Responses of cumin (*Cuminum cyminum* L.) to different seed priming methods under osmotic stress

Habib NOORI¹, Seyed G. MOOSAVI²*, Mohammadjavad SEGHATOLESLAMI², Mansour FAZELI ROSTAMPOUR²,³

¹Departement of Agriculture, Birjand Branch, Islamic Azad University, Birjand, Iran; habinoori83@yahoo.com
²Agricultural, Medicinal Plants and Animal Sciences Research Center, Birjand branch, Islamic Azad University, Birjand, Iran; moosavi_iaubir@yahoo.com (‘corresponding author); mjseghat@yahoo.com
³Horticultural Crops Research Department, Sistan Agricultural and Natural Resources Research and Education Center, AREEO, Zabol, Iran; mansour_fazeli@yahoo.com

Abstract

A common problem with vegetable production in drought areas is low crop stand, but germination data are limited and inconsistent for cumin. Different priming methods positively affect the enhancement of seed germination and seedlings growth, especially under stress conditions. The objective of this study was to assess the effects of different priming treatments (unprimed seeds as control, hydro-priming, salicylic acid, jasmonic acid, paclobutrazol, and chitosan) on cumin seed germination indices and physiological traits under osmotic stress (0, -5, and -10 bar; induced by polyethylene glycol-6000). Seed germination of cumin was reduced by 9.77% and 23.95% under osmotic potential -5 and -10 bar, respectively, compared with non-stressed conditions. Nevertheless, priming enhanced germination indices and improved photosynthetic pigments and activity of peroxidase, catalase, and superoxide dismutase enzymes at all potential osmotic levels compared with non-primed seeds. Seed treated by jasmonic acid showed the highest seedling vigor index and chlorophyll and carotenoids content under stress and non-stress conditions. Under the high level of osmotic potential (-10 bar), jasmonic acid treatment was caused increasing by 59.3%, 55.19%, 54.26%, 57.52%, and 47.72% of seedling vigor index, total chlorophyll, chlorophyll a, b, and carotenoids content, respectively. In conclusion, the jasmonic acid priming can modify the negative effects of the osmotic stress by improved physiological traits resulting in enhanced germination parameters.

Keywords: antioxidant enzyme activity; drought stress; jasmonic acid; photosynthetic pigments; polyethylene glycol; seedling vigor index

Abbreviations: Unprimed seeds (UPd); hydro-priming (HPd); salicylic acid (SA); jasmonic acid (JA); paclobutrazol (PBZ); chitosan (CH); final germination percentage (FGP); germination rate (GR); mean germination time (MGT); mean daily germination (MDG); seedling length (SL); seedling vigor index (SVI); peroxidase (POD); catalase (CAT); superoxide dismutase (SOD)
Introduction

Cumin (Cuminum cyminum L.) is most important an annual plant, and the medicinal plant grows in countries, e.g., Iran, India, and other Asian countries with medicinal properties including numerous stimulus appetites; strengthen the stomach, anti-flatulence, which has low germination, vigor, storage substances, and weak establishment in the soil. Weak cumin seed vigor is one problem with cumin production, which is followed by increased sensitivity to environmental stresses (Piri et al., 2019).

Drought stress is a severe agronomic problem in arid and semi-arid areas of the world, especially in Iran, and is one of the critical factors reducing plant growth and productivity (Ansari et al., 2012). Osmotic stress, in addition to reducing seedling growth by creating secondary stress, such as reactive oxygen species (ROS) get to cause the change in the synthesis compounds routes, secondary metabolites and can be through oxidative damage to lipids, proteins, and nucleic acids disrupt normal cell metabolism and damage to the cellular membrane that eventually led to cell death (Piri et al., 2019). Osmotic stress has profound effects on seed or plant physiology in general, productivity, and growth. Plant physiological processes, such as photosynthesis, enzymatic antioxidants activity such as catalase and peroxidase, non-enzymatic antioxidant accumulation such as proline and malondialdehyde accumulation depend on the rapidity, severity, and duration of the drought. Drought stress may cause damage to cells either directly or indirectly through the formation of ROS (Qi et al., 2018).

The seed germination process constitutes the most vital physiological function of seeds and is considered a precondition for most crops’ successful cultivation (Ghiyasi et al., 2019). Optimum seed germination secures a desirable crop stand in the field (that is, final plant density with plants of uniform size per unit area), which plays an essential role in the agronomical production process (Ghiyasi et al., 2019). The process (seed germination) is controlled by several eco-physiological factors, among which temperature, oxygen, and water availability are crucial ecological factors. In particular, soil’s water potential is a critical factor in seed germination in semi-arid climates. The seed is often sown into the soil with inadequate water for rapid germination. Furthermore, environmental stresses such as drought and osmotic stress negatively affect the final germination percentage, germination rate, seed vigor, and seedling growth (Ghiyasi et al., 2019). Rebeý et al. (2012) reported that the decreasing cumin germination with increasing drought levels. The germination and seedling stages in the plant life cycle are more sensitive growth stages to environmental stress such as drought and salinity than the adult stage for most species.

Seed priming has been shown to improve many plants’ seed germination indices (Arafa et al., 2021). It may constitute a useful tool for overcoming biotic and abiotic stresses such as drought, assuring a high and successful planted seed establishment (Mourradi et al., 2016). Mostly primed seeds demonstrate a faster and more harmonized germination, and the emerged seedlings are more vigorous and tolerant to abiotic stresses than seedlings that emerged from unprimed seeds (Ansari et al., 2012). Different priming methods include hydro-priming, osmo-priming, halo-priming, thermo-priming, and hormone-priming those various reports on other plants this priming is presented by researchers (Aghighi Shahverdi et al., 2017). Nazar et al. (2011) reported that the salicylic acid (SA) pre-treated mungbean plants exhibited changes in physiological processes to maximize nitrogen and sulfur use through the higher activity of nitrate reductase and ATP-sulfurylase and synthesis of glutathione.

