

Original Article

Osmotic stress and specific ion toxicity effects on seed germination and early seedling growth of eggplant (*Solanum melongena* L. cv. MTe 2) incurred by various salts

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Abstract

Eggplant is an important crop but no study has been done regarding salt tolerance of eggplant (*Solanum melongena* L. cv MTe 2). Thus, the present work aimed to compare the effects of types and concentrations of salt solutions on seed germination and early seedling growth of MTe 2 eggplant. Ten sterilized MTe 2 eggplant seeds were treated with different concentrations (10, 25, 50, 100, 150, 200 mM) of NaCl, KCl, MgCl₂ and MgSO₄ in Petri dish with deionized water as control. The germination and seedling growth were significantly reduced as concentration of salt increased in NaCl and KCl. The germination performance and seedling growth were enhanced in 25 mM KCl. The seeds were unable to germinate even at the lowest concentration of MgCl₂ and MgSO₄. Recovery test revealed that while NaCl and KCl imposed an osmotic stress and induced seed dormancy, MgCl₂ and MgSO₄ imposed a specific ion toxicity.

Keywords: eggplant, seed germination, different salts, osmotic stress, specific ion toxicity

1. Introduction

Globally, approximately 25% of the agricultural land is salt-affected and this remains a major problem in arid and semi-arid regions (FAO & ITPS, 2015; Karan & Subudhi, 2012). In saline soils, sodium (Na⁺) ions are the main ions and may coexist with other salts, such as chlorides (Cl⁻) and sulphates (SO₄²⁻) of calcium (Ca²⁺) and magnesium (Mg²⁺). An electrical conductivity (EC) exceeding 4 dS/m corresponds to 40 mM NaCl/1.25 g/kg salt level (IRRI, 2011; Sheldon, Dalal, Kirehhof, Kopittke & Maenzies, 2017). Saline habitat is a major limitation by the environment that affects physio-

logical and ecological characteristics of plants, such as germination, developmental growth, reproduction and geographical distribution (Rahimi, 2013). Depending on the composition of salts in the soil, osmotic effects or ion toxicities may affect crop plants.

Seed germination is a critical and decisive phase in the life cycle of a plant, as the survival and continuity of a species depend on the ability of the plant to germinate and establish itself as a seedling in its environment (Deng *et al.*, 2014). Delay and reduction in seed germination and later seedling emergence may lead to non-uniform stand establishment and reduced yield (Wojtyla, Lechowska, Kubala, & Garczarska, 2016). Salinity stress is a well-known, major abiotic factor that provokes disorders in seeds and in its entirety affects germination, seedling development, crop growth and productivity, by disrupting homeostasis in water potential, water uptake and ion distribution.

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Many salinity studies have solely focused on NaCl, but in reality salt-affected soils are composed with many different types of salts as well. The responses of an eggplant depend on variety, developmental stage, genotype, and environment in which it is cultivated. Currently, no prior study is available regarding the salt tolerance of eggplant (*S. Melongena* L. cv. MTe 2). Eggplant is important as the most common crop in Asian countries and worldwide. Not only its edible fruit is consumed in various ways, but the anthocyanin contents provide tremendous health benefits (Lim, 2013; Li, Wang, Luo, Zhao, & Chen, 2017). Thus, the present study was carried out to compare the effects of different types and concentrations of salt on seed germination behavior and early seedling growth of eggplant (*S. melongena* L. cv. MTe 2).

2. Materials and Methods

2.1 Plant materials and location of study

Seeds of eggplant (*S. melongena* L. cv. MTe 2) were purchased from Malaysian Agricultural Research and Development Institute (MARDI), Serdang, Selangor, in July 2015. The study was conducted in Cellular Biology Laboratory in the Department of Biology, Faculty of Science, Universiti Putra Malaysia, from January 2016 to August 2017.

