short report / kratko saopštenje
Ratar. Povrt. 50:1 (2013) 49-53

Maize Germination Parameters and Early Seedlings Growth Under Different Levels of Salt Stress

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received: 4 December 2012, accepted: 2 April 2013
published online: 28 May 2013

doi:10.5937/ratpov50-3042

Summary: Increased salt concentration has a negative effect on germination parameters and early seedling growth. The objective of this study was to evaluate germination parameters and early seedlings growth of maize under different salinity conditions (0, -0.3, -0.6, -0.9 and -1.2 MPa). Germination was tested in double rolled filter paper, moistened with different NaCl solution, in the dark at 25°C. Osmotic stress had significant effects on all examined characteristics, except on final germination. Under the highest osmotic stress, mean germination time and time to 50% germination were 14 h and 30 h longer than at control. Root and shoot length under those conditions (-1.2 MPa) were 76% and 87% shorter compared to control, while root and shoot weight were reduced (81% and 87%). Minor reduction in germination energy was recorded under stress. These results showed different responses of germination parameters and early seedling growth in the observed genotype under different salinity conditions.
Key words: abiotic stress, germination, maize, salinity, seedlings

Introduction

High salt content in the soil is one of the most important environmental factors limiting global crop production. Out of 230 million hectares of irrigated agricultural land approximately 45 million hectares are salt-affected (Athar & Ashraf 2009). The various negative effects of high salt concentrations on plant metabolism can be observed from the cellular to the whole plant level. Through the osmotic effect, salinity reduces the ability of plants to absorb water, which results in growth reduction (Munns 2002). Increased concentrations of salt cause disorders in ion absorption (Karimi et al. 2005). Increased absorption and accumulation of certain ions from the soil can result in specific ion toxicity (Nawaz et al. 2010). The most common salt in saline soils is sodium chloride (Zörb et al. 2004). Sodium can affect plant growth of many halophytes, influence the metabolism of C_4 plants and replace potassium (Subbarao et al. 2003). Sodium toxicity is primarily caused by Na^+ competing with K^+, which alters K^+/Na^+ ratio in the cytosol (George et al. 2012). Chlorine is widespread in the nature, and plants easily absorb chloride ions. Chloride ions play an important role in PS II, membrane potential stabilization and turgor and pH regulation. External Cl concentrations higher than 20 mM can be toxic in susceptible plant species, whereas in tolerant species external concentrations four to five times higher can show no effect on plant growth (Broadley et al. 2012).

Increased salt concentration has a negative effect on germination parameters. For example, Jamil et al. (2006) showed a negative effect of increased salt concentration on germination rate and early seedling growth of four plant species: sugar beet (*Beta vulgaris*), cabbage (*Brassica oleracea capitata* L.), amaranth (*Amaranthus paniculatus*) and pak-choi (*Brassica compestris*). Asaadi (2009) reported decreased seed germination of *Trigonella foenum-graecum* under high salinity conditions.

One of the three most important crops in the world, maize is classified as a moderately salt-sen-

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Acknowledgements:
This study presents results of the project TR31073, supported by Ministry of Education, Science and Technological Development of the Republic of Serbia.

sitive crop. Negative effect of increased salt concentration on maize seed germination and early growth has previously been reported (Carpici et al. 2009). Seed germination and optimal early seedling growth are major factors that influence appropriate crop establishment - one of the most important determinants of grain yield.

The aim of this study was to evaluate germination parameters and early seedling growth of maize under different salinity conditions.

Materials and Methods

Maize inbred line 21 NSHt, developed at the Institute of Field and Vegetable Crops from Novi Sad, Serbia was used as seed source. Seeds were sterilized in 5% NaOCl solution for 5 minutes, and after that rinsed under tap water. Germination was tested in double layer rolled filter paper (30x30 cm) moistened with different NaCl osmotic solutions (0, -0.3, -0.6, -0.9 and -1.2 MPa) calculated according to Coons et al. (1990). Testing was carried out in the dark at 25°C, in four replicates with 50 seeds per replicate. Rolls of filter paper were put in plastic bags. Filter papers were replaced every two days to avoid change of osmotic potential due to water uptake and evaporation. Number of germinated seeds was recorded daily for 7 days. Seeds were considered germinated when the radicle was 2 mm long.

