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Research paper

The effects of NACL, KCL and MGCL2 on the germination of *Brassica rapa* var. *parachinensis* seed

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Abstract

This study was conducted to determine seed germination on the mechanisms of salinity stress in *B. rapa* var. *parachinensis* (Hong kong choy sum). The type of experiment was a completely randomized design with three salinity treatment NaCl, MgCl2, and KCl and minimum four replications was used. Salinity treatment consisted of 0 (control), 50, 100, 150, 200, mM concentrations. The experiment was carried out under 70% relative humidity at 23°C with a photoperiod of 8:16 h light/dark. The germinated seeds were counted every day and germination percentage, seed vigor, seed tolerance, length of seedling and biomass seedling was measured. The data were analyzed by one-way ANOVA multiple comparison test followed by Duncan's multiple range test.

Keywords*: Brassica; Germination Percentage; Salt Tolerance; Seed Vigor.*

1. Introduction

Seed germination and seedling establishment are the most important stages in plant growth and development on salinity habitats. Seeds that have efficiency to respond to environmental conditions and change their germination behavior will be more likely to survive and become established. Tolerance to salinity during germination is critical for the establishment of plant growth in saline soil of arid and semi-arid regions (Ungar, 1995). The frequency and amount of precipitation, as well as the ability of seed species to germinate on low osmotic potential are the most important factors for successful seedling establishment (Bybordi and Tabatabaei, 2009). In addition, Shainberg (1975) reported that the main salt components of saline are Na^+, Mg^{2+}, K^+ and Cl^-. Therefore, the aim of this research is to investigate the effects of individual salts, NaCl, $MgCl_2$ and KCl at concentration of 50mM, 100mM, 150 mM and 200 mM on the germination and seedling growth of Hong kong choy sum, *B. rapa* var. *parachinensis.*

2. Materials and methods

Seeds of *B. rapa* var. *parachinensis* was obtained from Putra Agricultural Park, UPM. The experiment was conducted at the Department of Biology, Faculty of Science, Universiti Putra Malaysia from November- February, 2015. Mature, healthy and equal sized seeds were selected and surface sterilized with 5% sodium hypochlorite for ten minutes (Panuccio et al., 2014). 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. Four different concentrations of NaCl, $MgCl_2$, and KCl which were 50, 100, 150, and 200 mM were used as treatments and deionized water was used as the control. 10 seeds were placed on Whatman filter paper in sterilized petri dishes (9 cm diameter) containing 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 23 ± 1°C. The experiment was conducted in a completely randomized design with four replicates and repeated thrice. Seeds were considered germinated when radicle had extended at least 2 mm. The number of germinated seeds was recorded daily until day 9. On day 9, the length of hypocotyl, radicle and the biomass of each seedling were measured by selecting five seedlings randomly from each petri dish according to the method described by Li (2008).

Germination percentage, salt tolerance and seed vigor were determined by the following formula:

a) Germination Percentage (GP %) was calculated using a formula by (Kandil et al., 2012):

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

b) the salt tolerance rate was calculated with using standard formula according to Kaymakanova (2009):

$$\text{Salt tolerance (ST)} = \frac{\text{Seedling dry weight of treated}}{\text{Seedling dry weight in control}} \text{ X } 100$$

c) Seed vigor was calculated using the following equation (Abdul-Baki and Anderson, 1973):

$$\text{Seed vigor} = \frac{\text{(length of hypocotyls+length of root) germination percent}}{100}$$

2.1. Statistical analysis

All data were analyzed using SPSS windows version 21. Data were subjected to one-way analysis of variance (ANOVA) at significance level, $p<0.05$ to determine the significance difference between treatment and followed by Duncan's multiple range test ($p < 0.05$) for comparison of means.

