The effects of seed priming by ascorbic acid on some morphological and biochemical aspects of rapeseed (*Brassica napus* L.) under drought stress condition

Azadeh Razaji*, Maryam Farzanian², Saeed Sayfzadeh³

¹Department of Agronomy and Plant Breeding, Saveh Branch, Islamic Azad University, Saveh, Iran
²Department of Agronomy and Plant Breeding, Tabriz Branch, Islamic Azad University, Tabriz, Iran
³Department of Agronomy, Takestan Branch, Islamic Azad University, Takestan, Iran

Key words:

http://dx.doi.org/10.12692/ijb/4.1.432-442 Article published on January 11, 2014

Abstract: Ascorbic acid, rapeseed, drought stress.

Priming is one of the seed enhancement methods that might be resulted to increase seed performance (germination and emergence) under stress conditions such as salinity, temperature and drought stress. The objective of this study was to evaluate the effects of priming with ascorbic acid on improvement of morphological and biochemical characteristics of rapeseed (*Brassica napus* L.) under simulated drought stress. This study was conducted on factorial experiment on the basis of complete randomized design (CRD) with three replications. The first factor was drought stress on 4 levels (control, -4, -6, -8 and -12 bar) that was carried out by PEG 6000 and the second factor was ascorbic acid on 4 density (control, 55, 110 and 165 µm). Results indicated that with increasing in drought stress germination percentage, seedling fresh weight, seedling dry weight, shoot length, root length, and vigor index significantly decreased whereas catalase activity (CAT), peroxidase activity (POX) and Proline content increased as compared to control. However it is concluded that priming resulted improvement in germination components, seedling growth and enzymes activity of rapeseed on drought stress condition and boost the resistance of rapeseed to drought stress condition.

*Corresponding Author: Azadeh Razaji razajichardoli@gmail.com
Introduction

Canola is one of the most important oil seed crops which its production has been notably extended during recent years in Iran. Canola seeds are commonly planted in seedbeds having unfavorable moisture (because of the lack of rainfall at planting time). Drought stress is responsible for both inhibition and delayed seed germination and seedling establishment of canola in many areas of Iran. This stress adversely affects growth and development of crop and results in to low canola yield and economic return. Drought stress lowers plants production worldwide. The response of plants to stress conditions have involved a variety of physiological and biochemical processes, e.g., solute accumulation and the developments of enzymatic antioxidant systems (Ashraf and Foolad, 2007). Under different condition particularly environmental stress, reactive oxygen species, such as super oxide anion radicals, hydrogen peroxide, and hydroxyl radicals, are regenerated (Zhu, 2000). Reactive oxygen species can damage essential membrane lipids as well as proteins and nucleic acids (Noctor and Foyer, 1998). To be able endure oxidative damage under condition which favors increased oxidative stress such as drought, plants must possess efficient antioxidant system. Plant cells have evolved a complex antioxidant system, which is composed of low molecular mass antioxidants (gluthathione, ascorbate and carotenoids) as well as ROS-scavenging enzymes, such as super oxide dismutase (SOD), catalase (CAT), peroxidase (POX), ascorbate peroxidase (APX), guiacol peroxidase (GPX) and glutathione reductase (GR) (Apel and Hirt, 2004). Activity of antioxidant enzymes with detoxification and elimination of harmful effects of reactive oxygen species reduce the severity of oxidative stress (Mc Kersie et al, 1999). also under drought stress condition, many plants accumulate several kinds of compatible solute such as proline, glycine betaine, sugars and polyols (Ashraf and Foolad, 2007). The amino acid proline (Pro) is known to occur widely in higher plants and normally accumulates in large quantities in response to environmental stresses (Khedr et al, 2003). Proline accumulation in plant cells exposed to water stress is a widespread phenomenon and is often considered to be involved in stress resistance mechanisms, although its precise role continues to be controversial (Aspinall and Paleg, 1981; Hare et al, 1999). Plants employ antioxidant defense mechanisms against oxidative damage of reactive oxygen species. Proline and betain enhance antioxidant defense systems in plant responses to various oxidative stresses (Khedr et al, 2003; demiral and turkan, 2004 ; park et al, 2006; Molinari et al, 2007). Priming is a common method for increase germination rate and resistance to drought stresses (Taylor and Harman, 1990). Seed priming has been successfully demonstrated to improve germination and emergence in seeds of many crops, especially under stress condition (Mohammadi, 2009b). Reported that primed Brassica seeds may reduce the risk of poor stand establishment under unfavorable condition (Rao et al, 1987). Ascorbic acid (vitamin C) is an important metabolic involved in many cellular processes, including cell division (De Gara et al, 2003). Ascorbate has been shown to play multiple roles in plant growth, such as in cell division, cell wall expansion, and other developmental processes (Pignochi and Foyer, 2003). Ascorbic acid is an antioxidant molecule that acts as a primary substrate in the cyclical pathway for detoxification and neutralization of superoxide radicals and singlet oxygen (Noctor and Foyer, 1998). Increased cellular levels of ascorbic acid as an antioxidant can reduce oxidative stress by reducing reactive oxygen species. The present study was conducted to assess if the application of ascorbic acid could ameliorate the adverse effect of drought on Brassica napus plants. For this purposes some morphological and biochemical characteristics were measured.

