

## Effect of salt stress on germination and seedling growth in four maize genotypes

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### ABSTRACT

Studying seed germination and seedling growth under salt stress is important to select salt tolerance maize genotype. The present study was carried out to investigate the effect of different levels of NaCl concentrations on seed germination and seedling growth of four Maize genotypes. The laboratory experiment was conducted using two factors (salinity and genotypes) in completely randomized design with four replications at the Seed Technology Laboratory, Department of Seed Science and Technology, Bangladesh Agricultural University (BAU), Mymensingh. Six levels of NaCl were 0, 100, 150, 200, 250 and 300 mM, and four Maize genotypes were BARI Maize 5, BARI Maize 7, Mosaic Maize line, and Plain Maize line. There were 24 treatments combination (4 genotypes × 6 NaCl conc.). The results showed that the seed germination percentage, root and shoot lengths, fresh weight of shoot and root, dry weight of shoot and root, and salt tolerance index were significantly influenced by salt treatment. Results further revealed that seed germination and seedling growth were minimum at 300 mM NaCl and increased subsequently at lower NaCl concentrations *viz.* 250, 200, 150 mM NaCl and control condition. Genetic variation for seed germination and seedling growth were found. At 300 mM NaCl, none of the seeds of the four genotypes germinated except in BARI Maize 5 that indicated higher tolerance of this genotype to salt stress. It might be concluded that genetic differences for seed germination and seedling growth under salt stress existed among four tested maize genotypes with BARI Maize 5 having higher tolerance.

### INTRODUCTION

After wheat and rice, maize (*Zea mays* L.) is the third most important cereal crop and it is cultivated throughout the world including Bangladesh under a wide range of environmental condition. It is a newer crop in our cropping system whose growing areas in last few years were: 3100 ha in 1990, 10000 ha in 1995, 202000 ha in 2010 (Banik et al., 2010) and 312000 ha in 2013 with forecasted area of 404100 ha by 2020 (DAE, 2013; Prasanna et al., 2014). It is mostly grown in cool winter *Rabi* season in North-west and central part of Bangladesh. The Maize growing area in Bangladesh increased at approximately 20% per year since early 1990s. However, the annual deficit of Maize production was nearly around one million ton in 2009-2010 compared to its requirement (Banik et al., 2010).

Being cross pollinated, salinity tolerance may exist in maize (Paterniani, 1990). In fact, maize is

relatively salt-tolerant compared to other two important cereals, rice and wheat. Thus, the prospect of maize cultivation under salinity is higher than rice and wheat cultivation. To keep the pace of future demand, maize growing area can be expanded in agro-ecologically disadvantaged salinity affected coastal zone of Bangladesh.

Seed germination and seedling growth are two critical life stages often subject to high mortality rates due to various stresses. Typically, early plant establishment (germination and seedling) and the reproductive stage are the most sensitive in determining yield under stress (Barnabas et al., 2008). The adverse effect of salt stress appears on the entire plant at almost every stage of growth including germination, seedling development, vegetative and reproductive stages. However, tolerance to salt stress at every stage varies from species to species (Nawaz et al., 2010).

Even though a lot of studies have described reasons for shoot growth retardation and yield loss in maize in response to salinity (Hatzig et al., 2010; Munns et al., 2000; Pitann et al., 2009; Uddin et al., 2013; Uddin et al., 2014), genotypic variation in regards to seed germination and seedling growth under salt stress has been studied in a lesser extent (Bakht et al., 2011; Carpici et al., 2009). In best of our knowledge there is scanty of literature in favour of the evidence about seed germination and seedlings performance of maize in Bangladesh as influenced by salt stress. Keeping in view this idea a laboratory investigation was conducted for observing germination and seedling growth of 4 Maize genotypes with the aims to determine seed germination and seedling growth in four maize genotypes as affected by varying degree salt stress imposed by NaCl solution; and to assess genotypic variations of studied genotypes in terms of their relative performance under various levels of salt stress.

## MATERIALS AND METHODS

### Experimental layout

A petridish experiment was conducted in the laboratory of the Department of Seed Science and Technology, Bangladesh Agricultural University, Mymensingh, during the period of January 2014 to June 2014. The experiment was laid out following completely randomized design (CRD) having two factors *viz.* NaCl concentrations and maize genotypes. Six different concentrations of NaCl *viz.* 0, 100, 150, 200, 250 and 300 mM were tested on four maize genotypes namely BARI Maize 5, BARI Maize 7, Plain Maize line and Mosaic Maize line. Each treatment was replicated four times. Four Maize genotypes were used in the experiment. Among these four, seeds of two genotypes (BARI Maize 5 and BARI Maize 7) were collected from Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur, while seeds of other two genotypes (Plain Maize line and Mosaic Maize line) were generously gifted by Prof. Dr. Md. Abdur Rahim, Department of Horticulture, Bangladesh Agricultural University, Mymensingh from his BAU-USDA-ARS collection.

