INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 4, No. 1, p. 432-442, 2014

RESEARCH PAPER OPEN ACCESS

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

Azadeh Razaji[1*], Maryam Farzanian[2], Saeed Sayfzadeh[3]

[1]*Department of Agronomy and Plant Breeding, Saveh Branch, Islamic Azad University, Saveh, Iran*

[2]*Department of Agronomy and Plant Breeding, Tabriz Branch, Islamic Azad University, Tabriz, Iran*

[3]*Department of Agronomy, Takestan Branch, Islamic Azad University, Takestan, Iran*

Key words:

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

Abstract: Ascorbic acid, rapeseed, drought stress.

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

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

Introduction

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

Materials and methods

Laboratory studies

The experiment was carried out at the physiological laboratory of Faculty of Agriculture, Islamic Azad University, Saveh Branch. The cultivar of rapeseed was okapi. The experiment was a factorial method

with two factors arranged in a completely randomized design with three replications. The first factor was drought stress on5 levels (control, -4, -6, -8 and -12 bar) that was carried out by PEG 6000, the second was Ascorbic acid on 4 density (control, 55, 110 and 165µm). For ascorbic acid treatment, seeds of rapeseed were sterilized for 5 minute in sodium hypochlorite solution and in ethanol for 30 second and then rinsed by distilled water. Sterilized seeds were transferred in to sterile petri dishes contain filter papers and were added 10 ml ascorbic acid solution to each petri dish. Seeds of rapeseed were primed for 16 hours at 25 °C and dark conditions. Thereafter, the seeds treated with ascorbic acid solution rinsed with distilled water. Following this, the primed seeds were dried between two filter papers. Primed seeds were placed in petri dishes, on a layer of filter paper. Twenty five seeds were placed in each petri dish, the petri dishes were moistened with 5 ml of PEG 6000 solution at water potential of (0, -4, -6, -8 and -12 bar). The petri dishes were placed in germinator. The seeds were kept under aseptic condition for 7 days in 16h/8h light/dark cycle with a light intensity of 350 $\mu molm^{-2}s^{-1}$ and a relative humidity of 45% at 25±1°C. Seed germination was recorded daily up 7 days after the start of the experiment. A seed was considered germinated when radical emerged by about 2mm in length. Moreover germination percentage was determined in the end of test. Germination percentage was calculated with the following formula:

GP = 100 (n / N)

N = Total seeds number

n = Germinated seed number.

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

$$\text{Vigor Index} = \frac{\text{Germination percentage} * \text{Seedling lenght(cm)}}{100}$$

The remains of seedlings were frozen in liquid N_2 and stored under -80°C until biochemical analysis.

Extract preparation

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

Catalase activity

Catalase activity was estimated by the method of Cakmak and Horst (1991). The reaction mixture contained 100µl of crude enzymes extract, 500µl of 10 Mm H_2O_2 and1400µL of 25 Mm sodium phosphate buffer and the decrease in the absorbance was recorded at 240nm for 1 minute. Catalase activity of the extract was expressed as catalase units $min^{-1}mg^{-1}$ protein.

Peroxidase activity

Peroxidase activity was determined by the oxidation of guaiacol in the presence of H_2O_2. The increase in absorbance was recorded at 470 nm (Ghanati *et al*, 2002). The reaction mixture contained 100µl crude enzyme, 500µl H_2O_2 5Mm, 500µl guaiacol 28Mm and 1900µl phosphate buffer 60Mm (PH7.0).Peroxidase activity of the extract was expressed as peroxidase units $min^{-1}m^{-1}$protein.

