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یافته‌های نوین در علوم زیستی
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اثر تنش شوری بر جوانه زنی بذر، رشد اولیه گیاهک و ساختار تشریحی اندام‌های رویشی چغندرقند

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چکیده. چغندرقند از تیره تاج‌خروسیان دارای نیاکان شورپسند است. در این تحقیق، اثر غلظت‌های مختلف کلریدسدیم در مرحله جوانه‌زنی بذر گیاه چغندرقند و ساختار تشریحی گیاه بررسی شد. نتایج نشان داد که تنش شوری، جوانه‌زنی بذر و رشد اولیه گیاهچه چغندرقند را کاهش داد و باعث ایجاد تغییراتی در ساختارتشریحی گیاه مانند افزایش سنتز کوتین روی سلول‌های اپیدرمی برگ شد و همچنین تغییراتی در ضخامت سیستم آوندی، اپیدرم، پارانشیم برگ‌ها، ریشه‌ها و دمبرگ شد.

واژه های کلیدی. اپیدرم، برگ، پارانشیم، ریشه‌چه، سرعت جوانه‌زنی

The effect of salinity on seed germination, early seedling growth and anatomical structure of *Beta vulgaris*

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Abstract. *Beta vulgaris* belongs to the family Amaranthaceae and was found to have halophytic ancestors. The objective of this study was to investigate the effect of sodium chloride on seed germination, therefore the early stages of seedling growth of *Beta vulgaris* grown under different salinity levels (0, 100, 150 and 200 mM of NaCl) were studied. The experiment was laid out in a completely randomized design (CRD) with four replications. It was revealed that as concentration of NaCl increases, the rate and percentage of germination, length and fresh weight of radicle & plumule and seedling vigour index decrease. In conclusion, our results indicated that salt stress decreased *Beta vulgaris* seed germination and early seedling growth while induced changes in the anatomical characteristics such as increased level of cutin synthesis on epidermal leaves cells and altered the thickness of vascular system, epidermis and parenchyma in leaves, roots and petioles.

Keywords. epidermis, leaf, parenchyma, radicle, rate of germination

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INTRODUCTION

Soil salinization is one of the most serious threats to irrigated crop production in arid and semi-arid regions (Zakery-Asl et al., 2014). Land affected by salinization in arid and semi-arid regions of South Asia is about 42 million hectares (FAO, 1994). At least 20 % of the world's cultivated area and nearly half of the world's irrigated lands are salt- affected (Zhu, 2001). Moreover, around 33 Million hectares are in Iran, which affected nearly 55 % of agricultural lands. Therefore, salinity can be hazardous to plant growth in nearly every irrigated area of Iran that is affected by the salt accumulation. Soil salinity affects plant growth due to decreasing soil osmotic potential (osmotic stress), to interfere with normal nutrient uptake, inducing ionic toxicity, and associating nutrient imbalances (An et al., 2003). Salinity also affects water availability to plants mainly through reducing the soil water potential (Hasegawa, 2013). The decrease in water availability ultimately reduces the photosynthetic rate and hence the overall growth of the plant. 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 also quality of production (Sairam and Tyagi, 2004; Zeinali et al., 2002; Zakery-Asl et al., 2014). Seed germination is usually the most critical stage in seedling establishment, determining successful crop production (Bhattacharjee, 2008). The survey of the sensitivity and tolerance level of a variety at early seedling stages for favorable crop production in a saline condition (ºCHakim et al., 2010). Sugar beet (*Beta vulgaris* L.) is one of the salt tolerant crops. But it is reported to be less tolerant of salinity during germination, seedling emergence, and seedling growth stage (Jamil et al., 2006). Around 200000 ha of soils in different provinces of Iran (e.g., West and East Azarbayejan, Ardabil and Khorassan) are under sugar beet cultivation, and the effect of salinity in different stages is one of the problems in these regions. Khan et al., (2002) studied the effect of salinity and temperature on germination of *Salsola iberica*, they found that high salinity decreased germination rate. Katembe et al., (1998) studied the effect of salinity on germination and seedling growth of *Atriplex prostrata* and *Atriplex patula*. They suggested that the influence of NaCl is a combination of osmotic and ionic effects. Shahriary (2003) studied the effect of salinity stress on germination of *Atriplex veruuciferum* and *Atriplex lentiformis*; he reported high salinity inhibited *Atriplex* seed germination. Kamali et al. (2011) studied the germination of *Prosopis juliflora* and *P. specigera* under saline condition, and concluded that increasing salinity delayed germination and reduced both germination velocity and percentage. Jafari et al., (2013) surveyed the effect of salinity stress on *Matricaria comomilla* and *Thymus deanensis* seed germinations. Their results showed that high salinity caused a reduction in final germination percentage. Similarly, the negative effect of salinity on germination parameters and early seedling growth was reported by Saffan (2008), Hussein et al. (2009), Abdel-Monem et al., (2010), Yosefinia et al., (2012), Sozharajan and Natarajan (2014), Hassan et al., (2014) and Golizadeh et al., (2016). Salinity leads to anatomical modifications in plant anatomy and make them capable of minimizing the detrimental effects of salt stress. The effect of salinity on anatomical structure was discussed by Gadalla (2009) and Younis et al., (2014). Leaves and roots anatomy are affected by salt stress (Çavuşoğlu et al., 2007, 2008; Cecoli et al., 2011). High salinity mostly caused anatomical alterations such as a reduction of somata number (Çavişoğlu et al., 2007), decreased length of bundle, xylem rows, number of vessels and increased both spongy and palisade tissue (Hussein et al., 2012). El-Rodeney et al., (2012) studied the impact of different salinity levels on physiological changes and anatomical characteristics of root, stem and leaf of soybean. However, salt stressed plant showed an increase in leaf thickness in comparison to non-stressed plant (Kilic et al., 2007; Vijayan et al., 2008). Researchers found that salinity stress significantly increased cutin thickness and trichome density on epidermal cells of soybean plant (Dolatabadian et al., 2011). Peyrano et al., (1997) observed a reduction in root hydraulic conductivity in tomato under salinity. There are some studies about effect of NaCl salinity on germination parameters and early seedling growth of *Beta vulgaris* (Mohammadian, 1995; Ghoulam and Fares, 2001; Yavari et al., 2005; Dadkhah, 2007; Jafarzadeh and Aliasgharzad, 2007; Asadi Nasab et al., 2014, Khayamim et al., 2014). However, there was not previous study about effect of NaCl salinity on sugar beet anatomy and there were a few studies about seed germination parameters & early seedling growth of sugar beet. Therefore, the aim of this study was to evaluate germination parameters, early seedling growth and anatomical structure of *Beta vulgaris* L. under different salinity conditions.

