SEED GERMINATION AND RADICLE ESTABLISHMENT RELATED TO TYPE AND LEVEL OF SALT IN COMMON BEAN (*PHASEOLUS VULGARIS* L. VAR. *DJEDIDA*)

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ABSTRACT. Saline soils contain multiple types of salt, each of them may exert a different effect on seed germination and seedling growth. The aim of this study is to assess the effects of five types of salt on the seed germination and radicle establishment of common bean (*Phaseolus vulgaris* L. var. *Djedida*). The experiment was set out as a factorial experiment based on completely randomized design with four replications of ten seeds for the seed germination and twelve replicates for the seedling growth. We used five kinds of salts (NaCl, Na_2SO_4, $CaCl_2$, $CaCO_3$ and KCl) with concentrations of 0, 100, 200 and 300 mM. Seeds were incubated in Petri dishes at 25°C, in the dark, for 10 days. The results of analysis of variance indicated that the effects of salt types and concentrations, and their interaction effect were significant in all measured traits ($P < 0.01$). However, no significance effect was found on secondary roots number and seedlings dry biomass with $CaCO_3$ treatment solution. According to the results, the inhibitory effects of the five salt types differed significantly. Indeed, germination of common bean seeds by various salts were in the order of NaCl > KCl > $CaCO_3$ > Na_2SO_4 > $CaCl_2$. However, the taproot length, the number of secondary roots and the seedlings dry weight by various salts were in the order of $CaCO_3$ > KCl > NaCl > $CaCl_2$ > Na_2SO_4. The effect of salt concentration was also obvious. The reduction in dry biomass of cotyledons is proportional to germination rates and to the development of seedlings in dry biomass and in size. Seeds of *P. vulgaris* var. *Djedida* were able to germinate under all concentrations of the various types of salt. The lowest final germination percentage (FGP) was obtained under 300 mM of all salts recording the following values: $CaCO_3$ - 60%, NaCl - 60%, KCl - 52.5%, Na_2SO_4 - 50% and $CaCl_2$ - 27.5%. During

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germination stage, the radicle emergence would be controlled by the environment osmolarity, while the later growth of the seedling would be limited by the reserve mobilization.

INTRODUCTION

Food legumes play an important role in the cropping systems and in the diet of the population (Sinclair and Vadez, 2012). Among these legumes, bean or common bean (*Phaseolus vulgaris* L.) is one of the oldest species and a major part of humanity's diet (Bevilaqua and Antunes, 2015). In addition to its interest as one of the major legumes providing energy, beans are also an important source of protein in developing countries (Blair, 2013).

In Algeria, the low yields are mainly due to the environment fluctuations recorded during the various seasons (Beebe *et al*., 2013). Indeed, the Algerian climate is characterized by irregular rainfall over time and space, as well as a tendency towards more aridity and thus an increased impact of drought. These are considered as factors of a partial or total loss of production (Faramarzi *et al*., 2013).

Abiotic stresses, such as drought, salinity, extreme temperatures, chemical toxicity and oxidative stress are serious threats to agriculture and result in a deterioration of the environment (Kusvuran *et al.,* 2016). Abiotic stress is the leading cause of

crop loss in the world, reducing average yields of most field crops (Lobell, 2014). Drought and salinity are becoming widespread in many areas and can result in serious salinization of more than 50% of all arable land by 2050 (Galford *et al*., 2010). Abiotic stress results in a series of morphological, physiological, biochemical and molecular changes that affect plant growth and productivity (Zandalinas *et al*., 2018). Overall, more than 800 million hectares of land are affected by salinity (Munns, 2005), at the world level.

Saline soils contain multiple types of soluble salt components, each of which has a different effect on the initial growth of plants (Bernstein, 1958; Munns and Termaat, 1986; Shannon and Grieve, 1998; Kheloufi *et al*., 2016a), and the compositions of soluble salts in saline soils differ greatly among locations (Tobe *et al*., 2002; Kheloufi *et al*., 2017).

