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Full Length Article

Seed Dormancy and Germination Response of *Aegilops tauschii* to Exogenous Application of GA_3 and Warm Water

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Abstract

The invasion of *Aegilops tauschii* has seriously threatened the wheat production in China. The seeds of *A. tauschii* display obvious dormancy. In present study, seed dormancy and germination of *A. tauschii* were investigated by treating seeds with exogenous GA_3 and different hot water treatments. The results revealed that exogenous GA_3 and warm water soaking treatments were beneficial to dormancy breaking and germination of seeds. Among all treatments, 500 $mg \cdot L^{-1}$ GA_3 for 24 h and hot water treatments with 45ºC warm for 5 min significantly improved germination. During germination, SOD activity in seeds treated with GA_3 (500 $mg \cdot L^{-1}$) and warm water (45ºC) increased significantly. Increased SOD activity reduced the degree of oxidative damage of plasma membrane and resulted a continuous decrease in thiobarbituric acid reactive substance assay (TBARS) content. Thereby, seeds were prompted to develop in a direction conducive to germination. In addition, GA_3 (500 mg L^{-1}) and warm water (45ºC) treatments increased the endogenous levels of gibberellin (GA_3), auxin (IAA), and zeatin riboside (ZR), and decreased the endogenous ABA in seeds. This had resulted significantly higher ratios of GA_3/ABA, IAA/ABA and ZR/ABA than those ratios of distilled water (room temperature) treated plants. The effect eventually promoted the germination of *E. ulmoides* seeds. Based on the changes in enzyme activity and endogenous hormones during seed germination, it is concluded that 500 $mg \cdot L^{-1}$ GA_3 for 24 h and hot water treatments with 45ºC warm for 5 min significantly improved germination of *A. tauschii* seeds. © 2020 Friends Science Publishers

Key words *Aegilops tauschii* Coss.; Germination stage; Endogenous hormones; Enzyme activity

Introduction

Aegilops tauschii Coss., an annual grass species, is commonly known as ‘rough-spike hard grass’. The species originated in Eastern Europe and West Asia and is considered as one of the top ten malignant weed species (Wang 2017). In 1955, *A. tauschii* specimens were collected for the first time in the Xinxiang area of Henan Province, the People’s Republic of China. Nowadays, this weed has invaded more than 10 major wheat producing provinces, including Hebei and Shandong (Wang *et al.* 2019a). *A. tauschii* population has the characteristics of strong growth adaptability, high tillering coefficient, barren land resistance and a long emergence time. The seed shape and size are similar to those of wheat, which together make this weed extremely difficult to control in wheat fields (Wang *et al.* 2018).

Due to its serious threat to food security the plant has been listed as an alien Quarantine pest in the People’s Republic of China in 2007. Later on it was considered as one of the first batch of the “National Key Controlled Alien Species Directory” (published in 2013, Wang *et al.* 2019a, b). The damage caused by the species to wheat was surveyed by the Institute of Plant Protection of the Chinese Academy of Agricultural Sciences, in Hebei and Shanxi Provinces. Results showed that a large-scale damage by *A. tauschii* to wheat had occurred, and the yield of wheat was reduced by more than 50%. At present, *A. tauschii* has been known as the “wheat killer” and has been listed as a major quarantine object of the grain trade in many countries and regions (Wang *et al.* 2019b).

Liu *et al.* (1998) proposed that the seeds of *A. tauschii* had strong dormant characteristics. However, the existing literature on its dormancy characteristics is mainly focused their utilization, rather than its dormancy mechanism. This is because *A. tauschii*, a species closely related to *Triticum aestivum*, shows a strong pre-harvest sprouting resistance (Yan *et al.* 2003), and the pre-harvest sprouting seriously affects the quality and production of wheat (Yang *et al.* 2007). But the researches on dormancy mechanism and method of dormancy breaking of *A. tauschii* seeds have not been reported.

A large number of studies have shown that exogenous GA_3 treatment is helpful for seed dormancy and germination

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(Lu *et al.* 2014; Lai *et al.* 2017; Ma *et al.* 2018; Shang *et al.* 2019). In addition, soaking seeds in warm water has the effect of softening the seed coat and increasing the permeability of the seed coat. Therefore, warm water soaking at a suitable temperature is beneficial for seed germination (Wang and Zeng 1997; Chen *et al.* 2015, 2017). The plant hormones are the most important endogenous factors in regulating germination (Pawlowsk and Staszak 2016). The present study envisages the exploration of the changes in the enzymes and endogenous hormones involved in germination and dormancy breaking mechanisms of *A. tauschii* from both physiological and biochemical aspects, and provide a reference for its subsequent research.

