is an important endogenous growth regulator that has profound and various effects on regulating plant growth, development, and stress responses (Huerta et al. 2008; Sun et al. 2008). Specific roles of GA include the induction of seed germination, promotion of hypocotyls, stem elongation, regulation of pollen development and flower initiation, and wood formation (Mauriat and Moritz 2009; Richards et al. 2001; Ritchie and Gilroy 1998). Many previous studies have shown that exogenous GA reduces the minimum effective exposure time to the germination stimulant and promotes seed germination (Chae et al. 2004; Song et al. 2005a, b). A similar study found that paclobutrazol (an inhibitor of GA biosynthesis) applied during the conditioning period reduced seed germination (Zehhar et al. 2002). As is well known, germination growth is accompanied by water uptake and, thus, requires disequilibrium in water potential between the seed and its environment. For this reason, Liu et al. (1996) hypothesized that GA lowers seed water potential and may, thereby, promote germination.

Picea asperata is one of the dominant conifers of the subalpine forested areas in the western Sichuan province of China. However, very few spruce seedlings or saplings could be found among the undergrowths (Yin et al. 2007), which might be due to low seed germination percentage and high seedling mortality of the *P. asperata* forest. Wu et al. (2004) reported the impacts of light and temperature on the germination of *P. asperata*

seeds. Few studies documented the response of *P. asperata* seeds to the water conditions in this region. With the changing of management practices associated with subalpine coniferous plantations from timber- oriented to ecology- oriented, it is important to understand whether *P. asperata* plantations can self-regenerate, and, if so, how to control their natural regeneration (Yin et al. 2007). In addition, the understanding of seed germination physiology of a species can help evaluate safe-site availability because seed dormancy/germination traits determine the number and timing of seedling emergence (Shimono and Washitani 2004); however, few reports are available about the physiological properties of exogenous GA related to seeds and their response to water stress. As a result, in the present study, germination, osmotic adjustment, MDA, and the corresponding activities of catalase (CAT), ascorbate peroxidase (APX), and peroxidase (POD) of *P. asperata* seeds that had been pretreated with GA and subjected to water stress were studied. The present investigation also offers a reference opinion and a theoretical basis for improving the germination of *P. asperata* seeds and understanding the natural regeneration progress of *P. asperata* forest areas.

Materials and methods

Plant materials

Picea asperata seeds were harvested in October 2007 from the Miyaluo area (102°50′E, 31°50′N, 2,800 m a.s.l.), Sichuan province, China and kept at 4°C until treatment (March, 2008).

Experimental procedures

Germination tests were conducted from 26 June to 10 July 2008 as follows: *P. asperata* seeds of uniform size were surface sterilized with 10:1 water/bleach (5.25% w/v NaOCl) solution for about 15 min and then rinsed with distilled water. Disinfected seeds were soaked in either 100 mg l^{-1} GA solution or distilled water (control) for 24 h. Germination and early seedling growth of the cultivar were studied using distilled water (control) and under osmotic potentials of −0.2 (slight water stress) and −0.6 MPa (moderate water stress) for polyethylene glycol (PEG 6000) (Michael and Kaufaman 1973). In the present study, −0.2 and −0.6 MPa were designed as slight water stress and moderate water stress, respectively (Kaya et al. 2006; Zhu et al. 2005). Five replicates of 50 seeds each were tested for germination on two sheets of filter paper (previously moistened with 4 ml distilled water, which was periodically added) in 9-cm diameter glass Petri dishes. Incubation took place in a 16 h light/8 h dark photoperiod under a photon flux density of 120 μmol m^{-2} s^{-1} (determined by CI-301 PS, CID Inc., USA) and constant temperature regimes of 20°C. Germinated seeds were counted and placed into other moistened Petri dishes (a new Petri dish for each treatment) every 24 h throughout the incubation period. The germination criterion was the emergence of the radical through the seed coat. Germination index (Gi) was calculated on the basis of germination percentage as: $Gi = \sum Gt/Dt$, where Gt is the percentage of seed germinated at 2 days intervals, and Dt is the number of germination days (Huang et al. 2003). Seeds treated for 15 days were sampled for a determination of the biochemical parameters. The sample contained the germinated seeds and seedlings.