### Seed processing and germination assays

Seeds were soaked in aerated water for 24 hours to facilitate imbibitions for activating enzyme matrix responsible for germination. Surface moisture of seed were removed by blotting paper and then treated with the Thiram @ 5 g kg<sup>-1</sup> seed before placing them on the petri dish. Sodium chloride (NaCl) solutions of 0, 100, 150, 200, 250 and 300 mM were prepared from a stock solution of 1 M NaCl. Distilled water was used as a control (0 mM NaCl). 192 seeds of each genotype were placed in two folds of Whatman no. 1 filter paper placed in petri dishes following ISTA rules. Each petridish was moistened either with 4 mL of distilled water (control) or with one of the five levels of NaCl solution (100, 150, 200, 250 and 300 mM NaCl). The level of water and salt solution in petri dishes were assessed daily and applied time to time as per requirement. Germination percentage was recorded throughout the eight days whereas root and shoot length, root and shoot fresh and dry weight were recorded on day 8. Germination was assayed under room temperature (25 ± 2°C).

### Plant characters studied

From second day, the germinated seeds were counted daily at 10.00 am. At that time, those seeds were considered germinated whose radicle emerged out of the seed coat and measured ≥ 0.05 mm. The final germination percentage (GP) was calculated as follows:

$$GP = Ni / N * 100$$

Here, GP = Germination percentage,

Ni = Number of germinated seed on last day of counting

N = Total number of seeds placed for germination

The result is expressed in % by multiplying 100.

### Germination index (GI)

The germination Index (GI) was calculated as described in the Association of Official Seed Analyst (AOSA, 1983) by following formula:

$$\text{Vigour or Germination Index (GI)} = \sum ni / di$$

Where,

n<sub>i</sub> = number of seedlings emerging on day 'd<sub>i</sub>'

d<sub>i</sub> = day after seeding (placement) for germination

The seed lot having greater germination index is considered to be more vigorous.

### Salt tolerant index (STI)

$$\text{STI} = \frac{\text{Total dry weight of seedling in a certain salt treatment}}{\text{Total dry weight at control}} \times 100$$

### Fresh and dry weights of seedling

Fresh weight was taken just after harvest with the help of a digital scale. For getting dry weight, samples were placed in oven at  $78 \pm 2^{\circ}\text{C}$  for 48 hours. Afterwards, samples were removed from the oven, cooled and placed on digital precision

balance for getting accurate dry weight. Data were analyzed following SPSS software and MS Excel 2007.

### RESULTS AND DISCUSSION

In present study, it was found that NaCl solution of  $\geq 150$  mM had strong inhibitory effect on seed germination and germination index, seedlings performance in terms of length, fresh and dry weight of plant parts, and salt tolerance index (STI) tested in four maize genotypes (Bari Maize 7, Bari Maize 5, Mosaic Maize line and Plain Maize line).

Table 1

Seed germination and germination index at different days after seeding in four maize genotypes as influenced by various levels of NaCl solution.