Materials and Methods

Experimental details

A. tauschii seeds were collected from the experimental field (35° 18 'N, 113° 52' E) of the Xinxiang Academy of Agricultural Sciences, Henan Province, China in May 2019. The seed moisture content was about 7.50% and 1000-grain weight is 10.9~14.3 g. After drying, put them in sealed desiccators and store them at 25 ± 2ºC indoors. The experiment was carried out in the Horticultural Plant Laboratory, Henan University of Science and Technology, China in Oct. 2019. The entire *A.tauschii* seeds with consistent size were selected, sterilised with 5% sodium hypochlorite solution for 10 min, rinsed with distilled water 3 times, and then air-dried for use.

The treated seeds were divided into 2 batches, and were soaked separately. First, Batch of seed was soaked in 0 (CK), 100, 200, 300, 400, and 500 mg·L^{-1} of GA_3 solution, and for each concentration for 12 h, 24 h and 48 h at 22ºC ± 2ºC. Sec batch of seeds was subjected to five gradient constant temperature conditions (on water bath using beakers) at room temperature (CK), 40, 45, 50 and 60ºC at different soaking times (1, 2, 5 and 10 min). The seeds after soaking were dried in-door for further use.

Seed germination assay

Seed germination test was carried out by the sand culture method (Zhang *et al.* 2009). Fine sand was sterilised by treating in an oven at 120ºC for 120 min after washing. The treated seeds were placed in a petri dish (diameter 9 cm) covered with 60 g of fine sand (50 seeds per dish), and there were five replicates for each treatment. The plates were cultivated in a light incubator (Ningbo Jiangnan Instrument Factory, GXZ-280) in conditions of 25ºC /20ºC (day/night) and 12 h/12 h (light/dark).

Considering the dormancy characteristics of *A. tauschii* seeds and the method used by Liu (2014), seed germination test was carried out for 35 days. During this period, a moist condition of the fine sand was maintained and the number of germinated seeds was counted every day (the seed was considered germinated, when the length of the radicle was 1/2 of the length of the seed). After the end of the test, final germination was calculated.

Physical and biochemical indicators

According to the results of germination test, the best seed soaking method was selected to determine the physical and chemical indexes during germination. In germination, seeds treated with distilled water (room temperature; CK), 500 mg·L^{-1} GA_3 solution for 24 h, and 45ºC warm water for 5 min and samples were taken at 0 (CK), 7, 14, 21, 28 and 35 d. First, embryo and endosperm about 2.50 g excised from seed. The superoxide dismutase (SOD) activity and the content of thiobarbituric acid (TBARS) were measured using the nitrogen blue tetrazole photoreduction and the thiobarbituric acid method, respectively (Li 2000; Zou 2003). Sec, samples were taken at 0 (CK), 7, 14, 21, 28 and 35 d, embryo and endosperm 0.5~1.0 g excised from seed, store in -70ºC ultra-low temperature refrigerator, and measured the endogenous hormone content after all sampling is completed. Gibberellin (GA_3), auxin (IAA), abscisic acid (ABA) and zeatin nucleoside (ZR) contents were determined by enzyme-linked immunosorbent assay (EMSA) (Li 2000). The ratios of GA_3/ABA, IAA/ABA and ZR/ABA were also calculated.

Data processing

S.P.S.S. 18.0 was used to analyse the experimental data and to determine the significance of the differences among the treatments. The data of the experiment were plotted, wherever necessary, using Excel. Statistical values are expressed as means (± SE).

Results

Effects of soaking treatment on the germination of seeds

It was found that with an increase in the GA_3 concentration and the soaking time, the overall germination of *A. tauschii* seeds gradually increased (Fig. 1). Specifically, the germination of the seed that was soaked with 500 mg·L^{-1} solution for 24 h was the highest, reaching 96.33%, and was significantly different from the CK ($P < 0.05$). Soaking in warm water also significantly promoted the germination of *A. tauschii* seeds, but with the continuous increase of the water temperature and soaking time, the germination gradually decreased. In particular, the germination was highest when it was treated with 45ºC warm water for 5 min, reaching 98.33%, and it was significantly different from CK ($P < 0.05$).

