

Growth and Ionic Adjustments of Chaksu (*Cassia absus* L.) Under NaCl Stress

Khalid Hussain, Abdul Majeed, M. Farrukh Nisar,
Khalid Nawaz, Khizar Hayat Bhatti and Shahid Afghan

Department of Botany, University of Gujrat (UOG), Punjab, Pakistan

Abstract: Pot experiment was conducted to assess the growth and ion contents adjustment of Chaksu (*Cassia absus* L.) under NaCl levels as 0 (control), 8 dS/m and 10 dS/m. All growth attributes such as shoot and root length, shoot fresh and dry weights were decreased with increasing salinity levels. Na^+ , Cl^- and K^+ concentrations were increased with increasing salinity, while Ca^{2+} concentration was lower as salinity levels increased. Effect of NaCl was non significant on proline contents and total soluble carbohydrates increased sharply with increasing NaCl level. It was concluded that with increasing salinity levels there was a significant reduction in growth and biomass production in chaksu with varied accumulation patterns of ions.

Key words: NaCl • Growth • Ions • Proline • Chaksu

INTRODUCTION

Salinity is a worldwide problem causing serious constraints in arid and semi-arid regions of the world for growth and yield of crops [1]. Salinity causes not only differences between the mean yield and the potential yield, but also causes yield reduction. It affects the plant growth directly through its interaction with metabolic rates and pathways within the plants. It affects plant growth at all stages of development and sensitivity to salinity varies from one growth stage to another. Adverse effects of salt stress on germination, seedling growth as well as some physiological activities of a number of cultivated plant species have been extensively investigated [2-4].

Chaksu (*Cassia absus* L.) is a useful medicinal plant belongs to family leguminosae. It is regarded as useful enriching the blood as tonic, a bitter astringent for the bowels, applied locally to heal ulcers. It is useful in the diseases of eyes such as purulent conjunctivitis and ophthalmia [5].

Salinity stress disturbs the uptake and accumulation of essential nutrients [2, 6, 7]. Generally, Ca^{2+} and K^+ are decreased in plants under saline conditions [8, 9]. In contrast, Ashraf and Rauf [10] reported that under saline conditions concentrations of Na^+ , K^+ and Ca^{2+} increased significantly in all the parts of germinating from maize seeds primed with NaCl, KCl and $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, respectively. Alam and Naqvi [11] observed that plant height and dry matter yield were decreased

with increasing salinity at 85-days old plants of black seed under salinity levels of 1.95, 4.69, 9.38 and 14.06 dS m^{-1} NaCl. Salinity caused an increase in N, P, Ca^{++} , Na^+ , Fe^{++} and Mn^{++} and decrease in K^+ contents of the leaves.

It is generally accepted that the germination and seedling stage of plant life cycle is more sensitive to salinity than the adult stage [12]. Effect of salinity at different growth stages in wheat, sorghum and cowpea was investigated and it was found that the early seedling period was the most sensitive one in all the crops and reduction in growth was observed which decreased with increase in salinity [13].

In view of these studies, the principal objective to carryout the present study was to assess the growth and ionic adjustments made by chaksu under NaCl stress.

MATERIALS AND METHODS

Seeds of chaksu (*Cassia absus* L.) were obtained from the Shakarganj Botanic Garden, Jhang, Pakistan. Seeds were surface sterilized by dipping in 10 percent sodium hypochlorite solution for 10 minutes, then rinsed with sterilized distilled water and air-dried at an ambient temperature of 32 °C in the laboratory. Two levels of NaCl salt (0 and 100 mol m^{-3} NaCl) were applied after 14-days of germination. The experiment was laid out as Completely Randomized Design (CRD) with eight replicates. Plants were harvested at maturity.

Plants were uprooted carefully and washed in distilled water. Shoot and root length was measured with the help of scale meter in cm at final harvest. Plant samples were placed in oven at 75°C. After 4-days shoot and root dry weight (g/pot) was calculated with the help of electric balance at final harvest (42-days after treatment). The dried ground plant material (0.1g) was digested with sulphuric acid (H₂SO₄) and hydrogen peroxide (H₂O₂) according to the method of Wolf [14]. Shoot and root ion contents of Na⁺ and K⁺ were determined by emission spectrophotometry and of Ca²⁺ and Mg²⁺ by atomic absorption spectrophotometry. Data obtained was used to estimate the net ion transfer rates to the shoot according to the equation proposed by Salim and Pitman [15] at both harvests (21 and 42 days after treatments). Chloride was extracted by stirring ground-dried samples with 0.1 M NaNO₃ for 30 minutes. After extract clarification with activated coal, it was added 13.2 mM Hg(SCN)₂ in methanol and 20.2% (w/v) Fe(NO₃)₃ (4 + 1) and the absorbance determined at 460 nm [16].

Proline was also determined spectrophotometrically following the ninhydrin method described by Bates *et al.* [17] using L-proline as a standard. Approximately 300 mg of dry tissue was homogenized in 10 mL of 3% (w/v) aqueous sulphosalicylic acid and filtered. In 2 mL of the filtrate, 2 mL of acid ninhydrin was added, followed by the addition of 2 mL of glacial acetic acid and boiled for 60 min. The mixture was extracted with toluene and the free proline was quantified spectrophotometrically at 520 nm from the organic phase using a Shimadzu spectrophotometer (Duisburg, Germany).

