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Assessment of Sodium Chloride (NaCl) Induced Salinity on the Growth and Yield Parameters of *Cichorium intybus* L.

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ABSTRACT

The present study was done on the hypothesis that excess sodium chloride (NaCl) in the soil decreases the growth and yield of *Cichorium intybus* L. To investigate this hypothesis, a pot experiment was conducted in which chicory seeds were sown in garden soil-filled earthen pots and treated with three different doses of sodium chloride (45, 75, and 105 mM kg^{-1} soil) except the control, and each treatment was replicated three times. The results revealed that all the saline treatments significantly ($p \leq 0.05$) reduced the vegetative (including root and shoot length, dry weight, number of leaves, leaf area, number of branches, and photosynthetic pigments) and reproductive (mean fruit number/plant, mean seed number/fruit, and total seed yield/plant) growth parameters of Cichorium intybus. On increasing NaCl concentration in the soil, chlorophyll content significantly ($p \leq 0.05$) decreased while proline content in the fresh leaves increased significantly ($p \leq 0.05$). From the results, it is concluded that Cichorium intybus L. can tolerate a moderate level of sodium chloride stress (45-75 mM NaCl kg^{-1} soil) but is sensitive to high doses of sodium chloride stress (105 mM NaCl kg^{-1} soil).

INTRODUCTION

Plants tolerate several biotic and abiotic stresses of the environment for their survival (Teshome et al. 2020). Drought, salinity, flood, and extreme cold are the major abiotic stresses that plants have to tolerate (Gidhay Adhanom 2019, Nazir et al. 2019). Climate change increases the intensity of these abiotic stresses, which affect the survival and yield of plants (Chaudhry & Sidhu 2021, Corwin 2021, Zandalinas et al. 2021). Among these abiotic stresses, salinity is rapidly increasing in the environment and affects the fertility of agricultural lands (Hassani 2021, Ullah et al. 2021).

Soil salinity is a measure of salt content in the soil that is commonly expressed as electrical conductivity (EC) and measured in deci Siemens (dS). However, to express salt concentrations, molarity units, such as millimoles per liter, are frequently used ($mmol_c\ L^{-1}$). Contrarily, sodicity is defined in terms of the sodium adsorption ratio (SAR) or the exchangeable sodium percentage (ESP). Saline soil has an EC greater than or equal to 4 deci Siemens per meter ($dS\ m^{-1}$) at 25°C; A sodic soil has a SAR greater than 13 ($mmol_c\ L^{-1}$) or an ESP of 15 or more, and saline-sodic soils are those with EC greater than or equal to 4 $dS\ m^{-1}$ or 40 mM NaCl, and SAR > 13 ($mmol_c\ L^{-1}$) (Stavridou et al. 2017). Furthermore, few researchers considered soils with lower ESP values (6-8%) as sodic soils (Mohanavelu et al. 2021; Stavi et al. 2021). Soil salinization is the process through which salt concentration in the soil increases to levels that have an impact on environmental health, agricultural productivity, a farmer's financial stability, and quality of life (Kumar & Sharma 2020). Various anthropogenic activities, such as irrigation without proper drainage planning, increase saline areas around the world and reduce agricultural land available for crop production (Gidhay Adhanom 2019). Besides this, the domestic and industrial discharge, enriched with various kinds of salts, in freshwater bodies contaminated the water of these freshwater bodies, and when the water of these freshwater bodies is used in irrigation, it increases the salinity of agricultural soil and reduces its fertility, ultimately decreasing growth and yield of the crops (Petropoulos et al. 2017, Dietz et al. 2021).

