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Effects of Salicylic Acid Treatments on Seedling Growth of Red Cabbage under Salt Stress

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Abstract: ***High soil salinity is a limiting factor to red cabbage development and production. A study was conducted to investigate the effects of salicylic acid (SA) treatments (0.2 mg plant^{-1}) on the development of red cabbage seedlings grown under salt stress (200 mM NaCl). Seedling growth parameters and plant nutrient contents were measured. Salicylic acid treatments had positive effects on plant fresh biomass, dry biomass and root lengths. The plant fresh biomass value of 244.5 mg plant^{-1} under the control (zero application of SA) increased to 309.5 mg plant^{-1} with SA treatments. The fresh biomass of 205.0 mgplant^{-1} under saline conditions increased to 300.0 mg plant^{-1} with SA treatments. Similar, plant dry biomass values increased with SA treatments. Furthermore, SA treatments increased root length from 9.21 cm under salt stress conditions (200 mM) to 11.69 cm. In addition to improving plant growth parameters, SA treatments significantly reduced plant Na levels and significantly increased plant contents of K, Ca, P, Fe, Zn and B. SA treatments increased K/Na ratio (a significant indicator of plant tolerance to salinity) from 0.43 to 0.64 and Ca/Na ratio from 0.24 to 0.34.***

Keywords: *Brassica oleracea*, salicylic acid, dry biomass, root length

1. Introduction

Stress factors, especially soil salinity, inhibit or limit plant growth and development. Turkey is among the important agricultural countries of the world. However, about 1.5 million ha land area of Turkey is under the threat of soil salinity [1]. High soil salinity levels are toxic to plants and seriously hinder plant growth and development [2]. High soil salinity levels also reduce leaf sizes, thus reduce photosynthetic activity [3] and reduce enzyme activity and internal hormone production in plants [4]. Cabbages (*Brassica oleracea* L.) are moderately sensitive to saline conditions [5, 6], but there are great varietal variations in tolerance to salt, within the same cultivars and even among ecotypes of a variety [7, 8]. Reclamation of saline soils is both an expensive and a time-consuming process. Therefore, growing a plant on such problematic soils is possible only with certain practices or implementations. Improving soil organic matter content [9], applications of methyl jasmonate [10], 24-Epibrassinolid [11] and salicylic acid [12] like chemicals are the most common methods applied in plant culture over saline soils. Salicylic acid (SA) and similar hormones influence various physiological and biochemical functions of the plants and reduce or inhibit the negative impacts of biotic and abiotic stressors on plants [13, 14]. SA plays a regulatory role in plant tolerance to stress conditions [15-20]. The significance of salicylic acid under biotic and abiotic stress conditions and the role of salicylic acid in transcriptional regulation of defense genes of the plants against such stress conditions were comprehensively investigated and assessed by previous researchers [21]. Salicylic acid applications have been reported to significantly reduce the damage caused by salt stress in vegetable crops such as tomato [22], pepper [23], eggplant [24], sweet basil [25] and faba bean [26]. Red cabbages are widely grown in Turkey. They are quite rich in antioxidants and fibers and thus play a significant role in human diets as a winter vegetable. As it was in various other countries of the world, red cabbage has a great economic significance also in Turkey. Annual red cabbage production of Turkey was 186.826 tons in 2016 [27].

The present study was conducted to investigate the effects of salicylic acid treatments on plant growth and nutrient contents of red cabbage seedlings under salt stress.

