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Original article

Salinity effect on the germination of grain cultivars under *in vitro* conditions

Efecto de la salinidad sobre la germinación de cultivares de granos en condiciones *in vitro*

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ABSTRACT: Soil salinization is currently one of the most serious problems facing agriculture, since it significantly affects food production s; for this reason, it is necessary to study cultivars tolerant to these conditions for their inclusion in the country's production schemes. The study aimed to select different grain cultivars for their tolerance to salinity: chickpea (*Cicer arietinum* L.); common bean (*Phaseolus vulgaris* L.); cowpea (*Vigna unguiculata* (L.)Walp); wheat (*Triticum aestivum* L.) and pea (*Pisum sativum* L.). The tolerance of 15 grain cultivars to different concentrations of sodium chloride (NaCl) (50, 150 and 200 mM), was studied under in vitro conditions.For this purpose, the following were determined: the germination percentage (PG) and the germination speed index (IVG) of the seeds as described by the Maguire index. Of the crops studied the bean cultivars PRL-8, Caujerí-2170 and San Francisco-219, of cowpea INIFAT-93, INIFAT-94 and INIFAT- 202 and of wheat I-399 were selected by their tolerance to 200 mM of NaCl, by showing the best results in terms of germination percentage and germination speed index, which is why they constitute promising materials to be used in agroecosystems affected by this condition. All of them showed differences in the seed germination speed index.

Key words: agroecosystems, cultivars, tolerance, salinity, promising materials.

RESUMEN: La salinización de los suelos en la actualidad es uno de los problemas más graves que enfrenta la agricultura, ya que afecta significativamente la producción de alimentos; por esta razón se hace necesario el estudio de cultivares tolerantes a estas condiciones para su inclusión a los esquemas productivos del país. El estudio tuvo como objetivo seleccionar por su tolerancia a la salinidad, diferentes cultivares de granos: garbanzo (*Cicer arietinum* L.); frijol común (*Phaseolus vulgaris* L.); frijol caupí (*Vigna unguiculata* (L.) Walp); trigo (*Triticum aestivum* L.) y guisante (*Pisum sativum* L.). Se estudió en condiciones in vitro la tolerancia de 15 cultivares de granos frente a diferentes concentraciones de cloruro de sodio (NaCl) (50, 150 y 200 mM), para este fin se determinó: el porcentaje de germinación (PG) y la velocidad de germinación (VG) de las semillas según describe Maguire. De los cultivos estudiados se seleccionaron por su tolerancia a 200 mM de NaCl, los cultivares de frijol PRL-8, Caujerí-2170 y San Francisco-219, de caupí INIFAT-93, INIFAT-94 e INIFAT-202 y de trigo I-399, al mostrar los mejores resultados en cuanto a porcentaje de germinación e índice de velocidad en la germinación, por lo que constituyen materiales promisorios para ser utilizados en agroecosistemas afectados por esta condición. Todas ellos mostraron diferencias en el índice de velocidad de germinación de semillas.

Palabras clave: agroecosistemas, cultivares, tolerancia, salinidad, materiales promisorios.

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INTRODUCTION

In recent decades, the climate and food crisis has led us to rethink our diets and their effects on the environment, since climate change causes a series of unfavorable phenomena for the proper development of agriculture (1). Furthermore, the lack of essential nutrients such as nitrogen (N), phosphorus (P), and potassium (K) in soils with these characteristics further limits their fertility (2). Among these, soil salinization constitutes one of the most serious problems facing agriculture, as it significantly affects its productivity, making the survival of plants and other life forms difficult due to high levels of electrical conductivity, excess ionic salts, and low water potential (3). These effects also cause irreparable damage, totally or partially limiting the proper growth of crops (4).

It is estimated that there are approximately 830 million hectares (ha) with salinization problems, which is equivalent to more than 6 % of the total world area and approximately 20 % of the total arable area (5,6).

In Cuba, 14.9 % of the agricultural area is affected by salinity and sodicity, of which 15 % or more of the irrigated area is at risk of becoming saline (7); therefore, salt stress is considered a growing threat to the country's agricultural development.

A partial solution to soil salinization problems is the use of crops and varieties more tolerant to salinity, capable of activating defense mechanisms that allow them to maintain their functioning and better adapt to adverse conditions (8). At the same time, isolating genes responsible for tolerance to this stress and modifying plant genomes for greater productivity in saline areas (9). Hence the need to provide knowledge on the current situation and perspectives of agriculture oriented towards food production with emphasis on the characterization of productive scenarios (10) and the links between producers for the necessary transition towards sustainable and local agrifood systems (11).

