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Original article

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

Efecto de la salinidad sobre la germinación de cultivares de hortalizas 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 vegetables cultivars for their tolerance to salinity: of vegetables: Tomato (*Solanum lycopersicum* L.); Lettuce: (*Lactuca sativa* L.); Chinese cabbage: (*Brassica rapa* subsp. pekinensis (Lour.) Hanelt; Chinese beet: (*Brassica rapa* L. subsp. chinensis (L.) Hanelt; another of Broccoli: (*Brassica olearacea* var, Italica); Carrot: (*Daucus carota* L.) and Radish: (*Raphanus sativus* L.). The tolerance of 13 vegetables cultivars to different concentrations of sodium chloride (NaCl) (50 mM, 150 mM, 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 vegetables Chinese cabbage: N-100 Chinese beet: Aniela, another of Broccoli; Lettuce Chile 1185-3 and radish: PS9 and C 88, to present bigger germination percentage to values among 50-200 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. The rest of the cultivars didn't tolerate the ranges of salinity that were evaluated in the study.

Key words: seeds, tolerance, saline stress.

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 hortalizas: Tomate (*Solanum lycopersicum* L.); Lechuga: (*Lactuca sativa* L.); Col china: (*Brassica rapa* subsp. pekinensis (Lour.) Hanelt; Acelga china: (*Brassica rapa* L. subsp. chinensis (L.) Hanelt; Brócoli: *Brassica oleracea* var, Italica: Tropical F-8; Zanahoria: (*Daucus carota* L.) y Rábano: (*Raphanus sativus* L.). Se estudió en condiciones *in vitro* la tolerancia de 13 cultivares de hortalizas frente a diferentes concentraciones de cloruro de sodio (NaCl) (50 mM, 150 mM, 200 mM), para este fin se determinó: el porcentaje de germinación (PG) y el índice de velocidad de germinación (IVG) de las semillas según describe el índice Maguire. De los cultivos estudiados se seleccionaron como promisorios la Col china: N-100, Acelga china: Aniela, Brócoli: Tropical F-8, Lechuga: Chile 1185-3, Rábano: PS9 y C 88, por presentar mayor porcentaje de germinación a valores entre 50-200 mM de NaCl, 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. El resto de los cultivares no toleraron los rangos de salinidad que se evaluaron en el estudio.

Palabras claves: semillas, tolerancia, estrés salino.

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INTRODUCTION

Climate change and the severity of extreme meteorological phenomena, including droughts and heat waves, are conditions that favor an increase in groundwater for consumption and irrigation, which leads to greater depletion of the water table and facilitates salt seepage into the soil (1). On the other hand, the lack of essential nutrients such as nitrogen (N), phosphorus (P) and potassium (K) in soils with these characteristics further limits their fertility (2). Salinity also causes great damage, which totally or partially restricts adequate crop growth (3). Worldwide, it is estimated that approximately 830 million hectares (ha) have salinization problems, which corresponds to more than 6 % of the total world surface and about 20 % of the total arable land (4).

In Cuba, 14.9 % of the agricultural area is affected by salinity and sodicity and it is estimated that 15 % or more of the irrigated area is in danger of salinization (5), so that salt stress is a growing threat to the development of agriculture in the country, since it significantly affects its productivity (6). This phenomenon limits the development of horticultural crops, since it causes alterations both in plant growth and in the low absorption and distribution of nutrients to their different organs (7). This is an aspect that threatens food security and the increase of vegetable production in the country. In order to contribute to this problem, the possibility of producing vegetables on a small scale is being evaluated, with the use of available backyards and organoponics and on a larger scale with the provision of land in usufruct, among other ways. In recent years, these family vegetable systems have become a significant alternative to satisfy nutritional demands, both in rural and urban areas (8).

However, it is necessary to address the problems of soil salinization by searching for cultivars with greater tolerance to this type of stress, which will allow the production of a diversity of crops in these environments (9). For all these aspects, the present study aims to select different vegetable cultivars for their tolerance to salinity: Tomato (*Solanum lycopersicum* L.); Lettuce: (*Lactuca sativa* L.); Chinese cabbage: (*Brassica sativa* L.); Chinese cabbage: (*Brassica sativa* L.).); Chinese cabbage: (*Brassica rapa* subsp. pekinensis (Lour.) Hanelt; Chinese chard: (*Brassica rapa* L. subsp. chinensis (L.) Hanelt; Broccoli: (*Brassica oleracea* var, Italica); Carrot: (*Daucus carota* L.) and Radish: (*Raphanus sativus* L.).

