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ORIGINAL PAPER

SALT SENSITIVITY AND SOME PHYSIOLOGICAL AND MORPHOLOGICAL MECHANISMS OF ADAPTATION TO SALT STRESS IN CAMELINA*

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Abstract

Salinity is the most important abiotic stress factor that negatively affects agricultural production and quality. It necessitates a wider introduction of resistant species and the development of high-yielding varieties, especially in salinity-affected areas. Camelina is a plant that has an effective structure against abiotic stress factors. The research was carried out to determine the physiological and chemical changes caused by different salt concentrations in the camelina plant, and the salt concentration to which camelina is most sensitive. The research was carried out in greenhouse conditions. PI-650142 camelina genotype was used as the plant material, and 6 different NaCl concentrations (0, 35, 70, 140, 210 and 200 mM) were used as salt treatment. Plant height, leaf area, plant water content, relative water content, membrane permeability, amount of chlorophyll and carotenoids and biomass accumulation were evaluated. As a result, it was found that the applied NaCl doses showed significant differences on all the parameters evaluated. It has been determined that plants can tolerate salt stress up to 140 mM NaCl concentration, and there were serious negative changes in plant height, the weight of plant parts that make up the biomass, total biomass, leaf parameters and membrane permeability above the 140 mM NaCl concentration. These findings reveal that up to a certain level (140 mM NaCl concentration) in regions with salt stress, the camelina plant can be grown without encountering a serious problem in terms of morphological and physiological adaptation mechanisms.

Keywords: camelina, salinity stress, camelina biomass, NaCl concentrations, salt sensitivity

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INTRODUCTION

Should the current population growth rate continue, it is predicted that the world population will double in 2050 (Tester, Langridge 2010), and the need for food to feed this population will be at least twice what it is today. This situation shows that efficient use of available resources may not be enough in the future. Therefore, research on the active and efficient use of cultivation techniques, especially the development of new varieties in the agricultural sector, requires more attention in the upcoming years. In the context of global warming, the need for irrigation will increase even more in arid and semi-arid areas to protect yield and quality. It is estimated that the increase in irrigation demand may force the farmer to use lowquality irrigation water, which may consequently increase the salt content in soils, and 50% of the world's arable land will be affected by salinity (Bartels, Sunkar 2005). This rate may increase due to climate change, excessive use of groundwater, increased use of poor-quality water for irrigation and insufficient drainage.

Biotic and abiotic stress factors affect yield and quality. Salinity is the most important abiotic stress factor that negatively affects agricultural production. Salinity negatively affects the agricultural soil structure and many create adverse conditions for the plants grown in these types of soils (Machado, Serralheiro 2017). Salt stress can have a lethal effect on plants. It may deteriorate the photosynthetic metabolism or even disrupt the growth and development of a plant depending on the salt concentration (Kuşvuran 2012).

Measures to remediate effects of soil salinity are expensive and time-consuming. Among the techniques, the more effective is to breed plant varieties with higher salt stress tolerance, whose life cycle and yield are less affected in areas with salinity problems. As a matter of fact, increasing concerns about transportation fuel cost, carbon balance and energy supply have stimulated greater interest in salt-tolerant plant species that can be used as feedstock or firewood in recent years. Many oil plants, especially camelina, safflower and canola, are grown to obtain energy fuel, and the water needs of these plants are relatively low (Miyamoto et al. 2012).

Plants have developed mechanisms to change their morphology and physiology to buffer the negative effects of environmental factors (Visser et al. 2016). Abiotic stress conditions such as salinity are associated with reduced biomass accumulation in the plant, and different physiological and phenotypic changes. Plants with more water in their tissues in saline conditions are considered more tolerant to salt. It has been reported that salt treatments pose an osmotic effect, which results in plant cells losing the water content and decreasing their volume. At the later stages, the cells may regain their original size, but the growth rate of roots and leaves is lower than that of control plants (Munns 2002). In addition, plants are much more sensitive to salt stress in the early seedling period, and the first obvious sign of salt toxicity is decreased growth rate in roots and shoots (Beck et al. 2004).

