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The impact of sodium chloride on root growth, cell division, and interphase silver-stained nucleolar organizer regions (AgNORs) in root tip cells of *Allium cepa* L.

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ABSTRACT

To evaluate genetic damage, parameters including root growth, chromosomal aberrations, and interphase silver-stained nucleolar organizer regions (AgNORs) in root tip cells of *Allium cepa* L. were investigated after treatment with NaCl (40–160 mM). The results showed that NaCl caused a decrease in root growth. All concentrations of NaCl showed an inhibitory effect on dividing cells in root tips of *A. cepa* L. and caused a reduction in mitotic index values. Upon exposure to NaCl, roots exhibited various mitotic abnormalities, including c-mitosis, anaphase bridge, and chromosome stickiness. In addition, interphase cells with micronuclei, budding nuclei, and unequal-sized nuclei were observed. Moreover, total cell aberration increased with increasing NaCl concentration. For AgNOR parameters, the average number of AgNORs per nucleus decreased in roots treated at all NaCl concentrations. The singular AgNOR area and whole AgNOR area in the nucleus containing 1–3 AgNORs were inversely proportional to NaCl concentrations.

1. Introduction

Environmental stresses include high metal concentration in soil, the presence of air pollutants, high or low temperature, water deficits, and high soil salinity. Salinity is a significant stressor that reduces plant productivity of several horticultural crops. Most of the salt stresses in nature arise from sodium chloride salts (Levitt, 1972). When plants experience salinity stress, they develop different coping mechanisms that allow them to tolerate, avoid, or escape the stressor. These responses have been investigated at the morphological, anatomical, physiological and cellular levels. Furthermore, environmental stresses also impact cell cycle regulation by altering the endoreduplication process and changing cell proliferation (Granier et al., 2007). Cytological approaches are used for analyzing the changes in the proportion of mitotic phases and rate of cell division in response to environmental conditions. Cytological parameters are used to identify the effect of salt stresses on cell division in root tips to evaluate these effects.

Nucleolar organizer regions (NORs) contain tandem repeat genes called rDNA transcribed into ribosomal RNAs necessary for all cellular protein synthesis (Sumner, 2003). NOR argyphilia is due to a set of nucleolar proteins which are selectively stained by silver methods leading to silver-stained nucleolar organizer regions (AgNORs). After silver staining, NORs can be visualized as black (Ploton et al., 1986). During interphase, NORs are located in the regions of the nucleolus known as the fibrillar centre, and these areas retain nucleolin and protein B23, neither of which is directly involved in the transcription of ribosomal genes (Sumner, 2003). Silver-staining of NORs is a rapid method for visualization of ribosomal gene activities to evaluate protein synthesis rate. Previous investigations have shown that the amount of AgNOR protein is a good marker of cell proliferation activity (Trerè et al., 1989; Derenzini et al., 1990, 1994; Pession et al., 1991). Therefore, each silver-stained dot corresponds to ribosomal gene activity and the amount of silver-stained proteins. Research with important applications includes the application of interphase AgNOR measurement to assess cancer prognosis. Research shows that the interphase AgNOR value represents a valuable parameter for information about the progression of tumor disease in the host and on patient survival (Derenzini, 2000). While the toxicity of NaCl on the inhibition of plant growth has been reported extensively, detailed knowledge about salt stress responses at the cellular level, especially for cell division and nucleolus, is limited. To evaluate these effects, it is of interest to study chromosome behaviors and nucleolus changes and utilization of the *Allium* cytogenetic assay. Therefore, the aim of this work was to increase understanding about the effects of different concentrations of NaCl on root growth, the mitotic index, mitotic phases, mitotic abnormalities, AgNOR number, and AgNOR size by using root meristem cells of *A. cepa* L. The results from this research will enhance understanding of cytological damage mechanisms.

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2. Materials and methods

For this study, 0, 40, 80, 120, and 160 mM NaCl concentrations were used. Healthy onion bulbs of *A. cepa* L. were purchased from a local market. The roots were grown in tap water under laboratory conditions. When the radicle roots had protruded beyond the basal plate by at least 2 cm, the roots were then treated with different concentrations of NaCl for 48 h. Only healthy and newly emerged roots were selected for uniformity in size and shape and use for the test solution.

For cytogenetic analysis, the *A. cepa* L. root tips were cut and subsequently fixed in a freshly prepared mixture of absolute ethanol and acetic acid (3:1, v/v). To remove the fixative solution, the fixed root tips were washed in distilled water three times and macerated in an enzyme mixture containing 8% cellulose (Fluka), 6% pectinase (Fluka), and 1 mM EDTA (pH 4.2 adjusted with HCl) at 37 °C for 50 min. To determine the mitotic index, mitotic phases and the presence of chromosomal aberrations, chromosomes squashed in absolute ethanol and acetic acid were stained with 2% Giemsa solution (Merck, Co., Ltd.) for 10 min, rinsed with distilled water and dried. Some root tips squashed in absolute ethanol and acetic acid were stained with 50% silver nitrate and incubated in a moist chamber at 60 °C for 2 h to study AgNOR parameters. Finally, the slides were rinsed thoroughly in distilled water and dried.