Materials and methods

Laboratory studies

The experiment was carried out at the physiological laboratory of Faculty of Agriculture, Islamic Azad University, Saveh Branch. The cultivar of rapeseed was okapi. The experiment was a factorial method
with two factors arranged in a completely randomized design with three replications. The first factor was drought stress on 5 levels (control, -4, -6, -8 and -12 bar) that was carried out by PEG 6000, the second was Ascorbic acid on 4 density (control, 55, 110 and 165µM). For ascorbic acid treatment, seeds of rapeseed were sterilized for 5 minute in sodium hypochlorite solution and in ethanol for 30 second and then rinsed by distilled water. Sterilized seeds were transferred in to sterile petri dishes contain filter papers and were added 10 ml ascorbic acid solution to each petri dish. Seeds of rapeseed were primed for 16 hours at 25°C and dark conditions. Thereafter, the seeds treated with ascorbic acid solution rinsed with distilled water. Following this, the primed seeds were dried between two filter papers. Primed seeds were placed in petri dishes, on a layer of filter paper. Twenty five seeds were placed in each petri dish, the petri dishes were moistened with 5 ml of PEG 6000 solution at water potential of (0, -4, -6, -8 and -12 bar). The petri dishes were placed in germinator. The seeds were kept under aseptic condition for 7 days in 16h/8h light/dark cycle with a light intensity of 350 µmolm⁻²s⁻¹ and a relative humidity of 45% at 25°C. Seed germination was recorded daily up 7 days after the start of the experiment. A seed was considered germinated when radical emerged by about 2mm in length. Moreover germination percentage was determined in the end of test. Germination percentage was calculated with the following formula:

\[ GP = 100 \times \left( \frac{n}{N} \right) \]

\[ N = \text{Total seeds number} \]
\[ n = \text{Germinated seed number}. \]

To determine the radical and plumule length after 7 days, radicals and plumule produced in each petri dish were separated from the seeds, their length were measured with millimeter ruler. Seedling dry weight and seedling fresh weight were measured after the specified number of days. To deter mine the dry weight, seedlings were dried in aerated oven at 75°C until constant weight. Vigor index as described by Abdul-baki and Anderson (1973).

\[ \text{Vigor Index} = \frac{\text{Germination percentage} \times \text{Seedling length (cm)}}{100} \]

The remains of seedlings were frozen in liquid N₂ and stored under -80°C until biochemical analysis.

**Extract preparation**

Seedling (0.02 gr) were homogenized in a mortar and pestle with 3ml of ice-cold extraction buffer (25 Mm sodium phosphate buffer, PH 7.8). The homogenate was centrifuged at 18,000g for 30 minute at 4°C and then supernatant filtered through Watman paper. The supernatant fraction was used as crude.