Genotype	NaCl conc. (mM)	Germination % on								Germination index
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	
BARI Maize 7	0	28.1	34.4	34.4	34.4	43.8	71.9	84.4	100.0	3.95
	100	31.3	37.5	43.8	46.9	56.3	65.6	81.3	100.0	3.51
	150	21.9	31.5	46.9	56.3	62.5	68.8	81.3	100.0	3.18
	200	12.5	25.0	31.3	37.5	50.0	75.0	81.3	81.3	2.20
	250	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
BARI Maize 5	0	37.5	84.4	90.6	90.7	100.0	100.0	100.0	100.0	5.19
	100	15.6	59.4	71.9	87.5	100.0	100.0	100.0	100.0	4.10
	150	28.1	56.3	56.3	68.7	71.9	75.0	93.8	93.8	4.08
	200	15.6	53.1	59.4	65.6	71.9	71.9	78.1	81.25	3.32
	250	25.0	46.9	50.0	53.1	53.1	62.5	68.8	68.75	3.20
Mosaic Maize line	0	18.8	25.0	31.3	53.1	78.1	59.4	43.8	43.75	3.13
	100	46.9	78.1	78.1	78.1	78.1	84.4	87.5	100.0	5.21
	150	43.8	65.6	75.0	75.0	84.4	96.9	96.9	100.0	4.94
	200	40.6	59.4	68.8	75.0	81.3	87.5	87.5	87.50	4.56
	250	18.8	43.8	50.0	56.3	56.3	68.8	75.0	75.00	3.09
Plain Maize line	0	18.8	43.8	46.9	46.9	46.9	53.1	56.3	62.50	2.79
	100	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.000	0.00
	150	71.9	100.0	100.0	100.0	100.0	100.0	100.0	100.0	6.88
	200	37.5	75.0	84.4	93.8	100.0	100.0	100.0	100.0	4.94
	250	34.4	75.0	75.0	75.0	81.3	81.3	81.3	93.75	3.81
Significance	Genotype (G)	1289.1**	6057.0**	5995.7**	7517.6**	8110.9**	4084.2**	2722.9**	1293.4**	13.9**
	Salt conc. (S)	3406.9**	9308.4**	10248.6**	10088.6**	10760.1**	14256.9**	17466.8**	20197.9**	42.9**
	G×S	455.7**	515.9**	582.8**	741.3**	1165.9**	986.6**	776.4**	1048.6**	2.9**

**Note:** Seeds did not germinate at 300 mM in all genotypes (except BARI Maize 5), also at 250 mM in BARI Maize 7.

### Effect of salt stress on germination and germination index

The effect of salt stress to four maize genotypes, and their interaction on seed germination, seedling growth and germination index (GI) or vigour at 8 days after seeding was significant ( $P \leq 0.05$ ) (Table 1).

The final seed germination on day 8 varied between 11-100% under 100-300 mM NaCl stress compared to the control which had 100% seed germination (Fig. 1). Also, per cent emergence delayed with higher salt treatments (Fig. 1) (Plate 1). Under salt stress, at 8 DAS, GI and % germination were significantly higher in BARI Maize 5 than Plain Maize and Mosaic Maize genotypes, whereas BARI Maize 7 showed the lowest % germination and GI under salt stress (Table 1). The interaction effect between salt treatments and genotypes on % germination and GI was also significant (Table 1).

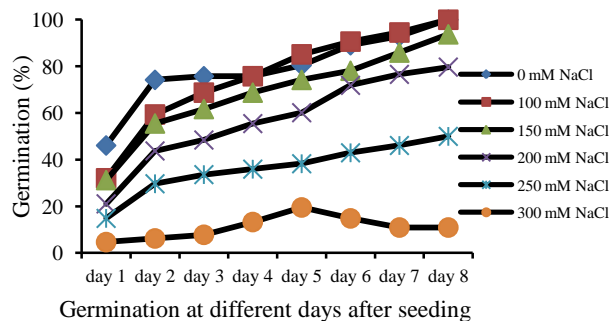


Figure 1  
Changes in germination at different days after seeding in various levels of NaCl solution.

Among the 24 different treatment combinations, germination index and % germination ranged between 0.00-5.19 and 0-100, respectively. The highest GI (6.88) was found in Plain Maize line at 0 mM NaCl and the lowest (0) in BARI Maize 7, Plain Maize and Mosaic Maize genotypes at 300 mM NaCl. High salt level interferes with maize seed germination (Mirosovljević et al., 2012, Caprici et al., 2009) either by creating osmotic potential external to the seeds preventing water uptake or through the toxic effects of  $\text{Na}^+$  and  $\text{Cl}^-$  ions on germinating seed (Khaje-hosseini et al., 2003). Salinity delays the onset, reduces the rate and increases the dispersion of germination events,

resulting in reduced plant growth and final crop yield (Ashraf and Foolad, 2005).

It is not surprising that in all the four maize genotypes, 100 mM NaCl (around  $10 \text{ dS m}^{-1}$ ) did not produce any inhibitory effect on germination and germination index. This implies that the salt level (around  $10 \text{ dS m}^{-1}$ ) that exerts medium stress for inhibiting at least 50% growth at early vegetative stage of maize plant (Uddin et al. 2013 and 2014; Hatzig et al. 2010) may not be a severe stress at seed germination. Most crop plants are salt tolerant at germination but salt sensitive during emergence and vegetative development when they are exposed to moderate level ( $\sim 100 \text{ mM NaCl}$  or  $\sim 10 \text{ dS m}^{-1}$ ) of salt stress (Grattan and Lächli, 2007). Present study also confirmed the fact that 100 mM NaCl is not inhibitory for Maize seed germination. However, in present study, 150 mM NaCl and all above order salt concentrations were found detrimental for seed germination.

