ELUCIDATING SOME PHYSIOLOGICAL MECHANISMS OF SALT TOLERANCE IN BRASSICA NAPUS L. SEEDLINGS INDUCED BY SEED PRIMING WITH PLANT GROWTH REGULATORS

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Key words: Abscisic acid; Antioxidant enzymes; Brassinosteroid; Ion accumulation; Salicylic acid.

Abstract

Present study was undertaken to elucidate some physiological mechanisms of induced salt tolerance by different plant growth regulators (PGRs) in rapeseed (Brassica napus L.) seedlings. Three salt stress levels were given; tapwater (0.7 dS m\(^{-1}\)), 6 dS m\(^{-1}\) and 12 dS m\(^{-1}\), which made by NaCl and CaCl\(_2\) as 2:1 molar ratio. The eight seed priming agents were included: dry seeds (no-priming), hydropriiming and six PGRs primings. The used PGRs were; abscisic acid (ABA), auxin (AUX), salicylic acid (SA), chlorocholine chloride (CCC), ascorbic acid (AS) and brassinosteroid (Brs). The results revealed that salinity, depending on level, reduced seedling emergence, shoot and root growth, potassium (K\(^+\)) concentration and enhanced sodium (Na\(^+\)) concentration and antioxidant enzymes. These reductions could be attributed to oxidative stress and/or ion balance disturbance due to salinity stress. It is documented by reduced K\(^+\) and increased Na\(^+\) in both root and shoot as well as enhanced antioxidant enzyme activity and H\(_2\)O\(_2\) in salt stressed rapeseed seedlings. Higher storage factor (SF) refers to a higher ions content kept in roots rather than transporting these to the shoot as salinity level increased. PGRs priming modulates some negative effects of salt stress on emergence, growth and physiological functions of plants. In triggering the ameliorating role of PGRs, it appeared that the ratio of Na\(^+\) to K\(^+\) is more important than their individual concentrations. Moreover, H\(_2\)O\(_2\) concentration was found to be a better key for estimation of the oxidative damage rather than antioxidative enzymes. Among the PGRs, SA and Brs showed better performance, and it seems that the main mechanism of action for SA was creating ion balance and changing ion partitioning in favor of roots. For Brs it appeared to be reactive oxygen species (ROS) scavenging by inducing higher activity of antioxidant enzymes, particularly catalase.

Introduction

Most crops are exposed to abiotic stresses and global climate change is adding to these adverse conditions (Ahmad et al., 2012; Mittler & Blumwald, 2010; Hasanuzzaman et al., 2012; Ozturk et al., 1997, 2012, 2019). Lately, salinity stress is posing a great threat in global crop production (Hasanuzzaman et al., 2012; Ozturk et al., 2014, 2019; Raza et al., 2019). Salinity produces significant negative effects on plant growth (Ozturk et al., 1992, 1994a, b, 2016, 2019; Khan et al., 2010; Altay & Ozturk, 2012; Hashem et al., 2019). It negatively effects plant metabolism, especially physiological and biochemical processes. Among these negative effects, besides interfering with the root function in water intake, nutrient intake and assimilation are major metabolic factors (Carillo et al., 2011; Hashem et al., 2019). However, soil salt accumulation varies considerably with time as well as place, and depends on several factors. Approximately 0.3 million hectares of agricultural land becomes unusable every year (Munns, 2002; Hashem et al., 2019). Salinity also affects at least 20% of irrigated land globally, with an increasing trend of loss up to 50% by the middle of the 21st century. The scenarios foreseen for the environmental resources and human health at global level due to soil fertility and crop yield losses are more disturbing (Mahajan et al., 2005; Santangeli et al., 2019). In view of this, great importance is attached to the development of strategies on the adverse effects of salinity on plants (Hashem et al., 2019; Raza et al., 2019).