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Plants affected by saline toxicity showed poor growth and reduced crop/seed yield by a significant amount (Egamberdieva et al. 2019, El-Sabagh et al. 2021). Scientists from various countries are attempting to find plants that can survive in moderate-to-high saline soil without compromising growth and yield to combat the food shortage caused by climate change (Petropoulos et al. 2017). In general, NaCl-induced toxicity reduces plant growth by damaging root cells, resulting in a lack of water uptake and osmotic stress, increasing the pH of the soil (alkalization), which causes poor uptake of essential nutrients that come with the water in the plants, resulting in an imbalance of mineral nutrition (excess Na^+ ions decrease absorption of K^+, NH_4^+, and Ca^{++} ions, and excess Cl^- ions decrease absorption of NO_3^- ions Na^+ induced salinity also reduced transpiration and photosynthesis in the plants by disturbing the stomatal conductance, altering the enzymatic activities of photosynthesis, CO_2 availability in mesophyll cells, and modifications in chloroplast structure (Arshi et al. 2010, Petropoulos et al. 2017).

Cichorium intybus L. (Chicory) plant is a member of the family Asteraceae commonly known as chicory. It is widely distributed in Asia, Africa, Europe, and South America (Abbas et al. 2015, Aisa et al. 2020, Mavrina et al. 2021). Chicory plant contains compounds like alkaloids, coumarins, flavonoids, inulin, unsaturated sterols, sesquiterpene lactones, and vitamins (Jangra & Madan 2018, Aisa et al. 2020, Das et al. 2020). Fresh chicory leaves have a laxative property, while dried chicory root powder mostly contains inulin, a polymer of fructose, and root powder is mainly used in the adulteration of coffee (Abbas et al. 2015, Street et al. 2013). Chicory leaves and seeds are used in traditional medicines for the treatment of fever, diarrhea, jaundice, renal problems, and gallstones due to their pharmacological properties (Aisa et al. 2020, Mavrina et al. 2021, Choudhary et al. 2021) as well as found useful in bio-monitoring of various heavy metals in the environment (Aksoy 2008). Chicory is also used as a forage crop due to its high nutritive value, and anti-helmentic and laxative properties. It improves the milk yield of dairy livestock (Street et al. 2013, Piluzza et al. 2014).

India is the home of more than 1.32 billion people and is projected to become the most populated country in the world. This population rise poses challenges related to food security in India (Sharma et al. 2020; Kumar & Sharma 2020). In India, approximately 6.727 million hectares of land are salt-affected and classified into two types: sodic soils and saline soils. In India, Uttar Pradesh and Gujarat State have the most saline soils (71.2%) and sodic soils (35.6%), respectively. Areas of the Indo-Gangetic region of India that are affected by sodic soils originated when groundwater containing high levels of carbonates and bicarbonates is irrigated. This, along with the execution of canal irrigation projects without sufficient drainage, has resulted in widespread salinity in India, which is expected to worsen with time (Kumar & Sharma 2020).

As a result, India must select and cultivate crops that can grow in saline or sodic soil. Therefore, the present research focused on evaluating the dose-dependent response of chicory to NaCl stress in terms of growth, biochemical (chlorophyll and proline contents), and yield parameters and is also helpful to determine the level of NaCl in the soil below which chicory can easily grow and provide a good yield.

MATERIALS AND METHODS

Study Area

Before initiating the experiment, soil samples were taken from several field beds to analyze the soil characteristics. All soil samples were analyzed at the Government Agriculture Farm's Soil Testing Laboratory in Aligarh, India. Table 1 lists the physicochemical characteristics of the soil utilized in the experiment.

Experimental work has been done in the natural greenhouse of the botany department of Aligarh Muslim University, Aligarh, India. Aligarh (27.88°N latitude and 78.08°E longitude) has a semi-arid and sub-tropical climate with sandy loam and alkaline soil. Most of the rainfall is received from July through September.

Plant Material and Experimental Design

Chicory seeds (Cichorium intybus L.) were procured from the herbal garden of Jamia Hamdard, New Delhi, and cleaned

Table 1: Physico-chemical characteristics of garden soil used in the study.

