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The effects of exogenous tyrosine supplement on spinach (*Spinacia oleracea* L.) cultivation under lithium stress

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ABSTRACT

In this study, the effects of exogenous Tyrosine (Tyr: 2.5 mM) application on the variations of growth rate parameters, enzymatic and non-enzymatic constituents, oxidative stress, and mineral content under lithium-applied (Li1: 6.44 mM; Li2: 19.32 mM) seedlings of the Anlani F1 spinach cultivar were investigated. Results showed that a higher Li led to a significant reduction in the growth rate parameters including shoot, root, and leaf length, the fresh weight of shoot, root, and leaf, and leaf blade sizes, whereas a lower Li dose resulted in an increase in those parameters. In contrast, the Tyr supply to the Li-applied seedlings resulted in a rise in these measured parameters. Similarly, chlorophyll and polyphenol contents and PAL, APX, CAT, POD, and SOD activities were higher in all exogenous Tyr-treated groups, including lithium-treated groups. Whilst nitrate content was higher in the Li-applied seedlings, NR activity was lower. Also, MDA and H_2O_2 were found to be higher in the Li-applied group, but exogenous Tyr supplements reduced their levels in the seedlings. Li, Ca, Na, Cl, Mn, Fe, Ni, Cu, and Zn accumulation were induced by Li doses and Tyr applications together with Li, but Tyr applications alone reduced all of their levels. Also, exogenous Try supplementations to the Li-applied group caused an important decline in the Li accumulation. As a result, a higher Li dose exhibited a negative effect on the growth rate, chemical constituent, and antioxidant compounds of the Anlani F1 spinach cultivar, but exogenous Tyr supplement improved those examined traits in the Li-applied seedlings.

1. Introduction

Spinach (*Spinacia oleracea* L.) is a popular leafy nutrient source, which is considered one of the healthiest vegetables in the daily diet of humans. The health effect of spinach is due to antioxidant molecules including pigments, phenols, vitamins, amino acids, enzymes, and minerals. In addition, having a low calorie and high water content increases its importance in health (Bostancı and Ulger 2022). As it is a cheap and readily available vegetable in almost all seasons, thisis an important advantage for the consumer as industrial and commercial properties are important for spinach producers. Having cultivars that can be grown in every season, spinach is a vegetable that is economically important for Türkiye. However, the nutritional quality and yield of spinach differ depending on the growing conditions, and the dosage and type of the fertilizer used (Turfan 2023). In recent years, due to wrong irrigation, unconscious and excessive fertilization, and spraying for good-looking crops, the soil properties are slowly deteriorating and therefore there has been a high yield loss in spinach production as well as other crops (Bostancı and Ulger 2022; Saddıque et al. 2022). Spinach is moderately tolerant of alkalinity in winter conditions and moderately sensitive in spring and summer. Most of the studies in spinach agriculture in Türkiye have focused on NaCl and heavy metal toxicity and the effects of exogenous organic/inorganic fertilization. However, there are no studies on lithium-induced saline-alkaline stress in spinach. Li is an alkali metal used in almost 20 industries including ceramic, glass, aluminum, oil, battery, phones, tablets, computers, electric cars, and autonomous robots and it has gained more importance in recent years (Robinson et al. 2018). The excessive accumulation of lithium-containing wastes in the soil triggers saline-alkaline stress which limits plant growth by repressing root growth, water and mineral uptake from the roots (Mulkey 2005; Hawrylak-Nowak et al. 2012). In addition, since it is a mobile element, it is readily transported to the leaves after being taken up by the roots, causing many problems such as chlorosis, disruption of photosynthesis metabolism and prevention of other biochemical reactions (Bakhat et al. 2020; Wang et al. 2021). In recent years, to diminish, alkalinity stress damage, exogenous aromatic amino acids applications have been widely used (Zhang et al. 2021). Tyrosine (Tyr), one of the aromatic amino acids, is a precursor for pigments, phenolic compounds, vitamins, and enzymes specialized secondary metabolites that are vital for plant adaptation to environmental change (Feduraev et al. 2020). Also, it is a fundamental component of the complex producing oxygen in photosystem II, as an electron carrier. Moreover, the degradation of Tyr by the Krebs cycle can provide nitrogen, carbon, and ATP that are required for plant growth, especially during periods of carbohydrate shortage (Hildebrandt et al. 2015). The positive effects of exogenous L-tyrosine application on the plant growth rate, nutritional quality, and stress resistance were observed following the foliar application of it in beetroot (El-Sherbeny et al. 2012), arugula Al-Mohammad and Al-Taey

(2019), and *Hibiscus sabdariffa* (Helaly and Ibrahim 2019). Although Tyr is an important aromatic amino acid, little is known about its specific defining roles in plant growth and development, and how tyrosine levels are controlled in plant tissues. In addition, there are studies in the literature on the vegetative growth of tyrosine, accumulation of beneficial metals, and alkali metals in plants, whereas no study has been found on how exogenous tyrosine application affects the response of plants to lithium. Also, no such study carried out in Türkiye was found. Thus, the present study aimed to determine the effect of foliar tyrosine treatment on spinach cultivation treated with lithium. The effect of exogenous Tyr application on the growth rate and biochemical characteristics of the Anlani F1 spinach cultivar treated with Li was investigated by making use of morphological parameters, bioactive chemical constituents, and mineral status.

