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Ameliorative Effect of Ascorbate on Growth and Oil Fatty Acid Composition of Soybean under Salinity

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THIS WORK aims at enhancing soybean growth and fatty acid composition of oil yield when grown under salinity by seed priming in AsA. Treatment of 10-day-old soybean seedlings with NaCl at 100 or 200 mM for 11 days significantly lowered K^+, K^+/Na^+ ratio and ascorbate (AsA) and inhibited the activities of catalase (CAT) and ascorbate peroxidase (APX), contrarily elevated Na^+, soluble sugars, MDA and H_2O_2. Meanwhile, total fatty acids were lowered in the yielded oil of mature seeds (115 days after sowing) concurrent with decreases in unsaturated fatty acids but increased saturated fatty acids (particularly palmitic and stearic). Nonetheless, seed priming in AsA improved growth coincided with raised K^+, K^+/Na^+ ratio and AsA as well as CAT and APX activities concomitantly with retractions in the accumulated Na^+, osmolytes and oxidative stress indices. Besides, saturated fatty acids were decreased but unsaturated fatty acids were increased synchronized with rises in total pool. This finding suggests the alleviation of salinity by priming in AsA through enhancing osmoprotection and antioxidants in addition to improving oil quality particularly omega fatty acids.

Keywords: Antioxidants, Ascorbate, Fatty acids, Salinity, Soybean.

Introduction

Soybean [*Glycine max* (L.)] represents an important oil crop. Salinity is hazardous to crops; it causes osmotic stress, ion toxicities and deficiencies in water and nutrients leading thus to plant growth reduction. Cl^- and Na^+ disrupt several physiological processes in crops. Na^+ blocks many cellular functions due to its competition with K^+ at many essential binding sites as well as interfering with the uptake of K^+ that would cause disruption in stomatal regulation (Munns, 2002; Andrés et al., 2014). The latter showed that guard cell vacuolar accumulation of K+ is a requirement for stomatal opening and a critical component in the overall K^+ homeostasis essential for stomatal closure.

Plants able to accumulate some osmolytes under osmotic stress such as soluble sugars that are osmotically active compounds which can lower the osmotic potential (Negrão et al., 2017). Due to salinity conditions, reactive oxygen species (ROS) are overproduced leading to oxidative stress, a state that has impacts to metabolism. It destroys proteins, lipids, and nucleic acids (Tuteja et al., 2009; Nemat Alla et al., 2020; Safwat & Abdel Salam, 2022). Plant have an array of antioxidant defense to prevent the formation of ROS or to limit their damaging effects (Nemat Alla & Hassan, 2006; Tuteja et al., 2009) either enzymatically or nonenzymatically (Azevedo Neto et al., 2006). The nonenzymatic antioxidants include AsA while the enzymatic antioxidants include CAT and APX.

AsA minimizes the oxidative stress damage via synergic function with other antioxidants; it is included in H_2O_2 detoxification (Reddy et al., 2004) in addition to its role for activities of several

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enzyme, (Foyer & Noctor, 2005). The higher the AsA content in plants, the better the protection against damage of oxidative stress. CAT and APX are among the enzymatic antioxidants act as scavengers for ROS. CAT decomposes H_2O_2 into water and O_2, whereas APX oxidizes AsA with the degradation of H_2O_2 (Hassan et al., 2021). These antioxidants might be insufficient under harsh conditions particularly in susceptible species, so these plants might need external supporters to enforce the defense system to withstand stresses (Perveen et al., 2018). Priming seeds in AsA may be very important for increasing the ability of plants to withstand salinity stress.

In oil crops, the contents of oil and the compositions of fatty acids are important attributes. Kajla et al. (2015) indicated that necessary seeds for good health are rich in essential fatty acids. Johnson and Bradford (2014) indicated that the quality of oil is worthy depending on essential fatty acids contents. These features are influenced by several factors; environmental, agronomic, genetic, etc. (Savoire et al., 2015). Since salinity influences metabolism of plants, fatty acid composition of the yielded oil should be also influenced. Therefore, a trial to investigate the impacts of NaCl on soybean was achieved. The investigation includes also if AsA as additive has a role for mitigating the salinity deleterious impacts. So, this investigation aims enhance soybean ability to cope with the harsh conditions of salinity by the application of AsA as seed priming to alleviate the impacts of NaCl stress on soybean growth for withstanding salinity and for improving fatty acid composition of the product.

Materials and Methods

Conditions of growth

Seeds of soybean [*Glycine max* (L.)] were soaked overnight either in water or in 0.5mM ascorbate (AsA) then sowed in plastic pots (40× 25 × 15cm) in quartz sand (pre-washed with hydrochloric acid) approximately 2cm-depth and spaced 5cm apart in 5cm adjacent rows and placed in greenhouse of the faculty of Science, Damietta University, at 30±2/16±2°C, day/night temperature with a 14-h photoperiod at 450-500µmol m^{-2} s^{-1} PPFD. Water was added for 7 days then Long Ashton nutrient solution was applied for 3 days followed by treatment with NaCl at 100mM and 200mM for 11 days. The pots were divided into 2 groups when seedlings were 21-day-old; the first was collected, washed, separated into shoots and roots, frozen in liquid nitrogen and stored at -80 °C for metabolic analyses whereas the second was left for seed production (after 115 days legumes were dried in sun for 10 days and seeds were used for fatty acids composition).

Determination of growth parameters, K^+, Na^+ and soluble sugars

Shoot height of 21-day-old seedlings, root length and fresh weight were recorded then samples were dried at 80°C for 2 days for dry weight determination. K^+ and Na^+ were extracted in ultrahigh purity water heated at 95°C for 1h and centrifuged for 20 min at 5000 ×*g*. Measurements were performed in supernatants using a Jenway PFP7 flame photometer (Hansen & Munns, 1988). The extraction of soluble sugars was performed in 80% ethanol then aliquots were mixed with anthrone reagent (8.6mM anthrone in 80% H_2SO_4), heated for 10 min, cooled in ice bath and absorbance was read at 623nm (Schlüter & Crawford, 2001).

