PERIÓDICO TCHÊ QUÍMICA
ARTIGO ORIGINAL

A PREPARAÇÃO DE SEMENTES MELHORA A GERMINAÇÃO E O CRESCIMENTO PRECOCE DE MUDAS DE NABO SOB ESTRESSE DE SALINIDADE

SEED PRIMING IMPROVES THE GERMINATION AND EARLY GROWTH OF TURNIP SEEDLINGS UNDER SALINITY STRESS

إعداد البذور يحسن الإنبات والنمو المبكر لشتلات اللفت تحت ضغط الملوحة

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RESUMO

Um experimento foi conduzido para melhorar o desempenho das sementes de nabo sob condições de estresse de salinidade. O impacto da priming com uma dosagem otimizada (5 g/l) de KCl e NaCl foi avaliado para aumentar o vigor das mudas e a tolerância ao estresse salino em mudas de nabo. Sementes preparadas com (5 g/l) de soluções de KCl e NaCl foram examinadas em diferentes níveis de salinidade (0, 50, 100, 150, 200) mM de NaCl em relação ao estágio inicial de crescimento. Os dados foram analisados no Windows SPSS versão 23 (ANOVA de uma via $p < 0,05$) para determinar a diferença significativa entre os tratamentos e os teste de Duncan, $p < 0,05$ para comparação das médias. Os resultados mostraram que o priming com KCl e NaCl foi eficaz na redução dos efeitos adversos da salinidade. Um aumento significativo ($P < 0,05$) na porcentagem de germinação, vigor das sementes, comprimento do hipocótilo e radícula, tolerância ao sal e peso seco das mudas de semente que iniciaram com (5 g/l) de KCl e NaCl em comparação com as sementes não preparadas foi gravado. Por outro lado, neste experimento, concluiu-se que se descobriu que o priming de sementes com KCl e NaCl era um melhor tratamento, especialmente na alta concentração de sais em comparação com as sementes não preparadas em caso de nabo para aumentar o vigor das sementes. e crescimento de plântulas sob condições estressantes ao sal.

Palavras-chave: *germinação, plântulas, nabo, preparação de sementes, sal.*

ABSTRACT

An experiment was conducted to enhance the turnip seed performance under salinity stress conditions. The impact of priming with an optimized dosage (5 g/l) of KCl and NaCl had been evaluated for enhancing seedling vigor and salt stress tolerance in seedlings of the turnip. Seeds prepared with (5 g/l) of KCl and NaCl solutions were examined at different salinity levels (0, 50, 100, 150, 200)mM of NaCl concerning the early growth stage. The data were analyzed using SPSS windows version 23 (one way ANOVA $p \leq 0.05$) to determine the significant difference between treatments and followed Duncan test, $p < 0.05$ for means comparison. The results have shown that priming with KCl and NaCl were effective in reducing the adverse effects of salinity. A significant ($P < 0.05$) increase in germination percentage, seed vigor, hypocotyl and radicle length, salt tolerance, and dry weight of the seedlings of seed that priming with (5 g/l) of KCl and NaCl compared to non-primed seeds was recorded. On the other hand, In this experiment, it was concluded that seed priming with KCl and NaCl had been discovered to be better treatment, especially in the high concentration of salts as compared to non-primed seeds in case of turnip for rising the seeds vigor and seedling growth under salt-stressed conditions.

Keywords: *germination, seedling, turnip, seed priming, salt.*

الملخص:

أجريت تجربة لتحسين أداء بذور اللفت تحت ظروف إجهاد الملوحة. تم تقييم تأثير التحضير بجرعة محسنة (5 جم / لتر) من (KCl) و (NaCl) لتعزيز قوة الشتلات وتحمل الإجهاد الملحي في شتلات اللفت. تم فحص البذور المحضرة بمحلول (5 جم / لتر) من محلول كلوريد البوتاسيوم و كلوريد الصوديوم

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بمستويات ملوحة مختلفة (0، 50، 100، 150، 200) ملي مولار من كلوريد الصوديوم فيما يتعلق بمرحلة النمو المبكر. تم تحليل البيانات بإستخدام نافذة الحزمة الاحصائية للعلوم الاجتماعية (SPSS) الإصدار 23 (تحليل التباين بأتجاه واحد ANOVA قيمة p≤0.05) لتحديد الفروق المعنوية بين المعاملات والمتبع بأختبار دنكن ذو المدى المتعدد، قيمة 0.05 > p للمقارنة. أوضحت النتائج أن الإعداد باستخدام كلوريد البوتاسيوم و وكلوريد الصوديوم كان فعالاً في تقليل التأثيرات السلبية للملوحة. تم تسجيل زيادة معنوية (P < 0.05) في نسبة الإنبات وقوة البذور وطول السويق وطول الجذير وتحمل الملح والوزن الجاف لشتلات البذور التي تم معاملتها بـ (5 جم / لتر) من KCl و NaCl مقارنة بالبذور غير المعاملة . من ناحية أخرى ، في هذه التجربة ، تم الاستنتاج إلى أن إعداد البذور بإستخدام KCl و NaCl قد تم اكتشافه على أنه افضل معاملة، خاصة في التراكيز العالية للأملاح بالمقارنة مع البذور غير المعاملة في حالة اللفت لتحسين قوة البذور و نمو الشتلات تحت ظروف الإجهاد الملحي.

