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Assessing the Impact of Proline and Abscisic Acid on Salt Stress Grown *BRASSICA NAPUS* at Germination and Seedling Establishment

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Abstract

Abiotic stress such as Salinity causes serious challenge to plant growth and development, so it causes negative influence on plant's growth and yield. Different growth regulators and osmolytes kept attention as productive strategy and help the plant for enhancing stress tolerance and plant' growth and yield improved under stress condition. This project investigates the performance of Brassica napus at germination and seedling stage after application of abscisic acid and proline with combination of salt stress. Experiment was designed in completely randomized manner and petri plates were arranged in different sets with 10 seeds/each and applied with different concentrations of NaCl solutions. Proline (10 -20mM) and ABA (50-100mg/L) was pplied separately and in combination (proline 10 x ABA 50) and (proline 20 x 100) in these sets. After 8 days experiment was terminated and different parameters (germination percentage, seedling growth, shoot and root length, fresh and dry biomass, vigor index, Reletive water content, (RWC) Water content (WC), Root Shoot Ratio (RSR), Shoot Weight Ratio (SWR) and Root Weight Rratio (RWR). Salt stress exhibited significant decrease in shoot and root length, fresh and dry biomass, vigor index, RWC, different ratios (SWR, RWR) and different physiological indices while RSR exhibited increase in comparison to control in all sets. According to this research the application of proline and abscisic acid alone showed increased performance in all studied parameters. Interactions of proline and ABA did not support germination and seedling establishment process.

This study concluded that application of proline (10mM) and ABA (100mg/L) improved germination and seedling establishment process very well as compared to other doses in Brasicca napus.

Keywords: Germination, Brassica napus, Proline, Vigor index, Abscisic acid, RWC

Introduction

Salinity, one of the major factors among abiotic stress factors which negatively affect plant growth, development and yield/production (Nejat & Mantri, 2017). This factor affects 50% of crop-land

and 20% of cultivated-land (Lakhdar *et al*., 2009). Under this stress condition, plants modify their mechanisms to exhibit response to such hazardous conditions at cellular, biochemical, physiological and molecular level survive under such conditions (Nakashima *et al*., 2009). In *B. napus* this stress factor also disturbs rate of CO_2 assimilation, root and shoot length and plant biomass (**Ahmad *et al*., 2009**). High concentration of salt when expose to plant, it negatively germination process, plant growth, different physiological processes e.g. transpiration, enzymatic activity, metabolic activites, respiration, photosynthesis, membrane properties, cellular homeostasis and hormone regulation results in production of Reactive oxygen species while severe stress condition causes plant death **(Hasanuzzaman *et al*., 2012)**.

Proline (Pro) is an osmo-protectant and performs as an osmolyte, energy source, proteinogenic amino-acid, carbon, nitrogen and anti-oxidant source (Ali *et al*., 2011). This amino-acid accumulation considered as a criteria for stress tolerance for selection in different plant species **(Ahmad *et al*., 2009).** Improvement in plant adaptation to salinity stress conditions done through application of proline which induce gene expression that respond to salt stress **(Khedr *et al*., 2003).**

ABA is a plant growth regulator (PGR) and act as growth suppressive agent in plants. This inhibition effect of ABA on plant-growth causes through partial cell-extensibility with reserve of cell-division cycle (**Finkelstein *et al*., 2002).** Application of ABA through leaves might be helpful in reducing the accumulation of chloride ions in citrus leaf, which results in reduction of release of ethylene hormone abscission of leaves under salinity stress condition **(Gomez *et al*., 2002).** Induction of this hormone (ABA) can also be used as adaptive strategy to exhibit response to adapt any plant to salinity stress condition and plant get salt tolerance **Garcia *et al*, (2015).** This tolerance was achieved through activation of anti-oxidant system **(Pandolfi *et al*., 2016)** with uptake of different ions, their translocation and photosynthetic process adjustment **(Janda *et al*., 2016).**

Brassica belongs to family Brassicaceae, consisting of approximately 100 species, e.g. rapeseed (*Brassica napus* L.), cabbage (*Brassica oleracea* L.) and mustard (*Brassica juncea* L.) grown for seasonings of forage, oil and vegetables **(Ashraf and McNeilly, 2004).** Specie *Brassica napus* grown to produce vegetable-oil having high quality for consumption of human, high protein containing food to feed fish, poultry, swine and cattle **(Canola Council of Canada, 2014d**). This species grown and produced for oil and fodder crop and its cultivation in saline environment is on high demand due to its high salt tolerance. Roots of this plant specie used as anti-scurvy, anti-inflammatory and diuretic for bladder, while seeds used as kidney and hepatic colic **(Saeidnia and Gohari, 2012).** To control this stress condition, reclamation of saline/sodic soil need to be carried out, in addition, other strategies were also adapted e.g. use of osmolytes, over-irrigation, used of growth regulators, fertilizers application molecular breeding mechanism and use of other different compounds to cope up with this condition. This study was designed to evaluate the adaptive-role of proline and ABA to reduce the effect of saline environment in *Brassica napus* at germination and seedling growth phase.

Material and Methods

The *Brassica napus L.* seeds were collected from Agriculture Research Institute, Tarnab Peshawar. Experiment was designed in completely randomized manner with 63 plates divided into 7 sets. Each set was divided into three subsets on the basis of NaCl treatment (control, 50mM, 150mM). Three replicas were maintained for each treatment (control, 50mM, 150mM). One set was kept spray control while other six were moisture with different concentration of proline (10, 20mM), ABA (50,100mg/L) and interactions of (proline 10 x ABA 50) and (proline 20 x ABA100). *Brassica napus* seeds were sterilized with mercuric chloride (0.1%) solution for 1 minute and then rinse with water thrice. Petri-plates were lined with filter paper, autoclaved and 5 seeds were arranged per plate. Plates were moistened with 5ml water/different concentration of NaCl, proline and ABA solutions. All sets were kept in incubator for germination for 7 days. Shoot and root lengths, fresh weight, dry weight, relative water content (RWC), vigor index, shoot weight ratio (SWR), root weight ratio (RWR), root shoot ratio (RSR) were measured after seven days of seed germination.

Relative Water Content

Relative Water Content (RWC) was determined and calculated through a method described by Barrs and Weatherly (1962).

RWC (%) = (FW-DW) / (TW-DW)*100

Vigor Index

Seedling vigour index (VI) was calculated in experimental seedling through a method described by Abdul-Baki and Anderson (1973).

Vigor Index (VI) = (Mean root length + Mean shoot length) x germination percentage.

Different Ratios

Different ratios in experimental seedling were calculated through different formulas, described by Hunt (1982).