Determination of H_2O_2, MDA and ascorbic acid (AsA)

Trichloroacetic acid (0.1%) was used for the extraction of H_2O_2 and MDA. The extracts were centrifuged at 12,000 ×*g* for 15min at 4°C. H_2O_2 was assayed in potassium phosphate (10mM, pH 7.0) containing 1M KI and absorbance was measured at 390 nm (Alexieva et al., 2001). The assay of MDA was conducted according to Heath & Packer (1968) and calculated by using the extinction coefficient 155mM^{-1} cm^{-1}. The extraction of AsA was carried out in 62.5mM phosphoric acid and centrifuged at 12000 ×*g* for 20min then eluted with 4.5 mM H_2SO_4 at a flow rate of 0.5mL min^{-1}. The assay of AsA took place according to Ahn et al. (1999) in sodium molybdate (0.66%) containing 0.05N H_2SO_4 and 0.025mM sodium phosphate followed by centrifugation at 4000 ×*g* for 5min and absorbance was read at 660nm.

Activity assay of catalase (CAT) and ascorbate peroxidase (APX)

The extraction of CAT was performed in phosphate buffer (50mM, pH 7) containing EDTA (2 mM) and β-mercaptoethanol (5mM) then followed by centrifugation for 10min at 12000 ×*g* at 4°C. The assay was taken place in phosphate buffer (50mM, pH 7.5) containing 200mM

H_2O_2 by determining the consumption of H_2O_2 at 240nm. APX was extracted in Tricine-KOH (0.1M, pH 8) containing 1mM dithiothreitol, 10mM $MgCl_2$, 50mM KCl, 1mM EDTA, 0.1% Triton X-100, and 0.28mM PMSF and assayed in phosphate buffer (50mM, pH 7.5) containing 40mM Na ascorbate and 200mM H_2O_2 at 270nm (Nakano & Asada, 1981).

Fatty acid composition

Seeds of the yield were grinded with *n*-hexane (60–80°C). Oil is converted into fatty acid methyl ester according to Danish & Nizami (2019) through mixing of an aliquot with methanolic NaOH (0.5N), heated for 3min at 60°C and followed by cooling. Six mL of 14% boron trifluoride solution was added, heated for 3min at 60°C. After cooling, 10mL isooctane were added, agitated and left for settling down then the upper layer was mixed with Na_2SO_4 then the extract was injected onto gas chromatography equipped with flame ionization detector (GC-FID Hewlett Packard, 6890). The oven and injector temperatures were 220 and 240°C, respectively. The oven program initial temp was 140°C for 5min with a rate of 4°C min^{-1}. The carrier gas was N at flow of 1mL min^{-1}. The column was 50% Cyanopropyl methylpolysiloxane, 30m DB-23, 0.32mm ID, 0.25µm film thickness. The syringe size was 10µL and the injection volume was adjusted at 3µL.

Statistical analysis

The experiment was repeated twice and samples were taken from both experiments for each analysis in triplicates (n= 6). SPSS version 22 was used for data analysis. The experiment was designed as a complete randomized block and the means (± SD) of values were calculated. Data were analyzed using ANOVA-least significant differences for each parameter separately of all treatments at every experimental time.

Results

Growth parameters, K^+, Na^+ and osmoprotectants

Figure 1 shows that salinity significantly decreased shoot height and root length of 21-old soybean seedlings in comparison with the control. Similarly, fresh and dry weights as well as water contents of both shoots and roots were decreased; the magnitude of decrease was more detected with 200mM than 100mM. Nevertheless, priming of seeds with AsA counterbalanced –to great extent- the impacts of NaCl induced in growth parameters such that reaching mostly to values of the corresponding control; the overcome was more pronounced for root fresh and dry weights in which some increases were even detected over the control values.

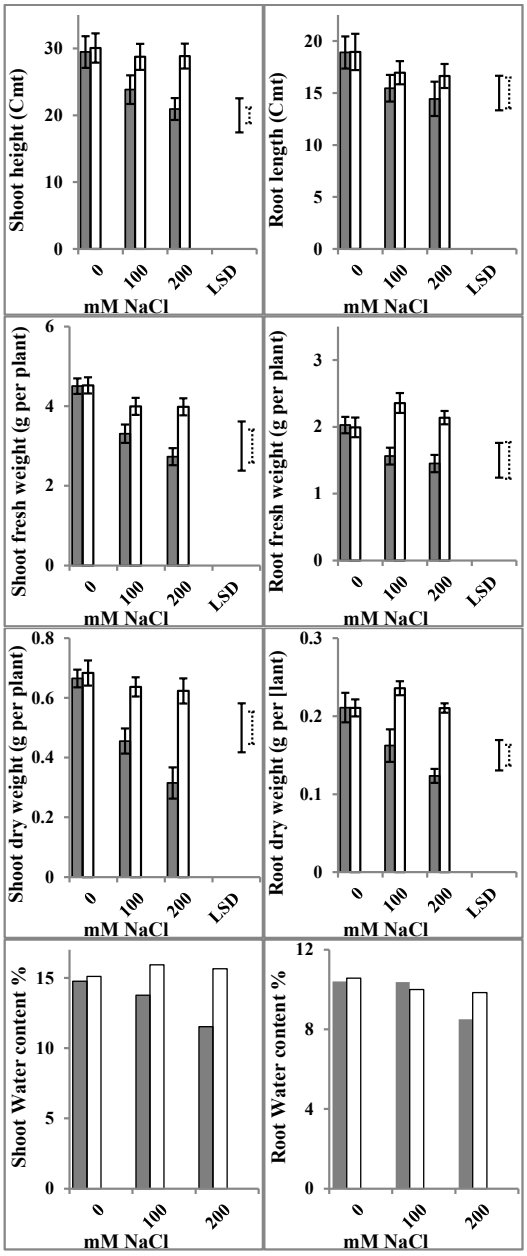

Fig. 1. Changes in length, fresh and dry weights and water content of shoot and root of 21-day old soybean seedlings emerged from grains soaked either in water (black bars) or in AsA (white bars) and both grown under NaCl treatment [Values are means ± SD. The full data were first subjected to analysis of variance (ANOVA) followed by

least significant differences (LSD). Vertical bars represent LSD at P≤ 0.05 for samples without AsA, entire; or with AsA, dotted). Values of water content are derived from the original means; neither ANOVA nor LSD was applied]