Free proline content

Free proline was extracted and determined according to the procedure used by Bates et al. (1973). Samples of 0.3 g were homogenized in 5 ml 3% aqueous sulphosalycylic acid and then were centrifuged at 3,000×*g* for 20 min. The supernatant was treated with ninhydrin dissolved in acetic acid and boiled for 1 h; next, absorbance at 520 nm was recorded with a calibration curve. Free proline content was expressed as mg g^{-1} seed.

Lipid peroxidation and soluble sugar

Lipid peroxidation was determined by estimating the malondialdehyde (MDA) content with slight modification (Heath and Packer 1968). Samples of 0.3 g were macerated in 5 ml of 0.1% trichloroacetic acid. The homogenate was centrifuged at 10,000×*g* for 5 min for every 1 ml of the aliquot of the supernate, 4 ml of 20% Trichloro acetic acid (TCA) containing 0.5% thiobarbituric acid was added. The mixture was heated at 95°C for 30 min, then cooled quickly on an ice bath; the resulting mixture was centrifuged at 10,000×*g* for 15 min. The concentration of MDA was calculated from the absorbance at 532 nm (correction was done by subtracting the absorbance at 600 nm for unspecific turbidity) by using the extinction coefficient of 155 mM cm^{-1}.

The total soluble sugars were extracted from samples by refluxing in 80% ethanol as described by Cerning and Guilbot (1973), and quantitative determination was carried out as described by Yemm and Willis (1954).

Enzyme assay

Enzyme extraction was conducted based on the method described by Chen and Sung (2001). Samples of 0.3 g were homogenized in a prechilled mortar and pestle in extraction medium at 4°C. The extraction medium contained 50 mM potassium phosphate buffer (pH7.4), 1 mM ethylenediaminetetraacetic acid (EDTA), 2% (w/v) polyvinylpyrrolidone (PVP), and 0.05% (v/v) Triton X-100. The homogenate was centrifuged at 12,000×*g* for 20 min at 4°C, and then the supernatant was kept at −20°C and used for enzyme activity and protein content assays. All assays were conducted at 4°C. Total soluble protein contents of the enzyme extracts were determined according to Bradford (1976) using BSA as a standard.

Following Luck (1974), catalase (CAT, EC 1.11.1.6) activity was determined in the homogenates by directly measuring the induction of H_2O_2 in absorbance at 240 nm, the reaction medium containing 50 mM potassium phosphate buffer (pH 7.0), 10 mM H_2O_2, and the 0.1 ml enzyme extract in a final volume of 3 ml at 25°C. CAT activity was determined using the extinction coefficient (40 mM^{-1} cm^{-1}) for H_2O_2.

Ascorbate peroxidase activity (APX, EC 1.11.1.11) was analyzed according to Nakano and Asada (1981) by monitoring the decrease at 290 nm (extinction coefficient 29 mM^{-1} cm^{-1}) for 1 min. The reaction mixture contained 50 mM sodium phosphate buffer (pH 7.0), 0.1 mM EDTA, 1 mM ascorbate acid, 2.5 mM H_2O_2, and 50 µl of enzyme extract.

Peroxidase activity (POD, EC 1.11.1.7) was based on the method described by Castillo et al. (1984): increase in optical density due to the formation of tetra-guaiacol. The 3 ml reaction mixture contained 10 mM guaiacol, 50 mM H_2O_2, 50 mM phosphate buffer (pH 6.0), and 50 µl enzyme extract. Absorbance was recorded at 470 nm and was calculated using the extinction coefficient 26.6 mM^{-1} cm^{-1}.