Proline content

Proline content was determined according to the method of Bates *et al* (1973). About 0.2 gr of the fresh seedlings was weighted and abraded in china mortar of 10 ml sulfa salicylic acid (3%). The achieved juice was centrifuged in a 10000 G device for 5 min. Then 2 ml of the solution from the centrifuged device with 2 ml of ninhydrin reagent (1.25 gr ninhydrin acid, 30 ml NH_3PO_4 (6M)) was incubated for 1h at 100°C. The reaction was stopped by placing the test tubes in cold water. The samples were vigorously mixed with 4 ml toluene. The light absorption of toluene phase was estimated at 520 nm using spectrophotometer. The proline concentration was determined using a standard curve, while the results

of measuring the proline content was calculated and presented with Mg/g.

Statistical analysis

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

Results

Analysis of variance

Analysis of variance (Table1) indicated that all of traits under study including germination percentage, seedling dry weight, seedling fresh weight, root length, shoot length, vigor index, CAT activity, POX activity and proline content were significantly influenced by drought stress ($p<0.01$). The evaluated traits also were significantly influenced by priming with ascorbic acid ($p<0.01$). The interaction effects two-way (seed priming* drought stress) were significant for the studied traits except seedling dry weight, seedling fresh weight and proline content.

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

S.O.V	Df	Germination Percentage	Seedling Dry Weight	Seedling Fresh Weight	Shoot Length	Root Length	Vigor Index	CAT Activity	POX Activity	Proline Content
PEG	4	3837.4**	24.3**	3884.3**	985.01**	1512.02**	71.4**	254.03**	1428.6**	134.4**
AA	3	416.2**	19.2**	1222.3**	440.5**	318.3**	15.2**	245.6**	496.7**	19.5**
PEG*AA	12	3.2**	0.95ns	3.3ns	4.132**	9.2**	0.2**	2.5*	7.7**	1.9ns
Error	40	1.006	0.82	2.7	1.2	1.1	0.05	1.2	1.6	1.26
C.V		1.436	15.6	3.4	4.1	3.6	5.5	6.2	4.9	10.12

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

Effects of drought stress

All of the traits under study including germination percentage, seedling dry weight, seedling fresh weight, root length, shoot length and vigor index decreased when drought stress level were increased from 0 to -12 bar whereas CAT activity, POX activity and proline content increased when drought stress level were increased from 0 to -12 bar (Table2).

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

PEG (bar)	Germination Percentage (%)	Seedling Dry Weight (mg)	Seedling Fresh Weight (mg)	Shoot Length (cm)	Root Length (cm)	Vigor Index	CAT Activity (units min^{-1} mg^{-1} protein)	POX Activity (units min^{-1} mg^{-1} protein)	Proline Content (Mg/g FW)
0	89.89a	7.43a	69.79a	37.44a	41.18a	7.09a	12.39e	13.43e	7.13e
-4	86.22b	6.90a	63.84b	34.80b	39.56b	6.43b	13.70d	17.66d	8.43d
-6	66.72c	5.78b	41.87c	23.34c	29.39c	3.55c	19.24c	24.03c	11.70c
-8	57.95d	4.86c	35.14d	21.22d	21.07d	2.51d	20.62b	30.98b	13.08b
-12	48.45e	3.97d	28.86e	16.39e	15.52e	1.52e	23.09a	40.89a	15.54a

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

Effect of ascorbic acid

In seedling that were treated by ascorbic acid increased germination percentage, seedling dry weight, seedling fresh weight, root length, shoot length and vigor index whereas decreased CAT activity, POX activity and proline content as compared to control. The best results were obtained

from the seeds treated with 165µm ascorbic acid (Table3).

Table 3. The main effects of Ascorbic acid on the studied traits.

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

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

Priming with ascorbic acid showed a significant effects on germination percentage, shoot length, root length, vigor index, CAT and POX activity under drought condition (table4). The maximum germination percentage was achieved when seedlings were primed with110 and 165 and µm ascorbic acid under normal condition and maximum shoot length, root length and vigor index were achieved when seedlings were primed with 165µm ascorbic acid under normal condition. The minimum germination percentage, shoot length, root length and vigor index were observed in seeds untreated with ascorbic acid and -12 bar of PEG treatments. The maximum CAT and POX activity were observed in seeds untreated with ascorbic acid and -12 bar PEG treatment and minimum CAT and POX activity were achieved when seedlings were primed with 165µm ascorbic acid under normal condition.

