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Role of Glutathione and Polyadenylic Acid on the Oxidative Defense Systems of Two Different Cultivars of Canola Seedlings Grown under Saline Condition

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Abstract: The antioxidant thiol tripeptide glutathione is regarded as one of the major determinants of cellular redox homeostasis. In addition polyadenylic acid is a multiple units of AMP and it stabilizes mRNA during protein synthesis. Therefore, it becomes important to consider how glutathione and polyadenylic acid migitate the oxidative salt stress experienced by two different cultivars of canola (*Brassica napus* L. cv. Serw and cv. Pactol). It was observed herein that, the plant growth parameters are significantly reduced by salt stress. Furthermore, salt stress elicit an effect on pigments, total soluble carbohydrates, free amino acids as well as total soluble proteins. The levels of antioxidant compounds (glutathione, ascorbic acid, carotenoids, phenols, proline and glycine betaine) were changed in response to salt stress. Seed priming with reduced glutathione and polyadenylic acid improve seedling resistance probably by increasing antioxdant substances levels and enhancing the activities of antioxidant enzymes (superoxide dismutase, SOD, phenol peroxidase and oxidase ,GPX & POX as well as ascorbate peroxidase and oxidase, AXP & ASO).

Keywords: Canola; glutathione; Polyadenylic acid; Osmolytes, antioxidants.

INTRODUCTION

Salt stress is considered as one of the most important abiotic factors limiting plant growth and productivity (Sairam and Tyagi, 2004). Salt tolerance mechanisms are quite complex, involving osmotic adjustment, compartmentation of toxic ions (Sairam and Tyagi, 2004). Metabolite accumulation, ion homeostasis, redox control and scavenging activated oxygen species (ROS).

Salinity produces oxidative stress in plant tissues (Bartosz, 1997, Holmberg & Bulow, 1998 and Rout & Shaw, 2001). Though salt stress may limits gas exchange and there by $CO2_2$ supply to the leaf (Harris & Outlaw, 1991 and Fendina *et al,* 1994) resulted in the over – reduction of photo synthetic electron transport chain (Osmond & Grace, 1995). This stimulates the generation of active oxygen species, such as singlet oxygen, superoxide anion, hydrogen peroxide and hydroxyl radical (Asada, 1994 and Gossett *et al,* 1994).

The levels of active oxygen species are regulated by their rates of generation, their rate of reaction with target substances, such as proteins, lipids, and/or nucleic acids, their potential rate of degradation and their rate of scavenging / buffering by enzymatic and/or non enzymatic antioxidants (Hodgs, 2003). Several enzymes are in volued in the detoxification of reactive oxygen species resulted under salt stress. Superoxide dismutase (SOD) (EC. 15.1.1) is the first defense enzyme which converts superoxide to H_2O_2 that can be scavenged by catalase CAT (EC 1.111.1.6) and different classes of peroxidases, POD (EC. 1.111.1.7) (Bowler *et al.*, 1992; Sairam., *et al.* 2004). A close relation between antioxidant capacity and NaCl tolerance has been demonstrated in numerous crops such as rice (Dionisio-Sese & Tobita, 1998).

Moreover, the recent studies reported varying responses of plant antioxidant enzymes specific for species and tissues (Gosset *et al.,* 1994; Ben Amor *et al.,* 2005).

Glutathione play an important role in the protection against oxidative stress. It is involved in the ascorbate / glutatione cycle and in the regulation of protein thiol – disulphid redox status of plants in response to abiotic and biotic stress (Mullineaux and Rausch, 2005).

It was reported also, that addition of adenylic, guanylic or cytidylic acids, RNA or RNA hydrolysate improve the growth of *penicillium notatum*. Adenine, also, increased growth of pea in saline medium (Kessler, 1961). Levitt (1980) deduced that, osmotic stress induces changes in the composition of polynucleotides which may lead to adaptive response reaction by serving as primer for RNA-depent RNA polymerase.

Finally, seed priming (osmocondioning) increases seed vigour as well as accelerate germination, improve stress resistance and enhance plant growth an productivity (Pattan *et al* 2001 and Burguieres *et al.* 2006).

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MATERIALS AND METHODS

The experimental plant used in the present work was canola (*Brassica napus* L.). Two different cultivars of canola cv. Serw and cv. Pactol) were tests for its sensitivity toward NaCl stress. Canola seeds were obtained from Egyptian Ministry of Agriculture, Giza, Egypt. The two applied substances used in seed priming are reduced glutathione and polyadenylic. They obtained from Sigma Company (ST Louis, Mo 63178 USA). The current studies were carried out to elucidate the roles of water soluble antioxidant reduced glutathione (GSH) as well as mRNA stabilizer polyadenylic acid (Polyadenylic acid made up of multiple units of AMP found in mRNA molecules and it stabilizes mRNA during protein synthesis as mentioned in Encarta msn Dictionary). on the oxidative defense systems of two different cultivars of canola grown under saline conditions. Also, to elucidate their roles in nullifications of salt injuries. Preliminary experiments were done to test the salt sensitivity of the two cultivars as well as to choose the proper concentrations of glutathione and polyadenylic acid.

A Lot of canola seeds of the two cultivars was soaked in water (control) as well as another two lots were soaked in 100 mg/L of reduced glutathione (GSH) and polyadenylic acid (Poly A) for 24 hrs at 25 °C (priming). The air dried untreated (control) and treated seeds were sown in plastic pots, each containing two Kg of soil composed of mixed sieved air-dried clay and washed sand (1:1 by weight). After ten days from sowing, the plants subjected to the desired salinization levels (100 mM and 200 mM NaCl).

