IJABBR- 2014- eISSN: 2322-4827

International Journal of Advanced Biological and Biomedical Research

Journal homepage: www.ijabbr.com

Original Article

Effect of Seed Priming on Germination and Seedling Growth of the Caper (*Capparis Spinosa*) Under Drought Stress

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A R T I C L E I N F O

Article history:
Received: 03 June, 2014
Revised: 30 June, 2014
Accepted: 16 July, 2014
ePublished: 30 August 2014

Key words:
Caper
Priming
Stress
Germination

A B S T R A C T

Objective: To evaluate the effect of chemical stimuli on germination and seedling growth of Caper (*Capparis Spinosa*) under drought stress. **Methods:** Factorial experiment in a completely randomized design with four replicates per treatment were performed. First factor of priming include three levels of acetylsalicylic acid (300, 200 and 100 mg/L), gibberellic acid levels (500, 250 and 125 ppm), ascorbic acid levels (300, 200 and 100 mg/L) and distilled water as a control and the second factor was drought stress levels (0, -0.3, -0.6, -0.9 and -1.2 MPa). **Results:** The results showed that priming increases germination rate, germination percent, root length, seedling length, shoot length and seed vigor index under drought stress. Among of levels used in priming, acetylsalicylic acid 200 mg/L has greatest effect on the germination of plants under drought stress. According to the survey results, priming with salicylic acid compared with other acids have more effect on germination characteristics of Caper under drought stress. **Conclusions:** Thus, using of this method can be useful for improving seed germination characteristics of plants in arid and semi-arid regions.

1.INTRODUCTION

Plants play an important role in providing food for humans (Abdolshahi et al., 2013; Rad et al. 2013). During their life cycle, plants endure a different of abiotic stresses such as high and low temperatures, drought, salinity, heavy metals, UV and highlight, Allelochemical substances, chemical compounds which can have a profound effect on viability, growth, morphology, and reproduction (Rad et al., 2013; Miri et al., 2013; Rad et al. 2014 a; Rad et al., 2014 b). Water shortage is a major problem for agricultural production in the world. Iran with an average of 240 mm of rainfall is located in arid regions of the world (Kazemepoor and Orvin, 1998). Increasing drought decreases germination rates that it can be as an adaptation mechanism of native plant to drought condition and amount of reduction depends on plant species and the drought intensity (Maraghni et al., 2010; Zeng et al., 2010). One way of increasing germination in stress condition is priming method (Maraghni et al., 2010). Seed priming is the process of regulating germination by managing the temperature and seed moisture content; in order to maximize the seed's potential. .Seed priming can improve germination rate, reduce time of germination and seedling emergence and improve plant establishment. There are evidence regarding the use of chemical stimuli in accelerating growth and germination (Imani et al., 2014). Growth

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hormone is normally used for seed priming, including auxin, abscisic acid, polyamines, ethylene, salicylic acid and ascorbic acid (Demiral and Turkan, 2005). Primed seeds with gibberellic acid usually increases the emergence, growth and extensive of root systems. In addition, seed priming with gibberellic acid accelerates flowering, maturity and yield of plants (McDonald, 2000; Senaratna et al., 2000). One way to combat free radicals in seeds is antioxidants vitamins, such as ascorbic acid (vitamin C). Vitamin C is a water-soluble vitamin and eliminates the negative effects of free radicals superoxide and hydroxyl (McDonald, 2000; Rad et al., 2014 c). Seed priming increases the glucose and proline content and can be improves quality of the germination and germination index in dry conditions. However, increasing in drought, germination stopped and it causing serious damage to seedling plants (Sun et al., 2010). Li et al.,(2011), investigated the seed germination of pyrethrum (*Tanacetum cinerariifolium*) and they reported that with increasing salinity, drought and light germination was decreased. Also they are reported that hydropriming improve germination percentage, reduce mean time to germination in all osmotic potential. Their results showed that germination in distilled water increased from 52% to 59% and from 16% to 29% at the highest concentration Salinity level (Li et al., 2011). Fang et al., (2012) showed that under drought conditions in two species of *Salix paraqplesia* and *Hippophae rhamnoides*, height, base diameter and number of leaves reduced. In this condition, these plants increased root to shoot ratio and therefore they are able to use water in dry conditions (Fang et al., 2012). Environmental stresses such as drought damage plants during germination which damage to their qualitative and quantitative performance and causing weakness and heterogeny in plant growth (Fetri et al., 2014). So, understanding drought tolerance threshold for seed germination is essential and can be an effective step towards increasing the permanent vegetation, soil and water conservation and identify the degree of drought resistance in plants.

