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Effects of Low Temperature on Seed Germination, Early Seedling Growth and Antioxidant Systems of the Wild *Elymus nutans* Griseb.

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ABSTRACT

Low temperature is a limiting factor of seed germination and plant growth. In this study, two independent experiments were conducted to investigate the effects of low temperature on germination, early seedling growth and antioxidant systems in two provenances of *Elymus nutans* Griseb. (Damxung, DX and Gannan, GN). The seeds and early seedlings of DX were more tolerant to low temperture than those of GN. Low temperature (5°C) negatively affected seed Germination Percentage (GP), Germination Energy (GE) and the First Germination Time (FGT) in both provenances of *E. nutans*. Low temperature also increased Mortality Percentage (MP) when compared with control plants grown at 25°C. Low temperature treatments significantly reduced length and fresh/dry weight of shoots and roots in the two accessions after 29 days of exposure to 5°C. In the second experiment, after 5 days of low temperature treatment, DX exhibited significantly higher Chlorophyll (Chl) and Carotenoid (Car) content as well as increased activities of Peroxidase (POD), SuperOxide Dismutase (SOD), Catalase (CAT). In contrast we observed lower Electrolyte Leakage (EL) and reduction of the amounts of MalonDiAldehyde (MDA), Hydrogen peroxide (H_2O_2) and superoxide radical ($O_2^{\bullet-}$). We conclude that GN is more susceptible to low temperature than DX due to more severe oxidative damage resulting from Reactive Oxygen Species (ROS) and lower antioxidant enzyme activities.

Keywords: Antioxidant systems, Early seedling growth, *Elymus nutans* Griseb., Germination, Low temperature.

INTRODUCTION

Low positive temperature is an important limiting factor in plant growth, photosynthesis, uptake of water and nutrients, as well as plant productivity (Tartoura and Youssef, 2011). Extreme temperatures give rise to an oxidative damage in plants. Cold is a severe environmental stress that disrupts the metabolic balance of cells, resulting in excessive accumulation of Reactive Oxygen Species (ROS) produced as a result of oxidative damage, which includes Hydrogen peroxide (H_2O_2), singlet Oxygen (O_2^1), superoxide ($O_2^{\bullet-}$) and the Hydrogen radical (**(**$HO^{\bullet}$) (Mittler, 2002). To alleviate oxidative damage, plants have evolved protective enzymatic and non-enzymatic defense systems to detoxify ROS and reduce oxidative stress. These strategies include the production of antioxidant enzymes, lipid-soluble antioxidants and water-soluble reductants (Mittler *et al.*, 2004; Duan, 2012).

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Temperature is one of the most critical factors affecting the germination of seeds (Verma *et al.*, 2010). The capacity of seed germination and seedling establishment can partly determine the regeneration of grassland plant communities (Vera, 1997). Previous studies also demonstrated that variations in germination of different species were related to altitude (Vera, 1997; Giménez-Benavides and Milla, 2013; Mondoni *et al.*, 2011). If germination patterns change in response to habitat, it could be expected that seeds from higher elevations would germinate better under low temperature conditions (Cavieres and Arroyo, 2000).

Elymus nutans Griseb., is a perennial species in *Elymus* L. of Triticeae. It is distributed throughout Mongolia, China, India, Turkey, Himalayas and Siberia. In China, it is found in the north, northwest and southwest regions, particularly on the Qinghai–Tibetan Plateau from 3,000 to 5,000 m (Jiang, 2007). *E. nutans* has been traditionally used as a typical native forage plant, collected and dried in the long cool season (Chen and Jia, 2002). Recently, it has been used in cultivated pastures in alpine areas, owing to its high adaptability, good nutrition, high yield and good tolerance to cold, drought and biotic stress (Jiang, 2007). Thus, an investigation of seed germination of wild *E. nutans* at high altitude is important to protect the fragile ecological environment of Tibet. This species plays a pivotal role in animal husbandry and environmental sustenance in China (Chen and Jia, 2002). However, to date, there is little information about the response of the wild seeds and seedlings of *E. nutans* to low temperature. In this study, we used two provenances of *E. nutans* (Damxung, DX and Gannan, GN), that contrast in low temperature tolerance, to elucidate the effects of low temperature on germination, early seedling growth and antioxidant systems. We tested the hypotheses that: (i) Low temperature led to decrease in germination percentage and germination energy in both provenances of *E. nutans*, with tolerant DX having higher values than sensitive GN seeds, (ii) DX exhibited less oxidative damage when exposed to low temperature by raising antioxidant enzyme activities to scavenge excessive accumulation of ROS under low temperature.

MATERIALS AND METHODS

Plant Material

Seeds of *E. nutans* (Damxung, DX) were collected in 2012 from wild plants growing in Damxung County, Tibet China (30° 28.535′ N, 91° 06.246′ E, altitude 4,678 m). *E. nutans* (Gannan, GN) seeds were obtained in September 2012, from Lanzhou Xinglong Grass Industry Technology Service CO. Ltd. They were planted in Gannan (33° 51.043′ N, 101° 40.139′ E, altitude 2,500 m), Gansu, China. The seeds were cleaned and stored at 4°C in paper bags until the start of the experiments.

Seeds Germination Experiment

Seeds were surface sterilized in 0.1% (w/v) sodium hypochlorite and rinsed several times in distilled water. They were germinated in 9 cm Petri dishes on top of 4 layers of moistened filter paper. Each treatment consisted of five replicates with 50 seeds. During cold treatment, the Petri dishes were placed in a growth chamber with 5/5^{o}C day/night temperature regimes, 14-hours photoperiod [Photosynthetic Photon Flux Density (PPFD), 100 μmol m^{-2} s^{-1}] and 70% of relative humidity. Seeds treatment at 25^{o}C and the remaining growth conditions were kept the same as the experimental group. Light was provided by a fluorescent lamp. Germinated seeds were counted daily at both temperatures from the first day of germination until the day of maximum seed germination (Kumar *et al.*, 2011). Seeds germination was defined as root or shoot emergence and seeds were

considered dead when they were necrotic (Fernández-Torquemada and Sánchez-Lizaso, 2013). The experiments were considered to have finished, when no radical emergence was observed. Germination Percentage (GP) and Germination Energy (GE) were calculated using the formula cited by Kumar *et al.* (2011). The remaining non-germinated seeds were then crushed, and seeds with a hard white embryo were considered viable. The final germination percentage was calculated, with non-viable seeds excluded **(**Giménez-Benavides and Milla, 2013). At the end of the experiment, root and shoot length, fresh or dry weight of shoot and root and mortality percentage were determined for early seedlings.

