Journal of Advanced Laboratory Research in Biology
E-ISSN: 0976-7614
Volume 11, Issue 1, January 2020
PP 1-6
https://e-journal.sospublication.co.in

Research Article

Impact of different concentrations of Sodium chloride on the Root growth, Cell division and Chromosomal abnormalities in the root tips of *Allium cepa*

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Abstract: The experiment was conducted to study the inhibition of the root growth, cell division and cytotoxic effects of NaCl on onion bulbs (*Allium cepa*). The onion bulbs were treated with different concentrations of NaCl (0.06, 0.12, 0.24 and 0.48 molarity) for 72 hours in a glass beaker. The results, based on the different concentrations and exposure time showed that the mitotic index and the average onion root growth rate decreased significantly compared with the control. Treatment with 0.48 molarity NaCl concentrations has a less percentage of root growth (12.9%) whereas treatment with distilled water (control) has maximum root growth (40.63%). The mitotic index of control was (12%) while onion treated with NaCl was decreased to (9.11%) in 0.48 molarity. It was found that the chromosomal aberrations increased as the concentration of the NaCl increased when compared to control. The recorded chromosomal abnormalities were micronuclei, budding nuclei, unequal-sized nuclei, c-mitosis, anaphase bridge, and chromosome stickiness. The results showed that the higher concentrations of NaCl have more impact on the root growth, cell division and chromosomal abnormalities in the root tips of *Allium cepa*.

Keywords: *Allium cepa,* Root growth, Mitotic index, Chromosomal abnormalities, NaCl.

1. Introduction

The genus *Allium* is one of the world's biggest flora. As per The Plant List (2020) there are 887 species of *Allium* under family Amaryllidaceae found in the world including both wild and cultivated plants. *Allium* species are very important herbaceous plant, it is used worldwide as spices and as vegetables. In early classifications of angiosperms, *Allium* and related genera have been placed in the Liliaceae family. In the more recent and competent taxonomic treatment of the Monocotyledons, they are recognized as the distinct family Alliaceae close to the Amaryllidaceae (Dahlgren *et al*., 1985).

Low water availability, high and low temperatures and air pollution are serious threats to agriculture and directly affect growth and yield in both arid and semi-arid areas. In a similar way, salinity affects many physiological characteristics, such as growth rate, water relations, photosynthesis, ion homeostasis, senescence, and yield elements (Negrão *et al.*, 2017). Salinity significantly inhibits plant productivity of several horticultural crops. Sodium chloride salts cause most of the salt stresses in nature. When plants experience high salinity, they develop various coping mechanisms that allow them to tolerate, avoid, or escape the stressor. Those responses were examined at the morphological, anatomical, physiological and cellular stages (Teerarak *et al.*, 2009).

Classical cytogenetic techniques are usually used to detect changes in chromosomal morphology. In 1928 Stadler describes the impact of physical and chemical agents on chromosomes (Maluszynska & Juchimiuk, 2005). The effect of colchicine on root mitosis in *Allium cepa* was first described by Levan (1938). The nomenclature used for chromosomes morphology is suggested by Levan *et al*., (1964). The *Allium* test (Levan, 1938, 1949) is based on a chromosome analysis of the meristem cells of the *Allium cepa* apical root cells to determine the effect of genotoxic substances. In addition, the *Allium cepa* system provides important information for evaluating the clastogenic and/or aneugenic impact of an agent on the genetic material (Leme & Marin-Morales, 2009).

Onion (*Allium cepa* L.) root meristem cells are highly sensitive to genetic damage by chemicals, and the *Allium* test that involves the length of the root and chromosome aberration (CA) measurements, proven to be an effective model system for measuring the environmental cytogenetic potential of pollutants. The *Allium* test is an excellent indicator for analyzing

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Received on: 1 November 2019
Accepted on: 11 December 2019

antiproliferative effects on plant medicinal extracts (Firbas & Amon, 2014). Although favourable cytological features make genus *Allium* species attractive subjects for research, only about one-third of chromosome numbers are identified and detailed cytological information is very limited (Ramesh, 2015). For decades, the *Allium* chromosomes have been studied for their diversity in size, structure and number (Awe & Akpan, 2017).