2.2 Effects of different salts on germination and seedling growth of MTe 2

According to the method of Al-Hassan *et al.* (2016) with slight modifications, healthy and uniform sized MTe 2 seeds were surface sterilized with 70% ethanol for 30 seconds, followed by 10% sodium hypochlorite (NaClO) solution for 10 minutes. Four types of salt solutions, namely of NaCl (Sigma, USA), KCl (MERCK, Germany), MgCl₂ (MERCK, Germany) and MgSO₄ (MERCK, Germany) were prepared at concentrations 25, 50, 100, 150 and 200 mM, and deionised water served as control. Ten sterilized MTe 2 seeds were placed in a 9-cm diameter Petri dish containing two Whatman No.1 filter papers moistened with 5 ml of a salt solution (Deng *et al.*, 2014). The petri dishes were tightly sealed with parafilm and left to germinate at 25 °C and 12 hours light with 12 hours dark photoperiod for 10 days. The experimental designed was 4x6 factorial (four types of salt and six concentrations of salt solution) in a complete randomized design (CRD) with three replicates (ten seeds per replicate) for each treatment, and was repeated twice. The seeds were considered germinated with any visible radicle protrusion (Bahrami & Razmjoo, 2012).

After ten days, five seedlings from each salt treatment were selected for measurement of hypocotyl length, radicle length, seedling length, and fresh weight of seedlings. For measurement of biomass, the seedlings were oven-dried for 48 hours at 65°C (Achakzai, 2009). All the ungerminated seeds were rinsed and placed in sterilized distilled water under otherwise similar conditions, to determine the recovery of germination. Germination of the previously ungerminated seeds was monitored and recorded daily for three days.

Germination percentage was calculated as $GP = \frac{\text{Number of seeds germinated in salt treatment}}{\text{Total number of seeds}} \times 100$. Germination rate is a measure of the rapidity of germination and was calculated using a formula by Nasri,

Saidi, Kaddour & Lachaal (2015), $GR = \frac{\sum(n1/d1)}{d}$, where n is the number of germinated seeds and d is the number of days. Relative salt injury rate was calculated as $RIR = \frac{GP \text{ control} - GP \text{ salt treatment}}{GP \text{ in control}}$. Salt tolerance was calculated by the formula $ST = \frac{\text{Seedling dry weight in treatment}}{\text{Seedling dry weight control}} \times 100$. Seed vigor was calculated as seed vigor = $\frac{[(\text{length of hypocotyls} + \text{length of radicle}) \times (\text{germination percentage})]}{100}$. Meanwhile, high germination recovery indicated that the previous seed germination was inhibited by osmotic effect, while low germination recovery was caused by specific ion effect (Lin *et al.*, 2016). It was calculated as percent recovery = $\frac{(d-s)}{(n-s)} \times 100$, where d is the number of seeds germinated in distilled water, s is the number of seeds germinated in salt solution and n is the total number of seeds.

Seeds treated with 200 mM NaCl and 25 mM MgSO₄ were collected at day 10 and preserved in Davidson solution consisting of formalin, acetic acid and 70% ethanol (1:1:17 v/v) for 24 hours. Histological study was then carried out following Chehregani, Mohsenzade and Ghanad (2011).

2.3 Data analysis

Statistical analysis was carried out using SPSS Windows version 22 (SPSS Inc., USA). All data were subjected to Two-way analysis of variance (ANOVA) to test the effects of primary factors (type of salt and concentration of salt) and their interactions at p<0.05 confidence level. Significant differences among treatment means were identified using Tukey HSD (Honestly Significant Differences) multiple range test as a post hoc test.

3. Results

The interaction between a type of salt and its concentration significantly affected the germination percentage, germination rate and relative salt injury rate of MTe 2 seeds (two-way ANOVA, p<0.05). As the level of salinity increased, the germination percentage and germination rate were markedly reduced in NaCl and KCl treatments (Figure 1 and Table 1). As shown in Table 1, although the MTe 2 seeds were able to germinate at the high 150 mM concentration of NaCl and 200 mM of KCl, the seeds were unable to tolerate even the lowest 25 mM concentrations of MgCl₂ and MgSO₄ as the seedlings died after germination: hence, no germination data was recorded. The germination rate was highest when treated with 25 mM KCl, which suggests that 25 mM KCl favored seed germination. As the concentration of a salt increased, the time taken for germination to occur became longer. The reduction in germination rate was more pronounced at 150 mM and 200 mM concentrations of all salts, indicating that the germination process was severely delayed. The MTe 2 seeds experienced injury at the concentrations 100 mM, 150 mM and 200 mM of NaCl; while for KCl, the seeds experienced injury only at 150 mM and 200 mM. The relative injury rate increased significantly (p<0.05) with concentration. The highest relative injury rate was observed for 200 mM NaCl.