MATERIALS AND METHODS

Biological material: 14 cultivars were used in the study: Tomato (Solanum lycopersicum L.): cultivars T60, M 44 and FL-5; Lettuce: (*Lactuca sativa* L.): cultivars BSS13, Fomento 95 and Chile 1185-3; Chinese cabbage: (*Brassica rapa* subsp. pekinensis (Lour.) Hanelt: cultivar N-100; Chinese chard: (*Brassica rapa* L. subsp. chinensis (L.) Hanelt: cultivars aniela and PK-7; Broccoli: (*Brassica oleracea* var, Italica) tropical cultivar F-8; Carrot: (*Daucus carota* L.): cultivars B5 and NK-6 and Radish: (*Raphanus sativus* L.): cultivars PS9 and C 88, from the Central Germplasm Bank of the Institute for Fundamental Research in Tropical Agriculture "Alejandro de Humboldt", INIFAT.

Test to evaluate tolerance to different values of sodium chloride (NaCl) under controlled conditions: seeds of each of the vegetables used in the experiment were disinfected with 4 % sodium hypochlorite for 15 min and washed three times with sterile distilled water. The experimental unit corresponded to glass Petri dishes 140 cm in diameter and 20 cm high, with filter paper moistened with distilled water on the bottom of the dishes. Twenty-five seeds (experimental unit) of each cultivar were used inside the plates. 25 mL of different sodium chloride (NaCl) solutions (50 mM, 150 mM, 200 mM) (millimoles) were applied to each plate, and a control treatment with 0 mM where only sterile distilled water was added. Plates were arranged in a completely randomized experimental arrangement, at a temperature of 25 °C and a humidity of 80 %. For each variant, three replicates were performed.

Germinated seeds were counted daily from establishment to stabilization, with the data obtained were determined:

Germination percentage (GP): the experiment was monitored for 15 days and the appearance of radicle greater than or equal to 2 mm was considered as germinated seeds. For the calculation of germination percentages by treatment, equation (10) was used.

$$\mathrm{GP}(\%) = \text{Number of germinated seeds} \\ /\text{Number of seeds sown x100}$$

Germination Velocity Index (GVI): represents the germination velocity calculated through a weighted time of accumulated germination. Where G is the percentage of seedlings that germinated during the time interval t (11, 12).

Experimental design and statistical analysis: the results (GP, GVI) were subjected to an analysis of variance (ANOVA) with Duncan's Multiple Range tests (5 % probability of error) to detect differences between treatment means. The STATGRAPHIS Plus version 5.0 program was used for this purpose.

RESULTS AND DISCUSSION

When analyzing the results obtained, significant differences were observed between species and cultivars, with a reduction in germination percentages in some genotypes as salinity levels increased. As for the germination percentage (GP), in the 0 mM treatment almost all the cultivars studied showed 100 % germination, which demonstrates the quality of the seeds used in the trial, with the exception of carrot NK-6, which only reached 80 % in this indicator. In the germination speed index, the three lettuce cultivars evaluated and the radish C-88 stand out. However, when studying the NaCl level of 50 mM, the behavior of Chinese chard with cultivar PK-7 stands out, with significant differences with respect to the rest of the vegetables tested.

Figure 1 shows the results for tomato (*Solanum lycopersicum* L.), in terms of germination percentage (GP), in the treatment with 0 mM the three cultivars showed 100 % germination and when using doses of 50 mM NaCl, T60 and FL-5 showed superior results with respect to M 44,