### Influences of salt stress on seedling morphology and root-shoot masses

Root fresh and dry weight; shoot fresh and dry weight, plumule and radical lengths, and number of seminal roots seedling<sup>-1</sup> were significantly influenced due to various levels of salt stress in four tested maize genotypes (Table 2).

Number of seminal roots seedling<sup>-1</sup> ranged from 0.0 to 9.2. The highest number of seminal roots seedling<sup>-1</sup> was found in BARI Maize 5 at 100 mM NaCl whereas the lowest in BARI maize 7 at both of 250 and 300 mM NaCl, and also in Plain Maize line with 300 mM NaCl.

The highest radicle length seedling<sup>-1</sup> (12.3 cm) was recorded in Mosaic Maize line at 100 mM NaCl and the lowest on Mosaic Maize line and BARI Maize 7 in 250 and 300 mM NaCl, and also in Plain Maize line at 300 mM NaCl (Table 2). The maximum plumule length (15.77 cm) was found BARI Maize 5 with the control salt treatment (0 mM NaCl) and the no plumule was recorded by the combination of genotype of BARI Maize 7 with 250 and 300 mM NaCl also BARI Maize 5 and Plain Maize line with 300 mM NaCl.

Table 2

Number of seminal roots, root and shoot fresh and dry masses, plumule and radical lengths in four maize genotypes as influenced by various levels of NaCl concentrations.

Genotype	NaCl Conc. (mM)	No. of seminal roots seedling <sup>-1</sup>	Radicle length (cm)	Plumule length (cm)	Root fresh weight (g)	Root dry weight (g)	Shoot fresh weight (g)	Shoot dry weight (g)
BARI Maize 7	0	5.8	9.3	7.9	0.88	0.56	1.33	0.47
	100	4.2	9.3	7.1	0.75	0.20	1.28	0.39
	150	3.7	7.4	5.8	0.57	0.12	1.06	0.34
	200	3.6	2.5	2.2	0.28	0.04	0.22	0.06
	*250	-	-	-	-	-	-	-
	*300	-	-	-	-	-	-	-
BARI Maize 5	0	9.1	9.9	9.8	0.95	0.17	1.46	0.16
	100	9.2	8.1	6.9	0.91	0.16	1.05	0.14
	150	8.0	6.8	4.2	0.74	0.12	0.88	0.11
	200	6.4	4.3	2.3	0.63	0.12	0.88	0.11
	250	3.0	2.4	2.3	0.44	0.12	0.68	0.11
	300	1.2	0.8	0.0	0.21	0.10	0.38	0.06
Mosaic Maize line	0	2.9	10.6	15.7	0.76	0.14	2.45	0.21
	100	7.4	12.3	12.8	0.55	0.12	1.76	0.18
	150	6.7	7.6	6.4	0.23	0.08	1.14	0.13
	200	3.1	5.4	5.3	0.18	0.05	0.16	0.05
	250	1.7	2.6	0.8	0.11	0.03	0.11	0.02
	*300	-	-	-	-	-	-	-
Plain Maize line	0	2.3	11.0	8.9	0.62	0.10	1.28	0.08
	100	1.9	9.4	6.6	0.32	0.05	0.92	0.07
	150	1.7	6.7	2.5	0.30	0.05	0.38	0.03
	200	2.4	3.7	1.4	0.15	0.04	0.14	0.02
	250	0.6	2.1	0.9	0.11	0.03	0.069	0.02
	*300	-	-	-	-	-	-	-
Significance (F value)	Genotype (G)	92.995**	13.348**	61.983**	0.74**	0.07**	1.16**	0.13**
	Salt conc. (S)	74.848**	273.617**	275.012**	1.29**	0.10**	6.13**	0.13**
	G×S	8.498**	3.02**	10.33**	0.04**	0.034**	0.43**	0.03**

The interaction effect of variety and NaCl had significant effect on root fresh weight (Table 2). The highest (0.95 g) was found at 0 mM of BARI Maize 5 genotype and lowest was in BARI Maize 7 at 250 and 300 mM along with Mosaic line and Plain line at 300 mM of NaCl concentration. The root dry weight was significantly decreased with increasing concentration of NaCl (Table 2). The highest dry weight (0.24g) was found at 0 mM of NaCl and lowest (0.02) was on 300 mM of NaCl.