Some work has been done to understand the mechanism of ecological tolerances of plants against salt stress (Yang & Guo, 2018; Tan et al., 2019). The major salt tolerance mechanism in plants is given as osmolyte accumulation which triggers the high osmotic pressure balance from outside to the cell. These compatible osmolytes mainly include proline, glycine betains, mannitol, glucosyglycerol, sucrose and raffinose, which under the regulation of their synthesis-direction pathways increase (Liang et al., 2018; Yang & Guo, 2018). For a synthesis of some osmolytes Mitogen Activated Protein Kinase pathway and ABA signals are needed (Bahmani et al., 2015; Tan et al., 2019). Active and passive absorption allows penetration of high ion concentrations of Na\(^+\) and Cl\(^-\) to the cells from outside through ion channels, thereby disrupting the internal ion balance (Munns & Tester, 2008). The nonsselective cation channels of salt overly sensitive pathways and other important ion transport systems have been found together with key genes with function in ion efflux (Formentin, 2017; Tan et al., 2019). K\(^+\), Mg\(^2+\), Ca\(^2+\) and several other ions also affect influx or efflux of Na\(^+\)/Cl\(^-\) (Bahmani et al., 2015). The salt stress induces ROS negatively effecting proteins, lipids, DNA and carbohydrates. There is a triggering of SOD, APX, and CAT enzyme systems for ROS elimination as well as repair of damaged components (Liang et al., 2018). According to Kumar & Kesawat (2018) the calmodulin pathway also affects the strength of salt stress resistance. Although much progress made in understanding the salt tolerance...
mechanism and many genetic loci have been identified in plant genomes functioning in the alleviation of salt stress but more work needed for satisfactory salt tolerance in the crops (Munns & Gilliham, 2015; Islam et al., 2019).

In the light of these findings, in order to counteract harmful effects of salt-stress, seed pre-soaking with different priming agents seems to be an effective approach involving a pre-sowing treatment in osmotic solution, allowing water imbibition of seeds so as to induce first stage of germination, preventing radical protrusion through the seed coat (Ibrahim, 2016; Pirasteh-Anosheh & Emam, 2017). Many seed priming treatments have been used to accelerate the germination and seedling growth in crops under normal and saline conditions (Basra et al., 2005). For rapid and uniform emergence, priming seems to be a viable technology for high vigor followed by better yields in some crops (Draganic & Lekic, 2012). It reduces the time between seed sowing and seedling emergence together with the synchronization of emergence (Ibrahim, 2016). The loss of membrane integrity, changes in enzymatic activities, decline in protein and nucleic acid synthesis, and lesions in DNA are responsible for seed deterioration (Eisvand et al., 2010). These changes are frequently related to AOS-induced oxidative injury and may be modulated by seed priming (Ashraf et al., 2010; Pirasteh-Anosheh & Emam, 2017). Seed priming strategies include hydropriming, osmoconditioning, osmohardening, hardening, hornopriming, matripriming, and others (Kaya et al., 2006).

As phytohormones abscisic acid (ABA) and auxins (AUX) are mainly involved in the plant responses to salinity and other abiotic stresses. The importance of AUX is indicated with respect to seed germination process (Verma et al., 2016). Changes in ABA and AUX level can be important factors for determining plant response to stress conditions (Verma et al., 2016). Ascorbic acid (AS) is also potentially used for lining, due to its important vitamin and antioxidant structure.

To maintain the antioxidant capacity, for a protection of plants from oxidative stress high level of endogenous ascorbate is essential (Zhou et al., 2009). As an anti-gibberellin growth retardant chlorocholine chloride (CCC) plays a positive role in crop salt tolerance (Pirasteh-Anosheh et al., 2014). According to Ashraf et al., (2008, 2010) under different stress conditions salicylic acid (SA) plays an active role in plant signal transduction and brassinosteroids (Brs) play an essential role in plant growth and development, all implicated in physiological responses.

The positive effects of phytohormones are well reported, but little is known about the comparison of different PGRs such as SA, ABA, AUX, CCC, Brs and AS under saline conditions (Ozturk et al., 1994a, b; Ozturk et al., 1994c, 2006). Rapeseed (Brassica napus L.) is a crop cultivated in some regions where salinity is an issue (Hashem et al., 2019). This investigation was thus planned to find out the effects of presoaking of rapeseed seeds; in some phytohormones under varying salt stress levels; on the aim was to demonstrate the role of phytohormones in stress tolerance and enlighten results about the potential of plants to adapt to saline conditions.

Material and Methods

The experiment was conducted in 2016 under controlled environment in order to determine the effect of hornopriming on eco-physiological traits of rapeseed (Brassica napus cv. Talayeh) under salinity stress conditions. The experiments were carried out at Shiraz University- College of Agriculture, Iran. The outlay was in the form of a factorial experiment based on completely randomized design (CRD) with three replicates. In the salinity treatment 3 levels were used; tapwater (0.7 dS m⁻¹, as control), 6 and 12 dS m⁻¹. Other set included eight priming treatments using dry seeds (no-priming), hydropriming and six PGRs priming: abscisic acid (ABA), auxin (AUX), salicylic acid (SA), chlorocholine chloride (CCC), ascorbic acid (AS) and brassinosteroid (Brs).

