# Role of exogenous folic acid in alleviation of morphological and anatomical inhibition on salinity-induced stress in barley

**Semra Kilic, Hatice Tuğba Aca**

***Department of Biology, Süleyman Demirel University, Isparta, Turkey***

## Abstract

Soil salinity is a serious threat to agricultural ecological environment and agriculture sustainability. Ever increasing salinity negatively affects processes such as plant growth and development, ultimately causing diminished economic yield and quality of production, and it might cause a worldwide famine in the future. Thus, helping plants adapt to saline soils and increasing their yield and quality is a must. Our study focused on the enhancing role of exogenously applied folic acid (FA) in mitigation of toxicity caused by salt (NaCl). Barley seeds were pre-treated with 50 μM FA for 24 h and then exposed to salt. Morphological and anatomical changes in seed germination and seedling growth stages were compared between different treatments of salt in laboratory conditions. Adverse effects of salt in both germination and seedling growth stages depended on the concentration of salt treatment (0.0, 0.25, 0.275, 0.30, 0.325 and 0.35 M). It was shown that the application of FA effectively alleviated the salt-induced inhibition, and reduced the negative effects of salt on germination (germination index and vigour index), seedling growth (radicle and coleoptile lengths, fresh weight) and leaf (stomata and epidermis number, stomatal index, stomata sizes of adaxial and abaxial surfaces) parameters. Moreover, FA elevated all examined parameters of barley also under non-stress conditions. Especially, germination and vigour indices were significantly higher than the control. Our results suggest that exogenous FA is involved in the resistance of barley to salt-stress.

Correspondence: Semra Kılıç, Department of Biology, University of Süleyman Demirel, Faculty of Science and Arts, 32260, Isparta, Turkey.
Tel.: +90.246.2114115 - Fax: +90.246.2371106.
E-mail: semrakilic@sdu.edu.tr

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## Introduction

Plants have evolved to live in environments where they are exposed to a wide range of abiotic stresses such as drought, salinity, high temperature, and UV. Among these stresses, salinity is considered as one of the major abiotic stresses that has negative impacts on the plant growth (Ruiz-Lozano *et al*., 2012), especially reduction in photosynthesis, respiration and protein synthesis (Hasanuzzaman *et al*., 2013). Also, processes such as seed germination, seedling growth and vigour, vegetative growth, flowering and fruit set are adversely affected by high salt concentration, ultimately causing diminished economic yield and quality of production (Sairam and Tyagi, 2004). Increased salt stress causes water stress (physiological drought) resulting from the increase of osmotic potential by the increase of ions in the soil. On the other hand, exposure of plants to excess salt causes both ion toxicity-induced imbalances and ion imbalance in metabolism (Golldack *et al*., 2011). Especially, during water stress, which is triggered by salt stress, excess accumulation of leaf $Na^+$ and $Cl^-$ may negatively affect photosynthesis that is the driving force of plant growth and development (Nazar *et al*., 2011). Moreover, salt stress inhibits $CO_2$ assimilation by inducing stomatal responses (Lu *et al*., 2009). Although the inhibiting effects of salt stress on the growth and development of plants have been known for a long time, the mechanisms of whole plant responses to salt stress have not been completely explained yet.

