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Germination and Seedling Growth in Primed Seeds of Sunflower under Water Stress

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Author's contribution

This whole work was carried out by author SNF.

Original Research Article

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ABSTRACT

Successful establishment of plants largely depends on successful germination. This study investigated the effect of ascorbic acid (1 and 2mM) on germination percentage, germination rate, seed stamina index, hypocotyl and radical length, seedling fresh and dry weight of sunflower (*Helianthus annuus* L.) under various water potentials (0, -2 and -4 bar) induced by PEG_{6000}. The experimental design was a factorial arrangement based on completely randomized design with three replications that performed at the laboratory of agriculture faculty of Shahid Bahonar University of Kerman, Iran. All parameters were negatively responded with water stress but using AsA alleviated the harmful effect of stress on the traits. The concentration of 2mM of AsA on measured traits was more effective compared with the other levels. Therefore, seed priming with AsA can be considered an appropriate tool for improving germination characteristics of sunflower under water stress.

Keywords: Ascorbic acid; water stress; germination; seedling growth.

1. INTRODUCTION

Drought is a major limitation to agricultural productivity [1,2]. It is one of the most important manifestations of abiotic stress and major yield limiting factor in plants. It also exacerbates the effect of the other abiotic and biotic stresses [3]. In recent years, important research

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efforts have been focused on improving the yields of crop species under water stress [4]. Drought disturbs the plant growth due to loss of turgor [5,6].

Seed priming, a controlled hydration technique allows the pre-germination metabolisms without actual germination [7,8], is one of the most pragmatic and short-term approach to combat the effects of drought [9,10] on seedling emergence and stand establishment.

Treatment with initiators caused to enhancement of germination rate, emerge of seedlings [7,11] and improveing resistance or tolerance to cold [12], drought [13] and salt [14]. Seed priming has obvious effects on seed germination and osmotic stress resistance [15,16], during which the contents of compatible solutes, proline and soluble sugar, and the activity of protective enzymes, such as superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) [17,18] as important indicators will increases. The effects of initiators on seeds germination and resistance to unfavorable growth conditions have been studied in legumes [19], wheat (*Triticum aestivum* L.) [20] and onion (*Allium cepa* L.) [21].

Ascorbic acid (AsA) is one of the important metabolites involved in cell division, osmotic adjustment [22] and also plays vital role during the initial stages of germination [23]. Application of AsA through seed priming may thus be helpful in improving the stand establishment of wheat under drought [24]. Priming with ascorbic acid significantly relived the harsh effects of drought stress on seedling growth of safflower and it seems that ascorbic acid was able to enhance the tolerant ability of the plant to drought stress [25]. Ascorbic acid treated plants showed higher net photosynthetic rate, transpiration and stomatal conductance compared to non-treated ones and exogenously applied of ascorbic acid may be effective in ameliorating the adverse effects of drought stress [26]. Osmo priming with AsA improved the drought resistance of wheat due to proline accumulation and antioxidant action of AsA and phenolics, leading the tissue water maintenance, membrane stability, and better and uniform seedling stand and growth [24]. This study was conducted in order to evaluate the effect of priming with ascorbic acid on sunflower germination characteristics and seedling growth during water stress.

2. MATERIALS AND METHODS

2.1 Plant Material

The seeds of sunflower (*Helianthus annuus* L.) used in the experiment were provided from seed and plant research center. The organization is work about seed control and certification that placed at Kerman, Iran.

2.2 Asa Treatment

The seeds were surface-sterilised with a 3% sodium hypochlorite solution, rinsed in distilled water for three times and dried before the experiment. Afterwards, the seeds divided into three groups that soaked for 12h in 0, 1 and 2mM of ascorbic acid.

2.3 Water Stress Treatment

After a preliminary test for low water potential tolerance (caused by PEG_{6000}), four water potential levels were selected and used for germination trial. The PEG solutions had water potential of zero (control), −2 and −4 Bar.

