

Enhancement of Welsh Onion (*Allium fistulosum* L.) Seed Vigor by KNO₃ Priming

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ABSTRACT

Welsh onion (*Allium fistulosum* L.) is one of the important vegetable crops in China. However, the seeds of this crop are short-lived and their vitality tends to be easily lost in the course of storage. The present study was conducted to elucidate the effects of welsh onion seed priming with potassium nitrate (KNO₃) on seed vigor and on other such related mechanisms during twelve months of storage. The priming treatment improved the germination rate and vigor index of welsh onion. Membrane deterioration of welsh onion seeds was significantly alleviated by the priming treatments, as assayed by using soluble sugar content and electrical conductivity of the seed leachates. The accumulation of hydrogen peroxide (H₂O₂) and malondialdehyde (MDA) in primed seeds was reduced compared with non-primed seeds. The increased activities of antioxidant enzymes, such as dehydrogenase, superoxide dismutase (SOD), and catalase (CAT) in primed seeds correlated with seed vigor and lipid peroxidation. This study provided the theoretical basis for improving the initial quality of welsh onion seeds using KNO₃ priming.

Keywords: Antioxidant enzymes, Lipid peroxidation.

INTRODUCTION

Members of the genus *Allium* are rich sources of sulfur-containing compounds, alkaloids, and steroidal saponins, which are beneficial to human health (Hu *et al.*, 2006). Species belonging to genus *Allium* are widely distributed all over the world, and account for 3.0% of the total cultivated area of vegetables, and 3.4% of vegetable production in China in 2004. However, *Allium* seeds lose viability and vigor at faster rates than seeds of most other crops, even at relatively optimum storage conditions (Ellis *et al.*, 1996; Yanping *et al.*, 2000). Poor seed performance is one of the crucial factors that limit welsh onion (*Allium fistulosum* L.)

production and development. Several treatments (osmo-conditioning, matricconditioning, and hydropriming) have been carried out to improve seed quality. The results obtained using these technologies have been described by Taylor *et al.* (1988). Such treatments involve hydration-dehydration or pre-treatment of seeds using a wide variety of chemicals prior to natural or accelerated ageing (Bailly *et al.*, 1998; Demir and Oztokat, 2003; Wahid *et al.*, 2008). Priming is a common practice for seed enhancement in the seed industry, including non-controlled water uptake systems (methods in which water is freely available and not restricted by the environment) and controlled systems

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(methods that regulate seed moisture content preventing the completion of germination) (Taylor *et al.*, 1998). This practice is known to increase seed vigor, synchronize, and accelerate germination, confer stress resistance and antioxidant activity, and improve plant growth and productivity (McDonald, 2000). Primed seeds are held at the plateau phase of water concentration following a rapid increase in water uptake. A series of biochemical and physiological activities occur during this plateau phase, thereby improving the subsequent germination.

Deleterious effects of ageing have been associated with damages occurring at membrane, nucleic acids, and protein levels (Fujikura and Karssen, 1995). Soluble sugars and electrolyte leakage have been demonstrated to be involved in the deleterious effects occurring during dehydration and ageing, suggesting that they are directly linked to seed vigor (Lee *et al.*, 1995). Long time storage also results in increased lipid peroxidation, decreased levels of antioxidants, and reduced activity of several enzymes involved in hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) accumulation. Seeds are equipped with oxygen radical detoxifying enzymes, such as dehydrogenase, superoxide dismutase (SOD), catalase (CAT), and peroxidases (POD) to survive under stressful conditions (Bailly *et al.*, 2002; Lehner *et al.*, 2008). Thus, increased antioxidative activities, along with other mechanisms, may contribute to the decreased susceptibility to deterioration of primed seeds (Goel *et al.*, 2003; Amjad and Anjum, 2007).