Cytogenetic analysis consisted of the mitotic index, the proportion of mitotic phases, and scoring of aberrant cells. Mitotic index was calculated as the percent ratio of dividing cells and total number of scored cells. The proportion of mitotic phases was scored in the dividing cells of root tips. The percentage of each type of aberrant cell, such as budding nuclei, micronuclei, unequal-sized nuclei, c-mitosis, chromosome stickiness, and anaphase bridge was calculated according to previously described methods (Gabara et al., 2006; Glińska et al., 2007). Five replications were performed for each treatment and scoring was done from the three root tips from each replication. For AgNOR parameters, the number of AgNORs was estimated on 1000 random nuclei per treatment. The area of AgNORs was measured in 300 cells per treatment using a micrometer eyepiece and subsequently calculated with the formula $A\ (\mu m^2) = \pi r^2$. The statistical significance of the differences among values of root length, mitotic index, mitotic phases, total abnormalities, and AgNOR parameters in the treated samples and the control was evaluated by means of the Duncan's multiple range test at $P < 0.05$ level.

3. Results

3.1. Root growth

Onion bulbs were grown in tap water under laboratory conditions for two days. After radicle roots reaching a length of 2 cm, the roots were treated with different NaCl concentrations. Fig. 1 shows the effects of different concentrations of NaCl on root length. Control onion bulbs which had root length of 3.25 cm were grown in tap water and they exhibited better root growth, whereas treatment with 160 mM NaCl reduced the control root length to 2.43 cm. A significant difference in root length between the treatment with NaCl concentrations from 40 to 160 mM and the control plants was evident (Fig. 1). At higher concentrations of 120 and 160 mM NaCl after two days of salt application, the cap roots turned slightly brownish in color.

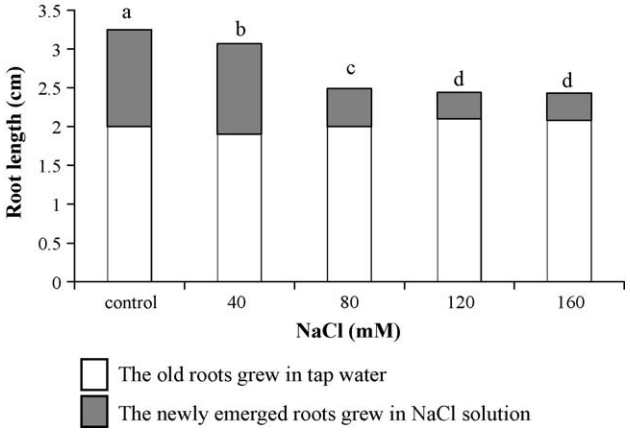

Fig. 1. The effect of NaCl on root growth. Different letters indicate a significant difference by Duncan's multiple range test at $P < 0.05$ level.

3.2. Mitotic index and frequency of mitotic phase

Table 1 shows the differences in mitotic index values of root tip cells of control and treated onion roots. The results of analysis of the mitotic index in the roots treated with NaCl for 48 h revealed that all NaCl concentrations could significantly inhibit cell division when compared to the control. The mitotic index being equal to the number of cells in mitotic phases decreased with increasing doses of treatment. There were 3–9% of the remaining cells in NaCl treated-plants entering mitotic phases. Additionally, the mitotic index values had the same trend in the rate of root growth. The percentage of dividing cells in four phases (prophase, metaphase, anaphase, and telophase) is shown in Table 1. The percentage values of particular mitotic phases of control in the experiment were 45.98 ± 5.32% for prophase, 29.97 ± 7.22% for metaphase, and 24.05 ± 2.06% for anaphase–telophase. There was no significant effect on the frequencies of mitotic phases between NaCl-treated and control plants.

3.3. Chromosome abnormalities

The NaCl effects evaluated by the *Allium* cytogenetic assays are shown in Table 2 and their photographs are presented in Fig. 2. In *A. cepa* L., the highest percentage of total abnormalities was 40.84 ± 2.36 and this was recorded in roots treated with 160 mM NaCl. The percentages of total abnormalities increased as the concentrations of applied NaCl increased. There were statistical differences between control and treated roots of *A. cepa* L. in total

Table 1
The mitotic index and frequency of mitotic phase in *Allium* root tips exposed to NaCl solution for 48 h.

NaCl concentrations (mM)	No. of counted cells	No. of dividing cells	Mitotic index	Mitotic phase (%)		
				Prophase	Metaphase	Anaphase–telophase
Control	2075	595	28.62 ± 3.94a	45.98 ± 5.32a	29.97 ± 7.22a	24.05 ± 2.06a
40	2003	166	9.22 ± 2.37b	48.89 ± 8.11a	24.19 ± 4.54a	26.92 ± 1.56a
80	2015	144	7.15 ± 1.55b	50.33 ± 6.43a	20.45 ± 6.23a	29.22 ± 6.15a
120	2002	71	3.53 ± 1.51b	55.09 ± 9.32a	19.64 ± 5.10a	25.27 ± 5.53a
160	2021	58	2.93 ± 1.81b	56.19 ± 6.73a	23.70 ± 4.29a	20.11 ± 4.71a

Mean within a column for each NaCl concentration followed by different letters (a, b) are significantly different according to Duncan's multiple range test at $P < 0.05$ level.