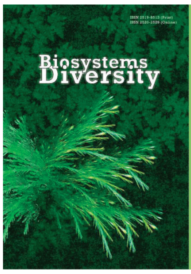

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Effect of degree of salinity on seed germination and initial growth of chickpea (*Cicer arietinum*)

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Chickpea (*Cicer arietinum* L.) is one of the main pulse crops cultivated mostly in the arid and semi-arid regions of the world, very often on saline lands. The problem is that it has not been clearly determined yet what is the safe salinity degree for obtaining uniform and vigorous sprouts of the crop without significant suppression in the parameters of initial growth and development. The goal of our study was to determine the effect of different NaCl concentrations in solutions on chickpea germination and initial growth to determine the safe degree of salinity for the crop cultivation. The study was carried out in greenhouse conditions of Kherson State Agrarian University. We studied the effect of five different gradually increasing degrees of NaCl solutions on the germination percentage and initial growth of chickpea (variety Rosanna, kabuli type) that was germinated in laboratory conditions in flasks filled with sand, at the temperature of 25 °C. A significant decrease in all the studied parameters was observed with the increase of salinity degree. However, we think that a considerable decrease of the crop germination and initial growth started with NaCl concentration of 1.79 g/L: germination percentage decreased by 33.9%, plant height – by 7.8 cm, root length – by 5.5 cm in comparison to the control variant (not saline conditions). Therefore, we conclude that the chickpea can be efficiently cultivated on slightly-saline lands. Besides, the results of linear regression analysis revealed that the most susceptible stage of chickpea growth and development is germination because this stage had strong close inter-connection with the degree of salinity. Further growth of the crop was less affected by the salinity stress. We recommend cultivation of chickpea on the saline lands only with a slight salinity level.

Keywords: chickpea; plant development; plant height; root length; salt stress.

Introduction

Chickpea (*Cicer arietinum* L.) comes from South-Eastern Turkey and Syria (Singh, 1997). It is a valuable leguminous crop with a great nutritional and dietary value due to the high content of protein, irreplaceable aminoacids and vitamins in the grain, that can help in solving the problem of malnutrition in a number of countries of the African and Asiatic region and helps in the treatment of some human diseases (Jukanti et al., 2012). In the period of 1985–1987, chickpea was cultivated in about 35 countries, mostly Asian, with the total grain yield of 7.1 Mt (Singh, 1990). And nowadays, the crop is cultivated on the area of 14.56 Mha with the total yield of 11.5 Mt, so, we see that there is an evident tendency to the increase of interest in chickpea cultivation throughout the world, especially, in the countries of North America, Australia, Europe (Merga & Haji, 2019).

The increased interest in the crop, widening of its cultivation area and environment has caused an increased concern in the development of the rational cultivation technology of the crop when it is grown for different purposes. One of the important subjects related to chickpea cultivation technology is its reaction to different degrees of soil salinity. It is believed that soil salinity is one of the main factors that results in the crucial decrease of productivity of crops (Priyadharshini et al., 2019). And chickpea is often cultivated not only in the arid climatic conditions of the Asian and African regions, but on the saline soils or on the lands irrigated with saline water, for example, in India, Pakistan and Australia (Vadez et al., 2007; Haileselasie & Teferii, 2012). The main cultivation areas for chickpea in Ukraine are located in the South of the country, and the plants are often exposed to salinity stress there. For example, there is the large saline and alkaline land tract of the Ingulets irrigation system basin in the South of Ukraine, where chickpea is cultivated on numerous farms (Lykhovyd & Kozlenko, 2018). Therefore, it is very important to understand the reaction of the crop to these unfavourable factors in order to provide sustainable production of qualitative chickpea grain.