Paclobutrazol (PBZ) [(2RS, 3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1H-1, 2, 4-trizol-1-yl)-pentan-3-ol], is one of the members of triazole family having growth-regulating property. The growth regulating properties of PBZ is mediated by changes in the levels of important plant hormones, including the gibberellins, abscisic acid, and cytokinins. The role of PBZ in plants is: alters growth regulation, improves plant water relation, improves membrane stability index, enhances plant photosynthetic pigments, alters the level of plant growth hormones, induces antioxidant activities, and increases the level of proline (Hajihashemi and Ehsanpour, 2013).
Chitosan (C\textsubscript{11}H\textsubscript{17}O\textsubscript{7}N\textsubscript{2}: CH) is a polyacetate polysaccharide produced as a natural polymer and from Alkaline N-deacetylation of chitin. It is known to be the second most abundant polymer on earth, which is the cell wall of some fungi, insects, and also algae are produced (Arafa et al., 2021). Researchers believe that this substance can increase antioxidant enzymes’ activity and acts as a neutralizing ROS (Li et al., 2019). Increasing chlorophyll content and catalase activity in stevia (Afshari et al., 2020) and stevia (Afshari et al., 2020), and proline content in cumin (Taheri et al., 2018) have been reported in various studies with CH application.

Jasmonic acid (JA) and its precursors and derivatives, referred to as jasmonates, are important molecules in the regulation of many physiological processes in plant growth and development, and especially the mediation of plant responses to biotic and abiotic stresses (Ruan et al., 2019). JA is a plant signal molecule involved in plants' defensive responses (Zalewski et al., 2010). Sheteiwy et al. (2020) reported that exogenous JA could alleviate stress conditions through regulating antioxidant activities. Ilyas et al. (2017) concluded that the application of JA and SA can enhance the growth of wheat plants under drought. In this context, the present work aimed to assess the effect of different priming technique on seed germination parameters of cumin under osmotic deficit (induced by PEG\textsubscript{6000}) and to study the photosynthetic pigments content, activity of some enzymes related to the antioxidant defense, and the proline content of the seedlings.

**Materials and Methods**

**Experimental design and seed material**

The study was a factorial experiment based on a completely randomized design (CRD) with three replications in which the experiment factors included three levels of drought stress 0, −5, and −10 bar induced by PEG\textsubscript{6000} and priming treatment: salicylic acid (SA), jasmonic acid (JA), Chitosan (CH), and paclobutrazol (PBZ), hydro-priming (HPd), and unprimed seeds (UPd) was considered as control.

The experiment was conducted at Biotechnology Research Institute, Faculty of Natural Resources, Zabol University, Iran, 2020. freshly matured cumin ('Sistan' ecotype) seeds were collected in June 2019 from Zabol city, Sistan and Baluchestan Province, Iran (31.2° N, 61.39° E, and 1385 m ASL). The mean seed dry weight per 1000 seeds was 3.5±0.2 g, and seed moisture ranged around 10.17%. Cumin seeds sterilization with sodium hypochlorite (5%) for 30 seconds and then washed with distilled water. According to the pilot experiments (separately four experiments), the best duration and concentration of seed priming with JA, SA, PBZ, and CH were 24 hours at the concentration of 0.01 mM, one mM, 0.5 mM, and 0.2% respectively. Also, the hydro-priming duration was 24 hours. These data were used in the experiment (data not shown).

Cumin seeds were entirely immersed in determining priming media concentrations (0.01 mM JA, one mM SA, 0.5 mM PBZ, and 0.2% CH) at 15 °C in darkness. At the end of the priming, the seeds were washed with distilled water and air-dried for 24 h. In each Petri dish, 50 seeds were put on Whatman paper, and based on various treatments was added Petri dish 10 ml of distilled water (as without drought stress) or PEG\textsubscript{6000} solution. The osmotic potentials of − 5 and − 10 bars were obtained by adding 202.12 and 295.7 g of PEG\textsubscript{6000} in 1000 ml of distilled water, respectively. The required amount of PEG\textsubscript{6000} was calculated by Michel and Kaufmann formula (Eq. 1) (Afshari et al., 2020):

\[
\Psi_s= - (1.18 \times 10^{-2}) \times C - (1.18 \times 10^{-4}) \times C^2 + (2.67 \times 10^{-4}) \times C^2 T + (8.39 \times 10^{-7}) \times C^2 T \ (\text{Eq.1})
\]

\(\Psi_s, C, \) and \(T\) are osmotic potential (bars), the concentration of PEG (g/l of distilled water), and temperature (°C), respectively. The distilled water potential is zero, so it was used as the control treatment (without drought stress).

The germination period was conducted in a growth chamber under controlled conditions with a temperature of 20 ± 2 °C and a photoperiod of 16 h light and 8 h dark. A seed scored germinated when radicle length reached 2- or 3-mm. Seed germination was counted daily, and terminated when no further germination occurred. At the end of the testing period (14 days) was calculated final germination percentage, germination rate, mean time germination, mean daily germination, and seedling vigor index according to formulas in Table
After two weeks of growth, each replicate's seedlings were collected and immediately frozen in liquid nitrogen and stored in the ultra-low freezer at −80 °C for physiological studies.