Thirty primed seeds of each group evenly placed on filter paper in sterilized 9-cm Petri dishes separately and 5ml of each PEG solution were added to related treatment. All Petri dishes were sealed to prevent the loss of moisture and avoid contamination, and then placed in a plant growth chamber (Conviron PGR-15) with a 14h photoperiod (300μmol m2 s1 PAR) at 25/22ºc (day/night) for 7 days. Germination was determined by counting the number of germinated seeds at 24-h intervals over a 7-d period and expressed as total percent germination. The seeds were considered to be germinated at the emergence of the radicle [27]. Radicle and hypocotyl lengths were measured 7 days after germination. The total dry weight were determined by drying the plant material in an oven at 75ºc for 24-h prior to weighing. Germination percentage, germination rate and seed stamina index were calculated using following formula.

$$G = (n/N)*100 \qquad [28]$$
$$RG = \Sigma\ (Ni/Di) \qquad [28]$$
$$SSI = [G * (HL+RL)]/100 \qquad [29]$$

G: Germination percentage, N: Number of seeds germinated, N: Total number of seed in each petri dishes, RG: Rate of germination (seed/day), Ni: Germinated seeds in each numeration, Di: Day of each numeration, SSI: Seed stamina index, HL: average of hypocotyls length, RL: Average of Radicles length.

2.4 Data Analysis

A factorial experiment based on a completely randomized design with three replications was carried out at the laboratory of agriculture faculty of Shahid Bahonar University of Kerman, Iran. All data were analyzed based on 2-way ANOVA analysis using SAS software [30]. The means comparison were done with Duncan's Multiple Range Test at $p<0.05$ level of probability.

3. RESULTS AND DISCUSSION

The results presented in Fig. 1 show that the germination percentage was reduced with decreasing water potential. So that, the lowest germination percentage was recorded at -4 bar water potential. Interaction of AsA and water potential on germination percentage was significant. AsA at the levels of 1 and 2mM caused to a significant increase in germination percentage at the all levels of water potential. The highest concentration of AsA (2mM) at the mentioned levels of water potential had the highest effect on germination percentage compared to the control.

The germination rate of Sunflower at the zero water potential and without AsA was 4.4 seed day^{-1}. Application of first level of AsA (1mM) significantly affected the germination rate of Sunflower in all levels of water potential and it could lessen the effects of low water potential on the trait. In general, the highest germination rate was recorded for 1 and 2mM of AsA at the zero water potential (control), while the lowest amount of the trait was belonged to the zero water potential that no pre-treated with AsA (Fig. 2).

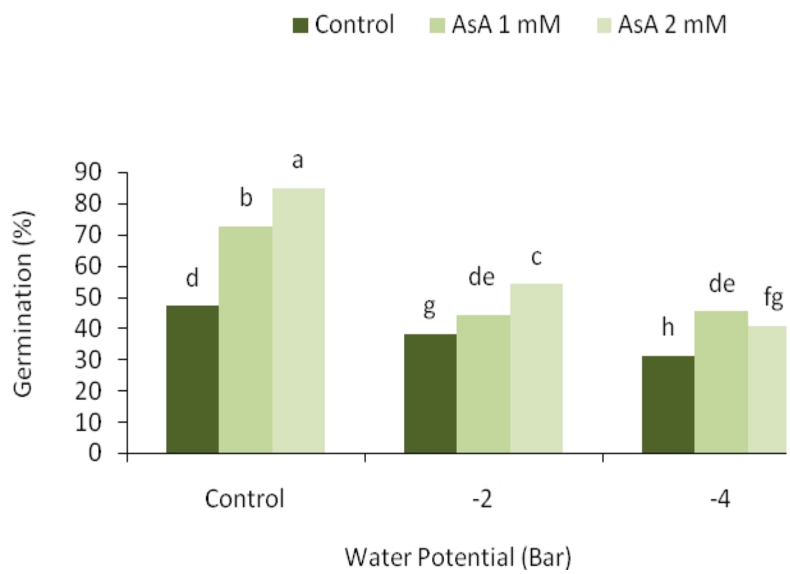

Fig. 1. The effect of AsA on germination percentage of Sunflower under water stress

