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STUDIES REGARDING THE INTERDEPENDENCE BETWEEN THE CITOGENETIC ASPECTS AND THE APLICATION OF STRESS FACTORS OVER THE TISSUES CULTIVATED "IN VITRO" AT TOMATOES

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***Key words**: haploid, embryogenesis, floral, bud, vitro*

INTRODUCTION

High soil salinity is now determined as one of the most major abiotic threat for productivity atall vegetable species, due to its increased occurrence in irrigated cultivation, that is the main cultivation systems in major part of world food production (Flowers, 2004). If drought or heat stress often occurs for intermittent periods of time, salt stress persists throughout most of the plants' growth cycles, transforming it in a major threat over the plant growth, development and ultimately production. Some authors concluded that salt stress may amplify the impact of pests and diseases on plants and decreasing the natural resistance. Thus, on may conclude that the salinity of soils is currently one of the major problemsin agricultural productionas it is directly associated witheconomic losses.

The studies shows that plantgrowth and development is disturbed during salt stress by the excessiveaccumulation of sodium, chloride, sulphate, bicarbonate, calcium, magnesium, potassium, copper, cadmium and nitrate. The studies also revealed that soil salinity decreases the water potential in plants anddisturbs cellular ion homeostasis with impact on the plant physiology that leads to inhibitionof many processes such as seed germination, vegetativegrowth and fruit setting [4].

Although soil salinity has a tremendous impact on the plant growth and development few datas are known regarding the effect of salinity over the chromosomes of tomatoes. The present study focused on the determination of chromosome behavior and frequency ofchromosomes aberration in root meristematic cells of tomatoes cultivated "in vitro" on MS medium suplemented with different concentrations of NaCland KCl.

MATERIALS AND METHODS

Plant growth conditions and biologic material

The biological material is represented by Siriana varietyand the experiments were accomplished at Vegetable Research and Development Station Bacau, during 2019-2020, in The Laboratory of Tissue Culture.

In order to test the impact of salt stress over the citogenetic structure of tomatoes plant, explants, represented by apexes of plantlets germinated in vitro were cultivated on basal culture media MS (Murashige Skoog, 1962)containing vitamins B5 (Gamborg, 1975),benzylaminopurine (BA)8.87 mM, 3% (w/v) sucrose, supplemented with different concentrations of NaCland KCl(100, 200, 300 mM).

Table 1. Experimental variants tested

No. crt.	Variant	NaCl	KCl
1.	V0	-	-
2.	V1	100	-
3.	V2	200	-
4.	V3	300	-
5.	V4	-	100
6.	V5	-	200
7.	V6	-	300

The pH was adjusted to 5.8 prior to the addition of 0.8% agar and autoclaved at 121°C (1.06 kg/cm^2) for 25 min. Cultures were then incubated at 26±1°C, a 16-h photoperiod, and 5000 lx light intensity.

Tissue culture in vitro

Seeds were washed thoroughly under running tap water and then treated with a surfactant, Tween 20 (10 drops per 100ml of sterilized distilled water), followed by surface sterilization with 0.1% mercuric chloride (w/v) for 15 min and repeatedly washed using sterilized distilled water. Under aseptic conditions, the seeds were inoculated on basal medium. After germination the apexes were carefully excised from plantlets and inoculated, in sterile conditions, on the experimental variants previously presented.

Periodic subcultures were accomplished on the same culture media and observations were carried out every 5, 10 and 15 days.

Cytological studies

The cytogenetic studies were carried out according to the data presented by the literature,

respectively Carr's Reagentstaining method.After hydrolysis for 7 minutes in HCl at 60^0C, on remove HCl from the vials and add 2-3 ml of Carr reagent. As a result of the chemical reaction that occurs between the aldehyde groups of the DNA released by hydrolysis and the basic fucsine, after 15-30 minutes, the meristematic region at the top of the roots is colored red-violet. To enhance coloration, the roots were left in the Schiff reagent for 1-2 hours, at room temperature.

The display of root tips was accomplished in a drop of acetic water, which is placed at the middle of the blade. Near the drop, with a spattered needle, the colored meristematic tip of a root is cut and dropped. Over the drop of acetic water containing the root tip is placed a glycerinated albumen glycerinate and quickly passed through the flame of a gas or alcohol lamp for coagulation of albumin (the use of glycerol albumin is not absolutely necessary, its role being merely to prevent dispersing chromosomes from the cells that break through the blade display).