The diploid chromosome number of *Allium cepa* is well established i.e., 2n = 2x = 16 (Okumuş and Hassan, 2000; Kim *et al*., 2002; Mukherjee & Roy, 2012).

2. Material and Methods

2.1 Onion preparation

Small onion (*Allium cepa*) bulbs of the similar uniform size, weighing about 10-13g, were denuded and scraped by removing the loose outer scales so that the root primordia are immersed in the specific distilled water (control) and NaCl concentrations (0.06, 0.12, 0.24 and 0.48 molarity).

2.2 Experimental procedure

In each experiment the exposure time of the onion bulbs was 72 hours at 22°C; they have been protected from direct sunlight. In order to minimize the effect of the daytime rhythms, the plants have been exposed to artificial medium intensity light. In another version of the experiment, the 5 onion bulbs had been placed directly into the experimental NaCl solution of different concentrations. The NaCl sample being studied has been divided into three parts which were applied successively to the roots of the onions for 24 hour period. So the roots got a fresh bath of the NaCl solution sample every 24 hours. The experiment was done after 72 hours and analysis of macroscopic and microscopic morphology of the tissue followed (Firbas & Amon, 2014).

2.3 Macroscopic parameters

Macroscopic parameters include the length of the root and other parameters, like as the normal root shape, number, color and turgidity after 72 hours.

2.4 Root harvest and slide preparation

Root tips from the germinated seeds were cut 1-2cm long and placed in a small specimen glass bottle and fixed in acetic alcohol at 4-6°C for 24 hours. The root tips were washed twice for 10 minutes each with ice-cold water and allowed to dry in a watch glass. In the watch glass 1 N HCl solution preheated to 60°C was added to the root tips for 5 minutes and the HCl was discarded. Repeat the HCl treatment a second time. Two root tips have been moved individually to clean microscope slides and from the growing tip cut 2mm. The tips have been kept and the remaining solution was discarded. 1% methylene blue stain was applied to each slide to cover the root tip for approximately 3 minutes. A glass coverslip was placed on the root tip and gently tapped with a pencil eraser to disperse the cells uniformly to form a monolayer to make the scoring process easier for normal and aberrant cells at various phases of the cell cycle (Vellaikkannu *et al*., (2017).

2.5 Microscopic parameters

The cytogenetic analysis consisted of the mitotic index, scoring of aberrant cells and the proportion of mitotic phases. Mitotic index was calculated as the percentage ratio of dividing cells and the total number of scored cells. In the dividing cells of root tips, the proportion of mitotic phases was scored. The percentage of each type of aberrant cells, such as budding nuclei, micronuclei, c-mitosis, unequal-sized nuclei, chromosome stickiness, and anaphase bridge was calculated in accordance with the methods previously described (Gabara *et al*., 2006; Glińska *et al*., 2007).

The slides were seen under the light microscope (Olympus) using the 100X objective lens with oil immersion. On one slide for each treatment, a total of 450 cells, classified into interphase or dividing cells (prophase, metaphase, anaphase and telophase) were scored. The mitotic index (MI) was expressed as the number of dividing cells per 100 cells scored.
Mitotic index (MI) is calculated by the following formulae:

$$\text{Mitotic index (\%)} = \frac{\text{Total Number of dividing cell}}{\text{Total number of cell examined}} \text{X } 100 \quad (1)$$

2.6 Statistical analysis

A one-way analysis of variance (ANOVA) with the Tukey test was used to analyze the relationship between different NaCl concentrations and chromosomal abnormalities. $p < 0.05$ is considered statistically significant.

Total chromosomal abnormality calculated by the following formulae:

$$\text{Total abnormality (\%)} = \frac{\text{Total number of aberrant cells}}{\text{Total number of cells in division}} \text{X } 100 \quad (2)$$

3. Results and Discussion

In this experiment, roots of *Allium cepa* are treated with different NaCl concentrations (0.06, 0.12, 0.24 and 0.48 molarity), distilled water as control and for selected time periods (24, 48 and 72 hours). The growth rate is different for each treated concentration and control. The growth rate of root tips of onion treated with distilled water (control) is 40.63% more than root tips of onion treated with NaCl concentrations (0.06, 0.12, 0.24 and 0.48 molarity) after 72 hours. The growth rate of root tips of onion treated with NaCl concentration of 0.06, 0.12, 0.24 and 0.48 molarity are

35.86%, 21.05%, 15.15% and 12.9% respectively. Five replications were performed for each concentration and control to calculate mean root length with standard error of the mean (SEM) at selected time periods (Table 1).