MATERIALS AND METHODS

Plant growth and Germination experiment

This study was carried out in an experimental greenhouse at the Higher Education Center of Miandoab, Urmia University, Urmia, Iran. Completely randomized block design with four

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replicates was used to investigate the different levels of salinity stress tests with concentrations of 0 (control), 100, 150, 200 and 250 mM sodium chloride, respectively. Mature, healthy and equal-sized seeds were sterilized in sodium hypochlorite 10 % (w/v) for 10 min and rinsed several times (5-6) with sterilized deionized water according to method reported by Panuccio et al., (2014) with slight modification. The seeds were placed in 90 mm petri dishes on one layer of filter paper Germination was carried out in a germination chamber with a regime of 12 h light at 25 ± 1°C. Distilled water or fresh salt solutions were added periodically keeping the filter paper wet during the 20 days of the experiment. Seeds were considered germinated when radicle had extended at least 2 mm (Redondo-Gómez et al., 2007). The length of both radicle and plumules, dry and fresh weight of both plumule and radicle were daily measured. Weight of radicle and plumule was calculated by digital scale with an accuracy of 0.001 g. Lenght of radicle and plumule was measured by a ruler.

Seeds were germinated in pots, 20 cm in diameter and 30 cm height, filled with sterile sand (previously rinsed with distilled water) and peat at 2:1 ratio, respectively. After emergence of the first true leaves, 15 days after germination, the numbers of plants were adjusted to six per pot and they were irrigated from the top with 300 ml of distilled water every other day. The pots (five plants) were arranged in a simple randomized design and each one was considered as one replicate with three pots per treatment. At 20 days after germination, a half strength Hoagland's nutrient solution was given once a week. Then, one month after sowing, five NaCl concentrations; 0 (control), 100, 150, 200 and 250 mM were applied. To avoid osmotic shock, NaCl concentrations were increased gradually by 50 mM every 2 days until the desired concentration. After 60 days of salt treatment, leaf, stem, root and petiole samples were harvested from control and NaCl-treated plants for evaluation of various parameters.

Germination percentage (GP), germination rate (GR) and seedling vigour index (SVI) for each treatment was calculated according to the following formula:

GP= (NG/NT)*100. In the formula, GP= germination percentage, NG=number of seeds germinated, NT=Total number of planted seeds (Kandil et al., 2012).

$(GR) - \sum_{i=}^{n} \frac{Si}{Di}$ in which, GR=germination rate (number of seed per day), Si=number of germinated seeds in each count, and Di=days to count, may days in which germinated seeds (Kader & Jutzi, 2004). Seedling Vigour Index (SVI) was calculated following a modified formula of Abdul-Baki and Anderson (1973). SVI=Germination percentage × seedling length.