The water soaked (untreated) seeds were grouped into 3 sets, the first set-used as control (no salt), while the anothers were subjected to 100 and 200 mM NaCl (used as refrences controls). The treated seeds were divided into e two sets to be exposed to 100 and 200 mM NaCl.

There after, the plants were daily irrigated to reach the desired salinization level. Plants were exposed to normal day length with natural temperature (a bout 26/13 + 2°C and 13 h photoperiod ; 70% humidity). After three weeks from seedling emergence, the plants were collected for measuring the growth parameters in termis of shoot height, root length as wll as fresh and dry weights of either the shoot or the root. Moreover, some plants are collected for measuring photosynthetic pigments, different metabolites, DNA, RNA, osmolytes (proline, glycine betain), phenols as well as the enzymatic and non enzymatic antioxidants.

Fresh and Dry Weights:

The green tops of untreated and treated canola seedlings as well as their roots were washed then dried in oven at 70 °C for 2 days, then the fresh and dry weights of shoots and roots were recorded.

Pigment Determination:

Chlorophyll a, Chlorophyll b and total carotenoids were extracted from one gram of fresh leaves in 85% acetone and measured spectrophotometrically according to Metzner *et al.* (1965) and their values were calculated according to the formula of Lichtenthaler (1987).

Carbohydrate Determination:

Total soluble carbohydrates determined using anthrone reagent (Fairbairn, 1953).

Total Soluble Protein Determination:

Total soluble protein levels were measured by using BIO-RAD protein assay dye reagent by the method of Bradford (1976).

Total Phenol Determination:

Total phenols of fresh green tops were extracted and estimated following the method described by Malik and Singh (1980).

Free- Amino Acid Determination:

Free amino acids were extracted according to the method described by Vartanain *et al* (1992) and estimated using ninhydrin reagent (Yemm and Cocking, 1955). Proline and glycine betaine were assayed according to the methods described by Greive & Maas (1984) and Bates *et al.* (1973) respectively.

Nucleic Acid Determination:

DNA and RNA were extracted with 10% cold percholoric acid following the method of Schmidt and Thannhauser (1945), and modfied by Kalinich *et al.* (1985).

Oxidation Products Estimation:

The degree of Lipid peroxide formation are assayed by measurming the peroxide value (P.V.) by recording the increase in absorption at 234nm due to increasing diene conjugation and the thiobarbituric (TBA) determination of malonaldehyde formation (St Angelo *et al.,* 1975 and Heath & Packer, 1968). The amount of MDA was calculated from the extinction coefficient of 155 mM^{-1} Cm^{-1} (Kwon *et al,* 1965). The content of conjugated dienes was calculated from the extinction coefficient of 2.74 x 10$^{4-1}$M cm^{-1} (Fishwick & Swoboda, 1977).

Enzymatic and non Enzymatic Antioxidants:

The water soluble antioxidants such as glutathione and ascorbic acid were determined by the methods of Scupp & Rennenperg (1988) and Kampfenkel *et al.* (1995) respectively.

Antioxidant enzymes were extracted from frozen canola green tops by using a known volume of phosphate buffer (PH7) (1:4 w/v). The crude extract were used for enzyme assay. Cu- Zn superoxide dismutase (Cu-Zn SOD) was measured according to Giannopolitis and Ries (1977). Polyphenol peroxidase (Px) activity was assayed according to the method described by Bergmeyer (1974), while polyphenol oxidase (PPO) activity was measured by the method of Gonzalez *et al.* (1991). Catalase activity assayed following the method of Chen *et al* (2000).

Ascorbate peroxidase (APX) and oxidase (AO) activities were measured by the method reported by Cao *et al.* (2004) and Maxwell & Batman (1967) respectively.

Statistical Analysis:

Analysis of variance was conducted using ANOVA one way varience test using Microsoft Excel 2000. Statistical P values were calculated to quantity levels of significance for each treatment type. The values of analysis of plants grown under 100 and 200 mM were used as a refernce controls for glutathione and poly (A) treated ones, as well as they compared also, with the untreated control (No salt) . Each treatment is an average of three different measurements.

RESULTS AND DISCUSSIONS

Plant Growth:

The growth of the two canola cultivars (*Brassica napus* L.) in termis of shoot height, root length as well as their fresh and dry weights are presented in Table (1). The growth of plants of both cultivars was greately influenced by high salinity. The height, fresh weight and dry weight of both cultivars roots & shoots of canola

Table 1: Effects of glutathione and polyadenylic acid on the growth parameters of two different cultivars of canola plants grown under salt stress conditions. Each value is a mean of ten replicates ± SD.