Leguminous crops are believed to be quite sensitive to salt stress, which is one of the main constraints of the enhancement of pulse production on saline lands (Farooq et al., 2017). It is well-known that chickpea is especially susceptible to salt stress at the reproductive stage of growth (Kotula et al., 2015), and firstly, the crop roots suffer (Tejera et al., 2006) resulting in worse productivity (Singla & Garg, 2005; Sohrabi et al., 2008). It has been proved that salinity stress causes decrease in the crop growth rate by 20%, plant height by 15%, and total biomass of the plants by 28% (Atieno et al., 2017). Irrigation with saline water considerably oppresses almost all physiological processes of the crop, resulting in worse yield and yield structure (Kumar, 2018). This is not a surprising fact because salt stress is caused by the osmotic stress that occurs in the root zone due to the high concentration of ions in the soil solution, and this condition leads to the difficulties in water and nutrition consumption by the crop (Munns & Tester, 2008). The higher the concentration of ions in the soil solution is, the worse the conditions for consumption of water and nutrients for the crop are. Some studies stated a significant decrease in the efficiency of water consumption by chickpea plants due to high soil salinity (Dang et al., 2008). However, there is another view on chickpea salinity stress. A previously conducted study determined that the crop could be efficiently used for phytoremediation at saline soils because of its capacity to uptake salts from the root zone at the rates of 2.52–2.55 t/ha in the irrigated conditions (Lavrenko et al., 2018). But we have to know the particular conditions for successful use of chickpea in this direction. First of all, the information on the salt content levels in the soil, which are not harmful for the crop, is needed. Efficient use of the chickpea in phytoremediation is possible only under the certain salinity degrees, and it will be impossible at higher degrees, when the crop begins to feel stress, reduces growth or die. We have some additional information in regard to the nitrogen fixation abilities of chickpea under the salt stress, its grain and biomass productivity (Flowers et al., 2010). Of course, salt tolerance of the crop depends on the variety (Khalid et al., 2001), though at the same time, Soltani et al. (2002) proved that there is no difference in seed germination in the salt stress conditions related to the type of chickpea (kabuli or desi). However, Gholipoor et al. (2001) proved that kabuli type of chickpea was more sensitive to the increased salinity of soil (0.9–4.9 dS/m) than desi type. But all in all, we know very little about the reaction of the crop to salt stress at the beginning of its growth. The objective of our study was determining the effect of different NaCl concentrations in solutions on chickpea germination and initial growth to conclude what salt salinity levels are safe for the crop cultivation if used in phytoremediation on saline lands.

Material and methods

The work was carried out in greenhouse conditions of Kherson State Agrarian University. The main methods used in our scientific investigation were visual observation and weighing method for determination of plant biometric parameters.

Usually, effect of salts stress on the germination and initial growth of crops is studied using NaCl solutions, especially, taking into account the statement that chloride type of salinity is more harmful for the crop than other types (Manchanda & Sharma, 1989; Haileselasie & Teferii, 2012). However, sometimes other options are applied. The study of Sheoran & Garg (1983) showed that Na_2SO_4 is the strongest inhibitor of chickpea germination compared to other sorts of salts. We should also remember that germination in saline soil is slower and with lower percentage of sprouts than in the conditions of solutions with the equal degree of salinity (Esechie et al., 2002). This fact is important when we provide recommendations for agricultural producers, based on the results of laboratory experiments. In our study we used NaCl salt solution to determine the parameters of seed germination and initial growth of chickpea. NaCl solutions of the required concentrations were prepared by mixing different, gradually increasing, amounts of NaCl with distilled water.

The variants of the experiment were represented by the following NaCl concentrations:

– not saline (0.13 ± 0.03 g/L);
– slightly saline (0.61 ± 0.04 g/L);
– moderately saline (1.79 ± 0.27 g/L);
– highly saline (4.41 ± 1.04 g/L);
– extremely saline (7.47 ± 0.59 g/L).