Seedlings Stress Experiment

Seeds were first germinated in a growth chamber with 25/25^{o}C day/night temperature regimes, 14-hours photoperiod [Photosynthetic Photon Flux Density (PPFD), 100 μmol m^{-2} s^{-1}] and 70% of relative humidity. Following germination, the 14-day-old seedlings were transferred into the growth chamber at 5^{o}C (and the rest of the growth conditions were identical) (Aroca *et al.*, 2001). After 5 days of low temperature treatment, plants were harvested, frozen in liquid nitrogen, and then stored at -80^{o}C for further analysis (Fu *et al.*, 2014).

Determination of Shoot and Root Growth Characters

Five morphologically uniform seedlings were randomly chosen from each group. Shoot and root lengths were immediately measured after removal from the containers. The shoots of seedlings were cut at the growth medium line and their fresh weights were recorded. The roots of the seedling were carefully washed and the surface water was removed and their fresh weights were recorded. The shoots and roots were dried at 80^{o}C for 72 h and their dry weights were determined.

Relative Water Content

Relative Water Content (RWC) was determined by the modified method according to Korkmaz *et al.* (2010). Fresh Weight (FW) of leaf and root were measured and then immediately floated on distilled water in a petri dish for 5 h in the dark. Turgid Weights (TW) of leaf and root were obtained after drying excess surface water with paper towels. Dry Weights (DW) of leaf and root were measured after drying at 75^{o}C for 48 h. *RWC* was calculated using the following formula: $RWC\ (\%)= (FW-DW)/(TW-DW)\times 100$.

Leaf Chlorophyll (Chl) and Carotenoid (Car) Content Measurement

The contents of Chl and Car were determined spectrophotometrically using 80% acetone as a solvent (Lichtenthaler, 1987). The absorbance of extracts was measured at 470, 645, and 664 nm with a spectrophotometer.

Electrolyte Leakage (EL) Measurement

Electrolyte leakage was determined by the modified method according to Song *et al.* (2006). The fresh leaves (0.5 g) were washed in deionized water and placed in Petri dishes with 5 mL deionized water at 25^{o}C for 2 h. After the incubation, the conductivity was measured (EC_1). Then, the samples were boiled for 20 minutes and conductivity was read again (EC_2). The *EL* (%) was calculated as $(EC_1/EC_2)\times 100$.

Analysis of Lipid Peroxidation

Membrane lipid peroxidation was determined as the level of MalonDiAldehyde (MDA) produced using

10% trichloroacetic acid according to Li *et al.* (2011). The absorbance of the supernatant was measured at 450, 532, and 600 nm.

Determination of Hydrogen Peroxide and Superoxide Radical

Hydrogen peroxide contents were measured according to Veljovic-Jovanovic *et al.* (2002). H_2O_2 content was calculated from a standard curve prepared in a similar way and expressed as nmol g^{-1} *FW*. Superoxide radical production rate was determined by the modified method according to Elstner and Heupel (1976).

Assay of Antioxidant Enzymes

The fresh leaves (0.5 g) were homogenized with a mortar and pestle at 4°C in 5 mL 50 mM phosphate buffer (pH 7.8) containing 1 mM EDTA and 2% PVP. The homogenate was centrifuged at 12,000×g for 20 min at 4°C and the supernatant was used for the following enzyme activity assays. The protein content in the supernatant was determined according to the method of Bradford (1976) with BSA as standard.

The activity of Peroxidase (POD) was assayed by the method of Upadhyaya *et al.* (1985). The activity of SuperOxide Dismutase (SOD) was determined according to Beauchamp and Fridovich (1971) by following the photoreduction of Nitro Blue Tetrazolium (NBT) at 560 nm. The activity of Catalase (CAT) was measured by following the consumption of H_2O_2 at 240 nm according to Cakmak and Marschner (1992).

Statistical Analysis

Each experiment was repeated at least three times. Values were expressed as means±SE. Statistical analyses were performed by a two-tailed Student's t-test and Pearson correlation analysis with SPSS-17 statistical software (SPSS Inc., Chicago, IL, USA).

RESULTS

Effects of Low Temperature on Seeds Germination and Early Seedlings Development

For both *E, nutans* varieties, the First Germination Time (FGT) was observed on the 3rd day at 25°C, and the 17th day at 5°C. The maximum seed germination of DX and GN was found on 11th and 12th days at 25°C, respectively. The highest seed germination for both plants was obtained on the 26th day at 5°C (Figure 1-a). The seed Germination Percentage (GP) of DX at 25°C varied from 22.8% (3rd day) to 66.8% (11th day), while the GP of GN at 25°C ranged from 12.0% (3rd day) to 48.4% (12th day). DX and GN seeds were cultured for 17 days at 5°C germinated at 10.0 and 4.0% while 59.2 and 26.8% of GP occurred at day 26, respectively (Figure 1-a). A similar trend was observed for Germination Energy (GE) (Figure 1-b). Low temperature also seemed to affect Mortality Percentage (MP). Mortality values of DX seeds decreased ($t= 3.494$, $df= 4$, $P< 0.05$) under low temperature, but increased with GN seeds ($t= -3.141$, $df= 4$, $P< 0.05$; Figure 1-c).