Characteristics	Observation/value
Color	Light Brownish
Texture	Sandy loam
CEC (meq. 100 g^{-1} soil)	3.20
pH	7.1
EC (μ mhos.cm^{-1})	312
Total organic carbon (%)	0.800
Nitrate nitrogen(NO_3 N) g kg^{-1} soil	0.320
Phosphorus (P) g kg^{-1} soil	0.130
Potassium (K^+) mg.L^{-1}	24.10
Magnesium (Mg^{2+}) mg.L^{-1}	30.34
Sodium (Na^+) mg.L^{-1}	13.45
Calcium (Ca^{2+}) mg.L^{-1}	23.00
Bicarbonate (HCO_3^-) mg.L^{-1}	20.28
Carbonate (CO_3^{2-}) mg.L^{-1}	93.04
Sulphate (SO_4^{2-}) mg.L^{-1}	19.10
Chloride (Cl^-) mg.L^{-1}	34.55

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with a 0.01% $HgCl_2$ solution, then washed three times with double-distilled water (DDW). Earthen pots of 12-inch size were autoclaved for 30 minutes after being filled with 3 kg of soil. Five to eight seeds were sown with proper spacing in each pot. Three plants were kept in each pot, and after germination, those were almost the same height and leaf number.

Plants were treated with 45, 75, and 105 mM NaCl kg^{-1} soil and were applied in the form of a dilute aqueous solution of sodium chloride (NaCl) when plants were 3 weeks old. Each treatment/dose was replicated three times. Control pots received no sodium chloride. Irrigation was carried out using tap water as required. The experiment was conducted in a simple randomized block design (SRBD). The plants were harvested at full maturity (170 DAS). The sampling of plants was done at the vegetative stage, 80 days after sowing (80 DAS) and at maturity (170 DAS) to estimate different growth and biochemical parameters.

Estimation of Vegetative and Reproductive Growth Parameters

The sampling of growth parameters was done at 80, and 170 days after sowing (DAS). Quantitative data for the following parameters were gathered, (a) Root and Shoot length (in cm); (b) Root and shoot dry weight (in g); (c) Mean leaf number $plant^{-1}$; (d) Mean leaf area (cm^2); (e) Mean number of branches $plant^{-1}$; (f) Mean fruit number $plant^{-1}$; (g) Mean seed number $fruit^{-1}$; (h) Mean seed number $plant^{-1}$. Three replicates were used for all measurements. A decrease or increase in NaCl-treated plants was calculated in comparison to the control.

For sampling, plants were harvested at two growth stages, the root-shoot junction was cut with precision, and the lengths of their shoot and root were estimated on a metric scale and expressed in centimeters. For estimating plant dry mass, samples were dried in an oven at 65-70°C for 72 hours. The dry biomass of the shoot and root of each plant was recorded on an analytical balance (Wensar Weighing Scales Limited, Lucknow, India) and expressed in grams. The total number of fully expanded leaves counted from three replicates of each treatment was divided by the sample size to obtain the mean leaf number of $plant^{-1}$. The graph paper method (Pandey & Singh 2011) was used to calculate the leaf area of mature leaves. Data obtained from three replicates were summed and divided by three to obtain the mean value of each remaining vegetative and reproductive parameter.

Estimation of Chlorophyll and Proline Contents

Using Arnon's method (Arnon 1949), the chlorophyll content of fresh leaf samples was estimated. The absorbance of the prepared sample was read on a spectrophotometer (2700, Analytical Technologies Ltd., Labtronics, India) at 645 and 663 nm for chlorophyll against the blank (80% acetone). The Bates et al. (1973) method was used to estimate the proline content of the fresh leaves. Finally, the prepared sample was transferred to the ice bath and the mixture was extracted with toluene and read at 520 nm on a spectrophotometer (2700, Analytical Technologies Ltd., Labtronics, India) using L-proline as a standard.

Statistical Analysis

One-way analysis of variance (ANOVA) of data was done by using statistical software R ver. x64-4.1.2 (package library, Agricolae). To determine the significance of the difference of means at $P \leq 0.05$, Duncan's multiple range test (DMRT) was performed with the same software.

RESULTS

Vegetative Growth Parameters

All treatments except control caused a significant reduction in root and shoot length, root and shoot dry weight, mean leaf number, mean leaf area, and mean number of branches at both sampling stages (80 and 170 DAS). 105 mM NaCl kg^{-1} soil treatment caused the highest reduction of 50% and 39% in root length, 56% and 40% in shoot length, 41% and 39% in root dry weight, and 48% and 43% in shoot dry weight, 50% and 28% in mean leaf number, 41% and 37% in mean leaf area, and 41% and 29% in the mean number of branches over control at 80 DAS and 170 DAS, respectively (Table 2).