At the present time, approximately 20% of agriculture areas are salty (Flowers and Yeo, 1995), and it is estimated that by 2050 this ratio will reach 50% (Gupta and Huang, 2014). Therefore, ever increasing soil salinity throughout the world is an important abiotic stress, which is a threat to living organisms (Wang *et al*., 2011). Food demand increases in parallel with world population. And the necessity to meet this demand imposes the use of saline soils. Furthermore, many studies are currently being conducted on increasing plants' salt tolerance or decreasing the effects of salt in plants by growth hormone (or chemical) applications (Cavusoglu *et al*., 2007; Kilic *et al*., 2007; Shahid *et al*., 2014). However, only a limited amount of studies investigated the effects of substances, which decreases or slows the effects of salt (Stakhova *et al*., 2000), such as folic acid (FA) and a complex water-soluble vitamin B (Cossing, 2000). Whereas, FA, a natural antioxidant (Asensi-Fabado and Munné-Bosch, 2010), is the ideal growth regulator which increases leaf chlorophyll content (Sladky, 1959). It was determined that application of methotrexate, which is an inhibitor of folate biosynthesis, to *Pisium sativum* leaves deteriorates methylation reactions that regulate chlorophyll biosynthesis and significantly reduces chlorophyll biosynthesis in the leaves (Wilder *et al*., 2009). In another study, Farouk and EL-Saidy (2013) had applied 20 mg/L folic acid to sunflower seeds to increase their viability and germination rate, and observed significant increases in vigour parameters (shoot and root lengths, dry weights, and shoot to root ratio). FA increases proline biosynthesis under stressful conditions, thus helping the plant to gain endurance against stress (Burguieres *et al*., 2007). Moreover, FA binds to essential elements and increases their absorption from the soil (Michael, 2001). On the other hand, FA, form of the water-soluble vitamin B9 and is not biologically active, is to tetrahydrofolate and other derivatives after its conversion to dihydrofolic acid (Burguieres *et al*., 2007; Bailey and Ayling, 2009). Increasing interest of some plant researchers in folic acid derivatives is due to its multiple functions (Stakhova *et al*., 2000). With all these characteristics, FA plays important roles in many physiological processes. For example, folic acid application to *Pisum sativum* L. and *Hordeum vulgare* L. seedlings after flowering greatly increased the yield, weight and quality of pea and barley seeds (Stakhova *et al*., 2000). Methionine, which plays a role in folate photorespiration, increases the growth and development rate of plants by activating the biosynthesis of purines and thymidylate (Hanson and Roje, 2001). A positive correlation between folate biosynthesis and cell division was determined during seed germination. The increase in cell division was explained as a response to the increased demand of carbon one units (Cossins and Chen, 1997), which is necessary for nucleotide biosynthesis and cell metabolism induced, by increased folate biosynthesis (Jabrin *et al*., 2003). Different concentrations of sulfamethoxazole, an inhibitor of folic acid biosynthesis, were used to determine the effects of folic acid on the germination characteristics and seedling growth of wheat. Growth and development of wheat was most repressed at the highest sulfamethoxazole concentration (30  mol). This negative correlation showed that folic acid plays an important role in biochemical and physiological processes during growth and development of the seeds (Esfandiari *et al*., 2012). Furthermore, folic acid significantly improved the protein content of *Cicer arietinum* seeds during germination (Sibian *et al.,* 2016).

Even though salt stress causes morphological and physiological deformations in plants, we know very little about the effects of FA against salt stress in seed germination, seedling growth and leaf anatomy of barley. Modifications in germination and growth parameters of plants under various stresses lead to changes in morphological, anatomical, physiological processes, and their complex interactions (Taiz and Zeiger, 2006) and they can be determined by anatomical and morphological anomalies which may be the visible signs of these changes (Anastasov, 2010). Therefore, in this study, direct and indirect effects of salt on the growth and development of barley were evaluated *via* various growth parameters, and the potential mitigation effects of the exogenous application of FA on seed germination, seedling growth and stomatal responses of barley plants exposed to increasing salt concentrations were measured.

## Materials and methods

### Plant material and plantation

In this study, barley (*Hordeum vulgare* cv. Bülbül 89) seeds were used. The seeds were surface sterilised with 1% sodium hypochloride. Salt (NaCl) concentrations used were 0.0, 0.25, 0.275, 0.30, 0.325 and 0.35 M. Folic acid concentration used in the experiments was 50 μM FA (Burguieres *et al*., 2007) and NaCl concentrations were determined in a preliminary investigation. Adequate amount of seeds for application were pre-soaked in beakers containing distilled water (C) and 50 μM FA under room temperature for 24 h. Subsequently, for each experiment, 25 seeds with the same size were carefully selected and placed on a Whatman paper soaked with 20 mL distilled water and then incubated in 20°C for 7 days. Each treatment was replicated three times. The ledge of radicle through the seed coat was taken as the criteria of seed germination (Bewley, 1997).

For analysis of growth parameters (stomatal index, stomata length and width, leaf area), seedlings were transplanted into pots (45 $cm^3$ volume) with perlite and after 7 days of germination 5 seedlings were planted in each pot. They were cultivated in a growth chamber (25/20°C day/night temperature, 75% relative humidity, 16/8 h photoperiod, light intensity 160  mol PAR $m^{-2}s^{-1}$), and each pot was regularly added Hoagland's nutrient solution for 45 days.