Seed priming treatments using salts such as KNO_3 have been effective in improving watermelon, tomato, egg plant, and chili germination (Demir and Van de Venter, 1999; Venkatasubramanian and Umarani, 2007). However, literature reports on welsh onion seed priming effects are limited. A few reports exist on osmotic priming of aged onion seeds using polyethylene glycol-8000, resulting in a marked increase in the rate of

germination (Basra *et al.*, 1994); however, the exact effects and mechanisms of KNO_3 priming on welsh onion seed vigor still remain unclear. Furthermore, most of the studies on cellular and biochemical deterioration during seed ageing have been performed under accelerated ageing conditions, i.e., at high temperature and in saturating humidity (McDonald, 2000).

Zhu and Wang (2008) have reported that KNO_3 priming significantly improves accelerated ageing of welsh onion vigor. Although accelerated ageing has been recognized as a good predictor of seed storability, the mechanisms involved in seed priming under such extreme conditions differ from those occurring under ambient conditions.

The present study was conducted to determine the effect of KNO_3 priming on the germination rate and vigor index of welsh onion seeds, and to confirm, at a physiological level, the alleviation (caused by the priming treatment) of welsh onion seed aging during storage. These data would contribute to further understanding of the mechanism of priming treatment on aging seeds, and offer an effective measure to improve seed quality.

MATERIALS AND METHODS

The widely grown and commercially produced seeds of welsh onion (*Allium fistulosum* L. cv. Zhangqiu) were harvested from Shandong Province, China, with a moisture content of 7.8 % (on dry weight basis) (International Rules of Seed Testing Standard Methods, 1999).

According to our previous study, the priming of 2% KNO_3 solution (Seed: Solution ratio 1:10) for 24 hours was the optimum condition, and this priming condition significantly improved the seed vigor of welsh onion (Zhu and Wang, 2008). To prime the seeds, they were immersed in 2% KNO_3 solution at 25°C for 24 hours in the dark (Maude *et al.*, 1994; Zhu and Wang, 2008). Thereafter, the seeds were

rinsed with distilled water three times. The treated seeds were surface-dried and dried back to their original moisture content at room temperature (about 22°C, 45% relative humidity), the original moisture content having been determined by changes in seed weight. Four replicates of 10-g seeds of primed and control seeds (non-treated seeds) were tested every three months up to 12 months. All of the primed and non-primed seeds were sealed in polyethylene bags and stored at room temperature (25 to 30°C) for the next test (Rao *et al.*, 2006).

The quality of the seeds was examined according to the International Rules of Seed Testing Standard Methods (ISTA, 1999). Four replicates of 50 seeds each were placed on two layers of filter paper moistened with water equivalent to 2.5 times the substratum weight, and germinated on Petri plates at 25°C. Seedling counts were performed after seven days, and the mean percentage of normal seedlings was calculated for each lot. The complete morphological parts without lesions or defects were selected and considered as vigorous seedlings. The average seedling length of ten seedlings was measured for the calculation of seedling vigor index.

Four replicates of 100 seeds each were placed in 100-ml beakers separately, containing 75 ml of distilled water. The seeds were gently stirred to ensure that all seeds were completely immersed and evenly distributed. The beakers were then placed in an incubator at 20°C for 24 hours. After immersion, the seeds were gently stirred and the electrical conductivity of the soaking water was measured without filtration using a digital conductivity meter (JENWAY, Model 4070) (Rao *et al.*, 2006). The soluble sugar extract was measured using anthrone, and absorbance was read at 625 nm by a spectrophotometer (Beckman, DU640) (Yemm and Willis, 1954).

The MDA content was determined by the method of Heath and Packer (1968). Seeds were ground in 3 ml of 0.1% trichloroacetic acid (TCA) solution. The homogenate was centrifuged at 15,000×g for 10 minutes, and

0.5 ml of the supernatant fraction was mixed with 20% TCA (2 ml) containing 0.5% thiobarbituric acid (TBA). The mixture was heated at 90°C for 20 minutes, cooled, and subsequently centrifuged at 10,000×g for 5 minutes. The absorbance was recorded at 532 nm, and the value for non-specific absorption at 600 nm was subtracted.