**Catalase activity**

Catalase activity was estimated by the method of Cakmak and Horst (1991). The reaction mixture contained 100µl of crude enzymes extract, 500µl of 10 Mm H₂O₂ and 1400µL of 25 Mm sodium phosphate buffer and the decrease in the absorbance was recorded at 240nm for 1 minute. Catalase activity of the extract was expressed as catalase units min⁻¹mg⁻¹ protein.

**Peroxidase activity**

Peroxidase activity was determined by the oxidation of guaiacol in the presence of H₂O₂. The increase in absorbance was recorded at 470 nm (Ghanati et al, 2002). The reaction mixture contained 100µl crude enzyme, 500µl H₂O₂ 5Mm, 500µl guaiacol 28Mm and 1900µl phosphate buffer 60Mm (PH7.0). Peroxidase activity of the extract was expressed as peroxidase units min⁻¹m⁻¹protein.

**Proline content**

Proline content was determined according to the method of Bates et al (1973). About 0.2 gr of the fresh seedlings was weighted and abraded in china mortar of 10 ml sulfa salicylic acid (3%). The achieved juice was centrifuged in a 10000 G device for 5 min. Then 2 ml of the solution from the centrifuged device with 2 ml of ninhydrin reagent (1.25 gr ninhydrin acid, 30 ml NH₃PO₄ (6M)) was incubated for 1h at 100°C. The reaction was stopped by placing the test tubes in cold water. The samples were vigorously mixed with 4 ml toluene. The light absorption of toluene phase was estimated at 520 nm using spectrophotometer. The proline concentration was determined using a standard curve, while the results
of measuring the proline content was calculated and presented with Mg/g.

**Statistical analysis**

All data were analyzed using SAS software. Each treatment was analyzed in three replications. When analysis of variance (ANOVA) showed significant treatment effects, Duncan's Multiple Range Test was applied to compare the means at p<0.05.

**Results**

**Analysis of variance**

Analysis of variance (Table 1) indicated that all of the traits under study including germination percentage, seedling dry weight, seedling fresh weight, root length, shoot length, vigor index, CAT activity, POX activity and proline content were significantly influenced by drought stress (p<0.01). The evaluated traits also were significantly influenced by priming with ascorbic acid (p<0.01). The interaction effects two-way (seed priming* drought stress) were significant for the studied traits except seedling dry weight, seedling fresh weight and proline content.

**Table 1.** Analysis of variance of the traits under study.

<table>
<thead>
<tr>
<th>S.O.V</th>
<th>Df</th>
<th>Germination</th>
<th>Seedling Dry Weight</th>
<th>Seedling Fresh Weight</th>
<th>Shoot Length</th>
<th>Root Length</th>
<th>Vigor Index</th>
<th>CAT Activity</th>
<th>POX Activity</th>
<th>Proline Content</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Percentage</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PEG</td>
<td>4</td>
<td>3837.4**</td>
<td>24.3**</td>
<td>3884.3**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>3</td>
<td>416.2**</td>
<td>19.2**</td>
<td>1222.3**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PEG*AA</td>
<td>12</td>
<td>3.2**</td>
<td>0.95ns</td>
<td>3.3ns</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>40</td>
<td>1.006</td>
<td>0.82</td>
<td>2.7</td>
<td>1.2</td>
<td>1.1</td>
<td>0.05</td>
<td>1.2</td>
<td>1.6</td>
<td>1.26</td>
</tr>
<tr>
<td>CV</td>
<td>1.436</td>
<td>15.6</td>
<td>3.4</td>
<td>4.1</td>
<td>3.6</td>
<td>5.5</td>
<td>6.2</td>
<td>4.9</td>
<td>10.12</td>
<td></td>
</tr>
</tbody>
</table>

ns=Non significant, * and** significant at 0.05 and 0.01 level of probability, respectively.