3. Results

3.1. The effects of NaCL, MgCl2, and KCl on the seed germination of *B. rapa* var. *parachinensis*

The viability of a seed is expressed in terms of its germination percentage as determined in a standardized germination test (Van de Venter, 2001). The seeds responded differently at specific salt concentration as shown in Table 1. The seeds show higher germination percentage in NaCl than KCl and the lowest percentage germination in $MgCl_2$. The seed germination treated with 100 mM NaCl and 100 mM KCl shows the highest percentage of germination (100%) followed by 150 mM NaCl (97.5 %). Intent reduction in germination was obtained at 200 mM and above. On the other hand, increasing $MgCl_2$ concentration caused a decrement in the viability germination percentage of *B.* rapa var. parachinensis seed.

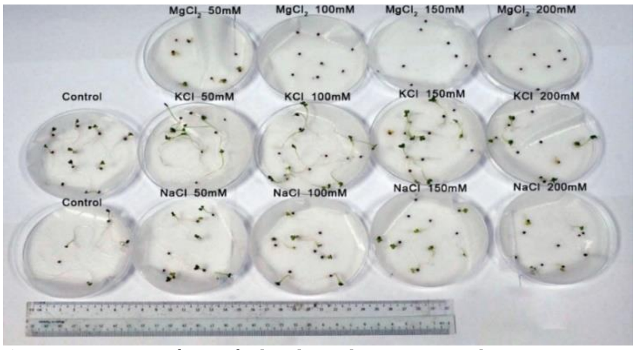

Fig. Error! No text of specified style in document. **1:** The Germination of *B. rapa* var. *parachinensis* seed in NaCl, KCl and $MgCl_2$ at Day 9.

Table 1: The Effects of NaCl, $MgCl_2$, and KCl on the Percentage of Seed Germination of *B. rapa* var. *parachinensis*

Concentration (mM)	Percentage of Germination (%)		
	NaCl	$MgCl_2$	KCl
0 (control)	95.0^a±2.9	95.0^c±2.9	95.0^b±2.9
50	90.0^{ab}±5.8	55.0^b±6.5	87.5^a±2.5
100	100.0^b±0.0	5.0^a±6.9	100.0^b±0.0
150	97.5^b±2.5	-	87.5^a±6.3
200	87.5a±6.3	-	85.0^a±2.9

Values are mean and standard error of measurement made on four replicates. Means in the same column with different alphabet (s) are significantly different at p≤ 0.05 according to Duncan comparison test

Seed vigor is the seed properties that determine the potential for rapid, uniform emergence and development of seedlings under a wide range of field conditions. Seedlings from high vigor seed are expected to emerge more uniformly than those from low vigor (Egli and Rucker, 2012). Vigor index represents the germination capacity and growing tendency of seedling (Deng et al., 2014). The vigor of *B. rapa* var. *parachinensis* seed significantly different when treated in three salinities at different concentrations (Table 2). Seed vigor is higher in KCl than NaCl while the vigor indexes of *B. rapa* var. *parachinensis* were significantly reduced as salinity increased. The highest seed vigor is in 50 mM KCl followed by 100 mm KCl. This result indicates that the capacity of germination and growing tendency of *B. rapa* var. *parachinensis* seed is higher when germinated in 50-100 mM KCl.

Table 2: Seed Vigor of *B. rapa* var. *parachinensis* as Respond In Different Concentration of NaCl, $MgCl_2$, and KCI

Concentration (mM)	Seed vigor NaCl	$MgCl_2$	KCl
0 (control)	5.7^{bc}±0.2	5.7±0.2	5.7^a±0.2
50	7.3^{cd}±0.2	-	9.7^b±0.3
100	6.4^{cd}±0.3	-	9.0^b±0.2
150	4.7^b±0.8	-	5.8^a±0.4
200	1.9^a±0.2	-	5.7^a±0.2

Values are mean and standard error of measurement made on four replicates. Means in the same column with different alphabet (s) are significantly different at p≤ 0.05 according to Duncan comparison test

3.2 The tolerance of *B. rapa* var. *parachinensis* seed on NaCl, $MgCl_2$, and KCl

The tolerance of seeds was studied with three salinities including NaCl, $MgCl_2$ and KCl on different concentrations (Table 3). *B. rapa* var. *parachinensis* seed have the highest tolerant in KCl in 100 mM (409.4%) followed by 50 mM (303.1%). Therefore, the seeds are tolerant with NaCl and KCl even though in high concentration (200 mM).

