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). Most crop species are sensitive to salinity, because after exposing plants to saline stress, crop productivity is reduced by 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.*

Keywords*: maize, in vitro, regeneration, genotypes, salinity*

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.

It is stated that in the last century, *in vitro* cultivation methods have become safe and fast ways of propagation of different species, especially horticultural species, elimination of phytopathogens, conservation of germplasm, obtaining high-performance genotypes, with a higher degree of adaptability to increasingly stressful environmental conditions.

These genotypes are able to ensure, through their production, not only the food needs of a growing human and animal population, but also allow the application of new cultivation methods, more economically and ecologically advantageous (Botău, 2006).

Vegetative propagation consists in increasing the number of specimens, starting from a single one. Thus, it is cloned regeneratively, *in vivo* or *in vitro*.

By vegetative multiplication, as many copies can be obtained (identical to the genotype subjected to regeneration), as the number of fragments which have stood at the basis of cloning. The clone is defined as "a group of plants, tissues or cells, genetically identical, descending (vegetatively or apomyctically) from a single organism" (PIERIK, 1984).

The capacity of vegetative propagation is a characteristic for many plant species, some of which can grow vegetatively naturally, while the vast majority of plants can be propagated vegetatively only artificially, through fragments of stems, roots or leaves. The results of research in the field have scientifically demonstrated that regenerative aptitude is closely correlated with the size of the fragments used as a starting point in vegetative propagation, with their nature, season and phenophase in which they were harvested or in which vegetative propagation is practiced. In this direction, using an uniform and healthy biological material represents the essential condition for an increased harvest.

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.

Salinity is one of the most important forms of abiotic stress, widely distributed in both irrigated and non-irrigated areas of the world. Salts 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 maize culture, which is reported as being one of the salt-sensitive species.

Most crop species, such as beans, eggplant, onions, peppers, corn, sugar cane, potatoes and cabbage 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 which shows that salinity induces water deficiency in many crop species, such as corn, sunflower, potato, and soybeans (KATERJI ET AL., 1996; KATERJI ET AL., 1998; KATERJI ET AL., 2004). A first response observed at plants subjected to saline stress is a decrease of plant water potential, leading to general toxic damage and reduced growth yield and productivity (GLENN AND BROWN, 1998; EL-HENDAWY ET AL., 2005; MANSOUR ET AL., 2005).

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).

The endogenous accumulation of proline that was observed in plants subjected to saline stress was used as an effective indicator for salt tolerance. Numerous tests have been performed on biochemical and physiological parameters, growth performance and yield, to

classify salt tolerance in some maize cultivars (NETO ET AL., 2004), wheat (EL-HENDAWY ET AL., 2005), rice (ZENG, 2005), tomatoes (JUAN ET AL., 2005), cowpea beans (MURILLO-AMADOR ET AL., 2006), coastal paspalum (LEE ET AL., 2008) and chickpeas (MALIRO ET AL., 2008).

MATERIAL AND METHODS

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. Then 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). Corn 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.

