



IMPACT OF NaCl, MgCl₂ AND CaCl₂ ON SEED GERMINATION AND SEEDLING GROWTH ON TURNIP (*BRASSICA RAPA RAPA*)

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Abstract

This study was conducted to compare the impact of NaCl, MgCl₂ and CaCl₂ on the germination of *B. rapa rapa* seed and seedling growth. Three varieties of salts (NaCl, MgCl₂, and CaCl₂) at different concentration (25, 50, 75 and 100 mM) and deionized water as control were used. A fifteen sterilized seeds were put in petri dishes containing 5 ml of deionized water or each salinity solution and kept in the growth chamber at twenty one ± 1°C. The experiment is conducted in a completely randomized design with nine replicates. The numbers of germinated seeds were recorded day by day till day tenth. On day 10th, the length of the hypocotyl, radicle and the biomass of seedlings were measured. Germination percentage, seed vigor and salt tolerance were calculated. Data were analyzed using SPSS windows version 21 (one way ANOVA 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 *B. rapa rapa* seed on NaCl, MgCl₂, and CaCl₂ is completely different. It indicates that the viability of (*B. rapa rapa*) seed to germinate relatively high in NaCl followed by CaCl₂ and MgCl₂.

Key Words: germination, seedlings, salt tolerance.

Introduction

Abiotic stresses such as high salinity, water scarcity, high or low temperature, and heavy metals that cause severe inimical effects on crop metabolism and thereby plant growth, development and crop productivity (Doupis *et al.*, 2011). In a saline environment adaptation of plants to salinity during germination and early seedling stages is critical for the plant stand to be established. Soil and water salinity hinder plant growth for two reasons: firstly, the existence of salt in the soil solution; lowers the plant's water absorption function, resulting in a decrease in the growth rate. This is a condition known as osmotic or water-deficit effect of salinity. Secondly, when there is excess of salt entering the plant, in the transpiration stream, the cells can be damaged in the transpiring leaves, and may further reduce growth. This is known as the salt specific or ion-excess effect of salinity (Yao & Fang, 2009).

The turnip or white turnip (*Brassica rapa* sub sp. *rapa*) is a root vegetable commonly grown in temperate

climates worldwide for its white, bulbous taproot. The word *turnip* is a compound of *tur-* as in turned/rounded on a lathe and *neep*, derived from Latin *napus*. Small, tender varieties are grown for human consumption, while larger varieties are grown as feed for livestock. In the north of England, Scotland, Ireland and eastern Canada (Newfoundland), turnip (or *neep*) often refers to rutabaga, a larger, yellow root vegetable in the same genus (*Brassica*) also known as swede (from "Swedish turnip"). Saltiness negatively affects the yield of the turnip edit by decreasing germination, seedling development establishment of the plant weak (Sato *et al.*, 2006). Salinity may impact the germination of seeds either by creating 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, MgCl₂, and CaCl₂ at concentration 25mM, 50mM, 75mM and 100 mM on the germination, seedling growth of *B. rapa rapa*.

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Materials and Methods

An investigation was conducted at the Plant Laboratory of Biology Department/University of Misan, Amarah, Iraq. The present experiment was conducted by Completely Randomized Design (CRD). Three types of salts (NaCl, MgCl₂ and CaCl₂) at four different concentrations (25, 50, 75 and 100 mM), the seeds were washed with sterilized distilled water for three times and air-dried before being used in the germination tests to avoid any fungal attacks. Five different concentrations of NaCl, MgCl₂ and CaCl₂ which is 25, 50, 75 and 100 mM and deionized water as a control was prepared separately. A fifteen seeds were placed on Whatman filter paper in sterilized petri dishes (9 cm 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 21 ± 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 2 mm. The number of germinated seeds was recorded daily until day tenth. On day 10th, the length of the hypocotyl, radicle and the biomass of each seedling were measured by selecting three seedlings randomly from each petri dish (Li, 2008).

Germination percentage (GP %) was calculated according to Ansari *et al.*, (2012) which is

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

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

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

Seed vigor was calculated according to Marcos (2015).

$$\text{Seed vigor} = \frac{(\text{Length of hypo cot yls} + \text{length of radicle}) \times \text{germination percentage}}{100}$$

Statistical analysis

Statistical analysis was performed using SPSS window version 21. One 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.