الكلمات المفتاحية: الإنبات ، الشتلات ، اللفت ، تحضير البذور ، الملح.

1. INTRODUCTION:

Turnip (*Brassica rapa*) belongs to family Cruciferae, and It is one of the most important dicotyledonous, cross-pollinated, and cool-season vegetable crops grown both for its enlarged roots and for the foliage (Pink, 1993). *Brassica* species are distinguished by their immense intraspecific diversity, exemplified by leafy plants, oilseeds, and crops with extended inflorescences and above-ground storage organs (Liu *et al.,* 2019). Soil saltiness is one of the abiotic adversely influence on seed germination, seedling establishment, and efficiency of numerous harvests by creating an osmotic potential outside the seed inhibiting the absorption of water, or by the toxic effect (Mustafa *et al.,* 2017; Bose *et al.,* 2017). Osmotic and saline stresses are in charge of the inhibition and declines of seed germination and plant development (Yohannes and Abraha, 2013).

The water absorption decreases during the imbibition stage, and salinity leads to excessive absorption of toxic ions by the seed (Abraha and Yohannes, 2013; Murillo-Amador *et al.,* 2002). From the beginning of agriculture, the man established contact with seed physiology and realized that many seeds do not germinate easily and uniformly. The capacity of seemingly (dead seed) to regenerate and grow a viable young and healthy seedling after germination had been fascinated by the ancient civilization, therefore, the scientists for decades trying to figure out several methods of seed priming in order to activate seeds and alleviate environmental stresses and common technique its water-based priming techniques, which is a pre-sowing treatment that partially hydrates seeds without allowing emergence (Evenari, 1984; Chen and Arora, 2011).

Seed priming is a pre-germination seed treatment that leads to a physiological state that enables the seed to germinate more efficiently mean seeds are held at water potential that permits imbibition but prevents radicle extensions (Ashraf and Foolad, 2005; Gupta *et al.,* 2008; Lutts *et al.,* 2016). Seed priming enhances seed performance by rapid and uniform germination, healthy and vigor seedlings, which resulted in faster and better germination and development in various harvests, this also helps seedlings to grow in stressed conditions (Mohammadi, 2009; Safdar *et al.,* 2019). Priming-induced increase in germination may be associated with a change in plant hormone biosynthesis and signaling. Priming has been reported to increase the gibberellins (GA)/abscisic acid (ABA) ratio, and this may be a direct consequence of a priming impact in gene expression pattern (El-Araby *et al.,* 2006; Schwember and Bradford, 2010). Several variables influence the priming performance and are highly on handled plant species and the priming technique selected. Physical and chemical factors such as osmotic and water capacity, priming agent, length, temperature, presence or absence of light, aeration and seed condition also affect the priming performance and decide the time and rate of germination, seedling vigor, and further plant growth (Hussain *et al.,* 2006; Varier, 2010).

Osmopriming means soaking seeds with low water content in an osmotic solution, rather than pure water. Because of the low water capacity of osmotic solutions, water enters seed gradually, which allows gradual impregnation of seed and activation of early germination phases but prevents radicular protrusion (Girolamo and Barbanti, 2012).

However, values of water potential, together with the duration of the priming treatment, should always be adjusted to species, cultivar, and sometimes seed lot. Different compounds are used in osmopriming procedures, including polyethylene glycol (PEG), mannitol, sorbitol, glycerol, and inorganic salts such as NaCl, KCl, KNO_3, K_3PO_4, KH_2PO_4, $MgSO_4$, and $CaCl_2$. Priming with salt solutions is often referred to as “halopriming” (Yacoubi *et al.,* 2013).

Seed priming with various salts, especially KCl and NaCl, has appeared to improve the germination and development of numerous harvests under stressed conditions (Naz *et al.,* 2014). The present study was carried out to investigate the impact of KCl and NaCl priming on germinations of seeds and seedling growth of turnip (*Brassica rapa rapa*) under salinity conditions.

2. MATERIALS AND METHODS:

2.1. Experiment site

In order to determine the effects of different salinity levels and Osmo priming on germination and seedling growth, the experiment was carried out in botany Laboratory, Department of Biology, University of Misan. The laboratory was approximately at 21°C, and the experiment was carried out inside safety cabins, the materials were used seeds, deionized distilled water, 5 g/l iter of NaCl and KCl, (50,100,150, 200mM) of NaCl, 70% ethanol, 5% Na_2HCl, the equipment was used Petri dishes, filter paper, and parafilm.