Chlorophyll and Proline Content

All treatments except the control caused a significant decrease in the photosynthetic pigments (chlorophyll a, chlorophyll b, total chlorophyll) and a significant increase in proline content at both sampling stages (80 and 170 DAS). 105 mM NaCl kg^{-1} soil treatment caused the highest decrease of 41% and 23% in chlorophyll a, 42% and 29% in chlorophyll b, and 42% and 29% in total chlorophyll content over control at 80 DAS and 170 DAS respectively (Fig. 1a-f). The same treatment caused the highest increase of 114% and 136% in proline content over control at 80 DAS and 170 DAS respectively (Fig. 2a and b).

Reproductive Growth and Yield Parameters

Plants treated with 45, 75, and 105 mM NaCl kg^{-1} soil showed a significant ($P \leq 0.05$) reduction of 08%, 17%, and 25% in fruit yield per plant over control at the harvesting stage or 170 DAS. Plants treated with the same doses of NaCl resulted in a statistically significant ($P \leq 0.05$) reduction of 05%, 10%,

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Table 2: Effects of different treatments of Sodium Chloride (45, 75 and 105 mM NaCl kg^{-1} soil) on growth and biochemical parameters of chicory at 80 DAS and 170 DAS.

Cichorium intybus L.	Different treatments of NaCl mM kg^{-1} of soil (80 DAS)				Different treatments of NaCl mM kg^{-1} of soil (170 DAS)			
Parameters (80 DAS)	CONTROL	45 mM	75 mM	105 mM	CONTROL	45 mM	75 mM	105 mM
Root Length (cm)	20.73± 1.65a	16.30 ±1.67 ab	13.63± 1.40bc	10.27±1.07c	26.73±1.97a	23.33± 1.76ab	19.50± 1.61bc	16.30± 1.57c
Shoot Length (cm)	74.50 ± 6.21a	55.07±5.90b	42.67± 3.84bc	32.5± 3.40c	140.60± 12.11a	117.00± 10.39ab	105.33±7.62bc	84.33± 6.69c
Root Dry Weight (g)	1.13 ± 0.12a	0.86± 0.07ab	0.75±0.07 b	0.66± 0.08b	4.95± 0.41a	4.28± 0.33ab	3.68±0.20 bc	3.00±0.29c
Shoot Dry Weight (g)	4.00 ±0.23 a	2.92± 0.24b	2.45±0.23 bc	2.06± 0.22c	15.57± 1.37a	12.90± 1.24ab	11.00±1.15bc	8.80±0.81c
Number of branches	4.00 ± 0.57a	3.33± 0.33ab	3.00± 0.57ab	2.33± 0.33b	8.00±0.58a	7.00±0.58ab	6.33±0.33b	5.67±0.33b
Number of Leaves	43.00 ±4.62a	34.33± 4.63ab	28.00± 4.61ab	21.33±4.09 b	80.00±5.77a	71.67±6.06ab	63.00±7.0ab	57.33±5.21b
Leaf area (cm^2)	45.42±5.13a	36.08±3.49 ab	31.33±2.60 b	26.50± 2.45b	76.33± 7.74a	65.00±5.51 ab	58.00±6.43ab	48.00±3.46b

Data represent the mean and standard error of three replicates. Different letters indicate significant differences between treatments at $p < 0.05$, ($n = 3$) through the DMRT test.

and 14% in seed yield per fruit over control. Plants treated with 45, 75, and 105 mM NaCl kg^{-1} soil caused a maximum significant ($P \leq 0.05$) reduction of 13%, 26%, and 35% in seed yield per plant over control at the harvesting stage or 170 DAS (Table 3).

