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Full Length Article

Impacts of Ascorbic Acid on Germination, Antioxidant Enzymes and Ultrastructure of Embryo Cells of Aged *Elymus sibiricus* Seeds with Different Moisture Contents

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Abstract

This study determined the effects of exogenous ascorbic acid (AsA) treatment on germination, membrane lipid peroxidation, activities of antioxidant enzymes and ultra-structure of embryo cells of Siberian wildrye (*Elymus sibiricus* L.) seeds with different moisture contents (4%, 10% and 16%) after ageing at 45°C for 48 h. Germination percentage and activities of enzymes declined, electrical conductivity value of seeds increased significantly as moisture content increased from 4% to 16%. However, ascorbic acid treatment had protective effects on alleviating the damage of ageing to seeds membrane integrity and structure of mitochondria, which was in accordance with the decrease of electrical conductivity and malondialdehyde (MDA) content, especially for seeds with 10% moisture content. Ascorbic acid treatment significantly (P<0.05) inhibited the loss of seed germination at 10% moisture content, and correspondingly electrical conductivity decreased and activities of catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR) improved. In addition, ultra structure observation of embryo cells showed that serious damage occurred in seeds with 16% moisture content, including the broken cellular and nuclear membrane, pyknotic nucleolus and swollen mitochondria. Although activities of CAT, APX and GR were improved by the exogenous AsA, it had no obviously protective effect on integrity of membrane system and mitochondria. Seed moisture of Siberian wildrye played a vital role in the function of AsA, which provided an efficient treatment to deal with the antioxidant effects of storage and ageing at seed moisture of 10%. © 2016 Friends Science Publishers

Keywords: Ascorbic acid; Germination; Antioxidant enzymes; Ultra structure of embryo cells; *Elymus sibiricus* L. seeds

Introduction

High-quality seeds generally determine the output and quality of agricultural production, and are also the basis of maintaining high energy and vigor during the period of storage to guarantee their application value (Deepa *et al.*, 2013; Hampton *et al.*, 2013). However, deterioration occurs in the process of seeds storage, which causes a drop of seed vigor, poor seedling emergence and bad influence on intracellular physiological reactions (McDonald, 1999; Kapoor *et al.*, 2011). Although the exact mechanisms of seed vigour losing are still under research, the lipid peroxidation has been considered as the main explanation for seed deterioration due to accumulation of reactive oxygen species (ROS) (Bailly *et al.*, 2008; Kodde *et al.*, 2012). Furthermore, deterioration is related to loss of membrane integrity, cell structural disruptions and other various cellular changes such as DNA degradation, protein denaturation and ultimately causes cell death (McDonald, 1999; Karuppanapandian *et al.*, 2011).

In order to protecting the subcellular components against oxidative stresses, seeds-themselves have evolved a set of special and effective defense system to scavenge the free radicals and deleterious compounds produced in the peroxidation to a minimum (Sung and Chiu, 1995). The protective mechanism comes into play by enhancing activities of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR) and increasing contents of non-enzymatic antioxidants including ascorbic acid (AsA) and glutathione (GSH) (Yahubyan *et al.*, 2009; Mallik *et al.*, 2011; Saruhan *et al.*, 2012). Among the antioxidant enzymes, CAT and APX provide the first line to defense hydrogen peroxide (H_2O_2); and SOD detoxifies superoxide anion to hydrogen peroxide and molecular oxygen by catalyzing the dismutation, which acts as a metalloprotein to work in the reactions in the cytosol, mitochondria, and chloroplasts (Yahubyan *et al.*, 2009; Mallik *et al.*, 2011). However, the effective defensive function is closely associated with the activities of these enzymes which are influenced by the extent of seed ageing (Bailly *et al.*, 1996).