The physico-chemical parameters of the soil used were; EC (dS m⁻¹) 0.60, pH 7.09, organic matter content 1.124 (%), nitrogen (%) 0.15, P (mg kg⁻¹) 720, and silty-loam texture. The 5 L plastic pots filled with soil, sand and humus (2:1:1) were used and 20 sterilized uniform seeds of rapeseed were sown. Both before planting and at the end of experiment soil sampling was done, the soil analysis results are presented in Table 1.

After an establishment of seedling in the greenhouse, thinning was done to 5 plants per pot. The pots were left at min. and max. temperatures of 14˚ and 28˚C and relative humidity was fixed at 55-60 percent. For salinity stress, salt was applied in 3 steps to avoid sudden stress shock. For each rapeseed priming treatment of seeds, a weighed quantity was soaked in 1 aerated distilled water/respective solutions for 12 h at 24±2°C. The seeds were soaked in distilled water for hydropriming, whereas for hormonal priming, seeds were soaked in 0.5 mM AS, 1 mM SA, 0.2 mM AUX, 10 μM ABA, 20 mM CCC and 3 μM Brs, respectively, 15 days after sowing using NaCl: CaCl₂ as 2:1 molar ratio. An aquarium pump was used for continued aeration, during the soaking period and the seeds were given two surface washings with distilled water after soaking, re-dried closer to the original weight under shade using forced air at room temperature, with untreated dry seeds as control.

Table 1. The results of soil analysis before planting and at the end of experiment.

<table>
<thead>
<tr>
<th></th>
<th>EC (dS m⁻¹)</th>
<th>pH</th>
<th>OM (%)</th>
<th>N (%)</th>
<th>P (mg kg⁻¹)</th>
<th>K (mg kg⁻¹)</th>
<th>Texture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before planting</td>
<td>0.86</td>
<td>7.12</td>
<td>1.13</td>
<td>0.16</td>
<td>18.1</td>
<td>333</td>
<td></td>
</tr>
<tr>
<td>0.7 dS m⁻¹</td>
<td>0.92</td>
<td>7.21</td>
<td>0.90</td>
<td>0.10</td>
<td>15.7</td>
<td>315</td>
<td>Silty loam</td>
</tr>
<tr>
<td>6 dS m⁻¹</td>
<td>7.52</td>
<td>7.06</td>
<td>0.97</td>
<td>0.12</td>
<td>15.9</td>
<td>310</td>
<td></td>
</tr>
<tr>
<td>12 dS m⁻¹</td>
<td>14.79</td>
<td>7.33</td>
<td>0.98</td>
<td>0.12</td>
<td>16.1</td>
<td>321</td>
<td></td>
</tr>
</tbody>
</table>
Emerged seed (ES) was counted and emergence percentage (EP) determined with the following equation, where \( G \) is the total sown seed.

\[
EP = \frac{ES}{G} \times 100
\]

A total of five seedlings was evaluated to record all observations and measurements. Samples were taken after 45 days of sowing. Five plants were harvested and shoot and root separated. The roots were suspended in water and hand cleaned using sieves of 0.5 mm mesh size. Plant height and root length were noted using a millimeter ruler. Root volume was noted as fresh root in 1 L beaker. After drying the samples at 70±2°C for 48 h in an air oven root and shoot dry weights were recorded.

For antioxidant enzyme assay, sampling was individually done from leaves at the same time. The leaves were immediately frozen in liquid nitrogen. The samples were freeze dried and stored at -80°C, ground using a pestle - mortar and ice-cold extraction buffer. Activity of SOD was measured with a spectrophotometer, based on the ability to inhibit photochemical reduction of nitroblue tetrazolium (Beauchamp & Fridovich, 1971). The method of Chance & Maehly (1955) was followed to record POD activity, as the ability to convert aromatic oil guaiacol to tetraguaiacol (\( \epsilon \) D 26.6 mM\(^{-1}\) cm\(^{-1}\)) and catalase (CAT) activity also was determined following Chance & Maehly (1955), which involves monitoring the disappearance of H\(_2\)O\(_2\) at 240 nm (\( \epsilon \) D 40 mM\(^{-1}\) cm\(^{-1}\)).

In the roots and shoots Na\(^+\) and K\(^+\) concentrations were determined using a flame photometer, and Storage factor used to quantify ion partitioning between roots and shoots, it was calculated using the equation introduced by Pirasteh-Anosheh et al., (2017) as follow:

\[
SF (\text{Storage factor}) = \frac{RI (\text{Root ion accumulation})}{T\text{AI} (\text{Total amount of ion absorbed})}
\]

where SF is storage factor, and RI and TAI are root ion accumulation and total amount of ion absorbed, respectively. SF code refers to the ratio of total absorbed ions remaining in the stem cells, which are not carried to the shoots. Almost all absorbed ions are carried to the shoots if SF value is zero, if it is 1 all absorbed ions are stored in the root (Pirasteh-Anosheh et al., 2017). That more Na\(^+\) accumulation in the roots and low access to shoots is a mechanism reported by Davenport et al., (2005), stressing a higher SF as an indication of high salt tolerance.