In Fig. 2, both concentrations of NaCl caused significant decreases in K^+ content, nonetheless, the highest concentration appeared more effective than the lowest one; meanwhile, Na^+ content was significantly increased. On the other hand, great diminish in the ratio of K^+/Na^+ was detected in the treated seedlings; the diminution was more obvious in seedlings treated with high than low concentration. Nonetheless, the use of AsA for seed priming led to a recovery from the effect of NaCl treatments on the changes of both K^+ and Na^+ contents. The application of AsA increased K^+ content and even some rises in magnitudes were detected over the control values while the vast increase of Na^+ content due to salinity was greatly decreased. Besides, AsA led to marked increases in K^+/Na^+ ratio although this increase did not compensate for reaching the control. Conversely, NaCl significantly increased soluble sugars but lowered insoluble sugars content with most likely slight changes in total sugars (Fig. 2). The effects of both concentrations of NaCl on all fractions of sugars (soluble, insoluble and total) appeared with no great differences. The application of AsA greatly counterbalanced the effects of NaCl on both soluble and insoluble sugars; AsA led to decrease soluble sugars and to elevate insoluble sugars and -to some extent- total sugars.

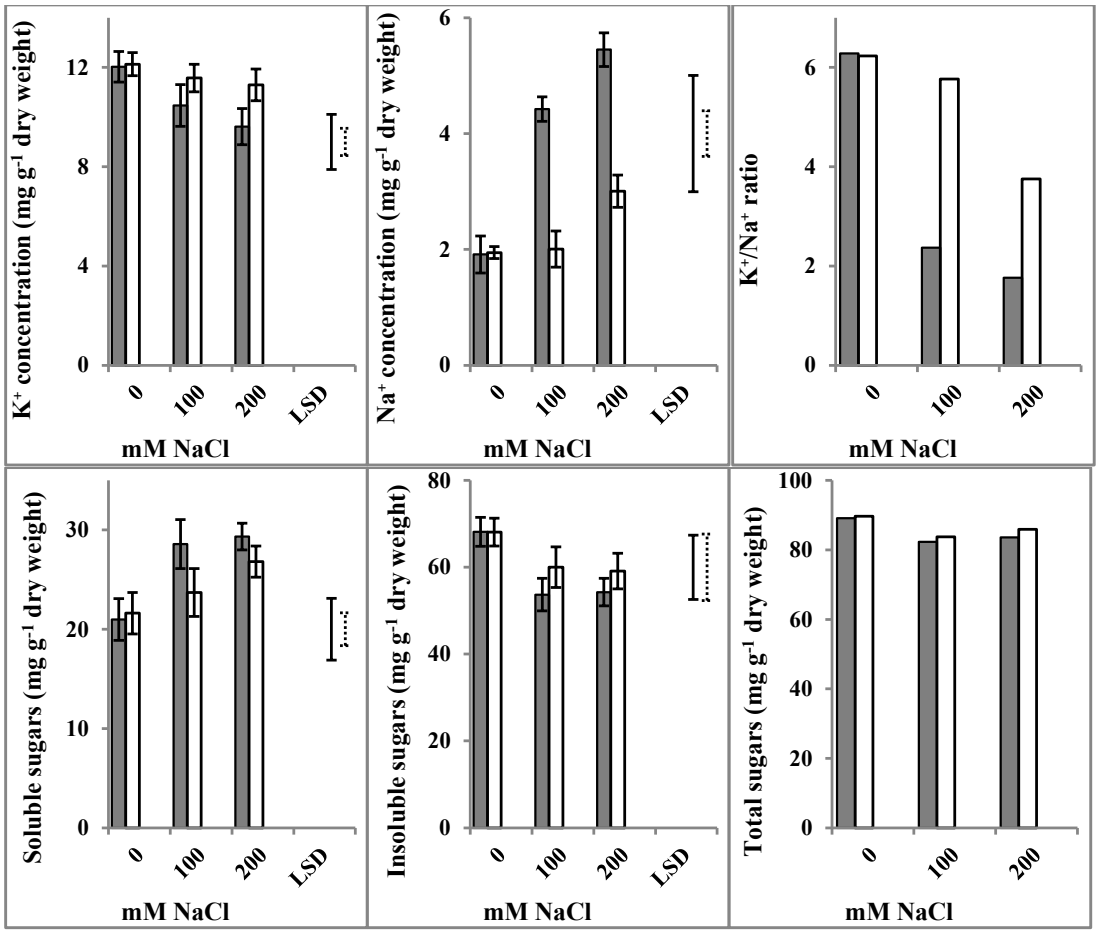

Fig. 2. Changes in contents of K, Na and sugars of 21-day old soybean seedlings emerged from grains soaked either in water (black bars) or in AsA (white bars) and both grown under NaCl treatment [Values are means± SD. The full data were first subjected to analysis of variance (ANOVA) followed by least significant differences (LSD). Vertical bars represent LSD at P≤ 0.05 for samples without AsA, entire; or with AsA, dotted). Values of K/Na and total sugars are derived from the original means; neither ANOVA nor LSD was applied]

ROS and antioxidants

Figure 3 shows significant accumulations of H_2O_2 and MDA were detected in soybean seedlings after treatment with NaCl at both applied concentrations; the magnitude of accumulation augmented with increasing NaCl concentrations. Conversely, NaCl concomitantly led to a significant decrease in the internal content of AsA; the decrease level was obvious at 200 mM than 100 mM. Nevertheless, the priming of soybean seeds with AsA resulted in diminution in the NaCl-induced accumulation of H_2O_2 and MDA, the retraction was most likely similar for both concentrations of NaCl whereas the internal AsA level was significantly augmented.