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In the antioxidant system, ascorbic acid (AsA) acts as an essential antioxidant substance to scavenge the ROS through playing a crucial role in ascorbic acid-glutathione cycle (AsA-GSH) (Talukdar, 2012). Ascorbic acid is an abundant small molecular compound widely distributed in plants and other organisms, and its regeneration and biosynthesis can be modulated in cells. Ascorbic acid is also expressed as the primary substance in the network of antioxidants and has been believed to play important roles in plant cell division and cell wall expansion (Pignocchi and Foyer, 2003). Exogenous application of AsA can increase adaptation to many detrimental environmental stresses such as heavy metals, drought, extreme temperature and pathogen attack (Shalata and Neumann, 2001; Ramírez *et al*., 2013) and is also demonstrated to alleviate seed oxidative stresses and enhance the seed vigour, germinability and seedling growth attributes (Fan *et al*., 2009; Ramírez *et al.*, 2013).

However, little information is available about the effect of AsA on ageing caused by moisture and high temperature in forage seeds. Siberian wildrye (*Elymus sibiricus* L.), a kind of perennial grass, is characterized by its high crude protein content, cold and drought tolerance and high palatability. It can be utilized to recover the degraded rangeland and provide the hay production. However, the utilization value is influenced by the seed quality and germinability, which usually decreases progressively due to the ageing during storage. The aim of this experiment was to determine impact of exogenous applied AsA to alleviate the damage of ageing on Siberian wildrye seed germination, activities of antioxidant enzymes, lipid peroxidation and the ultra-structure of embryo cells, and improve the resistance of seeds to storage and adverse environmental conditions.

Materials and Methods

Seed Material

Siberian wildrye seeds were provided by the Forage Seed Laboratory, China Agricultural University. Seeds germination percentage was 79% and original moisture content was 10.8%.

Determination of Seed Moisture Content

Seed moisture content was determined in accordance with ISTA (2013). Approximately 4.5 g seeds were put in a sample container and weighed and then oven-dried at 130°C~133°C for 1 h (two replicates). After cooling for 30 min in a desiccator, seeds were weighed again and moisture content was calculated.

Exogenous Application of AsA

Siberian wildrye seeds were sterilized for 10 min using 5% sodium hypochlorite solution. After surface sterilization, seeds were divided into two parts, and respectively treated by distilled water (the control) and 2.0 mM AsA for 24 h at 25°C. Soon afterwards, seeds were rapidly natural dried under shade and adjusted to 4%, 10% and 16% moisture content.

Adjustment of Seed Moisture Contents

Approximately 10 g seeds were weighed before treatment and the seed weight required to reach the corresponding moisture contents under natural conditions was calculated. Seeds after AsA treatments were placed into a desiccator with allochroic silica gel and weighed frequently until the required weight. Then seeds were immediately placed into an aluminum foil bag and sealed, incubated at 5°C for 24 h at least.

Seed Ageing Treatment

After regulation of seed moisture contents, the seed samples were aged at the controlled condition of temperature (45°C) for 48 h, and then used for the experiments.

Germination Test after Ageing Treatment

Germination was assayed according to the rules of ISTA (2013). Four replicates of 100 seeds each were germinated on filter papers moistened with distilled water in the germination incubator (GXZ-380B, China) at an alternative temperature with 8 h light period at 25°C and 16 h dark period at 15°C. The number of normal seedlings was counted after 12 d to calculate the seed germination percentage.

Antioxidant Enzymes Extraction and Activity Assays

The extraction procedures were carried out at 4°C. De-coated seeds (0.3 g) were ground and homogenized using a mortar and pestle in 6 mL phosphate buffer (50 mM, pH 7.0, 1.0 mM EDTA, 1% PVP). The homogenate was centrifuged at 18000 rpm for 20 min at 4°C. Then the resultant supernatant was used for the antioxidant enzymes assays.

Superoxide dismutase (SOD) (EC 1.15.1.1) activity was assayed essentially according to the method of Beauchamp and Fridovich (1971). Total 3.3 mL reaction mixture with 1.5 mL 50 mM phosphate buffer (pH 7.8), 0.3 mL 130 mM methionine, 0.3 mL 750 μM nitroblue tetrazolium (NBT), 0.3 mL 100 μM EDTA, 0.3 mL 20 μM riboflavin, 0.5 mL distilled water and 0.1 mL enzyme extract. The SOD activity was defined as suppression of 50% NBT photochemical reduction for an enzyme activity unit.