Cultivar	Parameter	Shoot			Root		
	NaCl Treatments	Height (cm)	Fresh Wt.(mg)	Dry Wt. (mg)	Length (cm)	Fresh Wt.(mg)	Dry Wt. (mg)
Serw	0.0 (Control)	13.60±1.2	227.0±4.5	17.5±5.0	4.06±0.6	15.0±2.0	2.8±.3
	100mM NaCl	10.00±1.0	219.0±6.0	16.4±4. 0	3.40±0.2^{a}	14.1±2.6	1.7±0.5
	200mM NaCl	8.32±0.6^{a}	167.0±4.0^{a}	8.5±5.0 ^{a}	3.30±0.1^{a}	9.6±3.0^{a}	1.2±0.9^{a}
	100mM NaCl+GSH	13.81±1.3^{a}	343.0±5.0^{b}	29.7±5.3 ^{a}	4.61±0.12^{b}	18.6±4.0^{a}	3.2±0.7^{a}
	200mM NaCl+GSH	11.23±0.23	240.0±3.0^{b}	13.8±5.1 ^{a}	4.10±0.1^{b}	12.0±2.0^{a}	1.8±0.4
	100mM NaCl+Poly A	13.0±2.0	333.0±7.0^{b}	35.0±1.6 ^{b}	4.33±1.0^{b}	14.3±0.9	5.2±2.0^{b}
	200mM NaCl+Poly A	12.0 ±1.0^{a}	281.0±4.0^{b}	27.3±3.0 ^{b}	3.80±±0.18	11.0±1.0^{a}	3.6±1.2^{b}
Pactol	0.0 (Control)	13.5±2.0	30.05±9.0	280±0.1	4.06±0.06	9.1±0.99	2.3±0.4
	100mM NaCl	9.7±1.7	27.1±5.0	270±2.0	3.40±0.2^{a}	9.0±0.9	1.5±0.2^{a}
	200mM NaCl	8.0±2.0^{a}	187.3±3.0^{b}	210±6.0^{a}	3.20±1.9^{b}	1.5±1.0^{a}	1.1±0.5^{b}
	100mM NaCl+GSH	13.9±0.9	317.0±5.0^{a}	290±4.6	4.61±0.12^{b}	3.9±1.0^{c}	2.6±1.0^{b}
	200mM NaCl+GSH	9.9±0.5	270.0±2.0^{c}	220±5.0 a	4.10±0.5^{a}	12.6±1.1^{b}	2.8±0.7^{b}
	100mM NaCl+Poly A	14.5±2.0^{a}	632.0±3.0^{a}	320±3.0	4.21±0.13^{a}	17.1±1.6^{c}	2.9±0.2^{b}
	200mM NaCl+Poly A	11.6±1.0	319.4±4.2^{b}	230±1.0	3.35±0.25	15.3±2.1^{b}	1.3±0.3^{b}

Letters a = * at P>0.05 b = ** at P>0.01 c = *** at P>0.001 absence of letter = Non significant

Table 2: Effects of reduced glutathione and polyadenylic acid on the photosynthetic pigments content (µg/g F.wt.) of two different cultivars of canola grown under salt stress conditions. Each value is a mean of three replicates ± SD.

Variety	Treatment	Chlorophyll a	Chlorophyll b	Corotenoids	Total pigments
Serw	0.0 (Control)	573.0±3.0	292.0±2.0	150.1±2.0	1005.1±5.1
	100mM NaCl	565.0±5.0	219.0±4.0^{c}	148.0±4.0	932.0±2.0^{c}
	200mM NaCl	560.0±5.0	210.0±2.0^{c}	127.2±2.0^{b}	897.2±2.8^{c}
	100mM NaCl+GSH	608.0±8.0^{a}	405.0±5.0^{c}	156.4±5.2	1169.4±1.8^{c}
	200mM NaCl+GSH	565.0±5.0	255.0±3.7^{c}	146.4±4.0	966.0±1.4^{c}
	100mM NaCl+Poly A	674.0±4.0^{c}	350.0±5.0^{c}	154.6±3.9^{a}	1178.6±1.0^{c}
	200mM NaCl+Poly A	575. 0±15.0	248.0±4.0^{b}	137.4±2.0	969.0±4.0^{c}
Pactol	0.0 (Control)	656.0±6.0	418.0±3.0	91.5±1.0	1165.5±2.9
	100mM NaCl	622.0±2.0^{a}	303.0±3.0^{a}	144.0±2.0^{c}	1069.0±9.5
	200mM NaCl	612.0±2.0^{b}	289.0±9.5^{a}	148.4±2.6^{c}	1045.4±5.0
	100mM NaCl+GSH	710.0±10.0^{c}	348.0±5.8	149.0±4.5^{b}	1207.0±2.0
	200mM NaCl+GSH	656.0±5.0^{b}	298.0±2.2	170.0±5.0	1124.0±2.0
	100mM NaCl+Poly A	715.0±5.0^{c}	342.2±2.0	146.0±1.0	1203.3±2.9
	200mM NaCl+Poly A	645.0±5.2^{a}	294.0±4.0	171.0±2.0^{a}	1100.9±2.0

Letters a = * at $P>0.05$ b = ** at $P>0.01$ c = *** at $P>0.001$ absence of letter = Non significant

seedlings were significantly reduced at high salinity (200 mM) (Table 1). Priming of seeds by soaking 100mg/L reduced glutathione and 100 mg/L polyadenylic acid greatly nulified the inhibitory effects of salinity on the previous growth parametrs in the two cultivars (Table 1). The inhibitory effects of salt stress on previously measured growth parameter were also, recorde by other investigators using various plant speies (Shaddad & Heikal; 1982, Soussi *et al.,* 1998 and Chaparzadeh *et al.,* 2004). The reduction in growth parameters of the stressed plants may be attributed to the osmotic effect resulting from salt stress thereby cause disturbances in the water balance of the stressesed plants, leading to stomatal closure, reduction of photosynthesis and consequently an inhibition of growth (Poljakoff – Mayber, 1982). Furthermore the retarded growth of salt – stressed plants may result from the accumulation of toxic ions, impaired uptake of essential nutrients and/or damage in cellular organelles (Torres – Schumann, *et al.,* 1989).

