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EVALUATION OF SALT TOLERANCE UNDER CONTROLLED CONDITION OF NINE CUBAN CULTIVARS OF SOYBEAN (*Glycine max* (L.) Merril)

Evaluación de la tolerancia a la salinidad bajo condiciones controladas de nueve cultivares cubanos de soya (*Glycine max* (L.) Merril)

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ABSTRACT. Salinity is a growing threat to the productivity of soybean cultivars (*Glycine max* L. Merril) and different strategies have been adopted to overcome this problem. Commercial exploitation of tolerant genotypes to salinity is a good alternative to obtain economical yields in these areas. For this reason, it is important to have rapid methods to assess the tolerance to this stress especially in the early stages of growth. The aim of this study was to evaluate the salt tolerance in nine soybean genotypes. Experiments were carried out in a greenhouse under controlled conditions. The rate of salinity tolerance of germination (IG), shoot length (ILB), root length (ILR), shoot dry matter (IMSB) and root (IMSR), as well as injury of cell membrane were measured in all genotypes treated with NaCl (chloride sodium) at concentrations 0 to 150 mM. The results obtained based on physiological criteria allowed the identification of AT22 and INCASoy36 as the most tolerant cultivars compared to other genotypes, thus those genotypes seem to be promising to achieve higher productivity in areas affected by salt.

Key words: soybean, salt stress, germination, membrane

RESUMEN. La salinidad es una amenaza creciente para la productividad de cultivares de soya (*Glycine max* (L.) Merril). Diferentes estrategias se han adoptado para superar el problema de la baja productividad. El empleo de genotipos tolerantes a la salinidad es una buena opción para obtener rendimientos económicos en estas áreas. Por esta razón, es importante disponer de métodos rápidos para evaluar la tolerancia a este estrés sobre todo en las primeras etapas de su crecimiento. El objetivo del presente estudio fue evaluar la tolerancia a la salinidad en nueve genotipos de soya. Los experimentos se desarrollaron en casas verdes bajo condiciones controladas. El índice de tolerancia a la salinidad de germinación (IG), longitud de los brotes (ILB), longitud de la raíz (ILR), materia seca de brotes (IMSB) y de la raíz (IMSR), así como la lesión de la membrana celular se midieron en todos los genotipos tratados con NaCl en concentraciones de 0 y 150 mM. Los resultados, a partir de los criterios fisiológicos, permitieron identificar a los cultivares AT22 e INCASoy36 como los más tolerantes en comparación con el resto de los genotipos, por lo que pueden considerarse como prometedores para elevar la productividad en las zonas afectadas por la sal.

Palabras clave: soya, estrés salino, germinación, membrana

INTRODUCTION

The high salt concenttration on the soil causes a negative effect on most of the important crops for human beings feeding. Around 800 million of hectares of the earth surface destined to agricultural purposes are affected by a high salt content[A], which surpasses the tolerance capacity of traditional crops. In recent decades, the impact and the severity of these conditions extended to coastal areas, where tides

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[A] FAO. *Global metwork on integrated soil management for sustainable use of salt-affected soils* [en línea], FAO Land and Plant Nutrition Management Services, Rome, Italy, 2005, [Consultado: 15 de junio de 2015], Disponible en: <http://www.fao.org/nr/aboutnr/nrl/en/>.

flood lands; to contaminated fields through fertilization practices where irrigation water had a high salt content and/or where a faulty drainage system was in place (1).

In Cuba, increased saline soils, in general, are associated to faulty drainage and consequently, 16 % of the agricultural surface is affected by this situation. Therefore, saline stress is considered an increasing threat for the agricultural development of the country (2).

Some authors predict that 50 % of world crop areas will be affected by saline stress by the year 2050 (3). For this reason, they refer to the need of quickly adopting new strategies to increase production in saline soils; the use of tolerant crops to salinity is one of the alternatives. Based on this, the variability of the available germplasm became a study focus and source of new varieties built on genetic differences (4). This approach means understanding plants response to different growth stages under saline conditions and the use of adequate techniques and selection criteria.