Germination energy (GE) and final germination (FG) were recorded on days 4 and 7, respectively (ISTA, 2008). Mean germination time (MGT) was determined using the formula (Ellis & Roberts 1981):

$$MGT = \sum Dn / \sum n$$

where D is the number of days counted from the beginning of germination and n is the number of seeds that had germinated on day D.

Time to 50% germination (T_{50}) was determined using the formula of Coolbear et al. (1984) modified by Farooq et al. (2005):

$$T_{50} = t_i + (N/2 - n_i)(t_j - t_i) / (n_j - n_i)$$

where N is the final number of germinating seeds, n_j and n_i are represent the cumulative number of seeds germinated at times t_j and t_i, respectively, when $n_i < N/2 < n_j$.

Seedling root length, shoot length, root fresh weight and shoot fresh weight were measured on day 7.

Data were processed using one-way analysis of variance. Means were compared using Duncan's multiple range test (Steel & Torrie 1980).

Results and Discussion

Osmotic potential of the NaCl solution had a significant effect on most maize seed germination parameters and early seedling growth (Table 1; Table 2). The largest changes in seed germination parameters and seedling characteristics occurred at the lowest osmotic potential conditions.

The final seed germination under different osmotic potential was between 96.5% and 97.5%, and there was no significant difference between all examined treatments. Increased salt stress had an adverse effect on maize seed germination energy. Germination energy significantly decreased (for 8% and 9%) compared to the control, under -0.9 and -1.2 MPa osmotic conditions of the NaCl solution. Similarly, Patanè et al. (2012) reported that increased salt stress had no negative effect on final germination; however, salt stress prolonged seed germination in sweet sorghum.

Significant differences in seed MGT and T_{50} were recorded for different osmotic potentials. MGT was the longest in the presence of the -1.2 MPa osmotic potential, and it was 14 h longer

Table 1. Influence of different osmotic potential on maize seed final germination (FG), germination energy (GE), mean germination time (MGT) and time to 50% germination (T_{50})
Tabela 1. Uticaj različitih vrednosti osmotskog potencijala na klijavost (FG), energiju klijanja (GE), srednje vreme klijanja (MGT) i vreme do dostizanja 50% klijavosti (T_{50}) semena kukuruza

Osmotic potential (MPa)	FG (%)	GE (%)	MGT (day)	T_{50} (day)
0	97.00 ^{a}	97.00 ^{ab}	4.61 ^{e}	1.68 ^{e}
-0.3	97.50 ^{a}	97.50 ^{a}	4.70 ^{d}	1.93 ^{d}
-0.6	97.50 ^{a}	92.50 ^{bc}	4.90 ^{c}	2.38 ^{c}
-0.9	97.50 ^{a}	90.00 ^{c}	5.03 ^{b}	2.60 ^{b}
-1.2	96.50 ^{a}	88.50 ^{c}	5.21 ^{a}	2.92 ^{a}

Different letters indicate significant difference at $P < 0.05$ level.

Table 2. Influence of different osmotic potential on maize seedling root length, shoot length, root weight and shoot weight

Tabela 2. Uticaj različitih vrednosti osmotskog potencijala na dužinu korenka, dužinu izdanka, masu korenka i masu izdanka klijanca kukuruza

Osmotic potential (MPa)	Root length (mm)	Root weight (g)	Shoot length (mm)	Shoot weight (g)
0	114.02 [a]	0.21 [a]	47.10 [a]	0.16 [a]
-0.3	82.70 [b]	0.16 [b]	37.42 [b]	0.13 [b]
-0.6	66.67 [c]	0.13 [c]	22.77 [c]	0.06 [c]
-0.9	46.42 [d]	0.08 [d]	9.53 [d]	0.03 [d]
-1.2	27.45 [e]	0.04 [e]	6.18 [d]	0.02 [d]

