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RESEARCH REGARDING IN VITRO REGENERATION OF SOME MAIZE GENOTYPES UNDER THE SALINE STRESS CONDITIONS

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Abstract: *Salinity is one of the most important forms of abiotic stress, widely distributed in both irrigated and non-irrigated areas of the world. Salty soils are soils with a high salinity content and are defined as soils that directly affect the growth and development of plants in the vegetative growth stage, prior to the reproduction stage, especially affecting crop species (Allakhverdiev et al., 2000; Sairam & Tyagi, 2004; Chinnusamy et al., 2005; Ashraf et al., 2008; Ashraf, 2009). Saline stress strongly affects the growth and development of plants, especially the corn plant, which is reported as a salt-sensitive species. Most crop species are sensitive to salinity, because after subjecting plants to saline stress, crop productivity is reduced by about 6-19%. In general, biochemical, physiological, morphological and anatomical characteristics of crop species directly affected by soil salinity are well established (Ashraf, 2004; Ashraf & Harris, 2004; Chinnusamy et al., 2005; Parida & Das, 2005). There are also numerous reports that salinity induces water deficiency in many crop species, such as corn, sunflower, potato, and soybean (Katerji et al., 1996; Katerji et al., 1998; Katerji et al., 2004). A first response observed in plants induced with saline stress is a decrease in plant water potential, resulting in decreased water use efficiency, leading to general toxic damage and reduced growth yield and productivity (Glenn and Brown, 1998; El-Hendawy et al., 2005; Mansour et al., 2005). During our experiment of in vitro testing of four inbred maize lines it was easily observed that the lines are sensitive to saline stress, and also, the decrease of the plant size is directly proportional to the increase in saline concentration. We mention that our results are presented also as master thesis (Negruț, 2020).*

Introduction

The purpose of the present paper was to test the germination capacity of four inbred lines/ maize genotypes, highlighting and comparing their growth and regeneration capacity under normal conditions (H_2O) and under saline stress (NaCl) conditions. Like all life forms on earth, corn has a life cycle. It begins in spring, with sowing, and ends in autumn, at harvest. Between them, the plant emerges, develops, reproduces and dies. The life cycle of maize is divided into several stages: germination, emergence, formation of vegetative organs, formation of reproductive organs, fertilization, grain filling and grain maturation (Gay, 1984). The germination value of maize seed, which is expressed by energy and germination quality, depends on a number of external and internal factors, which, depending on their quality, can positively or negatively influence the viability and genetic integrity of the seed. Among the external factors are mentioned the requirements of temperature, humidity, light, nutrients, corresponding to each stage of plant growth and development, and among the internal factors are highlighted cytological damage. The role of proline in osmotic cell adjustment, membrane stabilization, and detoxification of ion-induced injuries in plants exposed to saline stress is widely reported (Hare et al., 1999; Kavi Kishor et al., 2005; Ashraf & Foolad, 2007). There are several techniques to improve the accumulation of endogenous proline, which is beneficial to the defense mechanism against saline excess, such as exogenous applications (Santos et al., 1996; Hoque et al., 2007; Kaya et al., 2007), biosynthesis proline by overexpression of genes (Zhu et al., 1998; Han & Hwang, 2003) and inhibition of some regulatory genes (Nanjo et al., 1999).

Material and method

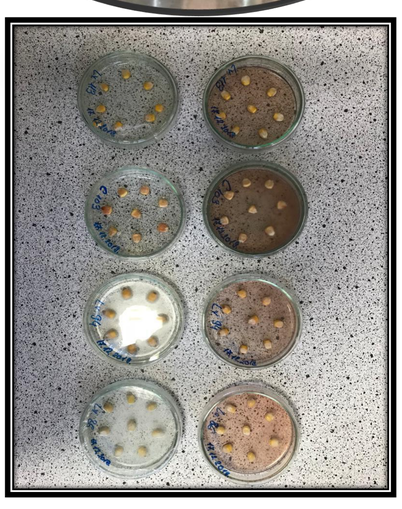
The experiment took place in November 2019, starting with testing the germination and regenerative capacity of biological material, under normal conditions (H_2O) and under conditions of saline stress (NaCl solution). These *in vitro* experiments were performed under sterile conditions, at a hood with laminated air flow, situated in the Vegetal Biotechnology Laboratory of University of Agricultural Sciences and Veterinary Medicine, Timisoara. Also we tried to observe and analyze the ability of growing and regeneration of these genotypes, in order to compare the results obtained.