2. MATERIALS AND METHODS

To study the effects of chemical stimulants on seed germination of Caper plant under drought stress, experiments was carried out in the biotechnology Center of University of Zabol, Iran. The seeds Capparis Spinosa was purchased from the Pakan Bazr Company, Isfahan, Iran.. At the first seeds was disinfected by sodium hypochlorite 5% and then were washed several times with distilled water. Then seeds were placed separately in chemical stimuli for 10 h with salicylic acid (100, 200 and 300 mg/l) and 24 h with gibberellic acid (125, 250 and 500 ppm) and 8 h with ascorbic acid (100, 200, and 300 mg/l) at 25 °C. Distilled water was used as control .After the soaking period, all the seeds were washed with distilled water and after that, seeds were placed on sterilized filter paper in 9-cm diameter Petri dishes under different drought level. To prepare different concentrations of drought, polyethylene glycol 6000 were used. To calculate the amount of required PEG for osmotic pressure (Michel and Kaufman, 1973), the following formula was used:

$$\psi s = -(1.18 \times 10-2)\,C - (1.18 \times 10-4)\,C_2 + (2.67 \times 10-4)\,CT + (8.39 \times 10-7)\,C_2T$$

ψs : Osmotic pressure in terms of Bar

C: The concentration of PEG 6000 g/kg water (g/kg H_2O)

T: Temperature in terms of centigrade

To prepare zero water potential (control), distilled water were used. Factorial experiment in a completely randomized design with four replications (25 seeds per replicate) in different level of drought (0, -3, -6, -9 and -12Mega - Pascal) was performed. The experiments were carried out in a programmed incubator at 25±2ºC and 16 hours light and 8 hours of darkness. For a period of 15 days, each day germinated seeds that root length was more than 2 mm were counted (Kolsarıcı, 2006). In this experiment, germination percentage, germination rate (Agrawal and Dadlani 1992, ISTA, 2002) and seed vigor index (Agraval, 2005) were calculated based on the following relationships:

$$GP = 100 \times (ni / s)$$

(Agraval, 2005)

$$GR = \sum ni / ti$$

(Agrawal and Dadlani, 1992)

GP: Germination percentage, S: Total number of seeds, ni Germinated seeds at time ti, GR: Germination rate, ti Number of days after germination. (Ashraf and Foolad, 2005)

Seed vigor index= final germination percentage × Seedling length

2.1. Statistical analysis

Statistical analyses were carried out using SPSS 11.5. All data were analyzed by one-way analysis of variance (ANOVA) to determine the treatments effects, and LSD test at 1% level was carried out to determine the statistical significance of the differences between treatments means.

3. Results

3.1. Percentage of germination

Analysis of variance showed that drought stress and chemical stimuli have significant effect on germination percentage at p≤0.01.Comparison of means showed that the use of chemical stimulants increase seed germination of *C. Spinosa* under drought conditions in compared to control. The maximum effect of priming on germination percentage was observed in salicylic acid (200 mg/L), salicylic acid (300 mg/L) and gibberellic acid 125ppm at-0.3 MPa of drought level. The results showed that there is no significant difference between gibberellic acid (125 ppm) and salicylic acid (300 mg/l). In -3.0 MPa of drought level.. Lowest germination was observed in the control (Fig. 1).

3.2. Germination rate

Chemical stimuli and drought levels have significant effect on the germination rate of *C. Spinosa* at p≤0.01.With increasing drought, germination rate significantly decreased. In all levels of drought, priming with salicylic acid (200 mg/l) had the maximum rate of germination. The results showed that various levels of gibberellic acid in compare to ascorbic acid had a greater role in reducing of the negative effects of drought stress (Fig. 2).