2. Material and Methods

Mohrenkkopf (Berk&Han) red cabbage cultivar was used as the plant material for the present study. The seeds were sown into 45-cell 5 x 9 rows viols filled with peat soil. 2-3 seeds were sown per cell. Following the homogeneous emergence, the number of plant per cell was thinned to 1 seedling. In present experiments, 0 mM (control) and 200 mM NaCl (Tekkim, TK.170540.01000, Bursa, Turkey) salt doses and 0 mg plant^{-1} (control) and 0.2 mg/plant salicylic acid (SIGMA, 27301, St. Louis, Missouri, USA) doses were applied. Salicylic acid was prepared by diluting to the 0.04 mg ml^{-1} from the stock solution (1 mg ml^{-1}). Five ml of diluted (0.04 mg ml^{-1}) salicylic acid was applied to each of the plant root regions when seedlings had their first true leaves. Following salicylic acid treatments, 5 days later, plants were subjected to salt stress. To prevent acute damages, 200 mM salt treatment was applied in 2-day intervals in 2 portions (firstly 100 mM and 2 days after other 100 mM). The same amount of distilled water was applied to control treatments. Experiments were conducted in randomized plots design with 3 replications and 45 plants in each replicate. Seedlings were left for normal growth and cultural practices were performed whenever required. The vegetative parameters were measured including (i) the fresh biomass, (ii) the dry weight and (iii) the root length. Nutrient contents were also determined. The fresh biomass per plant was obtained by dividing the fresh weight of the plants cut from the upper portion of the roots and weighted with a digital scale (e = ± 0.001 g) by the total number of plants. Fresh weighted plants were then dried at 72°C for 48 hours in an oven and weighted again with a digital scale (e= ± 0.001 g). Dry weight was divided by the number of plants to get dry biomass per plant expressed in mg plant^{-1}. Root lengths (cm) were measured with a ruler. Measurement of seedlings contents in sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), phosphorus (P), iron (Fe), zinc (Zn), manganese (Mn), boron (B) and copper (Cu) were performed by an inductively coupled plasma optical emission spectrometer (ICP-OES, Vista-Pro Axial, Agilent Tech., Mulgrave, AUSTRALIA). Prior to the measurement, 0.2 g of dry leaf sample was digested in a closed microwave digestion system (Marsexpress Cem Corp., Matthews, NC, USA) in the presence of 5 ml of concentrated nitric acid (HNO_3) and 2 ml of hydrogen peroxide (H_2O_2). Deionized water (H_2O) was used to top-up the volume of the samples to 20 ml. The analytical data was compared to the certified values of a standard reference material (SRM 1573a Tomato Leaf, National Institute of Standards and Technology, Gaithersburg, MD, USA). The data obtained were subjected to an analysis of variance using JMP v.10. statistical software (SAS Institute Inc., USA). Mean values of the parameters measured were compared using LSD test at 5% probability.

3. Results and Discussion

Results on the fresh biomass, dry biomass and root length values of red cabbage seedlings grown under salt stress are given in Table 1.

Table 1: Effects of salicylic acid on fresh biomass, dry biomass and root length of cabbage seedlings grown under salt stress

NaCl mM	SA mg plant^{-1}	Fw mg plant^{-1}	Dw mg plant^{-1}	Rl mg plant^{-1}
0	0	244.5 c	11.65 b	11.65 b
	0.2	309.5 a	16.81 a	16.81 a
200	0	205.0 d	9.21 c	9.21 c
	0.2	300.0	11.69 b	11.69 b
Means of Treatments				
NaCl	0	277.0 A	45.0 A	14.23 A
	200	252.5 B	35.4 B	10.45 B
SA	0	224.8 B	34.9 B	10.43 B
	0.2	304.8 A	45.4 A	14.25 A
LSD	NaCl x SA	3.45	1.51	0.97
	NaCl	2.44	1.07	0.68
	SA	2.44	1.07	0.68

LSD: Least significant difference; means followed by same letter are not statistically different; Fw: fresh biomass; Dw: dry biomass; Rl: root length

NaCl x SA interactions had significant effects on fresh biomass, dry biomass and root length of the plants. In fact, the fresh biomass, dry biomass and the root length were lowered by NaCl. SA treatment rose them, as clearly shown in Table 1. The greatest fresh biomass (309.5 mg plant^{-1}) was obtained from 0 mM NaCl + 0.2 mg SA treatments and the lowest fresh biomass (205.0 mg plant^{-1}) was obtained from 200 mM NaCl + 0 mg SA treatments. A similar case was also observed for dry biomass values of the plants. The highest dry biomass (52.1 mg plant^{-1}) was obtained from 0 mM NaCl + 0.2 mg SA treatments and the lowest dry biomass (31.9 mg plant^{-1}) was obtained from 200 mM NaCl + 0 mg SA treatments. When compared to the control, NaCl treatment reduced fresh and dry biomass of the seedlings. Salt stress-induced reduction of plant weight and limitations on plant growth and development were also reported for various plants by previous researchers [28-35]. Compared to its control SA treatments significantly increased fresh and dry biomass values. Significant roles of SA in plant growth and development were also reported in previous studies [36, 37]. Dry biomass values of NaCl x SA treatments were similar with the values under control conditions. As it was reported for wheat [38], maize [16], paddy [39] and tomato [40] present findings also revealed that salt stress-induced regress in plant growth could be reduced by applications of SA. SA treatments also increased root length from 9.21 cm under salt stress to 11.69 cm. Similar improved rooting with SA treatments were also reported by previous researchers [41-44].

