

# THE INFLUENCE OF GENOTYPE AND OSMOTIC STRESS ON GERMINATION AND SEEDLING OF MAIZE

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Original scientific paper

**Abstract:** The aim of this research was to estimate the influence of different NaCl osmotic solutions (0, -0.3 MPa, -0.6 MPa, -0.9 MPa, -1.2 MPa, -1.5 MPa) on seed germination, and early seedling growth in two maize hybrid different maturity groups (ZP 560 – FAO 500 and ZP 666 – FAO 600). Germination was tested in sterile plastic vessels on filter paper moistened with different NaCl solutions, in the dark at  $20 \pm 1^\circ\text{C}$ , in laboratory. Results of ANOVA indicated that hybrid ZP 666 had significantly higher root length (RL) (6.37 cm), shoot length (ShL) (2.06 cm), shoot fresh weight (ShFW) (43.86 mg), root dry weight (RDW) (7.56 mg), shoot dry weight (ShDW) (5.97 mg), seedling vigor index (SVI) (706.55) and dry matter stress tolerance index (DMSI) (55.85%) than hybrid ZP 560 (4.18 cm, 1.14 cm, 32.50 mg, 6.54 mg, 4.15 mg, 457.61 and 48.86%, respectively). Contrary, hybrid ZP 560 had significantly higher relative seedling water content (RSWC) (83.83%) and phytotoxicity of shoot (PhSh) (69.77%) than hybrid ZP 666 (81.16% and 62.51%, respectively). Generally, hybrid ZP 666 had better tolerance to salt stress than hybrid ZP 560. Germination energy (GE), germination (G), RL, ShL, root fresh weight (RFW), ShFW, RDW, ShDW, rate germination index (RGI), SVI, RSWC and DMSI were significantly decreased with the increase in osmotic stress induced by NaCl. Contrary, phytotoxicity of root (PhR) and PhSh significantly increased with the increase in osmotic stress.

**Keywords:** germination, early seedling growth, maize, osmotic stress

## Introduction

In Serbia, approximately 6 million tons of maize grain is produced on 1.2 million ha. Most of the maize production (about 80%) is used to feed livestock. Also, silage maize is used to feed livestock, primarily dairy cows. However, maize production depends by many abiotic factors. Salinity is one of the abiotic factors

that limits the plant germination and early seedling growth (*Almansouri et al., 2001*) and plant growth and productivity of crops (*Flowers, 2004*). Salinity land is soil contaminated with salts ( $EC_e > 4 \text{ dS m}^{-1}$  or 40 mM NaCl or osmotic potential  $< 0.117 \text{ MPa}$ ) (*Ashraf, 2009*). In Serbia has 4.6% of saline and alkaline soils (solonetz and solonchak) of 5,112,000 ha agricultural land (*Ličina et al., 2011*). High salt content in the soil reduces the ability of plants to absorb water, which results in growth crop reduction (*Munns 2002*), causing disorders in ion absorption (*Karimi et al. 2005*), increases absorption and accumulation of toxic ions (*Nawaz et al. 2010*). Maize is moderately sensitive to salinity (*Maas and Hoffman, 1977; Ouda et al., 2008*). However, maize hybrids differ in level of resistance to salt tolerance (*Radić et al., 2007; Carpici et al., 2009; Khodarahmpour, 2012*). Many results showed that maize seed germination significantly reduced with increase in salinity levels (*Rahman et al., 2000; Blanco et al., 2007; Carpici et al., 2009; Bakht et al., 2011; Khayatnezhad and Gholamin, 2011; Mirosavljević et al., 2013*). The reasons for this were osmotic problems in the first phase of salt stress and  $\text{Na}^+$  toxicity in the second phase (*Schubert et al., 2009*).

The aim of this study was to estimate the effects of various NaCl osmotic solutions (0, -0.3 MPa, -0.6 MPa, -0.9 MPa, -1.2 MPa, -1.5 MPa) on germination and early seedling growth in two maize hybrids (ZP 560 and ZP 666).

## Materials and Methods

The experiments were conducted under laboratory conditions in March 2014 (before sowing in the field) at the Institute for Animal Husbandry in Belgrade. Hundred seeds of each hybrids were placed in sterile plastic vessels (15 cm wide, 21 cm long and 4 cm high) on filter paper moistened with 10 ml different NaCl osmotic solutions (0, -0.3 MPa, -0.6 MPa, -0.9 MPa, -1.2 MPa, -1.5 MPa) prepared according to *Coons et al. (1990)*, and incubated at  $20 \pm 1^\circ\text{C}$  in the dark for 14 days. The seeds were stored in paper bags in laboratory room. Seeds were sterilized in 1% NaOCl during 5 min and washed 3 times in sterile distilled water. The experimental design was arranged in a Randomized Complete Block Design (RCBD) with four replications.

