



IMPACT OF NaCl, KCl, MgCl₂, MgSO₄ AND CaCl₂ ON THE SEED GERMINATION AND SEEDLING GROWTH OF CUCUMBER (*CUCUMIS SATIVUS* CV. MTi2)

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Abstract

This study was conducted to compare the effects of NaCl, KCl, MgCl₂, MgSO₄ and CaCl₂ on the seed germination and early seedling growth of MTi2. Five types of salts (NaCl, KCl, MgCl₂, MgSO₄ and CaCl₂) at different concentration (50, 100, 150, and 200 mM) and deionized water as control are used. A10 sterilized seeds were placed in petri dishes containing 5ml of deionized water or each salinity solution and kept in the growth chamber at 25 ± 1°C. The experiment is conducted in a completely randomized design with nine replicates. The number of germinated seeds was recorded daily until day 8. The length hypocotyl and radicle length and the biomass of seedlings were measured at day 8. Germination percentage, seed vigor and salt tolerant were calculated. Data were analyzed using SPSS windows version 22. Data are subjected two way ANOVA at p ≤ 0.05 to determine the significant difference between treatment and followed Tukey at p ≤ 0.05 for means comparison. Results show the response of MTi2 seed on five different salts is significantly different. It indicates that the viability of MTi2 seed to germinate relatively high in KCl followed by NaCl, CaCl₂, MgSO₄, and MgCl₂.

Key word: *Cucumis sativus* cv. MTi2, salinity, germination, salt tolerance, seed vigor,

Introduction

Plant exposes to abiotic and biotic stresses since decades ago that cause severe effects on metabolism, growth, development, and productivity (Vorasoot *et al.*, 2003; Kaur *et al.*, 2008; Thakur *et al.*, 2010; Doupis *et al.*, 2011). One of worldwide problem is salinity and caused about 323 million hectares' worldwide land salinized and is estimated to exceed 400 million hectares by 2025 (Hakim *et al.*, 2014; Flowers & Muscolo, 2015). Adaptation of plants to salinity during germination and early seedling stages is critical for the plant stand to be established. The common salt in soil are Na⁺, Ca²⁺, Mg²⁺, Cl⁻, SO₄⁻², and HCO⁻³ (Flower *et al.*, 1997; Hasegawa *et al.*, 2000; Ali, 2010). Cucumber (*Cucumis sativus*) belongs to the gourd, family Cucurbitaceae. It is a widely cultivated, creeping vine that bears cylindrical, fruits that are used as culinary vegetables (Grubben *et al.*, 2004). The cucumber originated from South Asia, but is currently found on most continents. This plant is an important

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greenhouse crop in semi- arid areas with saline ground water so it is necessary more research on the effect of salinity on germination of this plant (Sato *et al.*, 2006). Salinity has a negative effect on the yield of the cucumber crop by reducing germination, seedling growth establishment of the plant weak (Sato *et al.*, 2006). Salinity may impact the germination of seeds either by creasing an osmotic potential external to the seeds, preventing water uptake, the toxic effect of ions on the germinating seeds (Khajeh, 2003). Therefore, the aim of this research is to investigate the effects of individual salts NaCl, KCl, MgCl₂, MgSO₄ and CaCl₂ at concentration 50 mM, 100mM, 150 mM and 200 mM on the seed germination and seedling growth of *Cucumis sativus* cv. MTi2.