On the 7th day after sowing, the root length, stem height (Mulholland *et al*., 1996; Jacobsen *et al*., 2013) and number of germinated seed were calculated to determine germination and vigour indexes. The germination index (GI) (Tiquia, 2010) and vigour index (VI) (Hangarter, 1997) of each treatment were calculated using the following equations:

$$GI = (\%\ \text{Relative seed germination} \times \%\ \text{Relative root growth}) / 100 \quad (1)$$

where, *% Relative seed germination* is:

(Number of seeds germinated in salt concentration / Number of seeds germinated in control) × 100

and *% Relative root growth* is:

Mean root length in salt concentration / Mean root length in control × 100

$$VI = (\text{Seedlings length (cm)} \times \text{Germination percentage (\%)}) / 100 \quad (2)$$

Stomatal index was calculated number of stomata and epidermal cells counted in each field (1 $mm^2$) at independent measurement by superficial sections taken from upper (adaxial) and lower (abaxial) surfaces of leaves (Rengifo *et al*., 2002), based on average of 50 microscopic field. Stomata sizes (length and width) were defined using an ocular micrometre under light microscope (40 X object and 10 X ocular).

### Statistical analysis

The statistical analysis of variance (ANOVA) was performed on all experimental data reported in the present paper and statistical significance ($P \leq 0.05$). Duncan's multiple range test was applied for means of at least two independent assay with three replicate using SPSS Software 13.0. All experimental data are expressed as means ± standard deviation.

## Results

### Germination index, vigour index and other growth parameters

The effects of FA and salt, and their interaction with seed germination and seedling growth of the barley were examined in terms of GI and VI (Figure 1), and other growth parameters (radicle and coleoptile length, plant fresh weight) (Figure 2). With increasing salt stress, all growth parameters of barley dropped dramatically ($P \leq 0.05$). Moreover, growth of the barley plants was severely impaired by salt stress as demonstrated by all examined parameters. However, pre-treatment with FA alleviated the inhibitory effect of salt stress in a dose-dependent manner. Similarly, pre-treatment was accounted for marked enhancement of plant growth compared to control plants. For example, with the application of FA, the GI increased from 65.88 to 77.76, VI increased from 37.96 to 50.41 at 0.25 M salt stress, and at 0.275 M salt stress GI increased from 32.36 to 36.98, and VI increased from 24.19 to 27.76. While the most destructive effect on GI was a 97% decrease at 0.35 M salt concentration, FA pre-treatment decreased this value to 91%, and this result showed that FA had increased germination by 9%

even at the highest applied concentration of salt.

Salt stress reduced radicle length, coleoptile length and plant fresh weight by 90%, 83% and 74%, respectively, at the highest salt concentration (Figure 2). Whereas for FA applied plants, radicle length, coleoptile length and plant fresh weight increased by 54%, 32% and 17%, respectively, under similar salt stress. These results demonstrate that FA application alleviates the negative effects of salt stress in barley ($P \leq 0.05$).

### Stomatal responses

The effects of FA on photosynthetic apertures (stomata) in both adaxial and abaxial surfaces of the leaves of barley plants grown under salt stress are shown in Table 1. FA pre-treatment alleviated the inhibitory effect of salt stress on stomatal movements, stomata number, epidermis number, stomata sizes (width/length) and stomata index. Stomata indices of adaxial and abaxial surfaces of leaves were found to be lower in plants, which were not pre-treated with FA. The highest decrease in stomata index was observed on the abaxial surface (61%) of the leaf of barley plant treated with 0.30 M of salt, as compared to the control. Stomata and epidermis number of barley plants exposed salt decreased by 16%, 40%, 61% and 9%, 33%, 47% in relation to salt concentration, respectively. It suggests that salt decreases the number of stomata and epidermis of barley seedlings mainly by reducing stomata index. However, FA pre-treatment increased the stomatal index in both surfaces of leaves despite the salt stress. For instance, FA application increased stomata index on adaxial surfaces of barley leaves treated with 0.25, 0.275 and 0.30 M of salt concentration by 6%, 5%, 4% and 2%, respectively. A similar effect was observed on abaxial surfaces and this effect was found to be statistically significant ($P \leq 0.05$). FA's positive impact on stomata indices of both surfaces of leaves was in parallel with the positive changes in stoma and epidermis numbers. On the other hand, negative impact of increasing salt concentrations manifested as a decrease in stoma sizes in both surfaces of leaves ($P \leq 0.05$). This was most dramatically observed in the adaxial surface of the leaf treated with 30 M salt concentration as a 32% decrease in stomata widths. Effects of increasing salt concentrations on stomata lengths were similar on both surfaces (approximately 12% decrease) at the highest salt concentration, compared with control. In spite of that, FA pre-treatment positively affected the stomata sizes by significantly increasing them ($P \leq 0.05$). However, positive effects of FA decreased with increasing salt concentrations. For instance, stomata lengths of adaxial surfaces of barley leaves treated with 0.0, 0.25, 0.275 and 0.30 M of salt increased by 8%, 4%, 3% and 1%, respectively. The situation was similar in abaxial surfaces and increase rates were 11%, 8%, 7% and 4%.

