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Does Ascorbic acid mitigates the salinity Sufferences Assay for absolute growth indices of Canola (*Brassica napus* L.)

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An experiment was managed for study of alleviating potential of Ascorbic acid for salinity effects on Canola (*Brassica napus* L.). The experiment was conducted in pots arranged with complete randomization. Sandy loam soil was mixed thoroughly and pots were filled with six kg of soil. Seeds of the two Canola varieties namely AC-Exel and Cyclone were grown. After 15 days of germination, thinning of plants was carried out to keep three plants in each pot. After 25 days of germination, salinity treatments of 50.0 and 100 mM with 50mg L^{-1} foliar spray of Ascorbic acid was applied. Two sprays were applied at interval of fifteen days. After 40 days of emergence, studies were carried out taking three replicates of each treatment. The effects of Ascorbic acid and salinity treatments were highly influential in changing the root length, dry biomass of root, growth of shoot, biomass of shoot and biomass of leaves. However, the change in leaf area was not significant. The response of two varieties varied significantly to different treatments in term of root and shoot biomass. Salinity stress reduced the growth and biomass production while significant promotion by Ascorbic acid solely and as ameliorative role for both levels of salinity was noted. The mitigating effects of Ascorbic acid were clearer for high salinity level because higher salinity level, as stress, revealed clear differences.

Keywords: Ascorbic acid, Biomass, Canola , Growth, Salinity

INTRODUCTION

Among the limitations imposed on plant vital activities by external abiotic and biotic factors, photosynthesis got prime importance as it makes plant capable of converting energy into mass (Ali et al. 2020, Nazar et al. 2020, Zaheer et al. 2020a). Due to these factors, agriculture sector is facing problem in producing more food (Ali et al. 2021, Saleem et al. 2020b, Zaheer et al. 2020b). Among these, the major abiotic stresses like water stress, salinity and high temperature are influencing much crop production (Hashmat et al. 2021, Zaheer et al. 2020b). Salinity problem is increasing in the world and ia expected to cause 50% loss of cultivated land by the year 2050 (Alam et al. 2020).

The crop growth and productivity, in most of the soils under high salt contents, is adversely affected. Main sources of salinity are two in number: natural or primary, resulting from minerals weathering obtained from parent rock that is saline in nature (Ashraf, 1994), and due to interference of human beings such as overgrazing, irrigation, deforestation or extreme cropping secondary salinization occure (Ashraf, 1994; 2004; Ashraf and Foolad, 2007).

Ascorbic acid is a very important antioxidants (Smirnoff, 2000a). High concentration of

endogenous Ascorbic acid in plants is essential to antagonize the oxidative stress for regulating other plant metabolic processes. Exogenous Ascorbic acid, as a seed priming or foliar spray, can increase the endogenous Ascorbic acid (Chen and Gallie, 2004). Ascorbic acid is a non-enzymatic antioxidant of plants and prevents plant from oxidative stresses (Akhlaghi et al. 2018, Sharma et al. 2019).

Ascorbic acid is reported to be used in tolerance of heat stress (Alayafi, 2020); water stress (Ibrahim and Opabode, 2019); metal stress (Alamri et al. 2018); has antioxidant activity (Allahveran et al. 2018) and is involved in Ca^{+2} avalibility (Elkelish et al. 2019). Ascorbic acid promotes plant growth and enhances its potential to withstand against stress by synthesizing hydroxyproline category of proteins (Bilska et al. 2019). Ascorbic acid mediated improvement in salinity tolerance has been studied on many plants (Sajid and Aftab, 2009;Younis et al., 2010; Aly et al. 2012).

Canola (*Brassica napus* L.) is placed on second ranked among oilseed crops after soybean, Seeds of Canola supply meals with protein of about 35 to 40 percent and hold 40 percent oil 13 percent of the oil supply in the world is provided by Canola (Snowdon et al. 2007). Due to low erucic acid in Canola, exellent quality of edible oil is obtained from Canola. Oil is a significant source of fatty acids for the purpose of nutrition of humans and industrial product (Friedt and Lühs, 1998).

