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MORPHOLOGICAL AND CYTOGENETICAL RESPONSES TO SALT STRESS OF *GYNURA NEPALENSIS* DC. FROM DHAKA, BANGLADESH

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Abstract

Morphological and Cytogenetical responses of *Gynura nepalensis* DC. were investigated growing in saline stress environment. Plant height, leaf size, volume of interphase nuclei and changes in chromosomal morphology were studied to understand the cytogenetical and morphological responses of *Gynura* plant during salinity stress (NaCl). A significant difference between normal plants and stressed plants in respect of plant height, leaf size and volume of interphase nuclei was found. Reduction of plant growth was clearly observed in salt stressed plants rather than normal. Besides, induced salt stress affected mitotic activity in *Gynura* root meristem cells and significantly reduced the rate of mitotic cell division. Moreover, chromosomal breakage, chromosome fragmentation and sticky nature of chromosomes were observed during long time exposure to the salt stress conditions. These cytogenetical results suggest as probable reasons for morphological changes due to salt stress.

***Keywords*:** Salt stress, *Gynura* , Chromosomal abnormalities, Chromosomal breakage.

Introduction

At present, salinity is one of the egregious problems in agriculture and literally associated with economic losses. Bangladesh is thought to be in pole position as vulnerable countries of the world to climate change and sea level rise. The coastal areas and most of the southern areas of Bangladesh are very much prone to salinity. The higher salinity levels have adverse impacts on plant physiology and cytology. Ionic stress might be induced by NaCl or KCl, conducting specific ionic toxicities. However, salts also induce osmotic stress, due to the rise in salt concentration outside the cells, leading to inhibition

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of water uptake (Claeys et al., 2014). Salt stress inhibits plant growth and development by the excessive accumulation of sodium (Na^+), chloride (Cl^-), sulphate (SO_4^{2-}), bicarbonate (HCO_3^-), calcium (Ca^{2+}), magnesium (Mg^{2+}), potassium (K^+), and nitrate (NO_3^-) (Santa-Cruz *et al.*, 2002). Soil salinity decreases the water potential in plants and disturbs cellular ion homeostasis resulting in inhibition of many processes such as seed germination, vegetative growth and fruit setting (Romero-Aranda *et al.*, 2001). Successful improvement of salt tolerance has been reported for rice, rye, barley and canola (Francois, 1994; Flowers, 2004; Gopal and Iwama, 2007; Shahbaz and Ashraf, 2013), whereas in vegetables and medicinal crops the progress is slow (Keyvan and Setsuko, 2013). The responses of such crops plants to salinity may vary throughout their growth cycle and also correlated to environment, developmental stage of plant, and induced salt concentration (Flowers, 2004; Gopal and Iwama, 2007; Shahbaz and Ashraf, 2013).

The effect of salt stress on chromosome behavior is little known. Only few studies have been reported for the cytogenetic responses of cells subjected to induce salt stress effect (Kiełkowska, 2017). The genus *Gynura* Cass. belongs to Asteraceae, distributed in tropical Asia and Africa and it is currently one of the most important medicinal plants due to having its high efficient effects on antidiabetic response. This plant also used as traditional medicine for household remedy against various human ailments such as eruptive fevers, rash, kidney disease, migraines, constipation, hypertension and cancer (Perry, 1980). *Gynura* was used for possessing high medicinal and economic value and also having few large sized chromosomes (2n = 20) efficient material for cytological studies. Therefore, the aim of this research was to evaluate the response how induction of salt stress affects the cell cycle and chromosome morphology during long term exposure to salinity stressed environment in root meristematic cells of *Gynura* plant.

Materials and Methods

In this investigation, *Gynura nepalensis* DC. was used as plant material. These plant materials were collected from Shobuj Bangla Nursery, Begum Rokeya Avenue, Dhaka. Afterwards, these were maintained in the Botanical Garden, Department of Botany, University of Dhaka, Bangladesh. At the same time, three experimental pots were prepared with 5 Kg homogenous soil, having <0.14% nitrogen content, 2.90% organic matter, P^H 6.20, 15% sand, 62% silt, 31% clay and 2.60mg/m^3 particle density in each pot. Then fifteen branches of more or less similar lengths (15 ± 2 cm) were cut, and planted in three pots (five branches in each pot). After root induction, the plantlets were transferred in three experimental pots. One pot was maintained as normal, and salt treatments were carried out for other two pots maintained as control. We used different concentrations of sodium chloride (150 and 200 mM) for two different pots labeled as C-

1(for 150 mM) and C-2 (for 200 mM). The pots were irrigated every three days, alternatively with filtrated water and a (1× solution) nutrient solution, according to Etherton (1963), until they were 6 weeks old, but normal plants only received filtrated water. To avoid osmotic shock due to high concentrations, treatments were started on lower concentrations, then the concentrations were increased on a daily basis, until each group reached the targeted concentrations for the experiment. Then plants were irrigated with nutrient solution every three days (250 ml per day) with the addition of sodium chloride to the nutrient solution every two weeks. Each pot was washed with 500 ml filtrated water a week before irrigation with saline solution to prevent the continuous increase in osmotic potential resulting from the excessive accumulation of salts by the succession of irrigation procedures.