In Fig. 4, NaCl resulted in significant inhibition in CAT activity of soybean seedlings; the effect was greater under 200 mM NaCl than the low concentration. To a lower extent, APX activity was similarly inhibited; however, priming in AsA raised the activities of both CAT and APX reaching the control value; CAT exceeded the control levels.

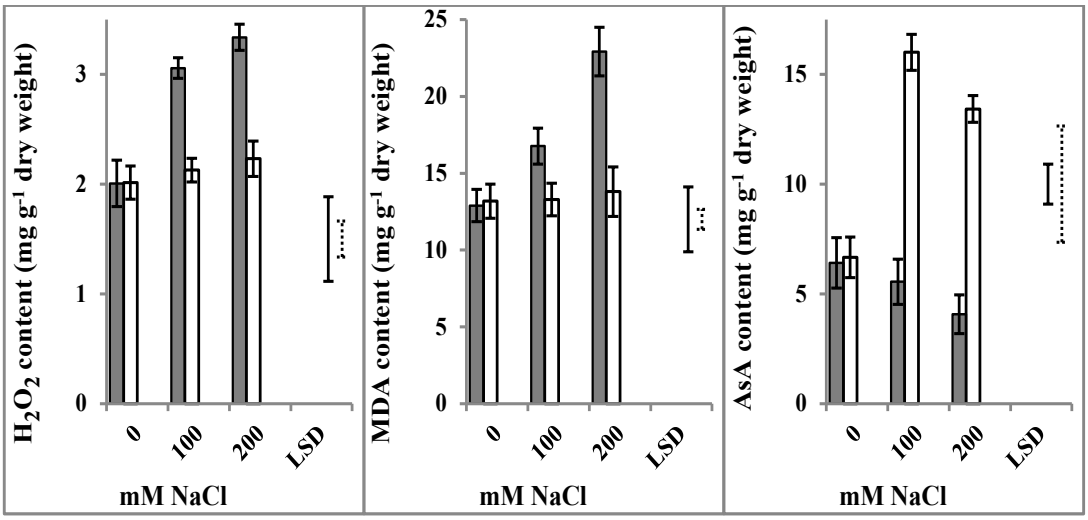

Fig. 3. Changes in contents of H_2O_2, MDA and internal AsA of 21-day old soybean seedlings emerged from grains soaked either in water (black bars) or in AsA (white bars) and both grown under NaCl treatment [Values are means ± SD. The full data were first subjected to analysis of variance (ANOVA) followed by least significant differences (LSD). Vertical bars represent LSD at P≤ 0.05 for samples without AsA, entire; or with AsA, dotted)]

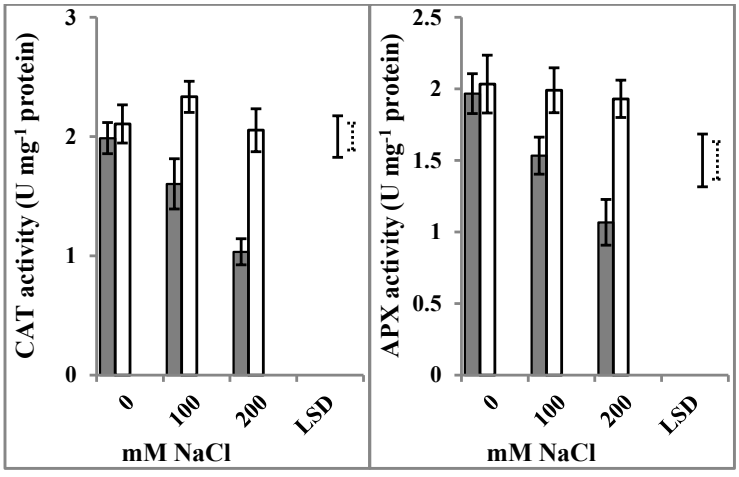

Fig. 4. Changes in catalase and peroxidase activities of 21-day old soybean seedlings emerged from grains soaked either in water (black bars) or in AsA (white bars) and both grown under NaCl treatment [Values are means ± SD. The full data were first subjected to analysis of variance (ANOVA) followed by least significant differences (LSD). Vertical bars represent LSD at P ≤ 0.05 for samples without AsA, entire; or with AsA, dotted)]

Fatty acid composition

Table 1 shows that NaCl disrupted fatty acid composition of soybean oil; caprylic acid completely disappeared after treatment with 200mM NaCl whereas capric and undecanoic acid appeared only in response to 200mM. Tridecanoic acid appeared only after treatment with 200mM NaCl either alone or under the AsA application. NaCl at both concentrations disappeared Myristoleic, the acid reappeared again after priming of seeds in AsA. In the same pattern, treatment with only 100 mM NaCl appeared pentadecanoic acid but not 200 mM; nonetheless, AsA re-appeared it with both concentrations. On the contrary, NaCl greatly decreased palmitoleic acid; AsA seemed to eliminate such decreases. On the other hand, the disappearance of gama-linolenic acid and cis-11,14-eicosadienoic by NaCl was overcome by priming in AsA. Also, AsA overcame the NaCl-induced lowering of linolenic, oleic, elaidic, cis-5,8,11,14,17-eicosapentaenoic, cis-8,11,14-eicosatrienoic and cis-11,14,17-eicosatrienoic. Contrarily, NaCl at both concentrations slightly increased stearic acid, 100mM greatly lowered cis-11-eicosenoic but 200mM resulted in its complete disappearance; AsA caused a recovery from the effect of both NaCl concentrations. 100 mM NaCl led to a complete disappearance of arachidic acid but AsA caused overcome. On the contrary, heneicosanoic, behenoic and tricosanoic acids appeared by NaCl whereas both nervonic and lignoceric acid were decreased while AsA counterbalances the impacts of 100 mM NaCl on nervonic acid but not lignoceric acid.