MATERIALS AND METHODS

The pot culture experiment was planned to study the ameliorative potential of Ascorbic acid for salinity effects on growth of Canola (*Brassica napus* L.). The selected soil was mixed and pots were filled with six kg of soil. Seeds of the two Canola varieties namely AC-Exel and Cyclone obtained from Ayub Agriculture Research Institute, Faisalabad (Pakistan) were sown. The pots were placed with complete randomization by arrangement. The germination took place after five days. After 15 days of germination, thinning of plants was carried out to keep 3 plants in each pot. Basic dose of organic manure and fertilizer was applied as a source of nutrient. All other agronomic practices were applied in accordance with the requirement of crop. After 25 days of germination, following treatments were applied

T_1 = Control.; T_2 = 50.0 mM salinity; T_3 = 100.0 mM salinity; T_4 = 50.0 mg L^{-1} Ascorbic acid spray; T_5 = 50.0 mM of salinity and 50mg L^{-1} Ascorbic acid spray;T_6 = Salinity level of 100 mM and 50 mg L^{-1} Ascorbic acid spray.

Sodium chloride salt was used for developing salinity. Ascorbic acid was sprayed twice with an interval of 15 days. The growth of stem, root and leaves of 40 days age plants were studied .Data were collected from three plants of each treatment. The data were analyzed statistically by applying (ANOVA) techniques based on Completely Randomized Design (CRD) with two factor factorial arrangements set at level of statistical significance of 5%. Representation of data were as means and standard deviation of each parameter and means were separated by using Duncan's Multiple Range test at 5% level of probability where treatment means, variety mean and the interaction among them were compared. By using MSTAT-C computer statistical program, significant F values were obtained and differences between individual means were tested with the use of LSD tests at significant level.

RESULTS

Shoot Length (cm)

Shoot length of Cyclone variety was more than that of Ac-Exel by 3.47%. Salinity level of 50 mM reduced the shoot length by 12.37%. While when the same level of salinity was accompanied with foliar 50 mgL^{-1} of Ascorbic acid, the shoot length was increased in by 5.88% instead of decreasing. Salinity levels of 100 mM reduced shoot length by 18.26%. With application of Ascorbic acid, 8.67% increase in shoot length was observed. Salinity of 50 mM along with application of Ascorbic acid also increased the shoot length by 9.90%. In short, salinity levels reduced the shoot length while the effect of salinity was nullified by Ascorbic acid.

Shoot Dry Weight (g)

Salinity level of 50 mM reduced the shoot dry weight by 14.44%. While when the same level of salinity was accompanied with foliar 50 mgL^{-1} of Ascorbic acid, the shoot dry weight was increased by 17.94% instead of decreasing. Shoot dry weight of Cyclone showed more increase than AC-Exel variety by 15.65%. Salinity level of 100 mM reduced dry weight of root by 24 %. By Ascorbic acid spray 53.02% increase in the shoot dry weight was noted.

Table 1: Root growth of 40 days old Canola (*Brassica napus* L.) grown under salinity stress [50.0 mM and 100.0 mM)] and exposed to foliar spray of Ascorbic acid [50.0 mg L^{-1}] at 15 and 25 days of age [Values represent means ± SE] Values in parentheses represent percentage increase (+) / decrease (-) over control