Materials and methods

A study was conducted at the Tissue Culture Laboratory of the Dept. of Biology, Universiti Putra Malaysia, Selangor. Five types of salts (NaCl, KCl, MgCl₂, MgSO₄ and CaCl₂) at four different concentrations (50,

100, 150, and 200 mM) and deionized water as a control was used. Sterilization of MT12 seeds was carried out according to method by Panuccio *et al.*, (2014) with slight modification. Mature, healthy and equal sized seeds were selected and were surface sterilized with 5% sodium hypochlorite for ten minutes. Subsequently, the seeds were rinsed with sterilized distilled water for three times and air-dried before being used in the germination tests to avoid any fungal attacks. A 10 seeds were placed on Whatman filter paper in sterilized petri dishes (9cm diameter) containing with 5 ml of deionized water or each salinity solution. The petri dishes were hermetically sealed with parafilm to prevent evaporation and kept in the growth chamber at 25 ± 1°C. The experiment was conducted in a completely randomized design with three replicates and repeated thrice. Seeds were considered germinated when the radicals had extended at least 2mm. The number of germinated seeds was recorded daily until day 8. On day 8, the length of the hypocotyl, radicle and the biomass of seedling were measured by selecting three seedlings randomly from each petri dish (Li, 2008).

Germination percentage (GP %) was calculated according to a formula by Kandil *et al.*, 2012 which is

$$GP \% = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds sown}} \times 100$$

The salt tolerance (ST) was calculated according to Kaymakanova (2009):

$$\text{Salt tolerant (ST)} = \frac{\text{Seedling dry weight of treated}}{\text{Seedling dry weight of control}} \times 100$$

Seed vigor was calculated according to Abdul-Baki and Anderson (1973):

$$\text{Seed vigor} = \frac{(\text{length of hypocotyls} + \text{length of radicle}) \times \text{germination percentage}}{100}$$

Statistical analysis

Statistical analysis was performed using SPSS window version 22. Two way analysis of variance (ANOVA) at confidence level, p ≤ 0. 05 was conducted to find the difference between salts and concentration, followed by Tukey at p ≤ 0.05 for mean comparison.

Results

The Impact of NaCl, KCl, CaCl₂, MgCl₂ and MgSO₄ on the germination MTi2 seed

The result shows the germination of MTi2 seed in different type and concentration of salts is significantly different (ANOVA, p < 0.05). Table 1 presented the germination percentage decreased with increased the concentration of salts except in 50 mM KCl. Each type of salt shows significantly different effect on the germination of MTi2 seed. MTi2 seed able to germinate in NaCl and KCl until high concentration, 200 mM. On the other hand, MTi2 seed unable to germinate MCl₂ and

Table 1: The germination percentage of *Cucumis sativus* cv. MTi2 seeds as a response to different type and concentration of salinity.

| Germination Percentage (%) | | | | | |
|----------------------------|-------------------------|--------------------------|------------------------|--------------------------|-------------------------|
| Concentration(mM) | NaCl | KCl | MgCl ₂ | MgSO ₄ | CaCl ₂ |
| 0 | 98.9 ^e ±1.1 | 98.9 ^e ±1.1 | 98.9 ^e ±1.1 | 98.9 ^e ±1.1 | 98.9 ^e ±1.1 |
| 50 | 95.6 ^e ±1.8 | 98.9 ^e ±1.1 | — | 85.6 ^{cde} ±3.4 | 92.2 ^{de} ±2.2 |
| 100 | 94.4 ^e ±1.8 | 95.6 ^e ±2.4 | — | — | 70.0 ^b ±3.3 |
| 150 | 88.9 ^{de} ±2.6 | 92.2 ^{de} ±2.2 | — | — | 20.0 ^a ±4.7 |
| 200 | 74.4 ^{bc} ±4.7 | 78.9 ^{bcd} ±3.1 | — | — | — |

Values are mean and standard errors of measurement made on nine replicates. Superscripts within the means of each column (a-e) with different letters indicate significant difference among means (Tukey HSD test, p<0.05).