Photosynthetic productivity is an important factor determining the plant's resistance to abiotic stresses, and one of the most obvious effects of salt stress on plants consists in changes in photosynthetic pigment biosynthesis (Maxwell, Johnson 2000). When a large amount of salt enters the plant cell, the structure of chlorophyll may change, the function of chloroplasts may be impaired, and photosynthetic performance may be adversely affected if the plant is unable to maintain lower concentrations in the cytosol (Tsai et al. 2019). Kaymak and Acar (2020) determined that as the salt concentration in the soil increased, the amount of chlorophyll-a, chlorophyll-b and carotenoids in the leaves of *Bituminaria bituminosa* decreased. Chutipaijit et al. (2011) suggested that the amount of carotenoids in rice leaves treated with 100 mM salt concentration decreased by 36% compared to the control, and that the changes in the amount of chlorophyll in plants under salt stress can be used as a sensitive indicator for cellular metabolic functions.

Camelina plant has an effective structure against abiotic stress factors such as high temperature and drought besides salinity (Gugel, Falk 2006). It is known that the oily and waxy structure of the cuticle layer in camelina prevents water loss out of the stomata and thus provides the plant resistance against various abiotic and biotic stress factors (Razeq et al. 2014). The development of salt-tolerant crops such as camelina is important to ensure the food security of the rapidly growing population. As a result of morphological and biochemical analysis performed on plants grown in NaCl concentrations ranging from 50 to 300 M in vitro to determine the salinity resistance in camelina, it was found that it produced more biomass as a plant defense mechanism against low salinity levels (Morales et al. 2017). In another study, it was observed that the survivability of camelina in salty soil conditions is high, the relative water content, plant water content and chlorophyll content of the plant decrease under stress conditions, and the amount of membrane permeability increases (Khalid et al. 2015). In addition, it was reported that low concentrations (25-50 mM) of NaCl applied during the seedling period of camelina had a positive effect on fresh and dry weights, and the critical salt concentration for seedling growth was 75 mM (Russo, Reggiani 2015). Few studies have been carried out so far to determine the morphological and photosynthetic changes that occur in the camelina plant under salinity stress. Identifying species that can grow in saline soils is a good way to use such barren land. The present study is a step in this direction. It can also contribute to a greater introduction of resistant species and the development of high-yielding varieties, seen as a pressing necessity to salinity-affected areas. For this reason, this research was carried out to determine some physiological and chemical changes that occur in the camelina plant at different salt concentrations, and to determine the thresholds of salt concentrations in terms of salt sensitivity and tolerance of the camelina plant.

MATERIALS AND METHODS

Plant material

PI-650142 camelina genotype was used in the research. In the ecological conditions of Samsun, Turkey, PI-650142 camelina genotype ensured the best adaptability among 45 camelina genotypes registered in USDA gene banks.

Method

The experiment was conducted under controlled greenhouse conditions with the temp. of 17.5°C during the day (06:00-22:00 h) and 16°C at night. In addition, sodium lamps were used in the greenhouse during the daytime period, in cloudy weather, and in when there was a shortage of light at dusk and dawn. Sowing was done in multiple vials containing 1/3 peat, 1/3 vermiculite and 1/3 soil mixture, and care was taken to sow 2 seedlings in each compartment in a vial. Seedlings that completed their emergence in vials were transferred to 1-liter pots (11 cm x 11 cm x 12 cm) with one plant in each pot. The experiment was carried out as 8 repetitions, and 6 different NaCl concentrations (0, 35, 70, 140, 210 and 280 mM) were applied in the experiment. Salt doses were applied to each pot with 100 ml of irrigation water at two-day intervals when the plants reached the stage of development of approximately 8 leaves 4 weeks after the transfer. Salt applications were repeated 7 times during the growing period and the salt treatments were terminated when the total amount of salt was reached (Table 1). After the salt doses had been applied, the plants were irrigated at field capacity (100 ml per day) for ten days. The experiment took approximately 65 days in total, and the plants were harvested before the budding period. At the end of the period, data on plant height, plant total mass weight, membrane permeability, total leaf area, plant water content, relative water content, chlorophyll a, chlorophyll b and carotenoid content were obtained.