2. Materials and Methods

2.1. Experimental design

Anlani F1 spinach cultivar was used as a study material in the present research, which is a species tolerant of low temperatures, fragility, and diseases as well as suitable for greenhouse growing and open-area growing (Turfan 2023). The present study was carried out between 12 Nov 2022 and 14 March 2023 under greenhouse conditions with 14/22±2°C, relative humidity of 68±5%, and photoperiod of 16 h. First, balcony-type pots (80 x 23 x 24) were filled with 25 L turf and soil mixture (3:1) and spinach seeds were planted. The study was designed with a random parcel experimental design with 3 repeats. The six treatment patterns were as follows: 1) Control (0), 2) Tyr (2.5 mM L-tyrosine), 3) Li1 (6.44 mM $LiCl_2$), 4) Li2 (19.32 mM $LiCl_2$), 5) Tyr-Li1 (6.44 mM $LiCl_2$ + 2.5 mM L-tyrosine), and 6) Tyr-Li2 (19.32 mM $LiCl_2$ +2.5 mM L-tyrosine). Four pots were used in each group and 6x3= 18 pots in total. They were irrigated with a nutrient solution twice a week until lithium (Li) and tyrosine (Tyr) applications were started. After the 15th day since germination, the number of plants in each pot were reduced to approximately 10 seedlings. Li applications ($LiCl_2$: Merck Lithium chloride CA 7447-41-8. EC: 231-213-3) were performed in the soil by dissolving in a nutrient solution (Hoagland and Arnon 1950) when seedlings had 4-5 leaves. L-tyrosine (Sigma-Aldrich, CAS 35424-81-8) application was performed on leaves with a tyrosine solution dissolved in a nutrient solution, while control plants were sprayed with nutrients. The applications were repeated twice a week for 8 weeks. Plants were harvested in the 9th week and some of them were used in morphological measurements, some in chemical analyses, and nutrient assaying. The determination of the doses of the chemicals was carried out according to the preliminary study with the viol. The stimulant doses of lithium (Li1) and tyrosine were considered at the concentration that caused a considerable increase in shoot, root, and leaf lengths and fresh weight of seedlings. The toxic dose of lithium (Li2) was taken into account in the concentration that caused the greatest reduction in shoot, root, and leaf development and fresh weight of seedlings. The nutrient components were: available magnesium 24.65%, available potassium 14.80%, available calcium 9.66%, available sodium 7.92%, available phosphorus 6.53%, available sulphur 2.4%, available lithium 114.74 mg kg^{-1}, available manganese 980.8 mg kg^{-1}, available iron 44230 mg kg^{-1}, available nickel 140.8 mg kg^{-1}, available zinc 144.8 mg kg^{-1}, and available copper 46.8 mg kg^{-1}. The pH value of the soil was found to be 6.38, and also electrical conductivity (EC) of 6.53 dS m^{-1}.

2.2. Assay

2.2.2. Morphological measurement

After treatments, all spinach seedlings were harvested for shoot/root, leaf length, fresh weight of plant and leaf measurements. The parameters were examined in 10 plants for each application. Shoot, root, leaf and leaf blade length (cm) were measured by a ruler. Fresh weight (g) shoot and leaf were determined by weighing with a precision balance. All chemicals and mineral analyses were performed with three replications. Measurements for plant growth rate parameters were carried out with ten replications.