Table 1. The computing relation of germination percentage, germination rate, mean germination time, mean daily germination, and seed vigor index characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Final germination percentage</td>
<td>$\text{FGP} = \frac{N \times 100}{M}$</td>
</tr>
<tr>
<td>Germination rate</td>
<td>$\text{GR} = \frac{\sum N_i}{T_i}$</td>
</tr>
<tr>
<td>Mean germination time</td>
<td>$\text{MGT} = \frac{\sum (D_N)}{\sum n}$</td>
</tr>
<tr>
<td>Mean daily germination</td>
<td>$\text{MDG} = \frac{\sum N_i}{\sum T_i}$</td>
</tr>
<tr>
<td>Seed vigor index</td>
<td>$\text{SVI} = \text{GP} \times \text{Mean (SL)}$</td>
</tr>
</tbody>
</table>

$N =$ sum of germinated seeds at the end of the experiment, $M =$ total planted seeds, $T_i =$ number of days after germination, $D_i =$ the number of days from the start of the test to the enumeration of $n$th, $\text{SL} =$ seedling length.

**Determination of chlorophyll content**

According to the Lichtenthaler and Buschmann (2001) method, 0.25 g of fresh seedling sample was extracted by using 5 ml 80% acetone. The extract was centrifuged at 11000 rpm for 10 min. Using a spectrophotometer (PerkinElmer-Lambda 25, USA), the extract optical density was measured at wavelengths 470, 646.8, and 663.2 nm to estimate photosynthetic pigments. The amount of chlorophylls was calculated according to the following equations:

- $\text{Chl a (µg/g FW)} = 12.7 \times \left( \frac{\text{OD of 663}}{1000} \right) - 2.69 \times \left( \frac{\text{OD of 645}}{1000} \right)$
- $\text{Chl b (µg/g FW)} = 22.9 \times \left( \frac{\text{OD of 645}}{1000} \right) - 4.68 \times \left( \frac{\text{OD of 663}}{1000} \right)$
- $\text{Total Chl (µg/g FW)} = 20.2 \times \left( \frac{\text{OD of 645}}{1000} \right) + 8.02 \times \left( \frac{\text{OD of 663}}{1000} \right)$

Whereas $W$: the fresh weight by grams for extracted tissue; $V$: the final size of the extract in 80% acetone; O.D: optical density at a specific wavelength.

**Determination of antioxidant enzymes activity**

A mortar and pestle crushed approximately 200 mg of frozen seedling tissue in liquid nitrogen. Then 1.2 ml of 0.2 M potassium phosphate buffer (0.1 mM EDTA and pH 7.8) was added to obtain a homogenized extraction. The extract was centrifuged at 15,000 × g for 20 min. The supernatant was separated, and the pellet was again extracted (Elavarthi and Martin, 2010). The combined supernatants were stored at -80 °C for enzymatic activity assay.

**Determination of peroxidase (POD) activity**

Peroxidase activity was assayed by the method proposed by Chance and Maehly (1995). An alcoholic liquid of the tissue extract (100 µl) was added to 3 ml of assay solution, including 3 ml of reaction mixture containing 13 mM guaiacol, five mM H$_2$O$_2$, and 50 mM sodium (Na)-phosphate (pH 6.5). An increase in the optical density at 470 nm for 1 min at 25 °C was recorded using a spectrophotometer.

**Determination of catalase (CAT) activity**

Catalase activity was determined according to the method described by Kar and Mishra (1976) and Aghighi Shahverdi et al. (2017). The 60 µl protein extract was added to Tris buffer (50 mM, pH=7) H$_2$O$_2$ 5 mM in the ice bath, then the absorbance curve was considered at a wavelength of 240 nm.
Determination of superoxide dismutase (SOD) activity
Superoxide dismutase activity was determined according to the method described by Beauchamp and Fridovich (1971). About 3 ml of the reaction mixture, containing 0.1 ml of 200 mM methionine, 0.01 ml of 2.25 mM nitro blue tetrazolium (NBT), 0.1 ml of 3 mM EDTA, 1.5 ml of 100 mM potassium phosphate buffer, 1 ml distilled water, and 0.05 ml of enzyme extraction, were taken in test tubes in duplicate from each enzyme sample. Two tubes without enzyme extract were taken as control. The reaction was started by adding 0.1 ml riboflavin (60 µM) and placing the tubes below a light source of two 15 W fluorescent lamps for 15 min. The reaction was stopped by switching off the light and covering the tubes with black cloth. Absorbance was recorded at 560 nm.

Determination of proline content
Proline was determined according to the method described by Bates et al. (1973). Approximately 0.2 g of fresh seedling tissue was homogenized in 10 ml of 3% aqueous sulfosalicylic acid. Then, this aqueous solution was filtered through Whatman’s paper No. 2. Finally, 2 ml of filtrated solution was mixed with 2 ml acid-ninhydrin and 2 ml of glacial acetic acid in a test tube. The mixture was placed in a water bath for 1 h at 100 °C. The reaction mixture was extracted with 4 ml toluene, cooled to room temperature, and the absorbance was measured at 520 nm with a spectrophotometer.

Statistical analysis
Distribution normality of achieved data was done according to the Kolmogorov-Smirnov and Shapiro-Wilk test. The studied traits (i.e., germination indices and seedling physiological traits) were statistically analyzed by the Statistical Analysis System software (SAS Institute, Cary, NC, USA, and Version 9.4). The differences among means were separated using the LSD test (least significant difference) at 0.05 statistical probability level.

Results
Final germination percentage (FGP)
As shown in Table 2, the effects of osmotic stress and priming treatments were significant on FGP (p≤0.01). Final germination percentage of cumin seeds was negatively affected by osmotic stress; therefore, the highest osmotic stress decreased 23.95% FGP compared to the without drought treatment. The highest FGP (66.8%) was achieved in the control treatment. Seed priming by JA had the greatest FGP (70.44%), and UPd treatment showed the lowest FGP (43.33%). According to the LSD test, non-significant variations (p≤0.05) were detected between the three types of priming treatment (SA, PBZ, and CH) for the FGP (Table 2).