Table 2 represents the percentages of fatty acid composition in the oil. The percentages of caprylic, capric, undecanoic, tridecanoic, myristoleic, myristic, pentadecanoic, palmitoleic, palmitic, heptadecanoic, gama-linolenic stearic, cis-8,11,14- eicosatrienoic, cis-11,14,17-eicosatrienoic, heneicosanoic, behenoic, tricosanoic, nervonic, and lignoceric acids were very low (traces to 5%). NaCl raised these percentages while AsA had no effect. Linolenic, oleic and elaidic acid represented from 11-13%, salinity decreased these percentages as well as the presence of AsA application although AsA led to some rises. No more than 6% are the percentages of cis-5,8,11,14,17-eicosapentaenoic, cis-11,14-eicosadienoic, cis-11-eicosenoic, arachidic, cis-4,7,10,13,16,19-docosahexaenoic, and cis-13,16-docosadienoic; however, salinity decreased these percentages while AsA alleviated them.

As a whole, NaCl increased total saturated fatty acids; the magnitude of increase was alike for both concentrations; however, AsA lowered this elevation (Table 3). Contrarily, NaCl greatly diminished the summations of omega-3, omega-7 and omega-9; the diminution was greater by 200 mM than by the lowest concentration. Besides, salinity completely disappeared both omega-5 and omega-6. AsA counterbalanced these decrements to reach mostly the control levels and even there were somewhat increases in the omega-5 and omega-7. Also, NaCl decreased the unsaturated fatty acids (mono- and poly), such that the total unsaturated fatty acids were declined, the higher concentration the more the magnitude of decrease was. Priming of seeds in AsA raised total and unsaturated fatty acids.

Table 4 indicates that total unsaturated fatty acids comprises 82% of total fatty acids; a percentage that decreased following treatment with 100 mM and 200 mM NaCl to 61% and 50%, respectively but AsA led again to elevations upto 78% and 67%, respectively. The unsaturated fatty acids are formed from the poly- (42%) and the mono-unsaturated ones (40%); the formers were more affected than the latter ones reaching 25% and 35%, respectively by 100mM NaCl and about 24% and 25%, respectively by 200mM NaCl. Such impact was mitigated by soaking of seeds in AsA so that NaCl became mostly with no effect; the polyunsaturated fatty acids percentages became 40% and 39% by 100mM and 200mM NaCl, respectively while the monounsaturated fatty acids percentages became 38% and 28%, respectively. The unsaturated fatty acids are composed from omega-3, omega-6 and omega-9 (29%, 12% and 34%, respectively in control samples), while omega-5 and omega-7 formed 1% and 5%. Salinity greatly affected these acids whilst AsA led to rises to reach that of control. Saturated fatty acids form about 17% of the total fatty acids; NaCl at 100mM and 200 mM elevated this percentage to 38% and 49%, respectively while AsA led to retraction to 21% and 32%, respectively. NaCl diminished total fatty acids but priming seeds caused overcome.

TABLE 1. Changes in fatty acid concentrations (mg g^{-1} oil) of soybean (cultivar Giza 21) emerged from grains soaked in either water or AsA and both were grown under treatment with NaCl at 100 mM (100) or 200 mM (200).

Fatty acids	Concentration (mg g^{-1} oil)				
	C	100-AsA	200-AsA	100+AsA	200+AsA
Caprylic (C8)	2.24±0.123	2.24±0.111	-	2.24±0.008	2.24±0.009
Capric (10)	-	-	0.25±0.002	-	-
Undecanoic (C11)	-	-	0.22±0.002	-	-
Tridecanoic (C13)	-	-	0.27±0.002	-	0.27±0.014
Myristoleic (C14)	0.59±0.004	-	-	0.71±0.066	0.78±0.048
Myristic (C14)	0.45±0.004	0.41±0.003	0.41±0.004	0.55±0.004	0.64±0.055
Pentadecanoic (C15)	-	0.33±0.003	-	0.33±0.002	0.74±0.046
Palmitoleic (C16)	2.62±0.211	1.72±0.166	0.88±0.077	2.98±0.214	0.79±0.063
Palmitic (C16)	1.19±0.098	1.37±0.112	0.91±0.087	1.22±0.133	-
Heptadecanoic (C17)	0.59±0.044	0.61±0.052	0.79±0.041	0.60±0.051	-
gama-Linolenic (C18)	2.86±0.201	-	-	2.19±0.154	1.65±0.133
Linolenic (C18)	5.93±0.512	2.71±0.201	1.37±0.093	3.31±0.283	2.33±0.212
Oleic (C18)	6.10±0.632	3.93±0.403	2.90±0.154	3.61±0.313	2.88±0.265
Elaidic (C18)	7.02±0.612	3.46±0.303	1.02±0.087	5.37±0.441	1.99±0.167
Stearic (C18)	1.02±0.088	2.96±0.207	1.96±0.154	1.18±0.105	-
cis-5,8,11,14,17-Eicosapentaenoic (C20)	3.46±0.305	1.49±0.117	1.46±0.122	2.48±0.203	2.46±0.221
cis-8,11,14-Eicosatrienoic (C20)	2.69±0.201	2.34±0.187	1.26±0.113	2.67±0.207	1.48±0.132
cis-11,14-Eicosadienoic (C20)	1.50±0.111	-	-	1.13±0.089	0.74±0.564
cis-11-Eicosenoic (C20)	2.15±0.203	1.10±0.087	-	1.55±0.117	1.15±0.101
cis-11,14,17-Eicosatrienoic (C20)	1.52±0.117	1.12±0.069	1.01±0.077	1.51±0.116	1.21±0.111
Arachidic (C20)	0.83±0.064	-	0.84±0.062	0.84±0.062	0.77±0.064
Heneicosanoic (C21)	-	1.09±0.098	1.09±0.087	0.20±0.013	1.09±0.092
cis-4,7,10,13,16,19-Docosahexaenoic (C22)	1.61±0.112	1.01±0.095	1.03±0.093	1.60±0.127	1.61±0.096
cis-13,16-Docosadienoic (C22)	2.34±0.198	-	-	2.00±0.153	1.72±0.108
Behenoic (C22)	-	-	2.02±0.101	0.80±0.685	0.98±0.078
Tricosanoic (C23)	-	1.29±0.108	1.23±0.107	-	1.29±0.112
Nervonic (C24)	2.68±0.231	1.68±0.111	1.68±0.114	1.98±0.147	1.68±0.121
Lignoceric (C24)	2.57±0.228	2.65±0.198	2.37±0.187	1.06±0.0854	2.57±0.204