Statistical analyses

Statistical analyses were conducted with SPSS11.0 statistical software package for Windows. The experiment was a split-plot design. Three levels of PEG treatment were taken as main plots and nested into two levels of GA pretreatment, which was vice plot. Thus, we had 6 treatments, and each treatment was duplicated 5 times. ANOVA were applied to test the difference between GA pretreatment and water stress treatment. Data in all of the figures and tables are shown as means ± SE.

Results

Germination percentage and index

In the distilled water and GA pretreatment, PEG-induced germination percentages were lower compared to those under controlled treatment (Fig. 1). However, the magnitude of the decrease was more pronounced under the −0.6 MPa conditions (decreased by 91.95 and 73.03%, respectively) than that under the −0.2 MPa conditions, where the decrease was 13.64 and 15.73%, respectively (Fig. 1, $P < 0.001$). In addition, GA-pretreated seeds showed a higher final germination percentage compared to seeds that had been pretreated with distilled water (Fig. 2, $P < 0.05$). Significantly interactive effect between GA and PEG treatment was not found in final percentage of germination (Fig. 1, $P > 0.05$).

The index of germination (Fig. 2) reached a minimum value under the −0.6 MPa conditions. In contrast to the control treatment, the germination index in both distilled-water-and GA-pretreated seeds decreased 88.06 and 79.82%, respectively, under the −0.6 MPa conditions. There was no statistically significant change in the control and the −0.2 MPa treatment.

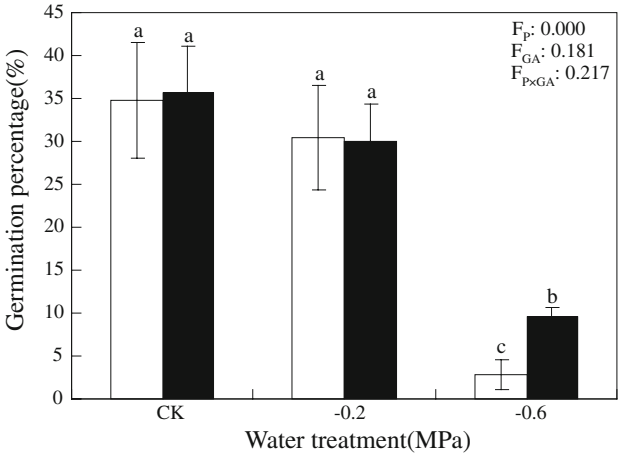

Fig. 1 Germination, osmotic adjustment and antioxidant enzymes activities of *Picea asperata* seeds pretreated by gibberellin under water stress. *Open square*, distilled water treatment; *filled square*, gibberellin pretreatment. *Different letters* above the *bars* indicated significantly differences between treatments ($P < 0.05$, $n = 5$). Data were shown as means ± SE. *F*p, PEG effect; F_{GA}: gibberellin effect. $F_{P\times GA}$, PEG × gibberellin interactive effect

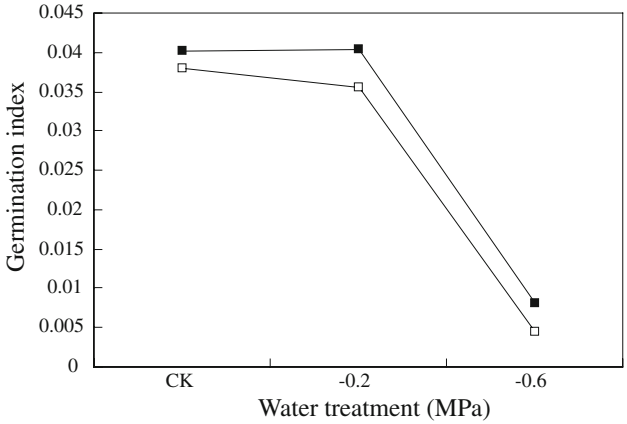

Fig. 2 Germination, osmotic adjustment and antioxidant enzymes activities of *Picea asperata* seeds pretreated by gibberellin under water stress. *Open square*, distilled water treatment; *filled square*, gibberellin treatment. The *different letters* above the *bars* indicated significantly differences from each other ($P < 0.05$, $n = 5$)