According to *ISTA (2008)* germination energy (GE) and germination (G) were determined after 4 and 7 day after sowing, respectively. Root length (RL), shoot length (ShL), root fresh weight (RFW), shoot fresh weight (ShFW), root dry weight (RDW) and shoot dry weight (ShDW) were evaluated after 14 days. Root and shoot dry weight were obtained after drying at  $80^\circ\text{C}$  for 24 hours (*Mandić et al., 2012*).

Also calculated rate germination index (RGI) according to *Islam et al. (2000)*, seedling vigor index (SVI) according to *ISTA (1999)*, relative seedling water content (RSWC) according to *Shalaby et al. (1993)*, phytotoxicity of shoot (PhSh) and root (PhR) according to *Chou (1976)* and dry matter stress tolerance index (DMSI) according to *Ashraf et al. (2006)*:

$RGI = (\text{No. of seeds germinated at 3 day} / \text{No of seeds germinated at 7 day}) \times 100$

$SVI = (\text{Root length} + \text{Shoot length}) \times \text{Germination percentage},$

$RSWC = ((\text{Fresh weight} - \text{Dry weight}) / \text{Fresh weight}) \times 100,$

$PhSh = ((\text{Shoot length of control} - \text{Shoot length of treatment}) / \text{Shoot length of control}) \times 100$

$PhR = ((\text{Root length of control} - \text{Root length of treatment}) / \text{Root length of control}) \times 100$

$DMSI = (\text{Dry matter of stressed plant} / \text{Dry matter of control plants}) \times 100.$

The data were statistically analyzed by ANOVA using program Statistica version 10. Duncan's Multiple Range Test was used to compare differences among treatment means at 5% level.

## Results and Discussion

Results of ANOVA indicated that hybrid had highly significant effect on RL, ShL, ShFW, RDW and ShDW (Table 1). Hybrid ZP 666 had significantly higher RL (6.37 cm), ShL (2.06 cm), ShFW (43.86 mg), RDW (7.56 mg) and ShDW (5.97 mg) than hybrid ZP 560 (4.18 cm, 1.14 cm, 32.50 mg, 6.54 mg and 4.15 mg respectively). Hybrids did not differ for GE, G and RFW although the values for these parameters were higher in ZP 666, except for GE. Hybrid ZP 666 has a longer root system, and proved that genotypes with longer root growth are resistant ability for drought (*Leishman and Westoby, 1994*).

The osmotic stress had significant effect on germination and early seedling growth. GE, G, RL, ShL, RFW, ShFW, RDW and ShDW were significantly decreased with increasing osmotic stress. The highest values of GE (34.88%), G (97.25%), RL (12.21 cm), ShL (4.65 cm), RFW (82.31 mg), ShFW (135.60 mg), RDW (10.66 mg) and ShDW (12.46 mg) were recorded at 0 MPa and the lowest at -1.5 MPa (0.25%, 13.00%, 1.28 cm, 0.24 cm, 16.90 mg, 5.09mg, 3.21mg and 1.47 mg, respectively). However for RL, ShL, RFW, ShFW parameters there was no significant difference between -1.2 and -1.5 MPa. With regard to EG and G treatment with -0.3 MPa can be the germination sensitivity threshold in the studied maize hybrids. At the higher osmotic stress levels of -0.3 MPa, EG and G were significantly decreased. Root and shoot growth were significantly reduced by increasing osmotic stress. RL, ShL, RFW, ShFW, RDW, ShDW were significantly reduced already at -0.3 MPa. Reduction of RL under osmotic stress occurs due to the growth inhibition and loss of turgidity. *Mirosavljević et al. (2013)* reported that osmotic stress had significant effects on mean germination time, time to 50%, RL, ShL, root and shoot weight, except on final germination. *Khodarahmpour (2012)* concluded that G, germination rate, RL, ShL, seedling length and SVI were decreased by increase in osmotic potential. *Demir and Arif (2003)* concluded that the root growth was more sensitive than shoot growth under salinity conditions. *Sozharajan and Natarajan (2014)* reported that salt stress decreased germination,

biomass and growth of maize seedlings due to ion toxicity, decrease osmotic potential and oxidative stress. High absorption of  $\text{Na}^+$  and  $\text{Cl}^-$  ions during seed germination can be due to cell toxicity that finally inhibits or slows the rate of germination (Taiz and Zeiger, 2002). Shonjani (2002) stated that germination rate, germination speed and RL of maize significantly decreased with increasing salt concentrations. Cicek and Cakirlar (2002) and Idikut (2013) stated that RL and ShL of maize were decreased with increasing of salt concentration level. Salt concentration decreased significantly G, germination index, RDW and ShDW of maize seedling (Carpıcı et al., 2009). Many results showed that increased salt concentration reduces germination rate, germination speed, RL and ShL in several species (Cicek and Cakirlar, 2002; Shonjani, 2002; Dağüstü, 2003; Dumlupınar et al., 2007; Taslak et al., 2007; Xiao-Xia et al., 2008; Turkyimaz et al., 2011).