2.2. Seed sterilization and soaking

Healthy turnip seeds were surface sterilized with 70% ethanol for 30 seconds and then with 5% sodium hypochlorite (Na_2HCl) solution for 20 min and then thoroughly washed three times with sterilized distilled water (sterilized by using autoclave). Subsequently, the seeds were soaking with 5 g/l KCl and NaCl solution separately for 24 hours at 21°C. After priming. The seeds were washed with sterilized distilled water after priming and dried at room temperature on filter paper for 24 h to their original moisture level, utilizing sensor balance before being used in germination tests. (Yohannes and Abraha, 2013).

2.3. Experimental design

The study was carried out in November/2019. A complete random design (CRD) used to study the effect of seed priming on germination and seedling growth of turnip under salt stress. This part of the experiment was carried out in the laboratory in 45 Petri dishes, that is, 15 for KCl, 15 for NaCl primed seeds, and 15 for unprimed (control) seeds and repeated thrice. Primed and unprimed seeds were placed in 9 cm diameter Petri dishes on two layers of filter papers (Whatman No. 1). 15th seeds were placed in each Petri dishes were containing 5 ml of five different saline solutions (0, 50, 100, 150, 200 mM of NaCl). The Petri dishes were closed with par-film to prevent evaporation and kept in the growth chamber at 21 ± 2°C. The experiment was conducted in a completely randomized design with six replications and 15th seeds per replicate. Seed germination was recorded daily up to day ten after the start of the experiment. When the radicle emerges by around 2 mm in length, a seed was considered germinated (Mohammed and Nulit, 2019[a]). The length of the hypocotyl and radicle of seedling were measured by selecting three seedlings randomly from each Petri dish, the seedlings were dried at 60°C for 48 h and then weighed (Li, 2008; Oliveira *et al.,* 2019). Germination percentage (GP %) was calculated according to Equation 1 by (Kandil *et al.,* 2012).

$$GP\% = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds sown}} \times 100 \qquad \text{(Eq. 1)}$$

Seed vigor was calculated according to (Gebremedhn and Berhanu, 2013) Equation 2, which is:

$$\text{Seed vigor} = \frac{(\text{L1} + \text{L2}) \times \text{germination percentage}}{\mathbf{100}} \qquad \text{(Eq. 2)}$$

whereas: L1= length of hypocotyls

L2= length of radical

2.4. Statistical analysis

Statistical analysis was performed using SPSS window version 23. One-way confidence-level variance analysis (ANOVA) (Naz, 2014), $p \leq 0.05$ was performed to find the significance deference among treatments followed by Duncan's multiple range test (DMRT) at $p \leq 0.05$ for mean comparison.

3. RESULTS AND DISCUSSION:

3.1. Germination percentage (GP %)

Figure 1 shows the impact of NaCl and KCl priming on turnip (GP %) at different saltiness concentrations, respectively, in 10 days. From both primed and unprimed seeds diminished significantly ($P \leq 0.5$) with increasing NaCl salinity level. However, this reduction in (GP %) was lower for primed seeds compared to unprimed seeds. Salinity reduced germination percentage for more than half due to an increase in salinity level from 0 mM to 200 mM. A similar decrease in germination percentage has been recorded with rising salt levels in turnip (Mohammed and Nulit, 2019a; Mohammed and Nulit, 2019b). Seed generally, increasing salinity causes a decrease in turnip germination; this might be due to the toxic effects of Na^{+} and Cl^{-} in the process of germination (Tobe *et al.,* 2004; Osman, 2018). It changes the imbibitions of water by seeds due to decline osmotic capacity of germination media (Ibrahim *et al.,* 2019), it causes toxicity which changes the activity of certain enzymes of DNA metabolism, changes the metabolism of protein, breaks down the hormonal balance, and reduce the usage of seed reserve food (Ashraf and McNeilly, 2004; Ashraf *et al.,* 2010). Primed seeds of turnip might have better competency for water absorption from the growing media that enabled metabolic activities in seeds during the germination process of a start much earlier than radical and hypocotyl appearance (Elouaer and Hannachi, 2012; Zaghdani, 2002).

Similarly, increased solubility of seed storage proteins such as the beta subunit of the globulin and enhanced antioxidative reduction in lipid peroxidation and activity in primed seeds facilitated germination (Patade *et al.,* 2009; Kazemi and Eskandari, 2012). According to Soeda *et al*. (2005), the faster germination was due to the synthesis of protein, DNA, and RNA during seeds priming. A high positive correlation between seed germination and the amount of total soluble protein, Tania *et al.* (2019), reported the increased germination ability and total protein in areas with low pollution levels of anthropogenic pressure in *Betula pendula* plant.