Shoot lengths of DX and GN at 5°C decreased by 88.8% ($t= 16.530$, $df= 4$, $P< 0.001$) and 91.7% ($t= 13.657$, $df= 4$, $P< 0.001$), respectively, when compared with control (Figure 2-a). DX and GN seedlings placed at 5°C exhibited smaller root lengths in comparison with those at 25°C (Figure 2-a). The stressed DX seedlings at 5°C showed a smaller attenuation in dry leaf and root weights, compared with GN (Figures 2-b and -c).

Effects of Low Temperature on *E. nutans* Seedlings Physiology

The lengths of shoot and root in stressed DX seedlings were longer than that in GN

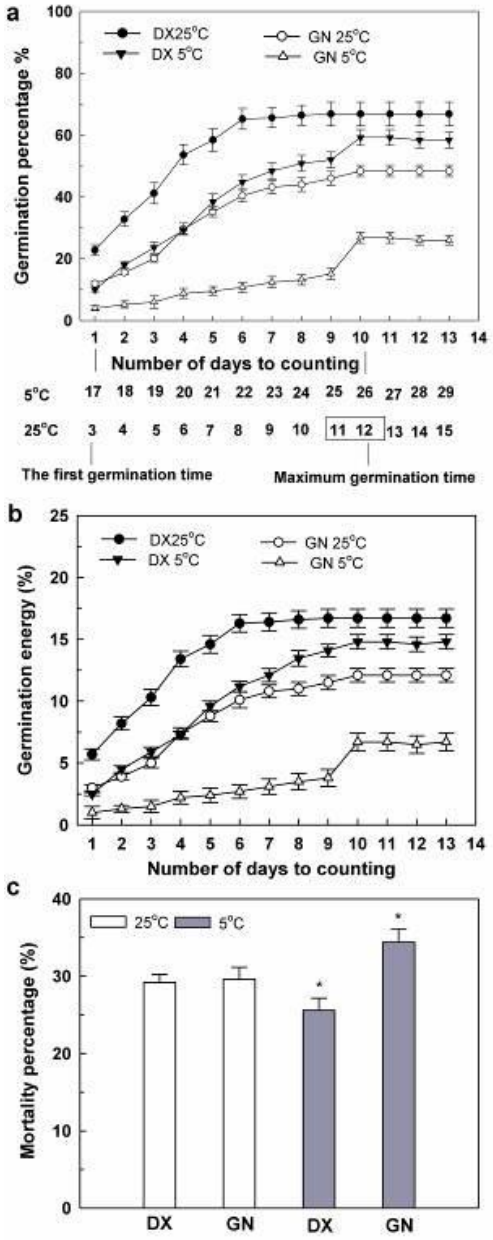

Figure 1. Germination percentage and corresponding days of germination (a), germination energy and corresponding days of germination (b) and mortality at the end of germination (c) of two *E. nutans* (DX, Damxung and GN, Gannan) seeds subjected to low temperature over the experimental period. Data shown are means±SE. One, two or three asterisks indicate significant difference between the treatments at $P< 0.05$, $P< 0.01$ or $P< 0.001$, respectively.

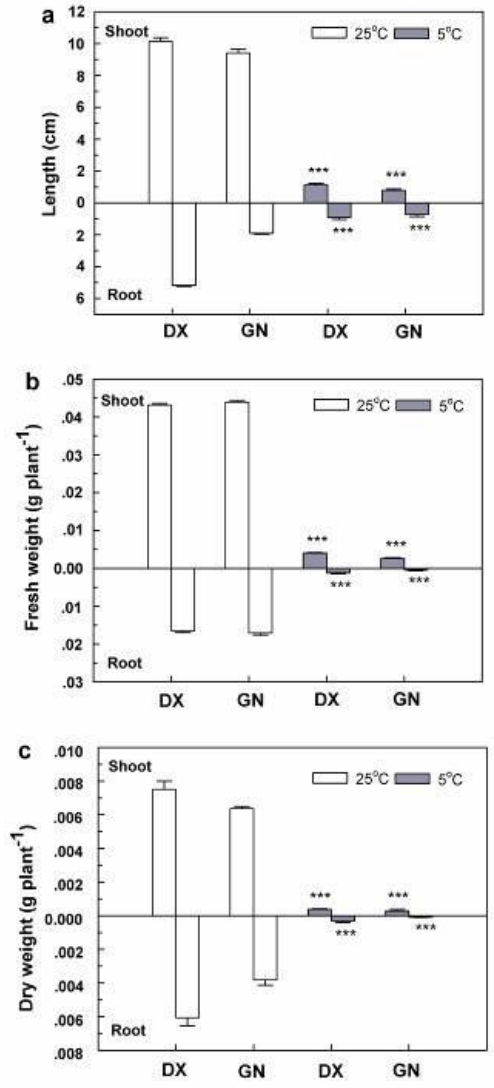

Figure 2. Shoot and root growth of germinated DX and GN seeds at the end of germination experiment.

(Figure 3-a). DX and GN shoot fresh weight decreased by 41.0% (t= 2.808, df= 4, P< 0.05) and 39.0% (t= 4.421, df= 4, P< 0.05) respectively, compared with controls, while a 6.0% (t= 0.520, df= 4, P> 0.05) and 54.6% (t= 10.929, df= 4, P< 0.001) decrease were observed in dry weight, respectively (Figure 3-b). A smaller decrease in fresh and dry weight of the roots was also observed in DX as compared to GN (Figure 3-c). After 5 days of low temperature treatment, a 12.4% (t= 0.869, df= 4, P> 0.05) and 6.8% (t= 2.833, df= 4, P> 0.05) decrease was observed in leaf and root *RWC* in DX, respectively. GN exhibited higher decrease in *RWC* of leaves and roots in response to low temperature (Figure 3-d).

After 5°C exposure, Chl contents in leaves of DX and GN decreased by 89.5% (t= 14.478, df= 4, P< 0.001) and 93.3% (t= 13.282, df= 4, P< 0.001), respectively (Figure 4-a). Low temperature at 5°C resulted in a significant decrease in Car contents of the two *E. nutans*, but DX was 72.3% (t= 3.082, df= 4, P< 0.05) higher than GN (Figure 4-b).