The interaction of hybrid and osmotic stress had significant effect on RL, ShL, ShFW and ShDW.

**Table 1** The effects of hybrid and different osmotic stress on germination energy (GE), germination (G), root length (RL), shoot length (ShL), root fresh weight (RFW), shoot fresh weight (ShFW), root dry weight (RDW) and shoot dry weight (ShDW) of maize

Parameters	Hybrid (A)	Osmotic stress, MPa (B)						Means
		0	-0.3	-0.6	-0.9	-1.2	-1.5	
GE, %	ZP 560	32.75	28.50	32.50	5.00	2.50	0	16.88
	ZP 666	37,00	32,50	17,00	7,50	0	0,5	15.75
	Means	34.88 <sup>a</sup>	30.50 <sup>ab</sup>	24.75 <sup>b</sup>	6.25 <sup>c</sup>	1.25 <sup>c</sup>	0.25 <sup>c</sup>	16.32
G, %	ZP 560	97,00	94,00	85,50	67,50	32,50	3,00	63.25
	ZP 666	97,50	94,00	80,50	54,00	43,00	23,00	65.33
	Means	97.25 <sup>a</sup>	94.00 <sup>ab</sup>	83.00 <sup>b</sup>	60.75 <sup>c</sup>	37.75 <sup>d</sup>	13.00 <sup>e</sup>	64.29
RL, cm	ZP 560	9.36	6.34	4.32	2.62	1.51	0.93	4.18 <sup>b</sup>
	ZP 666	15.06	9.32	6.16	3.60	2.46	1.62	6.37 <sup>a</sup>
	Means	12.21 <sup>a</sup>	7.83 <sup>b</sup>	5.24 <sup>c</sup>	3.11 <sup>d</sup>	1.98 <sup>e</sup>	1.28 <sup>e</sup>	5.28
ShL, cm	ZP 560	3.80	1.62	0.65	0.37	0.25	0.14	1.14 <sup>b</sup>
	ZP 666	5.50	2.27	2.91	0.73	0.60	0.35	2.06 <sup>a</sup>
	Means	4.65 <sup>a</sup>	1.95 <sup>b</sup>	1.78 <sup>b</sup>	0.55 <sup>c</sup>	0.43 <sup>c</sup>	0.24 <sup>c</sup>	1.60
RFW, mg	ZP 560	76,35	67,80	49,45	41,12	25,42	14,58	45.79
	ZP 666	88,28	55.85	47.15	37.62	27.30	19.22	45.90
	Means	82.31 <sup>a</sup>	61.82 <sup>b</sup>	48.30 <sup>c</sup>	39.38 <sup>c</sup>	26.36 <sup>d</sup>	16.90 <sup>d</sup>	45.84
ShFW, mg	ZP 560	11327	48.02	5.40	16.25	7.90	4.12	32.50 <sup>b</sup>
	ZP 666	157.92	49.82	25.20	10.30	13.88	6.05	43.86 <sup>a</sup>
	Means	135.60 <sup>a</sup>	48.92 <sup>b</sup>	15.30 <sup>c</sup>	13.28 <sup>c</sup>	10.89 <sup>e</sup>	5.09 <sup>e</sup>	38.18
RDW, mg	ZP 560	10.58	8.58	6.45	6.68	4.38	2.60	6.54 <sup>b</sup>
	ZP 666	10.74	9.15	9.39	6.83	5.46	3.82	7.56 <sup>a</sup>
	Means	10.66 <sup>a</sup>	8.86 <sup>b</sup>	7.92 <sup>bc</sup>	6.75 <sup>c</sup>	4.92 <sup>d</sup>	3.21 <sup>e</sup>	7.05
ShDW, mg	ZP 560	11.36	6.52	1.40	1.70	2.88	1.16	4.15 <sup>b</sup>
	ZP 666	13.55	7.43	6.37	4.35	2.36	1.78	5.97 <sup>a</sup>
	Means	12.46 <sup>a</sup>	6.93 <sup>b</sup>	3.89 <sup>c</sup>	3.02 <sup>cd</sup>	2.62 <sup>d</sup>	1.47 <sup>e</sup>	5.06
Factor	GE	G	RL	ShL	RFW	ShFW	RDW	ShDW
A	ns	ns	**	**	ns	**	*	**
B	**	**	**	**	**	**	**	**
AB	ns	ns	**	**	ns	**	ns	**