The exogenous application of glutathione and Polyadenylic acid mitigated partially or completely the adverse effects of salt stress on growth of the two cultivars. Glutathione regarded as one of the major determinants of cellular redox homeostasis (Mullineaux and Rausch, 2005), thereby glutathione is integrated into primary metabolism and influence the functioning of signal transduction pathways by modulating cellular redox state, it may be affected muclear gene expression which influenced by plant's external environment. Ruiz and Blumwald (2002) and Mullineaux & Rausch, (2005). reported that glutathione content in wild canola plants increased under salt stress, this suggests a possible protective mechanism against salt cnduced oxidative damage. However, poly (A) may affects, the synthesis of DNA, RNA, and gene expression (protein synthesis) or/and retard the activation of RNase stimulated under salinity, so increasing the growth parmaters under saline condition.

Photosynthetic Pigments:

Salt stress variously affected the photosynthetic pigments levels in the two canola cultivars. Imposition of salt stress at 100 and 200 mM cause reduction in the total pigments content of the two cultivar compared to that of the control unstressed plants (Table 2). Salinization induced a significant reduction in chlorophyll a&b levels in the two cultivars of canola. The carotenoids levels were significantly increased under salt stress in cv. Pactol however, reduced in the other cultivar particularly at high salinity levels (Table 2). Similar results obtained with pea by Weimberg, (1975) and Soussi, *et al.* (1998).

Priming of canola seeds with GSH and Poly (A), significantly increased chlorophylls a, b and carotenoids levels under salt stress compared to the reference control (100, 200 mM NaCl). Similar results have been reached by (Amini & Ehsanpour, 2005). The ameliorating effects of glutathione and polyadenylic acid on pigments levels

Table 3: Effects of reduced glutathione and polyadenylic acid on total soluble carbohydrates, total soluble proteins, total phenols, free amino acids, proline, Glycine betaine and nucleic acid levels in tw different cultivars of canola plants grown under salt stress. Each value is a mean of three replicates ± SD.

cultivers	Treatment	Total Soluble Carbohydrates mg/g D.wt	Total Soluble Proteins mg/gFwt.	Total phenols mg/g F.wt	Free amino acids mg/g F.wt	Proline µg/g F.wt	Glycine betain µg/g D.wt	DNA mg/g F.wt	RNA mg/g F.wt
Serw	0.0 (Control)	5.70±0.2	2.64±5.8	20.8±0.8	5.1±0.1	32.2±2.3	64.82±3.0	33.8±3.0	139.20±7.0
	100mM NaCl	4.20±0.2^{b}	1.51±0.8^{c}	8.42±0.4^{c}	8.6±0.2^{b}	39.17±2.2^{c}	66.0±2.0	27.1±2.9	117.60±3.0^{a}
	200mM NaCl	4.10±1.9^{c}	1.22±5.0^{c}	7.17±2.9^{c}	9.57±0.4^{c}	70. 42±6.4^{c}	70.7±5.0	24.0±0.8	90.824±4.0^{c}
	100mM NaCl+GSH	12. 42±0.4^{c}	7.2±0.25	29.63±1.0^{c}	30.24±0.39^{c}	49.78±3.5^{c}	79.5±4.0	45.2±6.0^{a}	143.6±3.0^{b}
	200mM NaCl+GSH	8.60±2.9^{c}	6.05±2.0^{c}	23.66±3.1^{c}	54.63±3.6^{b}	80.56±5.6^{c}	89.0±20	34.3±4.5	108.21±3.0^{b}
	100mM NaCl+Poly A	12.18±0.4^{c}	5.94±1.5^{c}	21.4±0.4^{c}	12.04±2.1^{c}	47.22±2.3^{c}	80.0±4.0^{a}	49.51±2.0^{b}	150.3±0.6^{a}
	200mM NaCl+Poly A	7.10±0.1^{c}	4.19±1.0^{c}	13.3±0.3	22.2±3.2^{c}	84.17±1.15^{c}	87.1±4.0^{a}	36.1±2.9	115.73±2.2^{b}
Pactol	0.0 (Control)	15.90±0.3	1.65±0.5	12.68±0.7	25.62±2.0	43.61±1.5	62.7±0.8	64.8±2.0	159.20±9.0
	100mM NaCl	16.70±0.2	1.84±0.90	8.92±8.0^{a}	13.90±0.1^{c}	48.59±3.6	31.3±1.3^{c}	50.4±3.6	135.20±4.8
	200mM NaCl	6.30±0.06^{c}	1.31±0.1	8.31±0.6^{b}	10.80±1.8^{c}	263.90±3.5^{c}	25.7±2.2	26.7±3.2^{b}	89.20±4.0^{b}
	100mM NaCl+GSH	20.10±0.1	7.7±0.70^{c}	31.76±1.0^{b}	18.64±3.6^{b}	60. 42±5.2^{b}	77.7±3.0^{c}	57.8±2.0	182.40±2.4^{b}
	200mM NaCl+GSH	9.80+0.8^{b}	3.12±0.20^{c}	11.35±1.5^{a}	67.25±4.9^{c}	250.0±9.6^{c}	37.93±1.0^{c}	34.5±4.5	145.60±5.8^{c}
	100mM NaCl+Poly A	17. 43±1.9	8.51±0.1^{c}	30.44±.43^{c}	15.12±1.1^{c}	91.11±1.0^{b}	65.1±2.0^{b}	58.0±6.0	230.60±2.9^{b}
	200mM NaCl+Poly A	8.20±0.199	4.9±1.9^{c}	10.85± 1.05^{a}	54.17±2.4^{c}	397. 23±1.0^{c}	37.1±1.0^{a}	38.5±3.3	162.80±4.0^{b}

of canola leaves may be due to their effects on either enhancing the photosynthetic activities and chlorophyll biosynthesis or retardation of chlorophyll degradation resulted from the oxidative stress.