**Effects of drought stress**

All of the traits under study including germination percentage, seedling dry weight, seedling fresh weight, root length, shoot length and vigor index decreased when drought stress level were increased from 0 to -12 bar whereas CAT activity, POX activity and proline content increased when drought stress level were increased from 0 to -12 bar (Table 2).

**Table 2.** The main effects of polyethylene glycol on the studied traits.

<table>
<thead>
<tr>
<th>PEG (bar)</th>
<th>Germination Percentage (%)</th>
<th>Seedling Dry Weight (mg)</th>
<th>Seedling Fresh Weight (mg)</th>
<th>Shoot Length (cm)</th>
<th>Root Length (cm)</th>
<th>Vigor Index</th>
<th>CAT Activity (units min⁻¹ mg⁻¹ protein)</th>
<th>POX Activity (units min⁻¹ mg⁻¹ protein)</th>
<th>Proline Content (Mg/g FW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>89.89a</td>
<td>7.43a</td>
<td>69.79a</td>
<td>37.44a</td>
<td>41.18a</td>
<td>7.99a</td>
<td>12.39e</td>
<td>13.43e</td>
<td>7.13e</td>
</tr>
<tr>
<td>-4</td>
<td>86.22b</td>
<td>6.90a</td>
<td>63.84b</td>
<td>34.80b</td>
<td>39.56b</td>
<td>6.43b</td>
<td>13.70d</td>
<td>17.66d</td>
<td>8.43d</td>
</tr>
<tr>
<td>-6</td>
<td>66.72c</td>
<td>5.78b</td>
<td>41.87c</td>
<td>23.34c</td>
<td>29.39c</td>
<td>3.55c</td>
<td>19.24c</td>
<td>24.03c</td>
<td>11.70c</td>
</tr>
<tr>
<td>-8</td>
<td>57.95d</td>
<td>4.86c</td>
<td>35.14d</td>
<td>21.22d</td>
<td>21.07d</td>
<td>2.51d</td>
<td>20.62b</td>
<td>30.98b</td>
<td>13.08b</td>
</tr>
<tr>
<td>-12</td>
<td>48.45e</td>
<td>3.97d</td>
<td>28.86e</td>
<td>16.39e</td>
<td>15.52e</td>
<td>1.52e</td>
<td>23.09a</td>
<td>40.89a</td>
<td>15.54a</td>
</tr>
</tbody>
</table>

Difference between averages of each column which have common characters are not significant at probability level of 5%.

**Effect of ascorbic acid**

In seedling that were treated by ascorbic acid increased germination percentage, seedling dry weight, seedling fresh weight, root length, shoot length and vigor index whereas decreased CAT activity, POX activity and proline content as compared to control. The best results were obtained...
from the seeds treated with 165µm ascorbic acid (Table3).
Drought is a major factor in reducing the growth and productivity of plants and involves different responses dehydration of the cells as a result of the low water potential and nutritional imbalance caused by low water potential and nutritional imbalance.

**Discussion**

**Drought stress**

Drought is a major factor in reducing the growth and productivity of plants and involves different responses dehydration of the cells as a result of the low water potential and nutritional imbalance caused by low water potential and nutritional imbalance.

![Table 3. The main effects of Ascorbic acid on the studied traits.](image)

<table>
<thead>
<tr>
<th>AA (µm)</th>
<th>Germination Percentage (%)</th>
<th>Seedling Dry Weight (mg)</th>
<th>Seedling Fresh Weight (mg)</th>
<th>Shoot Length (cm)</th>
<th>Root Length (cm)</th>
<th>Vigor Index</th>
<th>CAT Activity (units min⁻¹ mg⁻¹ protein)</th>
<th>POX Activity (units min⁻¹ mg⁻¹ protein)</th>
<th>Proline Content (Mg/g FW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>63.82d</td>
<td>4.57d</td>
<td>37.80d</td>
<td>20.73d</td>
<td>24.43d</td>
<td>3.12d</td>
<td>22.38a</td>
<td>32.06a</td>
<td>12.17a</td>
</tr>
<tr>
<td>55</td>
<td>67.50c</td>
<td>5.26c</td>
<td>43.35c</td>
<td>23.62c</td>
<td>26.79c</td>
<td>3.68c</td>
<td>19.88b</td>
<td>27.76b</td>
<td>11.78a</td>
</tr>
<tr>
<td>110</td>
<td>72.26c</td>
<td>6.12b</td>
<td>52.52b</td>
<td>29.65b</td>
<td>31.48b</td>
<td>4.72b</td>
<td>15.76c</td>
<td>22.97c</td>
<td>10.84b</td>
</tr>
<tr>
<td>165</td>
<td>75.81a</td>
<td>7.19a</td>
<td>57.93a</td>
<td>32.56a</td>
<td>34.68a</td>
<td>5.36a</td>
<td>13.36d</td>
<td>18.80d</td>
<td>9.60c</td>
</tr>
</tbody>
</table>