Different letters indicate significant difference at $P < 0.05$ level.

then mean germination time of the control. T_{50} was increased in presence of the -1.2 MPa osmotic potential, which was 30 h longer then the T_{50} of the control. Akbarimoghaddam et al. (2011) reported that the delay in MGT was a result of Na^+ accumulation in the seeds, which has an adverse effect on seed germination process in wheat. Chartzoulakis & Klapaki (2000) showed that salinity has physical-chemical effect on the paper seed, decreasing seed water uptake, resulting in a slower and/or lower rate of germination. T_{50}, MGT and GE are important parameters that indicate rapid and successful crop establishment. As a result of rapid seed germination, the period in which the seeds are exposed to the attack of pathogens is significantly reduced (Beckstead et al. 2007, Dalling et al. 2011).

Increased stress levels had a significant effect on maize seedling characteristics. Generally, with salinity level increase, root and shoot weight and length decreased. Root length and weight were sensitive to increased NaCl osmotic stress. In root length and weight, between all examined treatments significant difference was recorded. Root length was the most reduced in the presence of the highest negative osmotic potential value (-1.2 MPa), and was 76% shorter compared to control. Mean root weight varied between 0.21 and 0.04 g under different osmotic conditions. Minimum root weight, recorded at -1.2 MPa, was 81% reduced compared to the control. Roots are placed in a soil medium, and have a direct contact with the soil solution, and therefore root length and weight are important indicators of plant response to salt stress.

According to our results, shoot length and weight were sensitive to decreased NaCl osmotic potential. Significant decrease in shoot length and

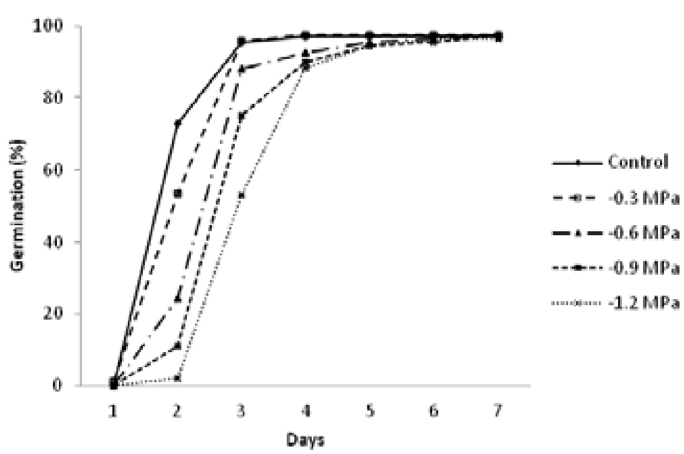

Figure 1. Cumulative germination of maize seed under different osmotic potential value of NaCl solution (0, -0.3, -0.6, -0.9 and -1.2 MPa)

Slika 1. Kumulativna klijavost semena kukuruza u uslovima raziličtih vrednosti osmotskog potencijala rastvora NaCl (0, -0.3, -0.6, -0.9 i -1.2 MPa) weight was recorded from the control to stress level of -0.9 MPa, while between salinity levels -0.9 MPa and -1.2 MPa significant difference was not found. The largest reduction of shoot growth was at the maximum salinity level (-1.2 MPa), with 87% reduction compared to the control. These results are consistent with the results of Khodarahmpour (2012) and Janmohammadi et al. (2008) who reported that root and shoot length were reduced following an increase in salinity. Bybordi (2010) showed that increased salinity reduced root and shoot length in canola. Shoot weight under -0.3, -0.6, -0.9 and -1.2 MPa NaCl solution treatments was reduced by 19%, 62%, 81% and 87% respectively, compared to the control. Iqbal & Ashraf (2010) also reported that salinity caused a decrease of shoot weight in two different wheat cultivars.