Table 2: Seed vigor of *Cucumis sativus* cv. MTi2 seeds as response to different type and concentration of salinity.

| Seed Vigor | | | | | |
|-------------------|------------------------|------------------------|------------------------|------------------------|------------------------|
| Concentration(mM) | NaCl | KCl | MgCl ₂ | MgSO ₄ | CaCl ₂ |
| 0 | 9.3 ^f ±0.26 | 9.3 ^f ±0.26 | 9.3 ^f ±0.26 | 9.3 ^f ±0.26 | 9.3 ^f ±0.26 |
| 50 | 8.3 ^e ±0.32 | 9.7 ^f ±0.30 | — | 2.5 ^b ±0.32 | 3.1 ^b ±0.21 |
| 100 | 5.1 ^c ±0.18 | 7.1 ^d ±0.25 | — | — | — |
| 150 | 2.2 ^b ±0.13 | 4.4 ^c ±0.13 | — | — | — |
| 200 | 0.8 ^a ±0.14 | 2.2 ^b ±0.1 | — | — | — |

Values are mean and standard errors of measurement made on nine replicates. Superscripts within the means of each column (a-f) with different letters indicate significant difference among means (Tukey HSD test, p<0.05).

MgSO₄ even in low concentration. MTi2 seed can germinate in moderate concentration of KCl, however, the germination percentage decreased drastically in 150 mM.

The effect of NaCl, KCl, MgCl₂, MgSO₄ and CaCl₂ on the vigor of MTi2 seed

Seed vigor is the seed properties which determine the level of activity and performance of the seed during germination and seedling emergence under a wide range of field conditions. Seedling from high vigor seeds are anticipated to develop more uniformly than seedling from low vigor seeds (Egli & Rucker, 2012). The vigor of MTi2 seed significantly different in five different salts at different concentrations as shown in Table 2. Vigor of MTi2 seed highest in KCl, followed in NaCl while lower in MgSO₄ and CaCl₂. The highest vigor MTi2 seed is in 50mM KCl (9.7). The finding reveals that the capacity for germination and tendency for growth of MTi2 seeds with the highest in 50mM KCl.

Tolerance of MTi2 seed in different type and concentration of salt

Results found that the tolerance MTi2 seeds in different types of salts are significantly different. The level of tolerance decreased as salt concentration increased as presented in Table 3. MTi2 seeds highly tolerate in KCl followed by NaCl, on the other hand, intolerant in MgCl₂ and MgSO₄. MTi2 seed moderate tolerate in CaCl₂. The tolerance level of MTi2 seeds can be concluded KCl > NaCl > CaCl₂ > MgSO₄ > MgCl₂.

The Effect of NaCl, KCl, MgCl₂, MgSO₄ and CaCl₂ on the Early Growth of MTi2 Seedlings

Fig. 1 shows increase the concentration of salt caused the reduction in the early growth of MTi2 seedlings except 50 mM KCl. The five types of salts had different effects on the growth of MTi2 seedlings. In all salts, the length of seedling significantly declined with increasing salts concentration except 50mM KCl. The highest seedling (9.8 cm) was found in 50mM KCl.

Fig. 2 shows that the dry weights of the seedlings decrease significantly with increasing salt concentration in all types of salts except 50mM the highest seedling dry weight in 50mM of KCl (0.0189g) followed by control (0.0187g) then 100mM KCl (0.0179), hence the lowest seedling dry weight observed (0.0094g) in 150mM CaCl₂.