Root shoot ratio (RSR) = Root dry wt/ Shoot dry wt

Shoot weight ratio (RWR) = Shoot dry wt/ Total dry wt

Root weight ratio (RWR) = Root dry wt/ Total dry wt

Stress Tolerance Index

Stress Tolerance Index (STI) of different parameters of experimental seedling were calculated through different formulas described by **Ashraf & Harris (2004).**

Plant Height Stress Tolerance Index (PHSI) = (Plant height of stressed plants / plant height of control plants) x 100

Root Length Stress Tolerance Index (RLSI) = (Radicle length of stressed plants / radicle length of control plants) x 100

Shoot Fresh Weight Stress Tolerance Index (SFSI)= (Shoot fresh weight of stressed plants / shoot fresh weight of control plants) x 100

Root Fresh Weight Stress Tolerance Index (RFSI)= (Root fresh weight of stressed plants / root fresh weight of control plants) x 100

Shoot Dry Weight Stress Tolerance Index (SDSI)=(Shoot dry weight of stressed plants / shoot dry weight of control plants) x 100

Root Dry Weight Stress Tolerance Index (RDSI)=(Root dry weight of stressed plants / root dry weight of control plants) x 100

Reduction Percentages

Reduction percentages of different parameters of experimental seedlings were calculated through different formulas as described by **Raun et al., (2002).**

Shoot Length Reduction Percentage (SLRP %) = [1 - (*shoot length* $_{salt\ stress}$ / *shoot length* $_{control}$)] x 100

Root Length Reduction Percentage (DWPR %) = [1 - (*root length* $_{salt\ stress}$ / *root length* $_{control}$)] x 100

Fresh Weight Reduction Percentage (DWPR %) = [1 - (*fresh weight* $_{salt\ stress}$ / *fresh weigh* $_{control}$)] x 100

Dry weight Reduction Percentage (DWPR %) = [1 - (*dry weight* $_{salt\ stress}$ / *dry weight* $_{control}$)]

Statistical Analysis

Data analysis was carried out through SPSS (Version 21) statistical software, where one way analysis of variance (ANOVA) was applied while mean values were compared through Duncan's Multiple Range Test (DMRT) at 5% probability level.

Results and Discussion

Shoot length

Saline environment for plant, cause disturbances in progression in plant with negative effects on plant's growth and yield quantitatively and qualitatively. When salt stress applied on plants, it creates osmotic pressure which further reduce water absorption process through roots, which results in reduced division of cells and differentiation process hence cause decreased length of radicle and **plumule (Sattar *et al*., 2010)**. It was found in present investigation, application of salt stress caused significant reduction in seedlings shoot length as compare to control in different sets of experiment (Figure 1). In different experiments, **Shahbazi *et al*. (2011)** on Brassica napus and **Fateme *et al*., (2010)** on chickpea, when they grew thess plants under saline stress environment, they observed reduction in shoot length of plants. **Vital *et al*., (2008)** after their studies discussed, that distress plant growth occur under salinity stress condition which was caused due to altered ion inter-change procedure, reduction in important nutrients quantity in plant with accumulation of toxic ions.

However, application of proline @ 10 and 20 mM alleviate negative effects of salt stress and significant increase was observed in said parameter in saline as well as non-saline conditions, while comparing with set without proline application (Figxure 1). Different osmolytes accumulated in plant body under different environmental stress conditions. According to **Ashraf *et al*., (1998)** proline was considered as basic osmolyte play an important role in plant growth regulation under stress condition. They discussed different important ways for plant tolerance under stress and this osmolyte is one of them.

Presently we found that addition of ABA @ 50 and 100 mg/l cause improvement in seedling's shoot length under normal and stressed condition, while comparing with set grown without ABA application (Figure 1). In different experiments of **Gurmani *et al*., (2013)**, they demonstrated role of ABA in salt tolerance in rice under salt stress. They observed reduction in sodium and chloride levels under such conditions. This growth regulator also works in different reactions under salt stress by conversion of ion homeostasis in cytoplasm in studied rice lines. **Yang et al., (2012)** also studied role of ABA after application of this regulator on turfgrass grown under salt stress and they observed ABA involved as alleviative strategy through reduce physiological damage as well as membrane stability maintainance. When proline and ABA applied both (proline 10 x ABA 50 mg/l) exhibited promotion in this parameter, while same interaction at high doses (proline 20 x ABA 100 mg/L) showed reduction in this parameter at non-saline and salinity stress conditions.

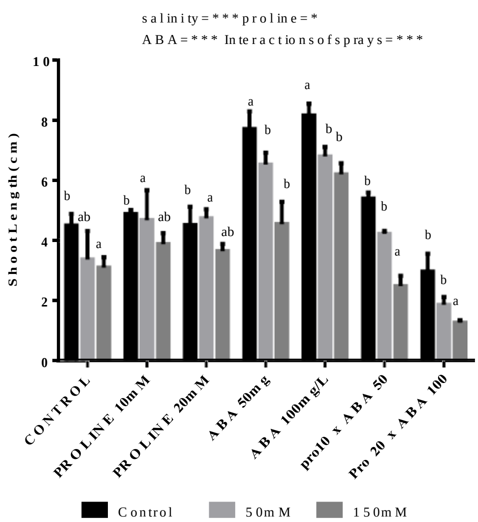

Figure 1.a.: Effect of salt (0, 50,150mM) and foliar application with different concentration of (proline), (ABA) and (interaction of proline and ABA) on shoot length of *Brassica napus*. (**=0.01, NS=Non significant, *=0.05)

Root Length

In plants, root considered as an important part for shoot growth under different environmental stresses. Growths occur as a result of vigorous translocation of different salts and ions from roots to different parts of shoot **(Asaadi, 2009)**. It was found in present investigation, application of salt stress caused significant reduction in seedlings root length as compare to control in different sets of experiment (Figure 1). **Ahmad *et al*., 2012)** peformed experiments on *B. juncea* under salt stressed conditions and observed reduction in plants shoot, root, biomass and disturbed distribution of carbon dioxide under stress environment. **Lo Gullo *et al*., (2007)** discussed that root growth and root surface area reduced under stress environment so as a result, reduction in water absorption process takes place.

An amino acid proline, considered as biochemical-indicator for salt-tolerance with different important functions in plants under environmental stress **(Szabados and Savouré. 2010)**. However, application of proline @ 10 and 20 mM alleviate negative effects of salt stress and significant increase was observed in said parameter in saline as well as non-saline conditions, while comparing with set without proline application (Figure 1). Enhancement in root growth after application of proline on plants under stress conditions due to role of this amino acid as osmo-protectant in stability of membrane permeability **(Bandurska, 2000),** provide turgid condition to leaves and resume growth **(Huang *et al*., 2000),** it's action as ROS-scavenger and improve salt tolerance in wheat **(Talat *et al*., 2013)** and sunflower **(Khan *et al*., 2014).**

Under stress conditions ABA had a very important and crucial role as a phytohormone. This hormone is regulated under drought stress condition and concentrated around the plant root **(Popova, 1995).** Presently we found that addition of ABA @ 50 and 100 mg/l cause improvement in seedling's root length under normal and stressed condition, while comparing with set grown without ABA application (Figure 1). **Jaschke *et al*., 1997)** discussed about this hormone after their studies that under saline environment application of this hormone cause improvement in plant growth, photosynthesis and translocation of different assimilates. Further, **Gurmani, (2007)** discussed progressive association between exogenous ABA application and plant stress tolerance. This tolerance in the presence of ABA created as a result of accumulation of calcium and potassium ions and other compatible solutes (e.g. proline and sugars) in vacuoles of root cells and counteract with chlorides and sodium ions.When proline and ABA applied in interaction (proline 10 x ABA 50 mg/l) and (proline 20 x ABA 100 mg/L) showed reduction in this parameter at non-saline and salinity stress conditions.