2.2.3. Chemical analyzes

The chlorophylls and lutein were homogenized using ethanol and the estimations were made using the methods described by Kukric et al. (2012). The amount of polyphenol was performed according to the method of Folin and Denis (1915). About 0.5 mL of an extract was introduced into test tubes followed by 2.5 mL of 10% Folin-Ciocalteu reagent and 2 mL of 7.5% Na_2CO_3. The mixture was allowed to stand for 30 minutes, and absorbance was recorded at 760 nm. The phenol content of the extract was determined using tannic acid standard curves. Phenylalanine ammonia-lyase (PAL) was assayed following the method of Dickerson et al. (1984). A 500 mg sample was homogenized with mortar and pestle in the extraction buffer (50 mM Tris-HCl buffer, pH 8.8, 5 mM EDTA, 5 mM ascorbic acid). The reaction mixture, containing 100 µl of crude enzyme extract, 500 µl of 50 mM Tris HCl (pH 8.8), and 600 µl of 1 mM L-phenylalanine, was incubated for 60 min at room temperature, and then the reaction ceased by the addition of 500 µl of 6 N HCl. The absorbance was recorded at 290 nm. The nitrate content was carried out by the salicylic acid method (Cataldo et al. 1975). The total amount of nitrate was calculated with the equation obtained from the NO_3 standard curve and the amount of nitrate in the samples was expressed as mg g^{-1}. Nitrate reductase (NR) activity was performed by following the method of Klepper et al. (1971). Antioxidant enzyme extraction was performed using a sodium phosphate buffer solution (50 mM, pH 7.6). 500 mg of fresh tissues were homogenized in a 5 Ml buffer containing 0.1 mM Na-EDTA (ethylenediamine tetraacetic acid disodium salt), then, were centrifuged for 15 min at 15000 rpm at 4°C. The resulting supernatant was used for the estimation of the activities of antioxidant enzymes such as ascorbate peroxidase (APX), catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD) (Zhang et al. 2006). APX activity was determined by following the decrease in absorbance at 290 nm, as the ascorbate was oxidized. Enzyme activity was measured in a reaction mixture containing 0.5 mM ascorbate, 100 mM sodium phosphate buffer (pH 7.0), and enzyme extract. The reaction was started by adding 30 mM H_2O_2. APX activity was expressed as µmol H_2O_2 mg $protein^{-1}$ min^{-1}. The CAT activity of seedlings was determined using a mixture containing 100 mM sodium phosphate buffer (pH 7.0), 30 mM H_2O_2, and 100 µL of crude extract in a total volume of 3.0 mL. One unit (U) of CAT activity was expressed as the amount of enzyme that caused an absorbance change of 0.001 per minute under assay conditions. The activity of POD was calculated using 3 mL reaction mixtures containing 2 mL buffer (400 µl 8 mM guaiacol, 100 mM sodium phosphate pH 6.4), 1 mL of 24 mM H_2O_2 and 0.5 mL of enzyme extract. Absorbance values were recorded twice at 30-s intervals at 470 nm. POD activity was expressed as U $µl^{-1}$ min^{-1}. SOD activity was measured by inhibition of the photochemical reduction of NBT (nitroblue tetrazolium). A 3 ml reaction mixture was prepared which comprised crude extract, phosphate buffer (pH 7.0), riboflavin, methionine, NBT, and EDTA. Test tubes were irradiated by a lamp having white fluorescence for 15 min. The absorbance of solutions was recorded at 560 nm. One unit (U) of SOD activity was expressed as a U mg^{-1} protein. The Malondialdehyde (MDA) content of samples was measured according to the Lutts et al. (1996) method. 500 mg of fresh tissues were extracted in 10 mL of 0.25% thiobarbituric acid (TBA) in 10% trichloroacetic acid (TCA). The extracts were boiled at 95°C for 60 minutes and quickly cooled in an ice bath. After centrifuging at 5000 rpm for 10 minutes, the absorbance was read at 532 nm and 600 nm. The level of MDA was calculated as µmol g^{-1} of fresh weight using the extinction coefficient of 155 mM^{-1}. Hydrogen peroxide (H_2O_2) was determined by the method of Velikova et al. (2000). Fresh tissues (500 mg) were homogenized with 0.1% (w/v) TCA. After centrifuging at 12.000 rpm for 15 minutes, 0.5 ml phosphate buffer (pH 7.0) and 1 ml potassium iodide were added to the supernatant. Its absorbance was noted at 355nm and H_2O_2 concentration was estimated by using the H_2O_2 standard curve. All the chemical measurements were carried out directly with the fresh samples as triplicated.

To determine elemental analysis (Li, Mg, P, S, K, Ca, Na, Cl, Al, Mn, Co, Fe, Ni, Zn, Cu, Cr, Cd, Pb, and I), some soil samples were taken before the planting experiments. Then soil and leaf samples were dried at 65 °C and powdered. These samples were used to determine the elemental analysis in Kastamonu University's Central Research Laboratory using the ICP-OES (SpectroBlue II) device. Each sample was analyzed in triplicate. The pH values of the soil samples were determined using a digital pH meter (Gülçur 1974).

2.3. Statistical analysis

All experimental data obtained from the effects of tyrosine application to the Acosta spinach variety under lithium stress on growth rate parameters, bioactive chemicals, and mineral status were subjected to multiple analyses of variance (MANOVA) using SPSS statistical software (SPSS for Windows, Version 16). Following the results of ANOVAs, Tukey’s honestly significance difference (HSD) test (α= 0.05) was also applied.

3. Results and Discussion

The effects of exogenous Tyr on the growth rate and leaf development of spinach seedlings treated with Li doses are presented in Table 1. All parameters significantly varied depending on the type and dose of application (P<0.0001).