Difference between averages of each column which have common characters are not significant at probability level of 5%.

Priming with ascorbic acid showed a significant effects on germination percentage, shoot length, root length, vigor index, CAT and POX activity under drought condition (table 4). The maximum germination percentage was achieved when seedlings were primed with 110 and 165 µm ascorbic acid under normal condition and maximum shoot length, root length and vigor index were achieved when seedlings were primed with 165µm ascorbic acid under normal condition. The minimum germination percentage, shoot length, root length and vigor index were observed in seeds untreated with ascorbic acid and -12 bar of PEG treatments. The maximum CAT and POX activity were observed in seeds untreated with ascorbic acid and -12 bar PEG treatment and minimum CAT and POX activity were achieved when seedlings were primed with 165µm ascorbic acid under normal condition.

**Table 4. Mean comparison of the drought stress level* seed priming interaction for the traits under study.**

<table>
<thead>
<tr>
<th>PEG (bar) (µm)</th>
<th>Germination Percentage (%)</th>
<th>Shoot Length (cm)</th>
<th>Root Length (cm)</th>
<th>Vigor Index</th>
<th>CAT Activity (units min⁻¹ mg⁻¹ protein)</th>
<th>POX Activity (units min⁻¹ mg⁻¹ protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>84.4d</td>
<td>32.38 de</td>
<td>36.63 d</td>
<td>5.82 d</td>
<td>16.2 f</td>
<td>17.51 i</td>
</tr>
<tr>
<td>0</td>
<td>87.5 c</td>
<td>33.66 d</td>
<td>36.94 d</td>
<td>6.17 d</td>
<td>14.17 g</td>
<td>15.03 i</td>
</tr>
<tr>
<td>0</td>
<td>93.5 a</td>
<td>40.81 b</td>
<td>44.26 b</td>
<td>7.95 b</td>
<td>10.66 h</td>
<td>12.74 j</td>
</tr>
<tr>
<td>0</td>
<td>94.1 a</td>
<td>42.89 a</td>
<td>46.91 a</td>
<td>8.45 a</td>
<td>8.52 i</td>
<td>8.43 k</td>
</tr>
<tr>
<td>-4</td>
<td>80.4 e</td>
<td>28.86 g</td>
<td>33.93 e</td>
<td>5.04 e</td>
<td>18.43 e</td>
<td>23.70 g</td>
</tr>
<tr>
<td>-4</td>
<td>85.5 d</td>
<td>31.78 ef</td>
<td>38.76 e</td>
<td>6.03 d</td>
<td>16.55 f</td>
<td>20.55 h</td>
</tr>
<tr>
<td>-4</td>
<td>87.5 e</td>
<td>38.83 c</td>
<td>43.08 b</td>
<td>7.13 c</td>
<td>10.73 h</td>
<td>19.19 i</td>
</tr>
<tr>
<td>-4</td>
<td>91.5 b</td>
<td>39.73 bc</td>
<td>42.49 b</td>
<td>7.52 e</td>
<td>9.83 hi</td>
<td>10.19 k</td>
</tr>
<tr>
<td>-6</td>
<td>60.4 i</td>
<td>18.33 j</td>
<td>23.67 g</td>
<td>2.53 h</td>
<td>23.76 bc</td>
<td>31.64 e</td>
</tr>
<tr>
<td>-5</td>
<td>63.5 h</td>
<td>19.3 j</td>
<td>27.35 f</td>
<td>2.96 g</td>
<td>21.7 d</td>
<td>25.85 f</td>
</tr>
<tr>
<td>-6</td>
<td>69.5 g</td>
<td>25.43 h</td>
<td>33.11 e</td>
<td>4.06 f</td>
<td>18.82 e</td>
<td>21.17 h</td>
</tr>
<tr>
<td>-6</td>
<td>73.5 f</td>
<td>30.33 fg</td>
<td>33.42 e</td>
<td>4.68 e</td>
<td>13.42 g</td>
<td>17.47 i</td>
</tr>
<tr>
<td>-8</td>
<td>51.5 k</td>
<td>13.32 k</td>
<td>16.57 h</td>
<td>1.53 jk</td>
<td>25.12 b</td>
<td>37.81 c</td>
</tr>
<tr>
<td>-8</td>
<td>55.5 j</td>
<td>18.76 j</td>
<td>17.74 h</td>
<td>2.02 i</td>
<td>22.49 cd</td>
<td>33.82 d</td>
</tr>
<tr>
<td>-8</td>
<td>60.4 i</td>
<td>24.31 h</td>
<td>22.26 g</td>
<td>2.81 gh</td>
<td>17.67 ef</td>
<td>26.59 f</td>
</tr>
<tr>
<td>-8</td>
<td>64.4 h</td>
<td>28.51 g</td>
<td>27.73 f</td>
<td>3.71 f</td>
<td>17.21 ef</td>
<td>25.71 f</td>
</tr>
<tr>
<td>-12</td>
<td>42.4 m</td>
<td>10.77 l</td>
<td>11.38 j</td>
<td>0.68 l</td>
<td>28.38 a</td>
<td>49.62 a</td>
</tr>
<tr>
<td>-12</td>
<td>45.5 l</td>
<td>14.61 k</td>
<td>13.16 i</td>
<td>1.26 k</td>
<td>24.5 b</td>
<td>43.56 b</td>
</tr>
<tr>
<td>-12</td>
<td>50.4 k</td>
<td>18.86 j</td>
<td>14.70 i</td>
<td>1.96 ij</td>
<td>20.9 d</td>
<td>38.16 c</td>
</tr>
<tr>
<td>-12</td>
<td>55.5 j</td>
<td>21.33 i</td>
<td>22.86 g</td>
<td>2.54 h</td>
<td>18.59 e</td>
<td>32.22 de</td>
</tr>
</tbody>
</table>