On the other hand, glutathione, plays a protective role in salinity tolerance by maintaining the redox status (Chaparzadeh *et al.,* 2004). However, poly (A) affects the stability of mRNA under salt stress, so, it may affect protein synthesis which required for chlorophyll biosynthesis.

Furthermore, carotenoids were significantly increased in treated plants under salt stress conditions. Caroteroids can protect the photosystems by reacting with lipid peroxidation products (Burton and Ingold, 1984).

Changes in Total Soluble Carbohydrates:

The accumulation of organic substances such as sugars and amino acid in tomato help to alleviate the salinity, mediated somatic stress (Amini & Ehsanpour, 2005). In the present study, a significant difference in total soluble carbohydrate between the two canola cultivars was observed (Table 3). Imposition of NaCl stress significantly reduced the total soluble carbohydrates level in cv. Serw cultivar as well as at the higher dosage of NaCl in cv. Pactol. However, 100 mM NaCl markedly increased total soluble carbohydrate in cv. Pactol. The change in soluble sugars content under salt stress has already been reported for a number of species (Ashraf & Tufial, 1993 and Amini & Ehsanpour, 2005). The reduction in soluble sugars may be attributed to the decline in the photosynthetic pigments or to reduction in the CO_2 assimilation rate, stomatal conductance (Downton, 1977).

The data herein obtained revealed that pretreatment of both cultivar seeds with GSH and Poly (A), stimulated the accumulation of soluble carbohydrates. A more pronounced increase in total soluble carbohydrate levels was obtained in the green tops pretreated with GSH. On the other hand total more soluble carbohydrates accumulated in cv. Pactol green tops as compared with the other cultivar (Table 3).

The interactive effects of glutathione and polyadenylic on accumulation of soluble sugars probably, attributed to the protective effects of glutathione on the photosythetic systems. GSH, also, plays a protective role in salinity tolerance by maintenance of the redox status (Chaparzadeh *et al.* 2004). Noctor & Foyer, (1998) and Mullineaux and Rausch (2005) also, stated that glutathione is integrated into primary metabolism and it can influence the functioning of signal transduction pathway by modulating cellular redox state. GSH may induce changes in photosynthesis and thereby may change the nuclear gene expression under the stress condition. Ascorbate and glutathione plays essential roles in plant metabolism and stress tolerance.

Polyadenylic acid is a salt derived from ADP and stabilizes the mRNA during protein synthesis, so it may affect gene expression (proteins) and thereby affects the cell metabolism.

Total Soluble Proteins:

Soluble proteins levels in the shoot tops of canola plants were significantly decreased under salt stress condition (Table 3). Protein content of cv. Pactol seedlings grown under 100 mM salt was little higher than cv. Serw. The decrease in total soluble proteins under salt stress was also observed by Ashraf and Waheed (1993). The decrease in total soluble protein under salt stress in canola green tops was referred to the decline in protein synthesis in cv. Serw which is accompanied by increasing of amino acid levels (Table 3). Conversely, the decrease in protein synthesis in cv. Pactol may be attributed to the disturbance in amino acid metabolism. Moreover, the enhancement effect of GSH and polyadenylic acid under salt stress may be due their effects on the gene expression as mentioned by Levitt (1980) who reported that adenine increased growth, nucleic acids and protein synthesis under drought condition.

Changes in Total Phenols:

Total phenols play a significant role in the regulation of plant metabolic processes and overall plant growth as well as lignin synthesis (Lewis & Yamamoto, 1990). On the other hand, phenols act as a substrates for many antioxidant enzymes, so, it mitigate the salinity stress injuries.

In the recent study, the total phenolics of canola seedlings were significantly lower compared to that of control unstressed plants. Priming of seeds by soaking in GSH and polyadenylic acid stimulate the accumulation of phenols under stress condition. The reduction in phenols levels under salt stress may be due to its oxidation by the antioxidant enzymes which withdraw phenols as their substrate and may also, due to the decline in its biosynthesis. Phenols protect the cells from potential oxidative damage and increase stability of cell membrane (Randhir *et al.,* 2003, Burguieres *et al.* 2006, Gaballah *et al.,* 2006).

Changes in Amino Acids:

Amino acids is a putative osmoprotective solute leading to lowering osmotic potential in several tissues exposed to stress. The exposure of canola plants to salt stress induced an accumulation of free amino acids which attained 2-folds of the control value in cv. Serw. In contrary, salinity significantly reduced the total free amino acids in cv. Pactol (Table 3). Similar results have been obtained by Jaeger & Prieb (1975). Furthermore, glutathione and polyadenylic acid significantly enhanced the stimulatory role of salt stress on the production of free amino acid in cv. Serw green tops as well as in cv. Pactol green plants. The highest level of amino acids was found in plants pretreated with GSH and Poly (A) and exposed to salt stress. The accumulation of amino acid is salt concentration dependent. The accumulation in amino acids in canola plants exposed to stress probably attributed to the disturbance in amino acid metabolism.