Result

Impact of NaCl, MgCl₂ and CaCl₂ on seed

germination.

The end result indicates the germination of *B. rapa rapa* seed in various 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. Each type of salt shows significantly different effect on the germination of

Table 1: The germination percentage of *B. rapa rapa* seeds as a response to different type and concentration of salinity

Germination Percentage (%)			
Concentration (mM)	NaCl	MgCl ₂	CaCl ₂
0	85.2 ^g ± 4.9	85.2 ^e ± 4.9	85.2 ^e ± 4.9
25	79.3 ^{fg} ± 3.4	54.8 ^{abcd} ± 5.3	71.1 ^{defg} ± 2.2
50	77.8 ^{efg} ± 5.7	48.9 ^{abc} ± 4.9	65.9 ^{defg} ± 5
75	65.2 ^{cdefg} ± 4.7	41.5 ^{ab} ± 4.6	60 ^{abcdef} ± 2.9
100	63 ^{bedef} ± 2.5	40 ^a ± 5.3	56.3 ^{abcde} ± 5.3

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

Table 2: Seed vigor of *B. rapa rapa* seeds as response to different type and concentration of salinity.

Seed vigor			
Concentration (mM)	NaCl	MgCl ₂	CaCl ₂
0	9 ^e ± 0.7	9 ^e ± 0.7	9 ^e ± 0.7
25	10.6 ^e ± 1.2	-	4.3 ^{cd} ± 0.5
50	8.6 ^e ± 0.9	-	3.1 ^{bc} ± 0.4
75	6 ^d ± 0.4	-	2.5 ^{bc} ± 0.3
100	4.6 ^{cd} ± 0.5	-	1.4 ^{ab} ± 0.1

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 3: Seed tolerance of *B. rapa rapa* seeds as a response to different type and concentration of salinity

Salt tolerant			
Concentration (mM)	NaCl	MgCl ₂	CaCl ₂
0	100.0 ^{cde} ± 12.7	100.0 ^{cde} ± 12.7	100.0 ^{cde} ± 12.7
25	137.9 ^e ± 14.4	-	68.2 ^{bc} ± 10.4
50	124.2 ^{de} ± 18.1	-	67.9 ^{bc} ± 6.9
75	86.6 ^{bcd} ± 14.1	-	63.3 ^{bc} ± 7.1
100	74.0 ^{bc} ± 15.1	-	38.5 ^{ab} ± 5.4

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).

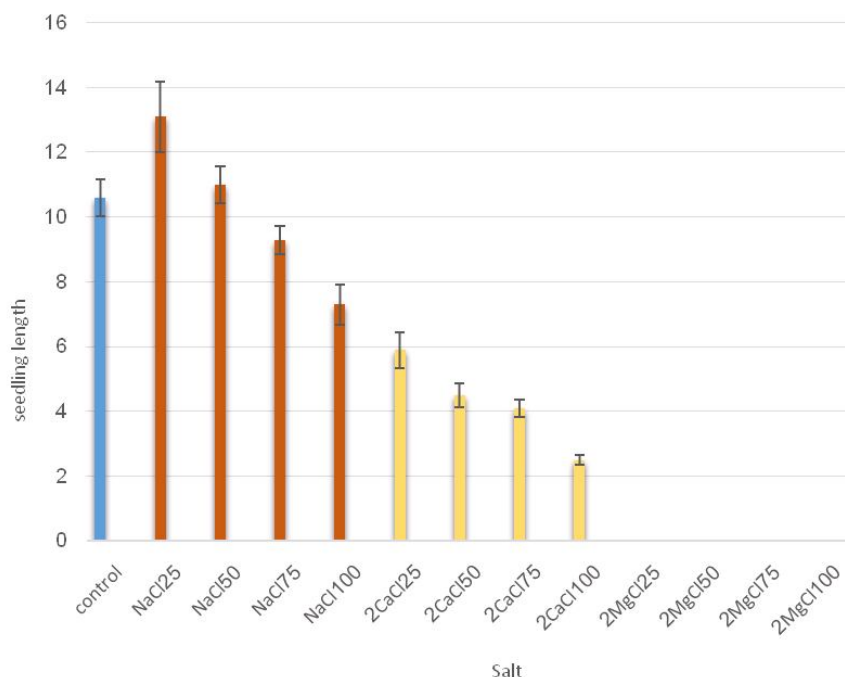


Fig.1: Seedling length of *B. rapa rapa* seedlings as a response to different type and concentration of salinity. Different letters indicate significant difference among means (Tukey HSD test, $p < 0.05$).