The H₂O₂ content was measured according to O'Kane *et al.* (1996). Seeds were ground and extracted using 3 ml of cold acetone. The homogenate was centrifuged at 10,000×g at 4°C for 20 minutes. Afterward, 0.5 ml of the supernatant fraction was mixed with 1.5 ml of CHCl₃ and CCl₄ (1:3, v/v) mixture. Subsequently, 2.5 ml of distilled water was added and the mixture was centrifuged at 1,000×g for 1 minute, and the aqueous phase was collected for H₂O₂ determination.

The total dehydrogenase activity was assayed according to the method of Kepczynska *et al.* (2003). Seeds were immersed in liquid N₂ and pulverized with mortar and pestle. Samples were then incubated in 7 ml of 0.1M sodium phosphate buffer (pH 7.2) containing 1.5% (w/v) of 2,3,5-triphenyltetrazolium chloride at 25°C for 24 hours. Afterwards, the samples were centrifuged for 6 min at 12,000×g, and the pellet was extracted three times with 15 ml of acetone. The supernatant fractions were combined and the solution absorption was measured at 510 nm. A standard curve was prepared from known concentrations of 1,3,5-triphenylformazan.

The SOD activity was assayed as described by Beauchamp and Fridovich (1971). The reaction mixture contained riboflavin, methionine, KCN, and nitroblue tetrazolium (NBT) salt dissolved in sodium phosphate buffer (pH 7.8). Approximately 3 ml of the reaction medium was added to 1 ml of the enzyme extract. The mixtures were illuminated in glass test tubes using two sets of Philips 40W fluorescent tubes in a single row. Illumination was performed to initiate the reaction at 30°C for 1 hour. Identical solutions kept under dark served as blanks. The absorbance was read at 560 nm in the



spectrophotometer against the blank. SOD activity is expressed in $U\ g^{-1}$ protein. One U is defined as the change of 0.1 in absorbance per hour per mg protein.

CAT activity was measured according to the method of Bailly *et al.* (1996), with minor modifications. The assay mixture contained 2.6 ml of $50\ mmol\ L^{-1}$ potassium phosphate buffer (pH 7.0), 0.4 ml of $15\ mmol\ L^{-1}\ H_2O_2$, and 0.04 ml of the enzyme extract. The decomposition of H_2O_2 was followed by the decline in absorbance at 240 nm. The enzyme activity was expressed in $U\ g^{-1}$ protein. One U is defined as $1\ mmol\ L^{-1}$ of H_2O_2 reduction per min per mg protein.

POD was assayed using the method of Goel *et al.* (2003). The assay mixture of POD contained 2 ml of $0.1\ mol\ L^{-1}$ phosphate buffer (pH 6.8), 1 ml of $0.01\ mol\ L^{-1}$ pyrogallol, 1 ml of $0.005\ mol\ L^{-1}\ H_2O_2$, and 0.5 ml of the enzyme extract. The solution was incubated for 5 minutes at $25^\circ C$, and then the reaction was terminated by adding 1 ml of $2.5\ mol\ L^{-1}\ H_2SO_4$. The amount of purpurogallin formed was determined by measuring the absorbance at 420 nm against a blank, prepared by adding the extract after the addition of $2.5\ mol\ L^{-1}\ H_2SO_4$ at zero time. The activity was expressed in $U\ g^{-1}$ protein. One U is defined as the change in the absorbance per 0.1 min per mg protein. In addition, the enzyme protein was estimated using the method of Bradford (1976) for all the enzymes.

Statistical analysis was performed using SPSS 16.0. Means were separated by calculation of least significant difference (LSD) at 5% level. The values are expressed as mean \pm SD of the four samples in each group.