Analysis of variance was used to evaluate the data obtained, and significant differences were determined between the treatment means using LSD test at \( p<0.01 \) or standard error (SAS software). MINITAB 16 was used to calculate the stepwise regression and correlation coefficients.

**Results and Discussion**

Hormonal priming significantly affected the emergence percentage and plant height under both normal and saline conditions (Fig. 1). Under non-saline conditions (0.7 dS m\(^{-1}\)), the highest emergence (100%) is achieved in seeds primed with SA, followed by Brs primed seeds (Fig. 1a). In saline conditions the highest emergence is observed in Brs priming treatment. Under non-saline conditions highest plant height (37.8 cm) is achieved in seeds primed with Brs (Fig. 1b). While, under saline conditions (i.e. 6 & 12 dS m\(^{-1}\)), Brs primed treatment has the highest plant height (29.1 cm & 18.5 cm, respectively), which non-significantly differed from the SA priming (Fig. 1b).

Salt stress significantly decreases root length in all priming and no-priming treatments (Fig. 2). These reductions are closely associated with salt stress severity. Priming with Brs shows the highest root length and volume as compared to non-priming or hydro primed under all salinity conditions. Under non-saline as well as under both salinity levels, the highest root length (25.1, 24.8 and 16.1 cm, respectively) (Fig. 2a) and root volume (9.4, 8.1 and 5.3 ml, respectively) (Fig. 2b) were attained in Brs treated seedlings, however root length is significantly similar to SA priming under 12 dS m\(^{-1}\) salinity (Fig. 2a), and root volume is significantly similar to SA and CCC priming (Fig. 2b). Although Brs priming has a positive effect on root length and volume, differences between salinity levels are kept even under Brs seed priming. During normal salinity condition (0.7 dS m\(^{-1}\)), lowest root length is recorded in ABA treated seedlings, which is significantly similar to AUX and AS priming. Under saline conditions, all priming treatments except priming with ABA & AS and AUX (under 6 dS m\(^{-1}\) salinity) improve root length when compared to control (Fig. 2a). Under non-saline conditions, all priming treatments improve root volume (Fig. 2b), whereas in salt stressed plants, especially 12 dS m\(^{-1}\) root volume significantly decreased in rapeseed in hydro-, ABA and AUX priming as compared to control.

All PGRs priming treatments improve shoot dry weight than control under both non-saline and saline conditions (Fig. 3a) however, no significant difference was seen between the treatments, especially in salt stressed plants. Under non-saline conditions, Brs and SA treated plants show an increase in the root dry weight by 14 and 12 percent respectively, as compared to the non-priming (Fig. 3b), other treatments have stimulation effects.

The activity of SOD, POD and CAT is differentially affected by salt stress (Fig. 4a-c). The activity of all three enzymes is significantly induced as affected by 6 dS m\(^{-1}\) salinity by 22.5, 11.4 and 5.0% respectively and by 12 dS m\(^{-1}\) salinity by 48.9, 67.9 and 13.2% respectively compared to the control (Fig. 3). It has also been observed that peroxide hydrogen (H\(_2\)O\(_2\)) enhances as salt stress is imposed (Fig. 4d). H\(_2\)O\(_2\) is greater in 6 and 12 dS m\(^{-1}\) salinity treatments than non-saline conditions by 24.9% and 52.4% respectively.