Proline:

There is a strong correlation between increased cellular proline levels and the capacity to survive the effects of high environmental salinity. It may also, serve as an organic nitrogen reserve (Sairam and Tyagi, 2004). In canola, proline accumulation was observed in the green tops of all stressed plants of the two cultivars compared with those of the control (unstressed) (Table 3). In addition, the increase in proline level was much higher in stressed plant pretreated with GSH or polyadenylic acid. With increasing NaCl, the proline content of the two cultivars increased significantly (Table 3). However, stressed plants of cv. Pactol accumulated much more proline compared to that of cv. Serw. This results may indicate that cv. Pactol more tolerant than the other cultivar. Similar results have been reached by Storey and Wyn Jones (1975) and Amini & Ehsanpour (2005). The higher level of proline content in canola shoots may be due to expression of gene encoding key enzymes of proline synthesis and low activity of the oxidizing enzymes which is controlled by osmotic and salinity stress (Amini & Ehsanpour, 2005). Finally, proline able to activate multiple responses that are component of the adaptation process (Maggio *et al.,* 2002). It was also, reported that proline and betaine act as free radical scavengers and/or enzyme protectants as well as compatible solutes (Okuma *et al.,* 2002 and Hoque *et al.,* 2007).

Glycine Betaine:

Betaine acts as a simple osmolyte and protect higher plants against salt stress not only by adjusting osmotic pressure (Pollard and Wyn Jones, 1979), but also, by stabilizing many functional units such as complex II electron transport (Hamilton and Heckathorn, 2001), membranes and proteins (McNeil *et al.,* 1999) and enzymes such as RUBISCO (Mäkelä *et al*., 2000). The accumulation of glycine betaine in cv. Serw was positively related

Table 4: Effects of reduced glutathione and polyadenylic acid on the lipid peroxidation products and water soluble antioxidant substances in two different cultivars of canola plants grown under saline conditions. Each value is a mean of three replicates ± SD.

		Lipid peroxidation products (µm/g Fwt.)		Antioxidant substances (µm/g Fwt.)	
Cultivar	Treatment	MAD	Conjugated dienes	Ascorbic acid	Total reduced glutathione
Srew	0.0 (Control)	2.00±0.3	36.78±0.9	266.6±1.6	26.4±0.6
	100mM NaCl	4.6±0.69	38.22±1.8	213.9±2.0	17.5±2.0^{a}
	200mM NaCl	5.92±0.5^{c}	40.0±4. 0	159.9±9.8^{c}	8.4±0.4^{b}
	100mM NaCl+GSH	2.24±0.9$_{a}$	30.7±4.2	273.3±3.2^{b}	39.5±3.0^{c}
	200mM NaCl+GSH	3.19±1.2$_{a}$	34.0± 2.5	167.9±1.3^{c}	25.6±1.6^{a}
	100mM NaCl+Poly A	2.88±1.05^{a}	31.2±2.0	233.3±3.30 ^{b}	27.8±1.8^{b}
	200mM NaCl+Poly A	3.96±0.3^{a}	35.9±1.7	168.6±5.0 ^{c}	19.8±1.1^{b}
Pactol	0.0 (Control)	4.21±0.1	39.24±1.5	249.3±10.0	27.1±0.8
	100mM NaCl	4.21±0.2	44.82±1.0	226.6±8.0	19.5±0.4
	200mM NaCl	4.8±0.2^{a}	58.6±1.6^{c}	178.6±12.0 ^{c}	11.8±1.01^{a}
	100mM NaCl+GSH	1.1±0.3^{c}	37.3±1.4^{a}	293.3±6.9 ^{b}	52.3±3.2^{c}
	200mM NaCl+GSH	1.5±0.5-8$_{c}$	43.0±2.3^{a}	189.0±1.2 ^{c}	47.9±4.0^{c}
	100mM NaCl+Poly A	1.8±0.1^{c}	38.2±1.0^{b}	238.6±6.0 ^{b}	40.0±5.1^{c}
	200mM NaCl+Poly A	2.3±0.15^{c}	45.5±2.0^{b}	187.0±8.0 ^{c}	28.7±1.6^{c}

Letters a = * at P>0.05 b = ** at P>0.01 c = *** at P>0.001 absence letter = Non significant

to the NaCl concentrations in contrast to that of cv. Pactol grown at the same condition. The priming with GSH and polyadenylic acid, significantly increased the levels of betaine in the two cultivars. The accumulation of amino acids as proline and betaine may be attributed to the hydrolysis protein or to the effects of salinity disturbing amino metabolism such as their biosynthesis.

Nucleic Acids:

Salinity disturbed nucleic acids metabolism and caused growth inhibition (Saakyan and Petrosyan, 1964). Salinity significantly reduced the level of both DNA and RNA in the two cultivars compared to the corresponding unstressed controls (Table 3). Presoaking of seeds in GSH and Poly (A) before sowing promoted the synthesis of DNA and RNA and/or prevented their degradation by nucleases enzymes. A higher levels of DNA and RNA were observed in canola seedlings exposed to either GSH or polyadenylic acid before the exposure to 100 mM NaCl . It was postulated that, the content of DNA in tomato increased in presence of 0.8% NaCl but both RNA and DNA decreased at injurious levels of NaCl due to its effects on the inhibition of synthesis and intensification of breakdown (Tsenov *et al.* 1973).