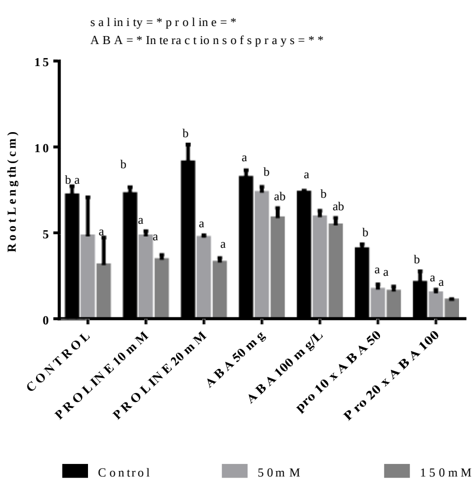

Figure 1.b: Effect of salt (0, 50, 150mM) and foliar application with different concentration of (proline), (ABA) and (interaction of proline and ABA) on root length (germination) of *Brassica napus*. (**=0.01, *=0.05)

Total Fresh Biomass

Under environemntal stresses especially salt stress reduces the growth and yield of different crops **(Krishnamurthy et al., 2007)**. This reduction in plant growth might be a result of low absorption and translocation rate of different ions e.g. nitrates in plants which in-turn lower the synthesis of nitrogen compounds, on the other hand, sodium ions level increased in high salt level **(Hamid et al., 2008)**. It was found in present investigation, application of salt stress caused significant reduction in seedlings fresh biomass as compare to control in different sets of experiment (Figure 2). **Behzadifar *et al*., (2013)** studied *Catharanthus roseus* under salt stress condition while **Mozafariyan *et al*., (2013)** worked on tomato plant under salt stress condition, they all observed reduction in fresh biomass of plant under stressed environment. **Yunwei *et al*., (2007)** explained that reduction in fresh biomass under salinity stress occur due to changes in biochemical and physiological activities which in-turn reduce leaf number and leaf area of plant.

An amino acid proline, under stress conditions in plants involved in regulation of osmotic pressure ad provide protection to enzymes from denaturation under such conditions. It also provides stability to molecular assembly and considered as well-known source of carbon and nitrogen, while worked as hydroxyl radical scavenger in different aquatic-macrophytes **(Bagdi & Shaw, 2013**). However, application of proline @ 10 and 20 mM alleviate negative effects of salt stress and significant increase was observed in said parameter in saline as well as non-saline conditions, while comparing with set without proline application (Figure 2). In a study **of Abideen *et al*., (2014)** on *Phragmites karka*

(Retz.) while exposing this plant to stress environment (100 mmol L-1 NaCl), promotion in plant biomass observed due to adjustment of leaf osmotic pressure with retain balance between different solutes e.g. potassium and sodium. In same study proline and sugar content remain unchanged in plants.

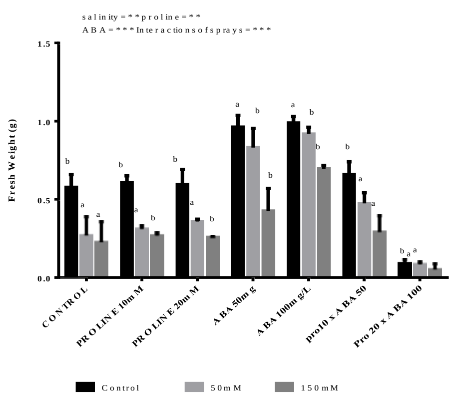

Figure 2: Effect of salt (0, 50, 150mM) and foliar application with different concentration of (proline), (ABA) and (interaction of proline and ABA) on fresh biomass (germination) of *Brassica napus*. (**=0.01, ***=0.001)

ABA, is one of the hormone in traditional five hormones category which is considered as most important and involved in growth regulation of plants and provide plasticity to plants in different developmental changes in plants **(Seung *et al.*, 2012).** Presently we found that addition of ABA @ 50 and 100 mg/l cause improvement in seedling's fresh biomass length under normal and stressed condition, while comparing with set grown without ABA application (Figure 2). ABA considered as stress hormone and provide stability to plant under such harsh conditions through its involvement in different processes **(Atkinson and Urwin, 2012)**. When proline and ABA applied in interaction (proline 10 x ABA 50 mg/l) exhibited promotion in this parameter, while same interaction at high doses (proline 20 x ABA 100 mg/L) showed reduction in this parameter at non-saline and salinity stress conditions.

Dry Weight

It is well documented, when plants grown under salt stress reduction in fresh and dry biomass of seedling was observed which is due to reduction of water absorption through seedlings (**Ashraf and Bhatti 2000).** It was found in present investigation, application of salt stress caused significant reduction in seedling's dry biomass as compare to control in different sets of experiment (Figure 3). According to **Mondal *et al.*, (2013)** reduction in dry biomass of any plant under stress environment caused as a result of reduced photosynthetic efficiency with low stomatal behavior and nutrient uptake of roots from soil. In another study, **Sharifi *et al.*, (2007)** discussed about the reasons of reduced dry biomass production under salinity, it may be caused as a result of low leaf number as well as reduced leaf area under such conditions. Plant height, root length, shoots and root dry matter of rice plant were affected significantly by salinity. Salinity decreased fresh weight and dry weight of seedlings **(Mansour and Ali, 2017).**

Under salinity stress environment an amino acid proline used as indicator in plants grown under such environment. However, application of proline @ 10 and 20 mM alleviate negative effects of salt stress and significant increase was observed in said parameter in saline as well as non-saline conditions, while comparing with set without proline application (Figure 3). **Maliro *et al.*, (2008)** observed reduction in total dry biomass in different crops (e.g. maize, wheat, rice, cowpea, tomato, seashore paspalum and chickpea) while after application of proline seedlings exbhited increased dry biomass. So, this amino acid helps the seedling to tolerate such stress through amelioration I different physiological and biochemical processes.