Difference between averages of each column which have common characters are not significant at probability level of 5%.
by reducing of nutrition absorb elements by unavailable moisture in the uptake and translocation (Ehsanpour and Fatahian, 2003; Zhu, 2002). Mohammadi and Amiri (2010) reported that with increase drought stress levels from 0 to -1.5 Mpa, germination percentage, root length and seedling dry weight reduced in rapeseed. Murillo Amador et al (2002) found that germination and emergence rate of two cowpea cultivars were delayed by PEG solution. Sadeghian and Yavari (2004) also reported that seedling growth severely diminished with increased drought stress. Murillo Amador et al (2002) also found that seedling growth of cowpea inhibited by NaCl and PEG, but higher inhibition occurred due of PEG. The decrease in seedling growth, under drought condition, maybe due to suppression of cell expansion and cell growth that is in response to low turgor pressure (Jaleel et al, 2008a; Ogbonnaya, 2003). Okcu et al (2005) has found that -0.6 Mpa osmotic potential decreased *Pisum sativum* seed vigor. This is an similar results with Murillo-Amador et al (2002). The reduction in plant growth of oxidative stressed plants, maybe attributed to the inhibitory effect of ABA which was induced by drought on cell division and/or cell expansion (Nabil et al, 1995). And/or resulted from the osmotic effect of oxidative stress which caused disturbances in water balance of stressed plants leading to stomatal closure, reduction in photosynthesis and consequently a retarded growth rate (Chaparzadeh, 2004). The decrease in dry weight of seedling by increasing the oxidative stress level could be ascribed to the decrease in photosynthesis output as indicated by the significant decrease chlorophylls and total carbohydrates in oxidative stressed plants. Other authors concluded that, reduction of dry weight may be due to a turgor limitation (Mengel and Arneke, 1982), or cell wall hardening by limited extension growth (Chazen and Neumann, 1994). It has been indicated that drought stress affects the physiology and biochemistry of plant cells under in vivo and in vitro conditions. In this context, increased proline and activities of CAT, POX, APX, GPX has been reported in plants grown under stress (Hoque et al, 2007). Drought simulated the accumulation of the ROS including H$_2$O$_2$ in plants cells. The metabolism of H$_2$O$_2$ is dependent on various functionally interrelated antioxidant enzymes such as CAT and POX. These enzymes are involved in elimination of H$_2$O$_2$ from stressed cells (Kim et al, 2005a; Nojavan and Khoshidian, 2006). Water deficit could cause oxidative damage, therefore, plant cells need different mechanisms, which enable the detoxification of excess ROS and keep the balance of formation and removal of ROS. The increase activities of CAT, POX , detected in this study are presumed to limit cellular damage and enhance the plants oxidative capacity to defend stress. CAT and POX activities coordinated with SOD activity play a central protective role in the O$_2$- and H$_2$O$_2$ scavenging process (Hoque et al, 2007a). The CAT and POX activity increased under drought stress when compared to control plants. Similar results reported under drought stress in wheat (Shao et al, 2005a) and tomato plants (Sanchez-Rodriguez et al, 2010). Redy et al (2003) reported that proline content increase in drought stress time. Sairam et al (1998) reported that in increasing proline cause increasing resistance on drought and salty. It is reported that increasing proline cause protecting turgor and the reduction of membrane damage on plants. So, osmo regulation is an adaption that increase the tolerance toward drought stress (Inze and Montage, 2000).