		0 mM Salinity + 0mg L^{-1} AsA	(50 mM Salinity + 0mg L^{-1} AsA)	100 mM Salinity + 0mg L^{-1} AsA	0 mM Salinity +50mg L^{-1} AsA	50 mM Salinity + 50mgL^{-1} AsA	100 mM Salinity + 50mgL^{-1} AsA	Mean
Root length(cm) LSD=2.33	AC-Exel	27.33±1.52	24.33±2.08	20.00±1.00	28.33±1.52	22.33±2.51	19.33±1.52	23.61±3.80 [a]
	%age		-10.97%	-26.82%	+3.65%	-18.29%	-29.27%	
	Cyclone	27.00±1.00	22.66±1.52	23.66±3.51	29.33±0.57	23.33±2.516	20.00±2.00	24.33±3.58 [a]
	%age Difference		-16.07%	-12.37%	+8.62%	-13.59%	-25.92%	+3.045%
	Mean (LSD=2.30)	27.16±1.16 [a]	23.50±1.87 [a]	21.83±3.06 [b]	28.83±1.16 [b]	22.83±2.31 [bc]	19.66±1.63 [c]	23.97±3.66
	%age Difference		-13.47%	-19.62%	+6.14%	-15.94%	-27.61%	
Root dry weight (g) LSD=0.48	AC-Exel	3.50±0.50 [cdef]	2.50±0.50 [f]	2.66±0.28 [ef]	5.33±1.15 [abc]	4.50±0.86 [cde]	3.50±0.50 [cdef]	3.66±1.75 [b]
	%age Difference		-28.57%	-24.00%	+52.28%	+28.57%	0.00%	
	Cyclone	3.66±0.76 [cdef]	3.10±0.65 [def]	2.16±0.907 [f]	6.83±0.76 [a]	6.50±0.50 [ab]	4.80±0.52 [bcd]	4.51±1.85 [a]
	%age Difference		-15.4644%	-40.9165%	+86.3884%	+77.4316%	+30.9836%	+23.087%
	Mean (LSD=0.05= 2.3075)	3.58±0.50 [bc]	2.80±0.61 [cd]	2.41±0.66 [d]	6.08±1.20 [a]	5.50±1.26 [a]	4.15±0.84 [b]	4.08±1.59
	%age Difference		-21.87%	-32.68%	+69.83%	+53.63%	+15.92%	

Means followed by dissimilar letters, are different at P = 0.05

Table 2: Shoot growth of 40 days old Canola (*Brassica napus* L.) grown under salinity stress [50.0 mM and 100.0 mM)] and exposed to foliar spray of Ascorbic acid [50.0 mg L^{-1}] at 15 and 25 days of age [Values represent means ± SE] Values in parentheses represent percentage increase (+) / decrease (-) over control

		0 mM Salinity + 0mg L^{-1} AsA	(50 mM Salinity + 0mg L^{-1} AsA)	100 mM Salinity + 0mg L^{-1} AsA	0 mM Salinity +50mg L^{-1} AsA	50 mM Salinity + 50mgL^{-1} AsA	100 mM Salinity + 50mgL^{-1} AsA	Mean
Shoot Length (cm) LSD=2.33	AC-Exel	56.00±2.00 [ab]	50.66±2.08 [cd]	45.33±1.50 [e]	58.66±1.50 [a]	50.33±3.5 [cd]	47.00±4.00 [de]	51.33±5.30 [a]
	%age		-9.53%	-19.05%	+4.75%	-10.12%	-16.07%	
	Cyclone	51.66±1.52 [bc]	43.66±3.21 [e]	42.60±2.00 [e]	58.33±1.50 [a]	51.00±4.00 [cd]	50.00±2.00 [cd]	49.55±5.84 [a]
	%age Difference		-27.1002%	-17.40%	+12.91%	-1.27%	-3.21%	3.47%
	Mean (LSD=0.05 =2.3075)	53.83±2.85 [b]	47.16±4.530 [d]	44.00±2.36 [e]	58.50±1.37 [a]	50.66±3.38 [c]	48.50±3.20 [cd]	50.44±5.57
	%age Difference		-12.37%	-18.26%	+8.67%	-5.88%	-9.90%	
Shoot dry weight (g) LSD=0.68	AC-Exel	8.66±0.57	7.50±0.50	7.16±0.76	11.83±1.04	7.16±1.04	8.66±1.52	8.50±1.85 [b]
	%age Difference		-13.39%	-17.32%	+36.60%	-17.32%	0.00%	
	Cyclone	10.83±1.89	9.20±0.30	7.66±0.28	13.33±0.57	8.83±1.04	9.16±1.04	9.83±2.05 [a]
	%age Difference		-15.05%	-29.20%	+23.08%	-18.46%	-15.38%	+15.65%
	Mean (LSD=0.05= 2.3075)	9.75±1.72 [b]	8.35±1.00 [cd]	7.41±0.58 [d]	4.58±1.11 [a]	8.00±1.30 [cd]	8.91±1.20 [bc]	9.16±2.04
	%age Difference		-14.44%	-24.00%	-53.02%	-17.94%	-8.71%	