Combined effect of genotype and NaCl concentration had significant influence on the shoot fresh weight (Table 2). The highest shoot fresh weight (2.45 g) was found in Mosaic Maize line at 0 mM NaCl concentration. Also, the interaction effect of genotype and NaCl concentrations was significant on shoot dry weight (Table 2). The maximum dry weight was found in BARI Maize 7 (0.47 g) in combination with 0 mM NaCl concentration.



Higher the salt tolerance index (STI) value better would be the tolerance. STI decreased with increasing salt concentration: 100 mM (89.42) > 150 mM (70.93) > 200 mM (42.17) > 250 mM (29.12) > 300 mM (17.03) (Fig. 3). Combined effect of genotype and salt concentration on STI was significant ( $P \leq 0.01$ ) (Fig. 2). Although STI increased with increasing salt concentration, this

trend was followed only in Mosaic Maize line and Plain Maize line. In BARI Maize 7, STI increased from 64.95 (at 100 mM) to 91.84 (at 150 mM) followed by a decline. Salt tolerance index (STI) is a relative parameter that can be used to differentiate genotypes for their degree to salt tolerance.

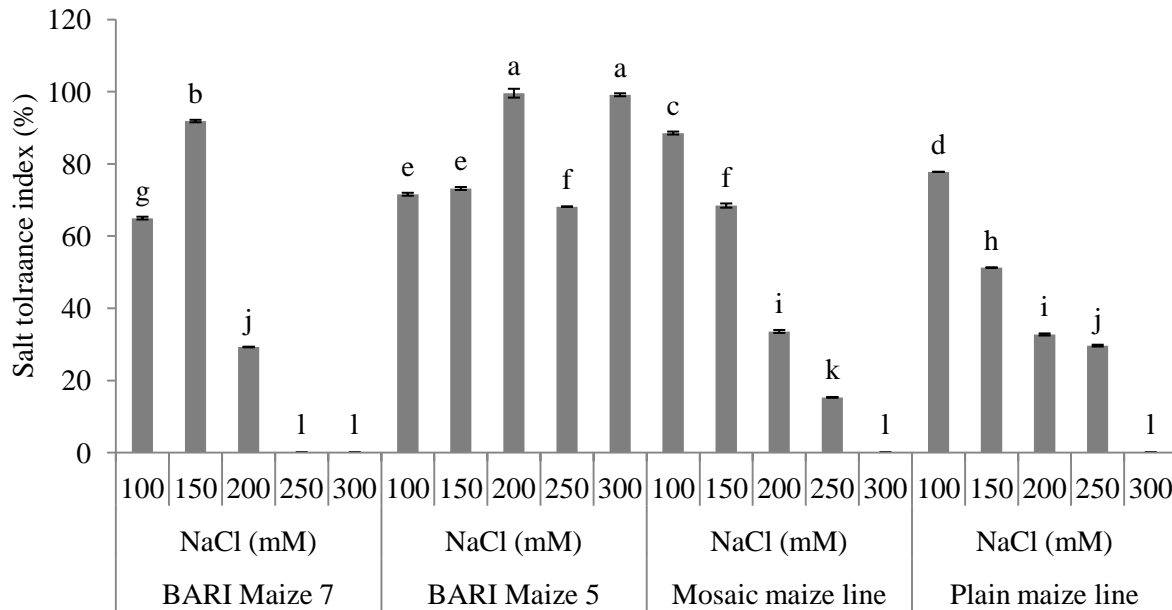


Figure 2 Changes in salt tolerance index at different salt concentrations. Vertical bars upon histogram indicate SEM (n = 4). Histograms sharing common letter do not differ significantly at  $P \leq 0.05$  as per Tukey

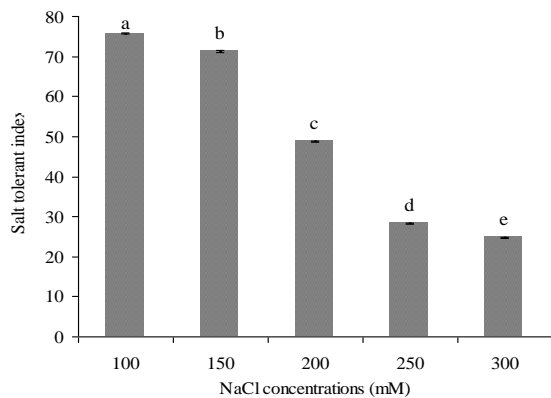


Figure 3 Salt tolerance index (STI) as affected by various levels of NaCl. Uncommon letter, above bar graph, differs significantly at  $P \leq 0.05$  by Tukey.