Soybean is an economically important crop planted on a wide range of environmental conditions worldwide. Though soybean germplasm shows a close genetic variability (5), there are differences to saline stress responses (6). These differences are expressed from moderate tolerance till salinity susceptibility (7). As many other species, soybean growth under saline environments brings about an osmotic result whose consequences include the disorganization of the membrane, metabolic toxicity, phosynthesis alternation and in extreme cases, plant death (8).

Seed germination is a process that is also affected when they are exposed to high salt concentrations. For this reason, the phenotype analysis of the developing plant is used as indicator to establish a selection criterion of the tolerant genotypes (7, 9). The study of the stability of the cell membrane (EMC) is another quick and simple way that allowed to study the tolerance to stress in different crops (10, 11, 12).

The present study has the objective of establishing the response differences of different soybean genotypes to high salt concentrations and select those with a promising performance to breeding and production programs. In so doing, the germination capacity, growth parameters and the membrane stability were used to select plants.

MATERIALS AND METHODS

Determination of the tolerance to salinity

Germinative stage. Nine soybean cultivars were used as plant material (AT22, DT02, DT84, DT96, INCASoy1, INCASoy24, INCASoy27, INCASoy35, INCASoy36) developed by the National Institute of Agricultural Sciences. The seeds from each cultivar were placed on Petri disehs for germination over a filter paper (Whatman no. 3) wetted with distilled water as control and a solution of (NaCl) at 150mM. This NaCl rate allowed differentiating the varieties INCASoy36, AT22 and DT84 in their response to salt stress in previous trials (data not shown). Ten seeds were placed in each dish and three replicates per treatment were made. Seeds were considered germinated when the radicle reached 5mm length. The experiment stayed under lab conditions for 8 days. The results were expressed in terms of the promptness index using the formula (13):

$$IP = nd2(l.00) + nd4(0.75) + nd6(0.50) + nd8(0.25)$$

where:

nd2, nd4, nd6 and nd8 are the number of germinated seeds in the second, fourth, sixth and eighth day respectively.

The tolerance rate to germinative stress was calculated according to the formula:

$$IG=(\text{IP of stressed seeds}/\text{IP of control seeds}) \times 100$$

Seedling stage. Germinated seeds were sown on plastic pots containing a mixture of organic matter and zeolite at 1:1 rate. Pots were placed in trays for irrigation and stayed in greenhouses with natural light and controlled temperature conditions (25± 2 °C). Two days after plant emergence, they were periodically irrigated by capilarity with the Hoagland nutrient solution (14) till reaching the vegetative stage V2 (seedling with the first trifoliate expanded leaf). This solution was changed twice a week. Later on, a NaCl solution at 25mM twice a day was added till reaching a concentration of 150mM. Five replicates were made per treatment made up of two plants/ genotype in each replicate.

After three weeks of treatment, variables like shoot length (LB) and root length (LR) were evaluated. Later on, plants were dried at 70 °C for 48 h and the accumulation of shoot dry matter and roots (IMSR) were measured (IMSB). From these data, the relative tolerance rate to salinity was measured with the following formula (15):

ILB (%)= (LB of stressed plants/ LB of control plants) x 100

ILR (%)= (ILR of stressed plants / ILR of control plants) x 100

IMSB (%)= (IMSB of stressed plants / IMSB of control plants) x 100

IMSR (%)= (IMSR of stressed plants / IMSR of control plants) x 100

Determination of the Cell Membrane Stability

Seeds germinated under the conditions described for seedlings. Fifteen days after emergence, trifoliate leaves were taken to the lab to evaluate the stability of the cell membrane (EMC), according to the methodology described **(10)**. For each variety, five leaf discs of 90 mm of diameter were taken. Leaf discs were treated with NaCl at 150 mM. Each treatment included three replicates and a totally randomized design was used. The EMC for each variety under study was calculated as the lesion of the membrane with the formula:

$$L (\%)= [1- \{1 - (T1/T2)\}_{/} 1 - (C1/C2)\}] \times 100$$

where:
T: conductivity of the treatments (NaCl 150mM)
C: conductivity of the controls (distilled water)
1 and 2: reading before and after the treatment of total death, respectively.