Seed vigor, percent recovery test and salt tolerance of MTe 2 seeds were significantly affected by the interaction between salt type and its concentration (two-way ANOVA, p<0.05). This study found that the vigor indexes were signi-

Table 1. Germination percentage, germination rate and relative salt injury rate of MTe 2 seeds by concentration and type of salt.

Parameter	Concentration of salt (mM)	NaCl	KCl	MgCl ₂	MgSO ₄
Germination percentage	0	100.00 ± 0.00 ^a			
	25	100.00 ± 0.00 ^a	100.00 ± 0.00 ^a	-	-
	50	100.00 ± 0.00 ^a	100.0 ± 0.00 ^a	-	-
	100	86.67 ± 2.11 ^b	100.00 ± 0.00 ^a	-	-
	150	30.83 ± 4.90 ^c	50.00 ± 3.65 ^b	-	-
	200	0.00 ± 0.00 ^d	31.67 ± 6.54 ^c	-	-
Germination rate	0	14.00 ± 0.09 ^a	14.00 ± 0.09 ^{ab}	14.00 ± 0.09 ^a	14.00 ± 0.09 ^a
	25	12.76 ± 0.14 ^b	14.51 ± 0.29 ^a	-	-
	50	11.47 ± 0.16 ^c	12.93 ± 0.49 ^{bc}	-	-
	100	10.31 ± 0.27 ^d	12.32 ± 0.24 ^c	-	-
	150	1.82 ± 0.06 ^e	9.07 ± 0.26 ^d	-	-
	200	0.00 ± 0.00 ^f	1.95 ± 0.18 ^e	-	-
Relative salt injury rate	0	0.00 ± 0.00 ^d	0.00 ± 0.00 ^c	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a
	25	0.00 ± 0.00 ^d	0.00 ± 0.00 ^c	-	-
	50	0.00 ± 0.00 ^d	0.00 ± 0.00 ^c	-	-
	100	0.13 ± 0.02 ^c	0.00 ± 0.00 ^c	-	-
	150	0.69 ± 0.05 ^b	0.50 ± 0.04 ^b	-	-
	200	1.00 ± 0.03 ^a	0.70 ± 0.07 ^a	-	-

Values are mean of six replicates ± standard error of measurement. “-”; the seedlings died after protrusion of radicle. Values with different superscript letters within the same column are significantly different at p<0.05, by Tukey comparison test.

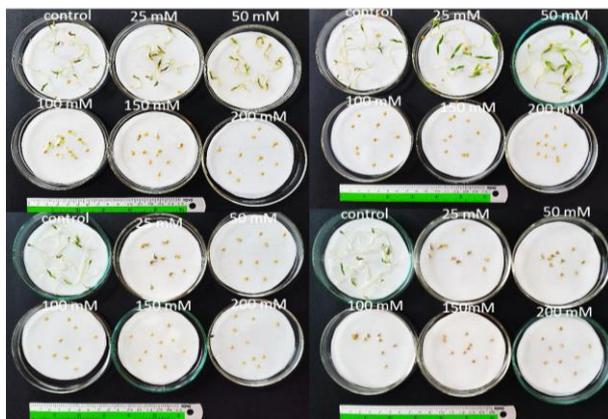


Figure 1. Germination of MTe 2 in various concentrations of NaCl, KCl, MgCl₂ and MgSO₄ at day 10.

ificantly reduced as level of salinity increased (Table 2). The highest germination and seedling performances were found when treated with 25 mM KCl as it produced the highest vigor index. Seed vigor was drastically reduced at 150 mM and 200 mM concentrations, regardless of salt type. High quality seeds are still viable at high concentrations and able to germinate again once the salinity decreases. Recovery test is used to determine whether the seeds died due to a specific ion effect, or were merely temporarily inactivated by osmotic stress (Lin *et al.*, 2016). The high recovery percentages presented in Table 2 for 100 mM, 150 mM and 200 mM NaCl; 150 mM and 200 KCl; and 150 mM and 200 mM MgCl₂ indicate that the previous seed germination was inhibited by osmotic effects, while the low germination recoveries at 25 mM, 50 mM and 100 mM MgCl₂; and all concentrations of MgSO₄ were caused by specific ion effects. The salt tolerance of MTe 2 seeds can be rank ordered as follows: KCl>NaCl>MgCl₂>MgSO₄. The salt tolerance significantly depended on the concentration of a salts, while also depending on the type of salt.