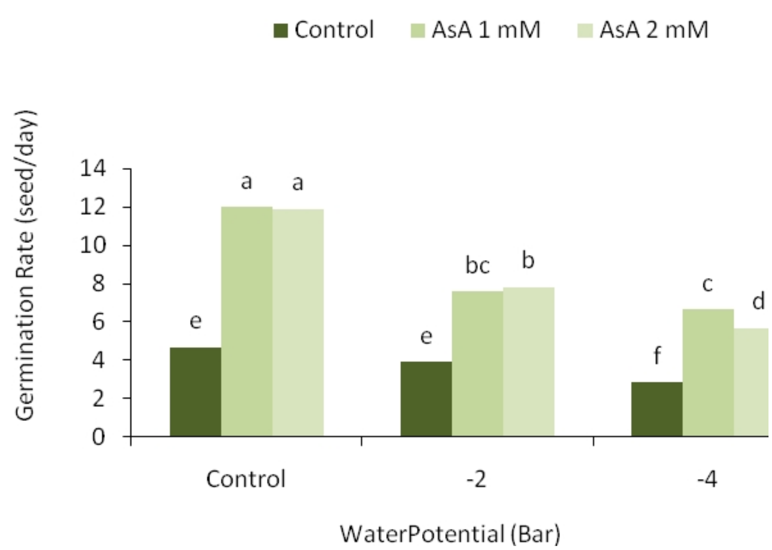

Fig. 2. The effect of AsA on germination rate of Sunflower under water Stress

Seed stamina index was significantly increased with the application of AsA. The higher concentration of AsA significantly affected SSI compared to the control. Although all the levels of water potential reduced the SSI significantly, but the lowest and highest reduction was belonged to -2 and -4 bar, respectively (Fig. 3).

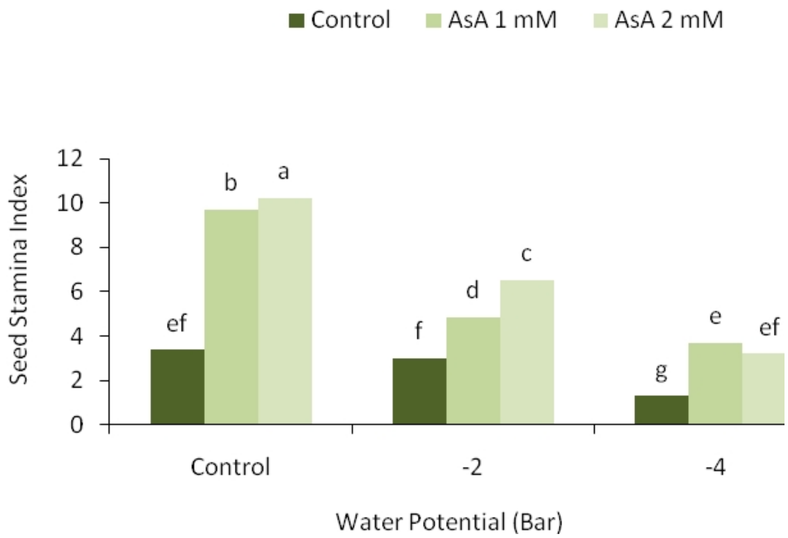

Fig. 3. The effect of AsA on seed stamina index of Sunflower under water stress

Priming presumably allowed some repairs of membrane damages which had been caused by deterioration. It has been reported that primed seeds showed better germination rate and higher vigor level than non-primed [14]. Nascimento and West [31] indicated that the improvement in germination and vigor of normal/low-vigor seed might be due to reserve mobilization of food material, activation and re-synthesis of some enzymes DNA and RNA synthesis start during priming. Priming can repair some damages that have been arisen from seed erosion and improve seed quality [32].

The reason of germination percentage reduction at the lower water potential may be due to slower rate of imbibition. From present investigations, it is quite clear that primeing seeds with various concentrations of ascorbic acid was effective in inducing stress tolerance at the germination stage in Sunflower. The mechanism of seed priming is to initiate the repairing system for membrane and the metabolic preparation for germination through controlling water absorption rate of seed [20]. As a result, the germination capability and resistance to unfavorable conditions of seed germination and growth can be promoted by seed pre-priming. Also the results of this experiment are same with results of Reiahi and Farahbakhsh [33] that worked on sorghum.

The response of hypocotyl length varied at different levels of water potential with different concentrations of AsA (Fig. 4). Hypocotyl length was decreased with reducing the water potential at the all levels of AsA. In addition, the results showed that the higher concentration of AsA (2mM) often had the same effect on this trait in comparison with level of 1mM AsA (Fig. 4).

The response of radicle length to different levels of water potential and AsA was closely same with hypocotyl length. The maximum radicle length (6.95cm) was observed when 1mM AsA applied at zero water potential while the minimum of the trait (2.62cm) resulted from the lowest level of water potential (-4 bar) that no pre-treated with ASA (Fig. 5).