Anatomical study experiment:

For anatomical studies, fresh samples of leaf, root and petiole were used in each case for experimental analyses and measurements. Anatomical studies were performed using an average four fresh specimens kept in glycerin and alcohol 96% (1:1). Free hand sections of leaves, roots and petioles were stained with Carmine – Methyl green. Slides were viewed and photographed with light microscope model BX40 Olympus. Measurements of various cells and tissues were taken with ocular micrometer.

Statistical analysis

The obtained data were analyzed using the statistical program SPSS (version 21.0). Analysis of variance was performed using one-way analysis of variance (ANOVA) at 5 percent probability level. The comparison of means by using Duncan's multiple range tests was performed.

RESULTS AND DISSCUSION

Effects of NaCl treatments on seed germination

The germination percentage of untreated seeds (control) was 58% while the germination percentage in seeds exposed to 100, 150, 200 and 250 mM NaCl treatments were 24, 9, 0 and 0%, respectively. The germination rate index of the control seeds was 0.54 while those in seeds exposed to 100, 150, 200 and 250 mM NaCl treatments were 0.28, 0.08, 0 and 0 no^{-1}day, respectively (Table 1). The highest seed germination percentage and rate related to 100 mm NaCl concentration (24.44 % and 0.28 no^{-1}days) and the lowest ones corresponded to 200 and 250 mM NaCl concentration (0 % and 0 no^{-1} day).

Salinity had highly significant effect on growth attributes of *Beta vulgaris* L. in petri dishes under different levels of NaCl stress. In general, our results showed that the percentage and rate of germination in all NaCl concentrations statistically reduced (Table 1). The highest percentage and rate of germination was noted at 100 mM NaCl concentration (2.4% and 0.28 no^{-1}day, respectively). The lowest percentage and rate of germination corresponded to 250 mM NaCl treatment (0% and 0 no^{-1}day, respectively) (Table 1). Comparision of means with Duncan test showed significant difference among NaCl treatments (Table 1).

Among the stages of the plant life cycle, seed germination and seedling emergence and establishment are key processes in the survival and growth of plant (Hadas, 2004). It is well established

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that salt stress has negative correlation with seed germination and vigour (Rahman et al., 1996).

Researchers suggested that a decrease in germination is related to salinity-induced disturbance of metabolic process leading to increase in phenolic compounds (Ayaz et al., 2000). It is postulated that germination rate and the final seed germination decrease with the decline of the water movement into the seeds during imbibitions (Hadas, 1977). Salt induced inhibition of seed germination could be associated to osmotic stress or to specific ion toxicity (Huang et al., 1995). Decrease and delay in germination in saline medium was also reported by these results are similar in line with Francois et al., 1984; Francois, 1985; Mauromicale et al., 2002; Rahman et al., 2000; Gulzar et al., 2001; Jeannette et al., 2002; Hagghani et al., 2008. These researchers observed decrease in percentage germination and seedling emergence with enhanced salinity.

Effect of NaCl on Plumule and Radicle length:

The results showed that plumule and radicle lengths decreased with the increase in NaCl concentrations (Table 1). The highest plumule length was observed at control and 100 mM NaCl treatments (58 and 2.93 mm, respectively). The maximum reduction in plumule length was observed in 200 and 250 mM NaCl treatments (Table 1). The highest and lowest radicle lenght was noted in 100, 200 and 250 mM (2.6, 0 and 0 mm, respectively) (Table 1). Plumule length was not affected by 100 and 150 mM, also 200 and 250 NaCl concentrations (Table 1) so that there was not significant difference between those treatments.

Abiotic stresses are reported to alter levels of plant growth hormones leading to decrease in plant growth (Gupta et al., 1993). The shoot and root length are the highly important characters for salt stress because roots are in direct contact with soil and absorb water from soil and shoot provide it to the rest of the plant. For this reason, root and shoot length provides an important clue to the reaction of plants to salt stress (Jamil, 2004). In this study, high salinity inhibited plumule and radical elongation of *Beta vulgaris*. This was consistent with the findings of Munns and Termaat (1986) who state that the growth of a plant is generally reduced by salinity. Sativa and Jakhar (2015) showed that plumule and radicle length of *Cicer arietinum* L. were significantly reduced by increasing salinity. Xiong and Zhu (2002) attributed that salt stress inhibited the efectiveness of translocation and assimilation of stored materials and might have caused a decline in shoot growth. The reduction in root and shoot development may be due to toxic effects of the NaCl used further more unbalanced nutrient uptake by the seedlings. Ability of a root system to control entry of ions to the shoot is crucial importance to plant survival in the presence of NaCl (Hajibagheri et al., 1989). Root elongation may be inhibited by high salinity due to slowing down the uptake of water by the plants (Werner et al., 1995).