Chromosome examination and photographing were performed on the Hund microscope at a magnitude of 1000x.

Statistical analysis

The data were analyzed by ANOVA (analysis of variance). The means were compared using the Duncan multiple comparison test at $P < 0.05$.

RESULTS AND DISCUSSION

The culture of apexes on media culture supplemented with different concentrations of NaCl and KCl showed the high impact that salt stress has over the growth and development of tomatoes tissues. On low concentrations the initiation of regeneration processes started and acted similar to the control variant, while as the concentration grows, the explant became yellowish, with dots of necrosis on the tissue of inoculum. – fig. 1.

Fig. 1. Dots of necrosis on explant tissue

In higher concentration (300mM), the explant gradually dies, while the apparition of new shoots at the base of the explant is heavily slowed down – fig. 2. The newly formed shoots were smaller and yellowish, with friable roots, definitely incompatible with living.

Fig. 2. Morphogenetic reaction of apexes cultivated on variants with the highest concentration of salt

On lower concentrations, namely 200mM at the base of the explant a callus masa appeared, which gradually covered the entire explant and strong roots were developed. The subcultivation of the callus masa on fresh medium culture allowed the development of new shoots but, comparing with the control variant, the shoots were presented abnormalities in development and even small brownish dots appeared.

At the end of exposure period, respectively after 15 days, the plants were taken out from the culture vessels. Roots of cultured plants were gently washed out from the remaining culture media by repeated washing in water and prepared for cytogenetic observations.

The results obtained showed that the mitotic index decreased with increasing salinity, the cultivation of tomatoes plantlets on medium with 300 mM of NaCl over 15 days conducted to an arrest of cells divisions in roots – fig. 3, which is also shown by the morphogenetic reation observed “in vitro”.

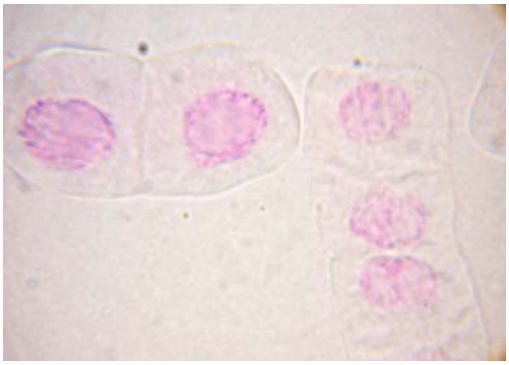

Fig. 3. Tomatoes cells after 15 days cultivation on medium with 300 mM of NaCl

While in the roots of control plants mitosis – fig. 4 was normal and and has not changed significantly over time, after 5 days, on the lowest (100 mM) concentration of salts the decrease was slight for KCl, while on plants cultivated on medium with NaCl the decrease in mitotic activity was more significant.

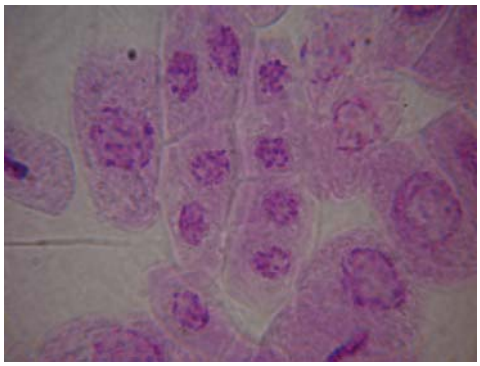

Fig. 4. Aspects of citogenetics in control plants

Over time, the exposure to salt both NaCl and KCl, causes the decrease in mitotic index and the appearance of cromosomal abnormalities – fig. 5.

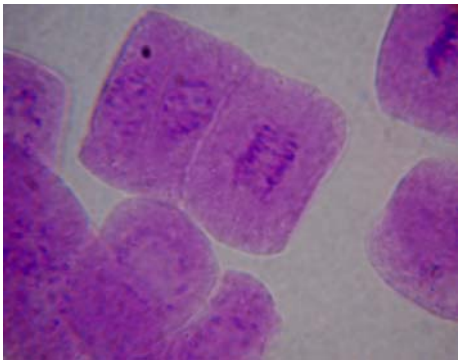

Fig. 5. Ana-telophase with multiple interchromatin bridges

The main abnormalities identified were: ana-telophases with with interchromatin bridges (simple and multiple), metaphases with lagging chromosomes, expelled chromosomes etc.