DISCUSSION

Along with sunlight, ample water and nutrient supply are required for proper plant growth (El-Ramady et al. 2014, Ahanger et al. 2016, Xu et al. 2021). The root is the first organ that has to face salt stress in the soil (Arshi et al. 2010, Bhatt et al. 2015, Chaudhry & Sidhu 2022). Excess sodium ion (Na^+) in the soil affects the soil texture; it increased pH (alkalization), which is produced by the presence of HCO_3^- and CO_3^{-2} and decreases the availability of organic matter and essential elements or ions to the roots, causing ion imbalances by inducing competition of Na^+ with K^+ and Ca^{++} (nutrient deficiency) and Cl^- with NO_3^{-2} (Stavi et al. 2021). Salinity also reduces nutrient availability to crops or ion toxicity (salt stress) by inducing Na^+ accumulation in the cytoplasm, or a combination of these factors reduces cell division and cell expansion in root and shoots, resulting in a decrease in root and shoot growth and biomass (Arshi et al. 2010, Upadhyay et al. 2012, Bhatt et al. 2015, Hafez et al. 2021). It was earlier reported that excess NaCl reduced leaf expansion, cell division, and cell elongation by reducing water entrance in the cell, affecting water balance (osmotic stress) in the plants, resulting a decrease in vegetative and reproductive growth parameters. Same results are found in the present study (Table 2, Table 3, Fig. 1a-f, Fig. 2a and b) (Jaleel et al. 2008, Machado & Serralheiro 2017, Hafez et al. 2021).

The current study discovered that a decrease in photosynthetic parameters of chicory was associated with Na^+ and Cl^- ions-induced phytotoxicity at both sampling stages and that it increases as NaCl concentration in the soil increases (Fig. 1a-f). NaCl stress decreases the CO_2 availability in the mesophyll cells by disturbing stomatal conductance, also producing reactive oxygen species (ROS) leading to oxidative stress which disrupts the activities of enzymes involved in photosynthesis like RuBP Carboxylase/oxygenase, damages chlorophyll structure by decreasing tocopherol content, protectant of chlorophyll in the cytoplasm (Machado & Serralheiro 2017, Hafez et al. 2021). Moreover, Na^+ and Cl^- ions accumulation (ionic stress) in the cytoplasm of plant cells damaged the chloroplast structure, causing modifications in the composition of the thylakoid membrane and other pigments resulting in the inhibition of electron transport (Singh et al. 2020). NaCl stress also decreases the rate of photosynthesis by promoting

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Table 3: Effects of different treatments of Sodium Chloride (45, 75 and 105 mM NaCl kg^{-1} soil) on yield parameters of chicory at 170 DAS.

Cichorium intybus L.	Different treatments of NaCl mM kg^{-1} of soil (170 DAS)			
Parameters (170 DAS)	Control	45 mM	75 mM	105 mM
Number of fruits (heads)	75.00±5.51a	68.67±4.33ab	61.67±2.60ab	56.00±4.73b
Seeds per fruit (head)	40.33±2.03a	38±1.53ab	36±1.15ab	34.67±1.20b
Seeds per plant	3025.00±73.98a	2609.33±71.27b	2220.00±24.25c	1941.33±100.37d

Data represent the mean and standard error of three replicates. Different letters indicate significant differences between treatments at $p < 0.05$, ($n = 3$) through DMRT test.