Germination rate (GR)
The effects of osmotic stress and seed priming treatment were significant on GR (p≤0.01). The highest GR was related to 0 and -5 bar osmotic treatments (4.37 and 4.05 seed per day, respectively). A significant GR reduction was observed under -10 bar (Table 2). Seed priming increased GR compared to UPd (control). Results showed the UPd seed (dry seed) and treated seed by JA had the lowest and highest GR (2.8 and 4.81 seed per day), respectively. Such as FGP; non-significant variations were detected between the three types of priming treatment (SA, PBZ, and CH) for the GR (Table 2).

Mean germination time (MGT)
As shown in Table 2, the effects of priming and the interaction of osmotic stress and priming were significant on MGT (p≤0.01). In contrast, according to the ANOVA test, the osmotic stress effect was non-significant. The MGT has been delayed significantly by -10 bar osmotic stress for the UPd seeds, while treated
seed by CH showed decreasing MGT (6.66%) under the high level of osmotic stress compared to the non-stress conditions. According to the MGT, there were non-significant differences between control and -5 bar osmotic stresses under all priming treatments (Figure 1).

### Table 2. Effect of osmotic stress and priming treatments on germination indices of cumin (*Cuminum cyminum* L.)

<table>
<thead>
<tr>
<th>Osmotic stress (bar)</th>
<th>Final germination percentage (%)</th>
<th>Germination rate (seed per day)</th>
<th>Mean germination time (day)</th>
<th>Mean daily germination</th>
<th>Seedling length (cm)</th>
<th>Seedling vigor index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>66.80±3.04 a</td>
<td>4.37±0.23 a</td>
<td>8.15±0.08 a</td>
<td>4.77±0.22 a</td>
<td>8.79±0.46 a</td>
<td>596.57±49.93 a</td>
</tr>
<tr>
<td>-5</td>
<td>60.27±2.84 b</td>
<td>4.05±0.22 a</td>
<td>8.21±0.06 a</td>
<td>4.30±0.20 b</td>
<td>6.65±0.27 b</td>
<td>406.23±30.71 b</td>
</tr>
<tr>
<td>-10</td>
<td>50.80±2.42 c</td>
<td>3.53±0.18 b</td>
<td>8.38±0.17 a</td>
<td>3.63±0.17 c</td>
<td>4.19±0.21 c</td>
<td>217.29±17.43 c</td>
</tr>
<tr>
<td>LSD (p&lt;0.05)</td>
<td>4.37</td>
<td>0.37</td>
<td>0.25</td>
<td>0.31</td>
<td>0.76</td>
<td>56.2</td>
</tr>
<tr>
<td>Priming treatments</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UPd (control)</td>
<td>43.33±2.91 d</td>
<td>2.80±0.17 d</td>
<td>8.65±0.20 a</td>
<td>3.1±0.21 c</td>
<td>5.23±0.6 c</td>
<td>226.2±37.6 c</td>
</tr>
<tr>
<td>HPd</td>
<td>50.56±2.27 c</td>
<td>3.42±0.22 c</td>
<td>8.29±0.11 b</td>
<td>4.01±0.15 b</td>
<td>5.71±0.4 b</td>
<td>288.6±44.2 c</td>
</tr>
<tr>
<td>SA</td>
<td>62.44±2.23 b</td>
<td>4.15±0.17 b</td>
<td>8.22±0.15 b</td>
<td>4.46±0.16 b</td>
<td>6.26±0.6 b</td>
<td>398.6±50.1 b</td>
</tr>
<tr>
<td>JA</td>
<td>70.44±3.30 a</td>
<td>4.81±0.28 a</td>
<td>8.01±0.04 b</td>
<td>5.03±0.31 a</td>
<td>7.70±0.8 a</td>
<td>564.8±90.7 a</td>
</tr>
<tr>
<td>PBZ</td>
<td>58.67±2.31 b</td>
<td>4.02±0.19 b</td>
<td>8.17±0.10 b</td>
<td>4.19±0.16 b</td>
<td>6.78±0.8 b</td>
<td>404.8±55.0 b</td>
</tr>
<tr>
<td>CH</td>
<td>61.56±2.80 b</td>
<td>4.13±0.15 b</td>
<td>8.19±0.14 b</td>
<td>4.40±0.20 b</td>
<td>6.76±0.7 b</td>
<td>428.8±62.3 b</td>
</tr>
<tr>
<td>LSD (p&lt;0.05)</td>
<td>5.64</td>
<td>0.48</td>
<td>0.33</td>
<td>0.40</td>
<td>0.98</td>
<td>72.64</td>
</tr>
</tbody>
</table>

Means followed by the same letter in each column are not significantly different according to the LSD test at 5% level (Means ± SD).

ns: non-significant, * and **: Significant at α=0.05 and α=0.01, respectively.

Unprimed seeds (UPd); hydro-priming (HPd); salicylic acid (SA); jasmonic acid (JA); paclobutrazol (PBZ); chitosan (CH); Coefficient of variation (CV)

### Figure 1. Interaction of osmotic stress and priming effects on mean germination time (MGT) of cumin seeds

Means followed by the same letter are not significantly different according to the LSD test at 5% level (Means ± SD).

Unprimed seeds (UPd); hydro-priming (HPd); salicylic acid (SA); jasmonic acid (JA); paclobutrazol (PBZ); chitosan (CH)
Mean daily germination (MDG)

According to the result analysis, MDG was affected by osmotic stress and priming treatments \((p \leq 0.01)\). Osmotic stress decreased MDG; therefore, the highest and lowest MDG (4.77 and 3.63 seed) were achieved in 0 and -10 bar treatments. In contrast, seed priming increased MDG, so that treated seed by JA had the highest MDG (5.03 seed), and UPd seeds showed the lowest MDG (3.1 seed) (Table 2).

Seedling length (SL)

As shown in Table 2, the SL was affected by osmotic stress and priming treatments \((p \leq 0.01)\). However, the interaction was not significant. The high level of osmotic stress (-10 bar) showed a considerable decrease of SL (52.33%) than the non-stress condition. Seed priming by JA, PBZ, and CH increased SL by 32.07, 22.86, and 22.63%, respectively, compared to the control.