We used chickpea variety Rosanna (included in the State Register of Plants Varieties of Ukraine) of kabuli type in our investigations. Seed germination was determined by the following procedure. Four randomly chosen samples of 50 seeds were placed into the flasks with pure sand. The sand with the seeds was watered with prepared in advance solute-ons of NaCl. The seeds were germinated at the temperature 25 ºC. The germination percentage was determined on the eighth day after placing of the seeds in the flasks. After determination of the germination percentage by the counting of the number of sprouts, we measured the height of the obtained chickpea plants and the length of their roots by using a ruler. We also counted the seeds with abnormalities. Normal sprouts of chickpea are those with well-developed and proportional main structural bodies (roots, cotyledons, buds, coleoptile, etc.) that have no defects and look healthy. Normally germinated seeds are those that have a well-developed germ root with the length not less than the diameter of the seed, and well-formed sprout with the length of half and more than the diameter of the seed. Germination percentage is calculated as the ratio of the number of normally germinated seeds to the total number of seeds.

Statistical data processing was performed by using linear regression analysis to develop the models of salinity effect on the parameters of germination and initial chickpea growth. The difference between the variants of the study was proved by using the standard procedure of one-factor ANOVA. All the differences were estimated at the probability level of $P < 0.05$. The values of the studied parameters and indexes are given as a mean value ± standard deviation (SD).

Results

The results of the study revealed that chickpea is highly sensitive to the increasing salinity of the environment. It was determined that the germination rate and initial growth of the crop were significantly affect-ted at the moderately, highly, and extremely saline conditions (Table 1). The least significant difference at the probability level of 95% with accordance to the results of ANOVA equaled 4.97% for the germination, 1.72 cm for the plant height, and 0.73 cm for the root length.

Table 1
The effect of water salinity degree at chloride type of salinity on the germination and seedling growth parameters of chickpea (mean values)

Water salinity degree	Soluble salts content, g/L	Germi-nation, %	Plant height, cm	Root length, cm
Not saline	0.13 ± 0.03	99.40^{a}	25.45 ± 0.85^{a}	11.75 ± 0.05^{a}
Slightly saline	0.61 ± 0.04	82.30^{b}	22.19 ± 2.27^{b}	8.51 ± 0.55^{b}
Moderately saline	1.79 ± 0.27	65.46^{c}	17.65 ± 3.38^{c}	6.24 ± 0.21^{c}
Highly saline	4.41 ± 1.04	41.45^{d}	4.60 ± 17.55^{d}	3.68 ± 0.95^{d}
Extremely saline	7.47 ± 0.59	12.68^{e}	0.69 ± 0.00^{e}	0.93 ± 0.16^{e}

Note: different letters within the columns indicate the significant difference between the variants of the experiment.

Although the studied parameters changed significantly when the crop was exposed to slight salinity stress, the difference between the not saline and slightly saline variants, especially, in the initial growth parameters of chickpea was not big enough to state that there was a dramatic suppression of the crop development.

Table 2
The results of linear regression analysis of interrelationship between the salinity of solution, germination percentage, plant height, and root length of chickpea

Interrelationship pairs	R^2	Interception	Slope	Regression model
Salinity – germination percentage	0.9848	85.30	–9.80	Y = 85.30–9.80X
Salinity – plant height	0.8509	21.24	–3.03	Y = 21.24–3.03X
Salinity – root length	0.9418	8.45	–1.03	Y = 8.45–1.03X

The results of linear regression analysis allowed us to develop three models of the interrelationship between the salinity of solution used for watering of the crop and the parameters of its growth and development. The strongest interrelationship was determined in the pair "salinity-germination percentage" with the R^2 (coefficient of determination) value of 0.9848. Much less interdependence was found to be present in the inter-relationship of the pair "salinity – plant height" with the R^2 value of 0.8509. This fact gave us the idea that the most susceptible period of chickpea growth to salinity is the stage of germination, while after successful germination the crop suffers less from salinity stress, which could be proved by the lower values of the R^2 for the stage (Table 2).

For the better presentation of the results of regression analysis and evaluation of their accuracy and reliability we performed graphic approximation of the developed models.