Electrolyte leakage in DX and GN seedlings increased 18.2% (t= -7.343, df= 4, P< 0.05) and 114.7% (t= -10.252, df= 4, P< 0.001) under low temperature, respectively (Figure 5-a). The lipid peroxidation levels, as determined by MDA concentrations, were higher in GN than that in DX under low temperature (Figure 5-b).

After 5 days of low temperature treatments, levels of H_2O_2 in DX leaves increased 7.8% (t= -2.772, df= 4, P< 0.05) and $O_2^{\bullet-}$ increased 0.8% (t= -0.234, df= 4, P> 0.05). In GN leaves the H_2O_2 levels decreased 16.8% (t= -8.621, df = 4, P< 0.01) and $O_2^{\bullet-}$ levels decreased 28.4% (t= -4.608, df= 4, P< 0.05) (Figure 6).

We observed higher activity of POD, SOD, and CAT in low temperature treated DX leaves, which were 65.9% (t= -16.567, df= 4, P< 0.001), 80.3% (t= -14.789, df= 4, P< 0.001), and 27.3% (t= -2.472, df= 4, P< 0.05) greater than control, respectively. In GN leaves, a 28.9% (t= -5.460, df= 4, P< 0.01) and 11.4% (t= -1.191, df= 4, P> 0.05) increase in activities of POD and SOD were observed, but a 25.5% (t= 4.810, df= 4, P< 0.01) decrease in CAT was obtained, compared to the control (Figure 7).

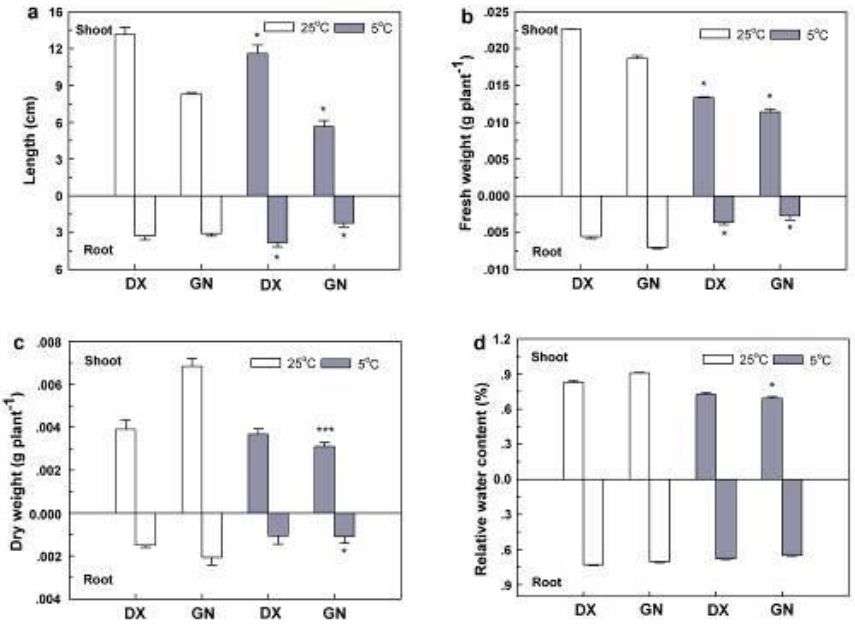

Figure 3. Changes in shoot and root length (a), fresh weight (b), dry weight (c) and relative water content (d) of shoot and root in DX and GN seedlings.

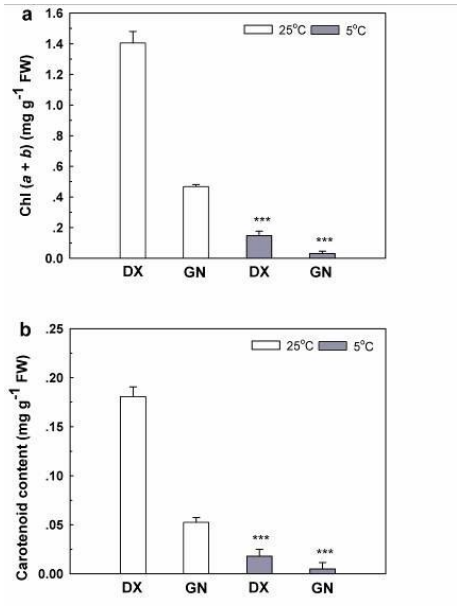

Figure 4. Changes in Chl (*a*+*b*) (a) and carotenoid content (b) of DX and GN seedlings.

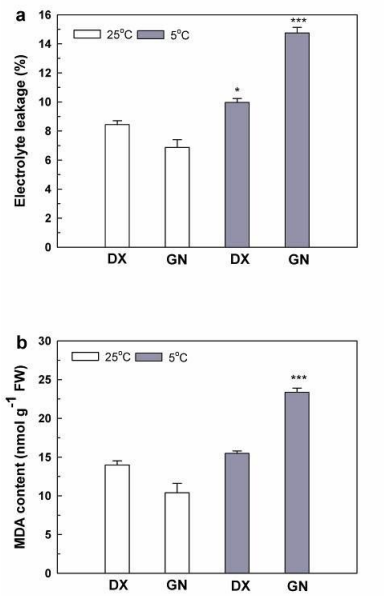

Figure 5. Changes in electrolyte leakage (a) and MDA content (b) of DX and GN seedlings.