Table 3: The Percentage of Tolerance of *B. rapa* var. *parachinensis* Seed on NaCl, $MgCl_2$, and KCl

	Percentage of Salt tolerance (%)		
Concentration (mM)	NaCl	$MgCl_2$	KCl
0 (control)	100.0^a ± 10.5	100.0 ± 10.5	100.0^a ± 10.5
50	275.0^d 13.0	-	303.1^c ± 23.8
100	219.6^c± 2.0	-	409.4^d±17.1
150	135.1^b±6.2	-	231.3^b±24.6
200	120.8^b±2.1	-	198.2^b±17.2

Values are mean and standard error of measurement made on six replicates. Means in the same column with different alphabet (s) are significantly different at p≤ 0.05 according to Duncan comparison test

3.3 The effects of NaCl, $MgCl_2$, and KCl on early growth of *B. rapa* var. *parachinensis* seedlings

The three types of salts had different effect on the growth on *B. rapa* var. *parachinensis* seedlings as shown in Figure 2 and Table 4. The length of seedling significantly increased with increasing KCl concentration. The highest seedling (10.6 cm) was found in treatment 50 mM KCl followed by 100 mM KCl which the length was 8.8 cm. Compared with NaCl, only 50mM and 100 mm showed the high length of *B. rapa* var. *parachinensis* seedling 7.6 cm, and 6.8 cm, respectively. In contrast, seedling growth declined with increasing NaCl concentration.

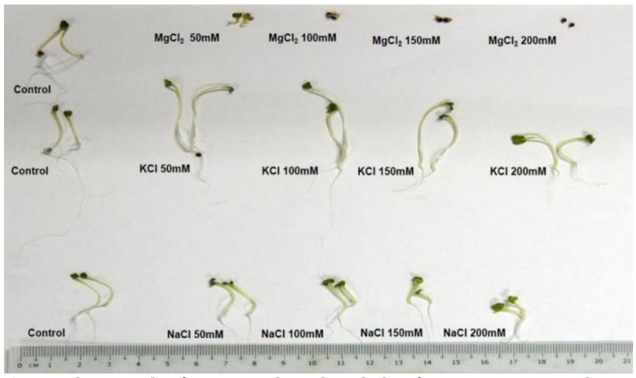

Fig. 1: The Length of Hypocotyls and Radicle of *B. rapa* var. *para*chinensis Seedling Germinated In NaCl, KCl and MgCl2 at Day 9.

Table 4: Mean Comparison of the Effects of NaCl, $MgCl_2$, and KCl on the Length *B. rapa* var. *parachinensis* Seedlings

Seedling length (cm) Concentration (mM)	NaCl	$MgCl_2$	KCl
0 (control)	6.6^c±0.44	6.6±0.44	6.6^a±0.40
50	7.6^c±0.42	-	10.6^c±0.37
100	6.9^c±0.43	-	8.8^b±0.17
150	4.6^b±0.47	-	6.8^a±0.14
200	2.7^a±0.26	-	6.7^a±0.12

Values are mean and standard error of measurement made on six replicates. Means in the same column with different alphabet (s) are significantly different at p≤ 0.05 according to Duncan comparison test

The length of hypocotyl and radicle are significantly different after treated with NaCl and KCl (Table 5). The length of radicle was significantly increased by 50 mM and 100 mM NaCl with mean length are 6.4 cm and 5.2cm, respectively compared with control and other concentration of NaCl. On other hand, increasing the concentration of NaCl decreased the length of hypocotyls of seedlings as shown in Table 5. KCl shows the adverse effect on hypocotyl's growth compared with NaCl and $MgCl_2$. The hypocotyl length significantly increased with increasing of KCl which is 50 mM KCl give the highest length (5.6 cm) followed by 100 mM (4.6 cm).