Data on Na, K, Ca, Mg, P, Fe, Zn, Mn, B and Cu contents of red cabbage seedlings grown under salt stress are presented in Table 2. Compared to the control NaCl treatment clearly increased Na contents. Such increases in Na contents with NaCl treatments were also commonly reported by previous studies [29, 32, 45, 46]. With regard to other macro and micro nutrients, it was observed that NaCl x SA interaction had significant effects on K, Ca, P, Fe, Zn and B contents of red cabbage seedlings (Table 2). NaCl treatments led to insignificant increases in K and Ca contents, but significant decreases in P and B contents. The interaction between soil salinity and P content is quite complex phenomenon and plant P respond to saline conditions (increase, decrease or insignificant change) vary based on plant species [47]. In present the study, the decrease in P contents under saline conditions was significantly temperated by SA treatments and even an increase was achieved in P contents when compared to the control (zero SA). NaCl treatments reduced plant boron contents by about 40% (0.58 µg plant^{-1} in control and 0.35 µg plant^{-1} under saline condition). The decrease in green herbage boron concentrations with salinity may be related to reduced transpiration flow hindering passive transport of boron [48]. Increase or decrease in plant boron contents under saline conditions are also related to genotypic differences, plant age, climatic conditions, and especially to the analyzed section of the plants [49]. Reduced salt stress-induced decreases in boron uptake with SA treatments and similar boron concentrations of NaCl x SA treatments to those of the control conditions reflect the positive impacts of salicylic acid on plant growth and development. As compared to NaCl treatments, NaCl x SA treatments significantly increased plant K, Ca, P, Fe, Zn and B contents. Considerable increases were also observed in other elements, but those increases were not found to be significant. Ahmed et al. [50], reported that SA treatment increased the K and B contents of tomato plants in saline condition. Similar findings were also reported for barley [15], maize [16] and paddy [39]. When compared only to NaCl treatments, NaCl x SA treatments significantly increased Zn (from 0.83 to 1.27 µg plant^{-1}) and B (from 0.35 to 0.57 µg plant^{-1}) contents of the plants. Such increases revealed the significant roles of these elements in preservation of root cell membranes through SA. Thusly, Zn was reported to control integrity and permeability of root cell membranes [51, 52]. Similarly, boron was reported to play a significant role in both structural and functional integrity of bio-membranes, especially of plasma bio-membranes [53].

Table 2: Effect of salicylic acid on nutritional content of red cabbage seedling grown in saline condition

NaCl mM	SA mg plant^{-1}	Na mg plant^{-1}	K mg plant^{-1}	Ca mg plant^{-1}	Mg mg plant^{-1}	P mg plant^{-1}	Fe µg plant^{-1}	Zn µg plant^{-1}	Mn µg plant^{-1}	B µg plant^{-1}	Cu µg plant^{-1}
0	0	0.08c	0.96c	0.56c	0.09	0.17c	0.67c	0.89b	0.46	0.58b	0.11
	0.2	0.05c	1.54a	0.86a	0.12	0.28a	1.10a	1.23a	0.64	0.89a	0.16
200	0	2.36a	1.01c	0.59c	0.08	0.14d	0.70c	0.83c	0.82	0.35c	0.11
	0.2	2.16b	1.37b	0.74b	0.11	0.20b	0.97b	1.27a	0.95	0.57b	0.15
Means of Treatments											
NaCl mM	0	0.06B	1.25A	0.71A	0.11A	0.22A	0.88A	1.06	0.55B	0.74A	0.13
	200	2.26A	1.19B	0.66B	0.09B	0.16B	0.83B	1.05	0.89A	0.46B	0.13
SA mg plant^{-1}	0	1.22A	0.98B	0.58B	0.09B	0.15B	0.68B	0.86B	0.64B	0.47B	0.11A
	0.2	1.10B	1.45A	0.80A	0.12A	0.24A	1.03A	1.25A	0.79A	0.73A	0.15B
LSD 5%	NaCl x SA	0.11	0.10	0.059	ns	0.013	0.076	0.048	ns	0.06	ns
	NaCl	0.049	0.035	0.024	0.012	0.007	0.031	ns	0.046	0.027	ns
	SA	0.049	0.035	0.024	0.012	0.007	0.031	0.033	0.046	0.027	0.007