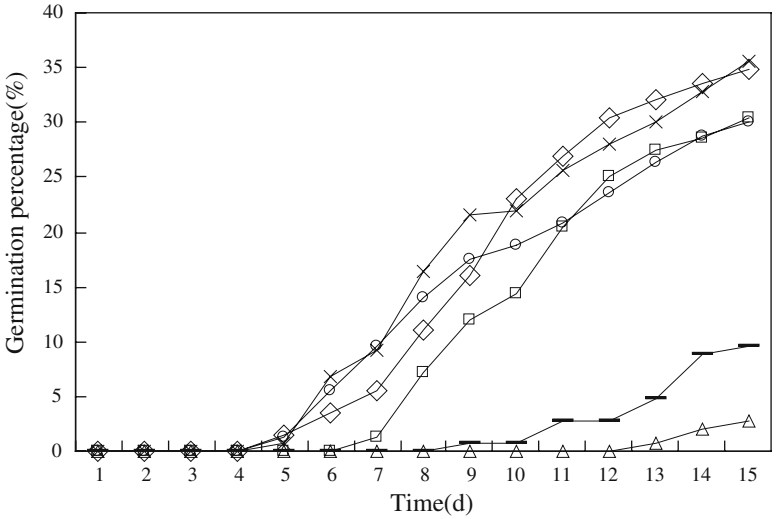

Fig. 3 Germination, osmotic adjustment and antioxidant enzymes activities of *Picea asperata* seeds pretreated by gibberellin under water stress. *Multi symbol*, gibberellin-stimulated seed and PEG 0 MPa, *circle*, gibberellin-pretreated seed and PEG −0.2 MPa, *solid line*, gibberellin-pretreated seed and PEG −0.6 MPa; *diamond*, distilled water pretreated seed and 0 MPa, *square*, distilled water pretreated seed and −0.2 MPa, *triangle*, distilled water pretreated seed and −0.6 MPa). The *different letters* above the *bars* indicated significantly differences from each other ($P < 0.05$, $n = 5$). To keep the figure clear *bars* standing for SE were omitted

Germination was initiated 5 days after PEG-treatment and reached approximately 35% after 15 days (Figs. 1, 3); a gradual increase in the germination percentage was found with all treatments from the 5th day to 15th day. As Fig. 3 shows, under both control and −0.2 MPa conditions, although the germination percentage in GA-pretreated seeds

initially increased more rapidly compared to the control treatment, the germination percentage in GA and distilled water pretreatments finally reached the same value by the end of the experimental period. However, under the −0.6 MPa conditions, GA-pretreated seeds had a higher germination percentage compared to the control conditions throughout the experiment (Fig. 3).

Free proline, MDA, and soluble sugar content

Under the −0.2 MPa conditions, free proline content in distilled-water- and GA- pretreated seeds increased by 26.34 and 40.00%, respectively, compared to the control treatment. However, under the −0.6 MPa conditions, it increased 94.74 and 215% as compared to the control treatment. These results indicated that PEG treatment increased free proline content significantly, but the increase under the −0.6 MPa conditions was more evident than that under the −0.2 MPa conditions. On the other hand, after the same PEG treatment, free proline content in distilled-water-pretreated seeds exhibited a lower value compared to GA-pretreated seeds.

The lipid peroxidation level in *P. asperata* seeds, measured as MDA, is provided in Table 1. The level of MDA after both distilled-water and GA pretreatments changed with decreasing PEG treatment. MDA content in distilled-water and GA pretreatments increased 22.22 and 157.14%, respectively, under the −0.2 MPa conditions, and it increased 38.89 and 285.71%, respectively, under the −0.6 MPa conditions. The interaction between PEG treatment and GA pretreatment was also significant on free proline and MDA ($P < 0.001$, Table 1).

Soluble sugar content increased with decreasing PEG treatment with both distilled-water and GA pretreatment. In addition, GA-pretreated seeds showed higher content in soluble sugar as compared to those under distilled water pretreatment ($P < 0.05$, Table 1). No significant interaction between PEG treatment and GA pretreatment was found in soluble sugar content.