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

PEG (bar)	AA (µm)	Germination Percentage (%)	Shoot Length (cm)	Root Length (cm)	Vigor Index	CAT Activity (units min^{1} mg^{-1} protein)	POX Activity (units min^{1} mg^{-1} protein)
0	0	84.4 d	32.38 de	36.63 d	5.82 d	16.2 f	17.51 i
0	55	87.5 c	33.66 d	36.94 d	6.17 d	14.17 g	15.03 i
0	110	93.5 a	40.81 b	44.26 b	7.95 b	10.66 h	12.74 j
0	165	94.1 a	42.89 a	46.91 a	8.45 a	8.52 i	8.43 k
-4	0	80.4 e	28.86 g	33.93 e	5.04 e	18.43 e	23.70 g
-4	55	85.5 d	31.78 ef	38.76 c	6.03 d	16.55 f	20.55 h
-4	110	87.5 c	38.83 c	43.08 b	7.13 c	10.73 h	19.19 i
-4	165	91.5 b	39.73 bc	42.49 b	7.52 c	9.83 hi	10.19 k
-6	0	60.4 i	18.33 j	23.67 g	2.53 h	23.76 bc	31.64 e
-6	55	63.5 h	19.3 j	27.35 f	2.96 g	21.7 d	25.85 f
-6	110	69.5 g	25.43 h	33.11 e	4.06 f	18.82 e	21.17 h
-6	165	73.5 f	30.33 fg	33.42 e	4.68 e	13.42 g	17.47 i
-8	0	51.5 k	13.32 k	16.57 h	1.53 jk	25.12 b	37.81 c
-8	55	55.5 j	18.76 j	17.74 h	2.02 i	22.49 cd	33.82 d
-8	110	60.4 i	24.31 h	22.26 g	2.81 gh	17.67 ef	26.59 f
-8	165	64.4 h	28.51 g	27.73 f	3.71 f	17.21 ef	25.71 fg
-12	0	42.4 m	10.77 l	11.38 j	0.68 l	28.38 a	49.62 a
-12	55	45.5 l	14.61 k	13.16 i	1.26 k	24.5 b	43.56 b
-12	110	50.4 k	18.86 j	14.70 i	1.96 ij	20.9 d	38.16 c
-12	165	55.5 j	21.33 i	22.86 g	2.54 h	18.59 e	32.22 de