As seen in Table 1, a higher Li dose caused a sharp decline in the shoot, root, and leaf development, but a slight increase occurred at the lower dose. The exogenous Tyr application to the non-stressed seedlings enhanced the length of the shoot, root, and leaf as well as their fresh weight. On the other hand, exogenous tyrosine supplement improved shoot, root, and leaf properties at low Li dose, but did not show the expected effect on the growth rate, except leaf weight and leaf length at a higher dose. Results showed that the growth rate traits were reduced significantly by the Li2 compared to the control. Also, the highest values of growth rate were obtained from the Tyr application followed by the Tyr-Li1 (Table 1). In a study examining the effects of lithium on the growth rate, Hawrylak-Nowak et al. (2012) exposed sunflower and corn plants to lithium stress and observed that lower Li doses increased the shoot and root development, and leaf development, whereas higher Li doses caused a significant decrease in shoot fresh weight, leaf surface area in both species. In another study, Kalinowska et al. (2013) showed that high Li concentrations suppressed the root growth of lettuce with metal toxicity from Li. On the other hand, the positive effects of Tyr application on the plant growth rate and leaf development are similar to the results obtained by Helaly and Ibrahim (2019) for okra, Feduraev et al. (2020) for wheat, and Tarasevičienė et al. (2021) for mints. The stimulatory effect of tyrosine on growth parameters may be due to increased pigment deposition in leaves, promoting increased photosynthetic gain (Perchlik and Tegeder 2018). Chlorophyll pigments, and lutein obtained from spinach seedlings are provided in Table 2, but secondary metabolism products are shown in Figures 1a, 1b. Compared to the control, the higher Li dose caused notable decrement in the chlorophyll a, chlorophyll b, and total chlorophyll content (Table 2). In contrast, there was a significant increase in the lower Li dose. Tyr alone enhanced all pigment content. Further, the Tyr supplement to the Li-applied group improved chlorophyll molecules, except chlorophyll a at the higher dose. Compared to the control, lutein content decreased in all applied groups, except the higher Li dose. Besides, there was an increase in the lutein in the Li-applied group upon the Tyr supply (Table 2). Similarly, while total phenolic content decreased in the Li-applied seedlings, it reached maximum levels by exogenous tyrosine supplementations to the Li-applied seedlings. In addition, the highest amount of TP was obtained from the tyrosine-applied group only (Figure 1a, 1b). Li applications caused a significant inhibition in the PAL activity, whereas its activity increased upon exogenous tyrosine supplement to Li-applied seedlings. The highest activity was recorded with the tyrosine only application followed by the Tyr-Li1, but the lowest activity was obtained from the Li2 group (Figure 1b).

Table 1. Effect of exogenous Tyr on the growth rate parameters of spinach seedlings under Li stress

Group	Shoot height (cm)	Root height (cm)	Fresh weight of plant (g)	Fresh weight of leaf (g)	Leaf height (cm)	Leaf blade width (cm)	Lead blade height (cm)	Leaf number per plan
0	10.89±0.01b*	9.31±0.07b	17.46±0.002b	1.87±0.05ab	8.60±0.06c	2.86±0.01b	3.80±0.02b	11.0±0.41b
Tyr	14.25±0.01c	12.90±0.14d	22.27±0.001d	3.48±0.10d	10.49±0.06d	4.40±0.06d	6.36±0.09d	12.5±0.29c
Li1	11.29±0.07b	9.99±0.06b	18.05±0.076bc	2.79±0.10bc	9.34±0.09cd	3.30±0.06c	4.28±0.07c	10.3±0.25ab
Li2	8.40±.06a	7.27±0.07a	13.95±0.002a	1.49±0.06a	5.84±0.01a	2.12±0.02a	2.71±0.06a	9.5±0.29a
Tyr-Li1	13.88±0.01c	11.47±0.08c	19.99±0.119c	3.27±0.07c	10.07±0.03d	3.85±0.07cd	4.91±0.06cd	11.3±0.25b
Tyr-Li2	11.87±0.10bc	9.81±0.09b	17.26±0.250b	2.51±0.06b	7.94±0.06b	2.77±0.01b	3.81±0.06b	10.5±0.29ab
F	1644.84	486.3	573.8	118.3	903.8	353.3	412.9	11.54
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

*: Means (±: n= 10) in the same column for each trait in each group with the same lower-case letter are not significantly different by ANOVA test at $P \leq 0.05$. 0: Control, Tyr: L-Tyrosine, Li1: 6.43 mM Lithium, Li2: 19.32 mM Lithium.

Table 2. Effect of exogenous Tyr on the chlorophyll pigments, carotenoids, and secondary metabolites in spinach seedlings under Li stress

Group	Chlorophyll a mg g^{-1}	Chlorophyll b mg g^{-1}	Total chlorophyll mg g^{-1}	Lutein mg g^{-1}
0	0.840±0.001bc*	1.436±0.001d	2.276±0.001c	1.42±0.002b
Tyr	0.978±0.001a	1.484±0.001c	2.462±0.001ab	0.73±0.002e
Li-1	0.781±0.001c	1.350±0.001e	2.126±0.002d	0.79±0.001d
Li-2	0.754±0.001d	1.241±0.001f	1.994±0.002e	1.52±0.002a
Tyr-Li1	0.857±0.001b	1.630±0.001a	2.486±0.001a	0.76±0.002d
Tyr-Li2	0.853±0.001b	1.509±0.001b	2.361±0.002b	0.87±0.001c
F	12503.9	8986.2	23092.8	91.65
P	<0.001	<0.001	<0.001	<0.001

*: Means (±: n= 3) in the same column for each trait in each group with the same lower-case letter are not significantly different by ANOVA test at $P \leq 0.05$. 0: Control, Tyr: L-Tyrosine, Li-1: 6.43 mM Lithium, Li-2: 19.32 mM Lithium.