Although most of these reports are basing on experiments with NaCl, it has been hypothesized that the other salts have similar effects on cellular function, but to different degrees (Tobe *et al*., 2004). More cations and anions associated with salinity are calcium (Ca^{2+}), magnesium (Mg^{2+}), sodium (Na^+), chloride (Cl^-), sulfate (SO_4^{2-}), and bicarbonate (HCO_3^-). In some instances, potassium (K^+) and nitrate (NO_3^-) may participate to salinity, and when pH is greater than 9, CO_3^{2-} becomes a problematic anion (Schabes and Sigstad, 2005). These effects of salts on the functions of the

cell membranes and the cell walls may affect the water potential of the cytosol and cellular extensibility, and thus, may affect seed germination and seedling growth (Kheloufi *et al.*, 2018).

Seed germination is the most important stage in a plant life cycle. Water, air, temperature and light are all essential for the seed germination process, starting from imbibition, activation and succeeding manifest-tation (Bradford, 1995; Bewley and Black, 2012; Finch-Savage and Bassel, 2015).

In glycophytes species, salinity can reduce the growth of plants or damage the plants through osmotic effect (it causes water deficit), toxic effects of ions and imbalance of the uptake of essential nutrients (Parihar *et al.*, 2015).

In the present study, we compared the effects of different types of salts on seed germination and seedling growth of common bean (*Phaseolus vulgaris* var. *Djedida*). This variety is among the most cultivated bean varieties in Algeria. It has already been the subject of several studies evaluating its tolerance to NaCl (Bouzid and Rahmoune, 2012; Adda *et al.*, 2014; Taïbi *et al.*, 2016) and even seawater (Mansouri and Kheloufi, 2017).

MATERIAL AND METHODS

Experimental design and treatments

This research project was performed in 2018, at the laboratory of the Department of Ecology and Environment, University of Batna 2, Algeria. Seeds of *P. vulgaris* var. *Djedida* (length: 12.9 ± 0.63 mm; width: 6.46 ± 0.51 mm; thickness: 5.69 ± 0.42 mm; mean ± SE; n = 50) were provided by SARL AGROSEED (Harvest date: 2016; seed conditioning: France; origin: USA; reference: DJ0512). The one-thousand seed weight was 273 g.

To evaluate salt tolerance during germination, a total of four replicates of ten seeds were disinfected with 1% NaOCl, rinsed with distilled water and immediately sown on 0.8% (water agar) in 9 cm Petri dishes.

The water agar was prepared with various salts: NaCl, Na_2SO_4, $CaCl_2$, $CaCO_3$ and KCl, with concentrations of 0, 100, 200 and 300 mM. Seeds were incubated at 25°C, in the dark, for 10 days, because the seeds germinate favourably under these conditions (Mansouri and Kheloufi, 2017). Experiments was performed in a completely randomized block. Germination counts were done daily to evaluate the kinetics of germination. Seeds were considering germinating only when 2 mm radicles emerged (*Fig. 1*).

At the end of the germination period, the final germination percentage, the length of the taproot, the number of secondary roots, the dry weight of cotyledons and seedlings (without cotyledons) were recorded. The common bean root system is characterized by a taproot. Roots arising from it are called secondary roots, those formed from them is called tertiary roots. Adventitious roots were formed at the base of hypocotyl (Díaz-Ruiz, 2012). Cotyledons and seedlings were weighed separately after drying in an oven at 80°C for 48 hrs. Twelve replications were used for the growth traits, *viz.* three replicates from each Petri dish.

In the germination tests, final germination percentage (FGP) for each

$$FGP\ (\%) = \frac{\Sigma ni}{N} \times 100$$

where, *FGP* is final germination percentage, *ni* is the number of germinated seeds at final day of test, and *N* is the total number of incubated seeds per test (Côme, 1970).