Discussion

Results show that each type of salt has different effects on the germination of MTi2 seed. Germination percentage of cucumber seeds were decreased with the increased concentration in all types of salts except 50mM KCl. Reduction in germination percentages of MTi2 seeds is in following order of treatment: KCl > NaCl > CaCl₂ > MgSO₄ > MgCl₂. A similar reduction in germination percentage with increasing salt levels was reported in pumpkin varieties (Aydinsakir *et al.*, 2013), cucumber cultivars (Baghbani & Kadkhodaie, 2013), two Acacia species (Abari and Bayat, 2011). Seed germination is necessary in plant development in order to achieve maximum seedling numbers that will produce higher seed yield (Aishah *et al.*, 2010) It is a critical growth stage often subjected to rising mortality ratio (Asaadi, 2009; Ratnakar & Rai 2013). Seed germination relies on the usage of reserved nutrient material in the seed (Hussain *et al.*, 2011). Thus, the adaptation to salinity when a seed is germinating and the seedling is growing is very essential for healthy plant growth. The different responses of MTi2 seeds and seedlings to different salt solutions are believed to have resulted from differences among various salt components in permeability of the membrane, toxicity and impact on the function of the cell wall or the plasma membrane or both. It was thought that high levels of KCl and NaCl create osmotic potential which prevents the uptake of water, which will change the germination response of MTi2. According to Munns & Tester (2008), the decrease in germination percentage could be due to two reasons, firstly, high accumulation of toxic salt ions which may reduce their water absorption potential, change certain enzymatic or hormonal activities inside the seed;

Table 3: Seed tolerance of *Cucumis sativus* cv. MTi2 seeds as a response to different type and concentration of salinity.

| Concentration(mM) | Salt tolerant | | | | |
|-------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| | NaCl | KCl | MgCl ₂ | MgSO ₄ | CaCl ₂ |
| 0 | 100.0 ^{ef} ±1.7 | 100.0 ^{ef} ±1.7 | 100.0 ^{ef} ±1.7 | 100.0 ^{ef} ±1.7 | 100.0 ^{ef} ±1.7 |
| 50 | 87.5 ^{de} ±1.8 | 101.3 ^f ±1.4 | — | 88.8 ^{def} ±4.4 | 77.2 ^{cd} ±4.3 |
| 100 | 76.8 ^{cd} ±1.7 | 95.9 ^{ef} ±1.9 | — | — | 60.1 ^{ab} ±3.1 |
| 150 | 70.9 ^{bc} ±2.2 | 82.2 ^{cd} ±1.6 | — | — | 50.1 ^a ±3.9 |
| 200 | 57.1 ^a ±1.6 | 74.3 ^c ±1.2 | — | — | — |

Values are mean and standard errors of measurement made on nine replicates. Superscripts within the means of each column (a-f) with different letters indicate significant difference among means (Tukey HSD test, p<0.05).

