Treatment with Different Sodium Salts Alters Growth and Photosynthetic Pigment Constituents in *Withania somnifera*

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Abstract In the present investigation, experiments were conducted to study the effects of different sodium salts viz., sodium chloride (NaCl), sodium sulphate (Na2SO4) and sodium carbonate (Na2CO3) on morphological parameters (germination percentage, root and shoot length, dry weight of root and shoot) and photosynthetic pigment contents (chlorophyll ‘a’, chlorophyll ‘b’, total chlorophyll and carotenoids) of *Withania somnifera* plants. The germinating *Withania somnifera* seeds were treated with 10, 20 and 50mM NaCl and Na2SO4 and 5, 10 and 15mM Na2CO3. The parameters were analysed on 25 DAS. All the treatments decreased the germination percentage; shoot length, root length and dry weight, chlorophyll and carotenoids contents significantly. This effect was very high in the Na2CO3 treated seedlings this was followed by Na2SO4 and NaCl treated seedlings. From the results of this investigation, it is clear that, the *Withania somnifera* were affected significantly in the Na2CO3 treatments and followed by Na2SO4 and NaCl treatments.

Key words: Sodium chloride, Sodium sulphate, Sodium carbonate, Morphological parameters, Photosynthetic pigment, *Withania somnifera*

INTRODUCTION

Every year more and more land becomes nonproductive owing to salt accumulation. Salinization plays a major role in soil degradation. It affects 19.5% of irrigated land and 2.1% of dry land agriculture in the world. In India, out of 9.38 million ha of salt-affected soils, 3.88 million ha are alkali soil and 5.5 million ha (including coastal lands) are saline soils [1-3]. The earliest response to salt stress in plants is a reduction in the rate of leaf surface expansion, followed by a cessation of expansion as the stress intensifies [4,5]. The deleterious effect of salinity on plant growth is attributed to the decrease in the osmotic potential of the growing medium, specifically the ion toxicity and nutrient ion deficiency [6]. The extent of plant growth depression under saline conditions varies with salt composition, salt concentration, the physiological stage of the plant and the plant species. The general pattern of plant response to salinity is growth suppression more or less proportional to the solute concentration [2-6].

Plants growing under such saline conditions accumulate various solutes by altering their metabolic pathways [3-5]. The use of saline soils and brackish groundwater for growing plants of varying economic importance like those used in traditional medicines assumes utmost importance [5-7]. A saline environment can reduce a wide number of responses in plants ranging from readjustment of transport and metabolic processes leading to growth inhibition. Na+ is the predominant soluble cation in most saline soils and water, particularly in the coastal area. Most crop plants exhibit considerable hypersensitivity to a saline environment because the inter cellular accumulation of Na+ is toxic to the cellular metabolism and for many salt sensitive plants, a major part of the growth inhibition was caused by excess Na+ in the soil [8,9]. High amounts of sodium disturb potassium (K) nutrition and when accumulated in cytoplasm, inhibit many enzymes [10]. These effects are also due to a combination of adverse osmotic gradients and inhibitory effects of salts and ions on cell metabolism and of nutrient imbalance and secondary stresses such as an
oxidative stress linked to the production of toxic reactive oxygen intermediates [11-14]. Ion uptake and compartmentalization are crucial not only for normal growth but also for growth under saline conditions, because stress disturbs ion homeostasis [9-16].

*Withania somnifera* Dunal, known as ashwagandha, has been an important herb in the Ayurvedic and indigenous medical systems for centuries in India [4]. In view of its varied therapeutic potential, it is the subject of considerable modern scientific attention [10]. A perusal of the literature showed that there is a lack of information on the responses of this plant to different sodium salts [4,10,17]. The present investigation was therefore undertaken to study the effects of NaCl, Na2CO3 and Na2SO4 on the growth and photosynthetic pigments in *Withania somnifera*.

**MATERIALS AND METHODS**

Seeds of *Withania somnifera* were surface sterilized with 0.2 per cent HgCl2 solution for 5 minutes with frequent shaking and then thoroughly washed with deionised water. The seeds were sown in plastic pots (300 mm diameter) filled with 3 kg of soil mixture containing red soil, sand and farmyard manure (FYM) at 1:1:1 ratio. All the pots were watered to the field capacity with tap water up to 19 DAS. On 20 DAS the pots were irrigated with tap water for control, 100 mM NaCl, 5 mM CaCl2 and 100 mM NaCl + 5 mM CaCl2 solutions. The plants were uprooted randomly on the 30 and 50 DAS and used for estimating the growth and pigments.

**Analysis of Growth:** The morphological parameters such as root and shoot length and dry weight of root and shoot were analysed on 30th day.