Effects of soaking treatment on physiological characteristics

During germination, the superoxide dismutase (SOD)

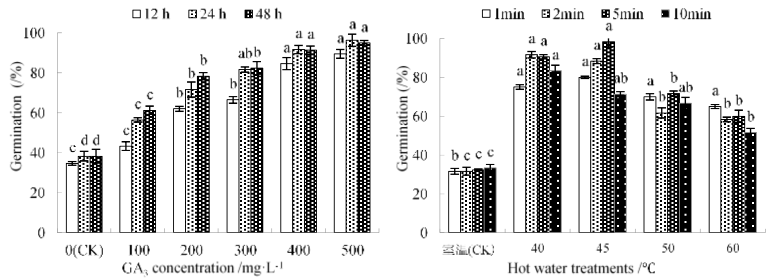

Fig. 1: Effect of different soaking treatment on germination of *A. tauschii* seeds
Note: Different letters indicate significant different among different concentration treatment ($P < 0.05$), the same as below

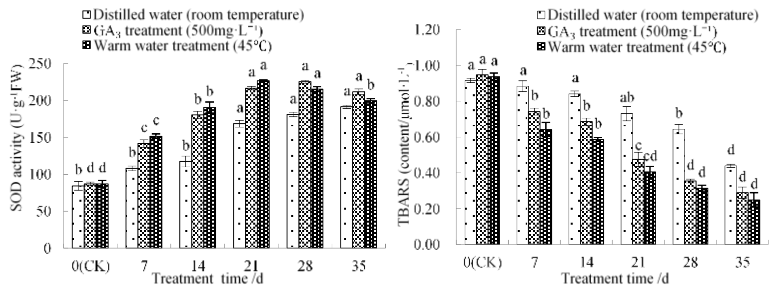

Fig. 2: Changes of the SOD activity and TBARS content in *A. tauschii* seed during germination stages with different soaking treatment

activity in seeds treated with distilled water (room temperature) continued to increase, while the SOD activity in GA_3 (500 mg·L^{-1}) and warm water (45ºC) treatment increased initially followed by a subsequent decrease (Fig. 2). The SOD activity of distilled water (room temperature) treatment was significantly different from that of CK on the 21^{st} day, while the treatments of GA_3 and warm water were significantly different on the 7^{th} day ($P < 0.05$). In addition, from the 5^{th} day, the SOD activity of GA_3 (500 mg·L^{-1}) and warm water (50ºC) treatment was significantly higher than that of distilled water (room temperature) treatment.

The contents of thiobarbituric acid (TBARS) in the seeds under different seed soaking treatments continued to decline during germination (Fig. 2). Among them, the content of TBARS treated with distilled water (room temperature) was significantly different from that of CK by 28^{th} day, while for the treatments of GA_3 and warm water, the TBARS contents were significantly different on the 7^{th} day ($P < 0.05$). From the 5^{th} day, the TBARS contents of GA_3 and warm water treatments were significantly lower than that of distilled water treatment.

Endogenous hormones

During the germination process, the endogenous GA_3, IAA and ZR content and GA_3/ABA, IAA/ABA and ZR/ABA of the seeds treated with GA_3 and warm water were significantly higher than the soaking with distilled water, but the IAA content were significantly lower than those treated with distilled water.

The endogenous GA_3 content of the different soaking treatments initially increased and subsequently decreased, and the peak appeared on the 14^{th} day (Fig. 3). Although endogenous GA_3 content declined slightly during the germination process, but was maintained at a high level until the 35^{th} day. The content of the endogenous IAA and ZR in seeds with different soaking treatments increased significantly compared with the CK at the 7^{th} day ($P < 0.05$). Subsequently, both the IAA and ZR decreased first and then increased. Although the ZR content decreased slightly, it remained at a high level during the entire process.

The ABA content of the seeds under different soaking treatments continued to decrease. The endogenous ABA content in seeds treated with distilled water was significantly different from the CK at day 14 ($P < 0.05$), while the endogenous ABA content of the GA_3 and warm water treatments were significantly different from the CK at day 7 ($P < 0.05$). During germination, the values of ratios of endogenous GA_3/ABA and ZR/ABA in the seeds treated with distilled water were significantly different from the CK until the 14^{th} day, while the ratios of GA_3 and warm water treatments were significantly different from the CK on the 7^{th} day (Fig. 4). The values of the ratios of endogenous IAA/ABA in the treatment with GA_3 and warm water increased, when compared with the CK on the 7^{th} day.