Salinity affects plants in various ways, including osmotic effects, specific-ion toxicity and/or nutritional disorders (Läuchli & Epstein, 1990). The degree to which one mechanism affects the plant over the other depends on a number of factors, including the species, genotype, plant age, ionic strength and composition of the salinizing solution and the organ concerned (Munns, 2002). *Allium cepa* was used as the experimental material in this study because chromosomal aberration observed in plants resembles the aberration produced in mammalian cells. A positive correlation existed between the aberrations induced by omnacortil in plant root tip cells and in cultured mammalian cells. This suggests that the root tip system of the plant can be recognized as an effective first-tier assay system for this study type (Alege & Ojomah, 2014).

Slide overview of each meristematic root tip for control and concentrated group (0.06, 0.12, 0.24 and 0.48 molarity) at 40X magnification as shown in Fig. 1. The effect of different NaCl concentrations on the mitotic index of the examined root tips and the total cells examined (450 cells), classified into interphase and dividing cells (prophase, metaphase, anaphase, and telophase) was shown in Table 2 and Fig. 2.

The percentage of mitotic index in the control group (12%) is higher than other treated groups (0.06, 0.12, 0.24 and 0.48 molarity of NaCl). Lower mitotic index (9.11%) is observed in the treatment with 0.48 molarity of NaCl. The mitotic index of other treated concentrations is significantly different in comparison with control. The percentage of mitotic index in 0.06, 0.12 and 0.24 molarity NaCl concentration is 11.55%, 11.33% and 10.88% respectively. Treatment with control and different NaCl concentrations, there is a significant difference between interphase and dividing cells (prophase, metaphase, anaphase, and telophase). Interphase has the highest number (409 cells) in the 0.48 molarity, while the control has the smallest number (396 cells). For other groups, the number of interphase cells was almost similar (0.06 molarity has 398 cells, 0.12 molarity has 399 cells and 0.24 molarity has 401 cells) Table 2.

Among dividing cells prophase has a higher number from metaphase, anaphase and telophase. Individually 0.24 molarity NaCl concentration has the highest number of prophase (33 cells) and 0.06 molarity NaCl concentration has the smallest number of prophase (24 cells). The other three dividing cells (metaphase, anaphase, and telophase) also differ between treated groups, 0.12 and 0.24 molarity NaCl concentration has the same and the smallest number of metaphase (4 cells only) while 0.06 molarity NaCl concentration has the highest number of metaphase cells (16) followed by control group (14 cells). The lowest number of anaphase seen in 0.48 molarity NaCl concentration (2 cells) followed by 0.06 molarity (6 cells), 0.24 molarity NaCl concentration and control group has the same number (8 cells). The largest number of anaphase has seen in 0.12 molarity NaCl concentration (9 cells). In telophase 0.48 molarity NaCl concentration has the lowest number (3 cells), 0.24 molarity NaCl concentration (4 cells) followed by the control group (5 cells), 0.06 molarity NaCl concentration has (6 cells) but the highest number of telophase observed in 0.12 molarity NaCl concentration (7 cells).

Cell division is one of the most important phenomena. Cell division controls organisms growth and chromosomes behavior is one of the unique characteristics. The treatment of *Allium cepa* root tips with different concentrations of NaCl induced six major types of chromosomal abnormalities, including micronuclei, budding nuclei, c-mitosis, unequal-sized nuclei, anaphase bridge, and chromosome stickiness.