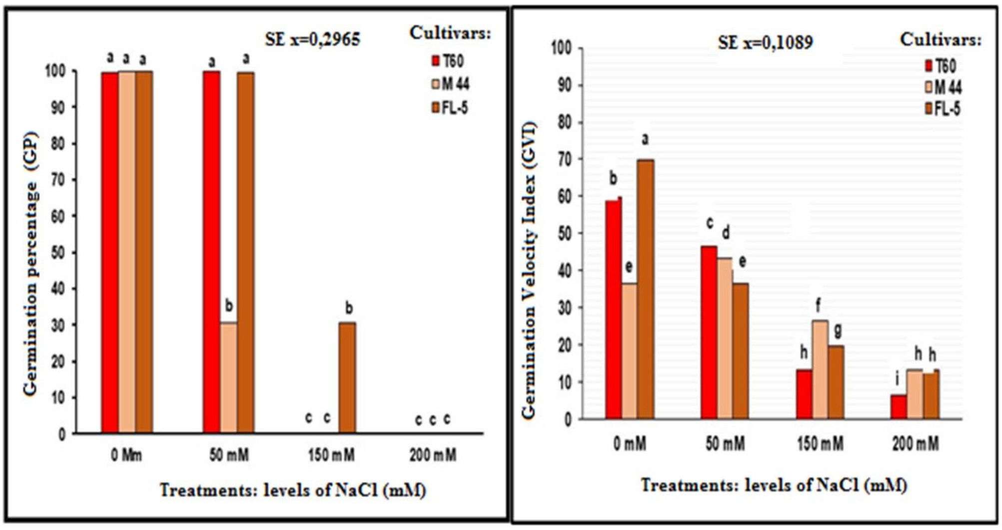

Figure 1. Germination percentage (PG) and Germination Velocity Index (GVI) of Tomato (*Solanum lycopersicum* L.) cultivars: T60, M 44 and FL-5, under NaCl concentrations (50 mM, 150 mM, 200 mM) and an absolute control.

with significant differences among them. The remaining salt levels evaluated did not show a favorable response in any of them, only in the case of FL-5, where 30 % of the seeds germinated at 150 mM NaCl, which is considered very low. For the germination speed index (GVI), it can be seen how the FL-5 cultivar stands out in the control treatment with 0 mM and consecutively T60 and in 50 mM T60, M 44 and FL-5, in that order, with significant differences among them. However, this indicator shows that the germination speed of the cultivars evaluated in general is relatively low, which could be due to different factors such as salt concentrations, as these increased, the GVI decreased considerably, among other aspects that could have influenced these results. Therefore, only T60 and FL-5 could be recommended for soils with a moderate salinity level; in other conditions of greater severity, these cultivars would not be of interest for further studies of this type.

Studies that evaluated the effect of salt stress on tomato seeds (*Solanum lycopersicum* L.), at different concentrations, showed that the germination rate decreased significantly (13). Similarly, other authors evaluating the impact of this culture at a concentration of 50 mM NaCl on Florada cultivar described how the germination rate was also reduced by 14 % with respect to the control (14).

Our results corroborate with those obtained previously and also an author observed that the germination rate of tomato seeds cv 'Rio Grande (15), decreased significantly compared to the control treatment by increasing NaCl concentrations. Similarly, as reported by some authors (16), not only in the germination of this crop, but they also state that salinity affected the other metabolic and photosynthetic processes in this crop.

In the results obtained (Figure 2) in the percentage of germination in Chinese cabbage: N-100, Chinese chard: Aniela and PK-7 and Broccoli: Tropical F-8, 100 % germination was observed in the treatment with 0 mM for the four cultivars. This behavior is maintained for all of them at 50 mM NaCl. These results, without significant differences among them, are maintained in the same way at concentrations of 150 and 200 mM NaCl in the cultivars N-100 and Aniela at these same concentrations, although with lower results than these, followed by the cultivar Tropical F-8. In the case of PK-7 the results were inferior to the rest with 60 % of germination at 150 mM NaCl and at 200 mM it did not germinate. Regarding the germination speed index, the best response was that of Chinese cabbage (N-100), which stood out from the rest of the cultivars in this indicator with 95 % germination at 50 mM NaCl, a trend that is maintained at 0 Mm. However, it should be noted that the best results for their completeness were those of Chinese cabbage (N-100) and Chinese chard (Aniela), which could be useful for studies of this type.

The generally good performance of all these cultivars may be due to stress tolerance mechanisms, almost exclusive to the Brassicacea family, where the synthesis of metabolites such as glucosinolates (GSLs) stands out (17). On this subject, it is well documented the correlation that exists when increasing salt levels, there is also an increase in the production of these metabolites, which allows greater tolerance to salinity to plant species that belong to this family (18).

Regarding studies on other species of the Brassica family, in this case cabbage (*Brassica oleracea* capitata L.), it is described in the same way how high levels of salt inhibit germination and growth (19).