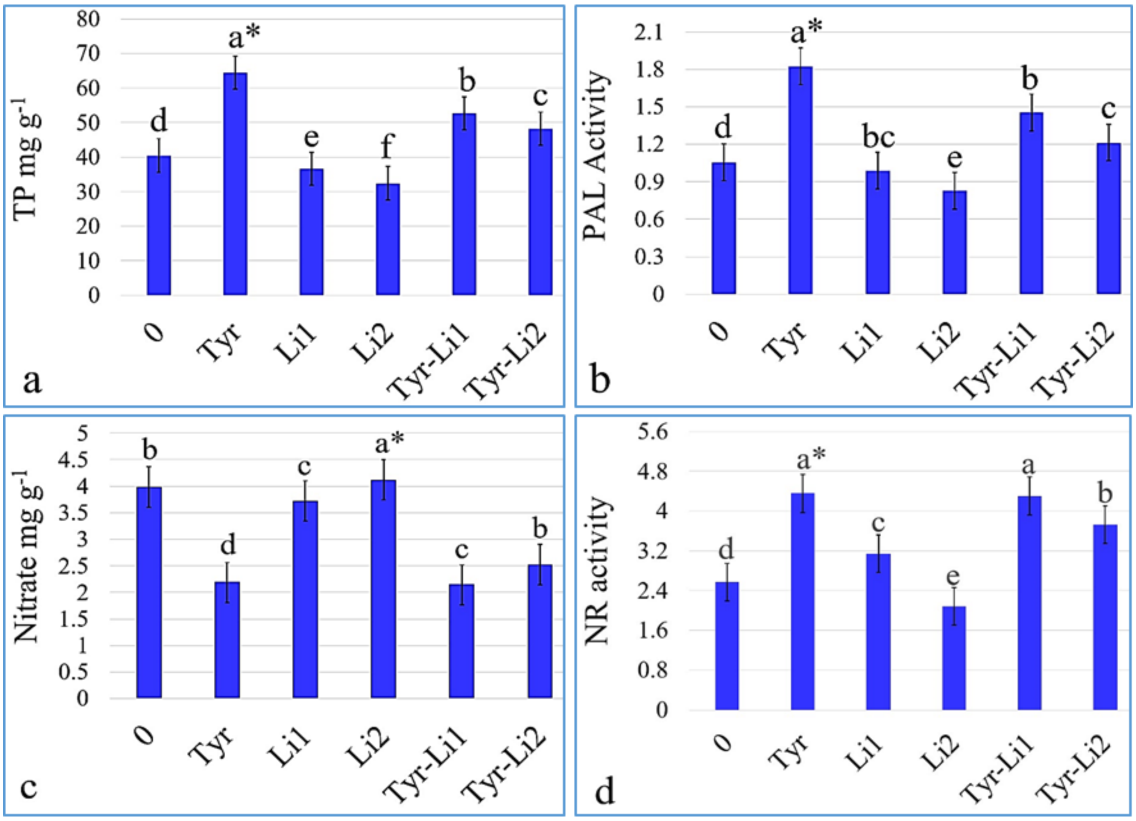

Figure 1. Variation of total phenolic (TP:1a), phenylalanine ammonia-lyase (PAL: 1b), nitrate (1c), and nitrate reductase (NR: 1d) levels in the seedlings. 0: Control, Tyr: 2.5 mM L-Tyrosine, Li1: 6.43 mM Lithium, Li2: 19.32 mM Lithium. Means (±: n= 3) in the same column for each trait in each group with the same lower-case letter are not significantly different by ANOVA test at $P \leq 0.05$.

The destructive influences of alkaline stress on leaf pigmentation, carotenoid, and secondary metabolite accumulation, in contrast, the ameliorative effect of amino acid supplements on stressed plants have been well-documented in many studies. Shams et al. (2016) in lettuce, Makus et al. (2006), and Bakhat et al. (2020) in spinach reported the pigment contents decreased at high lithium doses but increased at a lower dose. They suggested that a decline in pigment content may be connected with the degradation of chlorophyll caused by the replacement of Mg in chlorophyll molecules by Li. A high chlorophyll content in the spinach seedlings may be related to low NT content (Table 2, Figure 1c). It was reported that exogenous amino acids supply increases chlorophyll, carotenoid, and secondary compounds by adjusting nitrogen utilization (Al-Mohammad and Al-Taey 2019; Xu et al. 2019). The low level of lutein content of Li-applied seedling spinach was associated with low light intensity.