Catalase (CAT) (EC 1.11.1.6) activity was measured by the dynamic change in absorbance at 240 nm in 1 min due to the decline of extinction of H_2O_2. 50 μL of the supernatant was mixed with 3.4 mL phosphate buffer (25 mM, pH 7.0, mixed with 0.1 mM EDTA) and 200 μL 100 mM H_2O_2.

Ascorbate peroxidase (APX) (EC 1.11.1.11) activity was measured according to the method of Zhu *et al.* (2011). The assay depended on the decrease in absorbance at 290 nm in 1 min as ascorbate was oxidized. 3.4 mL 25 mM pH 7.0 phosphate buffer, 200 μL 10 mM H_2O_2, 200 μL 0.25 mM ascorbic acid and 200 μL enzyme extract were mixed for measuring.

Glutathione reductase (GR) (EC 1.6.4.2) activity was measured according to the modified method of Halliwell and Foyer (1978) by the rate of decrease in the absorbance of NADPH at 340 nm. The reaction mixture contained 0.1 mL enzyme extract, 2.6 mL 50 mM pH 7.8 phosphate buffer, 0.1 mL 5 mM $MgCl_2$, 0.1 mL 0.5 mM GSSG and 0.1 mL 1.0 mM NADPH.

Seed Leachates Electrical Conductivity Measurement

Electrical conductivity (EC) of seed leachate was carried out according to the method by Goel *et al.* (2003). Four replicates of 50 seeds each were weighted and rinsed several times with deionized water, surface dried with filter paper, then soaked in 50 mL of deionized water and kept in the incubator at 25°C for 24 h. Electrical conductivity (EC) measurement was performed using an automatic reading seed conductivity meter (DDSJ-308A, China). The conductivity was expressed as per gram seed conductivity value ($\mu S\ cm^{-1}\ g^{-1}$).

Determination of Malondialdehyde (MDA) Content

De-coated seeds (0.5 g) were ground and homogenized using a mortar and pestle in 8 mL 5% (w/v) trichloroacetic acid (TCA) at 4°C and centrifuged at 18000 rpm for 20 min. The supernatant was used for MDA determination according to the method of Heath and Packer (1968). The reaction mixture contained 2.5 mL 0.5% thiobarbituric acid in 5% (w/v) TCA and 3.0 mL supernatant. The reaction mixture was incubated at 100°C for 15 min, then immediately cooled to 25°C and centrifuged at 12000 rpm for 20 min. The MDA level was determined by measuring absorbance at 532 nm and 600 nm.

Ultra Structure Observation of Embryo Cells

Siberian wildrye seeds with exogenous AsA treatment and the control were selected randomly. The embryos were removed and fixed in a 4% glutaraldehyde solution for 24 h and placed in a refrigerator at 4°C. Then embryo samples were rinsed with 0.1 M phosphate buffer (pH 7.2), and post-fixed in 1% osmium tetraoxide at 4°C for 2 h. The samples were washed again with 0.1 M phosphate buffer (pH 7.2) after post-fixing, and then were dehydrated through a graded alcohol series of 30%, 50%, 70%, 80%, 90% and 95% and embedded in epoxy resin. Ultrathin slices were cut using the LKB8800 III ultra-microtome. The slices on copper grids were stained with 1% uranium acetate solution for 20 min followed by citrate solution for 5 min. Observations were then carried out using transmission electron microscopy (Hitachi H-7500).

Statistical Analysis

All data were analyzed from analysis of variance (ANOVA) which was performed using SPSS, version 17.0. Each treatment was analyzed in four replications. Duncan's multiple range test was applied to compare the treatment means of germination and physiological changes at P=0.05 and differences at $P<0.05$ were considered significant.