**Chlorophyll and Carotenoid:** Extraction and determination of chlorophyll and carotenoid was performed according to the method of Arnon [18]. Five hundred milligrams of fresh leaf material was ground with 10 ml of 80% acetone at 4°C and centrifuged at 2500 rpm for 10 minutes at 4°C. This procedure was repeated until the residue became colourless. The extract was transferred to a graduated tube and made up to 10 ml with 80% acetone and assayed immediately. Three milliliters aliquots of the extract were transferred to a cuvette and the absorbance was read at 645, 663 and 480 nm with a spectrophotometer (U-2001-Hitachi) against 80% acetone as blank. Chlorophyll content was calculated using the formula of Arnon [18] and expressed in mg g⁻¹ fresh weight (FW).

\[
\text{Total chlorophyll (mg/ml) = (0.0202) × (A.645) + (0.00802) × (A.663)}
\]

\[
\text{Chlorophyll 'a' (mg/ml) = (0.0127) × (A.663) - (0.00269) × (A.645)}
\]

\[
\text{Chlorophyll 'b' (mg/ml) = (0.0229) × (A.645) - (0.00468) × (A.663)}
\]

Carotenoid content was estimated using the formula of Kirk and Allen [19] and expressed in mg g⁻¹ FW.

\[
\text{Carotenoid = A.480 + (0.114 × A.663-0.638 × A.645).}
\]

**Statistical Analysis:** Statistical analysis was performed using one way analysis of variance (ANOVA) followed by Duncan’s Multiple Range Test (DMRT). The values are mean ± SD for seven samples in each group. P values ≤ 0.05 were considered as significant.

**RESULT AND DISCUSSION**

The results of effects of cobalt on morphological parameters of *Withania somnifera* are given in Table 1 and photosynthetic pigment content of *Withania somnifera* given in Table 2. Sodium salts treatment was proved to be not so favorable for the overall growth of *Withania somnifera*. The treatments decreased morphological parameters of *Withania somnifera* when compared control.

Morphological parameters such as root and shoot length and dry weight of root and shoot, of *Withania somnifera*, decreased with an increase in salt level in the soil. Similar decrease in plants height was observed under salt treatments in many plants [19-23]. The dry matter production of *Withania somnifera*, decreased with different salts level in the soil. The reduction in dry matter yield of plants at higher concentration of exogenous salt treatment was also observed in various plants [22-25].

The photosynthetic pigments such as chlorophyll ‘a’, chlorophyll ‘b’ total chlorophyll and carotenoid contents of *Withania somnifera* decreased with increasing salt level in the soil. Similar reduction in the pigment content by various metal treatments was recorded in many studies previously [24-27]. The decreased chlorophyll content at salt treatment was obviously due to retarded growth. The excess of salt treatment brought about a marked depression in photosynthetic pigments in *Withania somnifera*. These results are in accordance with previous reports [23-28] and it might be due to excess supply of salt resulting in interference with the synthesis of chlorophyll. The formulation of chlorophyll pigments depends on
Table 1: Effect of different sodium salts on growth parameters of *Withania somnifera* (30th day)

<table>
<thead>
<tr>
<th>Sodium salts</th>
<th>Root length</th>
<th>Shoot length</th>
<th>Root dry weight</th>
<th>Shoot dry weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.34 £</td>
<td>4.61 £</td>
<td>0.312 £</td>
<td>0.819 £</td>
</tr>
<tr>
<td>NaCl</td>
<td>4.09 ¥</td>
<td>5.18 ¥</td>
<td>0.386 ¥</td>
<td>0.897 ¥</td>
</tr>
<tr>
<td>Na2SO4</td>
<td>3.68 ££</td>
<td>4.54 ££</td>
<td>0.274 ££</td>
<td>0.761 ££</td>
</tr>
<tr>
<td>Na2CO3</td>
<td>3.21 £££</td>
<td>3.86 £££</td>
<td>0.234 £££</td>
<td>0.673 £££</td>
</tr>
</tbody>
</table>

Values are given as mean ± SD of six experiments in each group. Values, that are not sharing a common superscript (a,b,c,d,e,f) differ significantly at *P* ≤ 0.05 (DMRT)

Table 2: Effect of different sodium salts on chlorophyll and carotenoid content (mg g⁻¹ fresh weight) *Withania somnifera* (30th day)

<table>
<thead>
<tr>
<th>Sodium salts</th>
<th>Chlorophyll 'a'</th>
<th>Chlorophyll 'b'</th>
<th>Total Chlorophyll</th>
<th>Carotenoid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.719 £</td>
<td>1.143 £</td>
<td>1.862 £</td>
<td>0.093 £</td>
</tr>
<tr>
<td>NaCl</td>
<td>0.876 ¥</td>
<td>1.964 ¥</td>
<td>2.840 ¥</td>
<td>0.132 ¥</td>
</tr>
<tr>
<td>Na2SO4</td>
<td>0.648 * (-9.874)</td>
<td>1.012 £££</td>
<td>1.660 £££</td>
<td>0.086 £££</td>
</tr>
<tr>
<td>Na2CO3</td>
<td>0.517 £££</td>
<td>0.785 £££</td>
<td>1.392 £££</td>
<td>0.064 £££</td>
</tr>
</tbody>
</table>

Values are given as mean ± SD of six experiments in each group. Values, that are not sharing a common superscript (a,b,c,d,e,f) differ significantly at *P* ≤ 0.05 (DMRT)

The adequate supply of iron [24]. We have previously reported the effects of salinity [11] on photosynthetic pigment contents of *Catharanthus roseus*. The excess supply of different salts seems to prevent the incorporation of iron in protoporphyrin molecule, resulting in the

REFERENCES


