CYTOLOGICAL ANALYSIS OF ROOT TIPS AS A TOOL TO EVALUATE ABIOTIC STRESSES: AN APPLICATION WITH NaCl AND H_2O_2 TREATMENTS IN *Allium cepa* L.

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ABSTRACT: Usually, little attention is given to the consequences of abiotic stresses on cell division, cytological damage, and possible mutagenic and genotoxic effects. In this study, we tested a cytological approach to assess the occurrence of chromosomal abnormalities and possible disturbances in the mitotic cycle after salt stress conditions and exposure to H_2O_2. Bulbs of *Allium cepa* L. were treated with 75 mM NaCl, 150 mM NaCl or 80 µM H_2O_2 treatments and root tips were collected after 24 h. Histological slides were prepared and the occurrence of each mitotic phase and chromosomal abnormalities were analysed in an optic microscope. Our results showed lower mitosis rate and higher relative chromosomal abnormalities values after NaCl stress in comparison with the control. Root tips treated with 80 µM H_2O_2 displayed the highest mitotic index among all treatments and a relative chromosomal abnormalities value lower than the 75 mM NaCl treatment. In conclusion, our data reinforce the knowledge about the cytotoxic effects of NaCl in roots and highlight positive effects of low concentrations of H_2O_2 on root cell division. We estimate that cytological analysis of root tips is a simple, inexpensive, and efficient method to assess levels of different abiotic stresses in roots.

KEYWORDS: *Allium cepa* test; cytotoxicity; chromosomal abnormality; genotoxicity; DNA damage

INTRODUCTION: In addition to the low water availability and high temperatures, soil salinization is a major threat to agriculture in arid and semi-arid regions (Munns and Gilliham 2015). Recent data estimate that 45 million ha of the current 230 million ha of irrigated land, which correspond to 19.5%, have been already damaged by salt (FAO 2016), including the Brazilian semi-arid region. Salinity, similarly to other stressful conditions, impacts on different traits, such as relative growth rate, water relations, photosynthesis, transpiration, ionic relations, senescence, and yield components (Negrão et al. 2017). These traits are usually evaluated by using a broad variety of techniques related to plant morphology, anatomy, biochemistry, physiology, and molecular biology. Among these approaches, little attention is given to the consequences of abiotic stresses on cell division, cytological damage, and possible mutagenic and genotoxic effects. In this context, cytological analyses of cell division in root tips of *Allium cepa* L. (onion) have been demonstrated as a valuable method to evaluate these effects (Souguir et al 2011). The *Allium cepa* test has been used to evaluate DNA damages, such as chromosome aberrations and disturbances in the mitotic cycle since the 40s, mainly to assess mutagenesis and genotoxicity levels of a great number of chemical agents (Leme et al 2009). In plant physiology, cytological analyses of root meristem have been used, for example, to evaluate the occurrence of chromosome abnormalities induced by NaCl treatments (Chatterjee and Majumder 2010; Kiełkowska 2017; Mohammed and Ibrahim

2017) and effects of phytohormones and other signalling molecules on cell and genetic activity of different plants (Rza 2009). However, this simple, inexpensive, and efficient method has been neglected as a plant stress indicator. For example, the application of this test in studies of plants under stressful conditions like cold, heat, drought, heavy metals exposure, among others; and in studies about signalling molecules like H_2O_2 and phytohormones are scarce or inexistent. Therefore, here we used the *Allium cepa* test to assess the occurrence of chromosomal abnormalities and possible disturbances in the mitotic cycle after salt stress conditions and exposure to H_2O_2. We suggest this methodology as a good tool to evaluate abiotic stresses in plants.

METHODOLOGY: Bulbs of *Allium cepa* L. were kept in contact with water to allow initial root growth. After 48 h, roots were submerged in Hoagland and Arnon's solution as control, or the same solution containing 75 mM NaCl, 150 mM NaCl or 80 µM H_2O_2. The H_2O_2 concentration was chosen based on a result showing that 80 µM H_2O_2 was the most effective level in mitigating salinity effect for wheat seedlings (Wahid et al. 2007). Each treatment was composed of three biological replicates. The analysed apical meristem was obtained by harvesting root tips after 24 h in their respective treatments and fixation with Carnoy's solution composed of ethanol and acetic acid (3:1, v/v). The fixed roots were washed with distilled water and heated in HCl at 60 °C. After 10 min, the root tips were again washed with water and incubated in Schiff's reagent for 30 min in the dark. The root caps were put on slides with a drop of 45% acetic acid, pressed against cover slips and sealed with nail varnish. The sealed slides were stored at -20 °C until cell counting. Slides were examined at 100× magnification and 2000 cells were counted in each slide. The occurrence of each mitotic phase (prophases, metaphases, anaphases and telophases), chromosomal abnormalities and micronucleus were observed (Figure 1). The mitotic index (MI) was calculated according to the equation: *MI (%) = Total number of dividing cell/Total number of cells examined × 100*. Three slides were prepared for each treatment. Data were analysed by the Student's t-test ($p < 0.05$).