Table 1 The effect of PEG treatment on free proline, MDA and soluble sugar contents of gibberellin-pretreated *Picea asperata* seeds for 15-days

Treatment	Pro (mg g^{-1})	MDA (nmol g^{-1})	Soluble sugar (mg g^{-1})
H_2O			
CK	0.19 ± 0.03d	0.18 ± 0.02d	6.08 ± 0.37d
−0.2 MPa	0.24 ± 0.01c	0.22 ± 0.04 cd	7.04 ± 0.29 cd
−0.6 MPa	0.37 ± 0.02b	0.25 ± 0.03c	10.25 ± 0.19b
GA			
CK	0.20 ± 0.02d	0.21 ± 0.01 cd	7.16 ± 0.44 cd
−0.2 MPa	0.28 ± 0.04c	0.54 ± 0.05b	9.39 ± 0.62bc
−0.6 MPa	0.63 ± 0.04a	0.81 ± 0.05a	12.71 ± 3.18a
F_P	0.000	0.000	0.000
F_{GA}	0.000	0.000	0.010
$F_{p\times GA}$	0.000	0.000	0.627

F_P, PEG effect, F_{GA}, gibberellin effect, $F_{P\times GA}$, PEG × gibberellin interactive effects. Data were shown as means ± SE. Different letters in the table indicated significantly differences between treatments ($P < 0.05$, $n = 5$)

Antioxidant enzymes activities

The activities of CAT, APX, and POD were given in Table 2. A gradual increase in the activities of CAT, APX, and POD were observed under both GA and distilled-water pretreatment with decreasing PEG treatment. On the other hand, under the same PEG conditions, GA-pretreated seeds showed substantially lower activity in CAT and POD compared with distilled-water-pretreated seeds, but there were no statistically significant changes in APX activities with the same PEG treatment.

Discussion

Seed germination has been the critical stage for species survival. Water stress may result in delayed and reduced germination or may prevent germination completely (Hegarty 1977). The results obtained in the present study demonstrated that decreased water potential resulted in a delay and reduction of germination percentage (Fig. 3); similar results have been reported in lentils (Corchete and Guerra 1986; Zheng et al. 2005). However, the germination percentage drastically decreased and was delayed at water potential of −0.6 MPa (Figs. 1, 2) as compared to the control and −0.2 MPa treatment, which showed that slight water stress (−0.2 MPa water potential) has no effect on the germination of *P. asperata* seeds. The results also suggested that germination in *P. asperata* seeds is insensitive to water stress (Ma and Wang 1994). The results reported in the present study could be interpreted to mean that water does not play a key role for *P. asperata* seed germination; the knowledge of specific conditions that allow germination and early development would aid in understanding the ecological behaviors of *P. asperata*.

Seed germination has been promoted by gibberellin (GA) in many plant species (Peng and Harberd 2002). In the present study, GA-pretreated seeds showed higher germination

Table 2 The effect of PEG treatment on CAT, APX and POD activities of gibberellin–pretreated *Picea asperata* seeds for 15-days

Treatment	CAT(μmol $min^{-1}mg^{-1}$ protein)	APX(mmol $min^{-1}mg^{-1}$ protein)	POD(mmol $min^{-1}mg^{-1}$ protein)
H_2O			
CK	0.07 ± 0.01b	1.08 ± 0.16b	0.29 ± 0.01c
−0.2 MPa	0.08 ± 0.00a	1.25 ± 0.10ab	0.30 ± 0.01b
−0.6MPA	0.09 ± 0.01a	1.35 ± 0.10a	0.33 ± 0.01a
GA			
CK	0.05 ± 0.00d	0.61 ± 0.08c	0.23 ± 0.01e
−0.2 MPa	0.056 ± 0.01 cd	1.25 ± 0.08ab	0.26 ± 0.00d
−0.6MPA	0.06 ± 0.00c	1.37 ± 0.01a	0.28 ± 0.00c
F_P	0.004	0.000	0.000
F_{GA}	0.000	0.007	0.000
$F_{W\times GA}$	0.717	0.001	0.292