All PGRs priming in general increased the activity of SOD, POD and CAT (Fig. 4). However, SA and Brs priming markedly stimulated activity of antioxidant enzymes in rapeseed leaves, under saline or non-saline conditions. The highest antioxidant enzyme activity is attained in SA and Brs primed seedlings under non-saline and two salinity treatments (Fig. 4a-c). The seedling grown from dry seeds had the highest H\(_2\)O\(_2\) (Fig. 4d), which non-significantly differed to hydropriming. Priming with SA and Brs remarkably reduces H\(_2\)O\(_2\) concentration in leaves, so that H\(_2\)O\(_2\) concentration is less in SA-treated plants than non-priming by 50.1, 56.8 and 54.5% in non-saline, 6 and 12 dS m\(^{-1}\) salinity treatments respectively.
In general, salinity increased sodium ion (Na\(^+\)) and Na\(^+\)/K\(^+\) and decreased potassium ion (K\(^+\)) concentrations in both shoot and root tissues (Fig. 5), however PGRs priming modulated the negative effect of salt stress. No significant effect on root Na\(^+\) & Na\(^+\)/K\(^+\) in SA- and Brs priming 6 dS m\(^{-1}\) salinity and in other PGR primings. The negative effect on salt stress was less than no-priming. For example, the increasing amount in Na\(^+\)/K\(^+\) due to 12 dS m\(^{-1}\) salinity was the highest in no-priming (5.10 and 6.64 times in shoot and root, respectively) and the lowest in SA-priming (2.55 and 2.38 times in shoot and root, respectively). Under non-saline conditions, no significant difference observed between Na\(^+\) concentrations in shoot and root and Na\(^+\)/K\(^+\) in root, but Na\(^+\) concentration is highest in 6 and 12 dS m\(^{-1}\) no-priming (Fig. 5a-b). The results also showed a reduction in K\(^+\) due to salinity is less in PGRs treated plants (Fig. 5c-d). The reduction in K\(^+\) shoot and root due to 6 dS m\(^{-1}\) salinity is not
significant in CCC, SA and Brs priming. Highest K⁺ concentration in shoots and roots is observed in SA primed seedlings. Salinity increases Na⁺ and K⁺ storage factor (SF) in all of priming treatments, and these changes parallel with salinity intensity (Table 2). Na⁺ SF and K⁺ SF get reduced in 6 dS m⁻¹ salinity treatments and decrease more as salt stress intensifies to 12 dS m⁻¹. Under non-saline and both salinity conditions, the highest Na⁺ SF and K⁺ SF are observed in no-priming treatments, and lowest in SA-priming.

Correlation coefficients (Table 3) reveal that dry weight has a positive and significant relationship with emergence percentage, plant height, root length, root volume, root dry weight, shoot K⁺, root K⁺ and K-SF; but is negatively and significantly correlated with superoxide dismutase, peroxidase, H₂O₂, shoot Na⁺, root Na⁺, shoot Na⁺/K⁺, root Na⁺/K⁺, and Na-SF (Table 3). The results of stepwise regression reveal that root dry weight, Na⁺ storage factor, H₂O₂, shoot Na⁺/K⁺, catalase and root Na⁺/K⁺ are determining factors, being the most effective traits effecting growth, as assessed by shoot dry weight (Table 4). There is a negative and linear relationship between the antioxidant enzymes and H₂O₂ (Fig. 6a-c), as observed in all three salinity treatments. The fitted regression lines are more closed in 12 dS m⁻¹ salinity compared to 0.67 and 6 dS m⁻¹. They are more fitted for the relationship between catalase and H₂O₂ (Fig. 6a), compared to other two antioxidant enzymes (Fig. 6b-c).

There is a negative and linear relationship between H₂O₂ and shoot dry weigh (Fig. 6d). Arif et al., (2019) have reported that salt tolerance in Brassica species may vary. They have reported that salinity stress reduces growth, seed yield and oil production in these plants.

Our investigations have revealed that salt stress leads to significant reduction in the seedling growth and emergence of rapeseed. These finding are in agreement with the results of other studies showing emergence and growth are significantly decreased by salinity (Afzal et al., 2008). Higher emergence recorded compared to the untreated seeds in the case of SA pretreated seeds. Afzal et al., (2008) have reported that SA treatment increases the percentage of emergence following salinity treatments. An important role played by the seed priming in the improvement of germination, reduction in the seedling emergence time and improvement of the stand establishment as well as yield (Draganic & Lekic, 2012). SA and Brs treatments for seed priming induces rapid and uniform germination in rapeseed, resulting in a rapid emergence of seedlings. The initiation of metabolic events in primed seeds leads to an increase in the emergence following priming. Such a treatment may also lead towards the embryo development and/or lead towards a leaching of germination inhibitors from seeds (Ibrahim, 2016). The priming techniques result in an enhanced seedling vigor as indicated by high energy of emergence and emergence percentage.

Salinity reduces or delays the germination, root length, shoot height, root and shoot dry weight as well as leaf area index at the seedling stage in most crop traits. It affects all stages of plant growth, but germination and seedling stages are the most critical stages in crop species (Munns, 2002; Farhoudi & Sharifzadeh, 2006; Miyamoto et al., 2012; Benincasa et al., 2013; Haq et al., 2014; Dolatabadi et al., 2019).
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Fig. 5. Sodium (A and B) and potassium (C and D) in root and shoot as affected by different priming under varied salinity severity. The columns with similar overlap are not significantly different.

Table 2. Storage factor for sodium (Na-SF) and potassium (K-SF) in rapeseed plants treated by different seed priming grown under varied salt stress levels.