Seedling vigor index (SVI)

The effects of osmotic stress, seed priming treatment, and their interaction were significant on SVI. Generally, osmotic stress and seed priming treatments decreased and increased SVI, respectively. Seed priming by JA increased SVI under all levels of osmotic stress. The highest SVI was related to the JA priming under non-stress treatment (862.6), and the lowest (113.6) was UPd seed under high osmotic stress (Figure 2).

Photosynthetic pigment content

As shown in Table 3, the effects of osmotic stress, seed priming treatments, and their interaction were significant on total chlorophyll, chlorophyll a, and b content. The results showed that in all three osmotic stress levels, priming of cumin seeds increased the total chlorophyll content, which was much higher than in the drought control level and lower than in the high drought level. The highest total chlorophyll content (26.5 \(\mu g/g\) FW) was related to JA priming without osmotic stress. Seed priming by PBZ under -10 bar stress showed the lowest total chlorophyll content (4.15 \(\mu g/g\) FW). The control and hydro-priming treatments had the lowest this trait (4.05 and 4.75 \(\mu g/g\) FW, respectively) (Figure 3).
Table 3. Effect of osmotic stress and priming treatments on physiological characteristics of cumin 
(Cuminum cyminum L.)

<table>
<thead>
<tr>
<th></th>
<th>Total chlorophyll content (µg/g FW)</th>
<th>Chlorophyll a content (µg/g FW)</th>
<th>Chlorophyll b content (µg/g FW)</th>
<th>Carotenoids content (µg/g FW)</th>
<th>Peroxidase activity (U/mg protein.min)</th>
<th>Catalase activity (U/mg protein.min)</th>
<th>Superoxide dismutase activity (U/mg protein)</th>
<th>Proline content (µmol/g FW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osmotic stress (bar)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>17.89±1.55a</td>
<td>12.49±0.99a</td>
<td>5.41±0.6a</td>
<td>2.0±0.24a</td>
<td>5.8±0.92a</td>
<td>2.39±0.12b</td>
<td>8.65±0.43c</td>
<td>223.34±10.85c</td>
</tr>
<tr>
<td>-5</td>
<td>11.25±0.99b</td>
<td>8.58±0.96b</td>
<td>2.67±0.36b</td>
<td>2.13±0.37a</td>
<td>5.38±0.57a</td>
<td>4.12±0.23a</td>
<td>14.08±0.74b</td>
<td>327.77±13.05b</td>
</tr>
<tr>
<td>-10</td>
<td>6.24±0.6c</td>
<td>4.39±0.42c</td>
<td>1.85±0.22c</td>
<td>0.91±0.09b</td>
<td>14.79±2.4a</td>
<td>4.46±0.37a</td>
<td>24.27±1.4a</td>
<td>474.73±10.46a</td>
</tr>
<tr>
<td>LSD (p≤0.05)</td>
<td>1.08</td>
<td>0.89</td>
<td>0.58</td>
<td>0.38</td>
<td>2.81</td>
<td>0.67</td>
<td>2.67</td>
<td>25.29</td>
</tr>
<tr>
<td>Priming treatments</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UPd (control)</td>
<td>6.44±0.78c</td>
<td>4.79±0.63d</td>
<td>1.65±0.29d</td>
<td>0.86±0.2d</td>
<td>8.28±0.88b</td>
<td>4.07±0.6a</td>
<td>17.78±3.16a</td>
<td>369.54±38.99a</td>
</tr>
<tr>
<td>HPd</td>
<td>8.46±0.59d</td>
<td>7.98±1.11c</td>
<td>2.76±0.63c</td>
<td>1.56±0.32c</td>
<td>9.15±1.01b</td>
<td>3.72±0.61a</td>
<td>17.05±1.55a</td>
<td>342.1±29.7b</td>
</tr>
<tr>
<td>SA</td>
<td>10.34±1.47c</td>
<td>9.39±1.01c</td>
<td>2.95±0.5c</td>
<td>1.46±0.17c</td>
<td>5.78±0.91b</td>
<td>3.43±0.53a</td>
<td>15.84±2.14b</td>
<td>287.73±35.02c</td>
</tr>
<tr>
<td>JA</td>
<td>17.03±2.59a</td>
<td>12.49±1.64a</td>
<td>4.54±1.13a</td>
<td>2.63±0.44a</td>
<td>7.26±2.22b</td>
<td>3.31±0.33a</td>
<td>13.18±1.91b</td>
<td>361.08±39.18ab</td>
</tr>
<tr>
<td>PBZ</td>
<td>12.9±2.42b</td>
<td>9.53±1.76b</td>
<td>3.37±0.77bc</td>
<td>2.09±0.43b</td>
<td>13.73±3.39a</td>
<td>3.79±0.34a</td>
<td>16.58±2.52b</td>
<td>361.38±39.86ab</td>
</tr>
<tr>
<td>CH</td>
<td>12.26±1.55b</td>
<td>8.23±1.3c</td>
<td>4.03±0.45ab</td>
<td>1.35±0.34cd</td>
<td>8.23±3.06b</td>
<td>3.70±0.45a</td>
<td>14.95±2.6ab</td>
<td>330.5±36.9b</td>
</tr>
<tr>
<td>LSD (p≤0.05)</td>
<td>1.40</td>
<td>1.15</td>
<td>0.75</td>
<td>0.49</td>
<td>3.63</td>
<td>0.87</td>
<td>3.45</td>
<td>32.65</td>
</tr>
<tr>
<td>Statistical analysis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Osmotic (O)</td>
<td>512.4**</td>
<td>246.2**</td>
<td>51.9**</td>
<td>0.91**</td>
<td>9.13**</td>
<td>18.53**</td>
<td>943.8**</td>
<td>2392.47.1**</td>
</tr>
<tr>
<td>Priming (P)</td>
<td>134.1**</td>
<td>72.1**</td>
<td>11.0**</td>
<td>0.63**</td>
<td>1.95**</td>
<td>0.81ns</td>
<td>27.05ns</td>
<td>10320.6**</td>
</tr>
<tr>
<td>O × P</td>
<td>23.21**</td>
<td>13.08**</td>
<td>6.34**</td>
<td>0.32**</td>
<td>1.84**</td>
<td>1.87**</td>
<td>9.00ns</td>
<td>958.7**</td>
</tr>
<tr>
<td>Error</td>
<td>1.46</td>
<td>1.44</td>
<td>0.60</td>
<td>0.03</td>
<td>0.42</td>
<td>0.82</td>
<td>12.87</td>
<td>1150.5</td>
</tr>
<tr>
<td>CV (%)</td>
<td>12.38</td>
<td>14.15</td>
<td>23.53</td>
<td>15.42</td>
<td>23.94</td>
<td>24.88</td>
<td>22.89</td>
<td>9.91</td>
</tr>
</tbody>
</table>