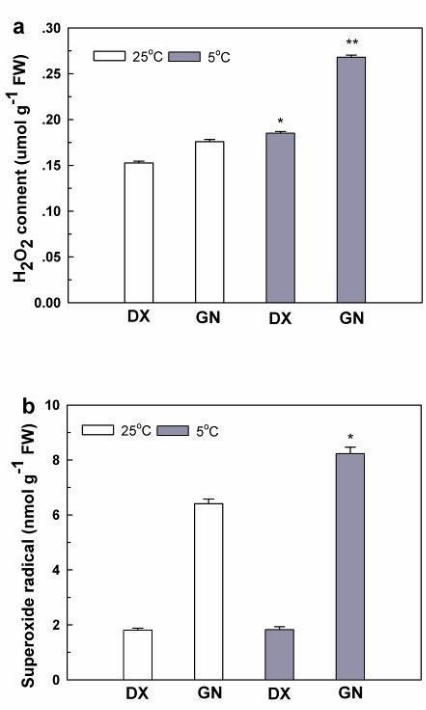

Figure 6. Changes in H_2O_2 (a) and $O_2^{\bullet -}$ (b) levels of DX and GN seedlings.

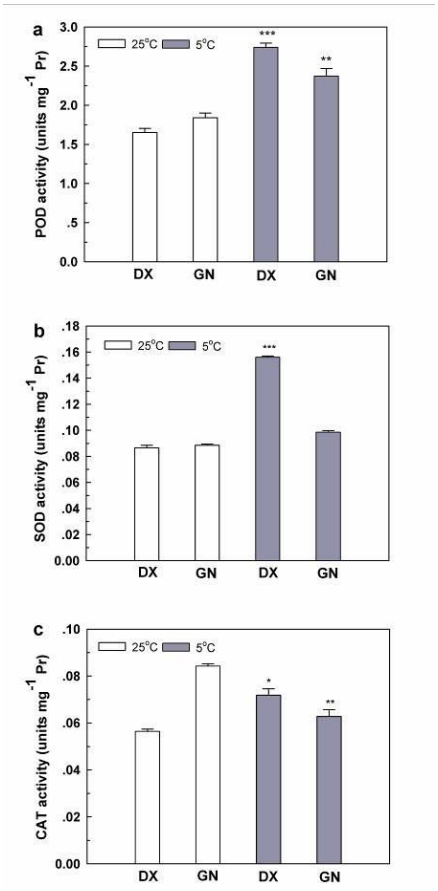

Figure 7. Changes in POD(a), SOD (b) and CAT (c) activity of DX and GN seedlings.

Correlation Analysis

Correlation analysis showed that there was a significant correlation between and among physiological indices and morphological parameters (Table 1). Chlorophyll content was positively correlated with shoot length and leaf fresh weight, but negatively correlated with POD. There was a significant positive correlation between shoot and root growth. The MDA content and *EL* in leaves showed significant negative association with shoot/root length and fresh/dry weight of shoot and root. The reduction in the accumulation of H_2O_2 and $O_2^{\bullet-}$ were paralleled with higher activities of antioxidant enzyme in the leaves of *E. nutans*. SOD activity was significantly positively correlated with POD in *E. nutans*, and the correlation coefficient was 0.867 ($P< 0.001$).

DISCUSSION

Temperature is a critical factor affecting seed germination (Verma *et al.*, 2010). To investigate the mechanisms of low temperature adaptation in *E. nutans*, we studied seed germination responses and evaluated them using the following parameters: Germination Percentage (GP), Germination Energy (GE), the First Germination Time (FGT), and Mortality Percentage (MP). We also looked at early seedling growth and the physiological responses of two accessions (DX and GN), contrasting in low temperature tolerance, under laboratory conditions. In this study, low temperature decreased GP and GE, and extended the time of seed germination in both plants (Figures 1-a and -b). Low temperature treated DX seeds exhibited a lower MP compared with control (Figure 1-c), contrary to previous reports in tomato by Foolad and Lin (1998). These observations are in agreement with our first hypothesis. Therefore, low temperature is not a prerequisite for the germination of high-altitude DX, and its strong negative effect on the germination of low-altitude GN may contribute to the altitudinal segregation of these two grasses.

Seeds (DX and GN) germinated under low temperature had reduced shoot and root lengths, as compared to the control (Figure 2-a). The negative effect of low temperature on root growth may cause a decline in the number of seedlings successfully established in the field (Fernández-Torquemada and Sánchez-Lizaso, 2013). The fresh/dry weight of shoot and root significantly decreased under low temperature in both *E. nutans*, while these reductions were more pronounced in GN than DX (Figures 2-b and -c). Thus, GN is more susceptible to low temperature than DX. These results were consistent with data obtained in the second seedling stress experiment.

Species have adapted to cold environments by evolving physiological and morphological means to enhance survival when subjected to extended cold periods (Guy, 1999). A 16.8% increase in root length was concomitant with an 11.8% decrease in shoot length in DX under low temperature. Whereas a significant decrease of root and shoot length was observed in GN (Figure 3-a). This is consistent with a prior investigation, which showed that insufficient root growth at low temperature limits maize seedling regrowth (Sowiński *et al*., 2005).

Photoinhibition induced by low temperature limits yield in many plants (Bertamini *et al.*, 2005). Low temperature lowered total Chl content in both DX and GN (Figure 4-a). The decrease in total Chl content was accompanied by the decrease of shoot fresh and dry weight in both *E. nutans* (Table 1). This agrees with the results reported in pepper (Korkmaz *et al.*, 2010). Carotenoids act as photoprotective pigments and their action helps cells to avoid the generation of singlet oxygen and quench the triplet-state Chl molecules to avoid Chl photooxidation (Young, 1991). A higher Car content was obtained in DX as compared to GN after low temperature exposure (Figure 4-b). Similar results were also reported by Aroca *et al.* (2001) who stated that chilling-

Table 1. Pearson Correlation analysis of different antioxidant enzymes (POD, SOD and CAT), lipid peroxidation (MDA), Electrolyte Leakage (EL), ROS accumulation (H_2O_2 and $O_2^{\bullet-}$), Chlorophyll (Chl) concentration and growth of *E. nutans* seedlings under cold stress.[a]