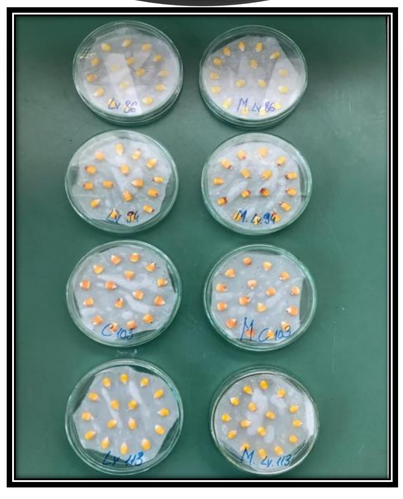
The solutions with different concentrations were sterilized by normal autoclaving (120˚, for 30 minutes). Maize seeds from the studied genotypes were sterilized by immersion for 2 minutes in 70 degrees ethyl alcohol, then in 0.1% mercuric chloride and washed with sterile distilled water. The kernels were germinated in Petri dishes under sterile conditions.

The seedlings resulted from aseptic germination were used as a source of nodal explants, the explantation being realized in sterile conditions, at the hood with air flow, near the flame of the gas bulb. With sterile instruments, forceps and scalpel, the part of the seedling was excised, continuing with the nodule (meristem), which was inoculated on MS medium supplemented with hormonal balance 0.3 mg / l NAA + 3 mg / l BAP and distributed in Erlenmeyer vessels.

The cultivation of inoculums for regeneration was carried out in the growth chamber, at a temperature of 24˚C and a photoperiod of 16 hours of light and 16 hours of darkness.

The results obtained during this monitoring experiment of plants inoculated under normal conditions (H_2O) and under saline stress (NaCl) conditions were statistically interpreted. The methods used for this interpretation were ANOVA and Student test. The following images presents aspects from different stages of the experiment.



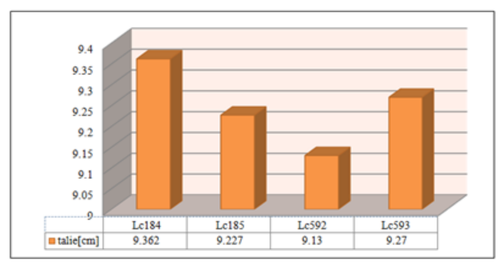


Results and discussions

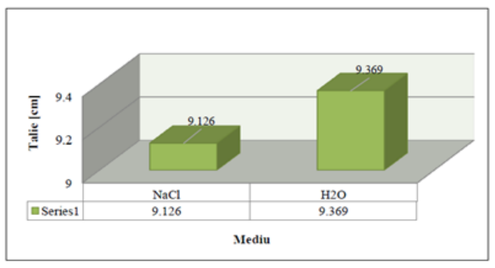
During our *in vitro* experiment conducted at USAMVBT we followed the germination percentage of the maize kernels for all studied genotypes, under conditions of saline stress (0.1% NaCl and 0.2%). After the first inoculation we observed that salinity diminishes the plant growth, but does not affect the germination, on the opposite, during our experiments, the germination percentage on saline medium (0.1% NaCl) is higher than in the martor case (H_2O). After the second inoculation, it was observed that the plants grow better under normal conditions, and under conditions of saline stress (NaCl 0.2%), the growth and development of the plants is below the martor (H_2O). These results justify us to say that saline stress is manifested at corn, started from a concentration of 0.2% NaCl, for the studied inbred lines.

Source	Degrees of freedom	Squares sum	Variance S^2	F test	Signif.
Repetition	9	68.345	7.594	0.4139	
Factor A	3	1.117	0.372	0.0203	ns
Error (a)	27	495.366	18.347		
Factor B	1	2.352	2.352	0.0877	ns
AB	3	110.603	36.868	1.3748	ns
Error (b)	36	965.425	26.817		
Factor C	1	3823.980	3823.980	253.2102	***
AC	3	194.222	64.741	4.2869	**
BC	1	0.225	0.225	0.0149	ns
ABC	3	24.637	8.212	0.5438	ns
Error (c)	72	1087.346	15.102		
Total	159	6773.619			