For cytogenetical study, healthy roots were collected on the 8th day from the start of treatment, and pre-treated with 8-hydroxyquinoline (0.002%) for 4 hours at 18°C followed by 15 minutes fixation in 45% acetic acid at 4°C and preserved in 70% alcohol for future use. They were then hydrolyzed in a mixture of 1N HCl and 45% acetic acid (2:1) at 65°C for 3 minutes. The root tips were stained and squashed in 1% aceto-orcein for 2 hours (Alam and Kondo, 1995). Afterwards, these slides were observed under a compound microscope (Nikon eclipse 100). For interphase nucleus volume evaluation, mean value was considered the diameter of interphase nucleus. Then, the final radius was obtained from the diameter. To calculate the nuclear volume, the following formula was employed: $4/3\pi r^3$, where 'r' is the nuclear radius used for volume calculation (Das and Mallick, 1993).

Results and Discussion

Plant growth

In the present investigation, the plant height and leaf size were studied to understand the response of *G. nepalensis* during salinity stress (Table). After 21 days exposure, C-1 and C-2 salt stressed plants height were 26.80 ± 4.52 cm and 20.96 ± 4.63 cm respectively, while normal plant's height was 55.60 ± 6.08 cm. The results showed a significance difference between normal plant and controlled plants, where plants height were reduced due to stress. The leaf size was also reduced in salt stressed plants and these were 71.27% and 57.39%, respectively. Significantly reduced plant height and leaf size indicated the reduction of plant growth due to saline stressed condition. The reduced plant growth may be for osmotic stress and disturbance of nutrient uptake which affected the plant's physiological metabolism.

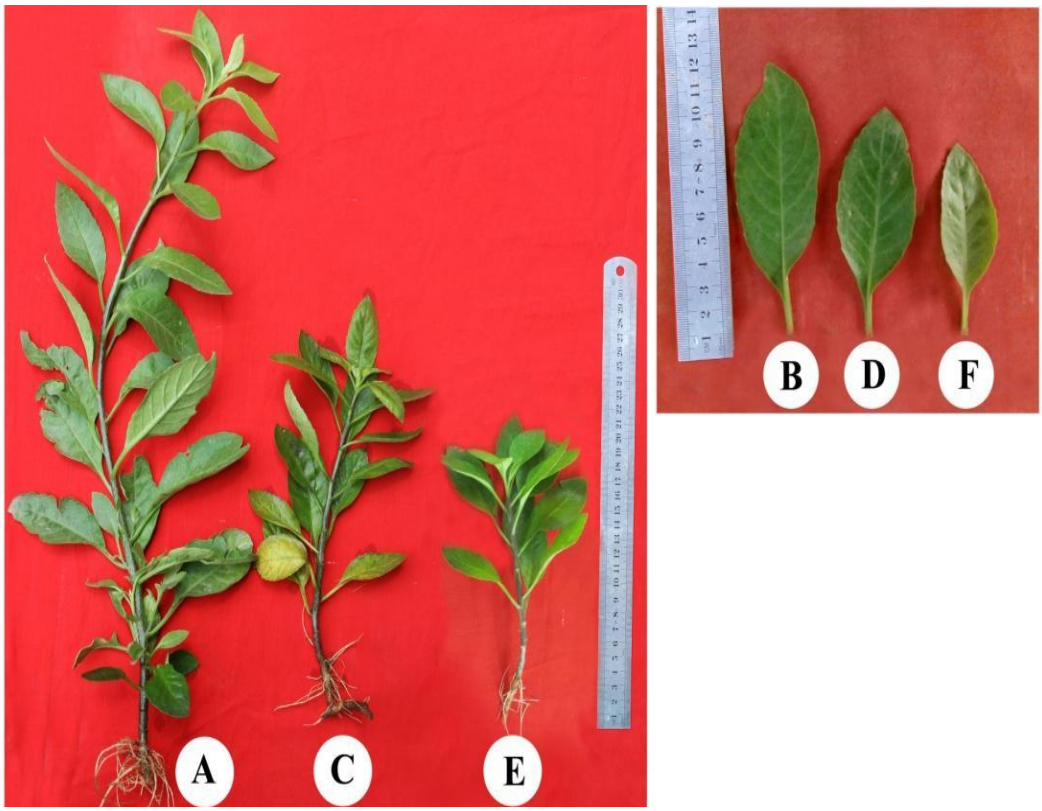

Fig. 1. Comparative growth of normal condition and salt stressed *Gynura* plants. A. normal *Gynura* plant, B. leaf of normal *Gynura* plant, C. C-1 stressed *Gynura* plant, D. leaf of C-1 stressed *Gynura* plant, E. C-2 stressed *Gynura* plant, F. leaf of C-2 stressed *Gynura* plant.