Figure 3 presents the approximation of the "salinity – germination percentage" model approximation, which is characterized by the highest accuracy by computational method of evaluation (R^2 value). Graphic approximation also showed that this model is quite reliable and has just a slight discrepancy between the true values of the germination percentage and the modeled ones (Fig. 1). However, the results of the graphic approximation of the model "salinity – plant height" showed that this model is good only at low salinity levels (first 30 pairs of approximation), and provides unreliable predictions with the increased salinity, especially, high and extremely high salinity (pairs 60–100). The model provides the wrong forecast beginning with the pair of 75 (extremely high salinity level) computing impossible negative values of chickpea plant height (Fig. 2). The model of chickpea "salinity – root length" performed better, however, it also fails to provide reliable predictions at the very low and extremely high degrees of salinity (Fig. 3).

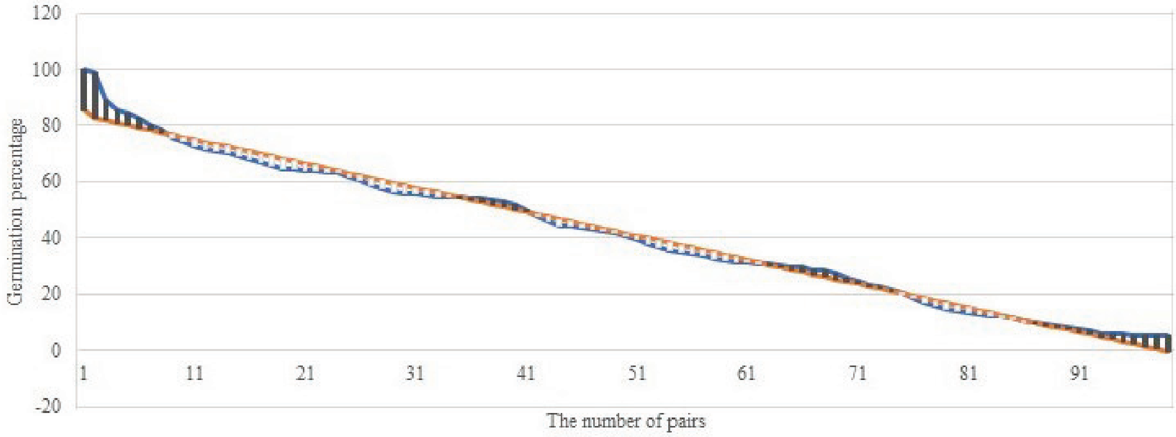

Fig. 1. The approximation of the linear regression model for chickpea: "salinity – germination percentage" (true values are blue, modeled ones are orange)

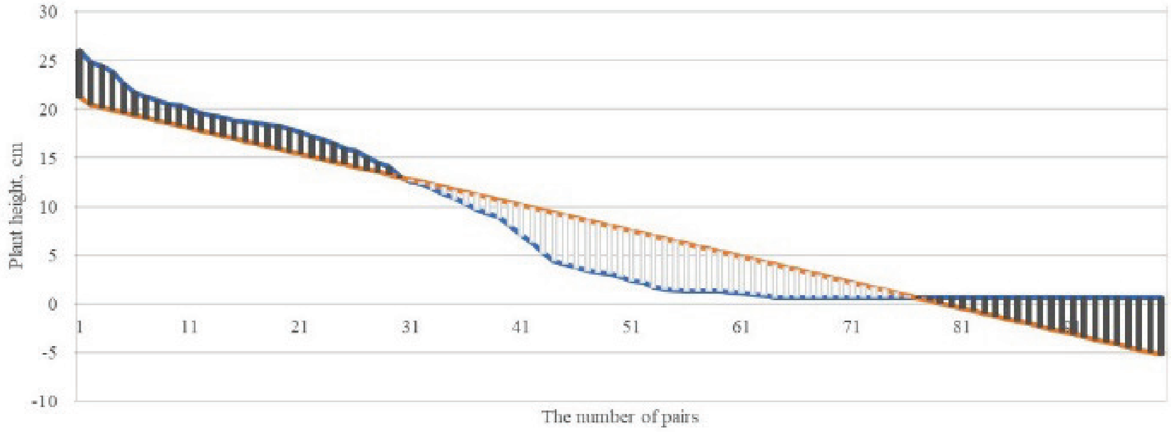

Fig. 2. The approximation of the linear regression model for chickpea: "salinity – plant height" (true values are blue, modeled ones are orange)