3.2. Turnip seedlings length

Shoot and root lengths are the essential factors in the plant for salt stress because roots absorb water due to direct contact with soil, and then shoots enable its supply in the whole plant. For this reason, shoot and root lengths provide important indications of a plant's response to salt stress (Dinneny, 2019). Figure (2) shows an increase in the concentration of salts caused the reduction in the early growth of turnip seedlings. The length of seedling (radicle+ hypocotyl) significantly declined with increasing salts concentration in both primed and un-primed seeds. However, this influence was more distinguished in radicle (Figure 3) and hypocotyl (Figure 4) lengths from unprimed seeds when compared to the primed ones. A similar study shows that the adverse impact of salinity on *Pisum sativum* was overcome by the application of potassium nutrient through seed priming with KCl (Naz *et al*., 2014). Seedlings' growth was affected by both osmotic and specific ionic of salinity (Nawaz *et al.,* 2010; Tabatabaei and Naghibalghora, 2014). Nasim *et al*. (2008) and Petropoulos *et al*. (2017) investigated that salinity inhibits the absorption of essential nutrients such as P and K, which could negatively affect seedlings growth. In this study, seed priming significantly improved turnip seedling growth at different salinity levels. The improvement in radicle and hypocotyl length in the primed seeds may be attributed to earlier germination induced by priming (Farooq *et al.,* 2005). During the period of priming, the embryo expands and compresses the endosperm, the compaction force of the embryo and the water activity on the walls of endosperm cell may alter the tissues to be flexible when dehydrated, Produce free space and facilitate rapid projection of root and seedlings after rehydration (Mohammadi, 2009; Yohannesu and Abraha, 2013).

3.3. Seed vigor

Seed vigor is the characteristics of seed that determine the level of activity and performance of seeds during germination and emergence of seedling under a wide range of field conditions. Seedlings of high-activity seeds are expected to develop more uniformly than seedlings of low-activity seeds (Talai and Sen-Mandi, 2010; Egli and Rucker, 2012). Figure (5) shows both primed with (NaCl or KCl) and unprimed seeds diminished significantly with increasing salinity levels. However, this reduction in vigor was lower for primed seeds compared to unprimed seeds. Similar past investigation on Canola (*Brassica napus* L.) and tomato (*Lycopersicon esculentum* Mill.) showed that seed priming significantly improved shoot and radicle length (Nawaz *et al.,* 2010).

3.4. Dry Biomass of turnip

Salinity treatments were applied on turnip showed a significant reduction in dry seedling biomass with the rise of stress level as compared with control in both unprimed and primed seeds (Figure 6). For primed seeds, dry biomass was higher than for unprimed ones at different levels of salinity stress. Mohammed and Nulit (2019a; 2019b) showed that salt stress decreases the weight of turnip seedling. They suggested that the decrease in growth of turnip seedling is due to ionic stress and osmotic stress of salts. By increasing salinity, the biomass of seedling decreased, which might be attributed to a decrease in the remobilization of the seed reserves from cotyledons to the embryonic axis

(Yohannes and Abraha, 2013). The factors that affected the growth rate of the embryonic axis also affected transfer from cotyledons to the embryonic axis and reserve remobilization (Sedghi *et al.,* 2010; Naz *et al.,* 2014). The efficiency of seed priming with KCl or NaCl in improving biomass weight under stressful conditions was also reported in sunflower, melon (Matias *et al.,* 2018; Oliveira *et al.*, 2019).

4. CONCLUSIONS:

This investigation provided useful knowledge on seed priming using a NaCl and KCl. Turnip's primed and unprimed seeds have different responses to saline solutions, especially in high concentrations. Seed priming with NaCl and KCl overcame these adverse effects of salinity. This priming significantly improved the germination and seedling growth of these plants. In unprimed seeds, declines in germination percentage and seedling growth with rising salinity levels were more apparent than in the primed seeds. Pretreatment with NaCl and KCl lead to a substantial improvement in the percentage of germination seed vigor seedling radical length, hypocotyl seedling length, and dry seedling biomass. Therefore, this research highlighted the benefit of applying seed priming techniques in salty stressful environments to reduce the adverse effects of salinity stress on germination and early development of seedlings under controlled conditions. However, more work is required to assess crop production (NaCl and KCl) on vegetative growth and yield under field conditions.