Presently we found that addition of ABA @ 50 and 100 mg/l cause improvement in seedling's dry biomass under normal and stressed condition, while comparing with set grown without ABA application (Figure 3). **Yoshida *et al*., (2003)** worked on *C. reinhardtii* under salinity stress and further plants were applied with ABA, this growth regulator provide strength to plant for tolerance through escalation in growth cells with improvement in different metabolic processes. When proline and ABA applied in interaction (proline 10 x ABA 50 mg/l) and (proline 20 x ABA 100 mg/L) showed reduction in this parameter at non-saline and salinity stress conditions

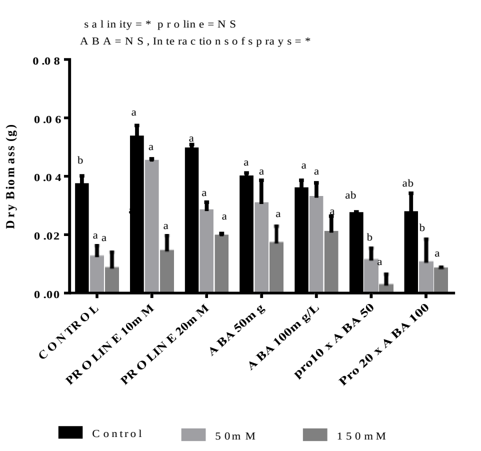

Figure 3: Effect of salt (0, 50, 150mM) and foliar application with different concentration of (proline), (ABA) and (interaction of proline and ABA) on dry biomass (germination) of *Brassica napus*. (**=0.01, NS=Non significant)

Vigor Index

Under stress factors especially drought and salt stress, vigor index (VI) of seedling considered and used as tolerance index to measure the effect of applied stress on growth of seedling **(Oliveira & Steiner 2017).** It was found in present investigation, application of salt stress caused significant reduction in seedling's vigor index as compare to control in different sets of experiment (Figure 4). In the study of Segatoleslami, (2010) on three medicinal species savory (*Satureja hortensis* L.), Chicory (*Cynaras colymus* L.) and Artichoke (*Cichorium intybus* L.) and Safarnejad and Hamidi, (2008) on *Foeniculum vulgare* observed reduction in vigor index of seedling when grown under salinity stress. In another study, **Ashraf *et al*., (2008)** reported reduction in vigor index of studied seedlings under salt stress conditions.

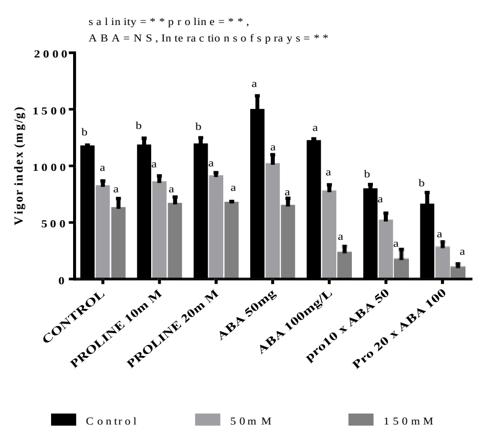

Figure 4: Effect of salt (0, 50, 150mM) and foliar application with different concentration of (proline), (ABA) and (interaction of proline and ABA) on vigor index (germination) of *Brassica napus*. (**=0.01)

However, application of proline @ 10 and 20 mM alleviate negative effects of salt stress and significant increase was observed in said parameter in saline as well as non-saline conditions, while comparing with set without proline application (Figure 4). According to **Ashraf and Foolad (2007),** application of proline on plants under normal and saline environment, this amino acid alleviates the harmful effect of stress through liberation of ions, preserve osmotic balance and provide defense to plant cell. Application of this amino acid enhances the values of index under stress condition and they observe this phenomenon in rice seedlings when grown under salinity and additionally provided by proline. According to **Guan *et al*., 2014)** when seeds treated with proline and other chemical like ascorbic acid, they accelerate metabolic rate of treated seed and provide strength to seedling which reflects as increase in vigor index value of seedling under stress condition in sorghum seeds. Presently we found that addition of ABA @ 50 and 100 mg/l cause improvement in seedling's vigor index under normal and stressed condition, while comparing with set grown without ABA application. When proline and ABA applied in interaction (proline 10 x ABA 50 mg/l) and (proline 20 x ABA 100 mg/L) showed reduction in this parameter at non-saline and salinity stress conditions.

Relative Water Content

Relative water content (RWC) considered as an important criterion to measure the effect of different environmental stresses especially salinity and drought. This is a reliable parameter to measure water status of plant, while leaf relative water content considered as a balanced between water status of plant and rate of transpiration with direct relation of cell volume of same plant **(Schonfeld *et al*., 1988).** It was found in present investigation, application of salt stress caused significant reduction in seedlings relative water content as compare to control in different sets of experiment (Figure 5). Ashraf, 2010) discussed after his experiments that RWC in different crop's leaves act as potential-index of plant water status which involved in different metabolic activities of cells. Reduction in this parameter results in damage in cell turgor, hence cause negative effect cell growth and crop yield. Reduction in this parameter under any stressed condition was observed due to low water absorption through root system due to root injury coupled with low substrate water potential (**Garg and Singla, 2009).**

However, application of proline @ 10 and 20 mM alleviate negative effects of salt stress and significant increase was observed in said parameter in saline as well as non-saline conditions, while comparing with set without proline application (Figure 5). The defensive mechanism of exogenous proline in plants under NaCl stress has been gradually reported in literature. **Okuma *et al*., (2004)** worked on durum wheat under salt stress conditions and further applied with 20 mM proline, they observed reduction in RWC under salt stress condition while application of proline exhibit improvement in this parameter under same stress environment. Presently we found that addition of ABA @ 50 and 100 mg/l cause improvement in seedling's relative water content under normal and stressed condition, while comparing with set grown without ABA application. **Kumar *et al*., 2008)** studied *Ilex paraguariensis*, chickpea and *Polypodium vulgarei* under salt stress and then applied with ABA, they observed improvement in reserve water status of cell after application of ABA under normal and stressed conditions. When proline and ABA applied in interaction (proline 10 x ABA 50 mg/l) and (proline 20 x ABA 100 mg/L) showed reduction in this parameter at non-saline and salinity stress conditions.

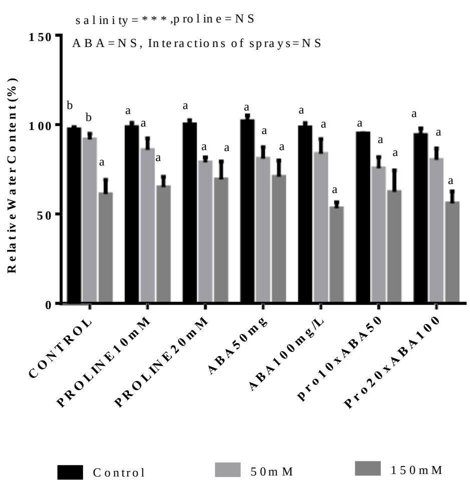

Figure 5: Effect of salt (0, 50, 150mM) and foliar application with different concentration of (proline), (ABA) and (interaction of proline and ABA) on RWC (germination) of *Brassica napus*. (***=0.001, NS=Non significant)

Ratios (RSR, SWR, RWR)

Ratio between the quantity of tissue of plant which function as growth to tissues having supportive functions. Increasing the proportion of plant shoot helps in capturing extra light energy, whereas root help in uptake of more nutrients efficiently from soil. It was found in present investigation, application of salt stress caused promotion in RSR and reduction in SWR and RWR as compare to control in different sets of experiment (Figure 6). When plant grow, relative length of shoot and root of seedling closely related to its total biomass production, while under stress condition this thing is considered as plant tolerance against stress in which it is present and it is directly related to high absorption rate of potassium ion with low concentration of sodium in shoots which positively correlated with K /Na ratio and Ca / Na ratio **(Krishnamurthy et al., 2007).** In study of **Moud and Maghsoudi, (2008)** on different crops under salt stress condition, they observed high values of root to shoot ratio with inhibitory effects of applied stress. This reflects higher ratio of absorption of water through roots and area of transpiration to the characteristics of the plant and useful for plant under dry-land environment when plant grow for long time during other stages of growth.