For this reason, selection criteria for tolerant genotypes are used as an indicator for their establishment under these conditions (9). However, working with cultivars with these characteristics under field conditions is a complex and expensive task, due to the levels of seeds required for this purpose. Nevertheless, evaluating the response of cultivars from the early stages of development allows for greater effectiveness in their selection and their salt stress tolerance indices (12).

In line with all these phenomena is the need to transition towards healthier, more diversified, and sustainable diets related to increasingly growing current food production and consumption systems. In this effort, grains play a key role due to their contribution of energy and nutrients globally and their health benefits (13).

For all these aspects, the present study aims to select, based on their salinity tolerance, different grain cultivars: chickpea (*Cicer arietinum* L.); common bean (*Phaseolus vulgaris* L.); cowpea (*Vigna unguiculata* L.); wheat (*Triticum aestivum* L.); and pea (*Pisum sativum* L.), for inclusion in food production programs in the country in areas affected by this type of stress.

MATERIALS AND METHODS

Biological material

Fifteen cultivars were used in the study: Chickpea (*Cicer arietinum* L.): Nacional-29 (N-29), Nacional 5-HA (N5-HA), Nacional 4x2 (4x2); Common bean (*Phaseolus vulgaris* L.): PRL-8, Caujeri 2170, San Francisco-219; Cowpea (*Vigna unguiculata* (L.) Walp): INIFAT-93, INIFAT-94, INIFAT-202; Wheat (*Triticum aestivum* L.): Cuba C 204, I 399, RM 04, RM 27; and Pea (*Pisum sativum* L.): Agro C-38, Afila 44, and Afila 64. These were obtained from the Central Germplasm Bank of the Instituto de Investigaciones Fundamentales en Agricultura Tropical "Alejandro de Humboldt", (Alejandro de Humboldt" Institute of Fundamental Research in Tropical Agriculture) INIFAT (according its acronyms in Spanish).

Trial to evaluate tolerance to different sodium chloride (NaCl) concentrations under controlled conditions

Seeds of each of the grains used in the experiment were disinfected with 4 % sodium hypochlorite for 15 minutes and washed three times with sterile distilled water. The experimental unit consisted of 140 cm diameter by 20 cm high glass Petri dishes, with filter paper moistened with distilled water placed at the bottom of the dishes. Twenty-five seeds (experimental unit) of each cultivar were placed inside the dishes. To each dish, 25 mL of different sodium chloride (NaCl) solutions (50, 150, and 200 mM) were applied, along with a control treatment (0 mM) where only sterile distilled water was added. The NaCl concentrations used in the study (50, 150, and 200 mM) were selected based on previous studies describing the germination response to a salinity gradient. Levels close to 50 mM are associated with mild stress, where the germination of most species remains above 70 %, allowing the detection of subtle differences between genotypes. In contrast, concentrations of 150 mM are considered moderate-to-high stress levels, where marked reductions in germination percentage and germination speed index are recorded, but sufficient data remain for statistical analysis of tolerance. Values of 200 mM NaCl are described as severe salinity, where decreases exceeding 80-90 % in germination and initial root and hypocotyl growth are observed, as well as significant alterations in ionic balance (increase in Na^+ and decrease in K^+, Ca^{2+}, and Mg^{2+}), allowing the evaluation of the response under extreme stress conditions (14). The dishes were arranged following a completely randomized experimental design, at a temperature of 25 °C and 80 % humidity. Three replicates were performed per variant.

Germinated seeds were counted daily from establishment until stabilization. The following parameters were determined from the data obtained

Germination Percentage (GP): The experiment was monitored for 15 days, and seeds were considered germinated when the radicle emerged to a length equal to or greater than 2 mm. Germination percentages per treatment were calculated using Equation 1, as referenced (15).

$$GP\ (\%) = \left(\frac{Number\ \ of\ \ germinated\ \ seeds}{Number\ \ of\ \ sown\ \ seeds}\right) \times 100$$

Germination Speed (GS): Calculated according to Equation 2, as referenced (16), where: GS = germination speed; G_i = number of seeds germinated at time i; N_i = time i (hours).

$$GS = \frac{G_1}{N_1} + \frac{G_2}{N_2} + \dots + \frac{Gi}{Ni} + \dots + \frac{Gn}{Nn} = \sum_{i=1}^{n} \frac{Gi}{Ni}$$

Experimental design and statistical analysis

The results (GP, GS) were subjected to analysis of variance (ANOVA) with Duncan's Multiple Range Test (5 % error probability) to detect differences among treatment means. The STATGRAPHICS Plus version 5.0 software was used for this purpose.