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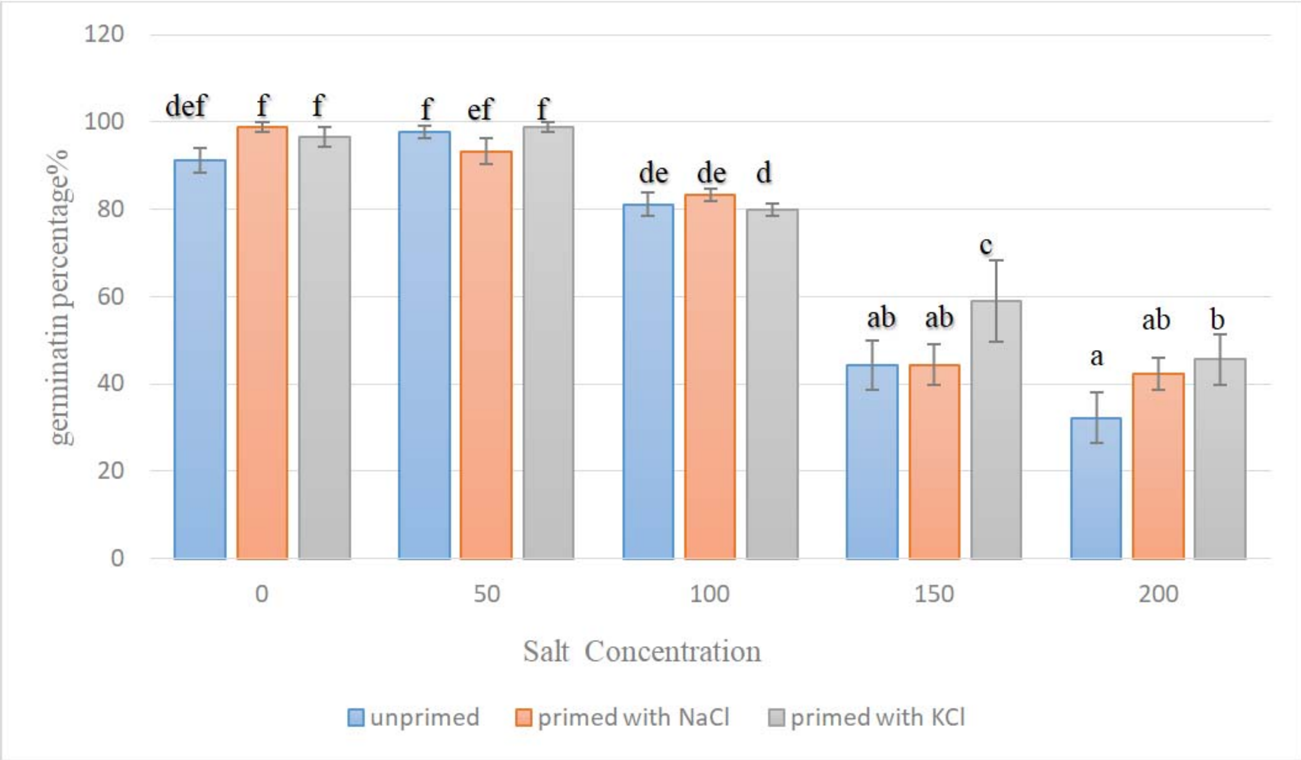

Figure 1. *Effect of seed priming with NaCl and KCl on germination percentage of Brassica rapa rapa under different concentrations of salt. Different letters indicate significant difference among means (Duncan's test, p< 0.05).*