## Discussion

In this study, the effects of salt stress on growth parameters of barley and its relation with FA were described. Salt stress reduced the growth of barley considerably and the effect was subsequently mitigated by the exogenous application of FA. Soil salinity is one of the most important abiotic factors threatening agriculture areas throughout the world

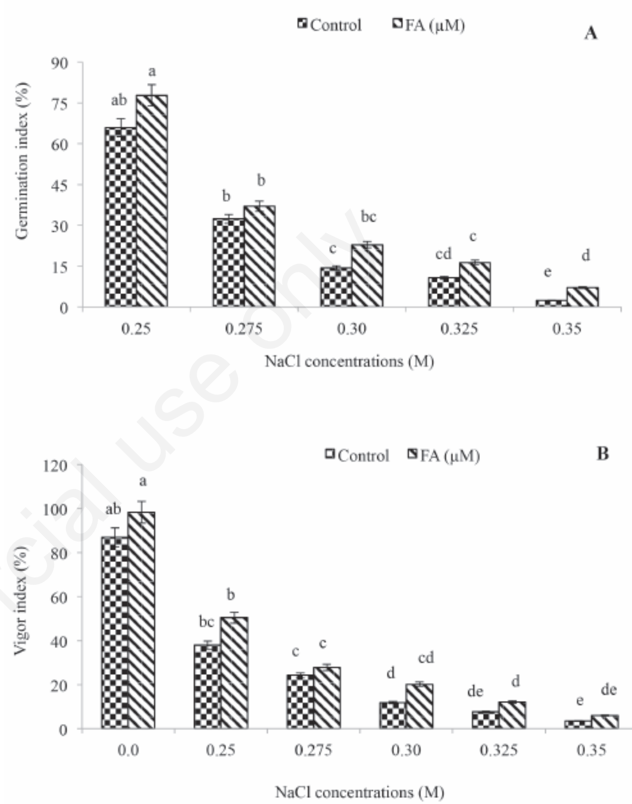


**Figure 1. Germination index (A) and vigour index (B) values of barley seeds exposed to various concentrations of salt after folic acid (FA) pre-treatment. Data represent the means and vertical bars indicate the standard error. The values that are followed by the same letter do not differ statistically at a significance level P≤0.05.**

**Table 1. The structure of adaxial and abaxial epidermis in leaves of barley plants treated with different concentrations of salt after folic acid pre-treatment.**