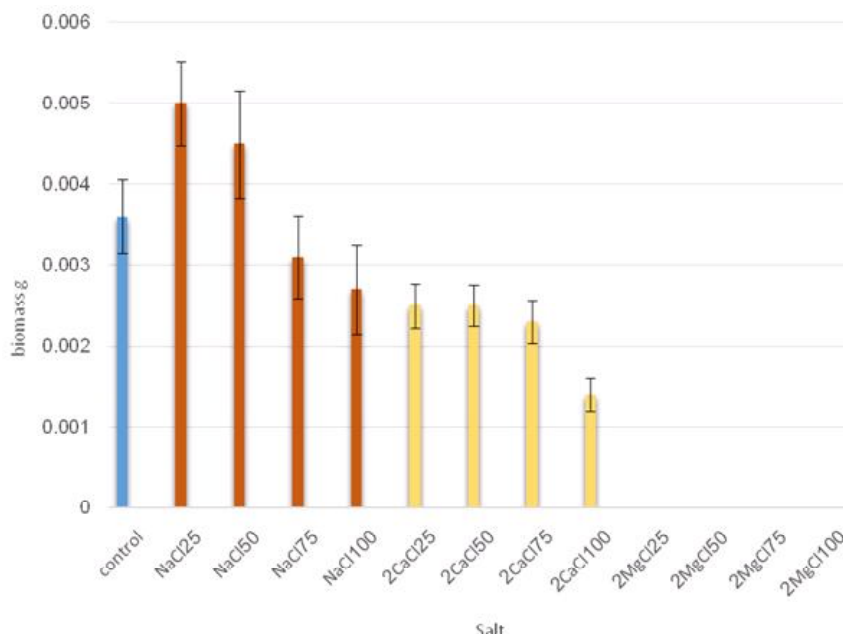


Fig. 2: Biomass of *B. rapa rapa* seedlings as response to different type and concentration of salinity. Different letters indicate significant difference among means (Tukey HSD test, $p < 0.05$).

B. rapa rapa seed. The seeds show higher germination percentage in NaCl than CaCl₂ and the lowest percentage germination in MgCl₂.

The effect of NaCl, MgCl₂, and CaCl₂ on the vigor level of *B. rapa rapa* 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 and Rucker, 2012). The vigor of *B. rapa rapa* seed significantly different in three different salts at different concentrations as shown in Table 2. Vigor of *B. rapa rapa* seed highest in NaCl while lower in CaCl₂ and MgCl₂. The highest vigor seed is in 25mM NaCl (10.6) followed by control (9). The finding reveals that the capacity for germination and tendency for growth of *B. rapa rapa* seeds with the highest in 25mM NaCl.

Tolerance of turnip seed in different type and concentration of salts

Results found that the tolerance turnip seeds in different types of salts are significantly different. The level of tolerance decreased as salt concentration increased as presented in Table 3. *B. rapa rapa* seeds highly tolerate in NaCl, moderate tolerate in CaCl₂, on the other hand, intolerant in MgCl₂. The tolerance level of *B. rapa rapa* seeds can be concluded NaCl > CaCl₂ > MgCl₂.

The Effect of NaCl, MgCl₂ and CaCl₂ on the Early Growth of *B. rapa rapa* Seedlings

The (Fig. 1) shows increase the concentration of salt caused the reduction in the early growth of *B. rapa rapa* seedlings except 25 mM and 50mM of NaCl. The three types of salts had different effects on the growth of *B. rapa rapa* seedlings. In all salts, the length of seedling significantly declined with increasing salts concentration except 25 mM and 50mM of NaCl. The highest seedling (13.1 cm) was found in 25mM NaCl following by 50mM NaCl (11 cm).