Means followed by dissimilar letters, are different at P = 0.05

Table 3: Leaf growth of 40 days old Canola (*Brassica napus* L.) grown under salinity stress [50.0 mM and 100.0 mM)] and exposed to foliar spray of Ascorbic acid [50.0 mg L^{-1}] at 15 and 25 days of age [Values represent means ± SE] Values in parentheses represent percentage increase (+) / decrease (-) over control

		0 mM Salinity + 0mg L^{-1} AsA	(50 mM Salinity + 0mg L^{-1} AsA)	100 mM Salinity + 0mg L^{-1} AsA	0 mM Salinity +50mg L^{-1} AsA	50 mM Salinity + 50mgL^{-1} AsA	100 mM Salinity + 50mgL^{-1} AsA	Mean
Leaf area (cm^2) LSD=2.33	AC-Exel	925.6±18.10	915.33±18.90	890±10.00	443.66±57.8	997.33±29.50	985±10.44	1524.16±23.96 [a]
	%age		-1.11%	-3.85%	-52.06.06%	+7.74%	+6.41%	
	Cyclone	979.00±11.53	964.33±27.61	951.66±20.84	1158±26.66	1022±27.87	997±24.35	1012±73.40 [a]
	%age Difference		-1.4984%	-2.7926%	+18.28%	-99.87%	+1.83%	33.60%
	Mean (LSD=0.05=2.3075)	952.33±32.22 [a]	939.83±34.18 [a]	920.83±36.80 [a]	2794.83±40.0 [a]	1009.666±29.00 [a]	991±9.71 [a]	1268.08±16.91
	%age Difference		-1.31%	-3.30%	+193.47%	+6.01%	+4.06%	
Leaf dry weight (g) LSD=0.56	AC-Exel	6.33±1.70	5.50±0.86	3.66±0.57	10.00±1.00	7.50±0.50	6.50±0.50	6.58±2.14 [a]
	%age Difference		-13.11%	-42.18%	-57.97%	+18.48%	+2.68%	
	Cyclone	7.50±0.86	6.33±0.20	4.33±0.70	10.76±0.23	7.00±0.50	7.00±0.86	7.15±2.03 [b]
	%age Difference		-15.60%	-42.26%	+42.66%	-6.66%	-6.66%	+8.66%
	Mean (LSD=0.05=2.3075)	6.91±1.39 [bc]	5.91±0.70 [c]	4.00±0.70 [d]	10.38±0.70 [a]	7.25±0.52 [b]	6.75±0.68 [bc]	6.86±2.08
	%age Difference		-14.45%	-42.19%	+50.17%	+4.82%	-2.40%	

Means followed by dissimilar letters, are different at P = 0.

Salinity of 50 mM level with Ascorbic acid also increased the shoot dry weight by 8.71 %. Reduction in shoot dry weight by salinity was compensated by Ascorbic acid.

Root Length (cm)

Root of Cyclone showed more growth than AC-Exel by 3.04%. Salinity of 50 mM level reduced the root length by 13.47%. While when the same level of salinity was accompanied with foliar 50 mgL^{-1} of ascorbic acid, the root length was increased by 15.94% instead of decreasing. 100 mM of salinity level reduced root length by 19.62%. Ascorbic acid increased 14% root growth when was applied. 50 mM of salinity level with 100 mgL^{-1} of Ascorbic acid also increase in the root length by 27.61 %. Reduction in root length by salinity was compensated by Ascorbic acid.

Root Dry Weight (g)

Root Biomass in term of dry weight of Cyclone was greater than that of AC-Exel by 23.08%. Salinity level of 50 mM reduced the dry weight of root by 21.87% %. While, when the same level of salinity was accompanied with foliar 50 mgL^{-1} of ascorbic acid, the root dry weight was increased by 53.612%. Salinity level of 100 mM reduced dry weight of root by 32.65%. An increase of 69.83% in the root dry weight was observed when foliar spray of Ascorbic acid was applied. Salinity stress of 50 mM accompanied with Ascorbic acid also increased the root dry weight by15.92 %. Salinity level reduced the root dry weight while the effect of salinity. was mitigated by Ascorbic acid.