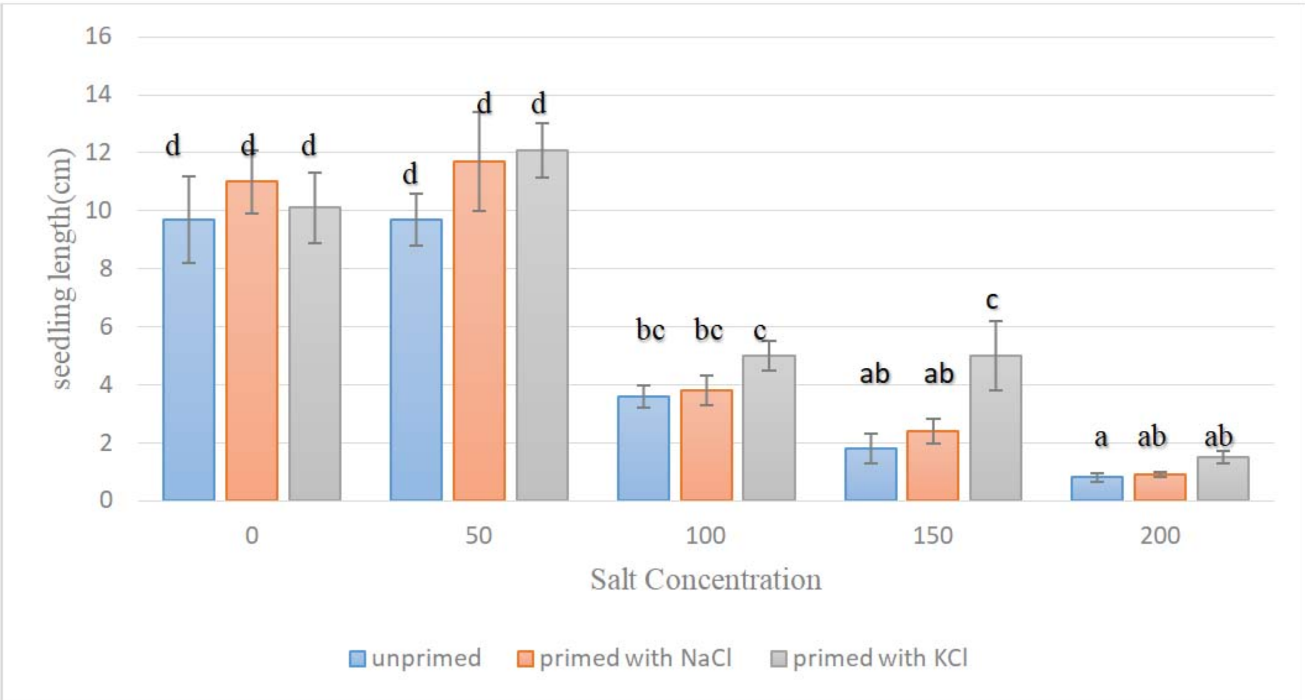

Figure 2. *Effect of seed priming with NaCl and KCl on seedling length of Brassica rapa rapa under different concentrations of salt. Different letters indicate significant difference among means (Duncan's test, p < 0.05).*

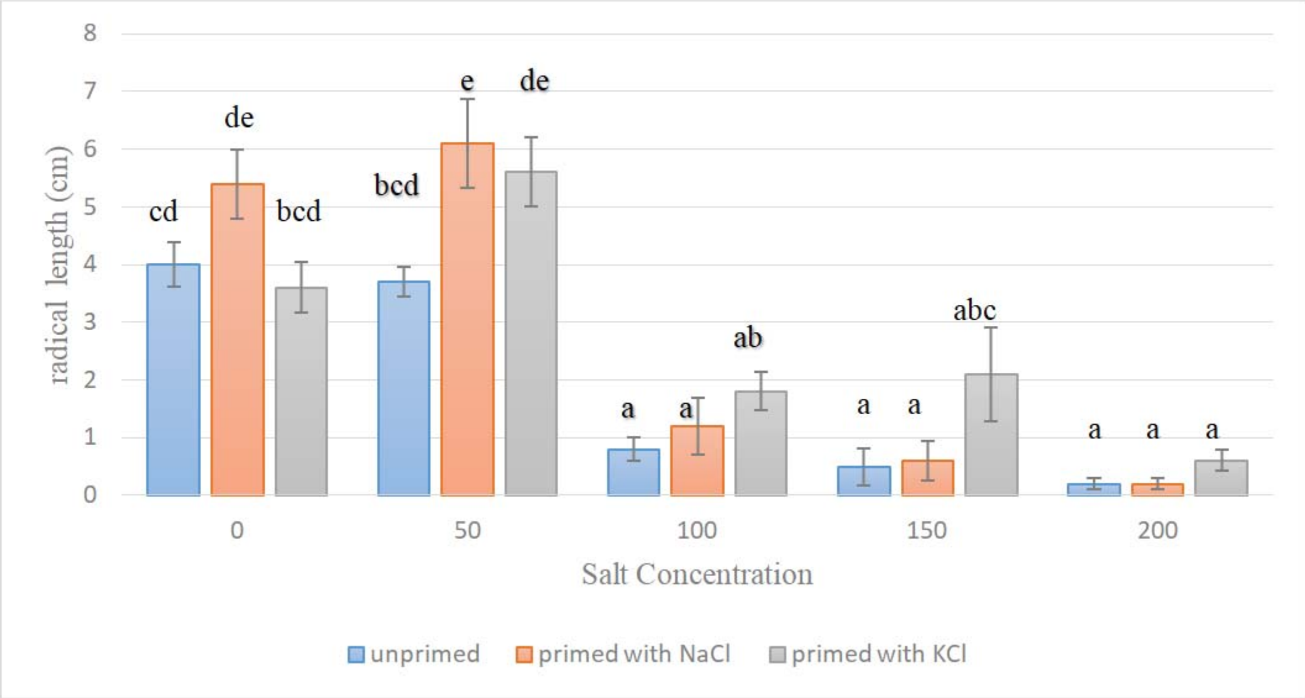

***Figure 3**. Effect of seed priming with NaCl and KCl on radical length of Brassica rapa rapa under different concentrations of salt. Different letters indicate significant difference among means (Duncan's test, $p < 0.05$).*