RESULTS AND DISCUSSION

When analyzing the results obtained, significant differences were observed between crops and species, with a reduction in germination percentages in some genotypes as salinity levels increased. In general terms, regarding germination percentage (GP), almost all cultivars studied showed 100 % germination in the 0 mM treatment, demonstrating the quality of the seeds used in the trial. Regarding the germination speed index (GSI), the bean cultivar PR-L8 and the wheat cultivar RM-27 stood out, achieving 90 % in this indicator in the 0 mM treatment.

Figure 1 shows the results for chickpea (*Cicer arietinum* L.) in terms of germination percentage (GP). No significant differences were observed between the control and the 50 mM sodium chloride (NaCl) treatment for cultivar Nacional-29 with respect to the others, a trend that remained at 150-200 mM NaCl concentrations for the same cultivar. The response of Nacional-5HA and 4X2 was similar, although with lower values than Nacional-29 in both germination percentage and germination speed index.

In chickpea, cultivar Nacional-29 stood out in germination percentage and germination speed index (GSI) compared to the others, although its best performance was at concentration values between 50-150 mM. However, other authors describe the sensitivity of this crop to salinity, which negatively affects not only its germination but also its reproductive processes (17). It is proposed that this stress decreases flowering and fruiting, resulting in effects on the ovule fertilization process. Nevertheless, differences exist among chickpea genotypes in salinity tolerance (18), an aspect also demonstrated in the results obtained in the present study for the evaluated variables, thus clearly indicating the need for cultivars with these attributes.

Figure 2 shows the results obtained for common bean (*Phaseolus vulgaris* L.). When evaluating germination percentage (GP), no significant differences were observed between the control and the three levels of sodium chloride studied. However, regarding germination speed index (GSI) at 50 mM, the cultivars PRL-8 and Caujeri-2170 stood out, although Caujeri-2170 excelled at all three salt levels studied. When assessing the results in common bean, it is evident that the three cultivars show promising results for further studies.

In this crop, other authors found that the use of sodium-alkaline salts limits root growth due to hydrolytic processes, where Na^+ acts on the meristematic regions, destroys plant cells, and inhibits hormone synthesis, thereby affecting plant growth (19).

In this regard, other studies evaluating salt concentrations similar to those used in our research on two bean cultivars demonstrated a decrease not only in germination percentage but also in germination speed as the concentrations of this element increased (20). When evaluating the response to salinity induced by NaCl, sodium sulfate (Na_2SO_4), and sodium bicarbonate ($NaHCO_3$) in common bean, it was observed that fresh biomass decreased with $NaHCO_3$, whereas NaCl increased it (19). These studies reveal the diverse responses that can be obtained in this crop and highlight the importance of further research in this field, as demonstrated by the potential of the three evaluated cultivars and their possible inclusion in soils affected by salinity.

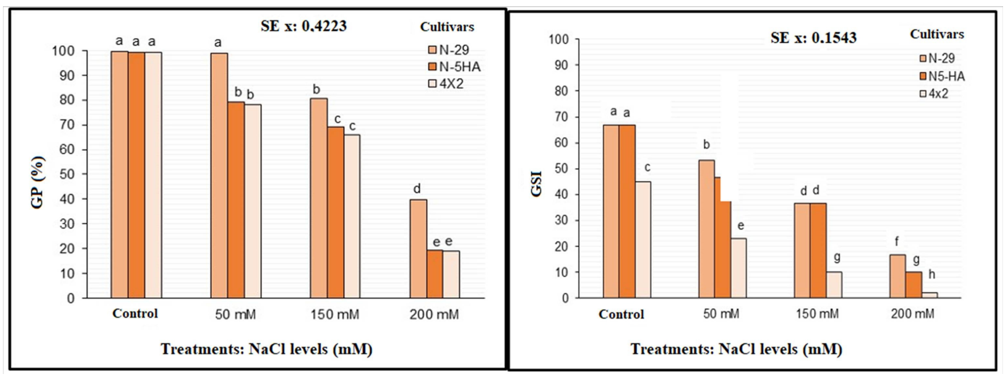

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Figure 1. Germination percentage (GP) and germination speed index (GSI) of chickpea (*Cicer arietinum* L.) cultivars: N-29, N5-HA, and 4x2, in the presence of different sodium chloride (NaCl) concentrations (50, 150, and 200 mM) and an absolute control