Statistical analysis

The experiments were conducted with four replicates of ten seeds (n=4*10) for the trait seed germination and with 12 replicates (n=12) for the morphological characteristics, and the results were expressed as mean ± standard deviation (SD). All the data were subjected to one-way and two-way analysis of variance (ANOVA) and Duncan's multiple range test (*P*< 0.05) using SAS Version 9.0 (Statistical Analysis System) (2002) software.

RESULTS AND DISCUSSION

The results of analysis of variance indicated that the effects of salt types and concentrations, and their interaction effect were significant in all measured traits (*P*< 0.01) (*Table 1*). Mean comparison of simple effects of different salt types and concentrations and their interaction are shown in *Table 2*.

The mean comparison results and the results of analysis of variance (*Table 2*) showed that among different salts, the lowest inhibitory effects on germination and the most measured traits was belonged to $CaCO_3$ and KCl, and the highest inhibitory was related to NaCl, Na_2SO_4 and $CaCl_2$.

salt types and levels were calculated by using the following formula:

On the other hand, it is important that effect of salt concentration was obvious. The highest inhibitory effect was related to 300 mM of all the salts (*Table 2*).

Germination kinetics

According to our results, the common bean seeds were able to germinate in the absence and/or in the presence of a low/high salinity, they are tolerant in the presence of salts; but, this tolerance differs from a treatment solution to the other one, because the type of salt plays a determining role grafting for the seed germination (Kheloufi *et al.*, 2016b). The results represented on *Fig. 2* indicate that under the saline conditions of the five kinds of salts (NaCl, Na_2SO_4, $CaCl_2$, $CaCO_3$ and KCl), the kinetics of germination expresses three phases: a first phase of latency, expressed by the imbibition of seeds; a second exponential phase where one attends an acceleration of germination and finally a third phase characterized by a stationary stage indicating an interruption of germination. Finally, whatever the type of salt used, the germinative capacity of salt-stressed seeds is reduced and this for the three concentrations used.

Under saline conditions operated by 100 mM of all the type of salt, the dynamics of the germinative capacity

are slightly disturbed. The seeds expressed their sensitivity starting from the concentration of 200 mM by expressing a very reduced germination rate with a very slow exponential phase and which lasts much longer to be stabilized at the 10th day of the experiment. The concerned salts by this observation were only NaCl, Na_2SO_4, $CaCl_2$ and $CaCO_3$ (*Fig. 2*). According to Rejili *et al*. (2010), the osmotic effects are translated by the inability of seeds to absorb sufficient amounts in water to return them to their critical threshold of moisture, necessary for the release of the process of germination; however, the toxic effects are bound to a cellular accumulation of salts, which cause disturbances of enzymes involved in the physiology of seed germination, prevent dormancy breaking of embryos and lead to a decrease in the capacity of germination.

Figure 1 - Experimental design

Final germination percentage

According to *Figure 3A*, interpretation of interaction effect of salt types and concentrations on germination percentage showed that maximum germination was obtained under non-saline conditions (control treatment) (87.5%). The lowest FGP was obtained under 300 mM of all salts recording the following values: 60% (NaCl), 60% ($CaCO_3$), 52.5% (KCl), 50% (Na_2SO_4) and 27.5% ($CaCl_2$). This reduction in the FGP induced by an increase of salinity stress have been described by numerous authors for the same leguminous species (Beltagi *et al*., 2006; Taffouo *et al*., 2010; Cokkizgin, 2012; Mena *et al*., 2015). Decrease in germination by increasing of salinity level was possibly due to reduced soluble osmotic potential, high toxic ions and seed nutrient imbalance (Hu and Schmidhalter, 2005). It is also assumed that in addition to toxic effects of certain ions, higher concentration of salt, reduces the water potential in the medium, which hinders water absorption by germinating seeds and thus reduces germination (Jamil *et al.,* 2006; Tavakkoli *et al*., 2010).