RESULTS

The initial percentage of germination in the non-primed control seeds was 91.2%, indicating the superior quality of the seed lot used in this experiment (Figure 1). The germination rate of welsh onion seeds decreased during the 12th month of storage,

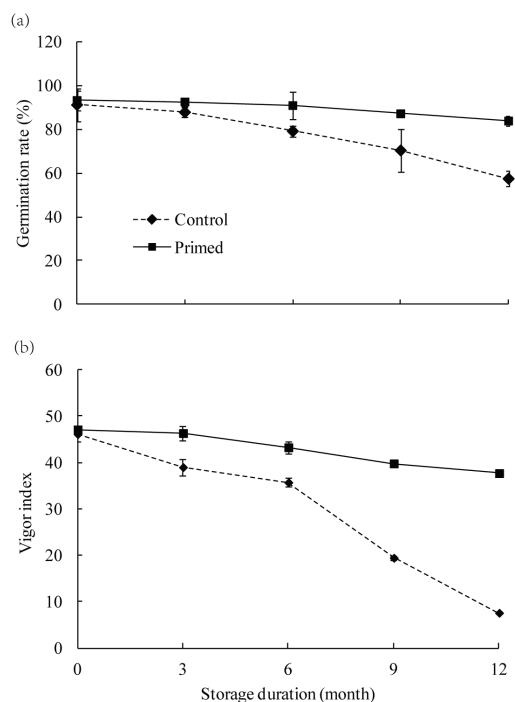


Figure 1. Effects of priming treatment on the germination rate and vigor index of seeds during 12 months of storage. Data are the means of three replicates with standard errors shown by vertical bars.

both in the non-primed control and in the primed treatment (Figure 1-a). The germination rate of the control treatment declined to 57.4% after 12 months of storage. The primed seeds had no difference at the beginning of the experiment compared with the control treatment, but the percentage of germination declined slightly during the 6th month. Priming enhanced the germination rate of the seeds by up to 26.3% after 12 months of storage. Similarly, the seedling vigor gradually reduced during storage (Figure 1-b). The loss of vigor was significantly higher in the non-primed control treatment seeds. These results confirmed that priming improved the viability and vigor of the seeds.

The soluble sugars content and the relative electrical conductivity of the seed leachates increased progressively by the priming treatment after the first three months of storage (Figure 2). The soluble sugars of the primed seeds were significantly lower than

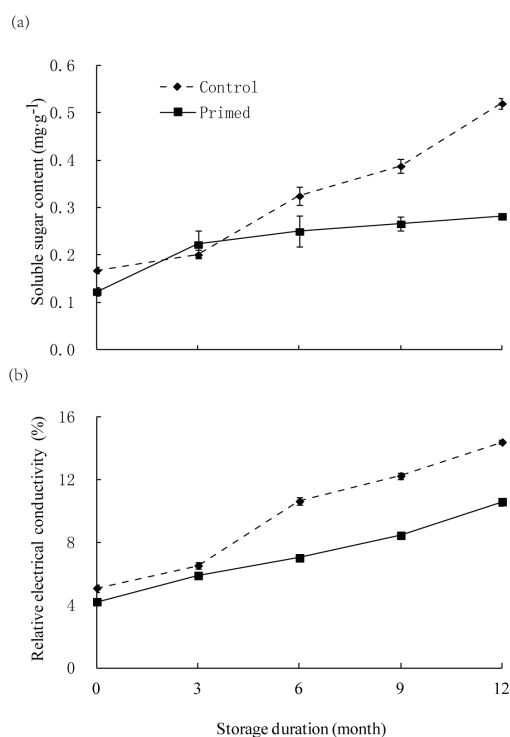


Figure 2. Effects of priming treatment on soluble sugar contents and relative electrical conductivity of seeds during 12 months of storage. Data are the means of three replicates with standard errors shown by vertical bars.

the non-primed control, the trend of decrease being evident with increasing storage time (Figure 2-a). The electrical conductivity of the primed seeds was significantly reduced in contrast to the non-primed control; however, a significant change was not observed in the first three months (Figure 2-b).