While (Fig. 2) shows that the dry weights of the seedlings decrease significantly with increasing salt concentration in all types of salts except 25 and 100mM of NaCl. The highest

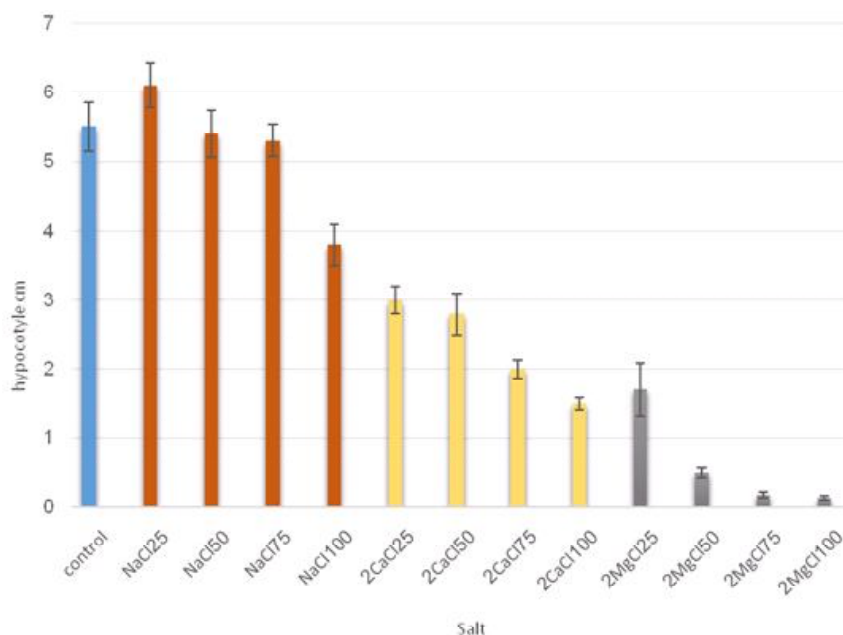


Fig. 3: Hypocotyle length of *B. rapa rapa* seedlings as a response to different type and concentration of salinity. Different letters indicate significant difference among means (Tukey HSD test, $p < 0.05$).

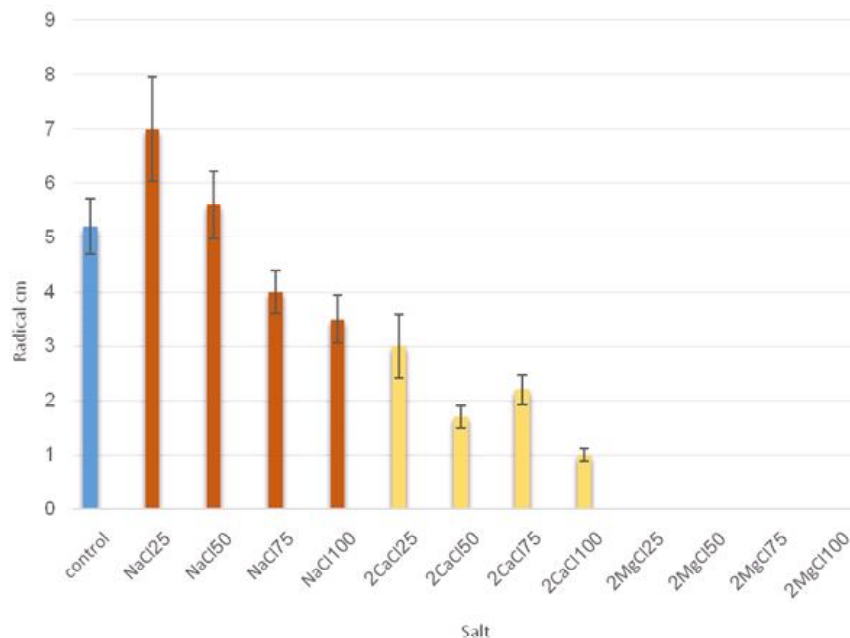


Figure 4: radical length of *B. rapa rapa* seedlings as a response to different type and concentration of salinity. Different letters indicate significant difference among means (Tukey HSD test, $p < 0.05$).

seedling dry weight in 25 mM of NaCl (0.005g) followed 50 mM of NaCl (0.0045g) then control (0.0036g), hence the lowest seedling dry weight observed (0.0014g) in 100 mM CaCl_2 .