TABLE 2. Changes in fatty acid percentages of soybean (cultivars Giza 21) emerged from grains soaked in either water or AsA and both were grown under treatment with NaCl at 100 mM (100) or 200 mM (200)

Fatty acids	Percentage				
	C	100-AsA	200-AsA	100+AsA	200+AsA
Caprylic (C8)	4.31	6.70	-	5.32	6.78
Capric (10)	-	-	0.99	-	-
Undecanoic (C11)	-	-	0.88	-	-
Tridecanoic (C13)	-	-	1.08	-	0.82
Myristoleic (C14)	1.14	-	-	1.70	2.35
Myristic (C14)	0.86	1.22	1.62	1.31	1.95
Pentadecanoic (C15)	-	0.99	-	0.78	2.23
Palmitoleic (C16)	5.03	5.12	3.51	7.07	2.38
Palmitic (C16)	2.28	4.09	3.65	2.89	-
Heptadecanoic (C17)	1.14	1.81	3.18	1.42	-
gama-Linolenic (C18)	5.51	-	-	5.21	5.00
Linolenic (C18)	11.42	8.09	5.48	7.87	7.05
Oleic (C18)	11.74	11.72	11.62	8.57	8.71
Elaidic (C18)	13.51	10.33	4.08	12.76	6.03
Stearic (C18)	1.97	8.84	7.87	2.79	-
cis-5,8,11,14,17-Eicosapentaenoic (C20)	6.66	4.44	5.87	5.88	7.43
cis-8,11,14-Eicosatrienoic (C20)	5.18	6.98	5.04	6.35	4.47
cis-11,14-Eicosadienoic (C20)	2.89	-	-	2.69	2.24
cis-11-Eicosenoic (C20)	4.13	3.29	-	3.69	3.48
cis-11,14,17-Eicosatrienoic (C20)	2.92	3.34	4.03	3.58	3.66
Arachidic (C20)	1.61	-	3.36	1.99	2.34
Heneicosanoic (C21)	-	3.25	4.37	0.47	3.29
cis-4,7,10,13,16,19-Docosahexaenoic (C22)	3.11	3.02	4.13	3.81	4.88
cis-13,16-Docosadienoic (C22)	4.50	-	-	4.74	5.21
Behenoic (C22)	-	-	8.11	1.89	2.96
Tricosanoic (C23)	-	3.85	4.92	-	3.90
Nervonic (C24)	5.15	5.01	6.73	4.71	5.07
Lignoceric (C24)	4.95	7.92	9.49	2.51	7.77

TABLE 3. Changes in the concentrations of total saturated and unsaturated fatty acids (mg g^{-1} oil) of soybean (cultivar Giza 21) emerged from grains soaked in either water or AsA and both were grown under treatment with NaCl at 100 mM (100) or 200 mM (200)

Fatty acids	Concentration (mg g^{-1} oil)				
	C	100-AsA	200-AsA	100+AsA	200+AsA
Total Fatty Acids	51.96	33.50	24.94	42.10	33.06
Saturated Fatty Acids	8.90	12.95	12.35	9.00	10.59
Sum of Omega-3	15.21	8.67	6.12	11.58	9.09
Sum of Omega-5	0.59	-	-	0.71	0.78
Sum of Omega-6	6.70	-	-	5.32	4.12
Sum of Omega-7	2.62	1.72	0.88	2.98	0.79
Sum of Omega-9	17.94	10.17	5.59	12.51	7.70
Monounsaturated Fatty Acids	21.15	11.88	6.47	16.21	9.26
Polyunsaturated fats Fatty Acids	21.91	8.67	6.12	16.90	13.21
Total Unsaturated Fatty Acids	43.06	20.55	12.59	33.10	22.47

TABLE 4. Changes in the percentages of total saturated and unsaturated fatty acids of soybean (cultivar Giza 21) emerged from grains soaked in either water or AsA and both were grown under treatment with NaCl at 100 mM (100) or 200 mM (200)

Fatty acids	Percentage				
	C	100-AsA	200-AsA	100+AsA	200+AsA
Total Fatty Acids	100	100	100	100	100
Total Unsaturated Fatty Acids	82.88	61.33	50.49	78.63	67.97
Polyunsaturated fats Fatty Acids	42.18	25.87	24.55	40.14	39.95
Monounsaturated Fatty Acids	40.70	35.47	25.94	38.49	28.02
Sum of Omega-3	29.28	25.87	24.55	27.50	27.50
Sum of Omega-5	1.14	-	-	1.70	2.35
Sum of Omega-6	12.90	-	-	12.64	12.45
Sum of Omega-7	5.03	5.12	3.51	7.07	2.38
Sum of Omega-9	34.53	30.35	22.43	29.72	23.29
Saturated Fatty Acids	17.12	38.67	49.51	21.37	32.03

Discussion

Salinity can inhibit plant growth via altering the water potential, imbalance of ions, impairing of both cell division and expansion in addition to production of ROS causing oxidative damage (Tuteja et al., 2009; Nemat Alla et al., 2020). Elevated Na^+ level lowers the concentrations of the other cations in the plant, can disrupt the plant cationic balance (Acosta-Motos et al., 2017). Besides, Jouyban (2012) found that the nutrient imbalances could be due to competition between Na^+ or Cl^- and other ions as K^+, Ca^{2+}, and NO_3^-. Thus, the decrease of K concomitant with the elevated Na level in the present results could confirm nutrient imbalances or even deficiencies. In this respect, salt stress lowered K and K/Na ratio (D'Amelia et al., 2018; Nemat Alla et al., 2020). Moreover, the increased K/Na ratio is related with the plant salinity stress tolerance (Kordrostami et al., 2017).

