Anatomical Changes Induced by NaCl Stress in Root and Stem of *Gazania harlequin* L.

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**ABSTRACT**

NaCl induces the stress that results in plant reduced growth due to changes in internal mechanism of the plant. The present study was designed to investigate the performance of *Gazania harlequin* grown under different salinity levels (25, 50, 75 and 100 mg l⁻¹ of NaCl). The experiment was laid out in the completely randomized design (CRD) with four replications. Microscopic studies for root and stem anatomical attributes showed that salinity plays an important role in growth inhibition of plants. Xylem and phloem areas of root and stem were observed in decreasing trend under salt stress condition and the cortex also. While minimum cortex and epidermis area was observed in plants treated with 100 mg l⁻¹. The study revealed that control (T₀) exhibited better results as compared to other treatments under saline conditions. Visual observations showed that the reduction of water uptake by the plants under high saline condition created osmotic conditions and ion toxicity which results in smaller cell size and cell injury.

**Keywords:** Cortex, epidermis, phloem, salinity, stress, xylem.

**INTRODUCTION**

Ornamental plants provide aesthetic pleasure, soothing effect; improve the environment and quality of life (Younis et al., 2002). Ornamental flowering species tend to have a greater demand worldwide, depending on people's preferences for a particular flowering species (Younis et al., 2010; Tariq et al., 2012). *Gazania harlequin* is an ornamental and flowering plant which is cultivated in parks and these annuals are usually used for decorative purpose. It starts blooming in early or mid and in some varieties in late summer. Environmental conditions affect the phronological characters, composition, growth and development of individual plant species (Younis et al., 2014a). Whenever the environmental features exceed from the optimum tolerance level of any plant species, it brings the plant under stress, and it adversely affects structural, developmental, biochemical and physiological processes in plants (Riaz et al., 2010).

Soil salinity and drought hindered plant production round the world (Pill et al., 1991; Tanji, 2002; Nadeem et al., 2012). A total of 880 million hectares of the global soils are salt-affected in Pakistan, about 6.7 million hectares of soils are salt-contaminated and of which 1.89 million hectares are saline, 1.85 million hectares are permeable saline-sodic, 1.39 million hectares are impermeable saline-sodic while 0.028 million hectares is acidic (Riaz et al., 2013). High salinity levels affect all phases of plant growth and development, forcing the plants to adapt a complex regulatory network to survive under stress conditions. The negative effects of stress on growth, morphology, anatomy, ultra-structure and metabolism were reported by different researchers on different plant species (Shae et al., 2008; Riaz et al., 2013). Among various abiotic stresses, NaCl induced salinity is the major factor that hampers the plant growth and development, decreases germination and ultimately reduces plant establishment and yield. Growth, yield and quality reduction under saline conditions occurred due to a decrease in the ability of plants to uptake water from the soil solution and the destruction of soil structure (Barret-Lennard, 2003).

Salts in soil can result in modifications of plant metabolic processes, culminating in stunted growth, reducing enzyme activities and
biochemical constituents (Muthukumarasamy and Panneerselvam, 1997). Salinity leads to anatomical modifications in plant body 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. (2014c). The negative effect of salinity on growth was reported by Hussein et al. (2009), Abdel-Monem et al. (2010) and Saffan (2008).

Most of the researches about salt stress were carried out in food crops, but less in ornamental plants. Therefore, it is the need of time to explore ornamental plants that are tolerant to salt stress; this will help landscape designers to select resistant plants in their designs in saline areas. Keeping in view the potential of plants to salt tolerance, the present study was carried out to assess the effect of salinity on the anatomy of Gazania harlequin L., to check the response of the plant cells and their tolerance level under salt stress conditions.

MATERIALS AND METHODS

Experimental Site and Design:

A pot experiment was conducted in the green house, University of Agriculture, Faisalabad, Pakistan, in order to investigate the effects of different salinity levels on possible anatomical changes in Gazania. The experiment was laid out in the completely randomized design (CRD) with four replications.