	RL	*SFW*	*SDW*	*RFW*	*RDW*	*LRWC*	*RRWC*	Chl	*REL*	MDA	H_2O_2	$O_2^{\bullet-}$	SOD	POD	CAT
SL	0.783**	0.583	-0.108	0.272	0.012	0.244	0.571	0.679*	-0.563	-0.528	0.574	-0.958**	0.231	-0.209	-0.255
RL		0.122	0.019	0.236	0.119	0.195	0.178	0.198	-0.600*	-0.575	0.611*	-0.841**	0.559	0.146	0.199
SFW			-0.018	0.635*	0.480	0.747**	0.792**	0.914**	-0.637*	-0.610*	-0.102	-0.353	-0.644*	-0.903**	-0.212
SDW				0.577*	0.645*	0.233	0.096	0.885**	-0.187	-0.131	-0.112	0.223	-0.172	-0.239	0.230
RFW					0.921**	0.874**	0.611**	0.502	-0.773**	-0.768**	-0.313	-0.089	-.0462	-0.714*	0.369
RDW						0.801**	0.364	0.314	-0.653*	-0.625*	-0.383	0.161	-0.534	-0.677*	0.479
LRWC							0.681*	0.516	-0.831**	-0.857**	-0.396	-0.068	-0.519	-0.750	0.392
RRWC								0.736**	-0.615*	-0.652*	0.117	-0.445	-0.293	-0.613*	-0.138
Chl									-0.475	-0.460	0.127	-0.530	-0.509	-0.791**	-0.409
REL										0.964**	0.097	0.457	0.122	-0.444	0.533
MDA											0.629*	0.738*	-0.802*	-0.491	-0.831*
H_2O_2												-0.622*	-0.796*	-0.535	-0.876**
$O_2^{\bullet-}$													-0.742*	-0.483	-0.844**
SOD														0.867**	0.114
POD															0.081

[a] *F*-values are present with significant differences: *$P < 0.05$, **$P < 0.01$.

tolerant varieties developed at low temperatures showed a greater amount of Car than chilling-sensitive ones.

Low temperature induces an oxidative burst, which leads to over-production of ROS, such as Hydrogen peroxide (H_2O_2) and superoxide anion ($O_2^{\bullet-}$). This causes membrane lipid peroxidation and cellular membrane damage (Xie *et al.*, 2008). MDA levels, a product of lipid peroxidation, could reflect the degree of lipid peroxidation (Tartoura *et al.*, 2011). Electrolyte leakage is an indicator of cell membrane injury (Korkmaz *et al.*, 2010). In this study, GN seedlings subjected to low temperature showed a significantly higher MDA content, *EL*, $O_2^{\bullet-}$ and H_2O_2 levels than that in DX (Figures 5 and 6). These results correlate well with the findings of Xu *et al.* (2012) in cold stored loquat fruit and Balestrasse *et al.* (2010) in soybean at 4°C treatment. The most common symptom of cold stress in intact plant tissues is water loss, finally leading to wilting during and after the low temperature exposure (Korkmaz *et al.*, 2010). The *RWC*, as indicated by the extent of dehydration, was used to assess cellular damage (Balestrasse *et al.*, 2010). DX exhibited lower *EL* and higher *RWC* compared with GN under low temperature (Figures 3-d and 5-a). This is in accordance with the results of our correlation analysis (Table 1). A similar result was also reported in pepper seedlings by Korkmaz *et al.* (2010).

Antioxidant systems in plants prevent or mitigate the membrane peroxidation resulting from ROS under stressful conditions such as chilling, drought or salinity (Xu *et al.*, 2008). Chilling-tolerant plants may initially contain or can generate more antioxidants during stress and/or produce fewer ROS than chilling-sensitive plants (Hodges *et al.*, 2004). A significant increase in POD, SOD, and CAT activity was observed in low temperature treated DX seedlings. In GN seedlings, a reduction in CAT activity and a smaller increase in SOD and POD activity were seen relative to control (Figure 7). SOD is located in the chloroplast, mitochondrion, cytoplasm, and peroxisome, and operates as the first line of defense against ROS (Liau *et al.*, 2007). Higher SOD activity can efficiently remove $O_2^{\bullet-}$, and leads to the production of H_2O_2. CAT and POD are important H_2O_2 detoxifying enzymes. The increased activity of POD and SOD in low temperature treated GN seedlings was accompanied by a decreased CAT activity, which may explain the higher MDA accumulation and H_2O_2 concentrations in GN. This agrees with the results reported in cucumber seedlings (Liang *et al.*, 2009; Bazrafshan and Ehsanzadeh, 2016). The results of our correlation analysis also showed a significant interaction between the antioxidant enzyme, MDA content, and ROS accumulation (Table 1). Based on these results, DX plants alleviated oxidative injuries through increasing antioxidant enzyme activities to scavenge newly produced ROS. This is consistent with our second hypothesis.

In conclusion, low temperature reduced seed germination percentage, extended the first germination time, and inhibited early seedlings growth in both *E. nutans* that were tested. In tolerant DX seedlings, increases in higher antioxidant enzymes activities and Chl and Car accumulation, along with lower lipid peroxidation at cellular membranes, resulted in a higher low temperature tolerance.

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REFERENCES

1. Aroca, R., Irigoyen J. J. and Sánchez-Díaz, M. 2001. Photosynthetic Characteristics and Protective Mechanisms against Oxidative Stress during Chilling and Subsequent Recovery in Two Maize Varieties Differing in Chilling Sensitivity. *Plant Sci.*, **161(4):** 719–726.
2. Balestrasse, K. B., Tomaro, M. L., Batlle, A. and Noriega, G. O. 2010. The Role of 5-aminolevulinic Acid in the Response to

Cold Stress in Soybean Plants. *Phytochem.,* **71(17-18):** 2038–2045.