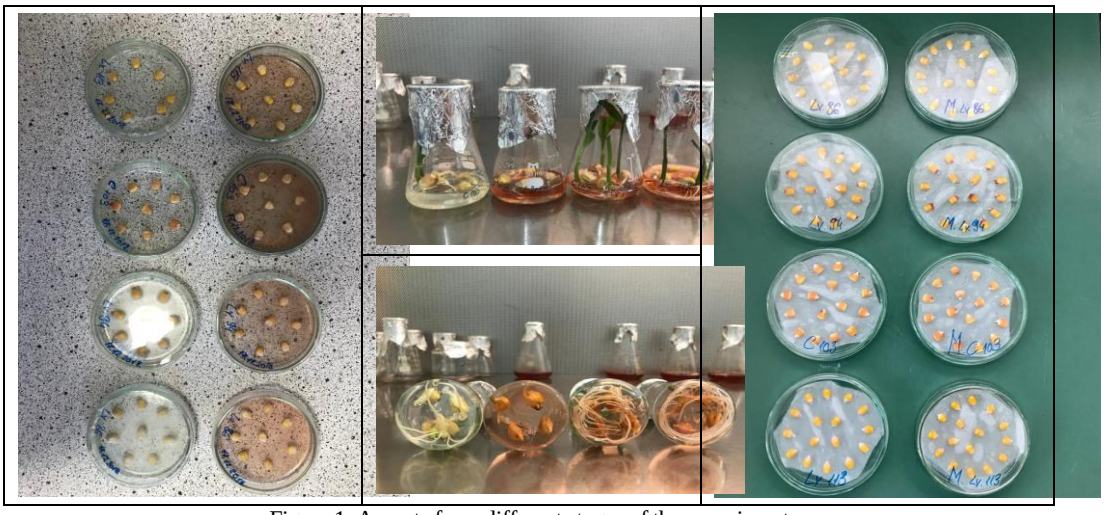

Figure 1. Aspects from different stages of the experiment

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%). The germination percentages are shown in the graph below (figure 2).

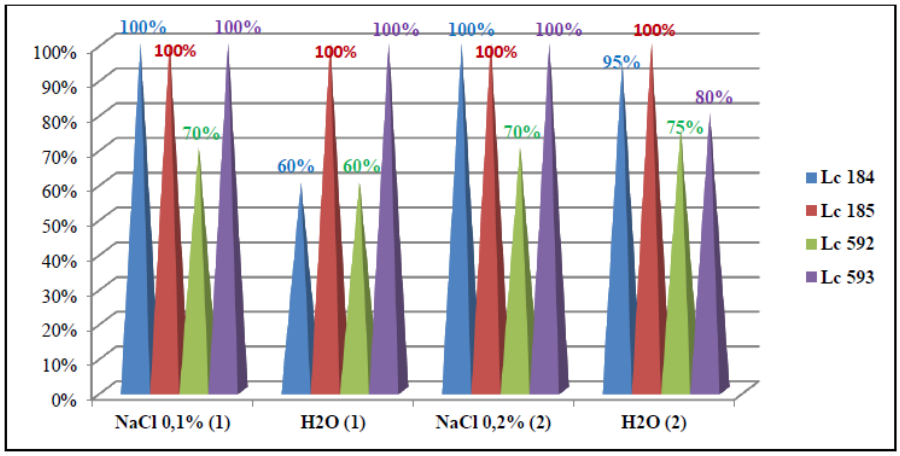

Figure 2. Graphical representation of the germination percentage for the studied lines

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.

Following these experiments, the Lc 185 line was highlighted by having the most pronounced vigor, as well as by the 100% germination percentage achieved both under normal and saline stress conditions. Based on this criterion, the order of the inbred maize lines is as follows: Lc 185, Lc 593, Lc 184 and Lc 592.

It has also been observed that maize is sensitive to in vivo testing because it is prone to infections with various pathogens. *Fusarium* and *Aspergillus* infections were present in the conducted inoculations.

Table 1

Analysis of variance (ANOVA)

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			

Coefficient of variation: 42.02%

For the calculation of ANOVA (analysis of variance) the experimental factors are A, B and C. Thus, factor A represents the inbred corn line (Lc 184, Lc 185, Lc 592, Lc 593), factor B indicates the solution used (H_2O and NaCl 0, 2%), and factor C shows the calendar date on which the measurements were tooked (3 December, respectively 11 December, 2019).

According to the coefficient of variation, the analyzed data from the table 1 had a variation of more than 20% (cv> 20%). The values of the F test for experimental factors A, B and C were deduced, dividing the variances of factors A, B and C by the variances of the respective errors (a), (b) and (c).

The results of the interpretation for the analysis of variance (F test) express a value:

- Very significant for the C factor (date of measurements);
- Distinctly significant for the A x C interaction;
- Insignificant for:
  - factor A (inbred line) and factor B (used solution);
  - interactions of order I (AxB, BxC) and order II (AxBxC).