Usually, plants resist salt stress by synthesis of certain organic compatible solutes that can help plants to survive extremist osmotic stress as soluble sugars because of their osmoprotectant properties (Negrao et al., 2017; Nemat Alla et al., 2020). The present investigation showed similar rises in soluble sugars. In addition, there is an antioxidant system for scavenging the generated ROS, which can destroy lipids, proteins and DNA. Of these ROS, MDA, $O_2^{\cdot-}$ and H_2O_2 were significantly augmented following treatment with NaCl (Nemat Alla & Hassan, 2020).

In the present study, H_2O_2 and MDA exhibited vast enhancement while CAT and APX activities were inhibited. CAT can scavenge H_2O_2 and APX can dismutase H_2O_2 to water and molecular oxygen using AsA as a vital electron source (Van Doorn & Ketsa, 2014; Nemat Alla et al., 2020). Therefore, the inhibited activities of CAT and APX would cause less efficient detoxification of H_2O_2 detoxification leading so to be accumulated with a concomitant increase in oxidative stress. In this account, there were significant decreases in the antioxidant enzymes activities in soybean plants exposed to 100 and 200mM NaCl (Amirijani, 2010).

AsA can act as a non-enzymatic antioxidant for the protectant of plant organelles and cells from the adversaries of ROS that over-accumulate because of oxidative stress (Naz et al., 2016). Mahajan & Sanejouand (2015) found that salinity affects level of AsA in sensitive more than in tolerant species. Moreover, NaCl stress produced a decline in the AsA content in leaves of salt-sensitive rice, but not leaves and roots of the tolerant cultivar (Lee et al., 2013). Thus, the alleviation of salinity upon AsA application might result from its importance in the effective regulation of plant antioxidative metabolism. From other side, Safwat & Abdel Salam (2022) indicated that exogenous proline and glycine betaine improved physiological parameters and reduced oxidative damage suggesting increased tolerance to oxidative damage caused by salinity via upregulating antioxidant defense system.

Although plants have an antioxidant system to tolerate stresses, salinity could cause impairing

of this system in sensitive species. So, exogenous additives might be helpful for supporting plants to tolerate stresses (Perveen et al., 2018). AsA has an important role in stress via protecting plants from salt-induced oxidative damage via the maintenance and/or increase of many antioxidant enzymes. AsA activates many enzymes and reserves physiological and signaling pathways and affects many enzymes, eliminating the oxidative stress via synergetic function with other antioxidants (Foyer & Nectar, 2005).

As shown, AsA effectively improved the growth characteristics of soybean seedlings under salinity stress so it could be suggested that AsA can alleviate the deleterious effects of salinity stress. Khan et al. (2011) indicated that the beneficial effects of AsA can be attributed to the involvement in the increasing the content of GA3 and IAA while lowering ABA content increases photosynthetic pigments. The priming of seeds in AsA, herein, counterbalanced the increases in H_2O_2 induced by NaCl indicating that AsA is efficient against the induced oxidative damage. This amelioration could arise from protection against uncontrolled oxidation and improved plant growth (Bartoli et al., 2006). Conversely, AsA led to a slight accumulation of soluble carbohydrates as compared with the corresponding saline-treatment alone. Such rises might have a vital role in plant osmotic protection and as scavengers of free radicals. Considering soluble sugars rises under salinity stress associated with the decrease in photosynthetic rate, the insoluble sugars levels would undergo declines.

Moreover, AsA enhanced K may be due to the positive effect on root growth that consequently increased the absorption level of different nutrients and alleviated the harmful effects of saline impacts. Besides, Khan et al. (2011) suggested that AsA could inhibit the stress-induced increase in the essential electrolytes leakage following peroxidative damage to cells membranes. In addition, the lowering of Na^+ by AsA was synchronized with elevation of K^+. This finding is affirmed by the conclusion that wheat plants protection against salt stress by AsA exogenous supply is carried out indirectly because of AsA effect on K^+ uptake that plays a vital role in plant metabolism (Athar et al., 2008). In general, the increased K^+ concurrent with decreasing Na^+ consequently raised the K/Na ratio indicating that AsA could mediate mitigation of salinity stress by adjusting the mineral balance. In addition, AsA application raised the endogenous AsA and lowered the levels of H_2O_2 and MDA; both effects could alleviate the hazardous effect of salinity stress.

These conditions would be highly destruction for sensitive plants without the support of external AsA. In accordance, application of AsA improves endogenous AsA, growth and CAT activity in salinized hydroponic wheat culture (Athar et al., 2009). So, in the present results, the inhibited activities of CAT and APX under the influence of salt treatment, was overcome upon AsA application. Generally, AsA supported soybean to withstand NaCl stress via increasing K and decreasing Na concomitant with rises in K/Na ratio and in the meantime diminishing ROS and elevating antioxidants.

The produced seed oil is composed of nearly 16% saturated fatty acids (foremost palmitic and stearic), 24% mono-unsaturated fatty acids (foremost oleic acid) and 60% polyunsaturated fatty acids (foremost linoleic and linolenic). Mostly, oil contents and fatty acid compositions are important attributes in oil crops determining seed oil quality. As a rule, seeds rich in essential fatty acids are necessary for good health (Kajla et al., 2015). Besides, Johnson & Bradford (2014) concluded that the quality oil is worthy depending on the contents of essential fatty acids. Many factors (environmental, agronomic, genetic, etc) influence such features (Primomo et al., 2002; Savoire et al., 2015). Herein, NaCl decreased total fatty acids in synchronization with decreases in the unsaturated fatty acids but increased the saturated ones. Total fatty acids declines were primarily due to the decrease in the unsaturated fatty acids. In accordance, saturated fatty acids such as stearic acid was increased by saline stress (Shaki et al., 2019). Such suggestion confirm that NaCl increases the saturated fatty acids but decreases the unsaturated fatty acids.