For NaCl, the level of tolerance was as follows: 0 mM & 25 mM>50 mM>100 mM>150 mM>200 mM. In treatments with KCl, the MTe 2 seeds’ salt tolerance was as follows: 50 mM>0 mM & 25 mM>100 mM> 150 mM>200 mM.

Plant growth was significantly affected by the interactions between type of salt and its concentration (two-way ANOVA, p<0.05). In general, MTe 2 showed a significant (p<0.05) decreasing trend with salt concentration in early seedling growth (Figure 2). This effect, however, had significant variation by type of salt and its concentration, and a different pattern of tolerant behavior than in germination phase. Figure 2 depicts the relationship between seedling length and concentration of each salt. NaCl caused a gradual decrease of seedling length as the salt concentration increased. No seed germination was observed for 200 mM NaCl, hence no seedling emergence. On the other hand, KCl had significantly (p<0.05) different effects on seedling length depending on the concentration of salt. Treatment with 25 mM KCl resulted in the longest seedlings among the treatments.

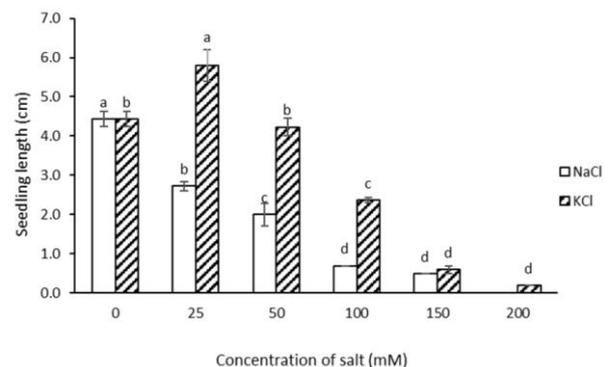


Figure 2. Seedling length of MTe 2 seedlings in various salt treatments. Bars represent mean of three replicates ± standard errors. Bars followed by different lowercase letters are significantly different (compared among NaCl or KCl treatments) at p<0.05, by Tukey comparison test.

Table 2. Seed vigor, percent recovery and salt tolerance of MTe 2 seeds by concentration and type of salt.

Parameter	Concentration of salt (mM)	NaCl	KCl	MgCl ₂	MgSO ₄
Seed vigor	0	4.43 ± 0.19 ^a	4.39 ± 0.19 ^b	4.43 ± 0.19 ^a	4.43 ± 0.19 ^a
	25	2.73 ± 0.12 ^b	5.80 ± 0.40 ^a	-	-
	50	2.00 ± 0.00 ^c	4.23 ± 0.22 ^b	-	-
	100	0.61 ± 0.00 ^d	2.37 ± 0.07 ^c	-	-
	150	0.15 ± 0.00 ^e	0.30 ± 0.05 ^d	-	-
	200	0.00 ± 0.00 ^e	0.06 ± 0.00 ^d	-	-
Percent recovery	0	-	-	-	-
	25	-	-	0.00 ± 0.00 ^d	0.00 ± 0.00 ^a
	50	-	-	0.00 ± 0.00 ^d	0.00 ± 0.00 ^a
	100	94.45 ± 3.21 ^a	-	20.00 ± 0.00 ^c	0.00 ± 0.00 ^a
	150	88.89 ± 0.00 ^a	90.08 ± 6.75 ^a	100.00 ± 0.00 ^a	0.00 ± 0.00 ^a
	200	84.18 ± 4.26 ^a	100.00 ± 0.00 ^a	85.00 ± 2.89 ^b	0.00 ± 0.00 ^a
Salt tolerance	0	100.00 ± 0.00 ^a	100.00 ± 0.00 ^b	100.00 ± 0.00 ^a	100.00 ± 0.00 ^a
	25	100.00 ± 0.00 ^a	121.43 ± 1.37 ^a	-	-
	50	73.81 ± 1.37 ^b	76.19 ± 1.94 ^c	-	-
	100	52.38 ± 0.00 ^c	25.00 ± 2.99 ^d	-	-
	150	36.90 ± 1.19 ^d	9.52 ± 0.00 ^e	-	-
	200	0.00 ± 0.00 ^e	7.14 ± 2.38 ^e	-	-

Values are mean of four replicates ± standard error of measurement. “-”; the seedlings died after protrusion of radicle. “-” Recovery test was not performed because all seeds germinated in those respective treatments. Values with different superscript letters within the same column are significantly different at $p < 0.05$, by Tukey comparison test.