| | NaCl (M) | Stomata number ($mm^2$) | | Epidermal cells number ($mm^2$) | | Stomatal index | | Stomata width (µm) | | Stomata length (µm) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | C | FA (µM) | C | FA (µM) | C | FA (µM) | C | FA (µM) | C | FA ( M) |
| Adaxial surfaces | 0.0 | $4.6\pm0.1^{a}$ | $5.5\pm0.6^{a}$ | $19.8\pm0.5^{a}$ | $22.2\pm1.2^{a}$ | $18.8\pm0.3^{a}$ | $19.8\pm0.1^{a}$ | 22.1±0.1a | $25.1\pm0.9^{a}$ | $45.1\pm0.8^{a}$ | $48.7\pm2.4^{a}$ |
| | 0.25 | $3.9\pm0.2^{b}$ | $4.6\pm0.4^{ab}$ | $18.1\pm1.1^{b}$ | $20.7\pm1.1^{ab}$ | $17.2\pm0.2^{ab}$ | $18.1\pm0.1^{a}$ | $21.3\pm0.2^{b}$ | $22.9\pm0.2^{ab}$ | $43.5\pm0.9^{b}$ | $44.9\pm1.2^{ab}$ |
| | 0.275 | $2.8\pm0.1^{c}$ | $3.3\pm0.1^{b}$ | $13.3\pm0.5^{c}$ | $15.1\pm1.3^{b}$ | $17.3\pm0.1^{b}$ | $17.9\pm0.2^{a}$ | $17.5\pm0.7^{c}$ | $18.7\pm1.2^{b}$ | $42.5\pm1.7^{c}$ | $43.6\pm2.1^{b}$ |
| | 0.30 | $1.8\pm0.7^{d}$ | $2.1\pm0.3^{c}$ | $10.5\pm0.2^{d}$ | $11.3\pm0.8^{c}$ | $15.1\pm0.4^{c}$ | $15.3\pm0.2^{b}$ | $15.1\pm0.4^{d}$ | $15.9\pm1.5^{c}$ | $40.1\pm1.2^{d}$ | $41.1\pm1.6^{c}$ |
| Abaxial surfaces | 0.0 | $4.3\pm0.5^{a}$ | $5.1\pm0.5^{a}$ | $20.9\pm0.2^{a}$ | $22.8\pm0.3^{a}$ | $17.1\pm0.4^{a}$ | $18.2\pm0.6^{a}$ | $20.1\pm0.5^{a}$ | $23.7\pm0.2^{a}$ | $46.9\pm1.1^{a}$ | $52.5\pm2.6^{a}$ |
| | 0.25 | $3.8\pm0.3^{b}$ | $4.4\pm0.4^{ab}$ | $20.2\pm0.4^{ab}$ | $21.7\pm0.4^{ab}$ | $15.8\pm0.3^{ab}$ | $16.8\pm0.5^{a}$ | $21.1\pm0.3^{ab}$ | $22.9\pm1.1^{ab}$ | $44.9\pm1.3^{b}$ | $48.5\pm1.7^{a}$ |
| | 0.275 | $2.9\pm0.1^{c}$ | $3.4\pm0.4^{b}$ | $16.5\pm0.6^{b}$ | $17.8\pm0.2^{b}$ | $14.9\pm0.1^{b}$ | $16.1\pm0.3^{a}$ | $18.9\pm0.8^{b}$ | $20.1\pm0.8^{ab}$ | $43.3\pm1.8^{c}$ | $46.5\pm1.4^{ab}$ |
| | 0.30 | $1.7\pm0.3^{d}$ | $1.9\pm0.6^{c}$ | $12.1\pm0.3^{c}$ | $12.8\pm0.4^{c}$ | $12.3\pm0.1^{c}$ | $12.9\pm0.2^{b}$ | $14.6\pm0.7^{c}$ | $15.1\pm1.1^{b}$ | $41.7\pm1.1^{d}$ | $43.1\pm1.5^{b}$ |

C, beakers containing distilled water; FA, folic acid. [a-d]The values that are followed by the same letter do not differ statistically at a significance level P≤0.05.