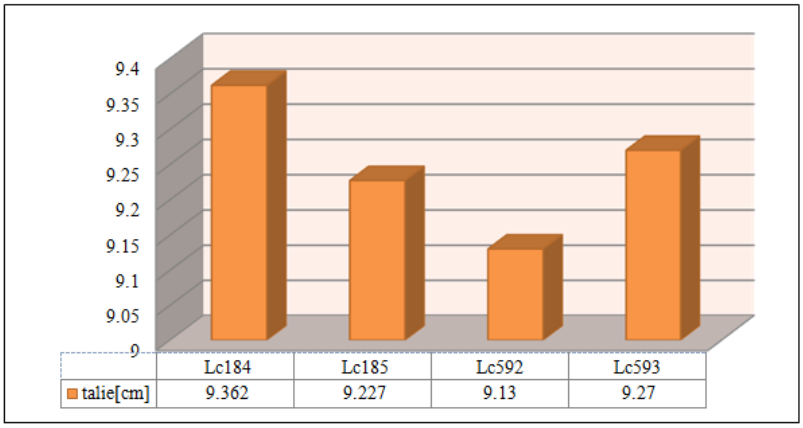

Figure 3. Graphic representation of factor A (maize inbred line)

The graph from the figure above shows the general average of the plants size (height) from all studied lines, respectively Lc 184, Lc 185, Lc 592 and Lc 593, an average that was realized, taking also into consideration the two experimental factors (B and C). From this representation we can see the percentage of growth of each genotype and the difference between them.

Even if the differences between the studied lines are insignificant, as it is demonstrated from the statistical interpretation (Student's test), we can conclude that in general, the lines Lc 184 and Lc 593 are more resistant to the saline stress than the lines Lc 185 and Lc 592.

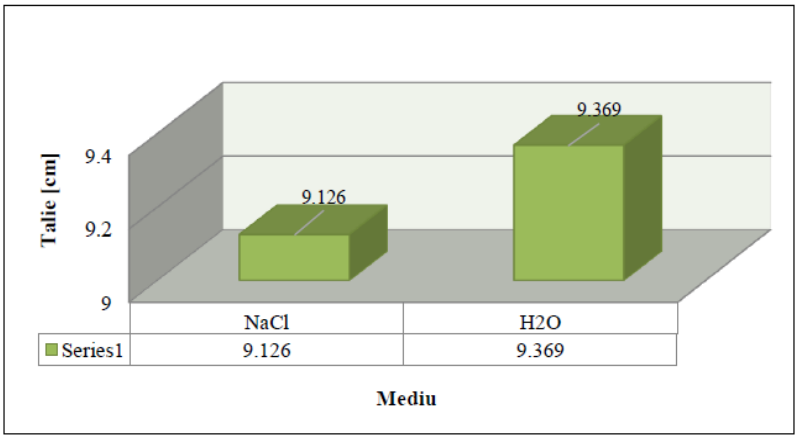

Figure 4. Graphical representation of factor B (tested solution)

From figure 4, comparing the growth of the treated plants with the two solutions (0.2% NaCl and H_2O used as martor), we observed that plants have a harmonious development in normal conditions, while the growth and development of the subjected plants to the saline stress (0.2% NaCl) is under the martor (H_2O). We tend to believe that this difference could possibly increase in a direct proportion to the increase of the salt solution concentration.

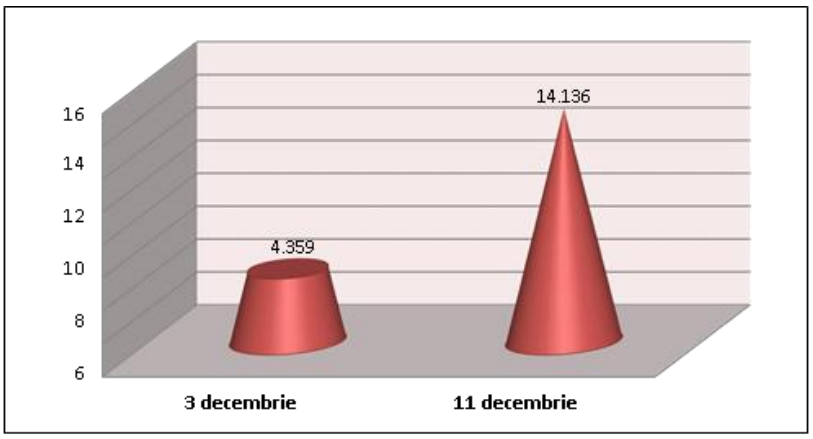

Figure 5. Graphical representation of factor C (measurement date)

Figure 5 indicates an approximately average growth values of maize plants at 3 December, respectively, 11 December. As these averages also include the martor, the values do not concretely show the resistance to saline stress, but they are representative because it express the average growth ratio within eight days. It was noted that during this period the plants grew on average by 10 cm.