According to **Veeranagamallaiah *et al.*, (2007)** under salt stress condition, proline considered as an initial resistance response which helps plant cells to conserve their osmotic pressure and described in salt-tolerant and salt-sensitive varieties of different crops. However, application of proline @ 10 and 20 mM alleviate negative effects of salt stress and significant increase was observed in said parameter in saline as well as non-saline conditions, while comparing with set without proline application (Figure 6). Under salt stress conditions, growth regulator ABA considered as stress hormone and plays important role in plant defense thoroughly with variation of plants **(Gurmani *et al.*, 2007).** Presently we found that addition of ABA @ 50 and 100 mg/l cause improvement in RSR and reduction occur in SWR and RWR under normal and stressed condition, while comparing with set grown without ABA application. The increase of ABA root concentration and root/shoot ratio measured under salt conditions. After experiments of Ghanem *et al*., (2011b), they discussed that increase in root to shoot ratio in different species considered as physiological response that modulate accumulation of different ions under any applied stress specially salinity stress. When proline and ABA applied in interaction (proline 10 x ABA 50 mg/l) and (proline 20 x ABA 100 mg/L) showed reduction all studied ratios at non-saline and salinity stress conditions.

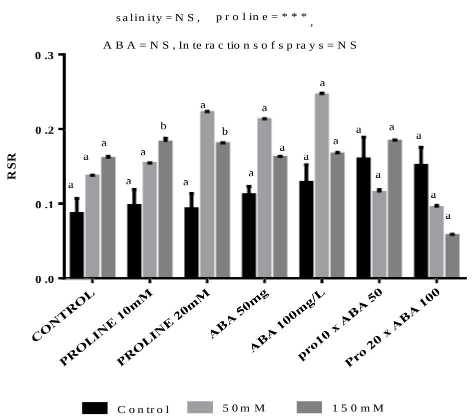

Figure 7: Effect of salt (0, 50,150mM) and foliar application with different concentration of (proline), (ABA) and (interaction of proline and ABA) on RSR (germination) of *Brassica napus*. (***=0.001)

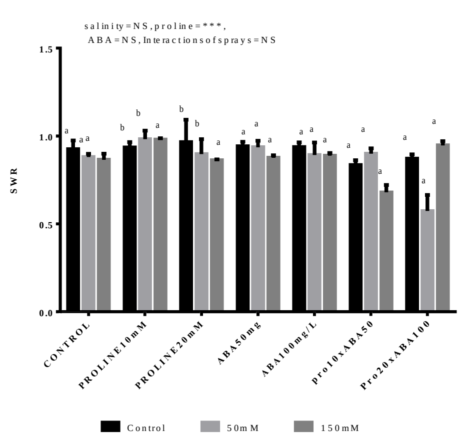

Figure 8: Effect of salt (0, 50, 150mM) and foliar application with different concentration of (proline), (ABA) and (interaction of proline and ABA) on SWR (germination) of *Brassica napus*. (***=0.001)

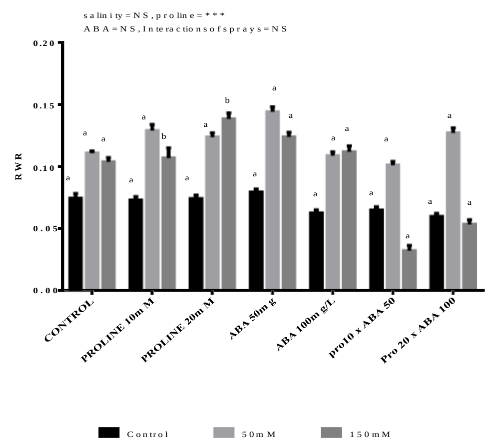

Figure 9: Effect of salt (0, 50, 150mM) and foliar application with different concentration of (proline), (ABA) and (interaction of proline and ABA) on RWR (germination) of *Brassica napus*. (***=0.001)

Salt Tolerance Index

Different physiological parameters with their salt tolerance indices considered as important tool to evaluate applied stress and other factors at early seedling stage because it is a simple evaluating criteria **(Flowers and Yeo, 1995; Shahzad et al., 2012; Hussain et al., 2013)**. It is well known and common phenomenon that salt application on plants cause reduction in plumule and radicle length, biomass and plant height. When Brassica was grown under salt stress, and applied with proline and ABA, response was evaluated after estimation of different physiological parameters with the help of different growth parameters. It was found in present investigation, application of salt stress caused reduction in different physiological indices (i.e. SLSI, RLSI, SFWSI, RFWTI, FWSI, SDWSI, RDWTI, DWSI) as compare to control in different sets of experiment (Table 1). However, application of proline @ 10 and 20 mM alleviate negative effects of salt stress and significant increase was observed in said parameter in saline as well as non-saline conditions, while comparing with set without proline application. Presently we found that addition of ABA @ 50 and 100 mg/l cause improvement in all studied physiological indices under normal and stressed condition, while comparing with set grown without ABA application (Table 1).

Table 1. Physiological stress tolerance indices of *Brassica napus* treated with ABA, proline, their interactions and different salt treatments.

Treatments/	**PHSI**	**RLSI**	**SFSI**	**RFSI**	**FSTI**	**SDSI**	**RDSI**	**DSTI**
Saliniy Levels								
50 mM NaCl								
Control	79.9 ± 3.1	64.3 ± 6.9	46.8 ± 2.8	55.5 ± 2.2	42.4 ± 1.8	24.3 ± 1.8	35.4 ± 3.1	23.4 ± 6.4
ABA (50ppm)	89.5 ± 10.2	84.6 ± 12.6	41.6 ± 2.4	125.5 ± 7.3	53.4 ± 8.8	16.6 ± 4	225 ± 87.3	19.4 ± 4.2
ABA (100ppm)	99.3 ± 5.5	55.1 ± 6.2	68.6 ± 5.1	511 ± 43.9	73.6 ± 8.6	57.8 ± 2.5	496 ± 84.4	77.5 ± 6.5
Proline (10mM)	84.9± 3.3	89.6 ± 4.3	80.4 ± 6.2	128 ± 20.4	85.4 ± 6.9	73.1 ± 2.6	281 ± 88.5	83.1 ± 12.6
Proline (20mM)	83.9 ± 7.3	80.6 ± 6.2	93.2 ± 1.9	91.3 ± 8.3	92.8 ± 2.4	79.3 ± 4.2	230.7 ± 80	91.5 ± 11.9
ABA (50ppm) x Proline (10 mM)	78.4 ± 1.6	41.3 ± 4.5	70.2 ± 3.3	79.6 ± 7.9	71.5 ± 3.8	95.3 ± 14.2	73.6 ± 4.4	89.9 ± 8.1
ABA (100ppm) x Proline (20 mM)	64.4 ± 4.2	77.7 ± 11.7	77.1 ± 6.9	112 ± 61.8	107 ± 19.5	85.1 ± 4.8	138.5 ± 8.9	117 ± 17.4
150 mM NaCl								
Control	71.2 ± 14.1	41.3 ± 8.5	38.8 ± 9.6	29.9 ± 1.7	33.8 ± 7.4	13.2 ± 8.4	25.7 ± 2.8	13.9 ± 8.7
ABA (50ppm)	64.6 ± 9.6	54.2 ± 8.9	47.4 ± 2.4	25.1 ± 5.1	35.3 ± 1.9	72.5 ± 7.8	270 ± 64.9	71.8 ± 4.1
ABA (100ppm)	76.2 ± 3.1	71.6 ± 9.1	39.4 ± 1.7	456 ± 67.6	45.7 ± 3.1	39.1 ± 3.6	332 ± 22.5	48.9 ± 12.3
Proline (10mM)	60.4 ± 13.3	72.6 ± 11.7	31.9 ± 3.5	138 ± 21.5	43.5 ± 3.3	41.6 ± 9.9	94.1 ± 7.1	46.8 ± 7.6
Proline (20mM)	76.7 ± 7.8	74.2 ± 6.9	66.6 ± 1.2	96.6 ± 10.3	70.3 ± 1.4	49.8 ± 1.2	163.8 ± 8.8	56.7 ± 1.1
ABA (50ppm) x Proline (10 mM)	45.9 ± 5.6	40.3 ± 10.1	50.5 ± 2.5	38.4 ± 2.3	48.4 ± 9.2	79.7 ± 5	189 ± 22.4	96.1 ± 11.8
ABA (100ppm) x Proline (20 mM)	44.8 ± 5.5	57.7 ± 11.2	65.7 ± 4.7	30.5 ± 2.1	60.2 ± 8.8	40.9 ± 3.4	38.7 ± 2.6	37.7 ± 1.4