3.3. Root length

The results of the variance analysis showed that the interaction between chemical stimuli and different levels of drought have significant effect on root length at p≤0.01 (Table 1). The results showed that root length was reduced with increasing drought stress but all the chemical stimuli increased the root length. The Maximum of root length was recorded in salicylic acid (200 mg/L) and gibberellic acid (125ppm) with 2.15 and 2.16 cm, respectively and the minimum root length was observed in control. Also the results showed that salicylic acid (200 mg/l) increased root length from 0.85 to 2.03 cm under -0.3 MPa of drought level. Furthermore all concentration of salicylic acid increased the root length in 0.6 MPa of drought level in compare to control. Gibberellic acid and ascorbic acid increased the root length at different level of drought (Fig. 3).

3.4. Shoot length

Temperature and chemical stimuli had significant effect on shoot length (Table 1).The results showed that shoot length decreased with increasing drought and all chemical stimuli significantly increased the shoot length. The maximum shoot was recorded in salicylic acid (200 mg/L) and salicylic acid (300mg/l) with 8.3 and 8.1 cm, respectively. It seems that among of the three studied acid, salicylic acid has the greatest effect on shoot length (Fig. 4).

3.5. Seedling length

According to Table 1 drought stress and chemical stimuli had significant effect on seedling length. For both primed and non-primed seeds, drought stress had significant effect on the seedling length (Fig. 5).

3.6. Seed vigor index

The results showed that chemical stimuli increased seed vigor. The maximum and minimum seed vigor indexes were observed in salicylic acid (200 mg/l) and salicylic acid (300 mg/l) with 910 and 828, respectively. The result showed that the acetylsalicylic acid had maximum effect on decreasing of the negative effect of drought stress. After acetylsalicylic acid, gibberellic acid had a positive role in reducing the negative effects of stress (Fig. 6).

4. DISCUSSION

Seed germination is negatively affected by drought stress (Rad and Rad, 2013). In this study, drought stress decreased germination percentage and germination. Seeds for germination process must absorb enough water. Soluble material in the medium such as polyethylene glycol reduces water absorption and subsequent seed germination is delayed or stopped (Tobe et al., 2000). Due to osmotic pressure loss under drought stress, water absorption process is disrupted and the alpha-amylase enzyme activity is inhibited (Afza, 2005). With increasing drought stress, root and shoot length decreased. (Bayoumi et al., 2008). Reducing in water absorption by seeds reduces secretion of the stress hormone and enzyme activity and it has negatively effect on seedling growth (Kafe et al., 2005). Also with increasing drought stress, seed vigor decreased. Similar results were reported by Yunial and Nautial (1998) in the study of *Ougeinia dalbergioides* germination. Overall priming improves seed germination under stress and non-stress conditions (Kolsarıcı, 2006, Murungu et al., 2003). Seed priming reduce time of germination and cause to accelerate seed germination. So, it is the reason of superiority of primed seeds in compared with non-primed seeds (Netondo et al., 2004). Seed priming increases antioxidant enzymes such as glutathione in primed seeds and this enzyme may reduce lipid peroxidation activity during germination and this can lead to increase germination (Hus and Sung, 1997, Baalbaki et al., 1995). Our results showed that the use of salicylic acid significantly increased germination and seedling growth in in *C. Spinosa* under drought stress. In agreement with the results, earlier reports (Borsani et al., 2002; Demiral and Turkan, 2005) have shown the germination and seedling growth was increased by using of salicylic acid. The mechanism that salicylic acid increases seed germination is not yet clearly understood. According to Nun *et al*. (2003), salicylic acid can inhibit the catalase activity. Catalase activity reduction leading to increased hydrogen proxide that it can enhance some germination of seeds. It is possible that 2-6 dihydroxi benzoic acids and salicylic acid stimulate the germination of seed via GA bio-synthesis and act as thermogene inducers (Shah, 2003). Also our results showed that gibberellic acid lead to increasing in all the germination studied traits in comparison with control. One of effective and positive reason for chemical stimuli such as gibberellic acid on the seed germination probably due to hormonal balance and decrease the proportion of growth inhibiting substances such abscisic acid (ABA). Gibberellin (GA) improves the synthesis and secretion of hydrolytic enzymes from aleurone cells. These enzymes then mobilize the endosperm storage reserves that fuel germination and growth (Cirac et al., 2004). In stress conditions, ascorbic acid acts as an effective antioxidant. Ascorbic acid can remove the free radicals in stress conditions especially oxygen radicals and it has important role in stimulating and cell expansion and nutrient absorption in the plant cells under environmental stresses which leads to avoid from the oxidation risk (Smirnoff and Wheeler, 2000, Smirnoff, 1996). Salinity and drought are two major abiotic determinants (Wang et al., 2009) on seed germination and plant growth. High salinity and severe drought could promote the salinization and desertification of land processes which are rapidly increasing on a global scale. For biological reclamation of this region, before sowing of seeds, chemical stimuli particularly gibberellin acid can be used for increasing seed germination percentage and seedling establishment. According to the survey of results, priming with salicylic acid in compared with other acids had a greater impact on Caper germination characteristics under drought stress. Thus, using of this method can be useful for improving seed germination characteristics of plants in arid and semi-arid regions.