Similarly, Makus et al. (2006), and Verhoeven et al. (2018) recorded that low light intensity stimulates the carotenoid mechanism of plants, especially in greenhouse conditions. The decrease in polyphenol content in the Li-applied seedlings may be related to the inhibition of PAL activity by high Li doses. Likewise, Shams et al. (2016), Feduraev et al. (2020), and Tarasevičienė et al. (2021) observed that polyphenol accumulation decreased under alkaline stress conditions by the inhibition of PAL and PPO activities, which are responsible for the synthesis of secondary compounds. However, the same researchers reported that exogenous amino acid supplements to plants under stress mitigate stress damage by stimulating secondary metabolism. Nitrate (NO_3), is one of the principal nitrogen (N) sources for all plants. The utilization of it by plants is closely related to nitrate reductase enzyme (Citak and Sonmez 2010). NT content and NR activity of seedlings were influenced negatively by a higher Li level (Figure 1c, 1d). While the highest NT was observed in the Li2 group, the lowest level was recorded with the Tyr-Li1, followed by seedlings treated with justtyrosine. On the contrary, NR activity was at the lowest level at Li2, followed by the control seedlings. Also, the exogenous Tyr supplement to the Li-applied seedling exhibited a positive effect (Figure 1d). Similar results were achieved by Citak and Sonmez (2010), and Perchlik and Tegeder (2018), who observed that the higher NR activity lowered NT content in plants, especially in cold seasons. They also reported that exogenous amino acid application suppressed NT accumulation in leaves by replacing nitrate, resulting in NT decreasing. Saline-alkaline stress may provoke the production of oxidative stress agents such as MDA, H_2O_2, and other molecules, which have a destructive effect on the cell membrane and components (Shams et al. 2016).

The increase in MDA and H_2O_2 content, especially at high lithium doses, in seedlings applied with Li depicted oxidative stress induced by lithium (Figure 2a, 2b). Also, exogenous Tyr supplement to the stressed seedling led to a considerable decrement in those molecules (Figure 2a, 2b). This increase in MDA and H_2O_2 has been observed in sunflower (Hawrylak-Nowak et al. 2012), spinach (Bakhat et al. 2020), lettuce (Shams et al. 2016), and arugula (Kusvuran et al. 2019) under salt-alkaline stress. Likewise, the improvement effect of foliar amino acid applications under stress conditions was demonstrated by Al-Mohammad and Al-Taey (2019), Helaly and Ibrahim (2019), and Saddıque et al. (2022). The decline in MDA and H_2O_2 in the Tyr-Li group may have resulted from antioxidant enzyme activations induced by Tyr.

Upregulation of APX, CAT, POD, and SOD enzymes during stressful conditions strengthens stress resistance by scavenging toxic compounds, therefore, they help to improve increasing yield and quality (Kusvuran et al. 2019; Saddique et al. 2022). In the spinach seedlings APX, CAT, POD, and SOD activity were importantly inhibited by a higher lithium application (Figure 3a, 3d). Whereas exogenous tyrosine supplement to stressed seedlings enhanced APX, CAT, and SOD activity under both Li applications, it reduced POD activity at higher Li levels (Figure 3a, 3d). Among the applications, the highest APX, POD, and SOD were recorded with an application of only Tyr, but the highest CAT activity was observed in the Tyr-Li1 applied group (Figure 3b). Inhibition of antioxidants, under severe saline-alkaline stress, has been recorded in lettuce (Kalinowska et al. 2013), muskmelon (Xu et al. 2019), and spinach (Bakhat et al. 2020). Further, the upregulation of antioxidants was recorded under stress conditions by a foliar amino acid supplement in lettuce (Shams et al. 2016), spinach (Bakhat et al. 2020), mints (Tarasevičienė et al. 2021), and Brassica (Zhang et al. 2019). Results displayed that tyrosine ameliorated the negative effects of lithium by regulating the antioxidant defense of the seedlings. Variations of Li, Mg, P, S, K, and Ca concentrations in the seedlings are presented in Table 3

The variation of mineral status of seedlings is presented in Table 3 and Table 4. The Li content of seedlings was found to be lowest in the control, and it was the highest at Li2. Contrary, Mg and K contents were lower in all applications. Also, there was a rise in the P and S contents in the Li doses and the Tyr-Li1 group. While the Ca in the seedlings decreased only in the Tyr application compared to the control, it reached its highest level at Li_2 (Table 3). While Na content was lower in the control group, Cl content was lower in the group which was provided just with Tyr. On the other hand, Mg, P, S, K, Ca, and Cl accumulation was reduced by the application of just Tyr, but the Li and Na were enhanced. Variations of the trace elements of the seedlings are shown in Table 4.