Germination of common bean seeds by various salts were in the

order of NaCl > KCl > CaCO$_3$ > Na$_2$SO$_4$ > CaCl$_2$ (*Table 2*).

Kaymakanova (2009) also showed that the three studied cultivars of *P. vulgaris* were inhibited stronger by Na$_2$SO$_4$ than NaCl treatment. This can be attributed to the fact that different salts have different inhibitory effects on induction of germination suppression

by lowering the osmotic potential of the medium (Moghaddam *et al.*, 2018).

Most studies showed that the effect of salts on the germination is primarily osmotic and few researchers showed that it could be both ionic and osmotic (Duan *et al.*, 2004; Joshi *et al.*, 2005; Zhang *et al.*, 2010).
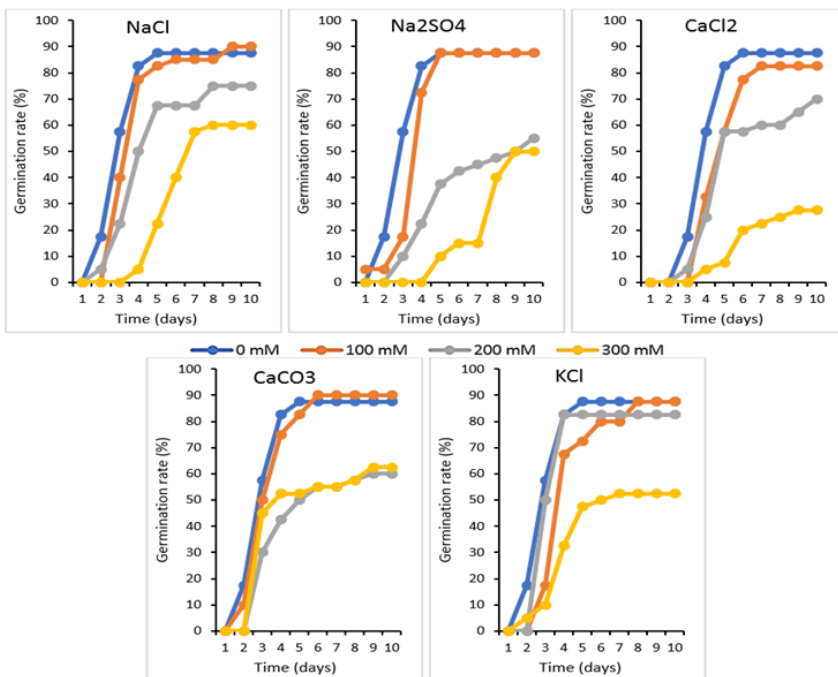
Figure 2 - Effect different type of treatment solution (NaCl, Na$_2$SO$_4$, CaCl$_2$, CaCO$_3$ and KCl) at various concentrations of (0, 100, 200 and 300 mM) on the germination kinetics of *Phaseolus vulgaris* var. *Djedida* after 10 days of incubation in Petri dish

Length of the taproot

The root length are the most important parameters for salt stress, because roots are in direct contact with soil and absorb water from soil and supply it to the rest of the plant. For this reason, root length provides

an important trait in response to salt stress (Munns, 2011; Kheloufi *et al.*, 2018). The length of the taproot showed a highly significant difference among the interaction effect of various salts and different salinity concentrations (*Fig. 3B*).

By increasing of the salinity levels, the length of the taproot decreased. The highest root length was recorded in control treatment (6.97 cm) (*Fig. 3B*). The inhibitory effect of salts on root length have been found in our previous studies with three leguminous crops, *viz.* common bean, pea and chickpea (Mansouri and Kheloufi, 2017). The taproot length by various salts were in the order of $CaCO_3 > KCl > NaCl >$ $CaCl_2 > Na_2SO_4$ (*Table 2*). Decrease in plant height and other growth parameters are the most distinct and obvious effects of salt stress, since inhibition of growth is probably the most general response of plants to stress (Munns and Tester, 2008). Bayuelo-Jimenes (2002) reported that salt-tolerant species (accessions) of *Phaseolus* maintained relatively high root growth even at 180 mM (nutrient solution) NaCl.