The inhibitory effects of NaCl have previously been recorded on the root growth of *Chrysanthemum morifolium* Ramat, four vegetables (*Beta vulgaris*, *Brassica oleracea* var. *capitata* L., *Amaranthus paniculatus* and *Brassica campestris*) and red raspberry (Hossain *et al.*, 2004; Jamil *et al.*, 2006; Neocleous & Vasilakakis, 2007). Karyotype analysis has been carried out extensively in plant phylogenetic and diversity studies for over 100 years (Ramesh, 2015). Soil salinity is a major abiotic stress in agricultural plants around the world. Information on the genetic diversity of crops is important for successful breeding program development (Barakat, 2003).

In anaphase–telophase cells, the bridge, stickiness, vagrant chromosomes, fragments, c-anaphase and multipolarity chromosome aberrations have been observed (Yildiz *et al.*, 2009). Different cytological abnormalities were scored, such as break, gap, exchange, multiple breaks, and chromosome fragments (Palanikumar *et al.*, 2011). The stick chromosomes induced abnormal uncoiling of chromosomes during anaphase to telophase (Qian *et al.*, 2006).

Table 3 and Fig. 3 showed that various chromosomal abnormalities induced by different NaCl concentrations in meristematic cells of *Allium cepa* root tips. In the control group (distill water) total chromosomal abnormalities are 0 (0%). The total chromosomal abnormalities in the 0.48 molarity NaCl concentration were 21 (51.2%). Micronuclei and budding nuclei have an approximate half-percentage 9 (21.9%), whereas c-mitosis and chromosome stickiness have the same proportion 3 (7.3%). Unequal-sized nuclei aberrations are 5 (12.1%). Anaphase bridge has the lowest percentage 1 (2.04%). On the other hand, 0.06 molarity NaCl concentration has only 1 (1.9%) of c-mitosis and chromosome stickiness. But the remaining aberrations (micronuclei and budding nuclei,

unequal-sized nuclei and anaphase bridge) have not occurred. Total abnormalities for 0.06 molarity NaCl concentration were 2 (3.8%). In 0.12 molarity NaCl concentration micronuclei and budding nuclei, unequal-sized nuclei and c-mitosis have the lowest percentage 1 (1.9%), followed by anaphase bridge 2 (3.9%). Chromosome stickiness has the highest percentage 3 (5.8%). Total abnormalities for 0.12 molarity NaCl concentration were 8 (15.6%). In 0.24 molarity NaCl concentration unequal-sized nuclei and anaphase bridge has similar percentage 1 (2.04%). Likewise, c-mitosis and chromosome stickiness have the same number 2 (4.08%). Micronuclei and budding nuclei have the highest percentage 4 (8.1%). Total abnormalities for 0.24 molarity NaCl concentration were 11 (22.4%).

Table 1: Inhibitory effect of different concentrations of NaCl on *Allium cepa* root growth.

Concentration	Mean root length (±SEM) at time (hour)			Change rate (100%) at 72 hr.
	24	48	72	
0 (control)	1.28±0.08	1.42±0.1	1.8±0.8	40.63%
0.06	0.92±0.03	1.05±0.5	1.25±0.5	35.86%
0.12	0.95±0.05	0.96±0.01	1.15±0.05	21.05%
0.24	0.825±0.04	0.85±0.07	0.95±0.05	15.15%
0.48	0.62±0.03	0.5±0.06	0.7±0.04	12.9%

Table 2: Effect of different concentrations of NaCl on the mitotic index of the examined root tip cells of *Allium cepa*.

Concentration	Total cells examined	Interphase	Prophase	Metaphase	Anaphase	Telophase	Mitotic Index (%)
0 (control)	450	396	27	14	8	5	12%
0.06	450	398	24	16	6	6	11.55%
0.12	450	399	31	4	9	7	11.33%
0.24	450	401	33	4	8	4	10.88%
0.48	450	409	29	7	2	3	9.11%

Table 3: Chromosome abnormalities induced by NaCl in *Allium cepa*.