Treatment with 50 mM KCl had no significant difference from the control treatment in seedling length. However, seedling length decreased significantly from 100 mM KCl onwards. In general, the reduction in seedling length due to salinity was more prominent with NaCl than with KCl, indicating that the seedlings were less tolerant towards NaCl than to KCl.

Figure 3 and Figure 4 show radicle and hypocotyl lengths of seedlings subjected to different salt types and salt concentrations. The effects of a salt concentration depend on the type of salt. In general, increasing the NaCl concentration resulted in gradual reduction in lengths of radicle and hypocotyl. Increasing the NaCl concentration from 25 mM to 50 mM reduced radicle length, but beyond that concentration no length of radicle was recorded, indicating that the growth of radicle was completely inhibited beyond 50 mM NaCl. On the other hand, the growth of hypocotyl treated in NaCl was observable, though the length continued to decrease significantly ($p < 0.05$) until treatment with the 150 mM concentration. Similar to NaCl, although radicle length of seedlings treated with KCl showed decrease from 50 mM until 200 mM, the hypocotyl was observable with length decreasing significantly from 50 mM to 100 mM only. These findings tell us that from 100 mM NaCl and 150 mM KCl onwards the seedlings were abnormal. Figure 5 presents the decreasing trend of seedling heights with the four types of salt. In the treatments with KCl, seedlings treated with 25 mM and 50 mM KCl were longer than control (deionized water), so no seedling height reduction was observed. As the concentration of salt increased, the seedling height reduction with both NaCl and KCl significantly increased, i.e. shorter MTe 2 seedlings were observed as concentration of salt increased, with exceptions for 25 mM and 50 mM KCl.

Seedling biomass was significantly affected by the concentration but not by the choice of type of salt (Figure 5). However, the interaction between type of salt and its concentration was significant at $p < 0.05$. This means that regardless

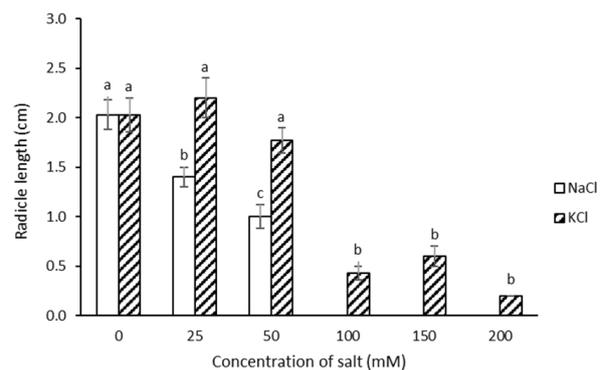


Figure 3. Radicle length of MTe 2 seedlings in various salt treatments. Bars represent mean of three replicates ± standard errors. Bars followed by different lowercase letters are significantly different (compared among NaCl or KCl treatments) at $p < 0.05$, by Tukey comparison test.

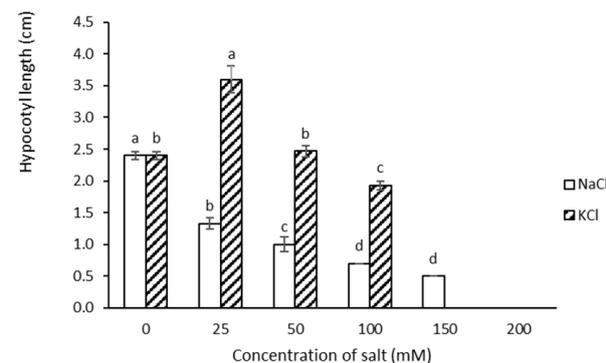


Figure 4. Hypocotyl length of MTe 2 seedlings in various salt treatments. Bars represent mean of three replicates ± standard errors. Bars followed by different lowercase letters are significantly different (compared among NaCl and KCl treatments) at $p < 0.05$, by Tukey comparison test.