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

Discussion

Drought stress

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

by reducing of nutrition absorb elements by unavailable moisture in the uptake and translocation (Ehsanpour and Fatahian, 2003; Zhu, 2002). Mohammadi and Amiri (2010) reported that with increase drought stress levels from 0 to -1.5 Mpa, germination percentage, root length and seedling dry weight reduced in rapeseed. Murillo Amador *et al* (2002) found that germination and emergence rate of two cowpea cultivars were delayed by PEG solution. Sadeghian and Yavari (2004) also reported that seedling growth severely diminished with increased drought stress. Murillo Amador *et al* (2002) also found that seedling growth of cowpea inhibited by NaCl and PEG, but higher inhibition occurred due of PEG. The decrease in seedling growth, under drought condition, maybe due to suppression of cell expansion and cell growth that is in response to low turgor pressure (Jaleel *et al*, 2008a ; Ogbonnaya, 2003). Okcu *et al* (2005) has found that -0.6 Mpa osmotic potential decreased *Pisum sativum* seed vigor. This is an similar results with Murillo-Amador *et al* (2002). The reduction in plant growth of oxidative stressed plants, maybe attributed to the inhibitory effect of ABA which was induced by drought on cell division and/or cell expansion (Nabil *et al*, 1995). And/or resulted from the osmotic effect of oxidative stress which caused disturbances in water balance of stressed plants leading to stomatal closure, reduction in photosynthesis and consequently a retarded growth rate (Chaparzadeh, 2004). The decrease in dry weight of seedling by increasing the oxidative stress level could be ascribed to the decrease in photosynthesis output as indicated by the significant decrease chlorophylls and total carbohydrates in oxidative stressed plants. Other authors concluded that, reduction of dry weight may be due to a turgor limitation (Mengel and Arneke, 1982), or cell wall hardening by limited extension growth (Chazen and Neumann, 1994). It has been indicated that drought stress affects the physiology and biochemistry of plant cells under in vivo and in vitro conditions. In this context, increased proline and activities of CAT, POX, APX, GPX has been reported in plants grown under stress (Hoque *et al*, 2007). Drought simulated the accumulation of the ROS including H_2O_2 in plants cells. The metabolism of H_2O_2 is dependent on various functionally interrelated antioxidant enzymes such as CAT and POX. These enzymes are involved in elimination of H_2O_2 from stressed cells (Kim *et al*, 2005a; Nojavan and Khorshidi, 2006). Water deficit could cause oxidative damage, therefore, plant cells need different mechanisms, which enable the detoxification of excess ROS and keep the balance of formation and removal of ROS. The increase activities of CAT, POX , detected in this study are presumed to limit cellular damage and enhance the plants oxidative capacity to defend stress. CAT and POX activities coordinated with SOD activity play a central protective role in the O_2^- and H_2O_2 scavenging process (Hoque *et al*, 2007a). The CAT and POX activity increased under drought stress when compared to control plants. Similar results reported under drought stress in wheat (Shao *et al*, 2005a) and tomato plants (Sanchez-Rodriguez *et al*, 2010). Redy *et al* (2003) reported that proline content increase in drought stress time. Sairam *et al* (1998) reported that in increasing proline causes increasing resistance on drought and salty. It is reported that increasing proline cause protecting turgor and the reduction of membrane damage on plants. So, osmo regulation is an adaption that increase the tolerance toward drought stress(Inze and Montage, 2000).

Ascorbic acid

Ascorbic acid is one of the most extensively studied antioxidant and has been detected in majority of plant species, organelles and apoplast and is synthesized in the mitochondria and transported to the other cell components through a proton-electrochemical gradient or through facilitated diffusion (Smirnoff, 2000). Ascorbic acid is an antioxidant molecule that acts a primary substrate in the cyclical pathway for detoxification and neutralization of superoxide radicals and singlet oxygen (Noctor and Foyer, 1998). Also it is one of the best indentified non-enzymatic compounds as antioxidant that plants bearing is increased to oxidative stresses (Smirnoff, 1996). Ascorbate has been shown to play multiple roles in plant growth, such as in cell expansion and other developmental processes (Pignochi and Foyer, 2003).

Reported that priming with ascorbic acid increased germination percentage, length of shoot and root, their dry weight and seedling total dry weight in sunflower and rapeseed and decreased CAT activity significantly than control treatment. Infact increase of root and shoot length by ascorbic acid might be due to the cell division and differentiation of meristem cells (Liso *et al*, 1988). Application of vitamins improved growth of plants by causing significant increases in the values of the above growth parameters of oxidative stressed plant. The inhibitory effects of high levels of oxidative stress were mitigated partially or completely alleviated. This probably by increasing the efficiency of water uptake and utilization as well as protecting the photosynthetic pigments, and the photosynthetic apparatus (Hassanein *et al*, 2009). Ascorbic acid on plant survival is associated with the partial inhibition of a few interactions in reactive oxygen species production (Shalata and Neuman, 2001). Hamad and Hamada (2001) observed on their experiment on wheat seeds, priming with ascorbic acid reduced harmful effects of drought stress on root and shoot fresh weight. Dolatabadian *et al*. (2009) reported that salinity increased CAT and POX activity in leaves and roots of rapeseed while the application of ascorbic acid reduced the activity of these enzymes in salinity condition. Drought combined with ascorbic acid improves the biological status of rapeseed, Biological improvement is related to reduce production of harmful substances (Barkosky and Einhelling, 2003). Ascorbic acid as an antioxidant, reduced catalase activity in pea under stress condition (Kukreja *et al*, 2005). Ascorbic acid has antioxidant properties that can remove superoxide ion and prevents the production o hydrogen peroxide, thus CAT and POX activity is reduced because these enzymes play a key role in removing hydrogen peroxide. Generally, it is concluded that ascorbic acid as an antioxidant, can reduce the harmful effects of oxidative stress and improve plants growth in stress condition (Dolatabadian *et al*,2009).