RESULTS AND DISCUSSION: In this experiment, roots of *Allium cepa* L. treated with 75 and 150 mM NaCl showed growth rate lower than the control group and those treated with H_2O_2 (data not shown). This is in accordance with the mitotic index values, which were 96% lower in roots treated with 150 mM NaCl and 60% lower in those treated with 75 mM NaCl, both compared to the control group (Figure 2A). Among the diverse losses resulting of salt stress, the growth impairment is one of the best-known effects of this stress in plants (Negrão et al. 2017). On the other hand, the 80 µM H_2O_2 treatment induced higher mitotic index, confirming the hypothesis that this molecule is involved with root growth under low concentrations (Livanos et al. 2012), specifically with increased cell division rate. Indeed, in addition to its harmful properties as a reactive oxygen species under high concentrations, H_2O_2 is also known as a signalling molecule. Under low concentrations, H_2O_2 was proved as a signalling molecule for different processes in plant metabolism, including plant cell division (Livanos et al. 2012). Most of the cytological studies about the effects of H_2O_2 in roots are performed by using high H_2O_2 concentrations aiming to induce oxidative stress. Thus, to the better of our knowledge, our result is the first evidence of H_2O_2 acting as a growth-inducing molecule observed through cytological analysis. Both salt and H_2O_2 treatments induced changes in the proportion of each mitotic phase (Figure 2B). For example, the 75 mM NaCl treatment induced lower occurrence of metaphase and higher occurrence of prophase. Among all the mitotic phases, only anaphase was observed in the 150 mM

NaCl treatment. However, this result can be taken as a mere consequence of the low occurrence of mitosis in that treatment and therefore is inconclusive. Interestingly, the changes in the percentage of each mitotic phase for the 80 µM H_2O_2 treatment were the opposite of that found in root tips treated with 75 mM NaCl, with increased occurrence of anaphase and decreased occurrence of prophase, both treatments compared to the control. The induction of changes in cell cycle by H_2O_2 and NaCl have been previously reported (Pokora et al 2017; Mohammed and Ibrahim 2017). The 75 mM NaCl and 80 µM H_2O_2 treatments also induced increased occurrence of chromosomal abnormalities, which were seven and almost ten times higher than in control, respectively. However, the relative percentage of abnormalities, measured through the "percentage of abnormalities/mitotic index" ratio, revealed that the 75 mM NaCl treatment induces 3.4 times more chromosomal abnormalities than the H_2O_2 treatment. The results observed in our NaCl treatments are in accordance with other studies, in which chromosomal abnormalities were observed after treatments with different NaCl concentrations (Teerarak et al. 2009; Chatterjee and Majumder 2010; Mohammed and Ibrahim 2017).

FIGURES:

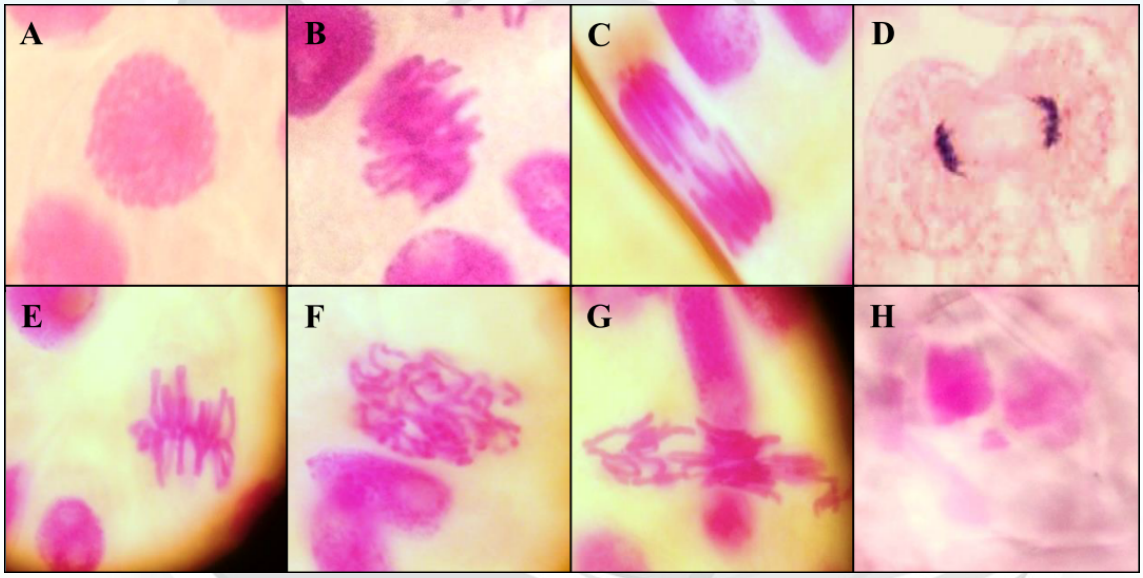

Figure 1. Root-tip meristematic cells of *Allium cepa* L. observed with magnification of ×100 showing the occurrence of prophase (A); metaphase (B); anaphase (C); telophase (D); and chromosomal abnormalities (E, F, and G), including micronucleus (H).