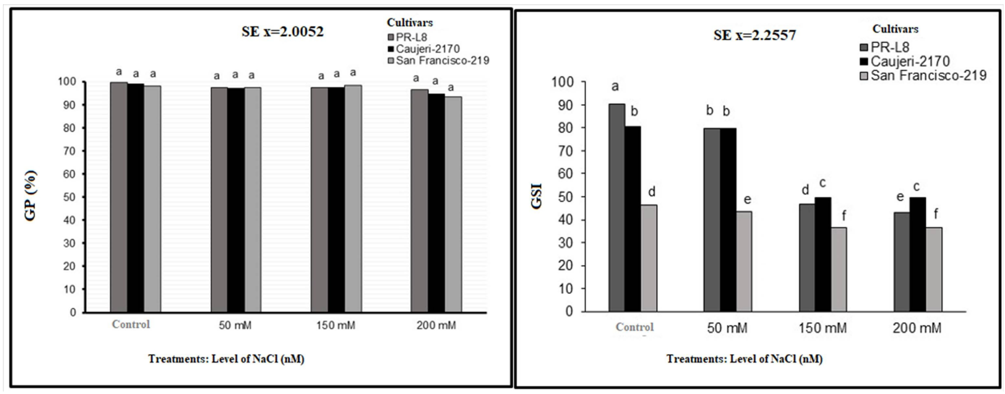

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Figure 2. Germination percentage (GP) and germination speed index (GSI) of common bean cultivars (*Phaseolus vulgaris* L.): PRL-8, Caujeri 2170, and San Francisco-219, under different sodium chloride (NaCl) concentrations (50, 150, and 200 mM) and an absolute control

Figure 3 shows the results for cowpea. Regarding germination percentage (GP), the trend was very similar to that observed in common bean, as no significant differences were found among cultivars INIFAT-93, INIFAT-94, and INIFAT-202, demonstrating their potential for further study under different salt concentrations. Regarding germination speed index (GSI) at 50-150 mM NaCl, INIFAT-94 and INIFAT-202 stood out, with no statistical differences between them, followed by INIFAT-93. However, the positive results of all three cultivars are noteworthy overall. This type of laboratory study promotes seed saving and also allows the evaluation of different species in a short period of time, serving as a basis for their inclusion in productive systems.

For cowpea, other studies describe varietal effects on both germination percentage and germination or emergence speed index, where differences in root length are also observed, an aspect that suggests the mobilization of reserve substances or their translocation during the crop growth stage (21).

Figure 4 shows the results for wheat. Regarding germination percentage (GP), no significant differences were observed between the control and 50 mM NaCl in the cultivars studied (Cuba C-204, I-399, RM-04, and RM-27). The same trend was observed at the 150 and 200 mM levels for cultivar I-399, with results similar to those found in both the control and the 50 mM level, demonstrating the superiority of this cultivar over the others. The same occurs with the germination speed index (GSI), where the same cultivar also stands out.

In this regard, researchers evaluated 400 wheat cultivars from different species during germination and the seedling stage. In these studies, they found a high genetic diversity that allowed them to select those that showed greater tolerance and susceptibility to saline levels (22). Likewise, in other studies, twenty genotypes were subjected to different salinity levels, which confirmed that Na^+ exclusion is related to ionic improvement (Na^+, K^+, and Na^+/K^+ ratio) as well as to the physiological traits present in different wheat genotypes under these stress conditions. Some of them, in the presence of high NaCl concentrations, manifested growth problems in root architecture, shoots, reduced leaf area, reproductive development, and maturation (8).