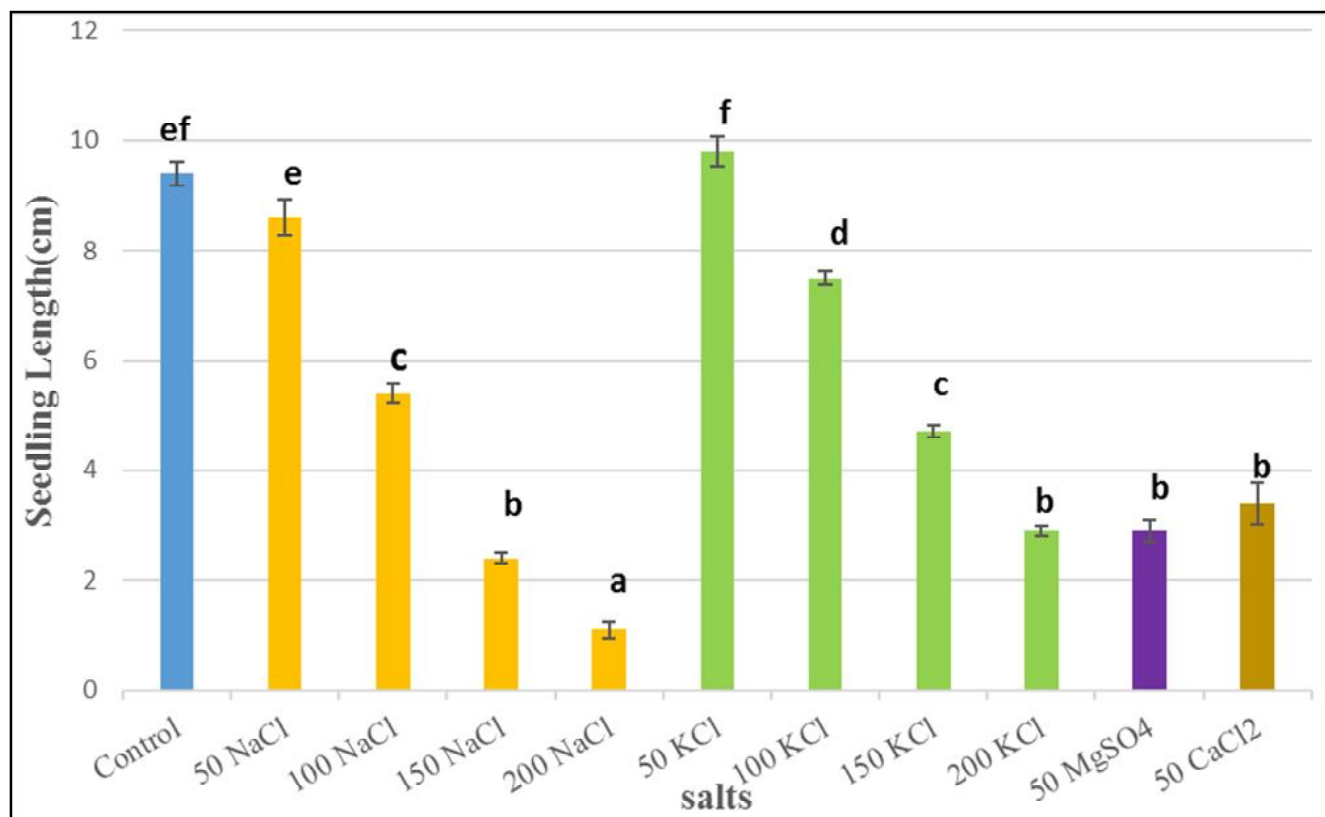


Fig. 1. Seedling length of MTi2 seedlings as a response to different type and concentration of salinity. Different letters indicate significant difference among means (Tukey HSD test, $p < 0.05$).

secondly, decreased water uptake through osmotic effect (Tobe *et al.*, 2004), salinity influence the functions of cell membranes and cell walls that influence the water potential of cytosol and cellular extensibility, eventually affecting seed germination and also seedling growth. Salt induced inhibition of seed germination could be attributed to osmotic stress or toxicity of specific ions (Flowers & Flowers 2005; Salama *et al.*, 2011). Result also found that increasing the concentration of salts led to decreasing germination percentage. Previous study as salinity enhances osmotic pressure, leading to reduction in water absorbent, division of cell and differentiation are inhibited, which adversely influences metabolism, induces leaf chlorosis, upsets the balance of the hormone level and physiological processes (Munns, Mittler, 2002; Ashraf, 2004; Ashraf *et al.*, 2010a). These factors or mechanisms delay the start of germination followed by prolonged seed germination duration (Kang & Saltvett, 2002) and eventually reducing the length of shoot and root (Keshavarzi, 2012). The significant impact of salinity on shoot and root lengths is because high salinity level in the external medium creates high osmotic potential and decreases the available water for cell growth. The decrease in the amount of available moisture could have an impact on cell elongation and thus affect cell expansion