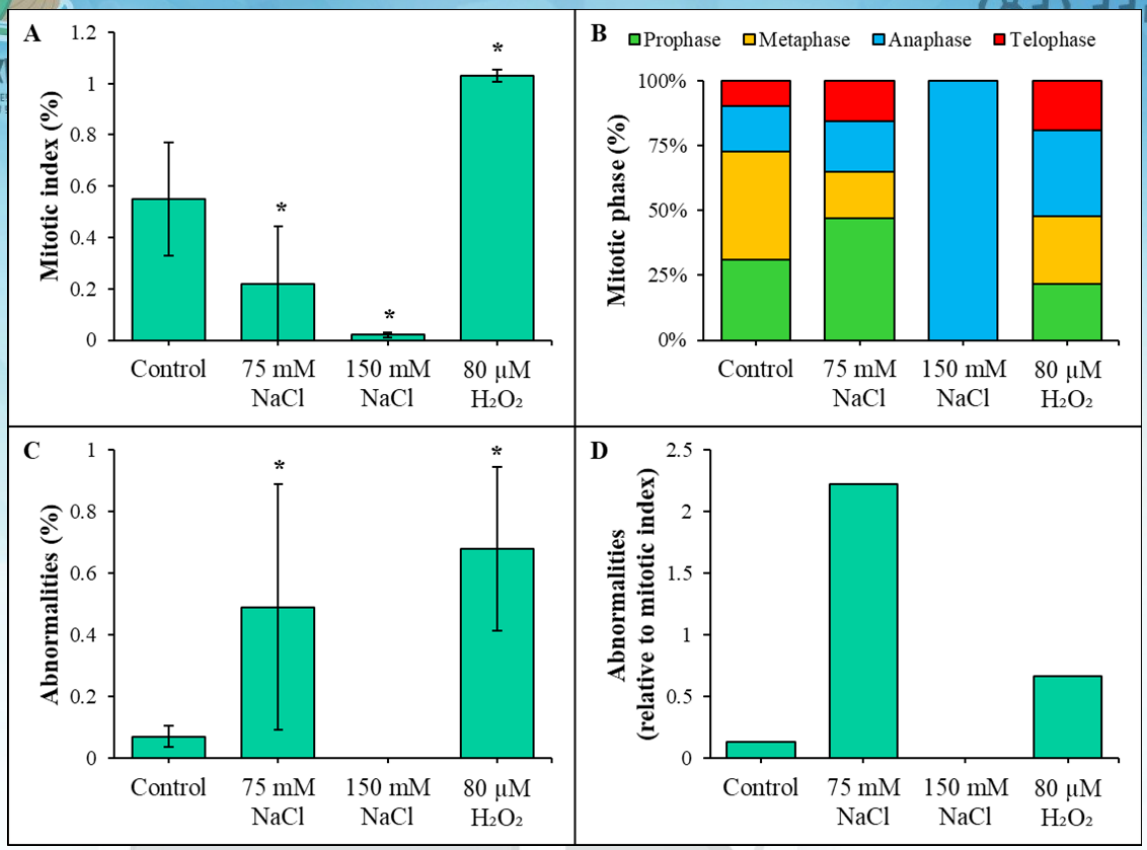

Figure 2. Mitotic index (A), percentage of mitotic phases (B; prophase, metaphase, anaphase, and telophase), percentage of chromosomal abnormalities (C), and relative occurrence of abnormalities (D) in root cells of *Allium cepa* L. treated with nutritive solution (control), or nutritive solution containing 75 mM NaCl, 150 mM NaCl or 80 µM H_2O_2.

CONCLUSION: Taken together, our results reinforce the knowledge that NaCl treatments decrease mitotic index, induce changes in the mitotic phases, and increase the percentage of chromosome abnormalities in roots. We also showed that low H_2O_2 concentrations induce higher mitotic index with lower chromosomal abnormalities occurrence than in NaCl-treated roots. More importantly, we conclude that cytological analysis of root tips is a simple, inexpensive, and efficient method to evaluate levels of abiotic stress in addition to other classic methods.

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