Effect of NaCl on Plumule and Radicle fresh weight:

Our results showed that fresh weight of both radicle and plumule of *Beta vulgaris* statistically decreased with the increase in NaCl concentrations (Table 1). The highest plumule and radicle fresh weight corresponded to 100 mM NaCl concentration (0.014 and 0.004 mg, respectively). The lowest fresh weight of radicle and plumule corresponded to 200 and 250 mM NaCl concentration (0 and 0 mg, respectively) (Table 1). Plumule and radicle fresh weights in seedling treated with NaCl concentrations became smaller than control group but, not significant differences were found between two groups (seedlings treated with 100 & 150 mM and 200 & 250 mM NaCl concentrations) (Table 1). Also, treatments of seedlings with 150, 200 and 250 mM NaCl concentrations had no significant effect on radicle fresh weight (Table 1).

During imbibition, movement of water occurs through aquaporins, which have reduced expression in the presence of salt (Boursiac et al., 2005). Studies have shown that NaCl treatment decreased some growth parameters such as fresh weight of root of plants (Yildirim et al., 2008). Accordingly, Mori et al., (2011) reported that salinity decreased the plumule fresh weight of plants.

Effect of NaCl on Radicle and Plumule dry weight:

Our results showed that dry weight of both radicle and plumule statistically decreased with salt increaese (Table 1). The highest weight of plumule and radicle corresponded to 100 mM NaCl concentration (0.005 and 0.05 mg, respectively). The lowest amount of dry weight of radicle and plumule corresponded to 200 and 250 mM concentrations of sodium chloride (0 and 0 mg, respectively) (Table 1). Plumule and radicle dry weights were affected by NaCl treatments, showing a decrease in comparison with the control group. Also, our results were in agreement with those obtained by other resrarchers (Redmann and Belyk, 1994; Osorio et al., 1998; EL-Melegi et al., 2004; François and Bahizire, 2007; Bahrami et al., 2012; Asadi Nasab, 2014).

As salinity levels increased, both fresh weight and dry matter of seedlings declined. Salt stress causes ionic imbalance (Zhu et al., 1997), with excess sodium and chloride ions having a deleterious effect on many cellular systems (Serrano et al., 1999),

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therefore plant survival and growth depends on adaptations to re-establish homeostasis. High salinity also inflicts hyper osmotic shock on plants, as chemical activity of water is decreased, causing a loss of cell turgor. Salt induced reduction in chloroplast stromal volume and generation of reactive oxygen species (ROS) also plays an important role in decreasing plant photosynthetic capacity and therefore growth (Price and Hendry, 1991).

Seedling vigour Index

Seedling vigour test was made on seeds produced from plants grown under varied levels of salinity. The results revealed significant difference in seedling vigor between seeds produced from control and saline grown plant except for 100 mM NaCl treatments (Table 1). The reduction gets stronger particularly at the higher levels of NaCl concentration (150 mM).

Seedling vigour is evaluated in terms of germination percentage and seedling length and has direct relationships with both parameters. Low seedling vigour might affect the plant yield in two ways: first, percentage of emerged seedlings in the field is less than expected, and consequently, plant density falls below the desired level, and second, the growth rate of seedling in such plants might be lower than the growth rate of plants produced from strong seeds (Roberts & Osei-Bonsu, 1988). Seedling vigour index and NaCl concentration were negatively correlated. Generally, the increase in salt concentration decreased the seedling vigour index of *Beta vulgaris* and this was in line with the findings of Khodarampour et al. (2011), Mensuh et al., (2006) and Mostafavi (2011).

Anatomical structures

Cross section of leaf, root and petiole of *Beta vulgaris* were analyzed to assess the effects of various salt concentrations to the anatomical adaptations of this plant to be under external salinity (Figs. 1-3). There was a significant alteration in anatomical feautures of leaf, root and petiole of *Beta vulgaris* plants imposed to various levels of salinity (Tables 2, 3, 4).

The statistical analysis confirmed that the increase in NaCl concentration; significant changes were noticed in root anatomical characteristics (Table 2). By examining the transverse sections of root from control and treated samples, it was observed that the thickness of epidermal layer was increased in NaCl-treated plants as compared to control group. Results showed that the thickest epidermal layer was observed in 200 (49.96 µm) and 250 mM NaCl concentrations (49.96 µm) (Table 2). The highest phloem thickness was observed in 100 mM NaCl concentration (200.03 µm). Xylem tissue thickness of the root reached the maximum (190.03 µm) in treated plants with 100 mM NaCl as compared to control group and then decreased at higher salinity. Cortical paranchyma became thicker in 100 mM NaCl concentration (400.03 µm). The protoxylem and metaxylem diameter became bigger than the control in 100 mM NaCl concentration (40.03 and 60.33 µm, respectively) (Table 2). The lowest epidermal layer thickness was noticed at 100 mM NaCl concentration (30.03µm) (Table 2). The mimimum thickness of phloem, xylem, cortical paranchyma & protoxylem and metaxylem diameter was observed in 250 mM NaCl concentration (100.33, 150.00, 179.96, 18.33 and 25.00 µm, respectively (Table 2).