and protrusion of embryo axis, which could hinder shoot and root growth (Munns and Tester, 2008). Ionic stress occurs when ions build up in cells, cause an imbalance in nutrient uptake and toxicity, higher leaf mortality, chlorosis, necrosis and decrease in cellular metabolic activities. It has been recorded that excessive Na^+ and Cl^- can possibly affect plant enzyme, leading to reduction in energy production and other physiological processes. Furthermore, the ion specific effect is described as the increase of toxic ions, e.g. (Na^+ , Cl^-) in the plant tissue with a decrease in useful ions, e.g. (K^+ , Ca^{2+}), thus decreasing plant growth (Khayatnezhad *et al.*, 2010; Karimi *et al.*, 2012). Seed vigor has the potential to determine the rapid and uniform emergence of seedlings and the establishment of crops (Mondo *et al.*, 2013). The vigor of germination depends on the capability of the plant embryo within the seed to maintain and coordinate its metabolic activity sequentially (Rajjou *et al.*, 2012). In this present study vigor of MTi2 seed is found to decrease with increasing levels in all types of salts. A previous study by Hokmalipour (2015) on fennel (*Foeniculum vulgare*), cumin (*Cuminum cyminum* L.) and chicory (*Chichorium intybus* L.) also found that the increased of salt concentration decrease the vigor of the seed. Shoot and root lengths are the most necessary parameters for