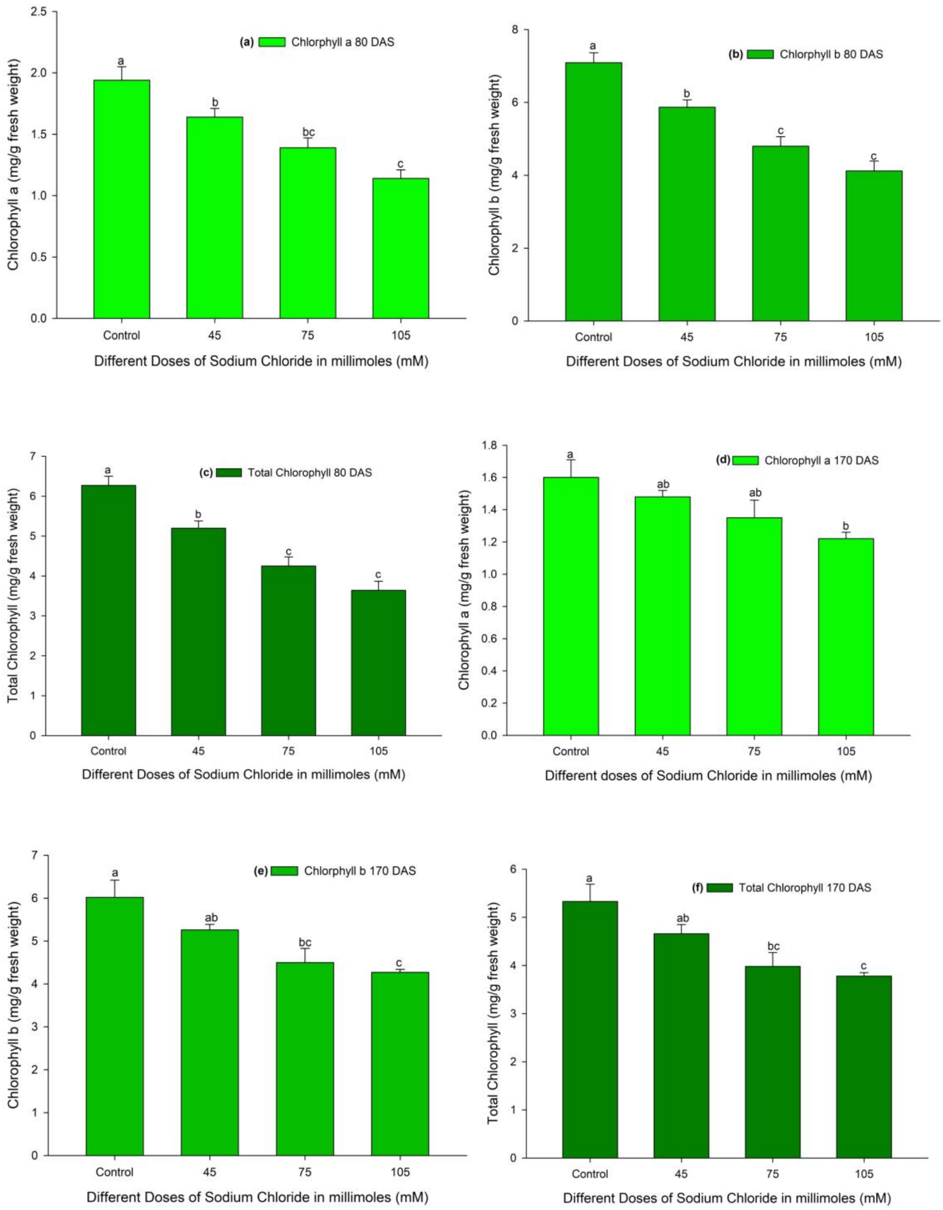

Fig. 1 a-f: Effects of different treatments of Sodium Chloride (45, 75 and 105 mM NaCl kg^{-1} soil) on chlorophyll content (a, b and total) of chicory at 80 DAS and 170 DAS. Data represent the mean and standard error of three replicates. Different letters above the bars indicate the significant differences between treatments at $p < 0.05$, ($n = 3$) through the DMRT test.

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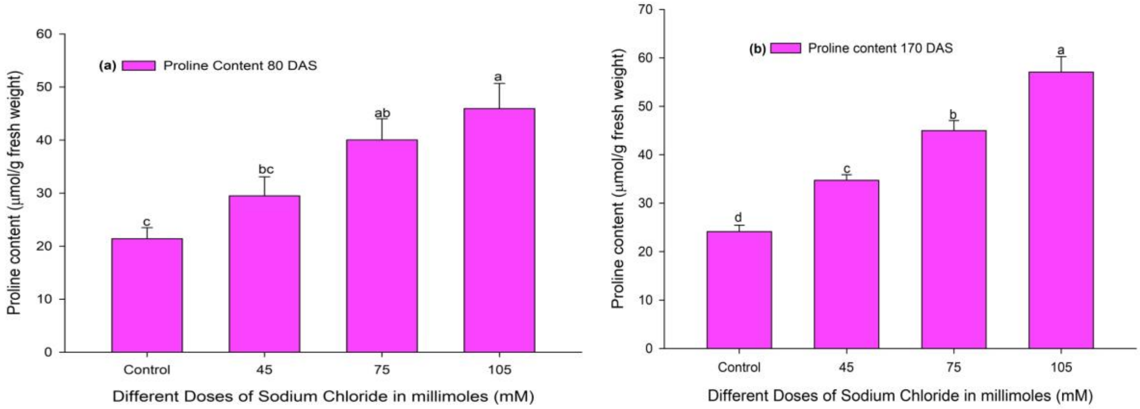