Table 1. The effect of salinity on seed germination characteristics and early seedling growth of *Beta vulgaris*.

Germination measurments	250 (mM)	200 (mM)	150 (mM)	100 (mM)	Control (No NaCl)	F
Germination percent	0.00±0.00^{c}	0.00±0.00^{c}	8.88±0.84^{bc}	24.44±0.69^{b}	57.776±0.77^{a}	15.71*
Germination rate	0.00±0.00^{c}	0.00±0.00^{c}	0.081±0.03^{c}	0.288±0.07^{b}	0.5413±0.15^{a}	24.81*
Seedling vigour index	0.00±0.00^{b}	0.00±0.00^{b}	35.53±0.00^{b}	134.216±0.00^{a}	752/208±0.00^{a}	45.36*
Plumule length	0.00±0.00^{c}	0.00±0.00^{c}	2.700±0.05^{b}	2.93±0.50^{b}	5.800±0.60^{a}	50.31*
Radicle length	0.00±0.00^{d}	0.00±0.00^{d}	1.07±0.11^{c}	2.60±0.26^{b}	7.370±0.06^{a}	110.22*
Plumule fresh weight	0.00±0.00^{c}	0.00±0.00^{c}	0.011±0.00^{b}	0.014±0.00^{b}	0.033±0.00^{a}	40.80*
Radicle fresh weight	0.00±0.00^{b}	0.00±0.00^{b}	0.002±0.00^{b}	0.004±0.00^{ab}	0.007±0.00^{a}	3.50*
Plumule dry weight	0.00±0.00^{d}	0.00±0.00^{d}	0.001±0.00^{c}	0.005±0.00^{b}	0.006±0.00^{a}	225.50*
Radicle dry weight	0.00±0.00^{d}	0.00±0.00^{d}	0.004±0.00^{c}	0.005±0.00^{b}	0.008±0.00^{a}	213.00*

The values are mean ± SD (n = 4). *: significant at $p < 0.05$, Means followed by different letters are significantly different at $p < 0.05$.

Table 2. The effect of salinity on root anatomical parameters of *Beta vulgaris* L.

NaCl Concentration (mM)	Metaxylem Vessel Diameter (µm)(	Protoxylem Vessel Diameter (µm)	Paranchyma (µm)	Xylem Tissue Thickness (µm)	Phloem Tissue Thickness (µm)	Epiderm Thickness (µm)
control	80.033±0.03[a]	60.033±0.05[a]	586.633±0.05[a]	200.033±0.05[a]	200.033±0.05[a]	30.033±0.06[a]
100	60.033±0.05[b]	40.033±0.04[b]	400.033±0.05[b]	190.033±0.06[b]	200.033±0.01[a]	30.03±0.05[b]
150	49.996±0.08[c]	30.033±0.07[c]	300.033±0.04[c]	169.033±0.05[c]	110.033±0.05[b]	39.96±0.08[b]
200	30.033±0.03[d]	25.033±0.05[d]	200.033±0.04[d]	170.033±0.05[c]	100.033±0.03[b]	49.96±0.08[c]
250	30.033±0.05[d]	18.033±0.05[e]	179.96±0.09[e]	150.033±0.03[d]	100.033±0.05[c]	49.96±0.05[c]
ANOVA	405000.80*	239220.00*	342170.80*	102.00*	253752.200*	89401.200*

The values are mean ± SD (n = 4). *: significant at p < 0.05, Means followed by different letters are significantly different at p< .05.

Table 3. The effect of salinity on petiole anatomical parameters of *Beta vulgaris* L.

NaCl Concentration (mM)	Protoxylem Vessel Diameter (µm)	Metaxylem Diameter (µm)	Xylem Tissue Thickness (µm)	Phloem Tissue Thickness (µm)	Paranchyma Thickness (µm)	Epiderm Thickness (µm)	Cuticle Thickness (µm)
control	25.33±0.70[a]	45.333±0.30[a]	130.33±0.30[a]	100.033±0.57[a]	350.00±0.28[a]	40.0±0.100[a]	1.00±0.00[a]
100	22.33±0.57[b]	37.33±0.40[b]	110.00±0.00[b]	90.33±0.89[b]	299.66±0.57[b]	35.0±0.100[b]	1.00±0.00[a]
150	18.66±0.20[c]	30.00±0.00[c]	90.33±0.57[c]	80.33±0.57[c]	250.00±1.00[c]	29.96±0.00[c]	1.00±0.00a
200	14.66±0.50[d]	25.66±0.90[d]	80.333±0.43[d]	75.333±0.57[d]	230.00±0.60[d]	20.0±0.30[d]	1.00±0.00[a]
250	11.66±0.60[e]	25.66±0.57[d]	70.333±0.50[e]	65.00±0.00[e]	200.33±0.57[e]	19.99±0.03[d]	1.00±0.00[a]
ANOVA	807.875*	276.300*	6499.000*	2085.250*	8768.52*	32781.95*	0.000*

The values are mean ± SD (n = 4). *: significant at p < 0.05, Means followed by different letters are significantly different at p< 0.05.