For the long time exposure to salt stress, reduced growth rate was observed of these stressed plants. This decreased plant growth might be due to the procurement of ions toxicity and less availability of capillary water. Decreased leaf area is one of the adaptive features of plant to deal with the harsh effects of salinity stress. Excessive sodium ions availability at the rhizosphere disrupts and shows strong inhibitory effect to potassium uptake in plants because of having similar chemical nature of sodium and potassium ions. We know potassium is one of the most abundant caution of plants that plays a vital role in many important cellular metabolism, membrane potential and cell turgidity (Shiwei Guo et al., 2013). Therefore, it could be suggested that plant growth might be inhibited due to excessive sodium ions availability and potassium ions deficiency.

Table. Effect of salt stress on plant height, leaf size, volume of interphase nuclei and divisional cell in *Gynura nepalensis* DC after 21 days exposure.

Treatment	Plant height (cm)	% to Normal	Leaf size (cm×cm)	% to Normal	Volume of Interphase nuclei (μm^3)	% to Normal	% of divisional cell	% to Normal
Normal	55.60 ± 6.08	100	11.50× 5.60	100	1556.1 ± 53.8	100	27	100
C-1	26.80 ± 4.52	48.20	10.20× 4.50	71.27	1374.6± 61.4	88.30	13	48.18
C-2	20.96 ± 4.63	37.69	8.80× 4.20	57.39	1088.3 ± 57.7	69.94	4	14.81

Cell division rate

Normal mitotic behavior was found in plants which were grown in normal condition. About 30% cells were in divisional state (prophase to telophase) in normal plants. On the other hand, after 21 days exposure the percentage of mitotic divisional stage (prophase to telophase) were reduced to 13% and 4% to that of normal in C-1 and C-2 salt treated plants, respectively. On the basis of above results it was clear that the rate of cell division was reduced with increasing the salt concentration. Kiełkowska (2017) also reported that salt and osmotic stress could lead to decrease in rate of mitotic activity. So, the present study supported the earlier findings. Induced stress affected mitotic activity in *Gynura* root meristem cells, and the given salt stress significantly reduced the rate of mitotic cell division in this stressed plants. In addition, most of the cells in this case, those entered into mitosis divisions were arrested in prophase stage. Above results suggested that salt or osmotic stress might have responsible for such abnormalities.

Interphasic nuclear volume

The mean volume of interphase nuclei of normal plants were approximately 1556.1 ± 53.8 μm^3 while 1374.6± 61.4 μm^3 and 1088.3 ± 57.7 μm^3 in C-1 and C-2 treated plant, respectively (Table). Salt treatment at a concentration of C-1 and C-2 had a significant effect on interphasic nuclear volume in *Gynura* root tip cells. The interphase nuclear volume of root meristem cells exposed to salt stresses for 21 days were approximately 88.30% and 69.94% to that of normal cells. So, significant decrease in nuclear volume of root meristems were