F_P, PEG effect; F_{GA}, gibberellin effect, $F_{P\times GA}$, PEG × gibberellin interactive effect. Data were shown as means ± S.E. Different letters in the table indicated significantly differences between treatments ($P < 0.05$, $n = 5$)

capacity compared to those that had been pretreated with distilled water under the same water conditions (Fig. 2). On the other hand, under the −0.6 MPa conditions, the germination percentage of GA-pretreated seeds was significantly higher than that of the distilled-water-treated counterparts. These results suggest that GA pretreatment may have partly removed the water-stress block to germination, which might be due to the fact that GA may induce production and activation of α-amylase and protease in the endosperm; this would result in improved germination capacities (Jones and Carbonell 1984).

Accumulation of proline under water stress protected the cell by balancing the osmotic strength of cytosol; preserving protein structures and enzyme activities; scavenging hydroxyl and other free radicals; and regulating cytosol acidity induced by unfavorable environmental conditions (Kishor et al. 1995). We found a significantly higher proline accumulation under water stress in both distilled-water- and GA-pretreated seeds (Table 1). Slow accumulation of proline occurred under the −0.2 MPa conditions (Table 1), which was consistent with similar findings for wheat (Song et al. 2005a, b). These results further confirmed that *P. asperata* seeds are insensitive to drought stress. On the other hand, GA-pretreated seeds have a higher proline accumulation under serious water stress from the PEG −0.6 MPa conditions. As we knew, the increase in proline content during osmotic stress may be essential for drought tolerance because it not only contributes to osmotic adjustment but also reduces the effects of drought stress damage by protecting enzyme structure, stabilizing membranes and scavenging hydroxyl radicals; therefore, accumulation of free proline content in GA-pretreated *P. asperata* seeds possessed a positive significance for seedling establishment.

Lipid peroxidation has considerable potential to damage membranes and may be a principal cause of seed deterioration (McDonald 1999); malondialdehyde (MDA) concentration could reflect the degree of damage resulting from adverse conditions. As showed in the present study, GA pretreatment had positive effects on seed germination (Figs. 1, 2, 3). Water stress resulted in detrimental effects on seed germination, but the combination of GA pretreatment and water stress alleviated the detrimental effect on seed germination (Figs. 1, 2, 3). Under water stress, a gradual decrease in germination capacities was concomitant with increased MDA concentration (Table 1). The water-stress-induced decrease in membrane stability indicates the extent of lipid peroxidation caused by active oxygen species (Menconi et al. 1995). In addition, a significant increase in the levels of MDA was observed in GA-pretreated seeds, suggesting that lipid peroxidation during water stress was enhanced in *P. asperata* seeds. The changes in the levels of MDA by seed pretreatment might result from treatment-induced changes in the activities of free-radical- and peroxide-scavenging enzymes.

Soluble sugars were usually utilized early in germination as an immediate energy source. This may be responsible for the decline in total soluble sugars in axes and cotyledons during initial germination (Nkang 2002). The extent of soluble sugar accumulation during the experiment was much higher in the seeds that had suffered from water stress than those in the control (Table 1). Likewise, similar to our results, Wang et al. (2004) reported that PEG treatment caused a significant increase in soluble sugar content of alfalfa and milkvetch seeds. The increase in the soluble sugar content was likely to result from decreased rates of export under drought stress conditions. On the other hand, GA-pretreated seeds had higher soluble sugar content than those of the untreated seeds under the same water conditions. The increase in soluble sugar, resulting from GA treatment, was reported in numerous species by many studies in the literature (Lovell and Booth 1967; Ning et al. 2006), demonstrating that the accumulation of soluble sugar content correlates

with the decrease of starch. In this study, the increase of soluble sugar accumulated the energy for seed germination.