In columns values are treatment means with ± SD.

PHSI = Plant Height Stress Tolerance Index, RLSI = Root Length Stress Tolerance Index, SFSI = Shoot Fresh Weight Stress Tolerance Index, RFSI = Root Fresh Weight Stress Tolerance Index, FSTI = Total Fresh Weight Stress Tolerance Index, SDSI = Shoot Dry Weight Stress Tolerance Index and RDSI = Root Dry Weight Stress Tolerance Index, DSTI = Total Dry Weight Stress Tolerance Index.

These findings are in agreement with **Islam and Karim (2010)** worked on different rice genotypes under salt stress condition, they observed reduction in germination percentage (GSI) under stress conditions. Ashraf, 2006) observed improved germination stress tolerance index (GSTI), plant height stress tolerance index (PHSTI) and reduced relative saturation deficit (RSD) in salt-tolerant crops, so cause improved crop growth and productivity in different salt affected areas. **Odjegba and Chukwunwike, (2012)** discussed that in plants, salt-resistance considered as quality of plant that endure the adversative properties in root-zone. Under stress conditions, Ahmed et al., 2014) used different screening tools e.g. specific leaf area, RWC (relative water content) and osmotic adjustment in cell for stress-resistant in different genotypes of bread wheat. **Bafeel, (2014)** worked on different sorghum varieties under salt stress, he observed significant negative effect on shoot growth and root growth was dissimilar between different varieties under normal and stress conditions. It was observed that C_4 varieties were salt sensitive than C_3 varieties that were salt-tolerant.

Conclusion

It is quite important aspect to develop comprehensive knowledge on plant response to stress environment by different approaches used to enhance plant growth and development. Present investigation suggests that salt stress had negative impact on studied parameters of Brassica seedlings. Further, application of proline and ABA (alone) with different doses cause improvement in studied parameters and improves germination process and seedling growth. Moreover, application of proline and ABA in different interactions failed to develop tolerance in brassica seedling.

References

Abdul-Baki, A.A. and J.D. Anderson. (1973). Vigor determination in soybean seed by multiple criteria 1. *Crop science*, *13*(6), pp.630-633.

Abideen, Z., H.W. Koyro, B. Huchzermeyer, M.Z. Ahmed, B. Gul, and M.A. Khan, 2014. Moderate salinity stimulates growth and photosynthesis of Phragmites karka by water relations and tissue specific ion regulation. *Environmental and Experimental Botany*, *105*, pp.70-76.

Ahmad, M.S.A., M.Hussain, M., Ashraf, Ahmad, R. and M.Y. Ashraf, 2009. Effect of nickel on seed germinability of some elite sunflower (Helianthus annuus L.) cultivars. *Pak J Bot*, *41*, pp.1871-1882.

Ahmed AAS., Uptmoor R, M.A El-Morshidy, K.A Kheiralla, M.A Ali N.E.M. Mohamed (2014). Some Physiological Parameters as Screening Tools for Drought Tolerance in Bread Wheat Lines (Triticum aestivumL.). World J. Agric. Res. 2(3): 109-114.

Ali, Q. and Ashraf, M., 2011. Exogenously applied glycinebetaine enhances seed and seed oil quality of maize (Zea mays L.) under water deficit conditions. Environmental and experimental botany, 71(2), pp.249-259.

Asaadi, A., 2009. Investigation of salinity stress on seed germination of Trigonella foenum-graecum. *Research Journal of Biological Sciences*, *4*(11), pp.1152-1155.

Ashraf MY, K Akhter, F.,Hussain, J. Iqbal (2006). Screening of Different Accessions of Three Potential Grass

Species from Cholistan Desert for Salt Tolerance. Pak.J.Bot.38(5): 589-1597.

Ashraf, M. and T., McNeilly, (2004). Salinity tolerance in Brassica oilseeds. *Critical Reviews in Plant Sciences*, *23*(2), pp.157-174.

Ashraf, M. and P.J.C. Harris (2004). Potential biochemical indicators of salinity tolerance in plants. Plant Sci., 166: 3-16.

Ashraf, M., (2010). Inducing drought tolerance in plants: recent advances. *Biotechnology advances*, *28*(1), pp.169-183.

Ashraf, M., H.R Athar, P.J.C. Harris, and T.R. Kwon, (2008). Some prospective strategies for improving crop salt tolerance. *Advances in agronomy*, *97*, pp.45-110.

Ashraf, M.F.M.R. and Foolad, M., 2007. Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environmental and experimental botany*, *59*(2), pp.206-216.

Ashraf, M.Y. and Bhatti, A.S., 2000. Effect of salinity on growth and chlorophyll content in rice. *Pakistan Journal of Scientific and Industrial Research*, *43*(2), pp.130-131.

Ashraf, M.Y., Y. Ali, and T.M. Qureshi. (1998). Effect of salinity on photosynthetic efficiency and yield of rice genotypes. *Pakistan Journal of Biological Sciences (Pakistan)*.

Atkinson, N.J. and P.E., Urwin, (2012). The interaction of plant biotic and abiotic stresses: from genes to the field. *Journal of experimental botany*, *63*(10), pp.3523-3543.

Bafeel S.O (2014). Physiological parameters of salt tolerance during germination and seedling growth of Sorghum bicolor cultivars of the same subtropical origin. Saudi J. of Biol. Scie. 21 (4): 300–304.