**Ascorbic acid**

Ascorbic acid is one of the most extensively studied antioxidant and has been detected in majority of plant species, organelles and apoplast and is synthesized in the mitochondria and transported to the other cell components through a proton-electrochemical gradient or through facilitated diffusion (Smirnoff, 2000). Ascorbic acid is an antioxidant molecule that acts a primary substrate in the cyclical pathway for detoxification and neutralization of superoxide radicals and singlet oxygen (Noctor and Foyer, 1998). Also it is one of the best indentified non-enzymatic compounds as antioxidant that plants bearing is increased to oxidative stresses (Smirnoff, 1996). Ascorbate has been shown to play multiple roles in plant growth, such as in cell expansion and other developmental processes (Pignochi and Foyer, 2003).
Reported that priming with ascorbic acid increased germination percentage, length of shoot and root, their dry weight and seedling total dry weight in sunflower and rapeseed and decreased CAT activity significantly than control treatment. Indeed, increase of root and shoot length by ascorbic acid might be due to the cell division and differentiation of meristem cells (Liso et al., 1988). Application of vitamins improved growth of plants by causing significant increases in the values of the above growth parameters of oxidative stressed plant. The inhibitory effects of high levels of oxidative stress were mitigated partially or completely alleviated. This probably by increasing the efficiency of water uptake and utilization as well as protecting the photosynthetic pigments, and the photosynthetic apparatus (Hassanein et al., 2009). Ascorbic acid on plant survival is associated with the partial inhibition of a few interactions in reactive oxygen species production (Shalata and Neuman, 2001). Hamad and Hamada (2001) observed on their experiment on wheat seeds, priming with ascorbic acid reduced harmful effects of drought stress on root and shoot fresh weight. Dolatabadian et al. (2009) reported that salinity increased CAT and POX activity in leaves and roots of rapeseed while the application of ascorbic acid reduced the activity of these enzymes in salinity condition. Drought combined with ascorbic acid improves the biological status of rapeseed, Biological improvement is related to reduce production of harmful substances (Barkosky and Einhelling, 2003). Ascorbic acid as an antioxidant, reduced catalase activity in pea under stress condition (Kukreja et al., 2005). Ascorbic acid has antioxidant properties that can remove superoxide ion and prevents the production of hydrogen peroxide, thus CAT and POX activity is reduced because these enzymes play a key role in removing hydrogen peroxide. Generally, it is concluded that ascorbic acid as an antioxidant, can reduce the harmful effects of oxidative stress and improve plants growth in stress condition (Dolatabadian et al., 2009).