At the end of the experiment, the plants were removed from the pots and their roots were cleaned with distilled water. Plant height, plant fresh

Table 1

| Salt concentration (mM) | Salt amount in each treatment (g) |
|-------------------------|-----------------------------------|
| 0 | 0 |
| 35 | 1.43 |
| 70 | 2.86 |
| 140 | 5.72 |
| 210 | 8.58 |
| 280 | 11.44 |

Salt (NaCl) concentration and salt amounts used in the research

weight, leaf fresh weight, stem fresh weight, root fresh weight and flower fresh weight were determined separately after the water had been removed from the plants by keeping them on blotting papers for 30 minutes. The data on the dry weight of the plant and its parts were obtained by weighing after drying in an oven at 70°C for 72 hours. Plant water content was calculated according to the following formula:

plant water content =
$$\left(\frac{\text{fresh weight} - \text{dry weight}}{\text{fresh weight}}\right) \times 100 \text{ (Turner 1981)}$$
 (1)

To determine the relative water content, approximately 0.3 g of fresh leaf samples from each potted plant were weighed and their wet weights were recorded. Each leaf sample was individually incubated in a 25 ml beaker filled with water for 1 night in dark conditions. At the end of the time, each sample was weighed and turgor weights were recorded. After each sample had been dried in an oven at 80°C for 24 h, they were weighed separately and their dry weights were recorded. Based on the data obtained, the relative water content of each sample was calculated using the formula below:

relative water content (RWC) =
$$\left(\frac{\text{fresh weight} - \text{dry weight}}{\text{turgor weight} - \text{dry weight}}\right) \times 100$$
 (Gonzales, Gonzalez-Vilar 2001) (2)

Membrane permeability was determined using an electrical conductivity (EC) meter according to Taiz et al. (2014). For each sample, 20 leaf discs of 1 cm diameter were placed in brown glass bottles, and after adding 10 ml of distilled water, the bottles were shaken for 24 h in a shaker at 100 rpm at 25°C in a climate chamber. At the end of the period, an EC meter was used to obtain the EC₁ values. Subsequently, samples were autoclaved at 120°C for 20 min and EC₂ values were read when cooled to room temperature. Using the data obtained, the membrane permeability was calculated using the following formula:

membrane permeability =
$$\left(\frac{\text{EC1}}{\text{EC2}}\right) \times 100$$
 (Lutts et al. 1995) (3)

All leaves of the plant in each pot were scanned in a scanner and using the Image-J Image Analysis Program, as described by O'Neal et al. (2002), an analysis was made and the leaf area of each sample was determined.

To determine the amount of chlorophyll and carotenoids, 1 cm diameter leaf discs were taken from a leaf on 2/3 of the main shoot of each plant. After the leaf discs were transferred to the tubes, some $CaCO_3$, 3-4 granules of quartz and 4 ml of ethanol (96%) were added to them. The leaves were pulverized and then centrifuged at 10.000 rpm. Measurements were made on the solution obtained after centrifugation at four wavelengths (710, 665, 649 and 410 nm) using a spectrophotometer. The amounts of chlorophyll and carotenoids were calculated according to Visser et al. (2016) using the data obtained after the above measurement.

Statistical analysis

The analysis of variance (ANOVA) of the experiment, which was carried out according to the randomized plot design, was performed using the SPSS program. The comparison of the different means was made according to the Duncan multiple comparison test (Gomez, Gomez 1984).

RESULT

According to the statistical analysis of plant height data, it was determined that NaCl doses resulted in significant (P<0.05) differences. Plant height decreased significantly with an increasing salt concentration, and the highest salt concentrations had the most negative effect on plant growth. On average, the longest plant height (43.50 cm) was obtained at the salt concentration of 0 mM (control), whereas the shortest plant height (17.62 cm) was obtained at the salt concentration of 280 mM. This result shows that there is a 59.47% decrease in plant height compared to control plants and that increasing salt concentrations negatively affect the physiological development (Figure 1a).