Total soluble carbohydrates (TSC) concentrations were determined according Brun [18]. Samples of 100 mg were homogenized with 10 mL of extracting solution (glacial acetic acid: methanol: water, 1:4:5, v/v/v). The homogenate was centrifuged for 10 min at 3,000 rpm and the supernatant was decanted. The residue was resuspended in 10 mL of extracting solution and centrifuged another 5 min at 3,000 rpm. The supernatant was decanted, combined with the original extract and made up to 50 mL with water. For measurement of TSC, a phenolsulfuric acid assay was used as described by Dubois *et al.* [19]. A volume of 0.5 mL of 5% (v/v) phenol solution and 2.5 mL of concentrated sulfuric acid were added to 0.5 mL aliquots. The mixture was shaken, heated in a boiling water bath for 20 min and cooled to room temperature. The absorption was then determined by spectrophotometry at 490 nm (Shimadzu spectrophotometer, Duisburg, Germany).

Analysis of variance technique was employed for carrying out statistical analysis of data collected [20]. Various treatment means were compared with Duncan's New Multiple Range (DMR) Test.

RESULTS AND DISCUSSION

Salinity had highly significant effect on growth attributes of chaksu (Fig. 1). Shoot and root lengths significantly decreased with increasing NaCl levels. The maximum reduction was observed at 10 dS/m. In control shoot length was 195.96 cm while at 10 dS/m it decreased up to 148.74 cm. In case of root length it decreased to 10.96 cm at 10 dS/m than control had 17.21 cm root length (Table 1). Similar pattern of reduction in shoot fresh and dry weights were noted in chaksu. Shoot fresh and dry weights were decreased with increasing NaCl level. The maximum shoot fresh was calculated in control (84.74g) and the minimum at 10 dS/m (41.42g). In case of shoot dry weight the maximum weight was present in control (20.85g), while it decreased up to 13.31g at 10 dS/m (Table 1). Similarly, Hussain *et al.* [1] found the reduction of growth in black seeds under salt stress.

Data pretended in Table 1 for different ion contents showed varied pattern of accumulations. Na⁺, K⁺ and Cl⁻ contents were increased with increasing NaCl level. The maximum accumulations for these ions were noted at 10 dS/m as compared to control. In control there were 5.89 mg/g of Na⁺ contents, while it increased up to 15.75 at NaCl level of 10 dS/m (Table 1). For K⁺ contents the maximum accumulation (16.45 mg/g) was present at 10 dS/m. Similarly, the maximum accumulations of Cl⁻ contents were calculated at higher level of NaCl (Table 1). These results are similar with the earlier findings in maize reported by Izzo *et al.* [21]. Similar results for accumulations of inorganic ions ((Na⁺, K⁺, Ca²⁺, Cl⁻, N) in salt sensitive and resistant pearl millet lines were described by Hussain *et al.* [2].

Effect of NaCl levels was statistically non significant on proline accumulation in chaksu. Ashraf [22] and Meloni *et al.* [23] reported that proline was not involved in the osmotic adjustment of black gram, sorghum and cotton cultivars, respectively. Total soluble carbohydrates (TSC) concentrations in root was increased sharply with increasing NaCl level (Table-1). This probably reflects the maintenance or even induction of root elongation at low water potentials, which can be considered as an adaptive response to salinity [24].

Table 1: Effect of different salinity levels on Chaksu

Parameters	0 dS/m	8 dS/m	10 dS/m	LSD value at 5 %
Shoot length (cm)	195.96 a	154.24 b	148.74 c	8.91
Root length (cm)	17.21 b	12.35 b	10.96 a	4.31
Shoot fresh weight (g)	84.74 a	58.20 b	41.42 c	7.54
Shoot dry weight (g)	20.85 a	14.95 b	13.31 c	3.21
Na ⁺ (mg/g)	5.89 c	12.51 b	15.75 a	4.32
K ⁺ (mg/g)	8.31 c	14.25 b	16.45 a	3.43
Ca ²⁺ (mg/g)	5.25 a	3.21 b	3.17 b	1.22
Cl ⁻ (mg/g)	6.47 a	14.25 b	15.18 c	2.33
Proline (µg g ⁻¹ DW)	14.56 a	15.16 a	14.78 a	3.64
TSC (µg g ⁻¹ DW)	400.45 c	525.75 b	598.23 a	25.94



Fig. 1: Pattern of growth in chaksu under NaCl stress

Addition of NaCl had an adverse effect on the growth of Chaksu. Salinity caused a significant effect on shoot and root lengths, shoot fresh and dry weights. The reason for growth reduction in Chaksu could be due to water shortage and ionic toxicity caused by salinity. The increase in plant growth may be due to turgor potential which is decreased by water deficit produced by high concentrations of the salts in the soil [25]. Assessment of pattern of accumulation of toxic ions in a species is vital importance to understand, whether the species uses partial exclusion or inclusion mechanism for tolerating toxic ions present in its growth medium [26].

CONCLUSIONS

It was concluded that with increase in salinity levels there was a significant reduction in growth and biomass production in chaksu with varied accumulation patterns of ions.

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