Means followed by the same letter in each column are not significantly different according to the LSD test at 5% level (Means±SD).

ns: non-significant, * and **: Significant at α=0.05 and α=0.01, respectively.

Unprimed seeds (UPd); hydro-priming (HPd); salicylic acid (SA); jasmonic acid (JA); paclobutrazol (PBZ); chitosan (CH); Coefficient of variation (CV)

Figure 3. Interaction of osmotic stress and priming effects on total chlorophyll content of cumin seedling

Means followed by the same letter are not significantly different according to the LSD test at 5% level (Means ± SD). Unprimed seeds (UPd); hydro-priming (HPd); salicylic acid (SA); jasmonic acid (JA); paclobutrazol (PBZ); chitosan (CH)
As shown in Figure 4, the trend of changes in chlorophyll a content was similar to the trend of total chlorophyll. Seed priming by JA increased chlorophyll a content by 54.26, 63.39, and 63.01% compared to the control under -10, -5, and 0 bar stresses, respectively. At all drought levels, the use of JA was more efficient than other compounds.

Results indicated that the use of JA under non-stress conditions, CH under -5 bar, and JA and CH under -10 bar showed the highest mean of chlorophyll b content. The highest chlorophyll b content was found in JA priming under non-stress conditions (8.93 µg/g FW). Dry seed (UPd) and PBZ priming treatments under the high level of osmotic stress had the lowest chlorophyll b content (1.1 and 1.14 µg/g FW, respectively) (Figure 5).

![Figure 4](image1.png)

**Figure 4.** Interaction of osmotic stress and priming effects on chlorophyll a of cumin seedling
Means followed by the same letter are not significantly different according to the LSD test at 5% level (Means ± SD).

![Figure 5](image2.png)

**Figure 5.** Interaction of osmotic stress and priming effects on chlorophyll b of cumin seedling
Means followed by the same letter are not significantly different according to the LSD test at 5% level (Means ± SD).
**Carotenoid's content**

Analysis of variance results showed that the effects of osmotic stress, priming treatments, and interaction of osmotic and priming were significant on carotenoids content ($p \leq 0.01$). The high level of osmotic stress (-10 bar) was caused by decreasing carotenoids content. No significant difference was observed between 0 and -5 bar osmotic stress levels. As shown in Figure 6, treated seed by JA under -5 bar stress caused the highest carotenoid content (4.00 µg/g FW), which showed an increase of 87% compared to the control treatment. This trait's lowest mean was observed in CH priming under -5 bar osmotic stress (0.44 µg/g FW).

**Antioxidant enzyme activities**

The effect of osmotic stress was significant on POD, CAT, and SOD activities ($p \leq 0.01$). Results showed the osmotic stress increased the activity of antioxidant enzymes. Under -10 bar stress, we observed 60.78, 46.41, and 64.35% increasing activity of POD, CAT, and SOD activities compared to the control, respectively. Moreover, the osmotic stress and priming treatment interaction were significant on POD and CAT activities (Table 3). Therefore, the PBZ treatment under the high level of osmotic stress showed the most increased POD activity (27.17 U/mg protein min), which increased 59.18% compared to the control treatment (Figure 7). The interaction results showed the highest CAT activity related to the UPd (control) seed under -10 bar stress (5.75 U/mg protein). The lowest mean the SA and JA treatments under non-stress conditions. According to the CAT activity, the priming treatment showed different effects under any level of osmotic stress. For example, no significant difference was observed between different priming treatments at control drought stress, while SA and UPd treatments had the highest CAT activity under -5 and -10 bar, respectively (Figure 8).

---

**Figure 6.** Interaction of osmotic stress and priming effects on carotenoids of cumin seedling

Means followed by the same letter are not significantly different according to the LSD test at 5% level (Means ± SD). Unprimed seeds (UPd); hydro-priming (HPd); salicylic acid (SA); jasmonic acid (JA); paclobutrazol (PBZ); chitosan (CH)
The variance analysis results (Table 3) showed significant effects for the osmotic stress and priming treatments ($p \leq 0.01$). Osmotic stress significantly increased proline content while different priming treatments negatively affected proline content. The highest proline content was related to the -10 bar (474.73 µmol/g FW) and UPd treatment (369.54 µmol/g FW). Furthermore, the JA and PBZ treatment had the highest proline content. In the two factors tested, SA treatment and non-stress conditions showed the lowest mean of this trait (287.7 and 223.34 µmol/g FW).
Correlation coefficients

There were significantly negative and positive correlations among seed germination properties as well as among seedling physiological characteristics. For example, the FGP was significantly and positively correlated with GR, MDG, SL, SVI, total chlorophyll, chlorophyll a, chlorophyll b, and carotenoids. Unlike, FGP was significantly and negatively correlated with MGT and activity of POD, CAT, and SOD enzymes, and proline content. Photosynthetic pigments were significantly and positively correlated with germination indices, while these traits were significantly and negatively correlated with seedling physiological parameters (Table 4).