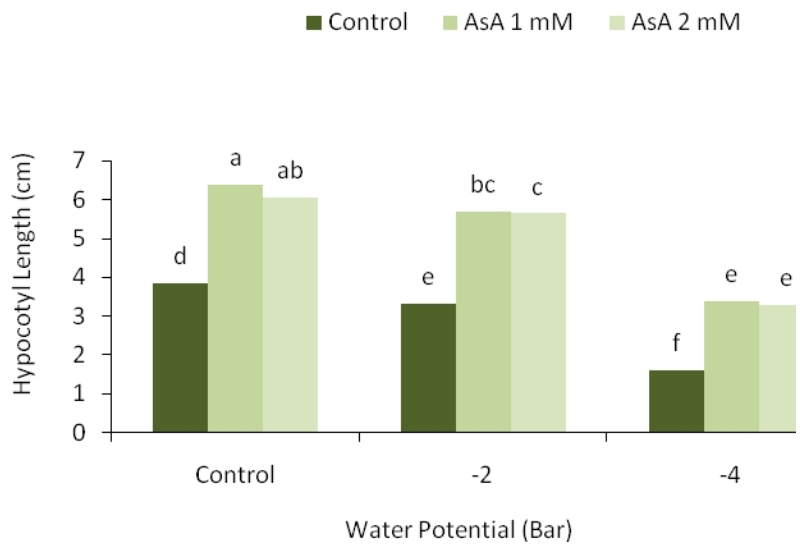

Fig. 4. The effect of AsA on hypocotyls length of Sunflower under water stress

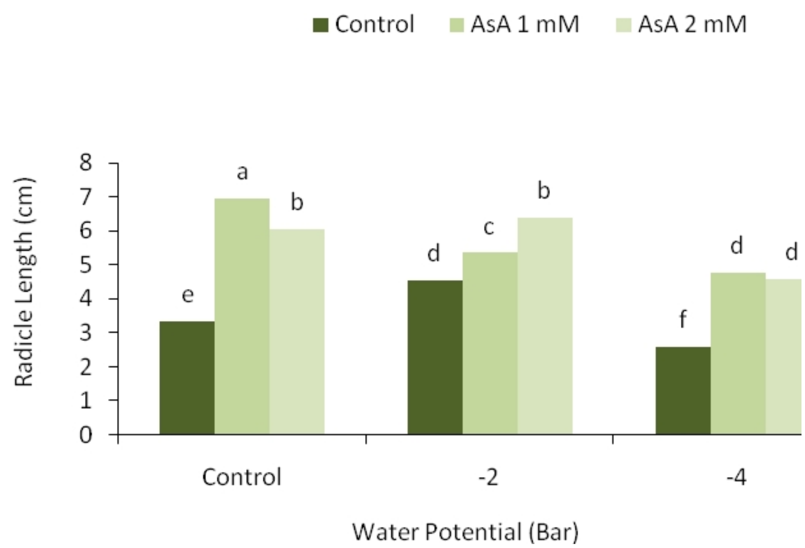

Fig. 5. The effect of AsA on radical length of Sunflower under water stress

The probable reason for early emergence of the primed seeds maybe due to the completion of pre-germination metabolic activities that making the seed ready for radicle protrusion and the primed seed germinated soon after planting compared with non-treated dry seed [34]. Similar to germination percentage, primed seeds had higher hypocotyl and radicle length compared with non-primed seeds. These positive effects are probably as a result of the stimulatory effects of priming on the early stages of germination process by mediation of cell division in germinating seeds [35,36].

The seedling fresh weight, responded differently to investigate factors (water stress and AsA). AsA at the 1 and 2mM increased FW significantly and had a same effect at the all levels of water potential (Fig. 6).

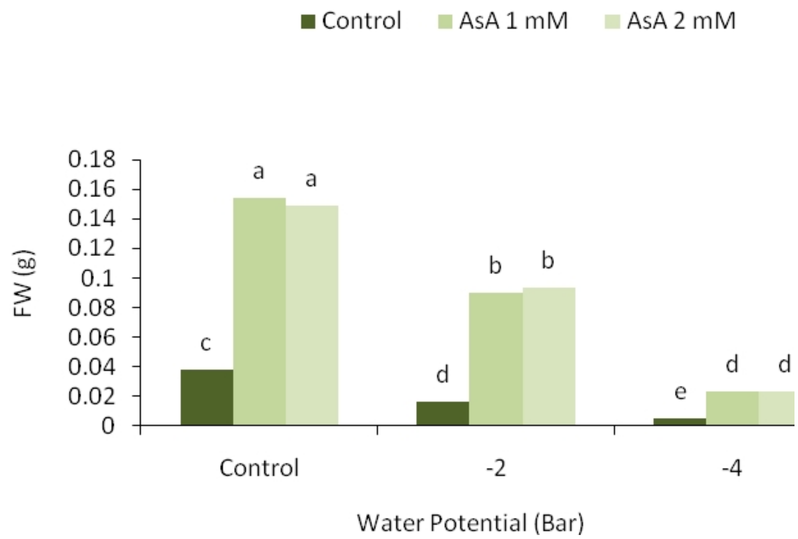

Fig. 6. The effect of AsA on seedling fresh weight of Sunflower under water Stress