<table>
<thead>
<tr>
<th></th>
<th>Na-SF 0.7 dS m⁻¹</th>
<th>Na-SF 6 dS m⁻¹</th>
<th>Na-SF 12 dS m⁻¹</th>
<th>K-SF 0.7 dS m⁻¹</th>
<th>K-SF 6 dS m⁻¹</th>
<th>K-SF 12 dS m⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>No priming</td>
<td>0.452 j-l</td>
<td>0.623 f</td>
<td>0.845 a</td>
<td>0.430 ij</td>
<td>0.616 e</td>
<td>0.794 a</td>
</tr>
<tr>
<td>Hydropriming</td>
<td>0.444 kl</td>
<td>0.555 g</td>
<td>0.794 ab</td>
<td>0.424 ij</td>
<td>0.560 f</td>
<td>0.744 ab</td>
</tr>
<tr>
<td>ABA</td>
<td>0.449 j-l</td>
<td>0.529 g-i</td>
<td>0.753 bc</td>
<td>0.420 ij</td>
<td>0.550 f</td>
<td>0.713 bc</td>
</tr>
<tr>
<td>AUX</td>
<td>0.450 j-l</td>
<td>0.539 gh</td>
<td>0.745 bc</td>
<td>0.425 ij</td>
<td>0.557 f</td>
<td>0.696 d-d</td>
</tr>
<tr>
<td>SA</td>
<td>0.433 l</td>
<td>0.449 j-l</td>
<td>0.645 ef</td>
<td>0.398 j</td>
<td>0.470 hj</td>
<td>0.628 e</td>
</tr>
<tr>
<td>CCC</td>
<td>0.450 j-l</td>
<td>0.497 h-j</td>
<td>0.734 cd</td>
<td>0.415 j</td>
<td>0.525 fg</td>
<td>0.684 ed</td>
</tr>
<tr>
<td>AS</td>
<td>0.450 j-l</td>
<td>0.530 g-i</td>
<td>0.739 c</td>
<td>0.430 ij</td>
<td>0.551 f</td>
<td>0.682 ed</td>
</tr>
<tr>
<td>Brs</td>
<td>0.447 j-l</td>
<td>0.485 i-k</td>
<td>0.688 de</td>
<td>0.426 ij</td>
<td>0.497 gh</td>
<td>0.660 de</td>
</tr>
</tbody>
</table>

[Means with similar letters had no significant difference (LSD 0.5%)]

Table 3. Correlations between the traits and shoot dry weight.

<table>
<thead>
<tr>
<th>Emergence percentage</th>
<th>Plant height</th>
<th>Root length</th>
<th>Root volume</th>
<th>Root dry weight</th>
<th>Superoxide dismutase</th>
<th>Peroxidase</th>
<th>Catalase</th>
<th>H₂O₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.859**</td>
<td>0.711*</td>
<td>0.732*</td>
<td>0.804**</td>
<td>0.701*</td>
<td>-0.682*</td>
<td>-0.678*</td>
<td>-0.647*</td>
<td>-0.835**</td>
</tr>
<tr>
<td>Shoot Na⁺</td>
<td>Root Na⁺</td>
<td>Shoot K⁺</td>
<td>Root K⁺</td>
<td>Shoot Na⁺/K⁺</td>
<td>Root Na⁺/K⁺</td>
<td>Na⁺ SF</td>
<td>K⁺ SF</td>
<td></td>
</tr>
<tr>
<td>-0.806**</td>
<td>-0.755**</td>
<td>0.818**</td>
<td>0.709*</td>
<td>-0.901**</td>
<td>-0.935**</td>
<td>0.894**</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

[ns: non-significant, * and ** are significant at 5% and 1% probability levels, respectively. SF: storage factor]
Plant height, root and root volume and shoot and root dry matter are reduced in salt stressed rapeseed plants, even in PGRs treated. Our results also indicate that the amount of reduction is associated with stress severity. The reductions in the shoot and root growth can be attributed to the effect of salinity on resource use efficiency, such as water and nutrition. Salt stress reduces the ability of plants to utilize such resources and results in a reduced growth (Gama et al., 2007). The plants may also show a strategy against salt stress that can significantly change the root hair characteristics and estimated root surface area (Arif et al., 2019).

Pirasteh-Anosheh et al., (2014) have shown that the lowest plant height and dry matter are observed in the plants under highest salinity level. Several investigators (Kumar, 1995; Maggio et al., 2004; Badruddin et al., 2005; Jamil et al., 2005) have reported negative effects such as decrease in plant height, size and yield as well as deterioration of seed quality, and the shoot/root ratio in Brassica crops.