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Table 1:
Variance analysis of studied traits in Caper seeds under drought stress

Square means						Freedom degree	Sources of variation
Seed vigor index	**Seedling length**	**Shoot length**	**Rootlet length**	**Germination rate**	**Germination percentage**		
116794.3**	11.24**	6.25 **	0.772**	4.06**	481.6 **	9	**Priming**
3091782.91**	480.49**	315.88**	17.82**	105.19**	37984.9**	4	**Drought stress**
12489.23**	0.92**	0.55**	0.091**	0.54**	57.16**	36	**Priming× Drought stress**
1807.10	0.065	0.023	0.013	0.014	12.68	150	**Error**

** Indicate significant difference at 1% probability level.

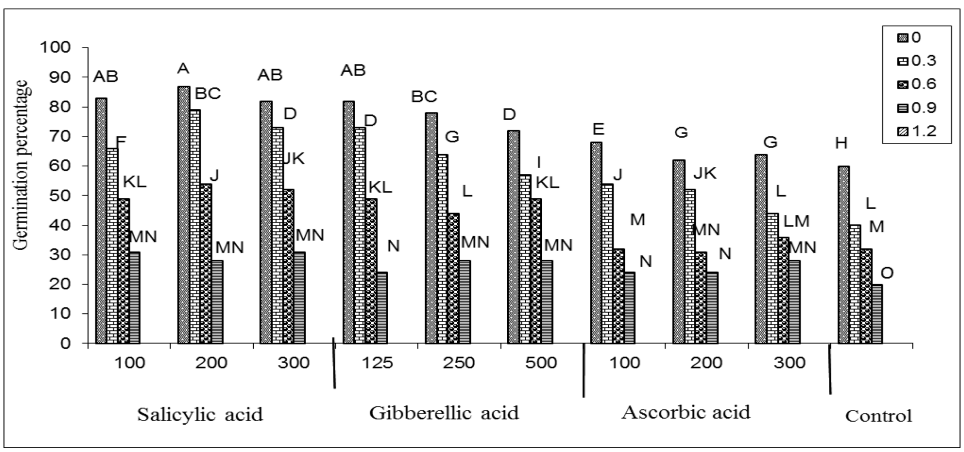

Figure 1. Effect of seed priming on germination percentage of *C. Spinosa* under different level of drought stress.

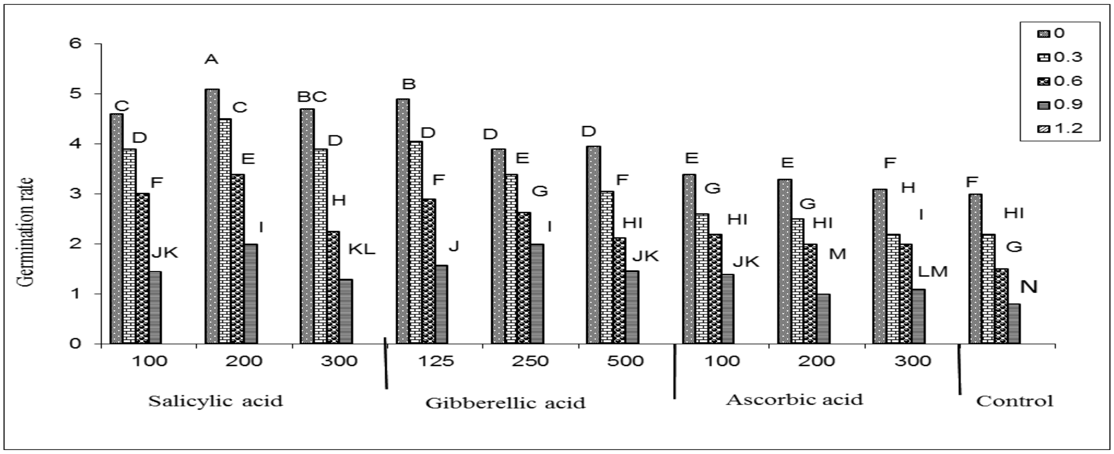

Figure 2. Effect of seed priming on germination rate of *C. Spinosa* under different level of drought stress.