Figure 3 shows the results in the cultivation of lettuce (*Lactuca sativa* L.), in the same in the germination percentage (GP), at concentrations from 0 mM to 150 mM NaCl, the three cultivars tested stand out. However, the cultivar Chile 1185-3 stands out with respect to the rest up to 200 mM. This orientation is maintained when analyzing the germination speed index, so it is considered the most promising cultivar to extend its results. Although, the rest of the cultivars studied also show positive results that place them in a favorable position for this type of studies.

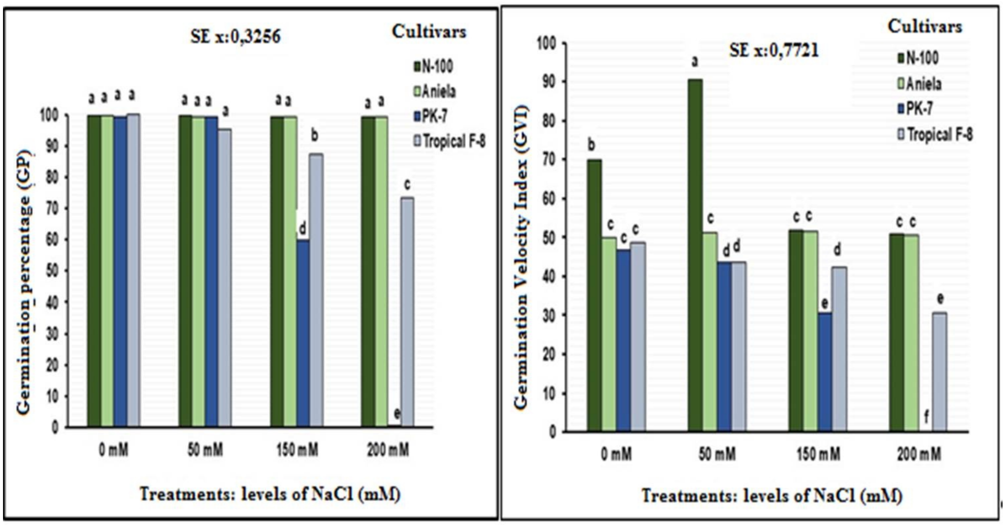

Figure 2. Germination percentage (GP) and Germination Velocity Index (GVI) of cultivars of Chinese cabbage: (*Brassica rapa* subsp. pekinensis (Lour.) Hanelt: N-100; Chinese chard: (*Brassica rapa* L. subsp. chinensis (L.) Hanelt: Aniela and PK-7 and on Broccoli: *Brassica oleracea* var, Italica: Tropical F-8, under NaCl concentrations (50 mM, 150 mM, 200 mM) and an absolute control.

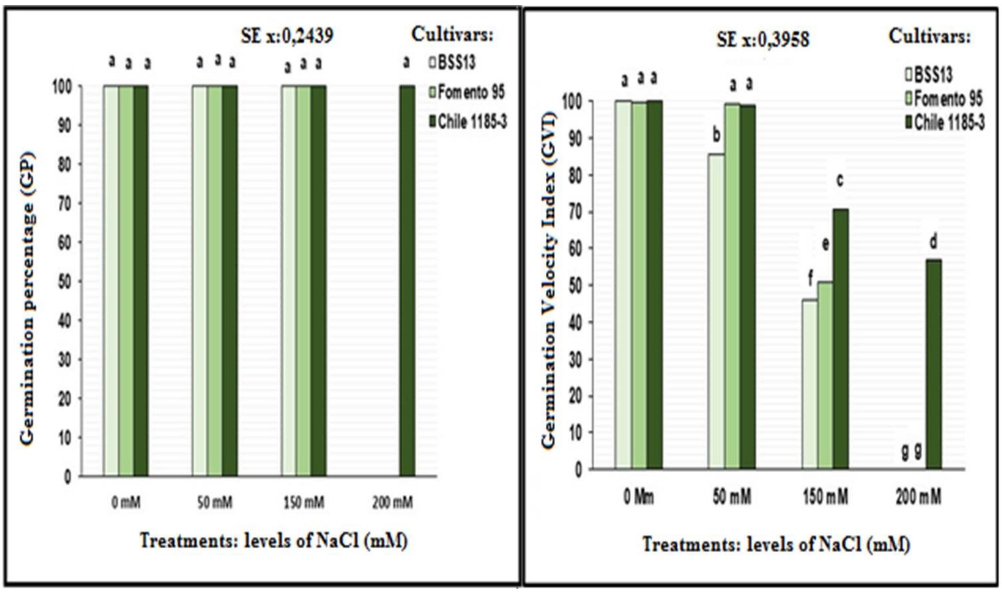

Figure 3. Germination percentage (PG) and Germination Velocity Index (GVI) of Lettuce cultivars: (*Lactuca sativa* L.): BSS13, Fomento 95 and Chile 1185-3, under NaCl concentrations (50 mM, 150 mM, 200 mM) and an absolute control.