Fig. 2 a and b: Effects of different treatments of Sodium Chloride (45, 75 and 105 mM NaCl kg^{-1} soil) on proline content in fresh leaves of chicory at 80 DAS and 170 DAS. Data represent the mean and standard error of three replicates. Different letters above the bars indicate the significant differences between treatments at $p < 0.05$, ($n = 3$) through the DMRT test.

stomatal closure by reducing K^+ ions concentration in the cell. Scientists discovered similar results in plants such as Cassia, Senna, Catharanthus, Oats, Millets, and Maize at various salinity levels (Arshi et al. 2006, Jaleel et al. 2008, Bhatt et al. 2015, Hafez et al. 2021).

Plants accumulate a high amount of proline in their organs/tissues to maintain osmotic balance and prevent cell membrane leakage to survive in any abiotic stress condition, such as salinity (Monteoliva et al. 2014). A significant increase in proline content was recorded in chicory grown under different levels of NaCl stress (Fig. 2a and b) at both sampling stages, suggesting that NaCl stress has imbalanced the osmotic equilibrium in chicory leaves. Earlier studies on chicory like Arshi et al. (2010), Sergio et al. (2012) and Petropoulos et al. (2017) confirmed that increasing NaCl stress increased proline contents in the leaves of the chicory plants, which is the result of an increase in protein degradation of plant tissues (Monteoliva et al. 2014). Leaf area plays an important role in biomass accumulation in plants, and a decrease in leaf area reduces the rate of photosynthesis leading to a lesser amount of biomass production in plants (Weraduwage et al. 2015). In this experiment, the significant ($p \leq 0.05$) decrease (Table 2, Fig. 1a-f, Fig 2a and b) in vegetative growth, leaf area, branching, and biomass of chicory by the lowest to highest treatment treatment of NaCl stress, i.e., 45 to 105 mM NaCl Kg^{-1} soil was due to a reduction in water balance, cell division and cell expansion, decrease in photosynthesis and photosynthetic pigments, increase in protein degradation, translocation, and assimilation of photosynthetic products (Arshi et al. 2010, Petropoulos et al. 2017, Hafez et al. 2021) and our results agree with findings of Sergio et al. 2012 which concluded that chicory plants can survive in mild salinity. Crop/seed yield is an important parameter of productivity and a high level of salinity decreases the crop/seed yield of the plants (Munns & Gilliham 2015, Stavridou et al. 2017, Ullah et al. 2021). In this experiment, results (Table 3) showed that each level of salinity decreased the crop/seed yield of chicory considerably. Poor seed or grain yield of cultivated crops harms farmers' economies (Petropoulos et al. 2017, Stavridou et al. 2017, Zandalinas et al. 2021). Our findings suggested that chicory showed poor growth and yield at 105 mM NaCl kg^{-1} soil treatments and the values of both parameters are directly correlated with a high concentration of NaCl in the soil.

CONCLUSION

The results of this study concluded that NaCl-induced toxicity inhibited the vegetative and reproductive growth of chicory plants in a concentration-dependent manner. At 105 mM NaCl kg^{-1} soil causes a considerable loss of 35 percent in total seed output $plant^{-1}$ which is a considerable economic loss for farmers. Finally, it is concluded that chicory can be grown in moderate saline soil (45 mM and 75 mM NaCl kg^{-1} soil) without compromising their growth and yield but at or beyond the level of salinity like 105 mM NaCl kg^{-1} soil, chicory starts to show poor growth and yield, which is not suitable or recommended to the farmers for cultivation, to save them from economic loss.

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