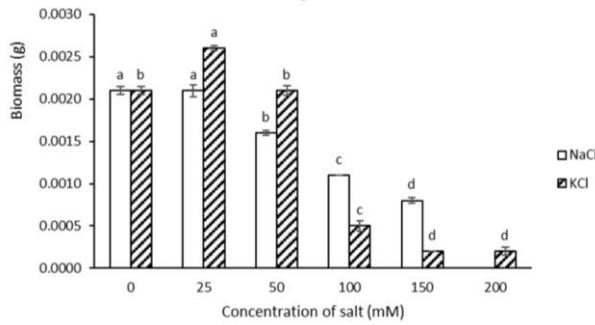


Figure 5. Biomass of MTe 2 seedlings in various salt treatments. Bars represent mean of three replicates ± standard errors. Bars followed by different lowercase letters are significantly different (compared among NaCl or KCl treatments) at $p < 0.05$, by Tukey comparison test.

of the type of salt, the seedlings showed a significant reduction in biomass. Seedling biomass decreased significantly ($p < 0.05$) in response to increasing salt concentration with all types of salt, but with exceptions for 25 mM and 50 mM KCl. KCl at 25 mM significantly increased seedling biomass, while 50 mM of KCl had no significant difference to the control treatment, as seen in Figure 5.

3.1 Seedling morphology

After ten-day treatments with the different salts, osmotic stress caused abnormal growth of the eggplant MTe 2 seedlings in moderate to high concentrations (50, 100, 150, 200 mM) of NaCl and KCl (Table 3 and Figure 6). The seedling symptoms included stunted radicle, and radicle split from the tip, spindly and decayed. Meanwhile the hypocotyls were short and thick, forming a loop or spiral. A progressive browning and leaf blister were visually noted. The cotyledons were small and browning was seen at the tips of cotyledons. On the other hand, the radicle in $MgCl_2$ and $MgSO_4$ showed brown color and complete wilting, which eventually lead to plant death. These results indicate that eggplant MTe 2 is sensitive to salt stress at the stage of early seedling growth.

3.2 Histological examination of MTe 2 seeds

Embryonic development of the seeds was affected by type and concentration of the salt (Figure 7). In the control treatment of ungerminated seeds they were not treated with

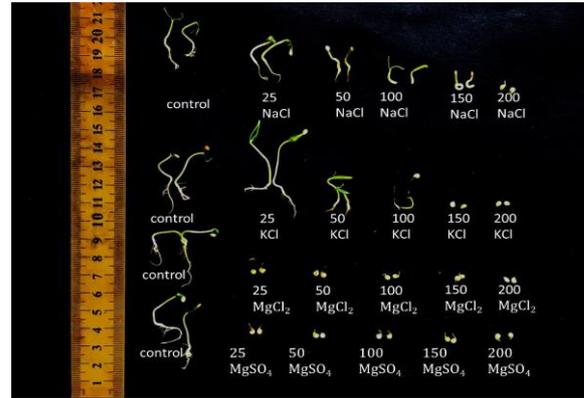


Figure 6. MTe 2 seedlings at day 10 in different concentrations of various salts.

any salt, thus showing dormant behavior of undeveloped radicle, hypocotyl, endosperm and testa. In 200 mM NaCl, the seed embryo showed slight changes in structure. As a seed started the imbibition process with increased metabolic activity and started to grow, it was observed that the radicle, hypocotyl and cotyledon started to elongate. However, the elongation of radicle was halted, and it was unable to break through the testa (seed coat) and remained in dormant state. Meanwhile, anatomical investigations on MTe 2 seeds treated with $MgSO_4$ revealed different findings. Even in the lowest concentration of $MgSO_4$, it was observed that the protrusion of radicle through the seed coat happened but the radicle tissues were severely affected destructively. The hypocotyl, cotyledon, and endosperm however, remained unaffected. The death of cells was observed to start at radicle, which ultimately caused unsuccessful germination and the seed was dead. Even after being placed again in distilled water, the seed was unable to recover and remained dead.

4. Discussion

Orlovsky *et al.* (2016) and Aydinsakir *et al.* (2013) demonstrated that germination and growth were affected by various salinity conditions. The concentration and type of salt used affect both stages. Results from the present study are in line with the prior findings. We found that both concentration and type of salt significantly modulated the effects on both seed germination performance and early seedling growth of MTe 2 eggplant.