The effect of AsA levels on DW was almost the same with FW. The results showed that the concentration of 1 and 2mM AsA were effective in improving the harmful effects of low water potential caused by PEG. At the zero level of water potential (control), 1mM AsA had the highest effect on DW while in -2 and -4 bar water potential, 2mM AsA was more effective than 1 mM AsA (Fig. 7).

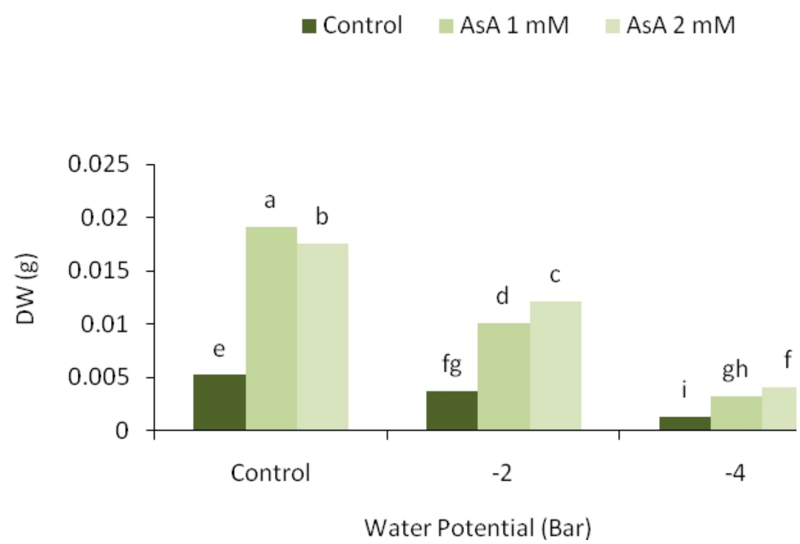

Fig. 7. The effect of AsA on seedling dry weight of Sunflower under water Stress

Reduction in plant biomass due to water stress has been widely reported [37,38,39]. In the present investigation, ascorbic acid improved growth of the treated seeds compared to non-treated ones which indicated that AsA helped seedlings to mitigate adverse effects of water stress. The results of the present study on the role of ascorbic acid in circumventing the adverse effects of water stress on seedling fresh weight are in line with some earlier reports. For example, ameliorative effect of ascorbic acid on fresh biomass of tomato seedlings when exposed to PEG or NaCl-induced osmotic stress was reported by Shalata and Neuman [40]. They found 0.5mM as the most effective concentration of ascorbic acid in the rooting medium.

This study investigated whether seed priming with AsA can improved drought resistance in sunflower. Water stress caused delay and erratic on germination and decreased the germination rate, seed stamina index, seedling dry and fresh weight, hypocotyls and radicle length of sunflower. Limited water availability during the imbibitions phase of germination is the primary reason for delayed and erratic stand establishment [41]. At cellular level, maintenance of higher water potential means increasing stomatal conductance under lower water condition [42], which increased root performance for water uptake [43] as has been indicated by increase in root length by seed priming with AsA.

Seed priming is a controlled hydration technique that allows the pre-germination metabolisms without actual germination [8]. It is one of the most pragmatic and short-term approaches to combat the effects of drought [10] and other environmental stresses [44] on seedling emergence and stand establishment. Primed seeds usually have higher and synchronized germination [8] owing to simply a reduction in the lag time of imbibitions [45], build-up of germination enhancing metabolites [10], metabolic repair during imbibition [46,47] and osmotic adjustment [7].

4. CONCLUSION

Overall, ascorbic acid application through seed priming was effective in overcoming the adverse effects of water stress in sunflower. Ascorbic acid treated seeds showed higher germination, germination rate, seed stamina index and growth contrasted to non-treated seeds. Thus, it may be concluded that exogenously applied ascorbic acid is effective in ameliorating the adverse effects of water stress. Information from this study provides a direct reference of seed priming technique in sunflower that can be useful for re-establishing projects.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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