Results

Effect of Exogenous AsA Treatment on Germination Percentage of Aged Seeds

Germination percentage of Siberian wild-rye seeds in control and AsA treatment both presented the declining trend with seed moisture content increased from 4% to 16%, and there were significant (P<0.05) differences between the level of seed moisture content (Fig. 1). Compared with control, AsA treatment could inhibit the loss of germination percentage after ageing, but only there was significant (P<0.05) difference at 10% moisture content, and no significant differences at moisture content of 4% and 16%. The decreasing of germination percentage in aged seeds could be alleviated significantly (P<0.05) by application of AsA at the 10% moisture content.

Effect of Exogenous AsA Treatment on Electrical Conductivity and MDA Content of Aged Seeds

For control and AsA treatment, electrical conductivity value of Siberian wildrye seeds at 10% and 16% moisture content both were significantly (P<0.05) higher than that at 4% moisture content (Fig. 2a). Also, under the conditions of 4% and 10% moisture content, electrical conductivity of seeds treated with AsA both were significantly (P<0.05) decreased than that in control, but there was no significant difference (P>0.05) at 16% moisture content (Fig. 2a).

Malondialdehyde (MDA) content of aged seeds was related with the level of moisture contents to a large extent in control and AsA treatments and it decreased significantly (P<0.05) with moisture content increasing from 4% to 16% (Fig. 2b). Malondialdehyde (MDA) content of seeds in AsA treatment was significantly (P<0.05) lower than that in control at moisture contents of 4%, 10% and 16%.

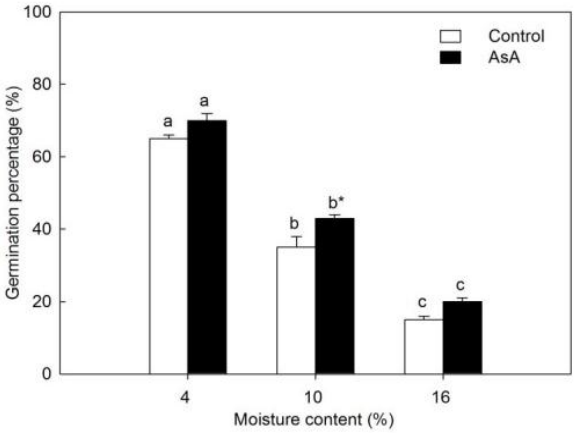

Fig. 1: Effect of AsA treatment on germination percentage of Siberian wildrye seeds aged with different moisture contents

Means ± SE from four replicates. Different letters indicate statistically significant differences between different moisture contents and asterisks indicate statistically significant differences within same moisture content (one-way ANOVA, $P<0.05$)

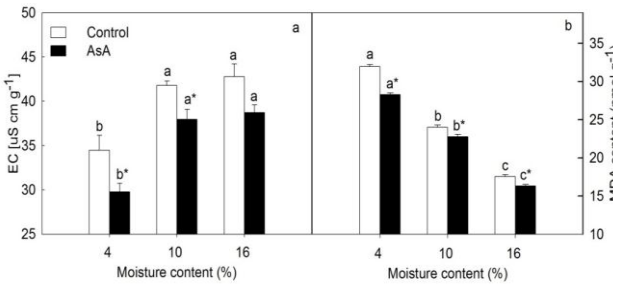

Fig. 2: Effects of AsA treatment on electrical conductivity (EC) (a) and MDA content (b) of Siberian wildrye seeds aged with different moisture contents

Means ± SE from four replicates. Different letters indicate statistically significant differences between different moisture contents and *asterisks* indicate statistically significant differences within same moisture content (one-way ANOVA, $P<0.05$)

Effect of Exogenous AsA on Antioxidant Enzyme Activities of Aged Seeds

The changes of enzyme activities in aged seeds of Siberian wildrye presented changing tendency as moisture content increased from 4% to 16%, according to the analysis results of SOD, CAT, APX and GR activities with AsA treatment and the control (Fig. 3). The SOD activity in AsA and control treatments both significantly ($P<0.05$) decreased with seed moisture contents increasing from 4% to 16% (Fig. 3a). However, its activity of seeds in AsA treatment was significantly ($P<0.05$) higher than that in control seeds at 4% and 16% moisture content and no difference was detected at 10% moisture content.