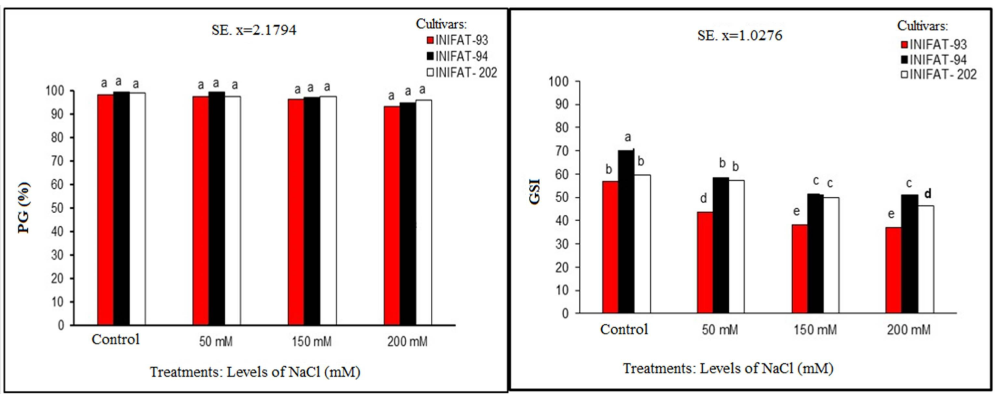

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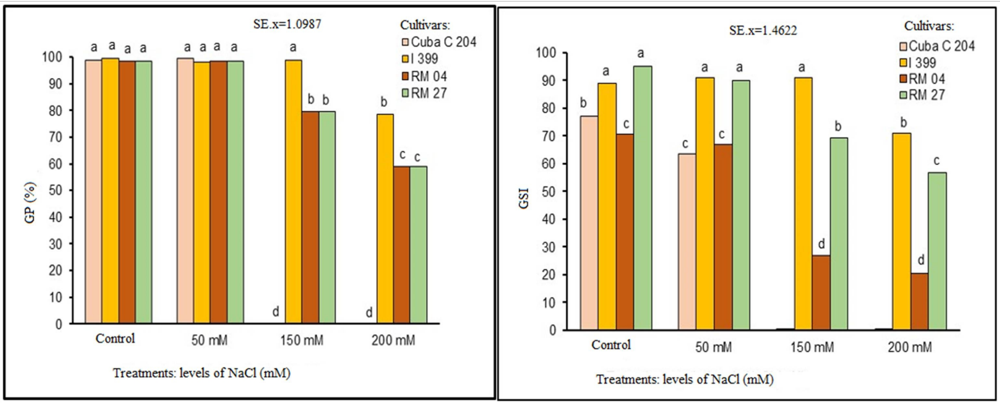
Figure 3. Germination percentage (GP) and germination speed index (GSI) of cowpea cultivars (*Vigna unguiculata* L. Walp): INIFAT-93, INIFAT-94, INIFAT-202, in the presence of different sodium chloride (NaCl) concentrations (50, 150, and 200 mM) and an absolute control



Means followed by the same letter in the same column are not significantly different according to Duncan's multiple range test (α = 0.05). n = 25

Figure 4. Germination percentage (GP) and Germination speed index (GSI) of wheat (*Triticum aestivum* L.) cultivars Cuba C 204, I 399 and RM 04, in the presence of different sodium chloride (NaCl) concentrations (50, 150 and 200 mM) and an absolute control

Similarly, they point out that the germination response was one of the variables with the highest degree of differentiation under this type of stress (23). They also state that salt stress affects plant water relations through osmotic and water stress, which is why some plants develop the osmotic adjustment process to maintain cell turgor and ensure growth, transport, accumulation, and compartmentalization of ions (21). All these studies support the results obtained in the present investigation and the importance of conducting them.

Figure 5 shows the results for pea. Regarding germination percentage (GP), all three cultivars showed excellent results at 50 mM NaCl, while at 150 mM, cultivar Afila 44 stood out with more than 70 %, followed by Afila 64 with lower values (60 %). At 200 mM, with only 40 %, Afila 44 and Afila 64 showed the same trend with no significant differences between them; therefore, they are not proposed for further studies.

In peas, it is described that when exposed to short-term salt concentrations, Na^+ increases in the xylem sap of non-acclimated plants, but if they are adapted, they reduce this flow and maintain a higher K^+/Na^+ ratio in the xylem. The functioning of PSII is altered, and ROS generation increases in leaves under light, forcing the activation of antioxidant defenses. These coordinated adjustments (control of ion flow, Na^+ sequestration, osmotic and antioxidant adjustment) are part of the multifactorial traits that determine tolerance or sensitivity to salinity in peas (24).