3. Bazrafshan, A. H. and Ehsanzadeh, P. 2016. Evidence for Differential Lipid Peroxidation and Antioxidant Enzyme Activities in *Sesamum indicum* L. Genotypes under NaCl Salinity. *J. Agr. Sci. Tech.,* **18(1):** 207-222.
4. Beauchamp, C. and Fridovich, I. 1971. Superoxide Dismutase: Improved Assays and an Assay Applicable to Acrylamide Gels. *Anal. Biochem.,* **44(1):** 276–287.
5. Bertamini, M., Muthuchelian, K., Rubinigg, M., Zorer, R. and Nedunchezhian, N. 2005. Photoinhibition of Photosynthesis in Leaves of Grapevine (*Vitis vinifera* L. cv. Riesling). Effect of Chilling Nights. *Photosynthetica*, **43(4):** 551–557.
6. Bradford, M. M. 1976. A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. *Anal. Biochem.,* **72(1-2):** 248–254.
7. Cakmak, I. and Marschner, H. 1992. Magnesium Deficiency and High Light Intensity Enhance Activities of Superoxide Dismutase, Ascorbate Peroxidase, and Glutathione Reductase in Bean Leaves. *Plant Physiol.,* **98(4):** 1222–1227.
8. Cavieres, L. A. and Arroyo, M. T. K. 2000. Seed Germination Response to Cold Stratification Period and Thermal Regime in *Phacelia secunda* (Hydrophyllaceae). *Plant Ecol.,* **149(1):** 1–8.
9. Chen, M. J. and Jia, S. X. 2002. *China Feeding Plant.* China Agriculture Press, Beijing, PP.119–120 (in Chinese)
10. Duan, M., Ma, N. N., Li, D., Deng, Y. S., Kong, F.Y., Lv, W. and Meng, Q. W. 2012. Antisense-mediated Suppression of Tomato Thylakoidal Ascorbate Peroxidase Influences Anti-oxidant Network during Chilling Stress. *Plant Physiol. Bioch.,* **58:** 37–45.
11. Elstner, E. F. and Heupel, A. 1976. Inhibition of Nitrite Formation from Hydroxylammo- niumchloride: a Simple Assay for Superoxide Dismutase. *Anal. Biochem*., **70(2):** 616–620.
12. Fernández-Torquemada, Y. and Sánchez-Lizaso, J. L. 2013. Effects of Salinity on Seed Germination and Early Seedling Growth of the Mediterranean Seagrass *Posidonia oceanica* (L.) Delile. *Estuar. Coast. Shelf S.,* **119:** 64–70.
13. Foolad, M. R. and Lin, G. Y. 1998. Genetic Analysis of Low Temperature Tolerance during Germination in Tomato, *Lycopersicon esculentum* Mill. *Plant Breed.,* **117(2):** 171–176.
14. Fu, J., Sun, Y., Chu, X., Xu, Y. and Hu, T. 2014. Exogenous 5-Aminolevulenic Acid Promotes Seed Germination in *Elymus nutans* against Oxidative Damage Induced by Cold Stress. *PLoS ONE,* **9(9):** e107152.
15. Giménez-Benavides, L. and Milla, R. 2013. Comparative Germination Ecology of Two Altitudinal Vicariant *Saxifraga* Species Endemic to the North of Spain. *Plant Biol.*, **15(3):** 593–600.
16. Guy, C. 1999. The Influence of Temperature Extremes on Gene Expression, Genomic structure, and the Evolution of Induced Tolerance in Plants. In: "*Plants Responses to Environmental Stresses. from Phytohormones to Genome Reorganization*", (Ed.): Lerner H. R. Marcel Dekker. New York, Basel, PP. 497–548.
17. Hodges, D. M., Lester, G. E., Munro, K. D. and Toivonen, P. M. A. 2004. Oxidative Stress: Importance for Postharvest Quality. *HortSci.,* **39(5):** 924–929.
18. Jiang, Y. Q. 2007. Forage and Manure Crops. In: "*Crops and Their Wild Relatives in China*", (Eds.): Dong Y. C. and Liu, X. Beijing, PP. 142–144. (in Chinese)
19. Korkmaz, A., Korkmaz, Y. and Demirkıran, A. R. 2010. Enhancing Chilling Stress Tolerance of Pepper Seedlings by Exogenous Application of 5-Aminolevulinic Acid. *Environ. Exp. Bot.,* **67(3):** 495–501.
20. Kumar, B., Verma, S. K. and Singh, H. P. 2011. Effect of Temperature on Seed Germination Parameters in Kalmegh (*Andrographis paniculata* Wall. ex Nees). *Ind. Crop. Prod.,* **34(1):** 1241-1244.
21. Li, Y., Zhao, H., Duan, B., Korpelainen, H. and Li, C. 2011. Effect of Drought and ABA on Growth, Photosynthesis and Antioxidant System of *Cotinus coggygria* Seedlings under Two Different Light Conditions. *Environ. Exp. Bot.,* **71(1):** 107–113.
22. Liang, W., Wang, M. and Ai, X. 2009. The Role of Calcium in Regulating Photosynthesis and Related Physiological Indexes of Cucumber Seedlings under Low Light Intensity and Suboptimal Temperature Stress. *Sci. Hortic-Amsterdam,* **123(1):** 34–38.