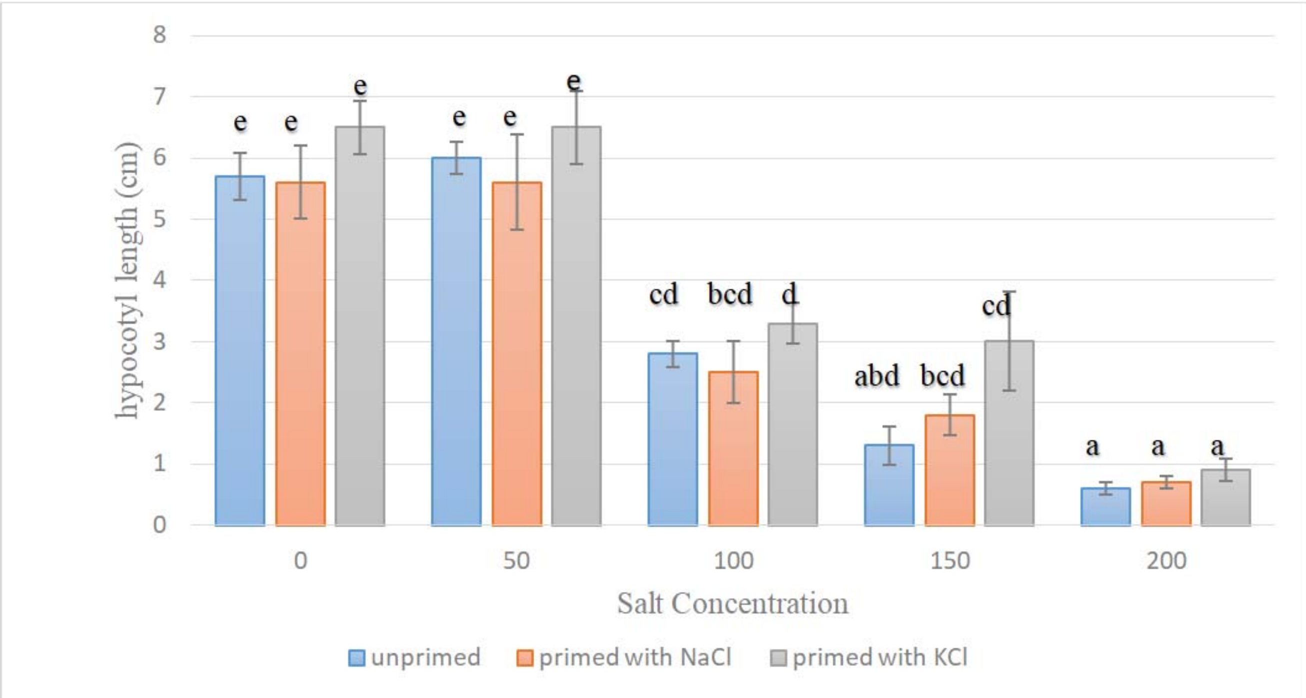

***Figure 4.** Effect of seed priming with NaCl and KCl on hypocotyl length of Brassica rapa rapa under different concentrations of salt. Different letters indicate significant difference among means (Duncan's test, $p < 0.05$).*