The CAT activity in control presented a significant ($P<0.05$) decrease with moisture content increasing from 4% to 16%; but its activity in AsA treatment significantly ($P<0.05$) increased as moisture content increased from 4% to 10%, and was also up to the maximum at 10%, then dropped to a lower level at 16% moisture content. Compared with control, AsA treatment significantly ($P<0.05$) enhanced this enzyme activity at moisture content of 10% and 16% (Fig. 3b).

Glutathione reductase (GR) activity in AsA and control treatments significantly ($P<0.05$) increased with moisture content increasing from 4% to 10% and was up to the maximum level at 10% moisture content, then significantly ($P<0.05$) decreased to the lowest level at 16% moisture content (Fig. 3c). Compared with control, AsA treatment contributed to a significant ($P<0.05$) increase in GR activity at moisture content 10%.

The changing of APX activity in AsA and control treatments was obviously different with seed moisture content increasing from 4% to 16% (Fig. 3d). The maximum APX activity in control was attained at 4% moisture content and the minimum at 10%. Also, there were significant ($P<0.05$) differences for APX activity in control between moisture content of 4%, 10% and 16%. Ascorbate peroxidase (APX) activity in AsA treatment was maximum at 16% moisture content and significantly ($P<0.05$) higher than at 4% and 10% moisture content. However, application of AsA made APX activity significantly ($P<0.05$) higher than in control seeds at moisture content of 10% and 16%.

Effect of Exogenous AsA Treatment on Ultrastructure of Embryo Cells of Aged Seeds

According to microscopic observation of embryo cells of Siberian wildrye seeds, the structure of embryo cells, cytoplasmic membrane, nucleus and mitochondria were discussed (Fig. 4). In control seeds with 4% moisture content, the results showed that the cellular structure was complete, with normal cytoplasmic membrane, intact nucleus, clear nuclear nucleolus, clearly visible nuclear membrane, normal mitochondria and cristae (Fig. 4a, g, m). As moisture content increased to 10%, the cytoplasmic membranes appeared to be broken, nuclear area blurred, the double structure nuclear membrane mis-shaped, but nuclear nucleolus was clear; in addition, parts of the mitochondria began to swollen and lose integrity, cristae became rare (Fig. 4b, h, n). Serious damage occurred in seeds with 16% moisture content, with diminished nuclear area, pyknotic nuclear nucleolus, discontinuously existent nuclear membrane, the mitochondria swelling seriously and becoming to vacuolar structure (Fig. 4c, i, o).

However, there were protective effects for AsA treatment on embryo cells of Siberian wildrye seeds, especially when moisture contents of seeds were 4% and 10%. For AsA treatment seeds with 4% moisture content, the structure of entire cells presented complete, cytoplasmic membranes, nucleus, nuclear nucleolus, nuclear membrane and mitochondria all were intact (Fig. 4d, j, p).