23. Liau, C. S., Cheng, T. J., Hsieh, Y. K., Lai, W. T., Lo, H. M., Ryu, S. J., Song, M. L., Tsao, W. L. and Yip, P. K. 2007. Reach Registry: An International Large Scale Registry on Atherothrombosis - Taiwan 1-Year Results. *Int. J. Cardiol.,* **122:** 65.
24. Lichtenthaler, H. K. 1987. Chlorophylls and Carotenoids: Pigments of Photosynthetic Biomembranes. *Method. Enzymol.,* **148:** 350–382.
25. Mittler, R. 2002. Oxidative Stress, Antioxidants and Stress Tolerance. *Trend. Plant Sci.,* **7(9):** 405–410.
26. Mittler, R., Vanderauwera, S., Gollery, M. and Van Breusegem, F. 2004. Reactive Oxygen Gene Network of Plants. *Trend Plant Sci.,* **9(10):** 490–498.
27. Mondoni, A., Probert, R. J., Rossi, G., Vegini, E. and Hay, F. R. 2011. Seeds of Alpine Plants Are Short Lived: Implications for Long-term Conservation. *Ann. Bot.London*, **107(1):**171–179.
28. Shi, Q. H., Bao, Z. Y., Zhu, Z. J., Ying, Q. S. and Qian, Q. Q. 2006. Effects of Different Treatments of Salicylic Acid on Heat Tolerance, Chlorophyll Fluorescence, and Antioxidant Enzyme Activity in Seedlings of *Cucumis sativa* L. *Plant Growth Regul.,* **48(2):** 127–135.
29. Song, L., Ding, W., Zhao, M., Sun, B. and Zhang, L. 2006. Nitric Oxide Protects against Oxidative Stress under Heat Stress in the Calluses from Two Ecotypes of Reed. *Plant Sci.,* **171(4):** 449–458.
30. Sowiński, P., Rudzińska-Langwald, A., Adamczyk, J., Kubica, I. and Fronk, J. 2005. Recovery of Maize Seedling Growth, Development and Photosynthetic Efficiency after Initial Growth at Low Temperature. *J. Plant Physiol.,* **162(1):** 67–80.
31. Tartoura, K. A. H. and Youssef, S. A. 2011. Stimulation of ROS-Scavenging Systems in Squash (*Cucurbita pepo* L.) Plants by Compost Supplementation under Normal and Low Temperature Conditions. *Sci. Hortic.- Amsterdam,* **130(4):** 862–868.
32. Upadhyaya, A., Sankhla, D., Davis, T. D., Sankhla, N. and Smith, B. N. 1985. Effect of Paclobutrazol on the Activities of Some Enzymes of Activated Oxygen Metabolism and Lipid Peroxidation in Senescing Soybean Leaves. *J. Plant Physiol.,* **121(5):** 433–461.
33. Veljovic-Jovanovic, S., Noctor, G. and Foyer, C. H. 2002. Are Leaf Hydrogen Peroxide Concentrations Commonly Overestimated? The Potential Influence of Artefactual Interference by Tissue Phenolics and Ascorbate. *Plant Physiol. Bioch.,* **40(6-8):** 501–507.
34. Vera, M. L. 1997. Effects of Altitude and Seed Mass on Germination and Seedling Survival of Heathland Plants in North Spain. *Plant Ecol.,* **133(1):** 101–106.
35. Verma, S. K., Kumar, B., Ram, G., Singh, H. P. and Lal, R. K. 2010. Varietal Effect on Germination Parameter at Controlled and Uncontrolled Temperature in Palmarosa (*Cymbopogon martinii*). *Ind. Crop. Prod.,* **32(3):** 696–699.
36. Xie, Z. X., Duan, L. S., Tian, X. L., Wang, B. M., Eneji, A. E. and Li, Z. H. 2008. Coronatine Alleviates Salinity Stress in Cotton by Improving the Antioxidative Defense System and Radical-scavenging Activity. *J. Plant Physiol.,***165(4):** 375–384.
37. Xu, M., Dong, J., Zhang, M., Xu, X. and Sun, L. 2012. Cold-Induced Endogenous Nitric Oxide Generation Plays a Role in Chilling Tolerance of Loquat Fruit during Postharvest Storage. *Postharvest Biol. Tech.,* **65:** 5–12.
38. Xu, P. L., Guo, Y. K., Bai, J. G., Shang, L. and Wang, X. J. 2008. Effects of Long-term Chilling on Ultrastructure and Antioxidant Activity in Leaves of Two Cucumber Cultivars under Low Light. *Physiol. Plantarum*, **132(4):** 467–478.
39. Young, A. J. 1991. The Photoprotective Role of Carotenoids in Higher Plants. *Physiol. Plantarum.,* **83(4):** 702–708.

اثرات دمای پایین بر جوانه زنی، رشد زودهنگام و سیستم آنتی اکسیدانی گیاهچه وحشی *Elymus nutans* Griseb

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چکیده

دمای پایین یک عامل محدود کننده جوانه زنی بذر و رشد گیاه است. در این مطالعه دو آزمایش مستقل به منظور بررسی تاثیر دمای پایین بر جوانه زنی، رشد گیاهچه زودرس و سیستم آنتی اکسیدان در دو پراوننسها .*Elymus nutans* Griseb (Damxung, DX and Gannan, GN) انجام شد. دانه ها و نهال های زودرس DX تحمل بیشتری به دمای پایین نسبت به GN دارند. درجه حرارت پایین (۵ درجه سانتیگراد) اثر معنی داری بر درصد جوانه زنی بذر (GP)، انرژی جوانه زنی (GE) و اولین زمان جوانه زنی (FGT) در هر دو پراوننسهای *E. nutans* داشت. همچنین در مقایسه با گیاهان کنترل در ۲۵ درجه سانتی گراد، درجه حرارت پایین باعث افزایش درصد مرگ و میر شد. کاهش معنادار طول و وزن تازه / خشک شاخه ها و ریشه ها در دو نمونه هایی که به مدت ۲۹ روز در معرض درجه حرارت پایین (۵ درجه سانتیگراد) بودند، نیز مشاهده شد. در آزمایش دوم، پس از ۵ روز قرارگفتن تیماردر دمای پایین، DX به طور قابل توجهی کلروفیل (Chl) و کاروتنوئید (car) بیشتر و نیز افزایش فعالیت peroxidase (POD) ، superoxide dismutase (SOD) و catalase (CAT) نشان داد. در مقابل ما نشت الکترولیت پایین (EL) و کاهش مقادیر malondialdehyde (MDA)، پراکسید هیدروژن (H2O2) و رادیکال سوپراکسید را مشاهده کردیم. ما نتیجه می گیریم که GN به علت آسیب های شدید اکسیداتیو ناشی از گونه های اکسیژن واکنش پذیر (ROS) و فعالیت های آنزیمی آنتی اکسیدان پایین تر، حساس تر از DX نسبت به دمای پایین است.