(Wang *et al*., 2011). Increasing salt salinity causes degradations in plant growth and irreversibly damages the plant. Soil salinity (Bewley, 1997) particularly affects the germination process (Fredj *et al*., 2013), which is one of the most important periods of plant growth, by delaying germination and decreasing germination rate (Ashraf and Foolad, 2005). A period of sensitivity to stress started with the imbibition of dry seeds during seed germination (Vertucci and Leopold, 1983). The negative impact of salt stress on seed germination occurs as a reduction of water absorption from the environment (Long *et al*., 2013) as a result of hyper-osmotic stress (Farsiani and Ghobadi, 2009) caused by the reduction of osmotic potential in soil solution by salt. We think that the decrease in water imbibition rate with increasing salt concentration could be caused by the reduction of seed weights, similar to our study. On the other hand, when sodium, which is a specific ion, reaches toxic levels in soil solution and plant structure, biological membranes and subcellular organelles get damaged, plant growth reduces, and abnormal growth is observed (Quintero *et al*., 2007). Various plant growth regulators and osmoprotectans are used to alleviate the detrimental effects of salt stress on plants. They act by either increasing nutrient intake and transfer, or protecting osmotic balance (Kaya *et al*., 2010). Our results showed that GI and VI of barley decreased with increasing salt concentrations. Exogenous application of FA significantly alleviated salt stress-induced growth inhibition. FA pre-treatment increased GI by 84%, 87%, 62%, 65% and 67% at 0.25, 0.275, 0.30, 0.325 and 0.35 M salt concentrations, respectively. VI was also increased by 87%, 75%, 87%, 58%, 62% and 58%, compared to control. B group vitamins act as precursors that regulates various metabolic activities like germination. But, micronutrient usability and vitamin content is at their highest during germination. Furthermore, germination can only happen if B group vitamins that act as precursors of enzymatic cofactors overcome oxidative stress which causes seed dormancy (Hotz and Gibson, 2007). Quinoa that had been raised in an environment enriched with B group vitamins including folic acid showed positive metabolic responses to these substances, thus they were determined as the best biomolecules to protect plants from oxidative damage (Pitzschke *et al*., 2015). In another study, folic acid application increased pea seedling vigour during germination (Burguieres *et al*., 2007). The decrease of GI and VI against increasing salt concentrations was reflected by other growth parameters. While barley plants exposed to 0.25 M salt concentration did not show significant deteriorations in radicle and coleoptile lengths and fresh weight ($P>0.05$), at 0.35 M the impact of salt was the most destructive ($P\leq0.05$). Therefore, the delay in growth of barley can be explained by suppression of germination under salt stress. The negative impact of salinity on plant growth has been also reported in several plant species (Wang *et al*., 2011; Long *et al*., 2013). Under salt stress, especially radicle growth is in parallel with decreasing mitotic index (Hesa, 2011). In contrast, the exposure of barley to salt in the presence of FA alleviated root and coleoptile growth inhibition when compared to the presence of salt alone. For instance, FA treatment increased radicle length by 92%, 96%, 67%, 60%, 46%, and coleoptile length by 32%, 27%, 18%, 9%, and 4% in increasing salt concentrations, respectively. FA showed the most alleviating effect at 0.25 M salt concentration. By increasing cell division and differentiation (Henning *et al*., 1997), FA significantly improved all examined parameters at the lowest salt concentration ($P\leq0.05$). However it was observed that the alleviating effect diminished as salt concentration increased. Our results suggest that FA can reduce chromosomal and mitotic abnormalities and alleviate salt stress which delays or supresses germination process. Another negative effect of salt toxicity was observed in plant fresh weight. Fresh weight reduced by 74% at 0.35 M salt concentration. However, FA application alleviated the effect and increased fresh weight by 32% at the same concentration. These results indicated that application of FA can alleviate the reduction in growth parameters of barley seedlings exposed to increasing salt concentrations ($P\leq0.05$).