Experiment Material and Imposition of Salinity Stress in the Pots:

After one month of sowing the seedlings of G. harlequin were transplanted in the pots (20 cm diameter and 22 cm depth) having a mixture of silt and leaf manure in the ratio of 1:1 as a growth medium. These transplanted seedlings were kept for 20 days to get settled before applying different salinity levels (25, 50, 75, 100 mgl⁻¹ of NaCl solution). The experiment was comprised of weighing the soil that was 1500 g (growth medium) in each pot and the salinity levels were developed artificially by adding the solution of 25, 50, 75, and 100 mgl⁻¹ NaCl salt along with the control. The EC of garden soil was determined as 2.5 dSm⁻¹ and taken as control. Data were collected after 90 days of transplantation.

Anatomical Parameter:

Plants were removed from the soil, their shoots and roots were separated. Root parts with length of 3 cm were cut and washed with distilled water to remove soil dust and other contaminations. The stem were prepared according to the method as adopted by Johansen (1940). Briefly, stem were dipped into 70 percent ethanol solution for 15 days after that the sections were fixed in Canada balsam and examined under microscope and consequently microphotography was done. Double staining dehydration procedure (safranin and fast green) was used for the preparation of permanent slides (Ruzin, 1999) to study various cells and tissues of root and stem. Measurements and micrographs were made using a digital camera (Nikon FDX-35) equipped with a Nikon stereo-microscope (Nikon 104, Japan). The anatomical parameters observed in this present study were: xylem area, phloem area, cortex area, epidermis area within the stem and root with a precision of μm².

Statistical Analysis:

Statistical analysis was carried out by using Fishers’ analysis of variance (ANOVA) techniques. The mean values were compared with least significance difference test (LSD) following Steel et al. (1997).

RESULTS

It was noticed that salinity brings about adverse effects on stem anatomy of Gazania. The statistical analysis confirmed that with the increase in salinity level, significant changes were noticed in different anatomical characteristics of stem. Stem area decreased consistently, but at the same time it increased remarkably by the increment of NaCl level. Xylem and phloem area of stem showed a decreasing trend under salt stress condition (Table 1) which indicates that with the increase in salinity level there was noticeably decrease in the xylem and phloem cell growth. Xylem area was maximum under normal soil (control) and phloem area was also at the highest level under normal soil (control). The phloem area was not significantly different in control, 25 mgl⁻¹, 50 mgl⁻¹ and 75 mgl⁻¹ treatments. The lowest xylem and phloem area of 21091.56 and 30558.00 μm², respectively was found at 100 mgl⁻¹ NaCl. This indicates that salinity induces structural changes in xylem and phloem of stems. The increasing salinity reduces the stems vascular area while cell thickness increased with salinity level compared to control treatment (Fig. 1). Cortical cell length and width also gradually reduced under salinity and therefore, it decreased its cell area (Fig. 1) and the significant results are shown in Table 1.

Epidermis cell area of Gazania rapidly increased by the induction of salt in the growing medium, but in the present experiment, it gradually decreased with further increase in salt content (Table 1). Minimum stem epidermis area of 39937.27, 23911.55 and 24394.84 μm² were observed at 50 mgl⁻¹, 75 mgl⁻¹ and 100 mgl⁻¹ NaCl respectively with no significance difference, whereas control and 25 mgl⁻¹ of NaCl were noticed with maximum stem epidermis area.
### Table 1. Effect of different salinity levels on stem anatomy of *Gazania harlequin*.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Parameters</th>
<th>Stem xylem area (μm²)</th>
<th>Stem phloem area (μm²)</th>
<th>Stem cortex area (μm²)</th>
<th>Stem epidermis area (μm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>91199.2 a³</td>
<td>177751.1 a</td>
<td>369793.7 a</td>
<td>142770.0 a</td>
</tr>
<tr>
<td>25 mg l⁻¹ NaCl</td>
<td></td>
<td>88234.8 ab</td>
<td>163079.5 a</td>
<td>358203.2 ab</td>
<td>76216.5 b</td>
</tr>
<tr>
<td>50 mg l⁻¹ NaCl</td>
<td></td>
<td>42283.7 bc</td>
<td>126144.7 a</td>
<td>301063.7 ab</td>
<td>39937.3 c</td>
</tr>
<tr>
<td>75 mg l⁻¹ NaCl</td>
<td></td>
<td>30925.80c</td>
<td>112835.5a</td>
<td>210061.1 bc</td>
<td>23911.5c</td>
</tr>
<tr>
<td>100 mg l⁻¹ NaCl</td>
<td></td>
<td>21091.56c</td>
<td>30558.0b</td>
<td>82472.2c</td>
<td>23494.8c</td>
</tr>
<tr>
<td>LSD Value</td>
<td></td>
<td>47181.1</td>
<td>73783.1</td>
<td>149256.2</td>
<td>35368.9</td>
</tr>
</tbody>
</table>

³ Means with the same letter are not significantly different at \( P<0.05 \).