Table 3. Morphology of seedlings by level and type of salinity.

Type of treatment	Concentration of treatment (mM)	Radicle	Hypocotyl	Cotyledon	Type of seedling
Control	0	Normal	Normal	Normal	Normal
NaCl	25	Normal	Normal	Normal	Normal with intact seedling
	50	Normal	Normal	Slight necrosis	Normal with slight defects
	100	Primary root stunted	Short and thick	Trapped in seed coat	Abnormal
	150	Primary root stunted	Very short and thick	Trapped in seed coat	Abnormal
	200	Primary root missing	Very short and swollen	Not developed	Abnormal
KCl	25	Normal	Long	Normal	Normal with intact seedling
	50	Normal	Normal	Normal	Normal with intact seedling
	100	Primary root stunted	Short	Trapped in seed coat	Abnormal
	150	Primary root stunted	Very short and swollen	Trapped in seed coat	Abnormal
	200	No germination	No germination	No germination	No germination

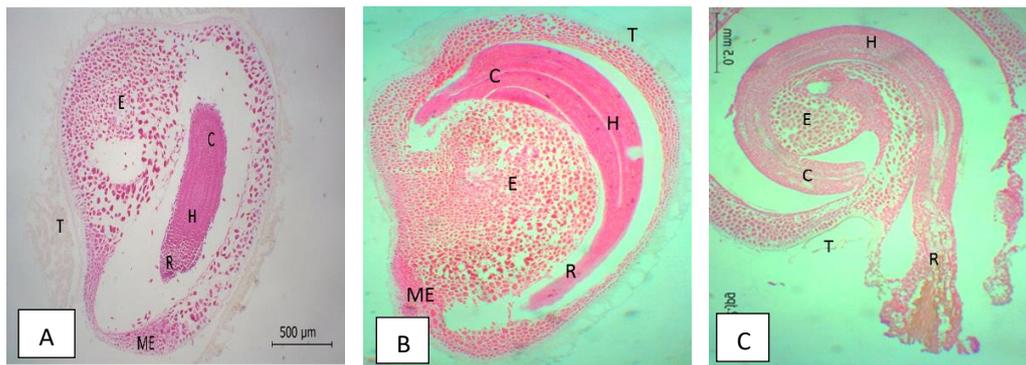


Figure 7. Comparison of cross-sections of MTe 2 seeds from the different treatments: (A) Control, (B) 200 mM NaCl and (C) 25 mM MgSO₄. (B) Inhibition of embryonic development caused dormant behavior of seed, induced by osmotic stress. (C) Inhibition of embryonic development was caused by ion toxicity on radicle. R=Radicle; H=Hypocotyl; C=Cotyledon; E=Endosperm; ME=Mycopylar endosperm; T=Testa. Magnification 4x.

In different types of salt, MTe 2 seeds could only germinate in NaCl and KCl, while germination was inhibited by treatments with MgCl₂ and MgSO₄, even at the lowest tested salt concentration (25 mM). According to Sheldon *et al.* (2017), depending on the salt used, salt stress induces a multitude of mechanisms within a plant (Deinlein *et al.*, 2014) as responses to osmotic stress, ion equilibrium imbalance, specific ion effects and toxicities, and oxidative stress (Panuncio *et al.*, 2014; Reddy *et al.*, 2015). Results from the present study are in line with these findings and confirm both osmotic and specific ion effects in seed germination performance, modulated by concentration and type of salt. The nature of the ions present in salt, permeability of the plasma membrane to them, and the interactions of the ions with seed metabolism also influence the overall germination performance of MTe 2 seeds.

Percent recovery test revealed that low germination percentage in treatments with moderately high to high concentration of NaCl and KCl and high concentration of MgCl₂ were caused by osmotic stress, whereas germination in low or moderate concentration MgCl₂ or in any concentration of MgSO₄ was inhibited by specific ion effects and oxidative stress. In the current study, the osmotic component of NaCl and high concentrations of MgCl₂ strongly inhibited hydration of embryo. The osmotic stress limited seed water uptake, cell division and expansion, and as a result, embryonic development was inhibited because the radicle was unable to break through the testa (seed coat) and remained in dormant state. The decrease in germination rate is an adaptive strategy of the seed to prevent germination in an adverse natural environment, in order to ensure the proper establishment of seedling. The nature of the ions and their interactions with seed also play important roles. MTe 2 seeds could germinate at the highest tested concentration of 200 mM KCl, but were less able at 200 mM NaCl. Germination rate also had significant differences, being higher in treatments with KCl than with NaCl. Based on analysis of the present data, MTe 2 seeds have higher salt tolerance towards KCl than to NaCl.