Storage tended to cause the accumulation of H₂O₂ and MDA in the seeds, with the trend of increase being evident with increasing storage time (Figure 3). Although priming and non-priming treatments had similar increasing ratio, the priming treatment alleviated the MDA content increase of the seeds during storage (Figure 3-a). The H₂O₂ content of the primed seeds decreased compared with the non-primed control at the start of the priming treatment, but did not significantly differ during the first six months. After six months of storage,

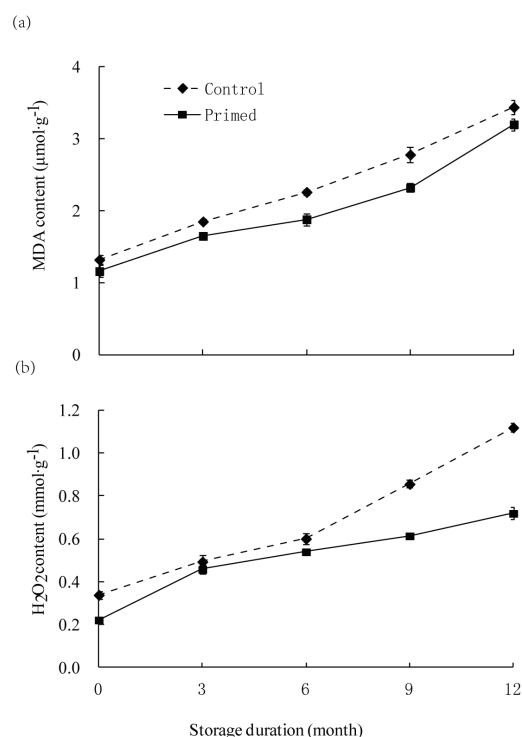


Figure 3. Effects of priming treatment on hydrogen peroxide (H₂O₂) and malondialdehyde (MDA) contents of seeds during 12 months of storage. Data are the means of three replicates with standard errors shown by vertical bars.

the priming treatment showed significant effects, markedly slowing down the increase of H₂O₂ content and causing the opposite effect to seed vigor (Figure 3-b).

Dehydrogenase, SOD, and CAT activities of the non-primed control and primed seeds decreased during the 12 months of storage. The priming treatment alleviated the reduction of these activities when compared with the non-primed treatment (Figure 4). At the start of the priming treatment, the primed seeds had higher SOD, CAT, and POD activities compared with the non-primed control treatment. After six months of storage, the differences became increasingly significant, which were correlated with seed vigor and lipid peroxidation. However, the slight reductions of POD activity were shown in the non-priming control and the priming treatments; the difference between treatments was less significant (Figure 4-d).