Discussion

Seed dormancy and germination are important characteristics for plants to survive and adapt to environmental changes (Kildisheva *et al.* 2019). During germination, endogenous hormones respond to various physiological changes in the seed by regulating the metabolism of a series of proteins and enzymes, thereby

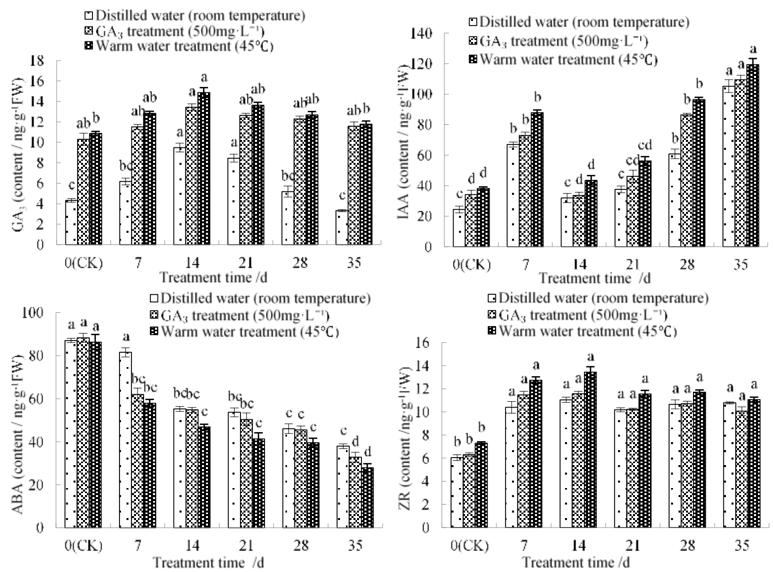

Fig. 3: Changes of endogenous hormone contents in *A. tauschii* seed during germination stages with different soaking treatment

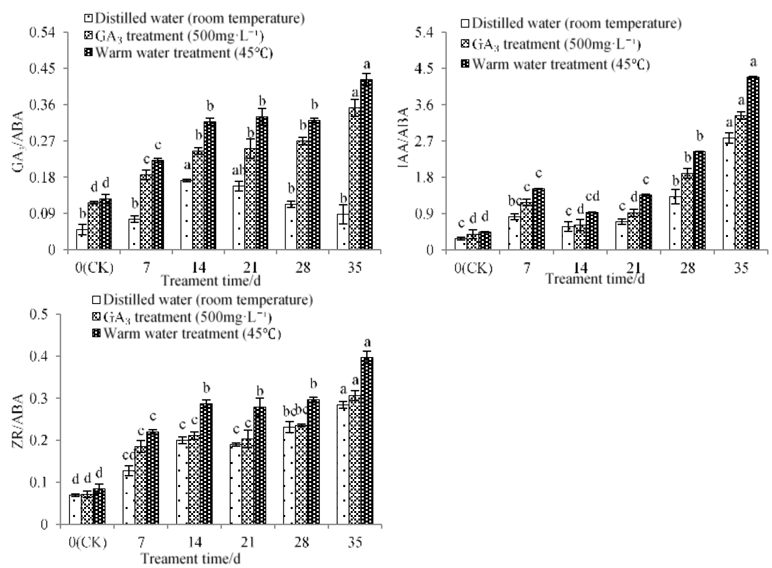

Fig. 4: The proportion of endogenous hormone contents in *A. tauschii* seed during germination stages with different soaking treatment

regulating the dormancy and germination of the seed. This study indicates that the exogenous GA_3 and warm water soaking treatments were beneficial to dormancy and germination of *A. tauschii* seeds. Among treatments with different levels of GA_3, highest germination was achieved in seeds soaked with 500 $mg \cdot L^{-1}$ GA_3 for 24 h. In addition, warm water also greatly promoted the germination of *A. tauschii* seeds. The germination rate was highest when treated with 45ºC warm water for 5 min but the germination gradually decreased with further rise in temperature. This could be because the seeds that entered the water-swelling stage were still in a high water temperature environment, and the seed embryos could get damaged at high temperatures, causing some seeds to die, and this eventually led to a decline in the germination rate.