Statistical analysis

The results of the analyses were processed using the software package SPSS 17.0 for Windows (16), using simple ANOVA. The statistical differences among means of the tolerance rates and the lesion of the cell membrane for p<0.05 were determined by Tukey's upon verifying the assumptions of the analysis of variance (normality, homogeneous variance and error independence).

RESULTS AND DISCUSSION

Evaluation of the Relative Tolerance to Salinity

Seed germination and seedlings early growth are sensitive stages to establish plant populations under saline conditions (7, 9).

When comparing the germinative tolerance rate (IG) for the evaluated cultivars, it was observed that INCASoy35 and DT96 showed the lowest germination percentage (78,51 % y 79,12 %, respectively) under saline stress (Table I), which indicates a moderate susceptibility to salinity. For genotypes AT22 and INCASoy36 the highest values were recorded (86,10 % and 85,28 %, respectively), unlike INCASoy35. The genotypes that show a high germinative capacity under saline conditions, usually show a higher biomass and yield and are classified as tolerant to salinity (17). This could be the case of the genotypes AT22 e INCASoy36 which leads to look at their yields and biomasses under conditions of saline stress to confirm the previous criterion.

Values of the tolerance rates to relative salinity of the nine evaluated soybean cultivars

Cultivars	Values of the tolerance rates (%)				
	IG	ILB	ILR	IPSB	IPSR
AT22	86,10 e	74,28 e	78,51 e	65,27 f	73,52 f
DT02	80,75 b	63,46 ab	70,00 b	51,43 a	65,41 bc
DT84	82,88 c	65,70 c	74,22 c	55,59 c	67,20 d
DT96	79,12 a	62,69 a	67,31 a	51,48 a	62,05 a
INCASoy1	84,29 d	71,16 d	75,84 d	59,25 d	71,18 e
INCASoy24	84,73 d	71,67 d	75,75 d	59,05 d	71,04 e
INCASoy27	81,08 b	64,81 bc	70,42 b	53,73 b	66,28 cd
INCASoy35	78,51 a	62,09 a	68,24 a	50,66 a	63,82 b
INCASoy36	85,28 de	73,46 e	78,34 e	61,02 e	73,01 f
Media	82,52	67,70	73,18	56,38	68,16
EE	0,60	1,17	1,28	1,05	0,99

* Means with different letters in the same column of the table indicate significant differences for p<0,05 according to Tukey's
IG: Germination rate; ILB: Shoot length rate; ILR: Root length rate; IMSB: Shoots dry matter rate; IMSR: Root dry matter rate
EE: Standard error of the mean

After 21 days of treatment with NaCl at 150mM tolerance rates to salinity were determined (ITS) for each cultivar. In general, the analysis of variance done to ITS showed significant differences for p<0.05 among the studied cultivars (Table). In soybean, the germination stage is much less sensitive to saline stress than the seedling stage (7). In turn, this study showed that salinity has inhibitory effects on seedling growth (Table) in comparison to the germination stage. It indicates that the evaluation of growth variables in early development stages is a determinant criterion for the selection of soybean genotypes tolerant to salinity.

For shoots tolerant rates (ILB) cultivars AT22 and INCASoy36 showed the highest values which indicates a higher tolerance to salinity. In turn, cultivars INCASoy35 and DT96 were the most sensitive ones, showing more chlorosis in their leaves and reduced biomass as a consequence of the high level of NaCl.