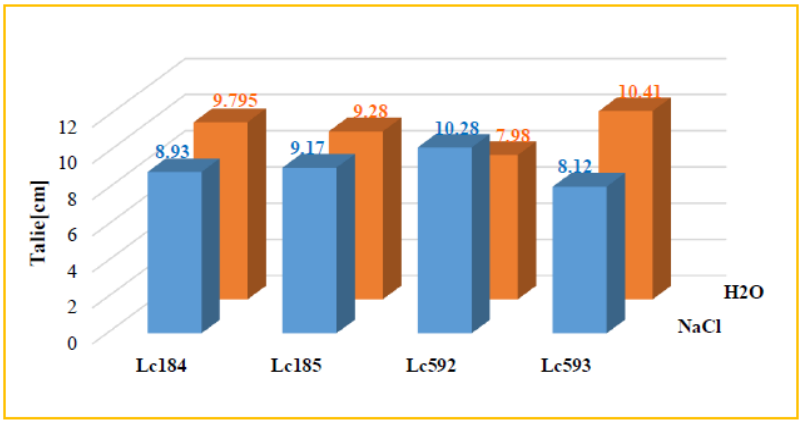

Figure 6. Graphical representation of experimental factors A and B

From the graphical representation (figure 6) we noticed that under saline stress conditions (NaCl 0.2%) plants growth and development have shown a small decrease, comparing to the martor. An important aspect which emerges from this experience is the fact that the Lc 592 line has an increased resistance to saline stress compared to the other studied lines (Lc184, Lc 185 and Lc 593), this line having also a good vigor.

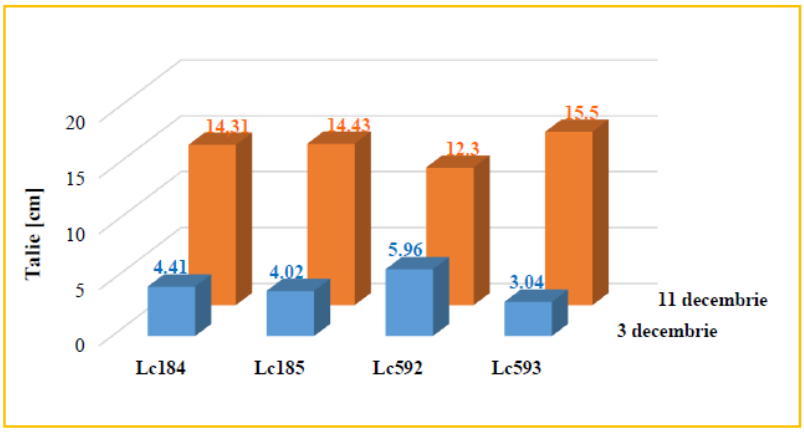

Figure 7. Graphical representation of experimental factors A and C

From the figure represented graphically above we can read the percentage of growth achieved for each inbred line in the time interval between the two measurements, respectively 3 and 11 December. Therefore, the Lc 184 line had an average increase of 9.90 cm; Lc 185 - 10.41cm; Lc 592 - 6.34 cm and Lc 593 - 12.46 cm. We can say thus that during their vegetation period, the line Lc 593 is the earliest one, followed closely by Lc 185 and Lc 184, while Lc 592 could be the last line arrived at maturity, with a longer period of vegetation.