The research proved that the tested NaCl doses had significant (P<0.05) effects on membrane permeability. Increasing salt concentrations increased the membrane permeability, and the lowest membrane permeability was obtained from the control treatment (17.98%), while the highest membrane permeability (75.94%) was obtained at the highest salt concentration (Figure 1b). This result reveals that increasing salt concentrations negatively affect the cellular structure and the cell permeability at the highest salt concentration increased 4.22-fold compared to the control.

The statistical processing of the data demonstrated that the NaCl concentrations caused significant (P<0.05) differences in terms of chlorophyll-a, chlorophyll-b and carotenoids. In these three parameters expressing the total amount of chlorophyll, the highest values were obtained at the 35 mM NaCl dose (1.183 mg g⁻¹ chlorophyll-a and 0.307 mg g⁻¹ chlorophyll-b) and 70 mM NaCl (0.833 mg g⁻¹ carotenoids), while the lowest values were obtained at 280 mM NaCl (0.508, 0.170 and 0.368 mg g⁻¹, respectively) – Figure 1*c*. It was determined that the decrease in the amount of chlorophyll, in general, was more pronounced at NaCl concentrations above 140 mM NaCl.

With respect to the leaf area data, the NaCl doses affected the leaf area significantly (P<0.05), and as the salt concentrations increased, significant decreases occurred in the leaf area compared to the control. Although the maximum leaf area was obtained in the control group (52.96 cm²), the smallest leaf area (9.02 cm²) was obtained at the 280 mM NaCl concentration while the highest salt concentration decreased the leaf area 5.87-fold compared to the control (Figure 1*d*).



Fig. 1. The variation of the investigated parameters at different NaCl concentrations: a – plant height, b – membrane permeability, c – the amount of chlorophyll-a, b and carotenoids, d – leaf area, e – relative water content, f – plant water content

Regarding the relative water content and plant water content, NaCl doses caused significant (P<0.05) differences in terms of both characters. As the salt concentration level increased, both parameters decreased. The highest relative water content (93.98%) and plant water content (87.68%) were obtained at the 0 mM NaCl concentration, and the lowest relative water content (49.89%) and plant water content (79.22%) were obtained at the 280 mM NaCl concentration. Compared with the control plants, it was determined that the loss in relative water content and plant

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water content at the highest salt concentration (280 nM) was 46.91% and 9.65%, respectively (Figure 1*e*). In terms of plant water content, it was demonstrated that the 280 mM NaCl concentration resulted in statistically different results than all the other NaCl concentrations (Figure 1*f*).

The best indicator of the adaptability of plants to the conditions they are in is the performance they display in biomass production. As a result of the research, it was found that as the salt concentration increased, the amount of biomass decreased, especially at concentrations higher than 70 mM NaCl. Biomass production at the 280 mM NaCl salt concentration (1.73 g) was determined to be 4.66-fold lower than the control (8.06 g) – Figure 2*a*). When plant parts were taken into consideration, such as the roots, stems, leaves and flowers, they gained biomass proportionally to the applied salt concentrations. It was determined that the reduction in root biomass was 50.69% and the decrease in flower biomass was 30.26% compared to the control. Depending on the applied salt doses, it was determined that changes in the leaf and stem biomass ratios differed compared to the control (Figure 2*b*).

DISCUSSION

Plants are exposed to many different stress factors, biotic and abiotic ones, and each activates a specific reaction mechanism, manifested in the metabolic, physiological and morphological characters of the plants (Viser et al. 2016). It has been reported that in many different plant species affected by salt stress, cell functions are disrupted, plant growth and development are inhibited, the mass of the root, stem and leaf organs change, and the leaf area as well as the amount of chlorophyll in leaves decrease, negatively affecting the plant's photosynthesis (Aziz et al. 2008, El-Danasoury et al. 2010, Khorasaninejad et al. 2010). It has been reported that camelina is more salt tolerant than soybean and safflower (Phang et al. 2009, Kaya et al. 2011). Besides, when the same salt concentrations tested in this research were applied to rapeseed, it was reported that the changes in leaf area, membrane permeability and relative water content were higher than the values obtained in this research (Korkmaz et al. 2020). This is important in that it shows that camelina plants should be preferred to soybean, safflower and rapeseed plants, especially in areas with salinity problems.