We found a significant increase in APX in the seeds of *P. asperata* under water stress that had undergone GA pretreatment and control treatment. Belmonte et al. (2005) reported that increased APX activity was observed in PEG-treated white *P. asperata* seeds. It seemed that a high level of APX may be involved in the removal of H_2O_2, which, if it accumulates in high levels, may be deleterious for the developing embryos. Previous research (Noctor and Foyer 1998) has discussed the crucial role played by APX during the detoxification processed.

CAT and POD have been implicated in the removal of H_2O_2. Catalase (CAT) participated in the elimination of H_2O_2 by its dismutation into water and oxygen (Smirnoff 1993). In our study, PEG treatment of −0.2 MPa had no significant effect on CAT activity in GA-pretreated seeds. Previous studies also reported that CAT activity was not affected by mild drought (Fu and Huang 2001). However, CAT activity under PEG −0.6 MPa conditions showed markedly higher levels compared to the control for both GA- and distill-water-pretreat seeds (Table 2). This result indicated that, compared to the control treatment, a higher level of CAT activity suggested more effective H_2O_2 dismutation capacity under water stress. POD was among the major enzymes that scavenged H_2O_2, which was produced through dismutation of O_2^- (Türkan et al. 2005). In this study, increased PEG stress corresponded to significantly increased POD activity of *P. asperata* seeds after both distilled water and GA pretreatment. This might be due to the fact that PEG treatment resulted in a greater capacity to decompose H_2O_2 more rapidly.

Our results indicated that an increase in antioxidant enzyme activities (CAT, APX, and POD) in *P. asperata* seeds suffering water stress corresponded to a decrease in germination (Fig. 1; Table 2). A similar increase in the activity of CAT, APX, and POD was reported in stressed seeds (Kopyra and Gwóźdź 2003). In contrast, some studies in the literature (Goel et al. 2003; Maity et al. 2000) demonstrated that germination of the seeds decreased progressively with artificial ageing, but a decrease in germination was accordant with a decrease in activity of CAT, APX, and POD. The discrepancy between our study and their results might exist in that orthodox seeds were devoid of antioxidant enzymes. In addition, stress conditions can create oxidative stress (Sgherri et al. 2000), and the plant may have possessed antioxidants such as ascorbic acid and enzymes such as SOD, APX, CAT,POD etc. that are able to endure oxidative damage. Yet under the same water conditions, GA-pretreated seeds showed lower activities of CAT and POD than did seeds that had been pretreated with distilled water. This result seemed to correspond to greater germination capacity in GA-pretreated seeds (Figs. 1, 2, 3), which might contribute to drought stress tolerance of GA-pretreated seeds.

In conclusion, based on the data obtained from the final germination percentage (i.e., the germination index), GA pretreatment did promote germination of *P. asperata* seeds. It was possible that the low sensitivity of *P. asperata* seeds to water stress was associated with its lower activity of proline; MDA and soluble sugar contents; and antioxidative enzymes (CAT, APX, and POD); as well as its greater germination capacity under the −0.2 PEG conditions compared to the −0.6 PEG conditions. Additionally, although serious water stress induced the inhibitory properties of *P. asperata* seed germination, GA pretreatment was able to alleviate the inhibitory effects resulting from drought stress. More definite results could be obtained by studying the influence of other environmental factors (such as light, temperature, litter aqueous extracts, etc.) on germination of *P. asperata* seeds.

Management implications

The conclusions of this study showed that *P. asperata* seeds were insensitive to water stress; only serious water stress could decrease the germination rate. Therefore, water is not a limiting factor for *P. asperata* seed germination in subalpine coniferous forest areas. In reforestation practice, policymakers should consider other environmental factors and take advantage of available human-aided possibilities. Land closure for reforestation can protect seeds from human activities, being trampled by livestock, and elevations in the temperature of the environment. The removal of thick litter may eliminate the physical obstacles associated with seed germination and seedling establishment. These measurements have been reported to have a positive effect for natural regeneration in coniferous forests (Béland et al. 2000; Narukawa and Yamamoto 2002; Yin et al. 2007).

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