Reactive oxygen species (ROS) are continuously produced by the metabolically active cells of seeds, and apparently play important roles in biological processes such as dormancy and germination. Strictly regulated concentrations of ROS are currently viewed as being essential for germination (Bailly *et al.* 2008). Cellular antioxidant systems maintain intracellular redox homeostasis, preventing the accumulation of toxic amounts of ROS while allowing ROS mediated signaling to occur (Foyer and Noctor 2009). SOD, as the first line of defense against ROS, can dismutate $O_2^{\bullet-}$ to produce H_2O_2 (Lai *et al.*

2017; Ma *et al.* 2018). In this study, compared with the CK, the increase in SOD activity in seeds treated with distilled water on day 21 was significant, and the SOD activities of GA_3 and warm water treated seeds were significantly different from the CK on day 7 ($P < 0.05$). In comparison with the distilled water treatment, GA_3 and warm water soaking treatments significantly increased the SOD activity in *A. tauschii* seeds, thereby reducing the damage of reactive oxygen species to the cell membrane system to a certain extent.

ROS can induce lipid peroxidation, and thiobarbituric acid-reactive substance (TBARS) is a final product of lipid peroxidation (Khoubnasabjafari *et al.* 2017). High TBARS levels are toxic to plant cells and cause programmed cell death, so the changes of TBARS content will have a certain impact on seed viability (Priestly 1986; Hou *et al.* 2009). The TBARS content of GA_3 and warm water treated seeds was significantly lower than that of distilled water treated seeds (Fig. 2). This is agreement with previous studies, which showed that soaking seeds with the appropriate concentration of GA_3 can increase the SOD activity and decrease the MDA content, which is conducive to seed germination (Huang *et al.* 2017; Ma *et al.* 2018; Zhu *et al.* 2018).

The importance of gibberellin (GA) on dormancy release has also been extensively reported (Hong *et al.* 2012; Hoang *et al.* 2014). The stimulation of GA on seed germination is mainly due to weakening the endosperm and increasing the embryo growth potential (Bewley 1997; Ogawa *et al.* 2003). The endogenous GA_3 contents of seeds in GA_3 and warm water treatments were significantly higher than that in the distilled water (room temperature) treatment (Fig. 3). It may be due to the continuous infiltration of external GA_3 or because soaking seeds in warm water caused more bound GA_3 in the seeds to gradually become free. While dormancy alleviation is strongly associated with a decline in abscisic acid (ABA) level (Chen *et al.* 2008). Exogenous GA_3 and warm water soaking treatment reduced the endogenous ABA content, thereby promoting the seed to develop in a direction favourable to germination (Fig. 3). This is agreement with previous studies on the seeds of *Paris Polyphylla* (Song *et al.* 2016; Su *et al.* 2018).

Studies have suggested that IAA can promote the termination of seed dormancy (Sun and Jia 2006; Han and Yi 2008) or has little relation with the termination of seed dormancy (Lu *et al.* 2014). ZR has an antagonistic effect on seed germination inhibitors, and to a certain extent it is negatively correlated with seed dormancy (Wu *et al.* 2015). The contents of endogenous IAA and ZR in the seeds treated with GA_3 and warm water were significantly higher than the seeds treated with distilled water, however, compared with the endogenous IAA (whose change was large), the ZR content in the different seed soaking treatments increased to a significant level on the 7^{th} day, but showed a slight decrease afterwards. It remained at an overall high level, which could be due to the relative promoting effect of ZR on the germination of *A. tauschii* seeds was more marked than IAA.

In contrast to a single endogenous hormone, changes in the balance between different hormones are more important for seed dormancy and germination; especially the ratio between promoting and inhibiting growth hormones (Duan *et al.* 2011; Su *et al.* 2018). In this study, the ratios of GA_3/ABA, IAA/ABA, and ZR/ABA were gradually enhanced in seeds treated with GA_3 and warm water were significantly higher than those treated with distilled water (Fig. 4). This is in consistent with the findings of Jin *et al.* (1997) who reported that the ratio of promoting hormones (GA_3, IAA, Z, ZR) content to that of ABA played a critical role in seed germination, with high germination percentage obtained when the promoting hormones/ABA ratio was high.

Conclusion

Exogenous GA_3 and warm water soaking treatments were beneficial to the dormancy and germination of *A. tauschii* seeds through modulations in different physiological and biochemical processes. Among treatments with different concentrations of GA_3, the germination rate of seeds soaked with 500 $mg \cdot L^{-1}$ GA_3 for 24 h was the highest. But in treatments with different temperatures of water, final germination was highest when treated with 45ºC warm water for 5 min.

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