Table 4. The effects of salinity on leaf anatomical parameters of *Beta vulgaris* L.

NaCl Concentration (mM)	Total Leaf Thickness (µm)	Mesophyll Thickness (µm)	Lower Epiderm Thickness (µm)	Upper Epiderm Thickness (µm)	Cuticle Thickness (µm)
control	839.996±0.06[e]	760.033±0.04[a]	58.033±0.05[a]	60.033±0.08[a]	10.033±0.00[e]
100	809.966±0.05[d]	730.033±0.05[b]	49.033±0.05[b]	55.033±0.09[b]	11.033±0.03[d]
150	800.033±0.06[c]	710.033±0.03[c]	40.033±0.09[c]	45.033±0.06[c]	15.033±0.04[c]
200	729.966±0.06[b]	630.000±0.05[e]	32.966±0.05[d]	38.033±0.05[d]	18.033±0.05[b]
250	670.032±0.03[a]	560.033±0.05[d]	25.966±0.05[e]	30.033±0.05[e]	22.033±0.07[a]
ANOVA	4272901.20*	95157180.25*	155430.00*	134370.00*	27885.25*

The values are mean ± SD, (n = 4). *: significant at p < 0.05, Means followed by different letters are significantly different at p< 0.05.

On the contrary, cross-sectional thickness of the petiole epiderm, paranchyma, phloem, xylem and metaxylem & protoxylem vessel diameter showed significant differences between all treatments (Table 3) decreasing from control to high NaCl concentration. There was no significant difference between two treatments (200 and 250 mM NaCl concentration) on epidermis thickness and metaxylem vessel diameter (Table 3). On regard to cuticle, all of the salinity treatments were not significantly different than the control. The highest and the lowest epidermis, paranchyma, phloem, xylem thickness and metaxylem & protoxylem vessel diameter were related to control and 250 mM NaCl treatment, respectively (Table 3).

Higher NaCl concentration (250 mM) induced increase the number of subsidiary vascular bundles of *Beta vulgaris* petiole (Fig. 2). There were 3 subsidiary vascular bundles and three main vascular bundles in petiole of control, 100 mM, 150 mM and 200 mM NaCl concentration treatment plants but at higher concentration level (250 mM) the number of subsidiary bundles was increased (up to 6 vascular bundles). Also, the petiole shape was different between control and salinity stressed plants (Fig. 2).

Results of leaf anatomical studies showed that salinity had noticeable effect on leaf structure of *Beta vulgaris* L. (Table 4, Fig. 3). Also, the statistical analysis showed that with increasing in NaCl concentration, significant changes were observed in leaf anatomical characteristics (Table 4). Results showed that the highest cuticle thickness was observed in 250 mM NaCl concentration (22.03 µm) while the lowest one was observed in 100 mM NaCl concentration (11.03 µm) (Table 4). The highest upper and lower epidermis, mesophyll and total leaf thickness was observed in 100 mM NaCl concentration (lowest NaCl level) (55.03, 49.03, 73.03 and 80.03 µm, respectively) (Table 3). The lowest upper and lower epidermis, mesophyll and total leaf thickness was observed in 250 mM NaCl concentration (30.03, 25.96, 560.03 and 670.0303 µm, respectively) (Table 4).