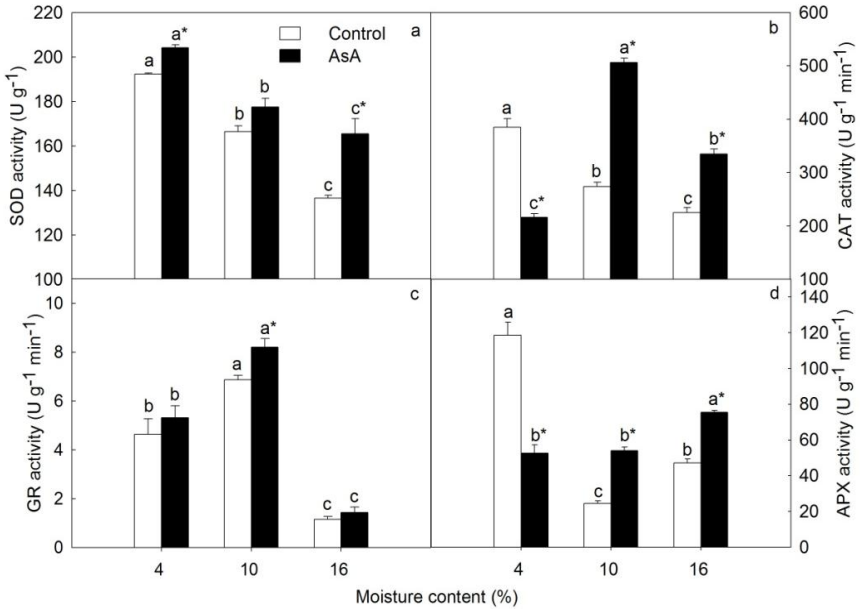

Fig. 3: Effects of AsA treatment on antioxidant enzymes activities [SOD activity (a), CAT activity (b), GR activity (c) and APX activity (d)] of Siberian wildrye seeds aged with different moisture contents

Means ± SE from four replicates. Different letters indicate statistically significant differences between different moisture contents and asterisks indicate statistically significant differences within same moisture content (one-way ANOVA, $P<0.05$)

As seeds moisture content increased to 10%, AsA treatment maintained the embryo cell in a good condition, with distinct nuclear area and clear nuclear nucleolus, double and visible nuclear membranes, normal mitochondrial membranes and part of cristae being distinct (Fig. 4e, k, q). In addition, AsA treatment had no ability to maintain cell integrity when seeds moisture content increased to 16%, nuclear nucleolus presented pyknotic, but it had a slight protective effect on nuclear membrane and mitochondrial membrane (Fig. 4f, l, r).

Discussion

Seed germination percentage is frequently used to express seed germination ability. However, it generally decreases as a consequence of ageing due to the overproduction and accumulation of oxidative radicals (Gill and Tuteja, 2010). Loss of seed germinability during ageing period is associated with the lipid peroxidation (Goel *et al.*, 2003) and has been widely recognized as a major cause of membrane integrity losing (McDonald, 1999). In this study, for aged seeds both in control and AsA treatment, the declined change tendency of germination percentage was exactly in accordance with the increase of electrical conductivity and the decrease of antioxidant enzymes activities as moisture content increased from 4% to 16% (Fig. 1, 2a and 3). Also, with increasing moisture content, cytoplasmic membranes became increasingly broken and the most sensitive mitochondria deteriorated with showing as swollen, deformed and even rupturing (Fig. 4m, n, o); nuclear nucleolus presented pyknotic (Fig. 4a, b, c); double structure of nuclear membrane blurred and even broken into fragments (Fig. 4g, h, i). All these suggested that the decline of seed germinability was related to the loss of membrane integrity and the rupture of cellular structure. While, as a product of lipid peroxidation, MDA is also the symptom of seed ageing (Lu *et al.*, 2013), and generally, MDA content increased with ageing extent deepening (Goel and Sheoran, 2003). But in our experiment for control and AsA treatment seeds, MDA content decreased progressively as moisture content increased from 4% to 16% (Fig. 2b), which had been reported in oat seeds with 4%~22% moisture content (Kong *et al.*, 2014). The decrease of MDA content demonstrated that seed ageing might occur without lipid peroxidation at high moisture content (16%) (Kibinza *et al.*, 2006). High moisture content caused serious damage to membranes, or might activate seed respiration to excessively consume substrate of lipid peroxidation such as lipid, which caused a shortage of targets for free radical, so that lipid peroxidation was insufficient to occur and MDA content decreased. But the accurate mechanism of inner physiological reaction and relationship between ageing and moisture content was not clear.

Ageing leads to oxidative stress due to the increasing ROS production, and ROS scavenging primarily depends on the efficiency of relevant antioxidant enzymes such as SOD, CAT, APX and GR (Bailly *et al.*, 1996; Thapa *et al.*, 2011). As effective antioxidant scavenger, AsA could improve the antioxidant enzymes activities when some species suffered from detrimental conditions, such as bean (Dolatabadian and Saleh-Jouneghani, 2009), corn (Darvishan *et al.*, 2013) and grass pea (Talukdar, 2012).