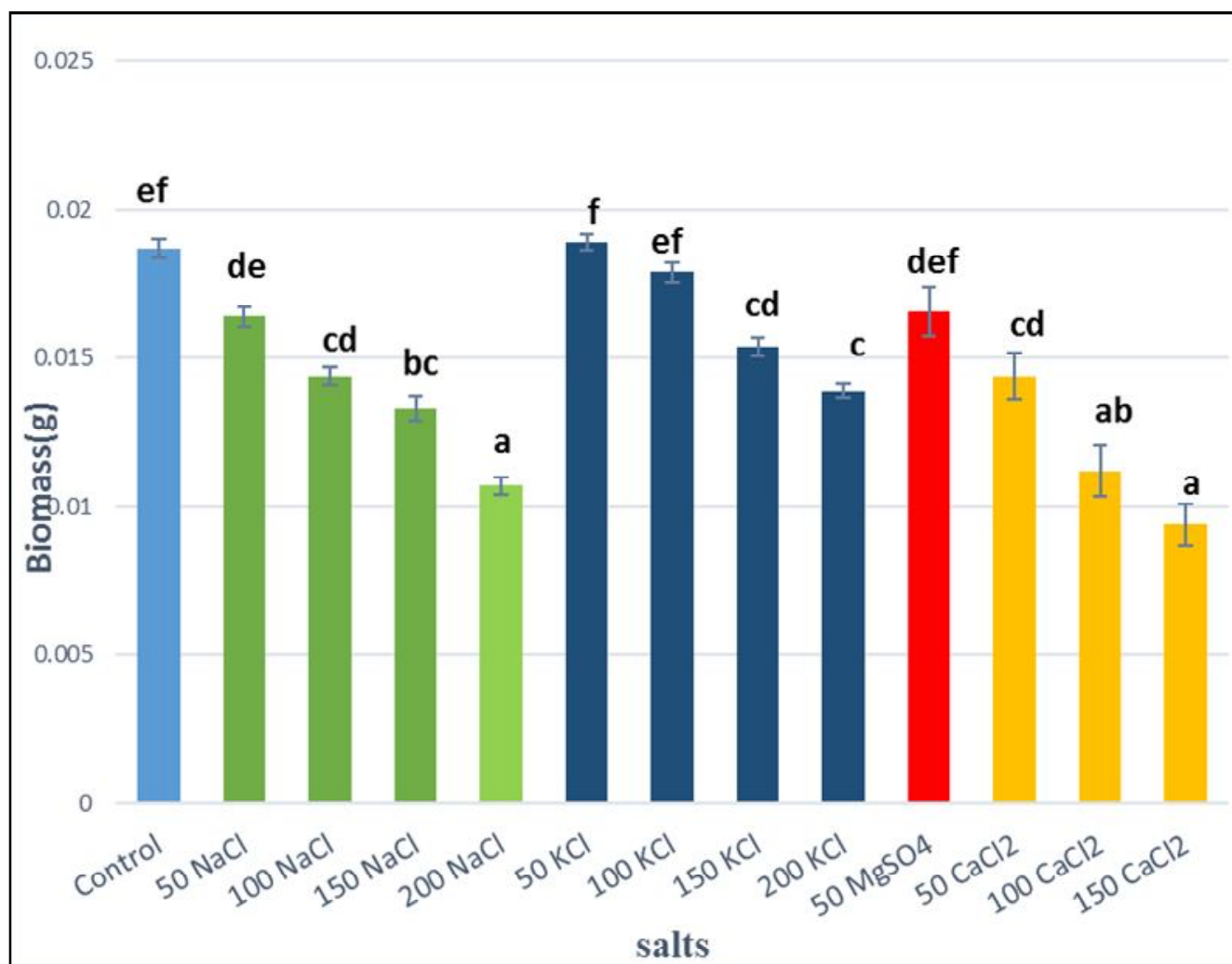


Fig. 2. Biomass of MTi2 seedlings as response to different type and concentration of salinity. Different letters indicate significant difference among means (Tukey HSD test, $p < 0.05$).

salt stress because of the observance of water by the roots from their direct contact with the soil for distribution to the whole plant. Consequently, shoot and root lengths provide the necessary indications of a plant's response to salt stress (Jamil and Rha, 2004). The reduction of biomass, seedling length were found to increase with increasing salinity level in all types of salts except in 50 KCl. A similar study by Baghbani *et al.*, (2013) reported that the values of biomass of the cucumber varieties were reduced with increased salinity. Also, the influence of various salt sources (NaCl, KCl and $CaCl_2$), and levels (0, 1, 3, 5, 7, 9 dSm^{-1}), on seed germination and seedling growth of four species of pumpkin to a reduction in the seedling lengths in all varieties when the EC of the solution exceeded 5 $dS m^{-1}$ (Aydivinsakir *et al.*, 2013). Moreover, Sohrabikertabadi *et al.*, (2013) found that *Cucumis melo* showed reduction in seedling lengths when NaCl levels were increased.

In this study, 50 mM KCl increased the germination

percentage and the vigor of MTi2 seed. The early growth and biomass of MTi2 seedlings also significantly higher than other salinity treatment. This present study found abnormal response toward $> 50mM$ of $MgSO_4$, with the seed transferring directly to cotyledon. Lianes *et al.*, (2013) had earlier indicated that with the presence of SO_4^{2-} in the medium, ion compartmentalization and osmotic change were decreased in the halophyte *Prosopis strombulifera*, causing water imbalance and signs of toxicity because of changes carbon metabolism (e.g. synthesis of sorbitol rather than mannitol, decreased sucrose production and protein content). $CaCl_2$ inhibits the radical of seedling in 100mM and 150mM. Moreover, this salt at 200mM prevents the seed from germinating and causes a significant decrease in all parameters of germination. This study found that Mg^{2+} , Ca^{2+} and SO_4^{2-} accumulate in the cytosolic solutes of seed had a toxic effect on MTi2.

Conclusion

Different types of salt give different effects on the germination responses and early growth of MTi2 seedlings. MTi2 seeds are able to germinate in KCl and NaCl until the high concentration (200 mM) which is % GP more than 50%. In addition, MTi2 seeds able to germinate in medium concentration of CaCl₂ (50-100 mM) and in low concentration of MgSO₄ (50 mM) but unable to germinate in any concentration of MgCl₂. The germination rates and the vigor of seed also higher in KCl and NaCl but lower in CaCl₂ and MgSO₄. Results also found Relatives Salts Injury Rate very low in KCl and NaCl but higher in CaCl₂ and MgSO₄. Therefore, it is thought that KCl and NaCl caused the ionic effect on MTi2 seed, but, MgSO₄, MgCl₂, and CaCl₂ caused the toxic effect on MTi2 seed. The tolerance level of MTi2 seed on different types of salts can be concluded as follows, KCl > NaCl > CaCl₂ > MgSO₄ > MgCl₂. Results also found that 50 mM KCl increased the germination percentage, germination rate and the vigor of MTi2 seeds.

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