Concerning this some researchers (20) describes how in studies carried out in the germination stage of *Lactuca sativa* L. they found that the length of the plumule and radicle decreased when the salinity level increased. In the present investigation, the results obtained were satisfactory in two of the cultivars studied, which shows their potential.

In the results in the radish cultivars (*Raphanus sativus* L.): PS9 and C-88, it can be seen that in the germination percentage (GP), with 0 mM both showed 100 % germination, which is maintained when evaluated against the three levels of NaCl investigated (Figure 4), so they are considered of interest for future work. When evaluating the germination speed index, differences were observed between them both at 0 Mm and at the three concentrations of NaCl evaluated, although at 0 mM C-88 predominates with significant differences in the three concentrations, so that work with any of them is not ruled out.

On the other hand, some authors evaluated the physiological behavior of radish plants (*Raphanus sativus* L.) subjected to salinity stress (21), showing that they did not show tolerance to this type of stress. Similarly others (22) emphasize the negative influence of saline treatments on this crop. Our results differ from these studies, since the Cuban cultivars evaluated showed a positive response to different levels of salinity, making them attractive for use in soils with this type of condition.

Figure 5 shows the results in the cultivation of carrot (*Daucus carota* L.) in the germination percentage (GP), in this case in the treatments with 0 Mm and 50 mM NaCl shows the best results in the cultivar B5 with 100 % germination with significant differences with respect to NK-6. This tendency is maintained in the rest of the concentrations, although with very low values of this indicator (20 - 30 %).

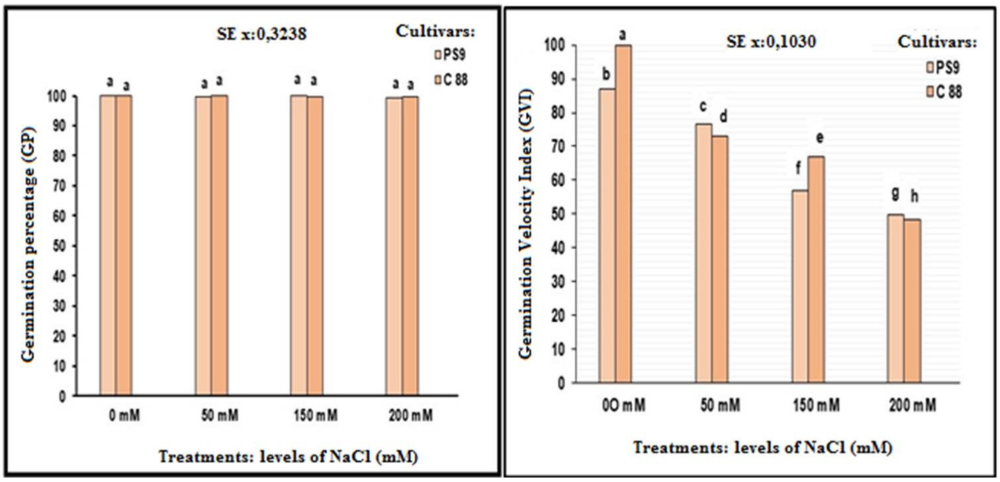

Figure 4. Germination percentage (PG) and Germination velocity index (GVI) of radish (*Raphanus sativus* L.) cultivars: cultivars PS9 and C 88, under NaCl concentrations (50 mM, 150 mM, 200 mM) and an absolute control.