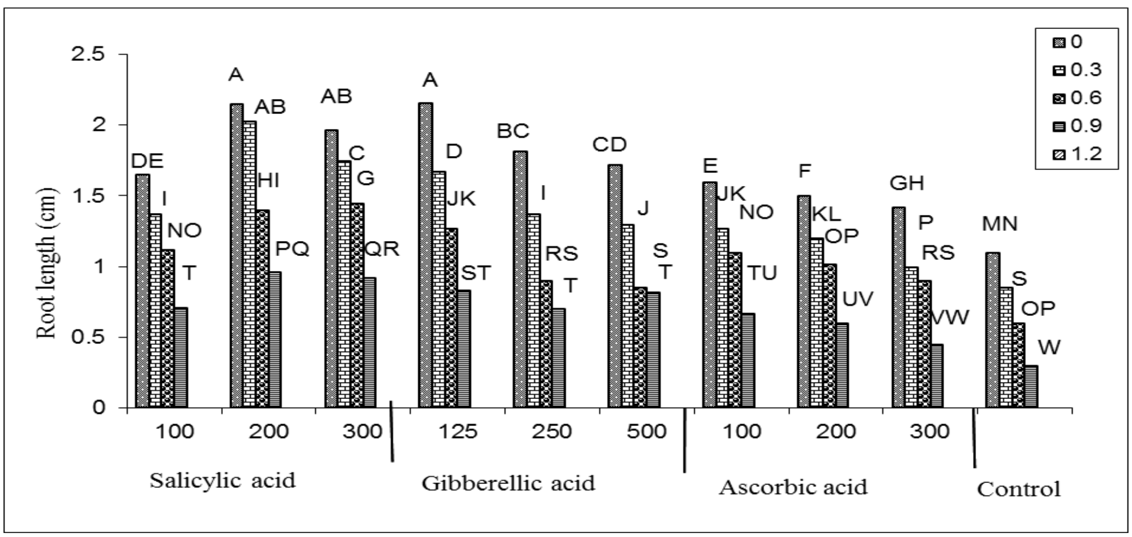

Figure 3. Effect of seed priming on root length of *C. Spinosa* under different level of drought stress.

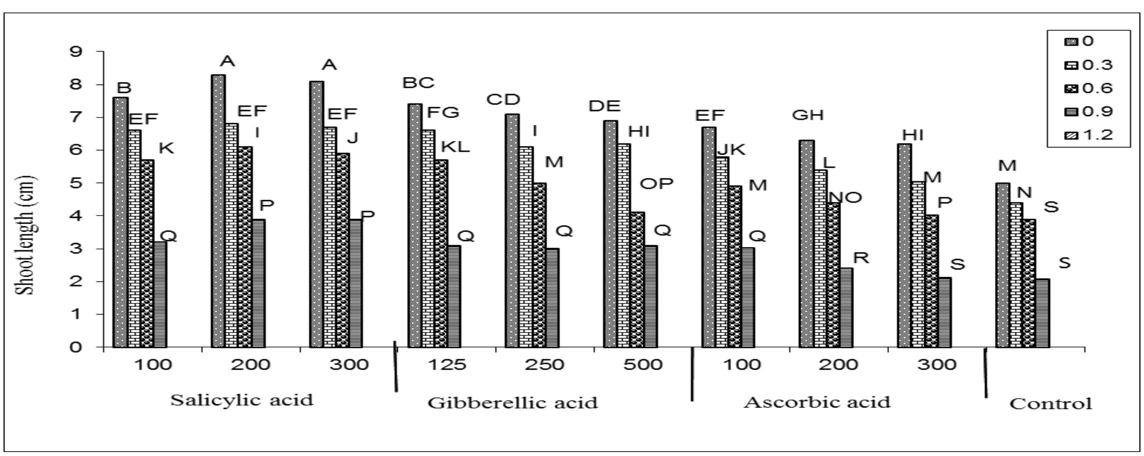

Figure 4. Effect of seed priming on shoot length of *C. Spinosa* under different level of drought stress.