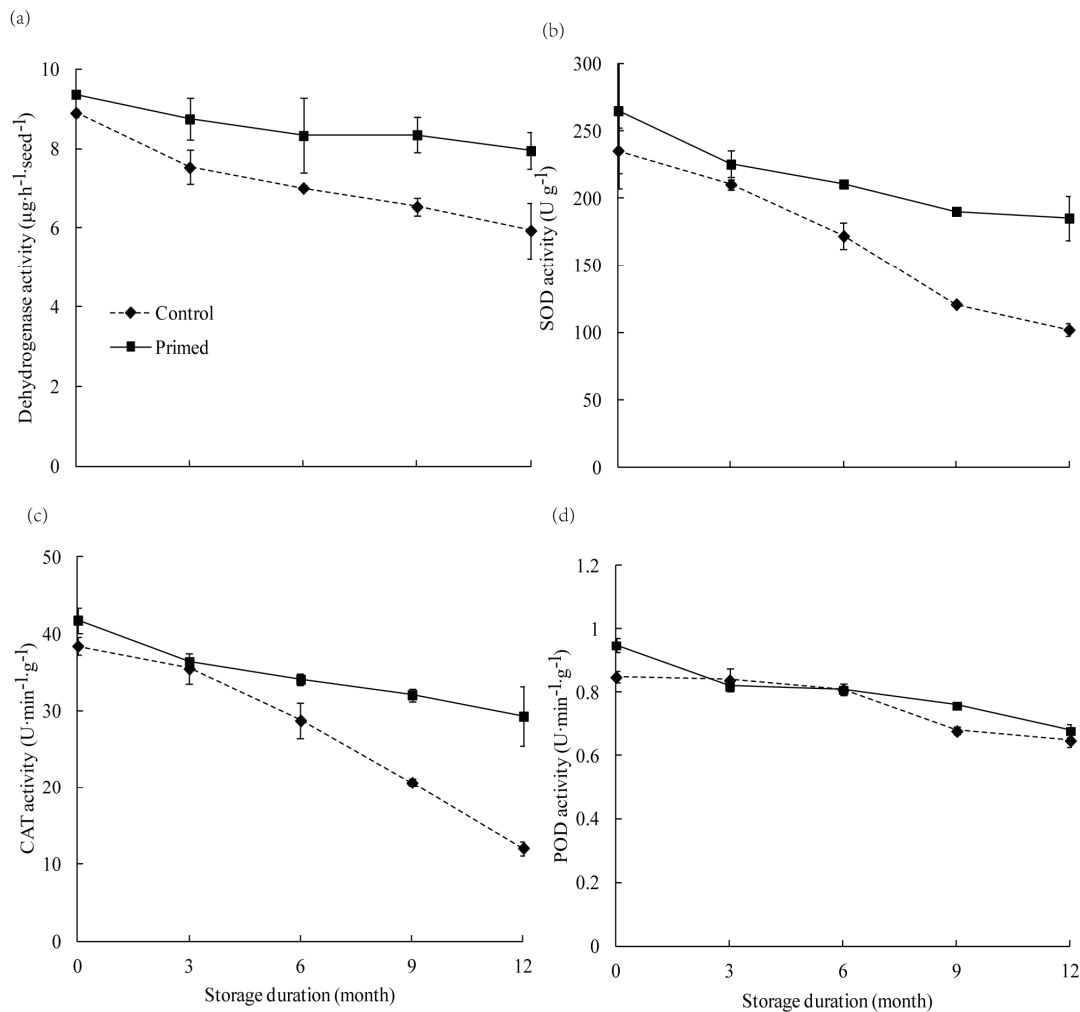


Figure 4. Effects of priming treatment on activities of dehydrogenase, superoxide dismutase (SOD), catalase (CAT), and peroxidases (POD) of seeds during 12 months of storage. Data are the means of three replicates with standard errors shown by vertical bar.

DISCUSSION

Results of the study showed beneficial effects of KNO_3 priming on welsh onion seeds and confirmed previous findings on the germination of onion seeds primed with polyethylene glycol-8000 (Basra *et al.*, 1994). Similar results have also been obtained with other species (Venkatasubramanian and Umarani, 2007). Seed vigor is defined as seed properties that determine the performance of the seeds during germination and seedling emergence

(Argerich and Bradford, 1989). In this study, vigor index decreased in the non-primed treatment by up to 83.6% after 12 months of storage, indicating that the seeds were of the short-lived type. The vitality of welsh onion seeds is easily lost in long time storage (Rao *et al.*, 2006). In the present study, the damage accumulated both in the non-primed control and in the primed treatment. Thus, KNO_3 priming was an effective and useful measure to diminish ageing effects on welsh onion seed germination.

Seed ageing is a natural phenomenon, which is the sum of a range of biochemical

processes that finally lead to death. The decline in viability of naturally or artificially aged seeds results mainly from damage to nucleic acid and the deterioration of cellular membranes (Hsu *et al.*, 2003). A number of metabolic processes accompany the loss of seed viability during ageing. The leachate conductivity and soluble sugars are directly proportional to the loss of seed vigor and viability (Lee *et al.*, 1995; Obendorf, 1997). The results presented here showed that increased electrolyte leakage and soluble sugar contents (Figure 2) were related to decreased germination rate and vigor index. Thus, priming treatment may relieve the damage on the cell membrane and decrease leachate conductivity and sugar contents during storage.