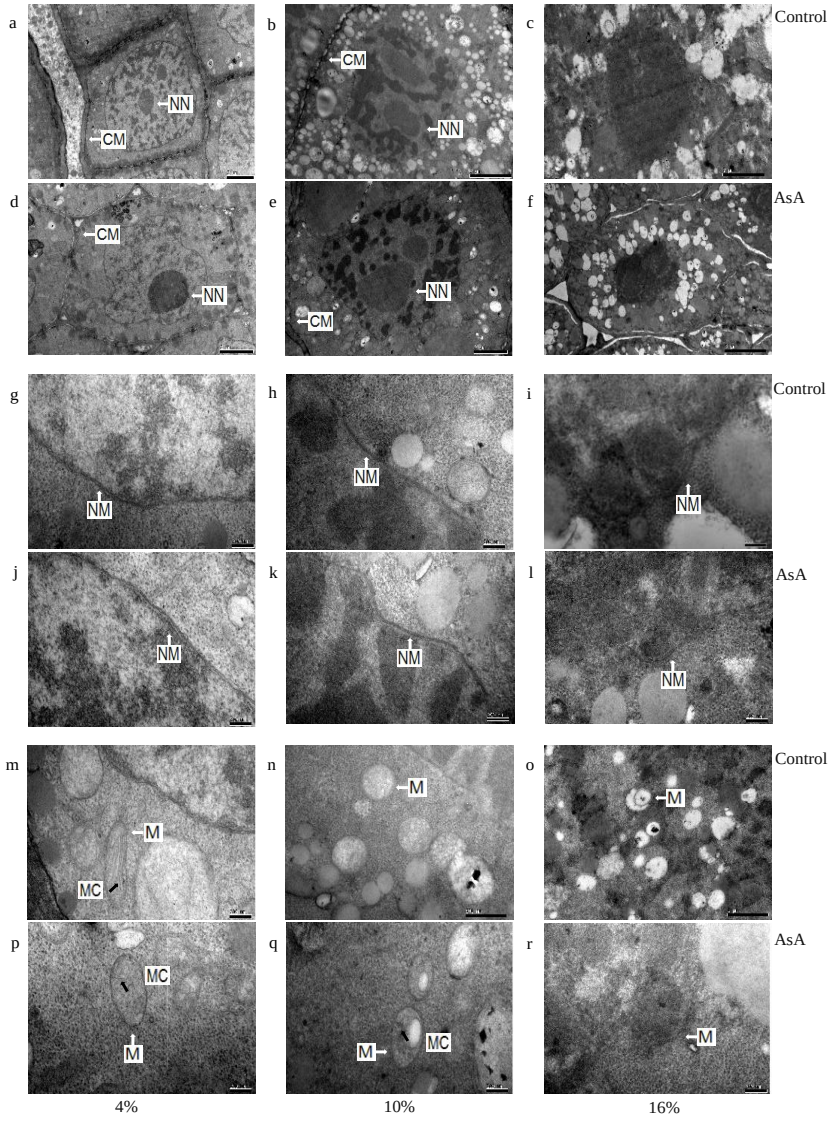

Fig. 4: Effects of AsA treatment on the ultra-structure of nucleolus (a-f), nuclear membranes (g-l) and mitochondria (m-r) of embryonic cells in Siberian wildrye seeds aged with different moisture contents

Moisture contents as indicated below, the control and AsA treatment as indicated on the right. a-e, bars = 2 μm; f, bar = 5 μm; g-l, m, p-r, bars = 200 nm; n, bar = 500 nm; o, bar = 1 μm. CM - cytoplasmic membrane; NN - nuclear nucleolus; M - mitochondria; MC - mitochondrial cristae; NM - nuclear membrane