Bagdi, D.L. and Shaw, B.P., 2013. Analysis of proline metabolic enzymes in Oryza sativa under NaCl stress. *Journal of environmental biology*, *34*(4), p.677.

Bandurska, H., (2000). Does proline accumulated in leaves of water deficit stressed barley plants confine cell membrane injury? I. Free proline accumulation and membrane injury index in drought and osmotically stressed plants. *Acta Physiologiae Plantarum*, *22*(4), pp.409-415.

Barrs, H. D. and P.E. Weatherley. (1962). A re-examination of the relative turgidity technique for estimating water deficits in leaves. Aust J Biol Sci., 15: 413-428.

Behzadifar, M., Chehrazi, M. and Aboutalebi, A., (2013). Effect of salt stress by using unconventional water on some morphological characters and ajmalicine alkaloid amount in the roots of Catharanthus roseus Cvs. Rosea and Alba. *Annals of Biological Research*, *4*(8), pp.229-231.

Canola Council of Canada. (2014d).What is Canola? [Online] Available: http://www.canola council.org/oil-and-meal/what-is-canola/ [2014].

Fateme, K., A. Rayhaneh, M. Akbar and E. Giti, (2010). Effect of hydro and osmopriming in two commercial chickpea (*Cicer arietinum* L.) cultivars on germination, growth parameters and nodules number in salt stress condition. Iran. J. Plant Biol., 2: 71-86.

Finkelstein, R.R., S.S Gampala,. and C.D. Rock, (2002). Abscisic acid signaling in seeds and seedlings. *The Plant Cell*, *14*(suppl 1), pp.S15-S45.

Flowers, T.J. and A.R. Yeo, (1995). Breeding for salinity resistance in crop plants: where next? Aust. J. Plant Physiol., 22: 875–884

García, M.J., F.J. Romera, C.Lucena, E. Alcántara, and R. Pérez-Vicente. (2015). Ethylene and the regulation of physiological and morphological responses to nutrient deficiencies. *Plant physiology*, *169*(1), pp.51-60.

Garg, N. and R., Singla (2009). Variability in the response of chickpea cultivars to short-term salinity, in terms of water retention capacity, membrane permeability, and osmo-protection. *Turkish Journal of Agriculture and Forestry*, *33*(1), pp.57-63.

Ghanem, M.E., I. Hichri, A.C Smigocki, A. Albacete, M.L. Fauconnier, Diatloff, E., C. Martinez-Andujar, S.Lutts, I.C.Dodd, and F. Pérez-Alfocea, , (2011b). Root-targeted biotechnology to mediate hormonal signalling and improve crop stress tolerance. *Plant cell reports*, *30*(5), pp.807-823.

Gomez-Cadenas, A., V. Arbona, , J. Jacas, E. Primo-Millo, and M. Talon, , 2002. Abscisic acid reduces leaf abscission and increases salt tolerance in citrus plants. *Journal of Plant Growth Regulation*, *21*(3), pp.234-240.

Guan, B., D. Cao, and J.B. Yu, 2014. Eco-physiological responses of seed germination of sweet sorghum to seed priming. *Chinese Journal of Ecology 33*(4), pp.982-988.

Gurmani, A.R., A. Bano and M. Salim, (2007). Effect of abscisic acid and benzyladenine on growth and ion accumulation of wheat under salinity stress. Pakistan Journal of Botany, 39(1), p.141.

Gurmani, A. R., A. Bano, N. Ullah, H. Khan, M. Jahangir, and T.J. Flowers, (2013). Exogenous abscisic acid (ABA) and silicon (Si) promote salinity tolerance by reducing sodium (Na+) transport and bypass flow in rice ('Oryza sativa'indica). *Australian Journal of Crop Science*, *7*(9), p.1219.

Hamid, M., M.Y., Ashraf, K.U. Rehman and M. Arshad. (2008). Influence of salicylic acid seed priming on growth and some biochemical attributes on wheat growth under saline conditions. Pak. J. Bot., 40(1): 361-367.

Hasanuzzaman, M., M.A. Hossain, J.A.T. da Silva, and M. Fujita, (2012). Plant response and tolerance to abiotic oxidative stress: antioxidant defense is a key factor. In *Crop stress and its management: Perspectives and strategies* (pp. 261-315). Springer, Dordrecht.

Huang, J., Hirji, R., Adam, L., Rozwadowski, K.L., Hammerlindl, J.K., Keller, W.A. and Selvaraj, G., 2000. Genetic engineering of glycinebetaine production toward enhancing stress tolerance in plants: metabolic limitations. *Plant Physiology*, *122*(3), pp.747-756.

Hunt, R. (1982), Plant growth curves: The functional approach to plant growth analysis. Edward Arnold Publ., pp.14-46.

Hussain, M., H.W. Park, M. Farooq, K. Jabran and D.J. Lee, (2013). Morphological and physiological basis of salt resistance in different rice genotypes. Intl. J. Agric. Biol., 15: 113–118.

Islam, M.M. and M.A. Karim, (2010). Evaluation of rice (Oryza sativa L.) genotypes at germination and early seedling stage for their tolerance to salinity. Agriculturists, 8: 57–65

Janda, T., Darko, É., Shehata, S., Kovács, V., Pál, M. and Szalai, G., 2016. Salt acclimation processes in wheat. *Plant*

Physiology and Biochemistry, *101*, pp.68-75.

Jaschke, W.D., Peuke, A.D., Pate, J.S. and Hartung, W., 1997. Transport, synthesis and catabolism of abscisic acid (ABA) in intact plants of castor bean (Ricinus communis L.) under phosphate deficiency and moderate salinity. *Journal of Experimental Botany*, *48*(9), pp.1737-1747.

Khan, A., Iqbal, I.R.A.M., Ahmad, I.F.T.I.K.H.A.R., Nawaz, H.U.M.E.R.A. and Nawaz, M.A.R.I.A., 2014. Role of proline to induce salinity tolerance in sunflower (Helianthus annus L.). *Sci Technol Dev*, *33*, pp.88-93.

Khedr, A.H.A., M.A. Abbas, A.A.A Wahid, W.P. Quick, and G.M. Abogadallah, (2003). Proline induces the expression of salt‐stress‐responsive proteins and may improve the adaptation of Pancratium maritimum L. to salt‐stress. *Journal of Experimental Botany*, *54*(392), pp.2553-2562.

Krishnamurthy, L., R. Serraj, C.T. Hash, A.J. Dakheel and B.V.S. Reddy. (2007). Screening sorghum genotypes for salinity tolerant biomass production. Euphytica, 156: 15-24.

Kumar, S., G. Kaur, and H. Nayyar, (2008). Exogenous application of abscisic acid improves cold tolerance in chickpea (Cicer arietinum L.). *Journal of agronomy and crop science*, *194*(6), pp.449-456.

Lakhdar, A., M. Rabhi, T. Ghnaya, F. Montemurro, N. Jedidi, & Abdelly, C. (2009). Effectiveness of compost use in salt-affected soil. Journal of Hazardous Materials, 171(1), 29-37.

Lo Gullo, M.A., P. Trifilò, and F. Raimondo. (2007). Hydraulic characteristics and water relations in pigment-less mutant shoots of an orange tree. *Tree physiology*, *27*(2), pp.209-217.