Salinization increase RNase activity in barley, tomato and Pea (Tal, 1977 and Kabanov & Chervina, 1973) respectively. The simpler nucleotides have also been reported to change as a result of salinization (Levitt,1980).

It was reported that GSH reacting directly or indirectly with reactive oxygen species, so contribute to maintain the integrity of cell structures such as proteins, lipids and nucleic acid from damage which induced by salt stress (levitt, 1980).

However, the enhancing effect of polyadenylic acid on nucleic acid may be attributed to adenine moiety which induced drought resistance only when applied at the early stage of development and this may influence DNA synthesis and promote RNA synthesis of pea seedlings (Kessler, 1961). On the other hand it may prevent or reduce the changes which occur in nucleotides under salt stress.

Lipid Peroxidation Products:

The extent of the salt – induced oxidative damage, assessed by measuring the levels of malondialdehyd (MDA) formation and conjugated dienes (Table 4). Salinization significantly exerted an increase in lipid peroxidation as well as conjugated dienes in the shoot tops of the two cultivars experienced salt stress.

Table 5: Effects of reduced glutathione and polyadenylic acid on the activity of antioxidant enzymes in two different cultivars of canola plants grown under salt stress. Each value is a mean of three replicates ± SD.

Caltivar	Treatment	Antioxidant enzymes (Unit/g F.wt)					
		SOD	CAT	POX	GPX	APX	ASO
Srew	0.0 (Control)	7.0±0.5	58.9±1.0	44.36±1.2	862.0±1.0	107.15±2.0	517.65±0.55
	100mM NaCl	6.81±0.3	57.3±1.9	31.58±3.0^{b}	443.2±3.0^{c}	110.9±2.0	113.05±3.0^{c}
	200mM NaCl	6.33±0.2	48.81±4.0	27.82±2.0^{b}	369.0±3.0^{c}	120.8±1.0^{a}	83.3±4.5
	100mM NaCl+GSH	7.5±0.2	385.31±5.0^{c}	124.1±1.0^{c}	1175.0±5.0^{c}	128.9±4.0^{b}	141.65±0.3^{b}
	200mM NaCl+GSH	7.2±0.19	95.9±1.9^{c}	118.8±1.2^{c}	1330.0±5.0^{c}	123.9±3.5	125.1±0.6^{c}
	100mM NaCl+Poly A	7.875±0.9	149.1±1.61^{c}	115.1±1.0^{c}	494.0±6.0^{c}	126.3±2.9^{a}	124.95±2.0^{a}
	200mM NaCl+Poly A	7.5±0.2^{a}	81.93±1.0^{c}	93.99±2.0^{c}	1017±3.0^{c}	121.4±1.4	99.81±5.0^{b}
Pactol	0.0 (Control)	7.9±0.4	523.0±3.0	34.59±2.0	836.0±6.0	99.3±0.52	43.6±2.98
	100mM NaCl	7.2±0.35	324.0±4.0^{c}	31.58±1.0	665.0±2.0^{c}	97.3±0.3	53.5±2.2
	200mM NaCl	6.05±0.75^{b}	87.6±0.7^{c}	20.31±8.6^{b}	449.0±1.0^{c}	96.2±0.2	50.3±2.1
	100mM NaCl+GSH	8.43±0.2^{a}	542.5±1.5^{c}	48.84±2.0^{c}	1052.0±2.5^{c}	142.9±3.0 ^{c}	85.5±5.0^{b}
	200mM NaCl+GSH	7.3±0.9^{a}	137.5±1.0^{c}	38.834±0.63^{c}	869.0 ±3.0^{c}	125.0±5.0^{b}	73.6±4.3^{a}
	100mM NaCl+Poly A	7.9±0.9	685.7±1.0^{c}	54.14±0.8^{c}	743.2±3.0^{c}	132.15±3.1^{b}	71.4±1.0^{a}
	200mM NaCl+Poly A	6.4±0.4	171.3±1.5^{c}	41.35±1.4^{c}	517.0±2.1^{c}	108.6±8.1	65.5±1.1

Letters a = * at P>0.05 b = ** at P>0.01 c = *** at P>0.01 absence letter = Non significant

The values of peroxidation products positively related to the concentration of NaCl applied, thereby there is a positive relation among the amount of lipid peroxidation products and the degree of membrane damages resulted from the injurious salt stress. Glutathione is a water soluble antioxidant which reacts directly or indirectly with the reactive oxygen species so, reduces stress injurious effects on membrane. Moreover, decreases in lipid peroxidation by glutathione and polyadenylic acid may be also, due to their effects on the activities of antioxidant enzymes and/or the high level of the endogenous GSH and ascorbic acid (Table 4). In addition, the presence of oxidation products such as MAD in biological systems is also, related to the beginning of peroxidation of unsaturated fatty acids. Every type of membrane is sensitive to oxidation process generated by free radicals. During Lipid peroxidation the double bonds of unsaturated fatty acids leading to diene conjugation (Heath & Packer, 1963). The increase in lipid peroxidation may be due to the incapability of antioxidants to neutralize and scavenge all the active oxygen species results from salt stress. The present results were agreed with the results of Ben Amor *et al.* (2005); Demiral & Türkan (2004) and Chaperzadeh *et al.* (2004).