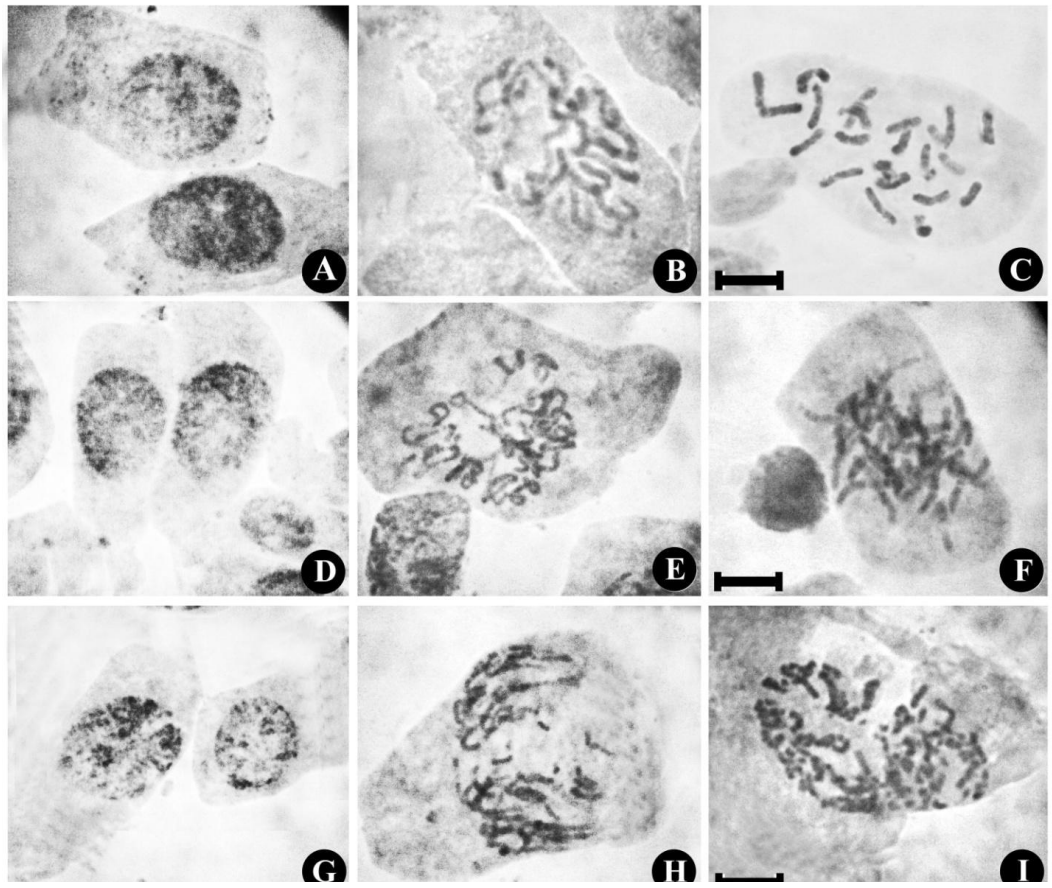

Fig. 2. Different stages of mitotic cell division of normal and salt stressed *Gynura* plants. A. Interphase nuclei of normal *Gynura* plant, B. Prophase chromosomes of normal *Gynura* plant, C. Metaphase chromosomes of normal *Gynura* plant, D. Interphase nuclei of C-1 stressed *Gynura* plant, E. Prophase chromosomes of C-1 stressed *Gynura* plant, F. Metaphase chromosomes of C-1 stressed *Gynura* plant, G. Interphase nuclei of C-2 stressed *Gynura* plant, H. Prophase chromosomes of C-2 stressed *Gynura* plant, I. Metaphase chromosomes of C-2 stressed *Gynura* plant. Bar = 10 µm (Magnification).

found with higher concentrations of salt stress exposure in compared to that of normal (Table). Yumurtaci and Uncuoglu (2009) reported the effects of salt on nuclear volume of wheat using sensitive and tolerant genotypes and they found that nuclear volume were reduced with increasing the concentration of salt stress while increased the size of nuclei under salt stress. In the present investigation, the decreasing nuclear volume under salt stress might be due to excessive hydration of nucleus (Mitchell and Van der Ploeg, 1982;

Dogan et al., 2012). Nuclear deformation and subsequent nuclear degradation might also be responsible for fragmentation of chromatin as a consequence of ion toxicity (Katsuhara and Kawasaki, 1996; Yazdani and Mahdieh, 2012).

Change in chromosome morphology

Regular and undisturbed mitosis were found in case of normal as well as controlled plants. In this study, the most frequent change in chromosome morphology was nuclear fragmentation and stickiness of chromosomes were observed in controlled plants. In the treatment of salt stress with C-1 pots, resulted in the occurrence of stickiness of chromosomes, and few chromosomes were also fragmented (Fig. 2E, 2F). Excessive chromosome fragmentation was observed in significant number of root tip cells in those plants treated with NaCl in C-2 labeled pots (Fig. 2H, 2I). Leme and Marin-Morales (2009) reported that stickiness of chromosome might have occurred due to inter-chromosomal linkages which could be coupled with excessive formation of nucleoproteins. The present investigation correlated and supported the earlier findings of Leme and Marin-Morales (2009). Thus, salt stress *i.e.* ionic toxicity responsible for sticky nature of chromosomes, chromosome fragmentation and chromosome breaks which lead the cells to death afterwards.

Conclusion

In conclusion, results obtained in this study suggested that *Gynura* root meristem cells nuclear volumes are mostly affected by changes in two different concentrations of salt stress due to ion toxicity and osmotic stress environment. And the results caused a decrease in the rate of mitotic cell division and increase the occurrence of chromosomal abnormalities in examined *Gynura* cells which are probably the main reasons for stunting growth.

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