Leaf area is one of the important indicators that give information about the vital activities of the plant. Under any stress, primarily the leaf area and therefore the photosynthetic area of the plants are affected. Plants exposed to salt stress close their stomatal cells and narrow the leaf area in order to balance the water loss in their bodies. This leads to a decrease in the rate of photosynthesis and plant growth (Siddiqui et al. 2015). The decrease in the leaf area as NaCl concentration increased determined in this experiment confirms this situation. However, unlike the leaf area, maximum



Fig. 2. The shares of flower, stem, root and leaf parts in plant biomass at different NaCl concentrations

chlorophyll values were obtained at the 35 mM NaCl concentration, decreasing above 35 mM. The critical NaCl level in terms of the amount of chlorophyll was determined to be 140 mM. A similar result was obtained in another study examining the effect of NaCl concentrations on the chlorophyll content of camelina, and significant reductions were reported after the 100 mM NaCl concentration (Morales et al. 2017). Similar results were obtained in studies on oil crops, such as soybean, safflower and sunflower, in which the effect of salt stress on chlorophyll was examined (Kao et al. 2006, Heidari et al. 2014, Komari et al. 2014). The reduction in the photosynthesis rate and chlorophyll content at high salt concentrations is considered a typical symptom of oxidative stress and leads to chlorophyll degradation (Marcińska et al. 2013). Growth in the leaf area decreases in saline conditions, photosynthesis is negatively affected due to increased leaf aging and there is a decrease in relative water content (Grant 2012). The relative water content has a positive relationship with the rate of photosynthesis and the amount of chlorophyll. The plant water content, relative water content and transpiration rate decrease significantly in plants exposed to salinity stress (Anjum et al. 2011). The findings obtained in the present research also confirm the reported results.

Too much soluble salts in the soil reduce the amount of water that plants can absorb from the soil. The reduction in plant water potential, a common response in such situations, can be offset by a reduction in osmotic potential as a result of increasing the solute content to maintain turgor potential. Increasing salinity reduces the water content potential of plants (Mugdal et al. 2010). With the loss of water in soil, the plant can absorb very little water from the soil and therefore the plant water content is further reduced (Bressan et al. 2008). There was a significant decrease in the plant water content depending on the increasing salt concentration in this research.

Due to the deterioration of the cell membrane of plants exposed to salt stress, the membrane permeability increases (Karlidag et al. 2011). It has been noticed that the membrane permeability increased as a result a worse water balance and inferior cell membrane functions in rapeseed plants exposed to salt stress, where the membrane permeability was 12.20% in the control group and 80.73% at the 100 mM NaCl concentration (Korkmaz et al. 2020). In the current study, 75.94% of the control's membrane permeability was obtained at the 280 mM NaCl concentration. This value shows that the camelina plant is more tolerant to cellular deterioration that may occur under the pressure of salt stress.

Biomass gain is largely dependent on photosynthesis, and is affected by leaf characteristics (Visser et al. 2016). It was found out that the plants exposed to a salt concentration higher than 70 mM differed greatly in the leaf area, relative water content and chlorophyll content compared to the control in the present research. Although the total biomass decreased up to the 70 mM NaCl concentration, it was concluded that such salt stress did not constitute a limiting value for camelina. As a matter of fact, it was reported that the biomass and plant height were higher in camelina at the 50 mM NaCl concentration compared to the control (Morales et al. 2017).

CONCLUSION

Salinity of arable and irrigable agricultural lands is a problem that is becoming more severe every year. It is estimated that the amount of salt in agricultural areas will double with climate change, the increase in the human population, and the corresponding increase in the demand for agricultural products. Salinity, one of the most important abiotic stress factors, is a major problem that negatively affects the morphological, physiological and chemical structure of plants. To solve this problem, there is a need to evaluate plants that have high tolerance to salt and where degradation/ /changes in plant structures under salinity conditions can be ignored. It was determined that the camelina plant could maintain its photosynthetic structure up to the 140 mM NaCl concentration, but there were serious changes in plant height, weight of plant parts that make up the biomass, total biomass, leaf parameters and membrane permeability above the140 mM NaCl concentration. On the other hand, it was determined that the camelina plant was adaptable to increasing salinity in arable lands.

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