NaCl concentrations	Micronuclei and Budding Nuclei (%)	Unequal-sized nuclei (%)	c-Mitosis (%)	Anaphase bridge (%)	Chromosome stickiness (%)	Total abnormal
0 (control)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
0.06	0 (0%)	0 (0%)	1 (1.9%)	0 (0%)	1 (1.9%)	2 (3.8%)
0.12	1 (1.9%)	1 (1.9%)	1 (1.9%)	2 (3.9%)	3 (5.8%)	8 (15.6%)
0.24	4 (8.1%)	1 (2.04%)	2 (4.08%)	1 (2.04%)	2 (4.08%)	11 (22.4%)
0.48	9 (21.9%)	5 (12.1%)	3 (7.3%)	1 (2.4%)	3 (7.3%)	21 (51.2%)

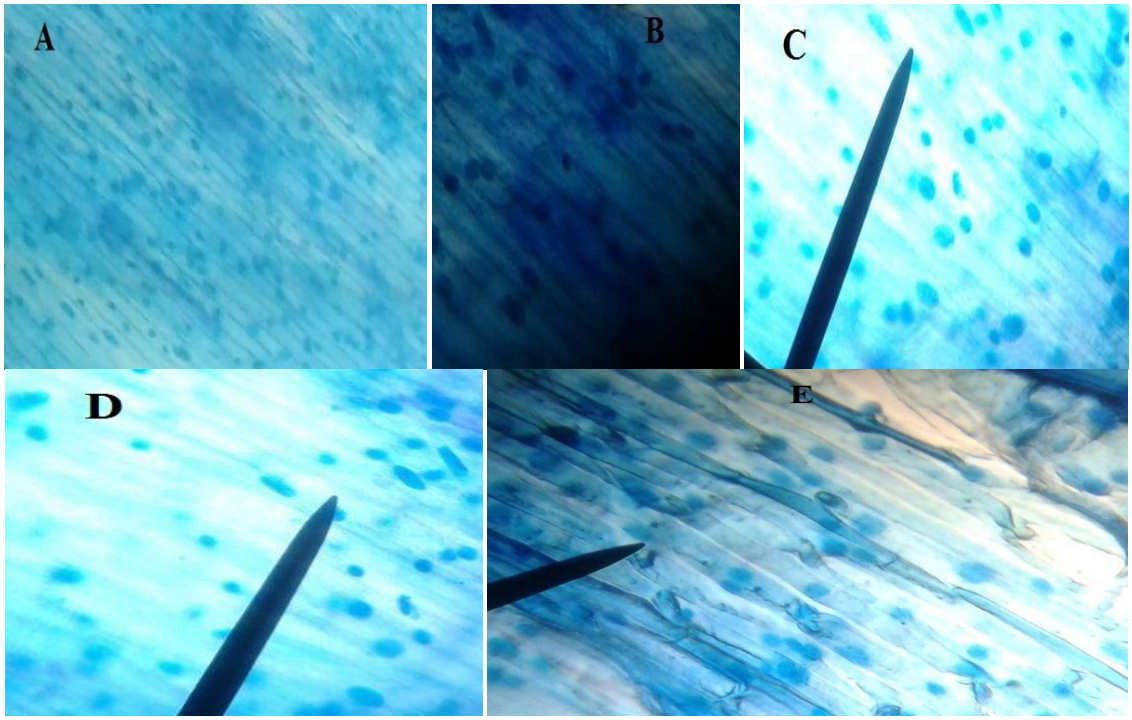

Fig. 1: Meristematic cells of root tips of *Allium cepa* in different state observed at magnification 40X. (A) - Control (B) - 0.06 molarity of NaCl (C) - 0.12 molarity of NaCl (D) - 0.24 molarity of NaCl (E) - 0.48 molarity of NaCl.