PGRs priming, especially Brs and SA modulate some negative effect of salt stress, however, Brs are more effective in root growth. Among another PGRs, CCC considerably affects root growth. The plants use large number of physiological processes for an adaptation to salinity conditions, which operate individually or synergistically. One of the most important biochemical strategies is induction of antioxidant enzyme system. Increase in dry weight of salt stressed plants in response to SA and Brs is related to the induction of antioxidant response and protective role of membranes (Gunes et al., 2007) which increase the tolerance to damage. These findings are supported by other studies as well (Ghoulam et al., 2001). It has also been shown that growth of shoots and roots are markedly reduced by salinity. In the seedlings raised from seeds primed with 50 ppm SA an increase in fresh and dry weight of shoots and root fresh weight are observed. SA application increases the plant tolerance to stress conditions, effecting dry weight reduction as in some cases. According to Ashraf et al., (2010) there is a stimulatory effect on shoot growth via more assimilate allocations to the shoot. In general, the role of PGRs in improve photosynthetic capacity, enhance soluble carbohydrates accumulation, increase ATP content and maintain optimum Na+/K+ ratio under saline conditions can be considered as the possible underlying mechanisms that allow carryover of priming effect from seed to seedling stages (Ashraf et al., 2008).

Table 4. The results of stepwise regression for determining the most effective traits on growth (shoot dry weight).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Partial R²</th>
<th>Model R²</th>
<th>F Value</th>
<th>Pr&gt;F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root dry weight</td>
<td>0.9012</td>
<td>0.9012</td>
<td>964.04</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Na+ storage factor</td>
<td>0.0348</td>
<td>0.936</td>
<td>170.74</td>
<td>0.0004</td>
</tr>
<tr>
<td>H₂O₂</td>
<td>0.0204</td>
<td>0.9564</td>
<td>95.22</td>
<td>0.0010</td>
</tr>
<tr>
<td>Soot Na⁺/K⁺</td>
<td>0.0182</td>
<td>0.9746</td>
<td>72.21</td>
<td>0.0012</td>
</tr>
<tr>
<td>Catalase</td>
<td>0.0076</td>
<td>0.9822</td>
<td>12.21</td>
<td>0.0021</td>
</tr>
<tr>
<td>Root Na⁺/K⁺</td>
<td>0.0074</td>
<td>0.9896</td>
<td>9.17</td>
<td>0.0031</td>
</tr>
</tbody>
</table>

Fig. 6. The relationship between the antioxidant enzymes activity: SOD (A), POD (B) and CAT (C) with H₂O₂ (D) under varied salinity levels.
Activity of major antioxidant enzymes like CAT, POD, and SOD is increased under salt stress. Application of exogenous PGRs as priming agents enhance the plant salt stress tolerance. Brs and SA priming modulates the negative impact of salinity on key intracellular antioxidant enzymes like CAT, SOD, and POD. Brs is more efficient priming agent in the salt stress modulation, but its role is little known (Ashraf et al., 2010). Under different stress conditions, exogenous application of Brs modifies SOD, CAT, POD, glutathione peroxidase and ascorbate peroxidase (APX) in plants (Hasan et al., 2008; Ashraf et al., 2010). During this research salicylic acid has proved effective as priming agent. It might be due to its non-enzymatic antioxidant nature. SA, AS, glutathione and tocopherol antioxidants following priming with their solutions, increase tolerance of plants exposed to abiotic stress (Draganic & Lekic, 2012). An exogenous application of SA has been reported to increase the activity of APX and SOD with a parallel decrease in the activity of CAT in some plants (Krantve et al., 2008). CAT and SOD activities under stress conditions are induced in the Agropyron elongatum seeds primed with ABA and gibberellin (GA) when compared to the unprimed seeds (Eisvand et al., 2010).

Our findings have also revealed that enhanced antioxidant enzymes are found following H₂O₂ reduction in rapeseed shoot and roots under non-saline and saline stress conditions (Fig. 6). These findings suggest that the oxidative damage induced by salinity is protected by SA application by directly effecting SA regulation in the redox balance (Pirasteh-Anosheh & Emmar, 2018). Seed priming with lower SA concentrations decreases the ROS but enhances the activities of antioxidant enzymes, giving a protection against oxidative damage (Choudhury & Panda, 2004). The capacity of SA to prevent oxidative damage has been shown in several studies (Ananieva et al., 2004; Kusumi et al., 2006; El-Tayeb, 2005; Pakar et al., 2016). A high level of endogenous ascorbate is essential to maintain the antioxidant capacity which gives protection to the plants against oxidative stress (Zhou et al., 2009). Ascorbic acid as an antioxidant may scavenge ROS and H₂O₂, thus acting in the APX mediated scavenging of H₂O₂ (Saed-Moucheshi et al., 2014). An oxidative damage occurs due to salt stress via higher H₂O₂ content (Pirasteh-Anosheh & Emmar, 2018). Plants generally eliminate superoxide (O₂⁻) using SOD, which is very important in preventing the metal ion reduction and synthesis of hydroxyl radicals, APX located in the thylakoid membrane also eliminates the H₂O₂ levels (Ashraf & Harris, 2004).