Oleic, linoleic and linolenic acids deceased due to salinity as also the other omega fatty acids indicating the deleterious effects of salinity on the quality of oil probably due to the effect on the metabolism of lipid. Primomo et al. (2002) indicated that salinity stress could change fatty acid composition of soybean cultivars seeds giving rise to reducing its total fatty acids. Besides, Bybordi et al. (2010) confirmed that

linoleic and linolenic acids percentages decreased due to salinity although there were increases in oleic acid.

Nonetheless, soaking of the seed in AsA counterbalanced these deleterious effects; AsA raised the unsaturated fatty acids concomitant with balancing in the saturated fatty acids and consequently led to rises in the total fatty acids. Kachroo et al. (2003) suggested that elevating percentage of linoleic and linolenic acids because of AsA application referring to increasing lipid membranes fluidity and oleoyl-phosphatidylcholine desaturase. Drought, as another abiotic stress, highly diminished the unsaturated fatty acids but increased the saturated ones while the application of stigmasterol elevated the levels of the unsaturated fatty acids but decreased the saturated ones (Hassan et al., 2021). So, the application of AsA led to improvement of fatty acids in seed oil. Mostly, the quality of oil depends on the essential fatty acid contents (Johnson & Bradford, 2014). Thus, salinity herein caused malfunction in the characteristics of soybean oil. It caused increases in the saturated fatty acids but lowered the differed omega acids with a consequent decline in the unsaturated ones.

Conclusions

The present results could conclude that salinity decreased the growth of soybean, K^+, K^+/Na^+ ratio, AsA and activities of CAT and APX but increased Na^+, soluble sugars, MDA and H_2O_2; the higher the NaCl concentration, the great the effects were. Besides, total fatty acid contents were decreased mainly due to the decreased unsaturated fatty acids although the saturated fatty acids were raised mainly due to the increased palmitic and stearic acids. Nonetheless, AsA made efficient growth synchronized with rises in K^+, K^+/Na^+ ratio and AsA as well as activities of CAT and APX concomitantly with retractions in the accumulations of Na^+, MDA and H_2O_2. Meanwhile, AsA better –to great extent- the quality of oil yield. AsA counterbalanced the deleterious influence of NaCl on fatty acid composition; AsA lowered the saturated fatty acids but elevated the unsaturated fatty acids synchronized with rises in the total ones indicating the relieve of AsA to soybean from salinity impacts through enhancing osmoprotection and antioxidants and improved the oil yield quality particularly ω fatty acids.

Competing interests: The authors report no conflicts of interest regarding this work.

Authors' contributions: Enas G. Budran and Mamdouh M. Nemat Alla have conceived and designed the experiments. Nemat M. Hassan and Mamdouh M. Nemat Alla have performed the statistical analysis. Enas G. Budran and Manal A. Abdelhamid have performed the experiments and result analysis. Nemat M. Hassan, Mamdouh M. Nemat Alla and Enas G. Budran have performed manuscript drafting and prearranging of the manuscript as well as reading and establishing the ultimate manuscript.

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التأثير التحسيني للمعاملة بالأسكوربات على النمو وتكوين الأحماض الدهنية الزيتية في فول الصويا تحت ظروف الملوحة

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يهدف البحث إلى تحسين نمو فول الصويا وتكوين الأحماض الدهنية لمحصوله الزيتي في ظل النمو تحت تأثير الملوحة عن طريق نقع البذور في محلول الاسكوربات. فقد تسببت الملوحة بتركيزي كلوريد الصوديوم (100 ملي مولار أو 200 ملي مولار) لمدة 11 يومًا لبادرات فول الصويا بعمر 10 أيام إلى خفض كل من محتوى البوتاسيوم ونسبة البوتاسيوم/الصوديوم والمحتوى الداخلي للأسكوربات، كما أدت إلى تثبيط أنشطة إنزيمات الكاتلاز وأسكوربات بيروكسيديز، بينما ارتفعت محتويات الصوديوم والسكريات الذائبة ومالوندای ألدهايد وفوق أكسيد الهيدروجين. وفي الوقت نفسه، انخفض محتوى الأحماض الدهنية الكلية في المحصول الزيتي للبذور الناضجة بالتزامن مع انخفاض في محتوى الأحماض الدهنية غير المشبعة بينما زاد محتوى الأحماض الدهنية المشبعة (خاصة بالمتيك واستياريك). أما نقع البذور في محلول الاسكوربات فقد أدى الى تحسين النمو بالتزامن مع ارتفاع محتوى البوتاسيوم ونسبة البوتاسيوم/الصوديوم والمحتوى الداخلي للأسكوربات بالإضافة إلى زيادة أنشطة إنزيمات الكاتلاز وأسكوربات بيروكسيديز متزامنا مع تقلص الارتفاع في محتويات الصوديوم والسكريات الذائبة ومؤشرات الإجهاد التأكسدي. وإلى جانب ذلك ، فقد نقصت الأحماض الدهنية المشبعة بينما زادت الأحماض الدهنية غير المشبعة بالتزامن مع ارتفاع في إجمالي الأحماض الدهنية الكلية. وتشير هذه النتائج إلى حدوث تخفيف من تأثير الملوحة عن طريق نقع البذور في محلول الاسكوربات من خلال تعزيز كل من الحماية من تأثير الأسموزية ومضادات الأكسدة بالإضافة إلى تحسين جودة الزيت وزيادة محتوى الأحماض الدهنية أوميجا بأنواعها.