Acknowledgment

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

References

Abdul-baki AA, Anderson JD. 1973. Relationship between decarboxilation of glutamic acid and vigor in soybean. seed Crop Science **13,** 222-226. http://dx.doi.org/10.2135/cropsci1973.0011183X001300020023x

Apel K, Hirt H. 2004. Reactive oxygen species: Metabolism, Oxidative stress and signal transduction, Annual Review of Plant Biology **55,** 373-399. http://dx.doi.org/10.1146/annurev.arplant.55.031903.141701

Ashraf M, Foolad MR. 2007. Roles of glycinebetaine and proline in improving plant abiotic stress resistance, Environmental and Experimental Botany **59,** 206-216. http://dx.doi.org/10.1016/j.envexpbot.2005.12.006

Aspinall D, Paleg LG. 1981. Proline accumulation: physiological aspects, in: Paleg LG, Aspinall D (Eds.), The Physiology and Biochemistry of Drought Resistance in Plants. Academic Press, Sydney 205-241. http://dx.doi.org/10.1111/j.13993054.1988.tb01997.x

Barkosky RP, Einhelling FA. 2003. Allelopathic interference of plant–water relationship by parahydroxy benzoic acid, Botanical Bulletin of Academia Sinica **44,** 53-58. http://www.as-botanicalstudies.com/

Cakmak I, Horst W. 1991. Effect of aluminium of lipid peroxidation, superoxide dismutase, catalase

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

De Gara L, Cde Pinto M, Moliterni VMC, D·Egidio MG. 2003. Redox regulation and storage processes during maturation in kernels of Triticum durum, Journal of Experimental Botany **54,** 249-258.
http://dx.doi.org/10.1093/jxb/erg021

Demiral T, Turkan I. 2004. Does exogenous glycinebetaine affect antioxidative system of rice seedlings under NaCl treatment, Journal of Plant Physiology **161,** 1089-1100.
http://dx.doi.org/10.1016/j.jplph.2004.03.009

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

Ehsanpour A A, Fatahian N. 2003. Effect of salt and proline on Medicago Sativa callus, Plant Cell Tissue Organ Culture **73,** 53-56.
http://dx.doi.org/10.1023/A:1022619523726

Ghanati , Morita FA Yokota H. 2002. Induction of suberin and increase of lignin content by excess boron in tabbaco cell, Plant Nutrition **48,** 357-364 .
http://dx.doi.org/10.1080/00380768.2002.10409212

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

Hare PD, Cress WA. 1999. Staden van J, Proline synthesis and degradation: a model system for elucidating stress related signal transduction, Journal of Experimental Botany **50,** 413-434.
http://dx.doi.org/10.1093/jxb/50.333.413

Hassanein RA, Bassuony FM, Baraka DM, Khalil RR. 2009. Physiological effects of nicotinamide and ascorbic acid on zea mays and plant grown under salinity stress.1- Changes in growth, some relevant metabolic activities and oxidative defense systems, Research Journal of Agriculture and Biological Science **5(1),** 72-81.