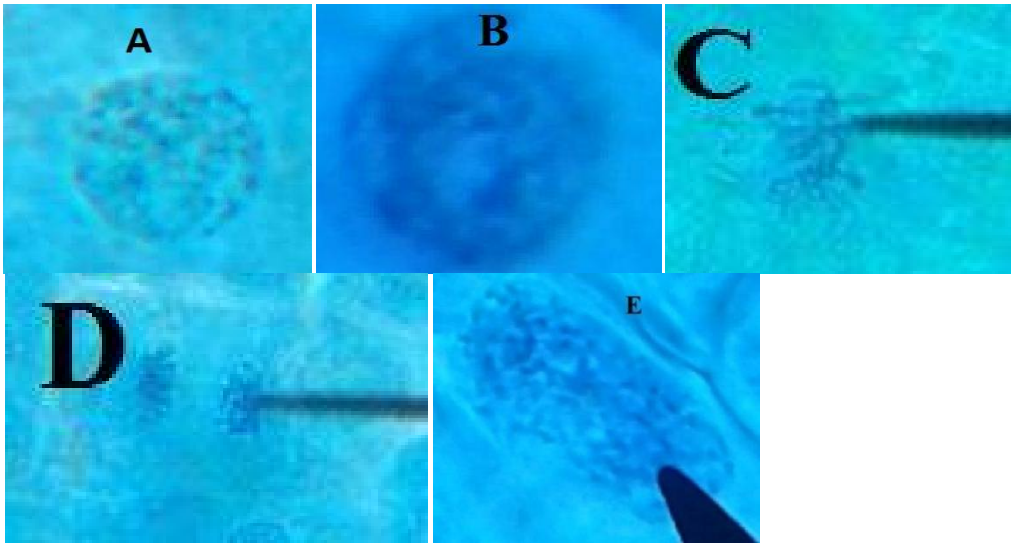

Fig. 2: Meristematic cells of root tips of *Allium cepa* in different stages of cell cycle observed at magnification 100X. (A) - Interphase (B) - Prophase (C) - Metaphase (D) - Anaphase (E) - Telophase.

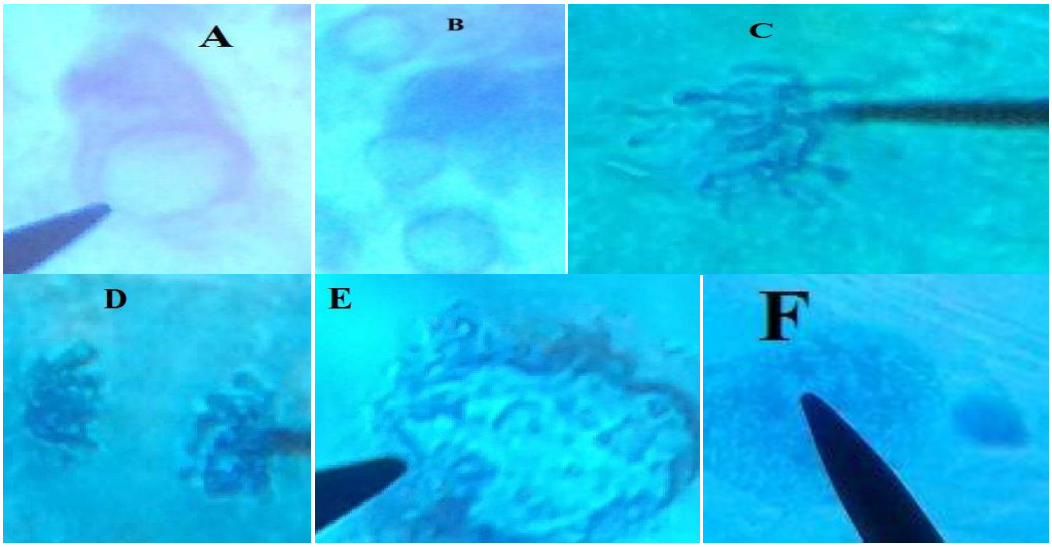

Fig. 3: Different types of chromosomal abnormalities induced by NaCl. (A) - Budding Nuclei (B) - Unequal-sized nuclei (C) - c-mitosis (D) - Anaphase bridge (E) - Chromosome stickiness (F) - Micronucleus.

The *p*-value was 0.002932 and confirmed that the different concentrations of NaCl have an effect on the chromosomal abnormalities and the results were statistically significant at *p*-value < 0.05. The *p*-value for mean root growth was 0.000682, indicated that the results were statistically significant. While for mitotic index *p*-value was 1, which means the results were statistically non-significant.

4. Conclusion

The results obtained from root growth (macroscopic) and cytological analysis (microscopic) of *Allium cepa* suggested that different concentrations of NaCl showed an inhibitory effect on root growth, decrease cell division and promote chromosomal abnormalities.

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