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**Fig. 1.** Transverse section of stem of *Gazania harlequin* plant grown (A) in the absence of NaCl, (B) in presence of 25 mg l⁻¹ NaCl, (C) in presence of 50 mg l⁻¹ NaCl, (D) in presence of 75 mg l⁻¹ NaCl and (E) in presence of 100 mg l⁻¹ NaCl.
It was also clear from the figure that by increasing the salinity levels, there was a significant decrease in the stem epidermis cell area (Fig. 1). Mean values of treatment revealed that control have higher cortex and epidermis cell area as compared to other salt treatments.

Salinity showed a subtractive effect on root anatomy of Gazania. The statistical data indicate that with the increase in salinity level, there was a significant decrease in root xylem, phloem, cortex and epidermis area (Table 2). The mean values of control have maximum root xylem phloem, cortex and epidermis area of 925428.03 μm², 347859.68 μm², 1210745.2 μm² and 536741.01 μm², respectively while the minimum xylem (227003.19 μm²), phloem (69318.95 μm²), cortex (81182.52 μm²) and epidermis area (75168.874 μm²) were observed in plants treated with 100 mgl⁻¹ NaCl. It was also clear that root xylem, phloem, cortex and epidermis area significantly decreased as compared to control (Fig. 2). Results indicate that lower salinity doses have a lower effect as compared to higher concentrations on root anatomy of Gazania.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Root xylem area (μm²)</th>
<th>Root phloem area (μm²)</th>
<th>Root cortex area (μm²)</th>
<th>Root epidermis area (μm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>925428.1a</td>
<td>347859.7a</td>
<td>1210745.2a</td>
<td>536741.0a</td>
</tr>
<tr>
<td>25 mgl⁻¹NaCl</td>
<td>751500.5ab</td>
<td>275939.9ab</td>
<td>894623.9ab</td>
<td>516734.8a</td>
</tr>
<tr>
<td>50 mgl⁻¹NaCl</td>
<td>577491.2abc</td>
<td>107448.4bc</td>
<td>570961.4bc</td>
<td>369151.2ab</td>
</tr>
<tr>
<td>75 mgl⁻¹NaCl</td>
<td>36853.3bc</td>
<td>104051.8bc</td>
<td>314562.4c</td>
<td>210343.4bc</td>
</tr>
<tr>
<td>100 mgl⁻¹NaCl</td>
<td>227003.2c</td>
<td>69319.0c</td>
<td>81182.5c</td>
<td>75168.9c</td>
</tr>
<tr>
<td>LSD value</td>
<td>468039.0</td>
<td>190187.0</td>
<td>571541.7</td>
<td>255597.5</td>
</tr>
</tbody>
</table>

¹ Means with the same letter are not significantly different at P<0.05.

DISCUSSION

In the present study, anatomical changes in root and stem of Gazania under different salinity levels were studied. The results of this study revealed that increasing the salinity level adversely affects the cell growth. Plants growing under saline conditions undergo different anatomical and cytological changes (Winter, 1988; Huang and Van Steveninck, 1990). These modifications are different in organ to organ at different levels of organization (Mills, 1989).