Table 5: Mean Comparison of the Effects of NaCl, MgCl2, and KCL on the Length of Hypocotyls and Radicals *B. rapa* var. *parachnesis*

Concentration (mM)	NaCl Hypocotyl length (cm)	NaCl Radicle length (cm)	$MgCl_2$ Hypocotyl length (cm)	$MgCl_2$ Radicle length (cm)	KCl Hypocotyl length (cm)	KCl Radicle length (cm)
0 (control)	$2.0^{c}\pm0.10$	$4.6^{c}\pm0.39$	2.0 ± 0.10	4.6 ± 0.39	$2.0^{a}\pm0.10$	$4.6^{b}\pm0.39$
50	$1.2^{b}\pm0.20$	$6.4^{d}\pm0.30$	-	-	$5.6^{c}\pm0.28$	$4.7^{b}\pm0.32$
100	$1.4^{b}\pm0.17$	$5.2^{c}\pm0.29$	-	-	$4.6^{c}\pm0.22$	$4.2^{b}\pm0.35$
150	$1.2^{b}\pm0.01$	$3.5^{b}\pm0.38$	-	-	$3.1^{b}\pm0.20$	$3.7^{a}\pm0.11$
200	$0.8^{a}\pm0.08$	$1.9^{a}\pm0.19$	-	-	$2.9^{b}\pm0.18$	$3.7^{a}\pm0.14$

Values are mean and standard error of measurement made on six replicates. Means in the same column with different alphabet (s) are significantly different at $p\leq 0.05$ according to Duncan comparison test

The biomass of the seedlings increased significantly in NaCl and KCl treatment compared with control and $MgCl_2$ (Figure 3). The biomass of seedling treated with KCl is higher than NaCl. Seedling biomass is 0.049 mg treated with 50 mM KCl and 0.066 mg treated with 100 mM KCl.

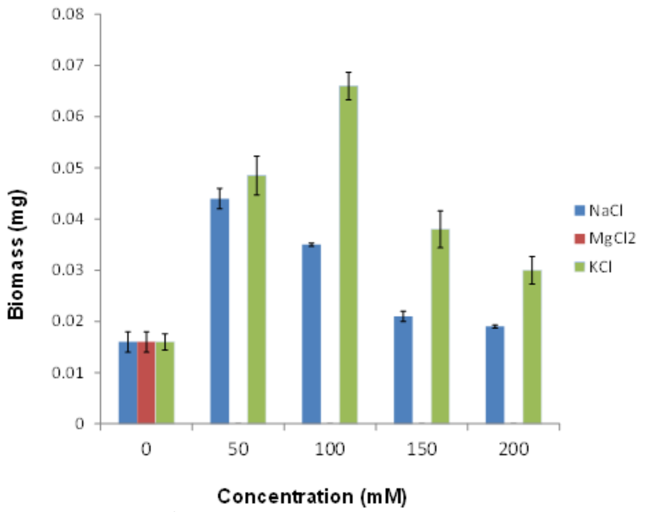

Fig. 3: Biomass of *B. rapa* var. *parachinensis* Seedlings as Respond to Different Types and Concentration of NaCl, KCL and $Mgcl_2$

4. Discussion

The effects of NaCl, $MgCl_2$, and KCl on the seed germination and seedling of *B. rapa* var. *parachinensis* were compared. Different responses with different salts at different concentration show evidences for existence of specific ionic effects. The result of high tolerance and high seed vigor proved that *B. rapa* var. *parachinensis* seed had viability to germinate in high concentration of NaCl and KCl. In this study, the percentage of seed germination increased until 150 mM NaCl and 100 Mm KCl but decreased at 200 mM NaCl and 150 mM KCl. The vigor of *B. rapa* var. *parachinensis* seed increased at 50 mM and 100 mM NaCl concentrations. Moreover, KCl at all concentration increased the vigor of *B. rapa* var. *parachinensis* seeds. Seed vigor is a characteristic that determine potential for fast and uniform seedling emergence and establishment of crops (Mondo et al., 2013). In addition, it is the seed properties that determine potential for fast and uniform emergence, and development of seedlings under a wide range of field conditions (Association of Official Seed Analysts, 2002). According to Hampton (2002), seed vigor has a high influence on establishment of initial population of plants as well as on their adequate development, what will affect crop yield.