**Acknowledgment**

According to the results obtained, drought stress decreased germination percentage, seedling fresh weight, seedling dry weight, shoot length, root length and vigor index and increased catalase activity, peroxidase activity and proline content. Priming with ascorbic acid significantly relieved the harsh effects of drought stress on germination percentage, vigor index, seedling growth, catalase and peroxidase activity of rapeseed and it seems that ascorbic acid was able to enhance the tolerant ability of the plant to drought stress.

**References**


and peroxidase activities in root tip of soybean (Glycine max), Plant Physiology 83, 463-468.

http://dx.doi.org/10.1093/jxb/erg021

http://dx.doi.org/10.1016/j.jplph.2004.03.009

Dolatabadian A, Modarres Sanavy SAM, Sharifi M. 2009. The effect of ascorbic acid on leaf feeding activity of antioxidant and proline accumulation in rapeseed (Brassica napus L.) in terms of salinity, Agriculture Sciences and Natural Resources 13(47B), 611-621.
http://dx.doi.org/10.1111/j.1439-037X.2008.00301.x

http://dx.doi.org/10.1023/A:1022619523726

http://dx.doi.org/10.1080/00380768.2002.10409212

Hamad A, Hamada A. 2001. Grain soaking pre sowing in ascorbic acid or thiamine versus the adverse effects of combined salinity and drought on wheat seedlings, in proceeding of the 12 th international congress on photosynthesis (Melbourne , Australia , Brisbane , Australia 18(23), 15 – 005 p.

http://dx.doi.org/10.1093/jxb/50.333.413


membrane integrity of cicer arietinum roots as affected by salinity, Biologia Plantarum 49, 305 – 308. 
http://dx.doi.org/10.1093/jxb/erg277

http://dx.doi.org/10.1111/j.14698137.1988.tb00284.x

http://dx.doi.org/10.1104/pp.119.3.839


http://dx.doi.org/10.1111/j.1399-3054.2007.00909.x

http://dx.doi.org/10.1046/j.1439-037X.2002.00563.x

http://dx.doi.org/10.1109/j.13993054.1995.tb02220.x

http://dx.doi.org/10.1146/annurev.arplant.49.1.249

http://dx.doi.org/10.3923/pjbs.2006.34.38

http://dx.doi.org/10.2135/cropsci2003.1114


Rao SC, Aker SW, Ahring RM. 1987. Priming Brassica seed to improve emergence under different
temperatures and soil moisture conditions, Crop Science 27, 1050-1053.
http://dx.doi.org/10.2135/cropsci1987.0011183X002700050045x

http://dx.doi.org/10.1023/A:1027353430164

http://dx.doi.org/10.1023/A:1001898310321

http://dx.doi.org/10.1016/j.plantsci.2009.10.001

http://dx.doi.org/10.1111/j.1439-037X.2004.00087.x


http://dx.doi.org/10.1093/jexbot/52.364.2207

Smirnoff N. 1996. The function and metabolism of ascorbic acid in plant, Annals of Botany 78, 661 – 669
http://dx.doi.org/10.1006/anbo.1996.0175

http://dx.doi.org/10.1016/S1369-5266(00)80070-9

http://dx.doi.org/10.1146/annurev.py.28.090190.001541

http://dx.doi.org/10.1104/pp.1243.941

http://dx.doi.org/10.1146/annurev.arplant.53.091401.14329