### Table 4. Correlation coefficients among germination and physiological characteristics of cumin under osmotic stress and priming treatments

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>0.96**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>-0.52**</td>
<td>-0.61**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.99**</td>
<td>0.96**</td>
<td>-0.52**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.68**</td>
<td>0.59**</td>
<td>-0.30*</td>
<td>0.68**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0.85**</td>
<td>0.78**</td>
<td>-0.36*</td>
<td>0.85**</td>
<td>0.94**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>0.77**</td>
<td>0.69**</td>
<td>-0.34*</td>
<td>0.77**</td>
<td>0.83**</td>
<td>0.88**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>0.74**</td>
<td>0.67**</td>
<td>-0.33*</td>
<td>0.74**</td>
<td>0.81**</td>
<td>0.86*</td>
<td>0.97**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>0.69**</td>
<td>0.62**</td>
<td>-0.29ns</td>
<td>0.69**</td>
<td>0.71**</td>
<td>0.78**</td>
<td>0.88**</td>
<td>0.74**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>0.49*</td>
<td>0.47*</td>
<td>-0.23ns</td>
<td>0.49*</td>
<td>0.56*</td>
<td>0.57**</td>
<td>0.67**</td>
<td>0.81**</td>
<td>0.25m</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>-0.32*</td>
<td>-0.25ns</td>
<td>-0.06ns</td>
<td>-0.32*</td>
<td>-0.50*</td>
<td>-0.47*</td>
<td>-0.43*</td>
<td>-0.45*</td>
<td>-0.32*</td>
<td>-0.38*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>-0.47*</td>
<td>-0.37*</td>
<td>0.32*</td>
<td>-0.47*</td>
<td>-0.65**</td>
<td>-0.60**</td>
<td>-0.55**</td>
<td>-0.52**</td>
<td>-0.52**</td>
<td>-0.23ns</td>
<td>0.34*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>-0.61**</td>
<td>-0.49*</td>
<td>0.32*</td>
<td>-0.61**</td>
<td>-0.84**</td>
<td>-0.77**</td>
<td>-0.72**</td>
<td>-0.71**</td>
<td>-0.62**</td>
<td>-0.45**</td>
<td>0.58**</td>
<td>0.80**</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>-0.60**</td>
<td>-0.47*</td>
<td>0.24ns</td>
<td>-0.60**</td>
<td>-0.76**</td>
<td>-0.73**</td>
<td>-0.68**</td>
<td>-0.62**</td>
<td>-0.66**</td>
<td>-0.28ns</td>
<td>0.35**</td>
<td>0.59**</td>
<td>0.81**</td>
</tr>
</tbody>
</table>

ns: non-significant, *: Significant at α=0.05 and **: Significant at α=0.01, respectively.


Discussion

In the current study, the effects of different priming treatments on seed germination and primary growth of cumin seedlings and physiological characteristics were investigated under osmotic stress. All priming treatments enhanced germination indices such as FGP, GR, MDG, SL, and SVI under stress and non-stressed conditions. Sadeghi and Robati (2015) reported that seed priming is a regular step before sowing in a few vegetables and flower crops in some countries. The seed priming mechanism is to initiate the repairing system for membrane and the metabolic preparation for germination by controlling the seed’s water absorption rate. Cumin seeds under JA priming have shown the highest FGP, GR, SL, and SVI under stress and non-stressed conditions. These results are supported by Yildiz et al. (2008), who found that pear seeds primed with JA not only improved germination indices but also reduced germination time.

Also, Sharma et al. (2018) reported that the JA seed treatment resulted in the significant recovery of chlorophyll content and seedling growth. Methyl jasmonate and JA have been isolated from many plant species and have been shown to affect many aspects of plant growth, including seed germination and seedling growth (Sharma et al., 2018). The increase in the seedling growth parameters with JA treatment might be due to the role of JA in enhancing the cell expansion, cell elongation, and differentiation of vascular tissues (Sharma et al., 2018). JA also plays a vital role in regulating plants’ primary root growth. Moreover, these results confirm the ability of JA to stimulate germination of embryos, described previously for other species (Yildiz et al., 2008). JA promoted alkaline lipase activity during the germination of seeds. This enzyme involves in the mobilization of lipid reserves in seeds. Mobilization of reserve lipids and proteins may play an important role in the increasing of embryo growth (Yildiz et al., 2008).
In this study, seed priming by JA under without PEG stress has been displayed as a profitable strategy for the cumin SVI to improve. SVI (SL × FGP), which are the important traits in the primary establishment of seedling were reduced with increased drought stress (Afshari et al., 2020). The positive effect of seed priming with JA was found with a higher FGP and SL in primed seeds than non-primed seeds. As a result, the SVI increased. Because this parameter is multiplied by the germination percentage by SL or weight (Aghighi Shahverdi et al., 2017; Abbasi Khalaki et al., 2019). PEG solution’s high concentration prevents water absorption and free radical production of oxygen, damage to the cell membrane, and changes in enzyme activity and ultimately reduces seed germination and SVI. SVI in JA primed seeds under high osmotic stress level was more than other primed or untreated seeds (Abbasi Khalaki et al., 2019).

In the present study, cumin seeds’ germination response was negatively affected by the PEG-induced drought stress. Drought stress affects germination indices by limiting water absorption by seed, transferring seed reserves, or directly influencing the embryo’s organic structure and protein synthesis. Poor and erratic germination might be attributed to the lower water uptake by seeds and the elevation of ROS levels under drought stress. The alteration of some enzymes and hormones found in the seed could reduce final germination under drought stress conditions (Abbasi Khalaki et al., 2019).