Applications of just Tyr caused a remarkable reduction in the Mn, Fe, Ni, Cu, and Zn content in comparison to the control and other groups, but a lower Li led to the greatest rise in Ni, Cu, and Zn. Mn and Fe content increased with Li doses, but just exogenous Tyr applications caused a decline in both elements in a higher Li dose, compared to Li-applied groups (Table 4). A decrease in the Mg and K under applications was associated with Li-induced limitations and interactions of tyrosine with cations (Ruan and Rodgers 2004; Bakhat et al. 2020). It was thought that the synergistic effect of this element with lithium might be effective in increasing the Na content (Siu et al. 2004; Franzaring et al. 2016). The high Cl content in the seedlings may be due to the chlorine elements in the applied lithium salt. Differences in the interactions of foliar aromatic amino acid applications with cations and anions have been already reported by Bakhat et al. (2020). In addition, the antagonistic/synergistic interactions between the elements, the mobility of the elements, and their functions in the plants may have influenced the amounts of these elements in the spinach leaf tissue (Hawrylak-Nowak et al. 2012; Al-Mohammad and Al-Taey 2019).

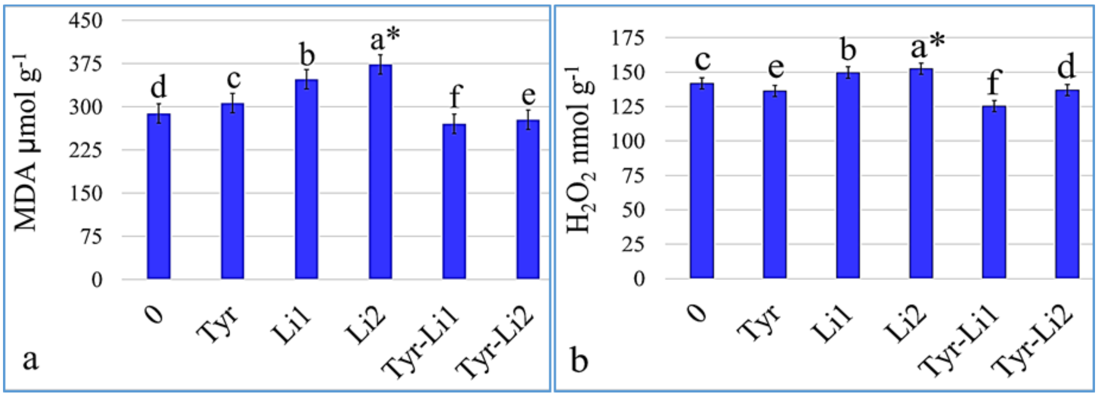

Figure 2. Variation of MDA (2a) and H_2O_2 (2b) levels in the spinach seedlings. 0: Control, Tyr: 2.5 mM L-Tyrosine, Li1: 6.43 mM Lithium, Li2: 19.32 mM Lithium. Means (±: n= 3) in the same column for each trait in each group with the same lower-case letter are not significantly different by ANOVA test at $P \leq 0.05$.

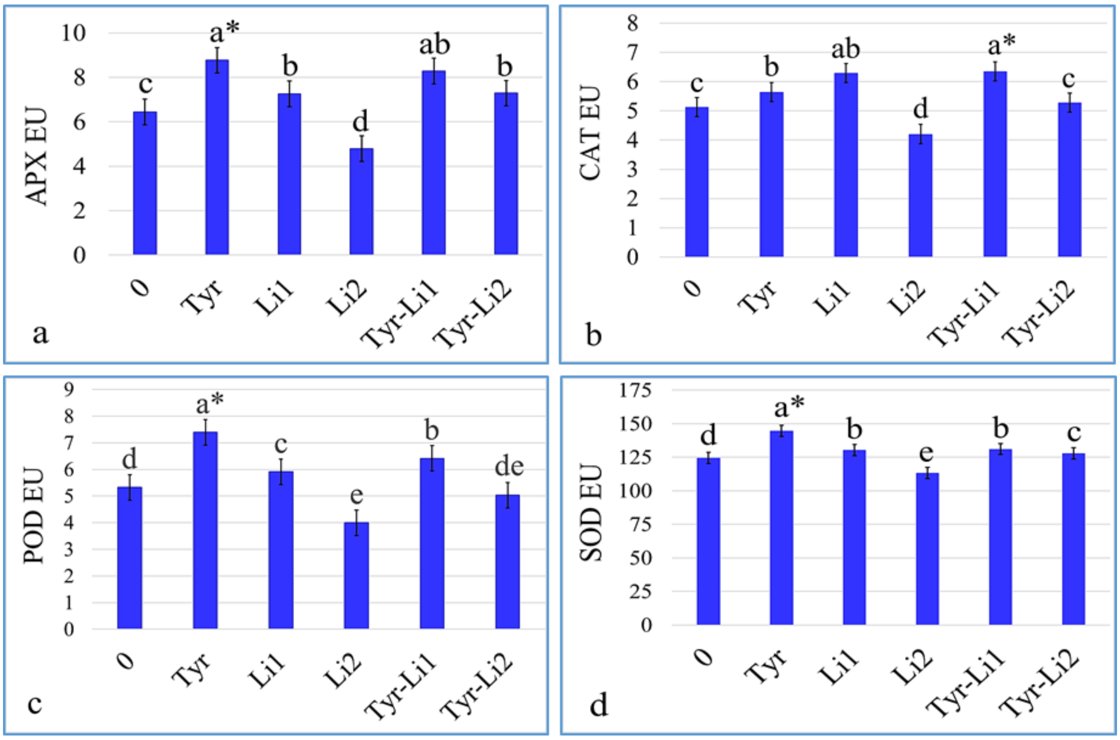

Figure 3. Variation of APX (3a), CAT (3b), POD (3c), and SOD (3d) activities in the spinach seedlings. 0: Control, Tyr: 2.5 mM L-Tyrosine, Li1: 6.43 mM Lithium, Li2: 19.32 mM Lithium. Means (±: n= 3) in the same column for each trait in each group with the same lower-case letter are not significantly different by ANOVA test at *P*≤0.05.