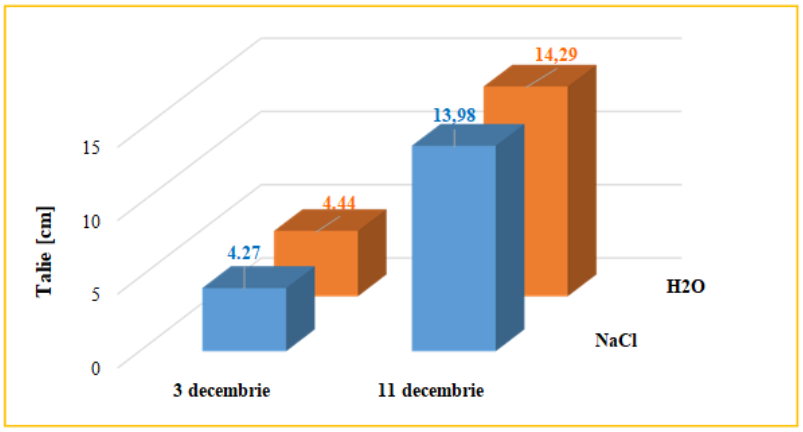

Figure 8. Graphical representation of experimental factors B and C

The graphical representation from figure 8 shows the difference between the values of our *in vitro* experiments, depending on the two measurements and the solution used. Even if the realized differences are insignificant, it still can be observed that under saline stress conditions, plant growth is lower than in normal conditions of development.

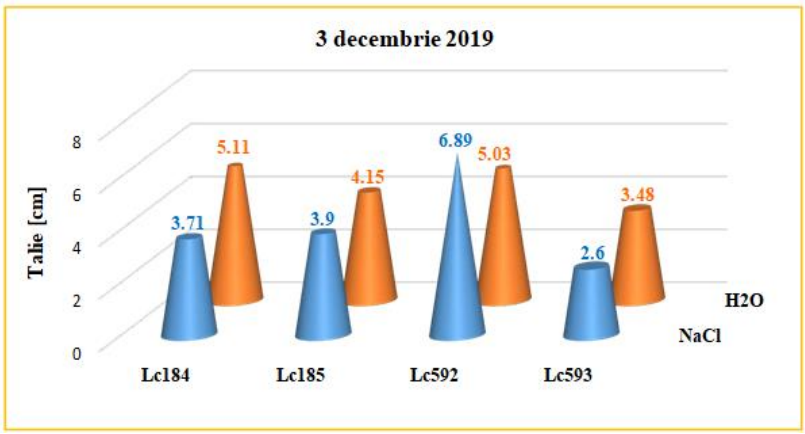

Figure 9. Graphical representation of experimental factors A and B (December 3)

From the graph figure 9, which shows the averages of the measurements from December 3, it is observed that the lines Lc 184, Lc 185 and Lc 593 develop better under normal conditions (H_2O), while under saline stress conditions (NaCl 0.2%), plant growth and development is a bit affected. At the same time, it is notable that the Lc 592 line is not only the most resistant to the saline stress, but also has the highest waist, exceeding even the average of the other lines, under normal conditions of development.

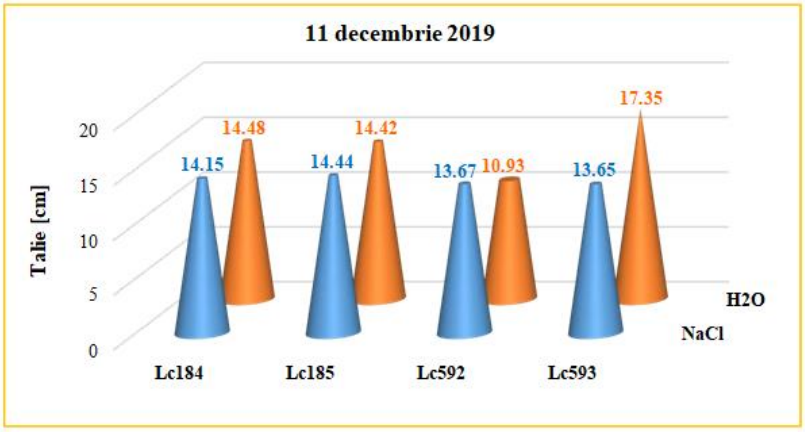

Figure 10. Graphical representation of experimental factors A and B (December 11)

The above graph shows the averages of the second measurement. From this one we can notice that the Lc 592 line maintains its growth ratio, being the most resistant line to the saline stress, out of the four studied.

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.

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