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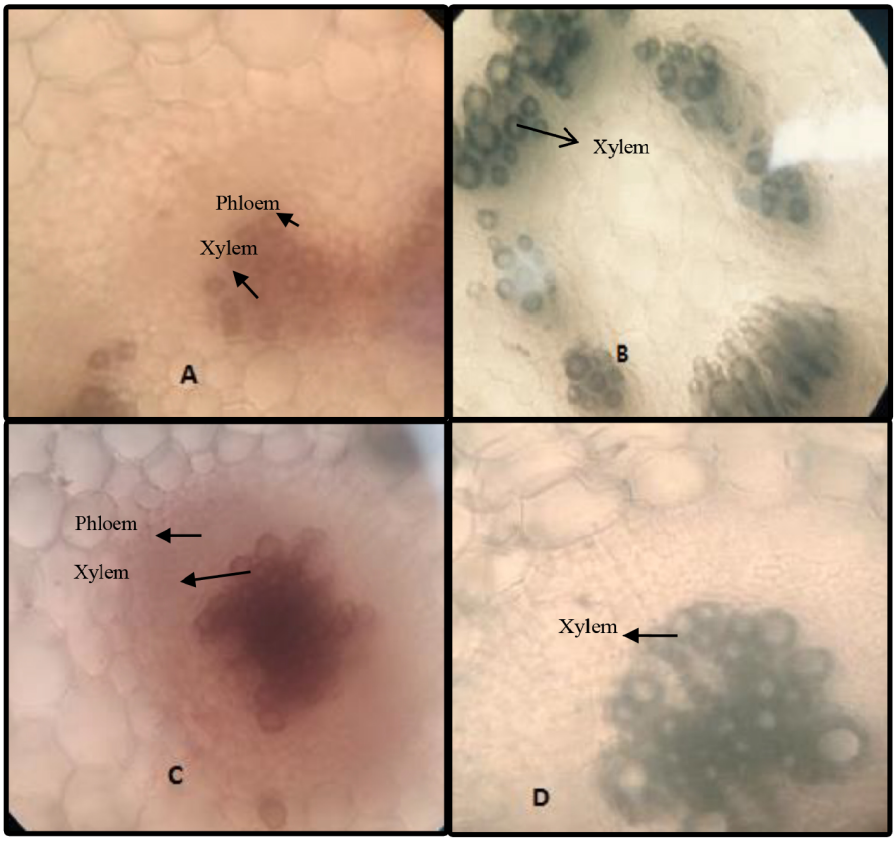


Figure 1. The effect of salinity on the root anatomy of *Beta vulgaris* L. plants treated with various levels of salinity. **A**. control (×40), **B**. 150 mM NaCl (×40), **C**. 250 mM NaCl (×40), **D**. 200 mM NaCl (×40).

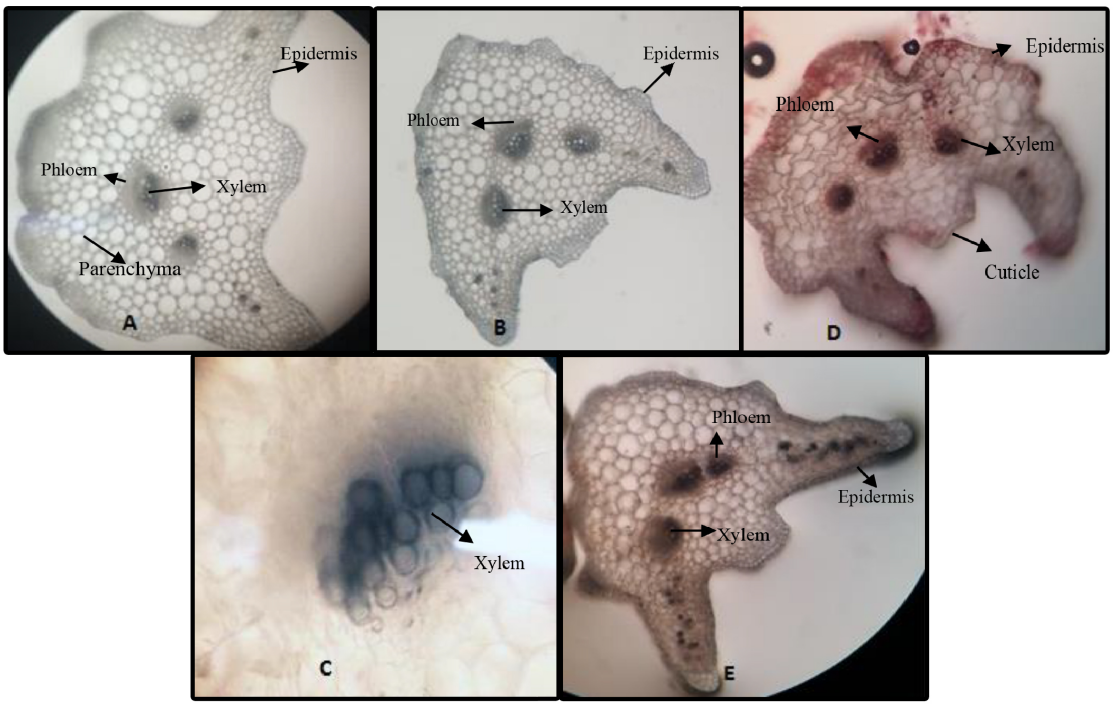


Figure 2. The effect of salinity on petiole anatomy of *Beta vulgaris* L. plants treated with various levels of salinity. **A**. control (×10), **B**. 100 mM NaCl (×10), **C**. 150 mM NaCl (×40), **D.** 200 mM NaCl (×10), **E.** 250 mM NaCl (×40).

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Plant roots are the first line of damage or defense due to the direct encounter with the saline soil solution (Rewald et al., 2012). Results of root anatomical studies showed that salinity had adverse effects on root anatomy of *Beta vulgaris* L. (Fig. 1). The increase in salinity resulted in a decrease in the phloem, xylem and cortical paranchyma thickness, protoxylem and metaxylem diameter, while epidermal layer thickness had reduction tendency from control to high NaCl concentration (200 and 250 mM) (Table 2, Fig. 1).