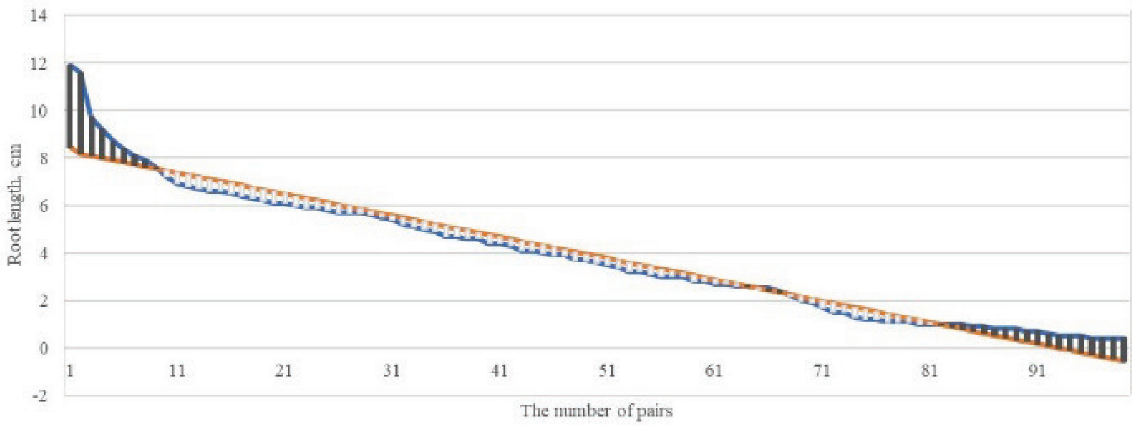

Fig. 3. The approximation of the linear regression model for chickpea: "salinity – root length" (true values are blue, modeled ones are orange)

So, we conclude that the developed models are more useful for theoretical understanding of the processes that take place in chickpeas at the initial stages of the crop growth under saline conditions, and we do not recommend usage of the above-mentioned models for practical purposes.

Discussion

Our experiment is not the first one that has been conducted with the aim of getting knowledge about the peculiarities of germination of crops under stress conditions. A number of foreign scientists have performed numerous investigations in the related field. For example, previous studies devoted to the investigation of sesame (*Sesamum indicum* L.) seed germination proved that the latter is significantly affected by different seed treatments used in the trials. It was determined that the best option of getting healthy sprouts of the crop with high germination percentage (91.5%) is treatment of the seeds with freshwater (Tizazu et al., 2019). And this fact is not really surprising. In regard to chickpea, Ullah et al. (2019) determined that Zn coating (5 mg/kg) of the crop's seeds dramatically increased germination rate of the crop, however, the treatment with Zn of higher concentrations resulted in suppression of the germination due to the osmotic disbalance. Besides, it is a well-known fact that seed germination of almost every crop is strongly dependent on temperature and water potential of the substrate in which it takes place (Bidgoli et al., 2018).