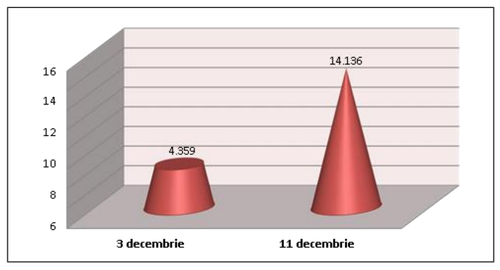
Analysis of variance (ANOVA)



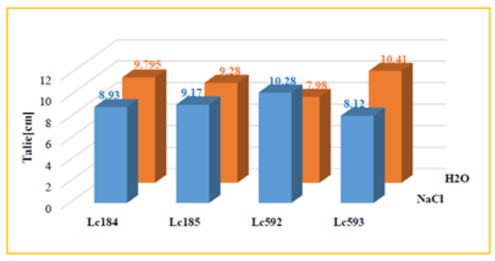
Graphic representation of factor A (maize inbred line)



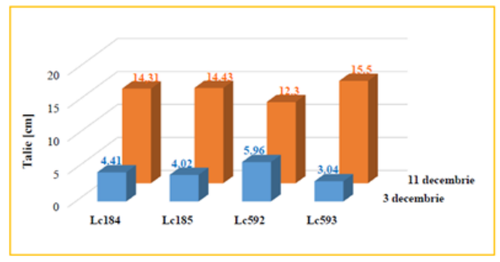
Graphical representation of factor B (tested solution)



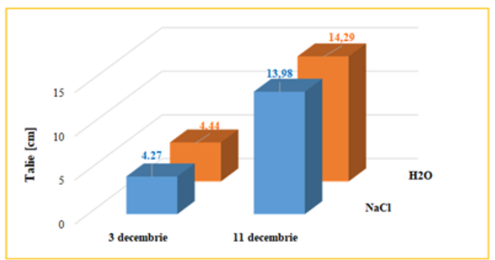
Graphical representation of factor C (measurement date)



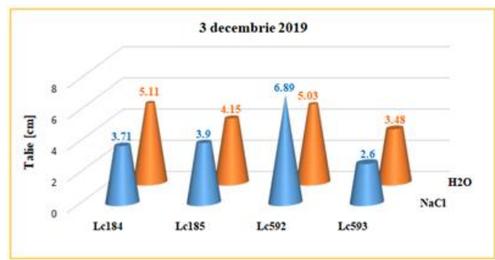
Graphical representation of experimental factors A and B



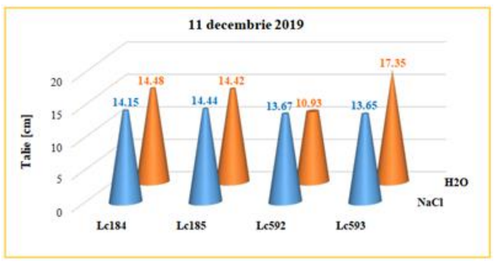
Graphical representation of experimental factors A and C



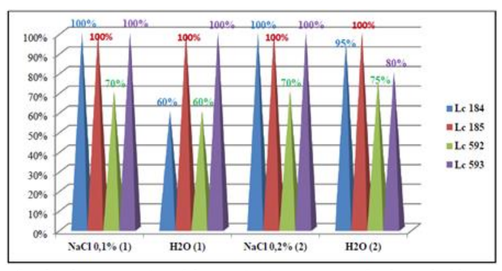
Graphical representation of experimental factors B and C



Graphical representation of experimental factors A and B (December 3)



Graphical representation of experimental factors A and B (December 11)



Graphical representation of the germination percentage for the studied lines

Conclusions

- Following the results obtained during our *in vitro* testing experience of the four inbred maize lines, we can affirm that these lines are sensitive to saline stress, starting with the concentration of NaCl 0.2%. Also, it can be said that the decrease in plant size is directly proportional with the increase in saline concentration.
- An important characteristic was highlighted at the line Lc 592, a line that has a good resistance to saline stress, compared to the other three studied lines, characteristic which recommends it for a future cultivation on soils with a certain degree of salinity.
- It is obvious that the genotype strongly influences the tolerance to saline stress conditions. Also, the genotype influences the *in vitro* behavior of the studied biological material.
- The obtained results show that the lines Lc 184, Lc 185 and Lc 593 had a good development under normal conditions (H_2O), which recommends using them further in the breeding process.