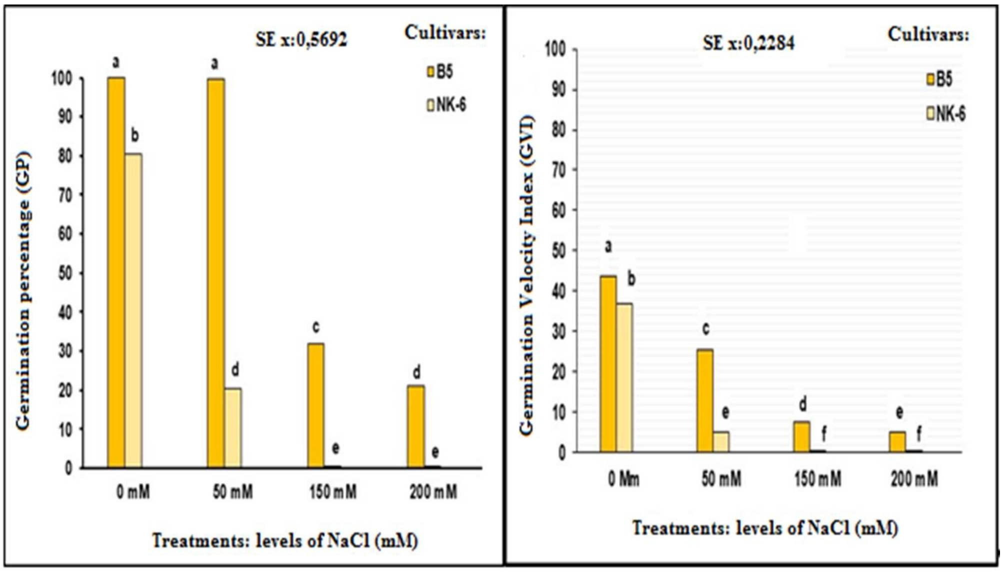

Figure 5. Germination percentage (GP) and Germination Velocity Index (GVI) of Carrot cultivars: (*Daucus carota* L.): B5 and NK-6, under NaCl concentrations (50 mM, 150 mM, 200 mM) and an absolute control.

The same tendency is seen in the germination speed index, although B5 stands out slightly with very low values in all cases, so that these cultivars are not proposed for studies with high salinity levels.

In this regard, it is described how salinity directly affects plant cells inhibiting germination and growth of different crops, which can be caused by different processes such as cellular homeostasis among others, which limits their photosynthetic capacity due to the restriction of CO_2 supply (23).

In the same way, in works where other vegetables are handled, it is defined as in some studies when exposing seeds to increasing isosmotic solutions of NaCl, higher germination percentages were detected (24), although, in other cases the effects for this concept are negative (25).

The results in the present research constitute an important contribution, since they facilitate the selection of promising cultivars to carry out this type of work in agroecosystems affected by salinity and thus increase the diversity of vegetables for food and agriculture in the country. Much more, the great variability shown by the cultivars in relation to salt concentrations is known, a result of great value that allows knowing the attributes of these and proposing them for studies of this type.

CONCLUSIONS

- The germplasm studied showed high genetic variability in terms of their level of tolerance to salinity, with differences between germination percentage and germination speed index.
- From 14 cultivars studied, five were tolerant to 200 mM, the highest concentration studied, two to 150 mM and four to 50 mM NaCl, which shows their potential for further studies. Only two cultivars did not tolerate the NaCl ranges studied.

Table 1. Cultivars tolerant to sodium chloride (NaCl) levels of 50-200 mM, according to germination percentage (GP)

Tolerates up to 200 mM NaCl	
Chinese cabbage (*Brassica rapa* subsp. pekinensis (Lour.) Hanelt	N-100
Chinese chard (*Brassica rapa* L. subsp. chinensis (L.) Hanelt).	Aniela
Broccoli: *Brassica oleracea* var, Italica	Tropical F-8;
Lettuce (*Lactuca sativa* L.)	Chile 1185-3
Radish (*Raphanus sativus* L.)	PS9
	C 88
Tolerates up to 150 mM of NaCl	
Lettuce (*Lactuca sativa* L.)	BSS13
	Fomento 95
Tolerates up to 50 mM of NaCl	
Tomato (*Solanum lycopersicum* L.)	T60
	FL-5
Chinese chard (*Brassica rapa* L. subsp. chinensis (L.) Hanelt..	PK-7
Carrot (*Daucus carota* L.)	B5
Intolerant to NaCl	
Tomato (*Solanum lycopersicum* L.)	M 44
Carrot (*Daucus carota* L.)	NK-6

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