CONCLUSIONS

One of the main constraint of now-a-days agriculture is environmental stress caused by the climatic changes. Salinity of soils is currently among the major stress that plants have to deal with, causing major economic losses. In the last years, different successful protocols for improving of salt tolerancehas been presented for rice, rye, barley, andcanola, but in vegetable crops few datas are available.

Thus, the present study focused on assessing the impact of salt stress over the citogenetics of tomatoes plants cultivated in vitro on media suplimented with different concentration of NaCland KCl(100, 200, 300 mM).Based on the results obtained on concluded that the mitotic index decreased with increasing salinity. The cultivation of tomatoes plantlets on medium with 300 mM of NaCl over 15 days conducted to an arrest of cells divisions in roots, results that are liniar with the one obtained in vitro, where the shoots ceased to grow and the eventually died. The exposure to salt both NaCl and KCl, causes the decrease in mitotic index and the appearance of cromosomal abnormalities in a thight connection with the quantity added to culture medium.

ABSTRACT

Tomato (*Lycopersiconesculentum* Mill) is an important member of the Solanaceaefamily due to its spread cultivation worldwide. The tomato is a diploid plant with 2n=2x=24 chromosomes. The tomato genome has been implemented to be used as a model for theSolanaceae family because of its short production period, simple diploid genetic structure, small genome size and the availability of both molecular breeding methodologiesand a wide diversity of genetic resources [2].

Due to its high food value the consumers request and the producers interesates is continuously increasing.In the same time, tomatoes production is affected by different types of stresses by both biotic and abiotic stress. Among biotic stress tomatoes are affected by intensive diseases caused by viruses, fungi, bacteria and also nematodes. The abiotic stresses, namely environmental stresses pose a tremendous pressure on the growth and production of crops [9]. Among them, the most agressive one are salinity of soil, high temperature and draught. Its vulnerability to insect and pest attacks is one of the factor causing the expand of production costs. Now-a-days the climatic changes dramatically increase the impact of all these biotic and abiotic stresses over the effectiveness of tomatoes production, which led to an intensification of plant molecular and in vitro breeding focused on the improvement ofstress tolerance of crops.

The objective of this study was to asses the impact of salt stress over the citogenetics of tomatoes plants cultivated in vitro on media suplimented with different concentration of NaCland KCl(100, 200, 300 mM).

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REFERENCES

1. BORSANI, O., VALPUESTA, V., and BOTELLA, M.A., 2003 - Developing salt-tolerant plants in a new century: a molecular biology approach, *Plant Cell Tiss. Organ Cult*., vol. 73, pp. 101–115.
2. DEINLEIN, U., STEPHAN, A.B., HORIE, T., LUO, W., XU, G., and SCHROEDER, J.I., 2014 - Plant salt-tolerance mechanisms,*Trends Plant Sci*., vol. 19, pp. 371–379.
3. HASEGAWA, P.M., BRESSAN, R.A., ZHU, J.K., and BOHNERT, H.J., 2000 - Plant cellular and molecular responses to high salinity, *Annu. Rev. Plant Physiol., Plant. Mol.Biol*., vol. 51, pp. 463–499.
4. LI, J.Y., JIANG, A.L., and ZHANG, W., 2007 - Salt stress induced programmed cell death in rice root tip cells, *J. Integrat.Plant Biol*., vol. 49, pp. 481–486.
5. MUNNS, R., 2002 - Comparative physiology of salt and waterstress, *Plant Cell Environ*., vol. 25, pp. 239–250.
6. MURASHIGE, T. and SKOOG, F., 1962 - A revised medium for rapid growth and bioassays with tobacco tissue culture, *Physiol. Plant*., , vol. 15, pp. 473–479.
7. ROMERO-ARANDA, R., SORIA, T., and CUARTERO, S., 2001 - Tomato plant-water uptake and plant-water relationshipsunder saline growth conditions, *Plant Sci*., vol. 160, pp. 265–272.
8. SANTA-CRUZ, M.M., MARTINEZ-RODRIGUEZ, F., PEREZ-ALFOCEA, R., ROMERO-ARANDA, R., AND BOLARIN, M.C., 2002 - The rootstock effect on the tomato salinity response depends on the shoot genotype, *Plant Sci*., vol. 162, pp. 825–831.
9. SHANNON, M.C. and GRIEVE C.M., 1999 - Tolerance of vegetable crops to salinity, *Scientia Horticulturae*, vol. 78, pp. 5–38.

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