Salt stress inhibits seed germination by limiting water absorption by the seeds (Dodd and Donovan, 1999). In addition, salt stress affects the mobilization of stored reserves (Bouaziz and Hicks, 1990) and the structural organization or synthesis of protein in germination embryos (Foolad and Lin, 1997; Machado Neto et al., 2004; Ramagopal, 1990). During germination under salt stress, seed require higher amount of water. This cause excessive uptake of the ions which results in toxicity in the seed. Therefore, water potential gradient between the external environment and the seeds inhibits the primary root emergence (Eneas-Filho et al., 1995).

Generally, this differential behavior of seeds according to the salt type generate different osmotic potential and the osmotic effect may well have a greater influence on germination. Interestingly, in this study seed *B. rapa* var. *parachinensis* was unable to germinate even at 50 mM $MgCl_2$. Therefore, NaCl and KCl have different effect on *B. rapa* var. *parachinensis* seed compared with $MgCl_2$ in which NaCl and KCl cause the osmotic stress on seeds, meanwhile, $MgCl_2$ cause toxicity on *B. rapa* var. *parachinensis*. Katembe et al. (1998) reported that high concentration of NaCl inhibited the water uptake, germination and seedling root elongation of *Atriplex prostrata*. Kaydan and Yagmur (2008) reported that high NaCl concentration lowered water uptake by seeds and consequently, germination decreased. NaCl affects the permeability of the plasma membrane and increases the influx of external ions and the efflux of cytosolic solutes in plant cells (Cuin et al., 2011). Previous report by Tobe et al. (2004) proved that germination of seeds for a variety of species decreased in response to increasing levels of salinity.

Magnesium ion, Mg^{2+}, plays important role in both the light and dark reactions of photosynthesis, as the central atom in the chlorophyll molecule and as a metallic cofactor in many enzymes including ATPases, protein kinases and rubisco. However, seed need only a small amount of Mg^{2+}. The current study found abnormalities in *B. rapa* var. *parachinensis* seedlings treated with $MgCl_2$ as low as 100 mM. Previous study by Brandenburg and Kleier (2011) found that low concentration of $MgCl_2$ (0.01 M) showed highest germination (95.83 %), height and weight but high concentration, 0.1 M $MgCl_2$ significantly reduced the germination (44.44%) of radish, height and weight of radish. This finding indicates that high concentration of $MgCl_2$ is toxic to radish. However, moderate concentration of $MgCl_2$ is beneficial to radish. Shaul (2002) reported that the concentration of $MgCl_2$ in plant cytosol is between 0.002-0.01M. This research discovered that *B. rapa* var. *parachinensis* seed was unable to germinate in 100 mM $MgCl_2$. Kobayashi et al. (2005) also reported $MgCl_2$ concentration as low as 0.03 M have been shown to reduce growth in rice and three species of Echinochloa. Tobe et al. (2003) reported that the toxicity of Mg^{2+} is several times stronger that Na^{+} on *Artemisia ordosia*, *A. adscensionis* and *Bassia dasyphylla*. Other than, creating osmotic potential which prevents the uptake of water, salinity have toxic effects of ions on embryo viability (Houle et al., 2001). Therefore, in the present study concluded that maybe Mg^{2+} accumulate in the cytosolic solutes of seed and led the toxicity effect on *B. rapa* var. *parachinensis* seed.

5. Conclusion

Different salts caused different effects on germination and the growth of *B. rapa* var. *parachinensis* seedling growth. The seeds are more tolerated to NaCl and KCl, but sensitive to $MgCl_2$. Apart from increasing the germination of *B. rapa* var. *parachinensis* seed, the pattern of seed vigor, salt tolerant, biomass, hypocotyl and radicle length by the effects of NaCl and KCl are different. NaCl was found to retard the hypocotyls length but increased the radicle length and biomass as high 100 mM NaCl, However, KCl at any concentration increased the seed vigor, the tolerant, the length of both hypocotyls and radicle and biomass. The present findings allow speculation that Hong kong choy sum, *B. rapa* var. *parachinensis* is a NaCl-tolerant and KCl-tolerant cultivar.

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