Anatomical modification of root was observed in *Beta vulgaris* plants treated with various levels of salinity. At higher saline condition (250 mm NaCl) the epidermal thickness increaesed. In accordance with our study, the epidermal thickness was increased in *Salvadora persica* with increasing salinity (Parida et al., 2016). The salt stress – induced increase in epidermal thickness of root reduces salt ion diffusion to the plant (Parida et al., 2016).

The diameter of root vascular bundles decreased as the external salinity increased. Xylems of plants encountering salinity tends to have vessels with lower diameter than unstressed groups (Junghans et al., 2006). Decreasing in the vessel diameter can be refered to reduce the hydraulic conductivity in the part formed during the stress period (Junghans et al., 2006). This modification in root xylem of the stressed plant confers safety to the vessel with significant conductivity (Junghans et al., 2006).

However, by reducing the vessel diameter of root, water uptake also reduced with the increase in the salinity consequently reduces photosynthesis (Junghans et al., 2006). Previous studies found smaller xylem vessels in stressed plants is in accordance with our results (Awasthi and Pathak, 1999; Boughalleb et al., 2009). Root parenchyma layer thickness was significantly affected by salinity (Table 2, Fig. 1). Parenchyma thickness was significantly decreased under salinity condition. Similar results were also presented by Akram et al., (2002), Hu et al., (2005) and Jafarian et al. (2012**)** on wheat. But in contrast with our results, Alshammary et al., (2004) and Parida et al. (2016), reported that with an increase in salinity there was an increase in parenchyma layer thickness.

On the contrary, cross-sectional thickness of the petiole epidermis, paranchyma, phloem, xylem and metaxylem & protoxylem vessel diameter showed significant differences among all treatments (Table 3) decreasing from control to high NaCl concentration. In cross-section of control plants, the petioles are a semi-circular contour, with an adaxial ditch, with latero-adaxial wings with enhancing of salinity, petiole had tall big wings and compressed. These results are in agrement with finding of Poscher (2005) on *Parthenium argentatum (*Asteraceae). This is the first study about effect of salinity on petiole structure of *Beta vulgaris*; therefore, comparision with other researches was impossible.

The cuticle of *Beta vulgaris* was significantly denser in leaves treated with NaCl concentrations than the control (Table 4). Our observations were consistent with finding of Longstrethand and Nobel (1979) and Hajibagheri et al., (1982). In salt stressed plants, the leaf epidermis thickness was smaller than control; the salinity effect was concentration dependent. This decrease in epidermal thickness may be attributed to the limited cell division and growth at higher salinity (Carcamo et al., 2012). Our results showed a gradual decrease in thickness of mesophyll with increasing salinity. In accordance with our results there was a decrease in the mesophyll thickness with increasing salinity in semi-mangrove plant *Myoporum botioides* (Xu et al., 2014). The significant decrease in the palisade tissue at extreme salinity (750 mM NaCl) might be an adaptation of this halophyte to minimize the photosynthetic energy utilization in the higher saline condition (Parida et al., 2016).

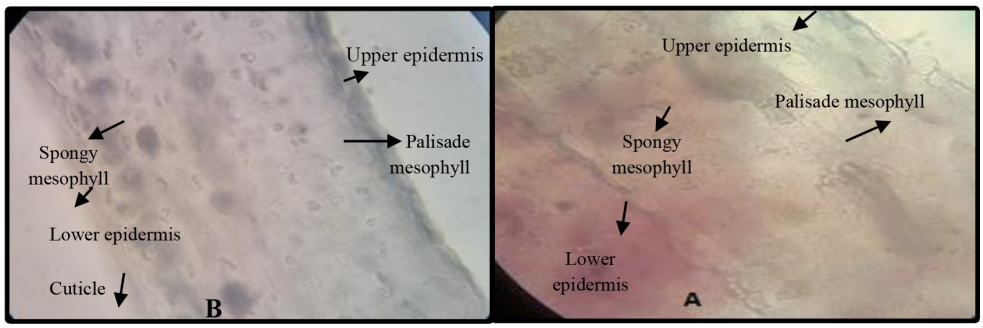


Figure 3. Effect of salinity on leaf anatomy of *Beta vulgaris* L. plants treated with various levels of salinity.**A.** 100 mM NaCl (×40), **B.** control (×40).

Conclusion

In conclusion, our results indicated that salt stress decreased *Beta vulgaris* seed germination & early seedlimg growth and induced changes in anatomical characteristics such as increase of cutin synthesis on epidermal leaves cells and also changes in vascular system, epidermis, parenchyma in leaves, roots and petioles.

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