Means followed by the same letter within a column are not significantly different by Duncan's Multiple Range Test at the 5% level; \*\* - significant at 1% level of probability, \* - significant at 5% level of probability and ns - not significant

Results of ANOVA indicated that hybrid had highly significant effect on SVI, RSWC, PhSh and DMSI (Table 2). Hybrid ZP 666 had significantly higher SVI (706.55) and DMSI (55.85%) than hybrid ZP 560 (457.61 and 48.86%, respectively). Contrary, hybrid ZP 560 had significantly higher RSWC (83.83%) and PhSh (69.77%) than hybrid ZP 666 (81.16% and 62.51%, respectively). Hybrids did not differ for RGI and PhR. Information on phytotoxicity is important parameters that are used to identify the phytotoxicity tolerance of the genotypes. Hybrid ZP 666 had relatively low phytotoxicity of shoot that indicates, it was better in tolerating higher NaCl concentration. The result of this study is in agreement with researches *Kinfemichael (2011)*, *Asmare (2013)* and *Mordi (2013)* who reported the presence of genetic variability between cultivars for tolerance and phytotoxicity effect of NaCl.

**Table 2. The effects of hybrid and different osmotic stress on rate germination index (RGI), seedling vigor index (SVI), relative seedling water content (RSWC), phytotoxicity of root (PhR), phytotoxicity of shoot (PhSh), dry matter stress tolerance index (DMSI)**

Parameters	Hybrid (A)	Osmotic stress, MPa (B)						Means
		0	-0.3	-0.6	-0.9	-1.2	-1.5	
RGI, %	ZP 560	10,77	2,37	1.63	0	1.63	0	2.73
	ZP 666	9.75	5.56	2.76	1.58	0	0	3.28
	Means	10.26 <sup>a</sup>	3.97 <sup>b</sup>	2.20 <sup>bc</sup>	0.79 <sup>c</sup>	0.81 <sup>c</sup>	0 <sup>c</sup>	3.00
SVI	ZP 560	1279,30	753,11	438,20	213,82	57,95	3,30	457.61 <sup>b</sup>
	ZP 666	2005,17	1087,79	729,69	233,20	137,83	45,60	706.55 <sup>a</sup>
	Means	1642.24 <sup>a</sup>	920.45 <sup>b</sup>	583.94 <sup>c</sup>	223.50 <sup>d</sup>	97.89 <sup>dc</sup>	24.45 <sup>e</sup>	582.08
RSWC, %	ZP 560	88.37	86.91	84.55	85.17	78.08	79.90	83.83 <sup>a</sup>
	ZP 666	90.07	84.06	78.03	76.58	80.39	77.85	81.16 <sup>b</sup>
	Means	89.22 <sup>a</sup>	85.48 <sup>a</sup>	81.29 <sup>b</sup>	80.88 <sup>b</sup>	79.24 <sup>b</sup>	78.87 <sup>b</sup>	82.38
PhR, %	ZP 560	0	33.00	54.65	74.92	83.57	89.59	55.45
	ZP 666	0	37.77	58.99	75.99	83.72	89.22	57.61
	Means	0 <sup>a</sup>	35.38 <sup>b</sup>	56.82 <sup>c</sup>	73.96 <sup>d</sup>	83.64 <sup>e</sup>	89.40 <sup>f</sup>	56.53
PhSh, %	ZP 560	0	56.48	83.26	89.84	92.98	96.07	69.77 <sup>a</sup>
	ZP 666	0	58.75	46.75	86.76	89.20	93.58	62.51 <sup>b</sup>
	Means	0 <sup>a</sup>	57.61 <sup>b</sup>	65.00 <sup>b</sup>	88.30 <sup>c</sup>	91.09 <sup>c</sup>	94.82 <sup>c</sup>	66.14
DMSI, %	ZP 560	100.00	67.94	36.89	38.06	32.83	17.47	48.86 <sup>b</sup>
	ZP 666	100.00	68.64	65.15	46.13	32.06	23.17	55.85 <sup>a</sup>
	Means	100.00 <sup>a</sup>	68.29 <sup>b</sup>	51.02 <sup>c</sup>	42.10 <sup>d</sup>	32.44 <sup>e</sup>	20.32 <sup>f</sup>	52.4
Factor	RGI	SVI	RSWC	PhR	PhSh	DMSI		
A	ns	**	*	ns	**	**		
B	**	**	**	**	**	**		
AB	ns	**	*	ns	**	**		