In the present study, in addition to JA treatment, priming of cumin seeds using CH and PBZ also increased germination parameters and seedling growth under stress and non-stress conditions. Various ameliorating agents such as PBZ (Hajihashemi and Ehsanpour, 2013; Afshari et al., 2020) and have been used for combating various abiotic stresses, including drought stress.

In the current study, the chlorophyll pigments were significantly decreased by PEG increase compared to the control seedlings. The reduction of chlorophyll content has been considered a usual symptom of oxidative stress under drought stress (Fathi and Tari, 2016). Reduction in photosynthetic pigments’ content might be due to the oxidative stress and enhanced activity of chlorophyllase enzyme under osmotic stress conditions. The seed priming treatments (especially JA and PBZ) moderate the adverse effect of osmotic stress on the chlorophyll pigments and had a significant stimulatory effect on the biosynthesis of chlorophyll. Therefore, the highest chlorophyll pigment contents were recorded in the seedlings raised from primed seeds by JA under any level of drought stress. Previous studies have shown that JA affects the chlorophyll content. Farhangi-Abriz and Ghassemi-Golezani (2018) reported that the JA treatment did not affect chlorophyll contents. It seems that the difference in the effect of JA on the content of photosynthetic pigments is highly dependent on plant species and, more importantly, the concentration of JA.

Carotenoids protect chlorophyll pigment from damage caused by photooxidation as a consequence of oxidative stress. JA also triggers the accumulation of carotenoids in a plant under insecticide and pesticide stresses (Sharma et al., 2018), and the current investigation, we noticed a significant enhancement in carotenoid accumulation after JA application under -5 bar stress (Figure 6). This JA-induced accumulation of carotenoids could be due to the JA-mediated up-regulation of critical genes’ transcription patterns (DXS, GGPS, PSY1, and PDS) involved in carotenoid biosynthesis (Sharma et al., 2018).

Tolerance to drought is strongly correlated to maintaining high antioxidant enzyme activities to avoid oxidative stress damages caused by ROS overproduced in the tissues in drought. Together, these compounds could neutralize the toxic effect of peroxide, superoxide, and hydroxyl radicals in the tissues (Mouradi et al., 2016). In the present study, the JA treatment by improving antioxidant enzyme activities reduced oxidative damages in cumin plants. Similarly, Farhangi-Abriz and Ghassemi-Golezani (2018) found that JA reduced lipid peroxidation in seedlings via decreasing oxidative stress by increasing antioxidant enzymes activities. It is not clear yet that how JA acts to modify the antioxidant systems. JA may influence enzyme activities through gene transcription changes, translation, or post-transcriptional modifications (Farhangi-Abriz and Ghassemi-Golezani, 2018). Sharma et al. (2018) reported that the activities of antioxidative enzymes and contents of non-enzymatic antioxidants were enhanced with the application of JA seed treatment. Farhangi-Abriz and Ghassemi-Golezani (2018) concluded that the JA improves glycine betaine and soluble proteins content, antioxidant enzyme activity, membrane stability index, and leaf water content. Ameliorative effects of
exogenous JA tended to be greater in drought-stressed sugar beet plants as it led to the enhancement of chlorophyll content and relative water content in water deficit conditions (Ghaffari et al., 2020).

Proline is known to act as an osmolyte/osmoprotectant agent under drought stress. It has an important role in the osmotic pressure adjustment, scavenging free radicals, stabilizing sub-cellular structures (e.g., membranes and proteins) and storing carbon and nitrogen (Afshari et al., 2020). The results of our study indicated a significant increase in the proline content under osmotic stress. While all priming treatments significantly decreased the accumulation of free proline in cumin seedlings compared to the control treatment (Table 3). In agreement with our results, Aghighi Shahverdi et al. (2017) and Afshari et al. (2020) reported that the seedlings had the higher proline content under stress conditions.

Conclusions

To conclude, the experiment results revealed that cumin seed has low germination and weak establishment, especially under osmotic stress. Seed priming moderately modified the adverse effects of drought stress and improved these growth parameters. Almost all seed treatments in this study gave a better performance than control (untreated), with apparent effectiveness of JA treatment in improving the germination percentage and early seedling growth and physiological traits. Therefore, for the development of cumin germination indices under drought stress, applying JA-induced physiological activities may enhance the germination characteristics. From the present study, it has been concluded that exogenous application of JA can aid plants in recovering from the negative impacts of oxidative stress caused by drought. JA also enhances the drought-tolerance potential of seedlings, resulting in a reduction of negative osmotic stress. However, more detailed studies on the JA mediated signaling mechanisms of various metabolic pathways are still needed to understand the exact mechanisms of enhanced drought-tolerance.

Authors‘ Contributions

Conceptualization, HN, SGM, MS, and MF; Data curation, HN and SGM; Formal analysis, HN; Methodology, HN and SGM; Software, HN and SGM; Supervision, SGM, MS, and MF; Validation, HN; Writing – original draft, HN, SGM, MS, and MF; Writing – review & editing, HN, SGM, MS, and MF. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

Acknowledgements

The authors gratefully acknowledge Dr. Maghsoudi and Ms. Khajeh, Biotechnology Research Institute of Zabol University, Iran, for her contribution in physiological part.
Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References


The journal offers free, immediate, and unrestricted access to peer-reviewed research and scholarly work. Users are allowed to read, download, copy, distribute, print, search, or link to the full texts of the articles, or use them for any other lawful purpose, without asking prior permission from the publisher or the author.

License - Articles published in Notulae Botanicae Horti Agrobotanici Cluj-Napoca are Open-Access, distributed under the terms and conditions of the Creative Commons Attribution (CC BY 4.0) License. © Articles by the authors; UASVM, Cluj-Napoca, Romania. The journal allows the author(s) to hold the copyright/to retain publishing rights without restriction.