Figure 1 shows different dynamics of cumulative germination, depending on NaCl solution osmotic potential. Increase in osmotic potential of NaCl solution resulted in decrease of germination rate. The greatest difference between treatments was on the second day.

Conclusions

The results of this study show that osmotic stress had a negative effect on mean germination time, time to 50% germination, germination energy, shoot length, root length, shoot weight and root weight. The highest concentration of NaCl had the most deleterious effects on germination parameters and early seedling growth. Osmotic stress had no significant effect on final germination. These results showed the negative response of germination parameters and early seedling growth in the observed genotype under different salinity stress conditions.

References

Akbarimoghaddam, H., Galavi, M., Ghanbari, A., & Panjehkeh, N. (2011). Salinity effects on seed germination and seedling growth of bread wheat cultivars. *Trakia J Sci, 9*(43-50).

Asaadi, A.M. (2009). Investigation of salinity stress on seed germination of *Trigonella foenum-graecum. Res J Biol Sci., 4*, 1152-1155.

Athar, H.R., & Ashraf, M. (2009). Strategies for crop improvement against salt and drought. An overview. In A.M. Ozturk & H.R. Athar (Eds.), *Tasks for Vegetation Science 44, Salinity and Water Stress*. (pp. 1-18). Heidelberg, Germany.

Beckstead, J., Meyer, S.E., Molder, C.J., & Smith, C. (2007). A Race for survival: can *Bromus tectorum* seeds escape *Pyrenophora semeniperda*-caused mortality by germinating quickly. *Ann. Bot., 99*(5), 907-14. pmid:17353206

Broadley, M., Brown, P., Cakmak, I., Rengel, Z., & Zhao, F. (2012). Function of Nutrients: Micronutrients. In P. Marshner (Ed.), *Marschner's Mineral Nutrition of Higher Plants*, Third Edition. (pp. 191-248). London, UK: Academic Press Elsevier.

Bybordi, A. (2010). The influence of salt stress on seed germination, growth and yield of canola cultivars. *Not Bot Hort Agrobot Cluj., 38*, 128-133.

Carpici, E.B., Celik, N., & Bayram, G. (2009). Effects of salt stress on germination of some maize (*Zea mays* L.) cultivars. *Afr J Biotechnol, 8*, 4918-4922.

Chartzoulakis, K., & Klapaki, G. (2000). Response of two greenhouse pepper hybrids to NaCl salinity during different growth stages. *Scientia Horticulturae, 86*(3), 247-260. doi:10.1016/S0304-4238-(00)00151-5

Coolbear, P., Francis, A., & Grierson, D. (1984). The effect of low temperature pre-sowing treatment under the germination performance and membrane integrity of artificially aged tomato seeds. *J Exp Bot., 35*, 1609-1617.

Dalling, J.W., Davis, A.S., Schutte, B.J., & Elizabeth, A.A. (2011). Seed survival in soil: interacting effects of predation, dormancy and the soil microbial community. *Journal of Ecology, 99*(1), 89-95. doi:10.1111/j.1365-2745.2010.01739.x

Ellis, R.A., & Roberts, E.H. (1981). The quantification of ageing and survival in orthodox seeds. *Seed Sci Technol, 9*, 373-409.

Farooq, M., Basra, S.M.A., Hafeez, K., & Ahmad, N. (2005). Thermal hardening: a new seed vigor enhancement tool in rice. *Acta Bot Sin., 47*, 187-193.

George, E., Horst, W., & Neumann, E. (2012). Adaptation of Plants to Adverse Chemical Soil Conditions. In P. Marshner (Ed.), *Marschner's Mineral Nutrition of Higher Plants*, Third Edition. (pp. 409-472). Netherlands: Academic Press Elsevier, Amsterdem.

International seed testing association (ISTA). (2008). *International rules for seed testing*. Zurich: ISTA.