Means followed by the same letter within a column are not significantly different by Duncan's Multiple Range Test at the 5% level; \*\* - significant at 1% level of probability, \* - significant at 5% level of probability and ns - not significant

The osmotic stress had significant effect on RGI, SVI, RSWC, PhR, PhSh and DMSI. RGI, SVI, RSWC and DMSI were significantly decreased with increasing osmotic stress. The highest value of RGI (10.26%), SVI (1642.24), RSWC (89.22%) and DMSI (100%) observed at 0 MPa and the lowest on -1.5 MPa (0%, 24.45, 78.87% and 20.32%, respectively). Contrary, PhR and PhSh were significantly increased with increasing osmotic stress and the lowest values recorded at 0 MPa (0% and 0%, respectively) and the highest at -1.5 MPa (89.40% and 94.82%, respectively). However for all studied parameters, except PhR and DMSI, between salinity levels -1.2 MPa and -1.5 MPa significant differences was not found. Similar, *Asmare (2013)* reported that the increase in NaCl concentrations significantly decreased seedling, shoot, and root vigor indices, and significantly increased PhR and PhSh of haricot bean. *Idikut (2013)* stated that SVI of maize were decreased with increasing of salt concentration level.

The interaction of salinity and genotypes had significant effect on SVI, RSWC, PhSh and DMSI.

## Conclusion

The results showed that hybrids significantly differ for RL, ShL, ShFW, RDW, ShDW, SVI, RSWC, PhSh and DMSI. Hybrids did not differ for GE, G, RGI and RFW. Hybrid ZP 666 had better tolerance to salt stress than hybrid ZP 560. Hybrid ZP 666 had higher values for root and shoot parameters, SVI and DMSI and lower for PhSh than hybrid ZP 560. These genetic differences are important for selecting hybrids for cultivation in salt-affected areas. The osmotic stress induced by NaCl had a negative effect on germination and early seedling growth of maize seeds. Root and shoot growth were significantly reduced already at -0.3 MPa. Other examined parameters, except PhSh and PhR, significantly decreased with increasing osmotic stress. PhSh and PhR were significantly increased with increasing osmotic stress. Treatment with -0.3 MPa can be the germination sensitivity threshold in the studied maize hybrids.

## Uticaj genotipa i osmotskog stresa na klijavost i klijance kukuruza

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### Rezime

Cilj istraživanja bio je da se odredi uticaj različitog NaCl osmotskog rastvora (0, -0.3 MPa, -0.6 MPa, -0.9 MPa, -1.2 MPa, -1.5 MPa) na klijavost semena i rani porast klijanaca dva hibrida kukuruza različite grupe zrenja (ZP 560 – FAO 500 and ZP 666 – FAO 600). Klijavost je testirana u laboratorijskim uslovima u sterilnim plastičnim kutijama na filter papiru natopljenom sa različitim NaCl osmotskim rastvorom, u mraku na  $20 \pm 1^\circ\text{C}$ . Rezultati ANOVA pokazali su da je hibrid ZP 666 imao značajno veću dužinu korena (DK) (6.37 cm), dužinu stabla (DS) (2.06 cm), masu svežeg stabla (MSvS) (43.86 mg), masu suvog korena (MSuK) (7.56 mg), masu suvog stabla (MSuS) (5.97 mg), vigor indeks klijanca (VI) (706.55) i indeks tolerancije suve materije na stres (ITSM) (55.85%) nego hibrid ZP 560 (4.18 cm, 1.14 cm, 32.50 mg, 6.54 mg, 4.15 mg, 457.61 i 48.86%). Suprotno, hibrid ZP 560 imao je značajno veći relativni sadržaj vode u klijancima (RSVK) (83.83%) i fitotoksičnost stabla (FS) (69.77%) nego hibrid ZP 666 (81.16% and 62.51%, respectively). Generalno hibrid hibrid ZP 666 imao je bolju toleranciju na stres soli nego hibrid ZP 560. Energija klijanja (EK), klijavost (K), DK, DS, masa svežeg korena (MSvK), MSvS, MSuK, MSuS, indeks klijanja (IK), VI, RSVK i ITSM su signifikantno smanjeni sa povećanjem osmotskog stresa indukovanog sa NaCl. Suprotno, fitotoksičnost stabla i fitotoksičnost korena su se značajno povećali sa povećanjem osmotskog stresa.

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