The tolerance rate of root length (ILR) also allowed to identify genotypes AT22 and INCASoy36 as the most tolerant ones, so they could be grown in soils of 15 dS m^{-1} salinity to produce more biomass. On the other hand, the most sesitive cultivars were INCASoy35 and DT96, which showed a maximum reduction of root growth (Table). It is indicated that tolerance to salinity is related to the efficiency of limiting the translocation of Na^+/ Cl^- from the roots to the leaves (6). For some soybean cultivars, tolerance to salinity is described through the retention of Cl^- in roots avoiding the accumulation in stems and leaves at toxic concentrations (18). These data suggest the effect on the optimum root development. For INCASoy35 and DT96 it is evident that their tolerance reduction to salinity is associated to a poor root development and therefore to a poor reduction of NaCl.

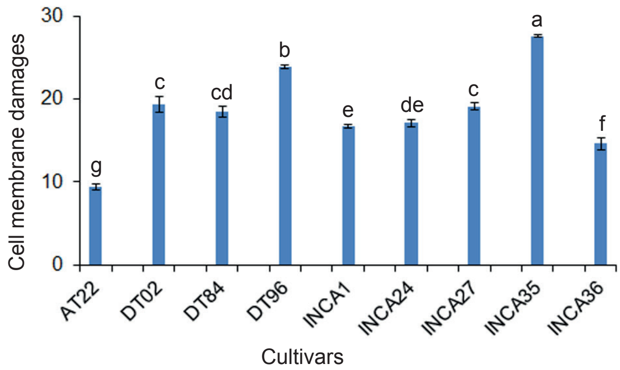
When the values reached for the tolerance rate were analyzed, the accumulation of shoots and roots dry matter (IMSB) (IMSR), showed higher values in this last parameter indicating that shoots were more affected by salinity than roots. These results coincide with those from other researchers (18), who evaluated the saline stress effect in soybean cultivars; however, studies done by other researchers revealed that salinity had an inverse effect[B] (19). On the other hand, as for rates like ILB and ILR cultivars AT22 and INCASoy36 showed the best results compared to the rest of the genotypes.

From these results, damaged growth rates caused by NaCl at 150 mM. Though for soybean the lethal ion is chlorine (Cl^-) similar results also refer to other crops where the sodium ion (Na^+) is the responsible for the death induced by saline stress (20, 21, 22). High concentrations of Cl^- and Na^+ on the soil inhibits plants growth because they affect the water uptake and some biochemical processes like the protein synthesis and the assimilation of carbon dioxide and nitrates (23). Thus, salinity causes an unbalance of the required organic and inorganic compounds in plants leading to a growth suppression.

Evaluation of the Stability of the cell membrane

Saline stress caused by high solute concentrations produces water deficit and ionic unbalance in plant cells that affects the structure and function of the membranes. Plant capacity is an important element of the tolerance mechanisms to salinity (6).

This study showed significant differences among evaluated genotypes (Figure) in relation to the percentage of the cell membrane lesion. Cultivars AT22 and INCASoy 36 showed the lowest damages to cell membranes (9,37 % and 14,75 % respectively), when compared to the rest of the genotypes. In turn, the result of measuring the electrolyte leak for the cultivar INCASoy35 showed a lower EMC. Cell membrane for this cultivar was higher than 25 % (Figure). This effect reduced tolerance to salinity according to the described correlation (24). These results confirm that EMC can be used as a physiological indicator to evaluate tolerance to salinity of the genotypes.



Means with different letters in the same column of the table indicate statistical differences for $p < 0.05$ according to Tukey's means comparison

Percentage of cell damages in the nine soybean cultivars subjected to stress with NaCl at 150 mM

CONCLUSIONS

The physiological variables studied in early growth stages allowed to see differences in the behavior of different soybean genotypes to saline stress. These differences showed a high degree of variability to stress response. Cultivars AT22 and INCASoy36 showed the highest tolerance rates (ITS) and a higher integrity of the cell membrane accordingly, which suggests they are more tolerant to salinity. Genotypes AT22 and INCASoy36 are attractive candidates for further studies and breeding programs and as such, they are recommended after this study. On the other hand, protocols used allowed a quick analysis and evaluation of the genotypes under saline conditions.

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