In our study, seed germination percentage remained at a higher level when moisture content was 4%. Also, compared with control, exogenous AsA treatment could significantly decline electrical conductivity value and MDA content (Fig. 2), and cellular structure could be protected showing that cytoplasmic membrane, nuclear membrane, mitochondria and cristae were all normal and complete (Fig. 4d, j, p). This indicated that seeds with AsA treatment could be kept higher vitality by maintaining the cytoplasmic membranes integrity due to function of AsA to scavenge ROS when seeds moisture content was at low level. However, among the antioxidant enzymes, SOD was the only one whose activity was significantly improved in AsA treatment seeds (Fig. 3a), as explained by McDonald (1999). Lipid peroxidation could indeed be prevented as moisture content was between 0.06 and 0.14 g H_2O g^{-1} DM, or even at a lower level. Superoxide dismutase (SOD) provided the first line to defense ROS by converting superoxide anion to H_2O_2, and exogenous AsA treatment could activate SOD enzyme to ensure its capability of scavenging ROS when less ROS produced by lipid peroxidation in seeds at 4% moisture content, which in return protected cytoplasmic membranes and seed vigour.

It had been reported that AsA could play a major role in ascorbate-glutathione cycle in mitochondria and APX was the most important enzyme in the regeneration of AsA

by using NADPH as an electron donor (Wu *et al.*, 2009). In addition, several isoforms of CAT were localized in cytosol, mitochondria and peroxisomes (Foyer and Allen, 2003). In our study, as seeds moisture content increased to 10%, exogenous AsA treatment could significantly inhibit the decline of germination percentage (Fig. 1); and correspondingly, the electrical conductivity value and MDA content in AsA treatment were significantly lower than in control seeds (Fig. 2). Meanwhile, AsA treatment protected seeds cellular structure against damage of ROS, as presented that cytoplasmic membrane, nuclear membrane, mitochondria and cristae were all normal and complete (Fig. 4e, k, q). This indicated that AsA-treatment seeds could be kept higher vitality by maintaining the membranes integrity, and similar results have been reported in some species such as pea (Hernandez and Almansam, 2002), *Arabidopsis thaliana* (Wójcik and Tukiendorf, 2011) and *Eragrostis ciliaris* (Zehra *et al.*, 2012). Moreover, CAT, GR and APX activities in AsA treatment seeds were significantly improved (Fig. 3b, c, d), which suggested that the three kinds of enzymes played important roles in scavenging ROS at this moisture content. As reported, CAT and APX were the major enzymes to scavenge H_2O_2 and localized in cytosol, mitochondria and peroxisomes (Foyer and Allen, 2003). Therefore, CAT and APX activities could be maintained by keeping the mitochondria structure integrity (Fig. 3b, d and 4q), and they devoted to catalyzing detoxification of H_2O_2 to water (Shigeoka *et al.*, 2002; Shunmugam *et al.*, 2013).

Close relationship was found between seed deterioration and the loss of membrane system integrity, which generally caused seed vigour to decline. It indicated that abnormal cell membrane and unregulated structure presented with the serious development of seed ageing. Cheng *et al.* (1991) had reported that mitochondria were susceptible to ROS and attacked first, and followed by the whole disintegrated during seed ageing. In our study, deterioration had much more detrimental effects as moisture content was 16%, and exogenous AsA had no obviously protective function on integrity of membrane system and mitochondria, so seed germination percentage decreased. Compared with control, exogenous AsA treatment had just kept the integrity of nuclear membrane and mitochondria partly (Fig. 4l, r), which was also the reason why APX and CAT activities were improved at 16% moisture content.

Conclusion

In conclusion, the application of exogenous AsA enhanced seed germinability and tolerance to deterioration by inhibiting the damage to membrane integrity caused by lipid peroxidation. As a non-ignorable factor, seed moisture content played a key role in the function of AsA, especially when it was 10%. Moreover, the inhibiting effect of AsA treatment on seed ageing was associated with the efficiency of relevant antioxidant enzymes. Therefore, exogenous AsA treatment on seeds before storage is an efficient technique to cope with the detrimental effects of storage and ageing.

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