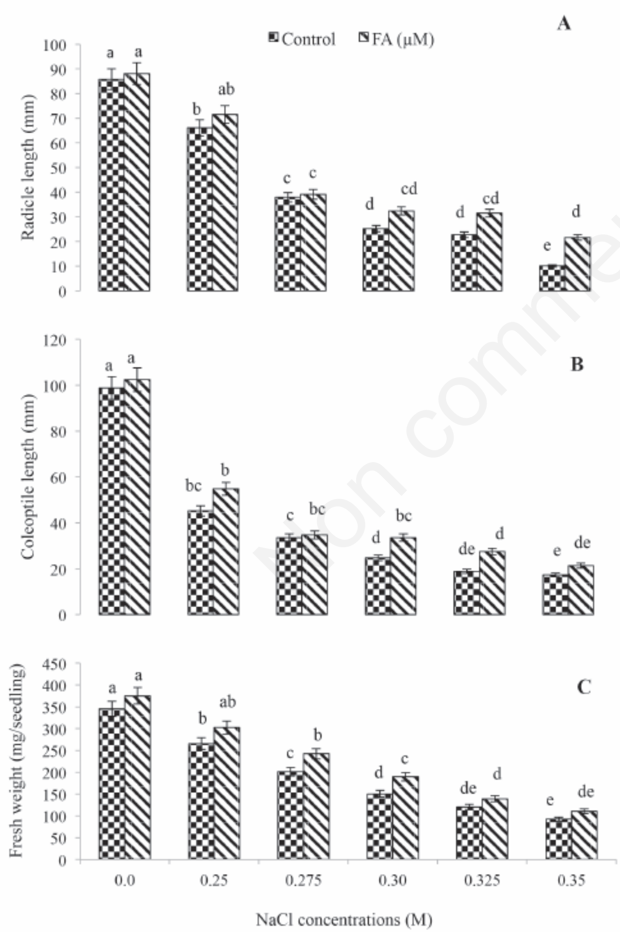


**Figure 2. Effects of folic acid (FA) on various characters of early seedling growth in salt stress. Radicle length (A), coleoptile length (B) and fresh weight (C). Data represent the means and vertical bars indicate the standard error. The values that are followed by the same letter do not differ statistically at a significance level $P\leq0.05$.**

Stomata are epidermal structures that are very important to plant's vital functions and productivity. Many environmental factors such as biotic and abiotic stresses can modulate stomatal reaction (Daszkowska-Golec and Szarejko, 2013). It was observed that stomata and epidermis numbers decreased on both surfaces of barley leaves as salt concentration increased. Especially, stomata number of the adaxial surfaces significantly decreased at the highest salt concentration ($P\leq0.05$). The effects were similar on stoma sizes (width/length) on adaxial surfaces. It is thought that salt stress can reduce leaf size by adversely affecting leaf cell growth (Szalai and Janda, 2009). On the other hand, being one of the most important structures of photosynthesis mechanism, which organizes plant growth, stomata fixate atmospheric carbon dioxide. Abnormalities in the shapes and numbers of these structures in plants exposed to salt stress can reduce carbon fixation capacity (Qu *et al*., 2012), and ultimately disturb the photosynthetic process (Farooq *et al*., 2015). Salt stress causes stomatal deformations by blocking potassium uptake from the environment due to presence of sodium ions at toxic levels (Sümer *et al*., 2004). The pretreatment of barley plants with FA considerably increased the photosynthetic apparatus and alleviated the damaging effect generated by salt stress ($P\leq0.05$). While salinity increased sodium concentration in maize plants, foliar application of kinetin- and indole-acetic acid increased potassium content in leaves (Kaya *et al*., 2010). It is thought that FA application can alleviate the adverse effects of salt stress on stomatal apertures through a similar effect. Because, FA binds essential elements, reduces environmental stress, and at the same time increases uptake of these elements (Michael, 2001). Moreover, a significant increase in potassium amount in sunflower plants exposed to drought stress was determined after FA application, and it alleviated the negative effects on quantitative and qualitative features of the plants (Poudineh *et al*., 2015). It is thought that exogenous FA treatment can restore ion balance of plants exposed to salt stress. While salinity decreased stomata number and sizes in both adaxial and abaxial surfaces, FA treatment significantly reduced the negative effects of salt. Stomata are apertures that regulates moisture balance, thus they are very important in maintaining the water balance of plants (Berry *et al*., 2010). Furthermore, stomatal density is proportional to gas exchange rate (Roth-Nebelsick *et al*., 2009). So, we think that increasing the number and sizes of stomatal apertures on both sides of leaves through FA treatment will have positive impact on photosynthesis rate.

## Conclusions

These results show that FA can alleviate the negative effects of salt stress and positively affect plant growth and development. GI, VI, radicle and coleoptile length, fresh weight, and stomatal activities in both surfaces of leaves were decreased with increasing salt concentrations, compared to control. The adverse effects of salt stress on the plant growth were alleviated when FA was applied. FA application significantly decreased the inhibitory effect on plant growth, and in this way, the application of FA stimulated the growth of plants. At the same time, the mitigation of salt-induced inhibition of all growth parameters by FA can be considered an important result as it protects the plant from oxidative damage by stimulating proline synthesis. Furthermore, exogenously applied FA alone also led to a positive effect in growth, as compared to control. The present study showed that the growth parameters of barley plants were enhanced by the FA addition under both non-salt stress conditions and salt stress conditions, proving the positive and beneficial effects of FA on barley growth.

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