Iqbal, M., & Ashraf, M. (2010). Changes in hormonal balance: A possible mechanism of pre-sowing chilling-induced salt tolerance in spring wheat. *J Agron Crop Sci., 196*, 440-454.

Jamil, M., Lee, D.B., Jung, K.Y., Ashraf, M., Lee, S.C., & Rha, E.S. (2006). Effect of salt (NaCl) stress on germination and early seedling growth of four vegetables species. *J Cent Eur Agr., 7*, 273-282.

Janmohammadi, M., Dezfuli, M.P., & Sharifzadeh, F. (2008). Seed invigoration techniques to improve germination and early growth of inbred line of maize under salinity and drought stress. *Gen Appl Plant Physiol, 34*, 215-226.

Karimi, G., Ghorbanli, M., Heidari, H., Nejad, K.R.A., & Assareh, M.H. (2005). The effects of NaCl on growth, water relations, osmolytes and ion content in *Kochia prostrate*. *Biol Plant, 4*, 301-304.

Khodarahmpour, Z. (2012). Evaluation of salinity effects on germination and early growth of maize (*Zea mays* L.) hybrids. *Afr J Agr Res., 7*, 1926-1930.

Munns, R. (2002). Comparative physiology of salt and water stress. *Plant Cell Environ., 25*(2), 239-250. pmid:11841667

Nawaz, K., Hussain, K., Majeed, A., Khan, F., Afghan, S., & Ali, K. (2010). Fatality of salt stress to plants: Morphological, physiological and biochemical aspects. *Afr J Biotechnol, 9*, 5475-5480.

Patanè, C., Saita, A., & Sortino, O. (2012). Comparative effects of salt and water stress on seed germination and early embryo growth in two cultivars of sweet sorghum. *J Agron Crop Sci*, (in press).

Steel, R.G.D., & Torrie, J.H. (1980). *Principles and procedures of statistics, a biometrical approach*. Second edition. (pp. 172-191). New York, USA: Mc Graw-Hill, Book Company. Chapter 8, second edition.

Subbarao, G.V., Ito, O., Berry, W.L., & Wheeler, R.M. (2003). Sodium-A Functional Plant Nutrient. *Crit Rev Plant Sci.,22*, 391-416.

Zörb, C., Schmitt, S., Neeba, A., Karl, S., Linder, M., & Schubert, S. (2004). The biochemical reaction of maize (*Zea mays* L.) to salt stress is characterized by a mitigation of symptoms and not by a specific adaptation. *Plant Sci.,167*, 91-100.

Parametri klijanja i rani porast ponika kukuruza u različitim nivoima sonog stresa

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Izvod: Povećane koncentracije soli imaju negativan efekat na parametar klijanja i rani porast klijanaca. Cilj istraživanja bio je ocena parametara klijanja i ranog porasta klijanaca kukuruza u uslovima različite zaslanjenosti (0, -0,3, -0,6, -0,9 i -1,2 MPa). Klijanje je testirano na filter papiru, nakvašenom rastvorom NaCl različitog osmotskog potencijala, u mraku na 25°C. Osmotski stres je imao značajan uticaj na sve ispitivane parametre, osim na klijavost. U uslovima najvišeg osmotskog stresa, srednje vreme klijanja i vreme dostizanja 50% klijavosti, bili su 14 h i 30 h duži u odnosu na kontrolu. Dužina korenka i izdanka u navedenim uslovima (-1,2 MPa) je bila za 76% i 87% kraća spram kontrole, dok je masa korena i stabla bila manja za 81% i 87%. Energija klijanja je smanjena u manjoj meri u uslovima stresa. Rezultati pokazuju razliku u reakciji parametara klijanja i ranog porasta ponika posmatranog genotipa u uslovima različitog nivoa osmotskog stresa.

Ključne reči: abiotski stres, klijanje, kukuruz, ponik, zaslanjenost