In case (Fig. 3) shows that the highest hypocotyl length found in 25 mM NaCl (6.1 cm) followed by control (5.5 cm) then 50 mM of NaCl (5.4 cm)

Whereas (Fig. 4) shows that the highest radical length (7cm) was found in 25 mM NaCl followed by 50mM NaCl (5.6 cm) then control (5.2 cm) the

lowest length of radical in 100 mM CaCl_2 (1 cm) however, the radical in treatment MgCl_2 suffered from degradation after germination.

Discussion

Results show that each type of salt has different effects on the germination of *B. rapa rapa* seed. Germination percentage of turnip seeds were decreased with the increased concentration in all types of salts. Reduction in germination percentages of *B. rapa rapa* seeds is in following order of treatment: $\text{NaCl} > \text{CaCl}_2 > \text{MgCl}_2$. A similar reduction in germination percentage with increasing salt levels was reported in Sweet Sorghum Cultivars (Almodares, *et al.*, 2007). Seed germination is an essential process in plant development to obtain optimal seedling numbers that results in higher seed yield (Kim and Park, 2008; Aishah *et al.*, 2010) It is a crucial growth stage typically subjected to rising mortality quantitative relation (Ratnakar and Rai 2013). Seed germination depends on the use of held supplement material in the seed (Begum *et al.*, 2010). Consequently, the adjustment to saltiness when a seed is developing and the seedling is developing is extremely fundamental for sound plant development. In the present study, The various type of salt solutions have significantly different effect on *B. rapa rapa* seeds 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 NaCl create osmotic potential which prevents the uptake of water, which will change the germination response of *B. rapa rapa*. According to Negrão, *et al.*, (2017), The decrease in the germination percentage may be due to two reasons: first, high accumulation of toxic salt ions that may reduce their water absorption potential, alteration of certain enzymatic or hormonal activity

within the seed; second, decrease in water absorption through osmotic effect (Munns and Tester, 2008). 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 and Flowers 2005; Salama *et al.*, 2011). Result also found that increasing the concentration of salts led to decreasing germination percentage. Past investigation as saltines improve osmotic pressure, leading to reduction in water absorbent, division of cell and differentiation are inhibited, which adversely impact metabolism, induces leaf chlorosis, upsets the balance of the hormone level and physiological processes (Martre *et al.*, 2002). These factors or mechanisms delay begin of germination followed by prolonged seed germination period (Finch-Savage *et al.*, 2006) and eventually reducing the length of shoot and root (Keshavarzi, 2012). The significant effect of salinity on shoot and root lengths as result of high salinity level within the external medium creates high osmotic potential and reduces the accessible water for cell growth. The decrease within the quantity of obtainable wetness may have an effect on cell elongation and so have an effect on cell enlargement and protrusion of embryo axis, which may hinder shoot and root growth. (Bewley and Black 2012). 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. Seed vigor has the potential to determine the rapid and uniform emergence of seedlings and the establishment of crops (Kaya *et al.*, 2006). 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 *B. rapa rapa* seed is found to decrease with increasing levels in all types of salts except 25 mM of NaCl also found that the increased of salt concentration decrease the vigor of the seed. Shoot and root lengths are the most necessary parameters for 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 (Parvaiz and Satyawati 2008). The reduction of biomass, seedling length were found to increase with increasing salinity level in all types of salts except in 25mM and 50mM of NaCl. In this investigation, the germination percentage, seed vigor, early growth and biomass of *B. rapa rapa* seedlings significantly in NaCl better than other salinity treatment.

MgCl₂ inhibits the radical of seedling in all concentrations. Additionally, the CaCl₂ causes a significant decrease in all parameters of germination. This have a look at found that Mg²⁺ and Ca²⁺ accumulate in the cytosolic solutes of seed had a poisonous effect on *B. rapa rapa*

Conclusion

This study found that different three types of salt have different influence on seed germination and early seedling growth of *B. rapa rapa*. Turnip seeds are able to germinate in all three type of salts (NaCl, CaCl₂ and MgCl₂) until the high concentration (100 mM). The vigor of seeds also higher in NaCl but lower in CaCl₂ and MgCl₂. Therefore, it is thought that NaCl caused the ionic effect on *B. rapa rapa* seed, but MgCl₂ and CaCl₂ caused the toxic effect on *B. rapa rapa* seed. *B. rapa rapa* seeds are more tolerant to NaCl, but sensitive to CaCl₂, and MgCl₂.

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