In general terms, regarding grain crops, it is stated that increasing NaCl levels causes a delay in seed germination, as well as in other indicators such as root length and plant height (4). Other studies also report that the sodium ion (Na^+) is responsible for salt-stress-induced death (25) and that high concentrations of Cl^- and Na^+ in the soil inhibit plant growth, as they affect water absorption and biochemical processes such as protein synthesis and the assimilation of carbon dioxide and nitrates (4). All these aspects cause substantial losses in plant growth and development (26), which equally affects yields (27). It is also explained how the high concentration of these compounds in the soil, water, and air generates an imbalance in the biogeochemical cycles and food chains of agricultural areas, leading to a decrease in productive capacity and difficulty in controlling pests, diseases, and weeds that have developed resistance (2). Studies of this type also demonstrate the role of enzymatic and non-enzymatic defense systems in salinity tolerance in cultivars that managed to grow optimally at 100 mM NaCl (8) and the importance of studying genotypes that tolerate stressful conditions (28).

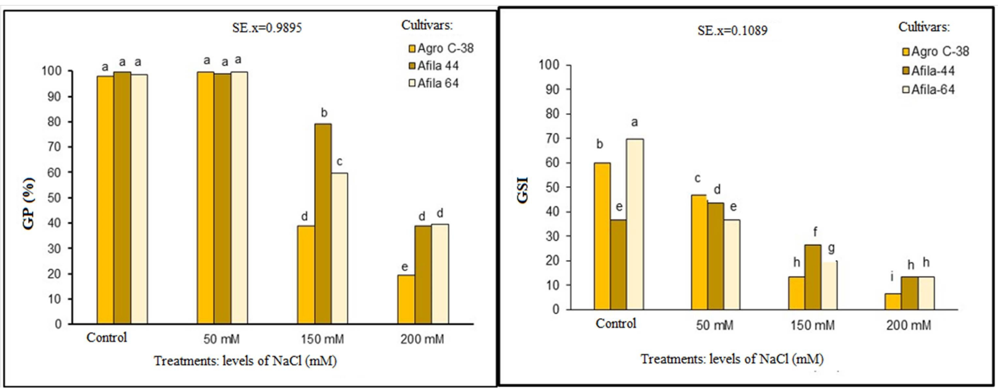


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Figure 5. Germination percentage (GP) and Germination speed index (GSI) of pea (*Pisum sativum* L.) cultivars Agro C-38, Afila 44 and Afila 64, in the presence of different sodium chloride (NaCl) concentrations (50, 150 and 200 mM) and an absolute control

When analyzing the overall results obtained regarding seed germination and germination speed index under the studied saline levels, differences were observed between crops and species, with a reduction in germination percentages in some genotypes as salinity levels increased. This allowed us to conclude the negative effect exerted by high salt levels in the soil and the importance of managing cultivars capable of tolerating these conditions, in order to continue studies and select the most promising ones to achieve positive results for their inclusion.

The Table 1 shows the tolerant cultivars against the different salinity ranges studied is presented.

Table 1. Tolerant cultivars to 50-200 mM sodium chloride (NaCl) levels based on germination percentage (GP)

Tolerant up to 200 mM NaCl	
Beans (*Phaseolus vulgaris* L.)	PRL-8
	Caujerí 2170
	San Francisco-219
Cowpea (*Vigna unguiculata* L.Walp)	INIFAT-93
	INIFAT-94
	INIFAT- 202
Wheat (*Triticum aestivum* L.)	I 399
Tolerant up to 150 mM of NaCl	
Chickpea (*Cicer arietinum* L.)	N-29
	N-5HA
	4X2
Wheat (*Triticum aestivum* L.)	RM 04
	RM 27
Pea (*Pisum sativum* L.)	Afila 44
Tolerant to up to 50 mM of NaCl	
Pea (*Pisum sativum* L.)	Agro C-38
	Afila 64
Wheat (*Triticum aestivum* L.)	Cuba C 204

The results obtained in this study represent a significant contribution, as they enable the selection of promising cultivars for use in salinity-affected agroecosystems, thus contributing to increasing grain diversity for food and agriculture in the country.

Los resultados obtenidos en la presente investigación constituyen una importante contribución, ya que facilitan la selección de cultivares promisorios para realizar este tipo trabajos en agroecosistemas afectados por salinidad y de esta forma contribuir a incrementar la diversidad de granos para la alimentación y la agricultura en el país.

CONCLUSIONS

1. There is high genetic variability among the germplasm studied regarding their level of tolerance to salinity, with differences in germination percentage and germination speed index.
2. From 15 cultivars studied, seven were tolerant to 200 mM (the highest concentration studied), six to 150 mM, and three to 50 mM NaCl, indicating their potential for further studies.

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