Hoque MA, Okcma E, Banu MNA, Nakamura Y, Shimoishi Y, Murata Y. 2007. Exogenouus proline mitigatesthe detrimental effects of salts tressmorethan exogenous betaine by increasing antioxidant enzyme activities, Journal of Plant Physiology **164,** 553-561.

Inze D, Montage MV. 2000. Oxidative stress in, Current Opinion in Biotechnology **6,** 153 – 158 .

Jaleel CA, Manivannan P, Lakshmanan GMA, Gomathinayagam M, Panneerselvam R. 2008. Alternations in morphological parameters and phytosynthetic pigment responses of carthamus roseus under soil water deficits, Colloids Surf B Biointer **61,** 298-303.

Khedr AA, Abbas MA, Abdel Wahid AA, Quick WP, Abogadallah GM. 2003. Proline induces the expression of salt-stress-responsive proteins and may improve the, adaptation of Pancratium maritimemum L. to salt-stress, Journal of Experimental Botany **54,** 2553-2562.

Kim DW, Rakwal R, Agrawal GK, Jung YH, Shibato J. 2005. A hydroponic rice seedling culture model system for investigating proteome of salt stress in rice leaf, Electrophoresis **26,** 4521-4539. PMID: 16315177 [PubMed - indexed for MEDLINE]

Kukreja, Nandval SAS, Kumar N, Sharma SK, Univ V, Sharma PK. 2005. Plant water status , H2O2 scavenging enzymes , ethylene evolution and

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

Liso RG, Innocenti AM, Bitonti A, Arrigoni O. 1998. Ascorbic acid-induced progression of quiescent center cells from G_1 to S phase, New Phytologist **110**, 469-471.
http://dx.doi.org/10.1111/j.14698137.1988.tb00284.x

Mckersie BD, Bowley SR, Jones KS. 1999. Winter survival of transgenic alfalfa overexpressing superoxide dismutase , Plant Physiology **119,** 839-847 .
http://dx.doi.org/10.1104/p.119.3.839

Mohammadi GR. 2009. The influence of Nacl Priming on seed germination and seedling growth of canola (Brassica napus L.) under salinity conditions, American – Eurasian . Journal of Agricultural and Environmental Science **5,** 696 – 700
.

Mohammadi GR, Amiri F. 2010. The effect of priming on seed performance of canola (Brassica napus L.) under drought stress, American-Eurasian Journal of Agricultural and Environmental Science **9(2),** 202- 207.

Molinari HBC, Marur CJ, Daros E, Campos MKF, Carvalho JFRP. 2007. Evaluation of the stress-inducible production of proline in transgenic sugrance (Saccharum sp.): Osmotic adjustment, chlorophyll fluorescence and oxidative stress, Physiology Plant **130,** 218-229.
http://dx.doi.org/10.1111/j.1399-3054.2007.00909.x

Murillo-Amador B, Lopez-Aguilar R, Kaya C, Larrinaga-Mayoral J, Flores-Hernandez A. 2002. Comparative effects of NaCl and polyethylene glycol on germination, emergence and seedling growth of cowpea, Journal of Agronomy and Crop Science **188,** 235-247.
http://dx.doi.org/10.1046/j.1439037X.2002.00563.x

Nabil M, Coudret A. 1995. Effect of sodium chloride on growth, tissue elasticity and solute adjustment in two Acacia nilotica subspecies, Plant Physiology **93,** 217-224.
http://dx.doi.org/10.1111/j.13993054.1995.tb02220.x

Noctor G, Foyer CH. 1998, Ascorbate and glutathione : Keeping active oxygen under control , Annual Review of Plant Physiology and Plant Molecular Biology **49**, 249-279.
http://dx.doi.org/10.1146/annurev.arplant.49.1.249

Nojavan AM, Khorshidi M. 2006. An investigation of vanillin imposed oxidative stress in corn (Zea mays L.) and the activities of antioxidative enzymes, Pakistan Journal of Biology Science **9,** 34-38.
http://dx.doi.org/10.3923/pjbs.2006.34.38

Ogbonnaya CL, Sarr B, Brou C, Diouf O, Diop NN, Macaulery HR. 2003. Selection of cowpea genotypes in hydroponics, pots, and field for drought tolerance, Crop Science **43,** 1114-1120.
http://dx.doi.org/10.2135/cropsci2003.1114

Okcu G, Kaya MD, Atak M. 2005. Effect of salt and drought stresses on germination and seedling growth of pea (Pisum sativum L.), Turkish Journal of Agriculture and Forestry **29,** 237-242.