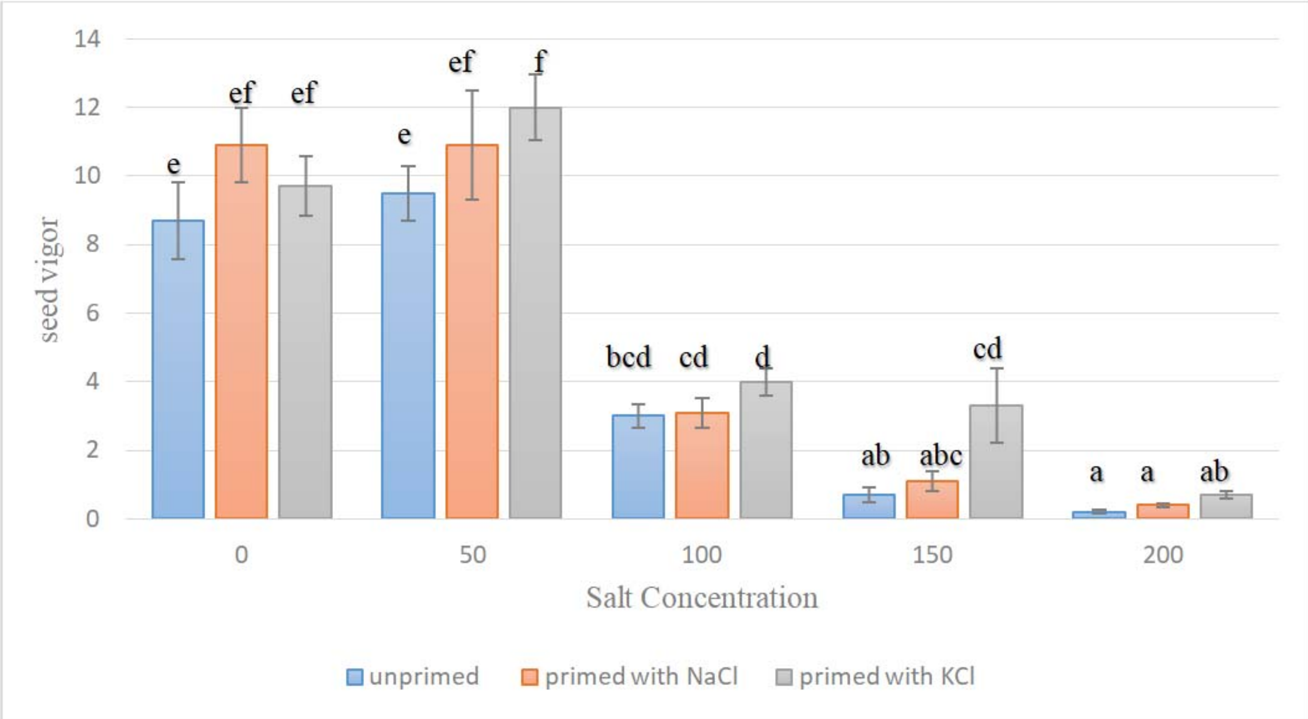

***Figure 5**. Effect of seed priming with NaCl and KCl on seed vigor of <u>Brassica</u> <u>rapa</u> <u>rapa</u> under different concentrations of salt. Different letters indicate significant difference among means (Duncan's test, $p < 0.05$).*

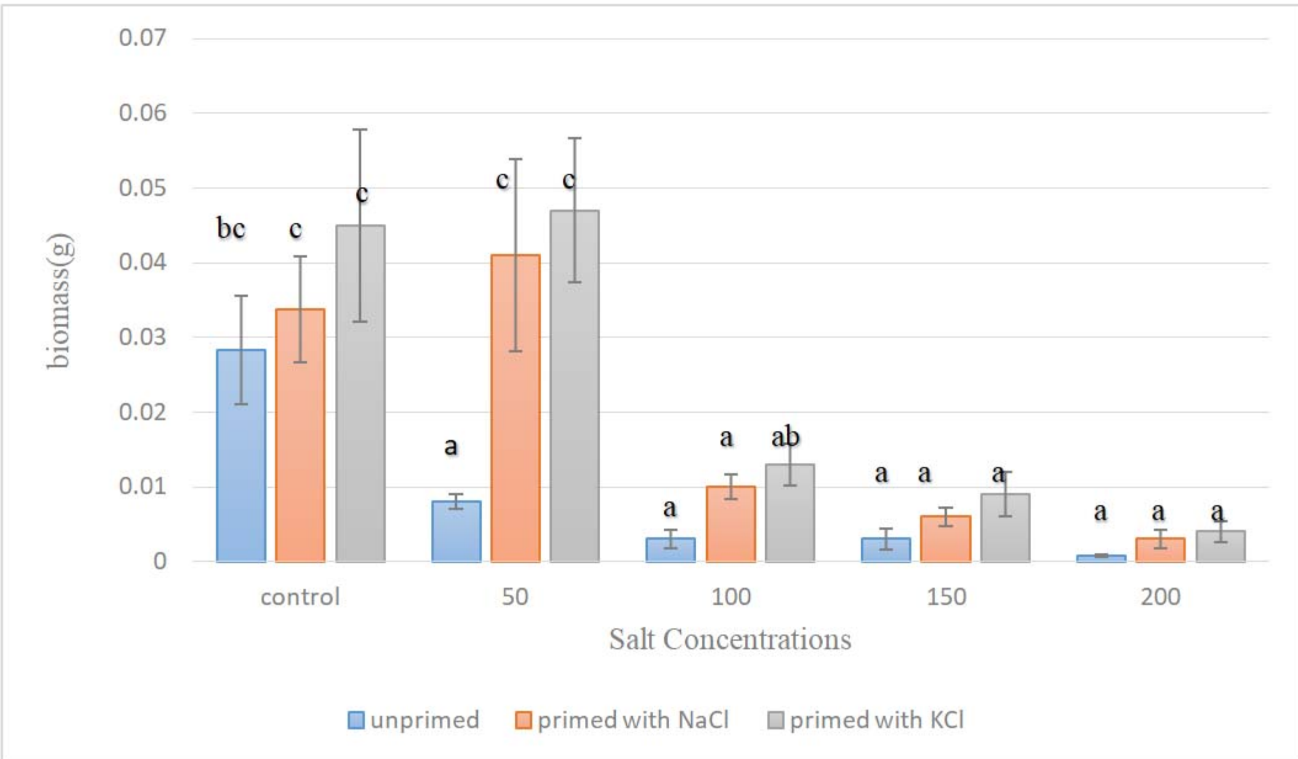

***Figure 6.** Effect of seed priming with NaCl and KCl on germination percentage of <u>Brassica</u> <u>rapa</u> <u>rapa</u> under different concentrations of salt. Different letters indicate significant difference among means (Duncan's test, $p < 0.05$).*