The ranking of STI for the studied four Maize genotypes was as follows: BARI Maize 5 > Mosaic Maize > BARI Maize 7 and Plain Maize. Thus, BARI Maize 5 performed much better in salt-stressed environment than other genotypes. Since there were genotypic differences, further work is necessary in order to determine the response of other maize genotypes to salinity during germination and to identify any suitable genotypes to cultivate under the saline prone areas of Bangladesh.

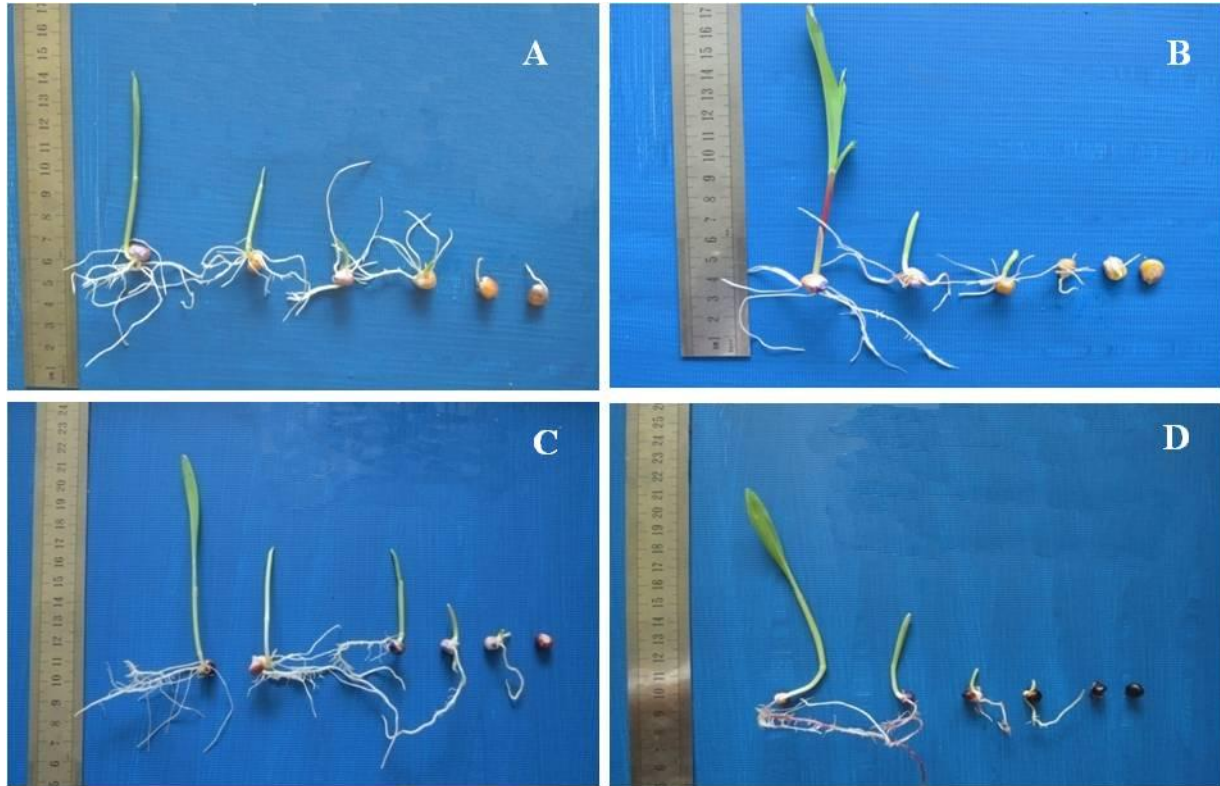


Plate 1

Influence of different concentrated NaCl solutions (from left 0, 100, 150, 200, 250 and 300 mM NaCl) on seed germination and seedling morphology in four Maize genotypes: (A) BARI Maize 5, (B) BARI Maize 7, (C) Mosaic Maize line and (D) Plain Maize line. In each photograph, the left placed seedling shows control treatment and the right five consecutive seedlings/seeds show their performance at 100, 150, 200, 250 and 300 mM NaCl, respectively, on day 7 after setting seeds for germination.

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