Although correlation analysis (Table 3) shows all three antioxidant enzymes along with H₂O₂ are negatively correlated with the shoot dry weight, however, stepwise regression indicates that H₂O₂ and CAT are the most effective traits in the shoot dry weight. POD responds more to salinity and PGRs priming compared to other antioxidant enzymes. POD activity has highest reaction to salt stress (Pakar et al., 2016). Increases in POD, CAT, APX, and SOD activities following high salt stress have been reported as 383.2, 48.1, 4.5, and 292.0 percent respectively. Pirasteh-Anosheh & Emmar (2018) have shown that the activity of antioxidant enzymes is negatively related with H₂O₂ content in both saline and non-saline conditions.

Significant negative effects on the yield of canola and oil percentage in the seeds have been recorded under salt stress. The data published by Musyimi et al., (2007) and Hashem et al., (2019) show that the effects of salinity on plants are complicated, comprising osmotic stress, ion toxicity, and mineral deficiencies, ending up with a reduction in the crop yields. In particular, negative influence of salinity in yield depicts an accumulation of more sodium ions and restriction of the availability of potassium required in many metabolic processes leading to the possible way of reducing yield (Wani et al., 2013; Kanwal et al., 2019). An accumulation of Na⁺ ions in sensitive cultivars is quicker than tolerant ones which ultimately cause cell death leading to plant death in rapeseed. According to Ashraf & McNilley (2004) and Kanwal et al., (2019) the plants tolerate the salinity by osmotic adjustment and through maintenance of Na⁺/K⁺ ion ratio by regulating the uptake of K⁺ and restricting Na⁺ ions from entering the cell.

Salt stress depends on the severity increased by the Na⁺ and Na⁺/K⁺ ratio. A decrease in K⁺ concentration in both root and shoot has also been recorded. Lower plant Na⁺ concentration is associated with salt tolerance in plants. It is generally associated with low uptake and accumulation of Na⁺ but higher uptake and accumulation of K⁺, as shown in barley, wheat, and rice (Kusumi et al., 2006; Kausar et al., 2013; Pirasteh-Anosheh et al., 2017). As mentioned by Wyn Jones et al., (1979) as well Pirasteh-Anosheh et al., (2017), Na⁺/K⁺ more than 1.0 means the plants are subjected to the salt stress, so in current research, the barley seedling under non-saline conditions and under 6 ds m⁻¹ treated by SA, Brs or CCC had normal growth, while they had not normal growth under all treatments in 12 ds m⁻¹. Sodium effects negatively on crops because of its toxicity. Its concentrations in plant tissues can be used as an important indicator for salinity tolerance (Singh & Usha, 2003). Changes in ion accumulation due to salt stress is enhanced by Na⁺ and reduced K⁺, which are offset by PGRs priming. Among the PGRs, SA followed by Brs are the most effective in priming for modulating the negative effect of salt stress. SA priming proves more effective than other PGRs which act in the reduced Na⁺ and Na⁺/K⁺ ratio, but increase K⁺ in both shoot and root. Under SA-seed priming a reduction of Na⁺ absorption and toxicity is seen, explaining the mitigation effect of SA on growth of rapeseed plants. Antagonistic relations between Na⁺ and K⁺ due to SA treatment shows it is involved in the modification of K⁺/Na⁺ selectivity under salt stress. This is seen in its lowering membrane damage and higher water content under salinity stress. It also alters the ion equilibrium in plant tissues (Kausar et al., 2013). According to Ashraf et al., (2008) some important ions could be effectively used as important selection criteria for salt tolerance, and in most crops K⁺ concentration could serve as an index for this. However, in our studies we found that Na⁺ storage factor following Na⁺/K⁺ ratio is more important than their individual concentrations. This argument was supported by the correlation coefficient and stepwise regression, meaning ion distribution is more important than ion accumulation. An excess transport of toxic Na⁺ and Cl⁻ ions to the shoot is the major reason for...
Salt tolerance in *Brassica napus* induced by seed priming


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