Leaf Area (cm^2)

Leaf area in Cyclone was more expressed than AC-Exel by 33.60%. Salinity level of 50 mM reduced the leaf area by 1.13%. While when the same level of salinity was accompanied with foliar 50 mgL^{-1} of ascorbic acid, the leaf area was increased by 6.01%. Salinity level of 100 mM reduced leaf area by 3.30%. An increase of 193.47% in the leaf area was noted when Ascorbic acid was applied. Salinity of 50 mM along with Ascorbic acid application increased the leaf area by 4.06 %. Salinity level reduced the leaf area while the negative effect of salinity was countered by ascorbic acid.

Leaf Dry Weight (g)

Leaf biomass was 8.66% more in Cyclone than AC-Exel variety. Salinity level of 50 mMreduced the leaf dry weight by 14.45%. While when the same level of salinity was accompanied with foliar Ascorbic acid, the leaf dry weight was increase in by 4.829% instead of decreasing. Salinity level of 100 mM reduced fresh weight of root by 42.19% while 50.17% increase in the leaf dry weight was noted when Ascorbic acid was applied. In plants of 50 mM salinity, Ascorbic acid increased leaf dry weight by 2.40 %.

DISCUSSION

In the current study, salinity stress reduces the root, shoot and leaf growth significantly (Table1). Reduction in growth might be due to the accumulation of Na+ and Cl^- ions which cause prohibition of necessary nutrients uptake. Another possible reason might be reduction in the rate of photosynthesis which is due to reduced amylase level or reduced chlorophylls contents. This may have shorten the accumulation of carbohydrate to the enlarging meristematic cells, resulted in decrease of roots and shoots growth. Salt stress can affects plants either by osmotic stress which limits the plant water supply or by ion toxicity that interferes with metabolic process and enzymes activity (Kaya et al. 2015, Parida and Das, 2005, Safdar et al. 2019, Zamin and Khattak, 2017). High salt contents in soil impose strong negative impacts on plant biomass (Li et al. 2020), physiology (Nizam, 2011), accumulation of mineral ions (Liu et al. 2020) and destruction of PSII reactions (Khan et al. 2013).

The enhancement in growth was observed by Indole Acetic acid (Table 1-3). Similar findings were reported by Mittler, (2002). This might be due to roots and shoots cell division enhancement. Ascorbic acid is reported to increase cytokinin in plant tissues. Ascorbic acid also improved the efficiency of photosynthesis due to expressively enhance in the level of chlorophyll. The Ascorbic acid might overcome the salt stress by minimizing the concentration of sodium and maximizing the potassium and magnesium levels (Shaddad et al. 1999). The protein contents in *Brassica napus* are reported to shows an enhancement due to exogenous appliance of the Ascorbic acid. The exogenous Ascorbic acid affects the assertion of cell wall loosening proteins, in plant growth. The expression of Ascorbate peroxidases (APXs) is reduced by Ascorbic acid during salt stress (Doorn and Ketsa, 2014). Ascorbic acid catalysis molecular oxygen species to overcome effects of salinity. Exogenous AsA as a co-factor might have moderated plant growth reduced due to salt stress by effective gibberellins synthesis and ethylene through specific Ascorbic acid-

dependantdioxygenase catalyzed reactions (Wang et al. 2013). AsA act as first line defender against ROS through ascorbate–glutathione pathway (Bilska et al. 2019, Sharma et al. 2019). Ascorbates act as cofactor for enzymes, such as violaxanthin de-epoxidase. This enzyme protects plant against ROS. Exogenous AsA improves antioxidant potential of plant through lipid peroxidation prevention and thus by decreasing malondialdehyde (MDA) concentration (Munir and Aftab, 2011, Zhou et al. 2016).

CONCLUSION

Salinity level of 50 mmole and 100 mmole, reduced the plant height, dry weight of stem, root and leaves. While the foliar spray of Ascorbic acid with concentration of 50mgL^{-1}, at the same salinity levels, significantly enhanced the plant height and dry weight of stem, leaves and root.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

GY designed the experiments MM performed the experiment and collected data. MK analysed data. AA helped in designing the experiment and reviewed the manuscript. All authors read and approved the final version.

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