LSD- Least significant differences; ns- not significant

Salicylic acid treatments resulted in significant decreases in Na content of seedlings under both control and saline conditions and led to significant increases in the other nutrient contents. While the average Na content of the plants without SA treatments was 1.22 mg plant^{-1}, average Na content of SA-treated plants was 1.10 mg plant^{-1} (Table 2). Such a case can be explained as plant growth-induced dilution with SA treatments. Similar findings were also reported in wheat [54]. Salicylic acid has various physiological roles in plants including growth, photosynthesis and nutrient uptake [55]. It was reported in the literature that plant nutrient solutions in soil got into the plants through the roots and a healthy and strong root system together with some other factors (SA application in present study) played a significant role in plant growth and development [56]. Compared to NaCl treatments, NaCl x SA treatments reduced Na levels, but increased K and Ca levels, thus increased K/Na ratio from 0.42 to 0.63 and Ca/Na ratio from 0.25 to 0.34 in saline conditions (Figure 1a and b).

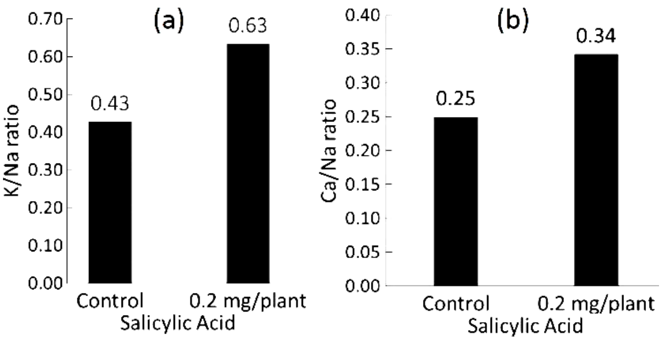

Figure 1: Effects of salicylic acid on K/Na (a) and Ca/Na (b) ratios of red cabbage seedlings under saline conditions

High K/Na and Ca/Na ratios were significant indicators of tolerance of plants to salt. Ashraf and Khanum [57] showed that K/Na and Ca/Na ratio were higher in salt tolerant sipring wheat line S24 than salt sensitive line Yecora Rojo. Zeng et al., [58] reported that the salt-tolerant rice lines led to the higher K/Na ratio than sensitive ones. Dasgan et al., [45, 59] pointed out that the higher K/Na and Ca/Na ratio the less salt injury in tomato and beans. Plant potassium uptake through active absorption and accumulation increase osmotic pressure and more water influent is provided to the plants. Therefore, potassium plays a significant role in preservation of plant water balance. Salt tolerant species can transport greater quantities of potassium from the roots to green herbage and hinder plant Na uptakes [45, 60, 61]. Higher Ca/Na ratios of the present study obtained through salicylic acid treatments under saline conditions was provided by prevention of disintegration in cell membrane permeability. Similarly, Tufail et al., [62] reported that SA increased both K/Na and Ca/Na ratio in maize in saline conditions.

4. Conclusions

Salt stress affected negatively the plant growth by means of decreasing some morphological parameters and changing plant nutrients' content in red cabbage. SA treatments had positive effects on growth of red cabbage seedlings under salt stress. The ameliorative effect of SA could be evaluated by the fact that it promotes plant's uptake K or Ca instead of Na in salt stress environment. In comparison to saline conditions (only NaCl treatments), NaCl x SA treatments significantly increased plant K and Ca contents and decreased Na contents. Subsequently, there was an increase of K/Na and Ca/Na ratios of the plants and hence an increase of red cabbage's tolerance to salt stress.

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