It is clear from above results that salinity elevation in soil has contrary effects on xylem and phloem area of the stem and root as their area tended to decrease under salt stress condition. It was due to the reduction of water uptake by the plants under high saline conditions and this reduction in water intake by the cells creates osmotic condition. Salinity affects plant growth through osmotic effects and ion toxicity (Hampson and Simpson, 1990). In ornamental plants, salinity causes reduction in cell turgor and reduces the rate of root and stem elongation (Fricke et al., 2006; Younis et al., 2014b) and this results in reduction of stem and root area. In saline conditions, reduced xylem and phloem area of stem and roots was earlier observed by Datta and Som (1973) and Reinoso et al. (2004) in Prosopis strombulifera. These results are in agreement with Nadia (1998), Soha (2006) and Baum et al. (2000) that xylem area of stem decreased due to a reduction in xylem width and length. Salinity reduced both its phloem area and sieve area at higher levels, and such findings were correlated with less tolerant species (Goncharova and Dobrenkova, 1981). Salinity caused the gradual changes in plant internal structure which reduces the xylem and the phloem area (Hameed et al., 2010). Xylem vessels also reduced under salt stress, as reported by Ling An et al. (2002). These studies are in contrary to the findings of Akram et al. (2002) and Hu et al. (2005) who reported decreased meta xylem area and cortical area, but there was no definite response of cortical thickness and its cell area of the stem.

Cortex area of root and stem was also significantly decreased under high level of salinity. Microscopic studies showed visual cell injury at 100 mgl⁻¹ of NaCl in stem while the root cells injury were observed at 50, 75 and 100 mgl⁻¹ of NaCl. The root cortex cells were injured as these cells controlled the salt transport into root. In stem cortex cells, 50 and 75 mgl⁻¹ of NaCl showed no injuries as the root cortex serves to reduce the transport to the stem. These cell injuries reduced the growth and area of cortex tissue in roots and stem. These results are supported by Casenave et al. (1999) findings, in which they observed smaller cortex in cotton seedlings at high salinity level. Walsh (1990) suggested that the thickness of...
cortical area increases up to a certain level, but under severe condition it turns to decrease. So, cortical cell area decreased at higher salinity levels, as reported by Akram et al. (2002), but increased cortical area under salt stress in the salt tolerant species may be critical under physiological drought for its better storage capacity (Baloch et al., 1998). As salt level increased, root diameter of *Gazania* decreased, as reported by De Villiers et al. (1995). In 1999, a botanist, Degano, relates succulence in root with the mechanisms of adaptation to saline conditions. Thicker meta phloem area of root was obtained under higher salinity levels which indicated its better adaptation to high salinity (Awasthi and Pathak, 1999). The less affected root area in the salt tolerant species may be for their better adaptation. These results of cortex match with the findings of Akram et al. (2002) who reported that cortical area of root decreased with increase of salinity levels.

Salinity generally reduces epidermal cell area in stem by reducing water uptake and induces osmotic condition (Akram et al., 2002). Increasing salt levels in roots caused reduction in epidermal area due to cell injuries. Halophytic or salt tolerant species generally possess thick epidermis and this

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**Fig. 2.** Transverse section of root of *Gazania harlequin* plant (A) in the absence of NaCl, (B) in presence of 25 mg/l NaCl, (C) in presence of 50 mg/l NaCl, (D) in presence of 75 mg/l NaCl and (E) in presence of 100 mg/l NaCl.
serves as an effective mechanism against water loss during limited moisture availability, but at high salinity levels thickness of epidermis decreased (Botti et al., 1998) however, in the salt tolerant species, epidermis thickness greatly increased, which showed its better adaptability because thick epidermis is a specific character of the salt tolerant species (Awasthi and Pathak, 1999). This characteristic is critical under limited moisture availability as thick epidermis is capable of checking water loss through stems (Nawaz et al., 2006, 2012a,b; Kanwal et al., 2012). Curtis and Lauchli (1987) observed that salt stress condition reduced the epidermis cell width and length due to that area of epidermal cell in the root of the plant.

CONCLUSION

In conclusion, it is clear that salt stress conditions affect all aspects of plant growth and development. Increasing levels of salts in soil has a negative impact on cell growth that it is expressed as anatomical and cytological changes. Salts in the root zone are responsible for the reduction of water uptake by the plants roots and this reduction in water intake by the cells creates osmotic condition in roots as well as in the stem. Reduced cell turgor and depressed rates caused by salt stress in root and stem resulted in the elongation of root and stem. Therefore, xylem, phloem, cortex and epidermis area of root and stem showed a decreasing trend with increasing salt stress condition. Despite from osmotic effects induced by salt stress the ion toxicity was also main obstacle in cell growth, as it was resulted in cell injuries. From the obtained results it is proved and clear that Gazania is a fairly tolerant plant species to salt stress and it can be recommended for the landscaping of moderately saline soils.

REFERENCES


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