Park EJ, Jeknic Z, Chen THH, 2006. Exogenous application of glycinebetaine increases chilling tolerance in tomato plants, Plant Cell Physiology **47,** 706-714. PMID:16608869 [PubMed - indexed for MEDLINE]

Pignochi C, Foyer CH. 2003. Apoplastic ascorbate metabolism and its role in the regulation of cell signaling , Current Opinion in Plant Biology **6,** 379-389 . PMID: 12873534 [PubMed - indexed for MEDLINE]

Rao SC, Aker SW, Ahring RM. 1987. Priming Brassica seed to improve emergence under different

temperatures and soil moisture conditions, Crop Science **27,** 1050-1053.
http://dx.doi.org/10.2135/cropsci1987.0011183X002700050045x

Redy TY, Reddy VR, Anbumozhi V. 2003. Physiological response of groundnut (Arachis hypogeal L.) to drought stress and its amelioration a critical review, Plant Growth Regulation **41,** 75-88. http://dx.doi.org/10.1023/A:1027353430164

Sairam RK, Deshmukh PS, Saxena DC. 1998. Role of antioxidant systems in wheat genotype tolerance to water stress, Plant Biology **41(3),** 387-394.
http://dx.doi.org/10.1023/A:1001898310321

Sanchez-Rodriguez E. Wilhelmi SMMR, Cercilla LM, Blasco B, Riso JJ, Rosales MA, Romero L, Ruiz JM. 2010. Genotypic differences in some physiological parameters symptomatic for oxidative stress under moderate drought in tomato plants, Plant Science **178,** 30-40.
http://dx.doi.org/10.1016/j.plantsci.2009.10.001

Sadeghian SY, Yavari N .2004. Effect of water-deficit stress on germination and early seedling growth in sugar beet, Journal of Agronomy and Crop Science **190,** 138-144.
http://dx.doi.org/10.1111/j.1439-037X.2004.00087.x

Shao HB, Liang ZS, Shao MA. 2005. Changes of anti-oxidative enzymes and MDA content under soil water deficit among 10 wheat (Triticum aestivum L.) genotypes at maturation stage, Colloid Surf B Biointerf **45,** 7-13. PMID: 16102947 [PubMed - indexed for MEDLINE]

Shalata A, Neumann PM. 2001. Exogenous ascorbic acid (vitamin C) increases resistance to salt stress and reduced lipid peroxidation, Journal of Experimental Botany **52,** 2207- 2211 .
http://dx.doi.org/10.1093/jexbot/52.364.2207

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

Smirnoff N. 2000. Ascorbic acid : metabolism and functions of a multi-factted molecule, Current Opinion Plant Biology **3,** 229 – 235.
http://dx.doi.org/10.1016/S1369-5266(00)80070-9

Taylor AG, Harman GE. 1990. Concepts and technologies of selected seed treatments, Annual Review of Phytopathology **28,** 321-339.
http://dx.doi.org/10.1146/annurev.py.28.090190.001541

Zhu JK. 2000. Genetic analysis of plant salt tolerance using Arabidopsis, Plant Physiology **124(2000),** 941-948.
http://dx.doi.org/10.1104/p.124.3.941

Zhu JK. 2002. Salt and drought stress signal transduction in plants, Annual Journal Plant Biology **53,** 247-273.
http://dx.doi.org/10.1146/annurev.arplant.53.091401.143329