Maliro, M.F., D. McNeil, B. Redden, J. F. Kollmorgen, and C. Pittock, (2008). Sampling strategies and screening of chickpea (Cicer arietinum L.) germplasm for salt tolerance. *Genetic Resources and Crop Evolution*, *55*(1), pp.53-63.

Mansour, M.M.F. and Ali, E.F., 2017. Evaluation of proline functions in saline conditions. *Phytochemistry*, *140*, pp.52-68.

Mondal, M., Puteh, A., Malek, M. and Rafii, M.Y., 2013. Salinity induced morphophysiological characters and yield attributes in rice genotypes. *Journal of Food, Agriculture and Environment*, *11*(2), pp.610-614.

Moud, A.M. and K. Maghsoudi, (2008). Salt stress effects on respiration and growth of germinated seeds of different wheat (Triticum aestivum L.) cultivars. *World J. Agric. Sci*, *4*(3), pp.351-358.

Mozafariyan, M., K. Saghafi, A.E. Bayat, and S. Bakhtiari, (2013). The effects of different sodium Chloride concentrations on the growth and photosynthesis parameters of tomato (Lycopersicum esculentum cv. Foria). *International Journal of Agriculture and Crop Sciences*, *6*(4), p.203.

Nakashima, K., Ito, Y. and Yamaguchi-Shinozaki, K., 2009. Transcriptional regulatory networks in response to abiotic stresses in Arabidopsis and grasses. *Plant physiology*, *149*(1), pp.88-95.

Nejat, N., & N. Mantri, (2017). Plant immune system: Crosstalk between responses to biotic and abiotic stresses the missing link in understanding plant defence. Current Issue in Molecular Biology, 23, 1-16.

Odjegba J.V., C.I Chukwunwike. (2012). Physiological responses of Amaranthus hybrids L. under salinity stress. Indian J. Innov. Deve. 1(10): 742-748.

Okuma, E., Y. Murakami, Y. Shimoishi, M. Tada, and Y. Murata, (2004). Effects of exogenous application of proline and betaine on the growth of tobacco cultured cells under saline conditions. Soil Science and Plant Nutrition, 50(8), pp.1301-1305.

Oliveira, C. E. S., F Steiner. (2017). Potassium nitrate priming to mitigate the salt stress on cucumber seedlings. *Scientia Agraria Paranaensis*, v. 16, n. 4, p. 454-462, 2017.

Pandolfi, C., E. Azzarello, S. Mancuso, and S. Shabala, (2016). Acclimation improves salt stress tolerance in Zea mays plants. *Journal of plant physiology*, *201*, pp.1-8.

Popova, L.P., Z.G. Stoinova, and L.T. Maslenkova, (1995). Involvement of abscisic acid in photosynthetic process in Hordeum vulgare L. during salinity stress. *Journal of plant growth regulation*, *14*(4), p.211.

Raun, S. Q. Xue K. Thlkowska. (2002) Effect of seed priming on germination and health of rice (*Oryza sativa* L) seeds. *Seed Sci Technol. 2002;*30:451–8.

Saeidnia, S. and A.R. Gohari, (2012). Importance of Brassica napus as a medicinal food plant. *Journal of Medicinal Plants Research*, *6*(14), pp.2700-2703.

Safarnejad, A. and H. Hamidi. (2008). Study of morphological characters of Foeniculum vulgare under salt stress.

Sattar, S., T. Hussnain, and A. Javaid. (2010). Effect of NaCl salinity on cotton (Gossypium arboreum L.) grown on MS medium and in hydroponic cultures. *The Journal of Animal & Plant Sciences*, *20,* pp.87-89.

Schonfeld, M.A., R.C. Johnson, B.F. Carver and D.W. Mornhinweg, (1988). Water relations in winter wheat as drought resistance indicators. Crop Sci., 28: 526-531.

Segatoleslami M J.(2010). Effect of salinity on germination of three species of medicinal savory (Saturejahortensis L.), Chicory (Cynarascolymus L.) and Artichoke (Cichoriumintybus L.). Iranian Journal of Agricultural Researches. 8(5):818–23.

Seung, D., J.P.M. Risopatron, B.J. Jones, and J. Marc, (2012). Circadian clock-dependent gating in ABA signalling networks. *Protoplasma*, *249*(3), pp.445-457.

Shahbazi, E., A. Arzani, and G. Saeidi, (2011). Effects of NaCl treatments on seed germination and antioxidant activity of canola (Brassica napus L.) cultivars. *Bangladesh Journal of Botany*, *40*(1), pp.67-73.

Shahzad, A., M. Ahmad, M. Iqbal, I. Ahmed and G.M. Ali, (2012). Evaluation of wheat landrace genotypes for salinity tolerance at vegetative stage by using morphological and molecular markers. Genet. Mol. Res.,11:679–692

Sharifi, M., M. Ghorbanli, and H. Ebrahimzadeh, 2007. Improved growth of salinity-stressed soybean after inoculation with salt pre-treated mycorrhizal fungi. *Journal of plant physiology*, *164*(9), pp.1144-1151.

Szabados, L. and A. Savoure,. (2010). Proline: a multifunctional amino acid. *Trends in plant science*, *15*(2), pp.89-97.

Talat, A., K.Nawaz, K.Hussian, K.H. Bhatti, E.H. Siddiqi, A. Khalid, , S. Anwer, and M.U Sharif. (2013). Foliar application of proline for salt tolerance of two wheat (Triticum aestivum L.) cultivars. *World Appl Sci J*, *22*(4), pp.547-54.

Veeranagamallaiah, G., P. Chandraobulreddy, Jyothsnakumari, G. and C. Sudhakar, (2007). Glutamine synthetase expression and pyrroline-5-carboxylate reductase activity influence proline accumulation in two cultivars of foxtail millet (Setaria italica L.) with differential salt sensitivity. Environmental and Experimental Botany, 60(2), pp.239-244.

Vital, S.A., R.W. Fowler, A. Virgen, D.R. Gossett, S.W. Banks, and J. Rodriguez, (2008). Opposing roles for superoxide and nitric oxide in the NaCl stress-induced upregulation of antioxidant enzyme activity in cotton callus tissue. *Environmental and Experimental Botany*, *62*(1), pp.60-68.

Yang, Z., Yu, J., E. Merewitz, and B. Huang, (2012). Differential effects of abscisic acid and glycine betaine on physiological responses to drought and salinity stress for two perennial grass species. *Journal of the American Society for Horticultural Science*, *137*(2), pp.96-106.

Yoshida, K., E. Igarashi, M. Mukai, K. Hirata, and K. Miyamoto (2003). Induction of tolerance to oxidative stress in the green alga, Chlamydomonas reinhardtii, by abscisic acid. *Plant, Cell & Environment*, *26*(3), pp.451-457.

Yunwei, D. J.Tingting, and D. Shuanglin, (2007). Stress responses to rapid temperature changes of the juvenile sea cucumber (Apostichopus japonicus Selenka). *Journal of Ocean University of China*, *6*(3), pp.275-280.