Although loss of seed viability results from cellular damage, different mechanisms may be involved in mortality, depending on ageing conditions. Lipid peroxidation and oxidative stress have been widely indicated as the major causes of seed deterioration during ageing (McDonald, 2000). Several studies demonstrated that loss of seed viability was associated with an accumulation of MDA and H₂O₂ (Bailly *et al.*, 1996; Lehner *et al.*, 2008). Our results showed that the non-primed control and the primed set-up had similar accumulation rate of MDA contents, with the priming treatment always alleviating the accumulation. The difference of H₂O₂ contents between the two treatments increased with storage time. These results, taken in conjunction with reduced ability to germinate, indicate that increased lipid peroxidation may explain the loss of vigor and viability of welsh onion seeds.

A previous study reported that improved seed vigor and the reduction in seed deterioration were due to quenching of peroxidative damage and the enhancement of the activities of peroxide scavenging enzymes (Goel *et al.*, 2003). The higher content of MDA and peroxide in welsh onion seeds may also result from ageing-induced inhibition of peroxide scavenging activity. Thus, the removal of peroxide from

aged seeds may be too slow to prevent the accumulation of peroxide (Bailly *et al.* 1996). In this study, we have established that the main enzymes involved in cell detoxification are dehydrogenase, SOD, CAT, and POD. These enzymes activities were parallel with decreases in germination, alongside increased levels of MDA and POD. Similar studies on the increase in the activities of antioxidant enzymes in primed seeds have also been reported recently (Chiu *et al.*, 2003; Hsu *et al.*, 2003).

In conclusion, KNO₃ priming of welsh onion seeds served as a viable technology to enhance germination and vigor. The results also support the hypothesis that the improvements in germination rate and vigor index of KNO₃ priming compared with non-primed controls were possibly caused by the decreased lipid peroxidation and increased antioxidative activities. Further investigations are required to elucidate the effects of seed priming under field conditions, and on the biochemical process of antioxidant function.

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بهبود بنیه بذر پیاز ولش (*Allium fistulosum* L.) با پرایمینگ با KNO₃

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چکیده

پیاز ولش (*Allium fistulosum* L.) یکی از سبزیجات مهم در چین است. با این وجود، بذرهای این گیاه عمر کوتاهی دارند و بنیه آنها در طول انبارداری از بین می رود. پژوهش حاضر برای روشن کردن اثرات پرایمینگ این بذر با نترات پتاسیم (KNO₃) روی بنیه بذر و دیگر سازوکار های مربوطه در طی ۱۲ ماه انبار داری انجام شد. تیمار پرایمینگ منجر به بهبود نرخ جوانه زنی و شاخص بنیه (vigor index) پیاز ولش شد. تخریب ممبران بذرهای پیاز ولش با پرایمینگ به طور معنی داری بهبود یافت که این امر با اندازه گیری قندهای محلول موجود در آبشویه (leachate) بذر ها و هدایت الکتریکی آبشویه مشخص شد. تجمع پراکسید هیدروژن (H₂O₂) و مالون دی الدیید (MDA) در بذرهای پرایم شده در مقایسه با بذر های تیمار نشده کاهش یافت. افزایش فعالیت انزیم های آنتی اکسیدانی مانند دی هیدرو ژناز، (SOD) superoxide dismutase و catalase در بذرهای پرایم شده با بنیه بذر ها و lipid peroxidation رابطه داشت. نتایج این پژوهش مبانی نظری را برای بهبود کیفیت اولیه بذرهای پیاز ولش با پرایمینگ بذر ها با استفاده از نترات پتاسیم فراهم آورد.