Changes in Antioxidants:

Salt stress accelerates the formation of active oxygen species. The lifetime of active oxygen species within the cellular environment is determined by the antioxidant system, which provides crucial protection against oxidative damage. The antioxidant system comprises numerous compounds of low molecular weights and enzymes (Noctor and Foyer, 1998). The level of endogenous total glutathione (GSH) and ascorbic acid were significantly decreased in the stressed green tops of the two cultivars. Such decreases were about 68.1%, 34.5%, at 200 mM NaCl in cv. Serw, however, reached about 56.4%, 52.4% in cv. Pactol (Table 4). The decreases in endogenous ascorbic acid and glutathione in stressed plants were also, observed by Sairam *et al.* (2005). The lower GSH, could be the result of an increased net glutathione degradation or of a decreased synthesis (Chaparzadeh *et al.* 2004).

Marked increases in both ascorbic acid and glutathione (GSH) were observed in canola plants pretreated with exogenous glutathione and polyadenylic acid (Table 4). The increased in endogenous ascorbic acid and glutathione were about 2-3 folds compared to stressed controls (100, 200 mM NaCl) concomitant with the reduction in MAD and conjugated dienes. This results on GSH are agree with Chaparzadeh *et al.* (2004). Under stress conditions, lower GSH could be the result of an increase net glutathione degradation or of a decreased synthesis (Chaparzadeh *et al.* 2004). Reduced ascorbate and reduced glutathione, are the two major water soluble antioxidants which scavenging reactive oxygen species to maintain the integrity of cell structures and the proper functions of various metabolic pathways (Kocsy *et al* 2001).

Antioxidant Enzyme Activities:

Salinity accumulates the ROS in plant cells. The phospholipids membranes are impermeable to charged Ō2 molecules therefore superoxide dismutase are present for the removal of Ō2 in the compartments where Ō2 radicals are formed (Takahashi & Asada, 1989).

Superoxide dismutase (SOD) is the first defense agent against ROS as it is the major scavenger of Ō2 (Almoguera *et al,* 1995). Results in this study showed that SOD activity in NaCl - stressed plants was nonsignificantlly lower than that in non-stressed control plants (Table 5).

Exogenous application of glutathione and poly adenylic acid markedly enhanced SOD activity in the plants of the two cultivars. Some researchers also, suggest that salt stress leads to a decrease in SOD activity in salt-sensitive plants but to an increase in salt-tolerance one (Shalata & Tal, 1998, Rout & Shaw, 2001 and Hoque *et al,* 2006).

Salinity stimulate 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 catalases and peroxidases. These enzymes ore involved in elimination of H_2O_2 from stressed cells (Kim *et al,* 2005).

Our results demonstrated that catalase (CAT) and guiacual peroxidase (GPX) activities were significantly decreased under stress conditions (Table 5). These results are also, supported by the findings of Sahalata *et al.* (2001). The reductions in catalase and peroxidase activities suggest that these enzymes were unable to completely neutralize H_2O_2 resulted from the oxidative salt stress.

However, presoaking of salts stress plants in GSH and polyadenylic acid promoted the activities of CAT and GPX (Table 5).

Priming of canola seeds with GSH and polyadenylic acid improve stress resistance (Pattan *et al* 2001). The increase in phenol peroxidase activity in stressed seedlings may be decrease the injurious effect of NaCl as well as it reacts with H_2O_2 and maintain the membrane integrity.

The ascorbate pool can be reduced by oxidative stress when regeneration capacity is over come (Cahaparzadeh *et al* 2004). The law ascorbate in stressed plants particularly at high salinity (Table 4) might be an indication of ascorbate peroxidase participation in ROS scavenging (Table 5). The activity of ascorbate peroxidase in cv. Serw is positively related to the dosage of NaCl. Similar results were reached by Demiral & Türkan (2004) and Sairam, *et al.* (2005).

On the other hand, ASX activity was non significantly reduced in cv. Pactol exposed to salt stress. However, exogenous application of GSH and polyadenylic acid significantly increased the activity of ascorbate peroxidase in stressed plants of the two cultivars.

Polyphenol oxidase (POX) and ascorbate oxidase (ASO) are among the major antioxidant enzymes involved in scavenging AOS (Levitt, 1980).

The activities of phenol oxidase (POX) and ascorbate oxidase were significantly deceased in stressed plants compared two the unstressed control plants the two cultivars. Similar results have been obtained by Moksimova & Matukihim, (1965) on stressed millet leaves. Exogenous, application of either glutathione or polyadenylic acid significantly increased the specific activity of POX and ASO in canola seedlings experienced salt stress.

Finally, there are some differences in the responsive behaviors of the tow cultivars towards salt stress which may be attributed to the genetic differences. We can deduced also, that application GSH, plays a protective role in salinity tolerance by increased the activities of the antioxidant enzymes as wells as antioxidant substances. GSH acts at the cellular level as affects the redox status of the cell and it may act on the gene level. However, polyadenylic acid perhaps works at the gene level, as a primer for RNA-polymerase. it may stabilizes mRNA during protein synthesis under salinity which induced changes in the polynucleotides.

More work need to be done to elucidate the mechanisms of the tow applied substances on the gene level.

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