It is a well-known fact that chickpea germination could be improved by the treatment of seeds with *Rhizobium* and *Trichoderma* (Sharma et al., 2018), although salinity of the soil or irrigation water leads to suppression of the bacteria symbiosis with the roots of chickpea and decreases the efficiency of such biological method of the crop growth improvement (Zurayk et al., 2008). Significant decrease in the effective symbiosis with *Rhizobium* strains was observed by Saxena & Rewari (1992) at the increase of NaCl concentrations 0.01–2.00%. As the results of our study indicate, high NaCl concentrations are very harmful for the crop beginning with the values of 1.79 ± 0.27 g/L (the decrease in the germination percentage is more than 30%). Esechie et al. (2002) also studied the effect of different salinity levels on the germination of chickpea. Their results are in agreement with ours: the higher the salinity of the substrate was 0.8–12.2 dS/m, the lower the germination percentage was. Chickpea germination and seedling growth were also proved to be strongly dependent on the salinity by the research of Soltani et al. (2002). They proved that the most susceptible to salinity degree index is germination uniformity, and the least one is total germination of the seeds. And we absolutely agree with this statement because our results showed the same feature: the most sensitive stage is germination (Table 2). The scientists also stated the clear tendency of better seedling establishment for a large-seed variety of chickpeas rather than at small-seed variety under the non-saline conditions of growing (Soltani et al., 2002). And, in addition, they proved that salinity tolerance of chickpeas is a genotype-dependent feature (Soltani et al., 2002), as Dua (1992) had mentioned earlier either. The same results were obtained earlier by the germination test conducted with two different chickpea varieties: the study revealed a considerable decrease in chickpea germination rate in regard to the increasing salinity of the substrate, and also proved a significant dependence of this parameter on the genotype of the particular variety (Goel & Varshney, 1987).

The study on the determination of chickpea germination and initial growth parameters revealed that an increase in the concentration of chloride salts from 4 to 16 dS/m resulted in considerable suppression of the crop germination and growth. The concentration level of 16 dS/m was lethal for all the studied chickpea varieties. Increased salinity of the germination conditions resulted in gradual decrease of the seed germination rate and seedling growth (Ozaktan et al., 2018).

The results obtained by Mamo et al. (1996) in their research on reaction of chickpeas to salinity stress revealed the same tendency: chickpea germination rates decreased significantly with the exposure to higher concentration of NaCl (0–8 dS/m).

However, none of the above-mentioned scientists mentioned the safe degree of salinity, when chickpea germination and seedling growth is suppressed but the crop is still able to develop normally. That is the difference in our investigations. According to the results of our study, we conclude that chickpea germination and initial growth is possible and will not undergo significant deterioration and disturbance in slightly-saline conditions (NaCl concentration of 0.61 ± 0.04 g/L). Besides, we made additional focus on the development of mathematical models of chickpea germination and initial growth that could be helpful for the improvement of theoretical knowledge in this subject. This approach was used earlier by other scientific groups to provide crop growth models of different crops, including corn, melon, alfalfa (Shani & Dudley, 2001), rice (Zeng & Shannon, 2000), and also chickpea (Kaya et al., 2008). But we have changed the parameters that were analyzed and modeled by the regression analysis, so, our research provides a new input for the global knowledge of chickpea reaction to salinity.

Besides, we should emphasize that we need to find out and propose the methods for improvement of salinity tolerance of chickpea. This question is the subject of our further scientific work. However, some studies related to the problem were carried out by foreign scientists, and now we can state that salt stress tolerance of the crop could be improved by inoculation of the seeds with *Bacillus subtilis* (BERA 71) strain of endophytic bacteria (Abd-Allah et al., 2018). And, of course, special work is conducted by plant breeders to discover the best ways, crossing combinations between different genotypes, pointing out salinity tolerance markers in the chickpea genotype to provide the best options for selection of new salt-resistant cultivars of the crop (Dudhe & Kumar, 2018).

Conclusions

Chickpea germination and initial growth are strongly affected by salinity stress. The most susceptible stage is germination. Chickpea can be cultivated without any considerable decrease of its growth and development in slightly saline conditions (NaCl concentration within the range of 0.61 ± 0.04 g/L), while the crop is exposed to strong suppression by the higher degrees of salinity. Therefore, we conclude that the crop has to be cultivated at the lands with no or slight salinity level.

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