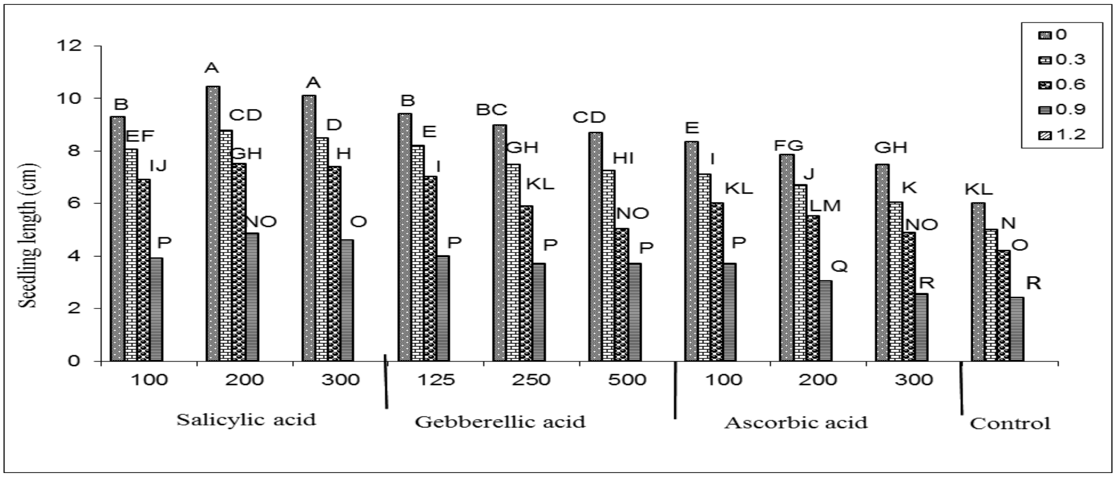

Figure 5. Effect of seed priming on seedling length of *C. Spinosa* under different level of drought stress.

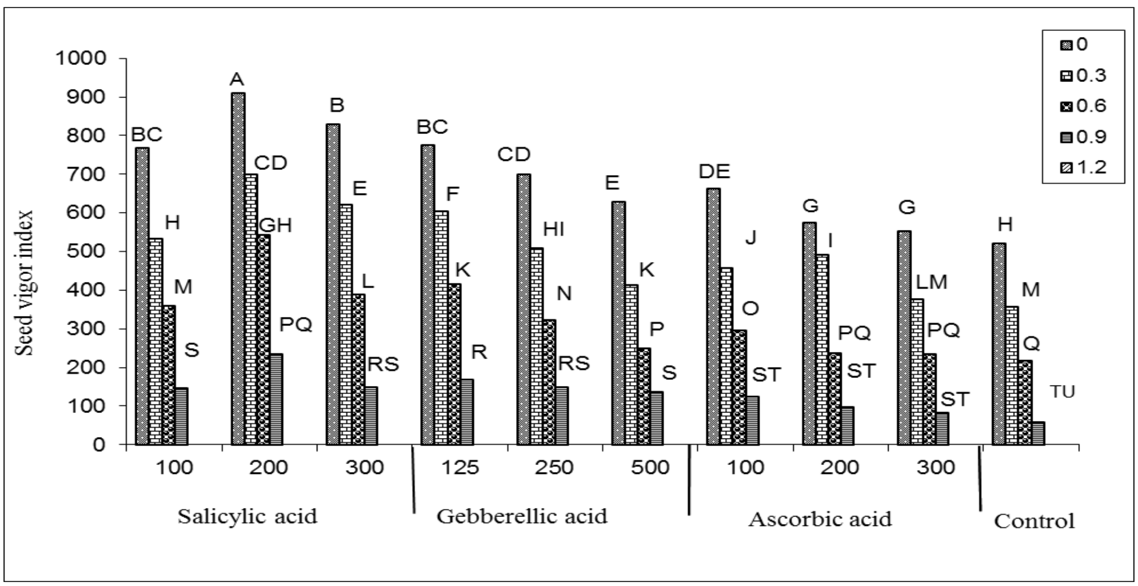

Figure 6. Effect of seed priming on seed vigor index of *C. Spinosa* under different level of drought stress.