Table 3. Variation of Li, Mg, P, S, K, Ca, and Na concentrations (mg kg^{-1})

Group	Li	Mg	P	S	K	Ca	Na
0	21±2.5f*	12786±120a	5545±30.6c	6545±60.6c	86556±88a	15479±52c	5067±45d
Tyr	80.6±7.6e	10548±104d	4234±28.8e	5067±44.7d	66789±66e	12345±122d	12071±120b
Li1	845±70.6c	12456±120ab	5878±35.6b	7234±60.2b	83481±82b	18770±170b	8145±75c
Li2	1268±120ab	12430±120ab	6244±408a	7685±60.8a	81345±80bc	20356±202a	12456±125b
Tyr-Li1	612±60.8d	12054±120b	5566±33.2c	7066±55.6bc	82345±80b	17123±160bc	14223±140a
Tyr-Li2	1014±102	11884±115c	5346±304d	6495±40.4c	75669±76d	19245±190a	12355±122b
F	18232054	495646	689575	6059	30079386	7814416	13184637
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

*: Means (±: n= 3) in the same column for each trait in each group with the same lower-case letter are not significantly different by ANOVA test at *P*≤0.05. 0: Control, Tyr: L-Tyrosine, Li-1: 6.43 mM Lithium, Li-2: 19.32 mM Lithium.

Table 4. Variation of Cl, Mn, Fe, Ni, Cu, and Zn concentrations (mg kg^{-1})

Group	Cl	Mn	Fe	Ni	Cu	Zn
0	5678±45c*	96.7±5.6f	2545±20.6e	34.56±2.4c	13.57±0.8c	115.48±10.8b
Tyr	2880±23d	90.8±4.8g	2044±18.8f	30.35±2.2d	10.24±0.6d	80.68±2.4d
Li1	10566±104bc	175.5±12.6b	4456±36.6b	48.77±6.4a	18.80±1.2a	126.56±11.7a
Li2	13466±133a	150.4±10.5cd	3423±28.8c	40.56±5.4b	15.48±0.9b	107.46±9.8c
Tyr-Li1	11456±112b	125.5±8.8e	3054±25.7d	33.46±3.6c	12.99±0.6c	114.57±10.3b
Tyr-Li2	10567±104bc	180.3±13.6a	5026±38.8a	40.67±3.8b	15.47±0.8b	124.55±11.7a
F	21249159	8915	3093776	1103408	136044	7252572
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

*: Means (±: n= 3) in the same column for each trait in each group with the same lower-case letter are not significantly different by ANOVA test at *P*≤0.05. 0: Control, Tyr: L-Tyrosine, Li-1: 6.43 mM Lithium, Li-2: 19.32 mM Lithium.

4. Conclusion

In this study, it was concluded that higher lithium levels negatively affect spinach growth rate, accumulation of pigments, secondary metabolites, and activity of enzymes. The exogenous supplement of tyrosine had a positive effect in improving all these attributes in the seedlings, even in lithium-treated groups. Incremental values of growth parameters, including shoot, root, and leaves by Tyr supplements were parallel with the enhanced synthesis of chlorophylls. The amounts of chlorophyll, lutein, secondary constituents, and the activity of antioxidant enzymes were decreased by the higher lithium level, but MDA, H_2O_2, and nitrate content increased. However, an external supplement of tyrosine reduced these examined constituents by increasing the activity of enzymes, and secondary compounds. A Tyrosine supplement to seedlings inhibited nitrate accumulation by stimulating nitrogenous compounds but increased NR activity.

The Mg, K, P, S, and Ca contents were lower in the Tyr-applied group, but the Cl, Mn, Fe, Co, Ni, Cu, Zn, Cr, Si, Al, Ag, Cd, I, and P concentrations were reduced by the Tyr and the Li1-Tyr groups. On the other hand, while Li doses increased the Li contents in seedlings, exogenous Tyr supplements caused a notable decrement in Li accumulations. It seems that with the application of exogenous tyrosine to the plant, the synthesis of tyrosine-derived compounds that increase yield and quality, increase resistance to stress, and are also beneficial for human health, can be stimulated. In conclusion, it can be said that exogenous Tyr supplements showed a positive effect on the growth rate of the Anlani F1 spinach cultivar, therefore, exogenous Tyr applications in barren, calcareous, and alkaline soils can be an alternative in spinach cultivation.

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