Meanwhile, early seedling growth of MTe 2 was found to be adversely affected by salt stress, though different salt concentrations had different significant effects. During the early germination phase, seeds experienced osmotic stress and this directly affected the latter phase of seedling growth,

decreasing seedling height, radicle and hypocotyl lengths, and biomass. In salt stress environment the effects of low water potential are countered with uptake of electrolytes, but this could also lead to excessive ion uptake. Non-halophytes in adapting to high internal ion concentration maintain turgor by compartmentalization of ions into vacuoles and neutral solute in cytoplasm; or by avoidance mechanism. The avoidance of excess ion concentration is carried out by controlling the uptake of ions and transport to hypocotyl (Al-Hassan *et al.*, 2016). This will result in an increase in shoot volume, which explains the succulence and short hypocotyl observed during early MTe 2 seedling growth. Decreased biomass is caused by alteration of de novo protein synthesis due to inhibition of fatty acid and carbohydrate metabolizing enzymes during embryo elongation (Ruffino, Rosa, Hilal, Gonzalez & Prado, 2009; Hasanuzzaman *et al.*, 2013).

The present study revealed that germination rate, seed vigor, salt tolerance, seedling length and biomass were significantly increased upon treatment with 25 mM KCl, compared to the other salt treatments including control. This was presumably due to the dominant regulatory role of K⁺ in osmoregulation, turgor provision, and many enzymatic processes. Potassium is the major cation in vacuoles and functions as an osmoregulator maintaining high cell turgor pressure, hence providing structure and enhancing cell expansion, which affect the elongation for plant growth.

MTe 2 seeds were not tolerant to MgCl₂ and MgSO₄, as shown by the inability to germinate at any tested salt concentration, even the lowest 25 mM. Percent recovery test revealed that this may be due to specific ion effects of MgCl₂ and MgSO₄. Histological study showed that extracellular toxic ions crept into the plant system and disrupted physiological activities and biochemical processes in the developing MTe 2 seeds.

Toxic ions can impair electron transport in mitochondria and the photorespiration pathway, inducing oxidative stress (Hasanuzzaman *et al.*, 2013). Though magnesium (Mg²⁺) is an essential nutrient for plant growth, it is also toxic at excessive concentrations. An excess of Mg²⁺ in a plant can inhibit growth and photosynthesis (Shaul, 2002). High concentration of MgCl₂ (100 mM) resulted in low germination rate of radish cv. Cherry belle (*Raphanus sativus*) and over a period of 28 days resulted in subsequent death (Branderburg

& Kleier, 2011). Another study reported that 5 g/L of MgSO₄ delayed germination and decreased the production of alfalfa (*Medicago sativa* L.) (Abid, Haddad & Ferchichi, 2008). The toxic effects of MgCl₂ and MgSO₄ include damage to cellular membrane integrity. Then exosmosis of intracellular ions and organic matter are caused impairment of ion selectivity. While osmotic stress only caused exclusion of NaCl ions, toxicity of MgCl₂ and MgSO₄ caused disruption to physiological activity and biochemical processes of seed (Orlovsky *et al.*, 2016; Saed-Moucheshi, Shekoofa & Pessarakli, 2014).

In conclusion, increasing concentration of a salt adversely affects the overall germination performance and early seedling growth. An exception is the case of 25 mM KCl, which treatment was found to improve the germination performance and early seedling growth of MTe 2. Germination rate, seed vigor, salt tolerance, seedling length and biomass of MTe 2 increased in 25 mM KCl compared to control and other treatments. Further analysis of a percentage recovery test revealed that treatments with NaCl and KCl invoked osmotic stress condition, while MgCl₂ and MgSO₄ are toxic and inhibit seed germination of MTe 2 eggplant. The salt tolerance of MTe 2 seeds by type of salt was ranked as follows: KCl>NaCl> MgCl₂> MgSO₄.

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