Table 1 - Analysis of variance for the traits investigated for *Phaseolus vulgaris* var. *Djedida* in response to salinity stress using different type of treatment solution (NaCl, Na₂SO₄, CaCl₂, CaCO₃ and KCl) at various concentrations of (0, 100, 200 and 300 mM)

Parameters	Source of variables	Degree of freedom	F de Fisher	*P*
FGP	TRT	3	56,48	< 0,0001
	TYPE	4	3,43	0,0136
	TRT × TYPE	12	2,74	0,0051
LTR	TRT	3	383,59	< 0,0001
	TYPE	4	39,25	< 0,0001
	TRT × TYPE	12	15,64	< 0,0001
SECROOT	TRT	3	70,27	< 0,0001
	TYPE	4	25,48	< 0,0001
	TRT × TYPE	12	8,10	< 0,0001
COTYDW	TRT	3	157,81	< 0,0001
	TYPE	4	37,42	< 0,0001
	TRT × TYPE	12	7,30	< 0,0001
SLDW	TRT	3	165,92	< 0,0001
	TYPE	4	173,03	< 0,0001
	TRT × TYPE	12	23,19	< 0,0001

TRT - Salt concentration, FGP - Final germination percentage (n=4*10), LTR - Length of the taproot (n=12), SECROOT - Number of secondary roots (n=12), COTYDW - Cotyledon dry weight (n=12), SLDW - Seedling dry weight (n=12)

Number of secondary roots

Developing a robust root system is crucial to plant survival and competition for soil resources (Schenk, 2006). There was a significant difference between various salts and each salinity level on secondary roots. The results of interaction effect showed that lateral roots were recorded mostly in control and 200 mM of $CaCO_3$ and KCl (*Fig. 3C*).

On the other hand, *P. vulgaris* seeds preserved the ability to produce secondary roots by increasing concentration to 300 mM up to 12

only in $CaCO_3$. While, under the same level, the inhibitory effects of Na_2SO_4, $CaCl_2$, NaCl and KCl were higher (*Fig. 3C*). According to the same figure, the highest value of secondary roots belonged to 100 mM KCl. High salinity produces in plant a physiological drought. According to this, common bean duplicated the number of secondary and tertiary roots with modified architecture in response to drought (Nuñez-Barrios *et al.*, 1998). No significance effect was found on secondary roots number under $CaCO_3$ (*Table 2*).

A deep and extensive root system has been advocated to increase productivity of food legumes under drought and salinity conditions. Root length could be an important trait to assist *in vitro* selection of salinity resistant varieties of beans with an improved capacity to acquire water (Mena *et al.*, 2015).

Duan *et al*. (2013) found that primary and lateral roots have intrinsically different response programs to salinity, with lateral roots having a stronger suppression than primary roots after salt treatment. Under high salinity, the production of abscisic acid (ABA) is induced and it is necessary for suppression of lateral roots.

The ability of the root system to control entry of ions to the shoot has crucial importance to plant survival in the presence of salt (Chinnusamy, 2005). In addition, high salinity may inhibit seedling and root elongation by slowing down the water uptake by the plant (Munns, 2002).

Cotyledons dry weight

During the germination process, the mobilization of seed reserves is initiated from the cotyledons to the embryonic axis (Bewley and Black, 1985). This process continues until the seedling stage, in which the primary sources are exhausted and the seedling becomes autotrophic to form leaves that carry out photosynthesis, which are the most important source of photosynthate of the plant (Munier-Jolain *et al.*, 1998). The embryonic axis consists of the plumule and radicle, which demands nutrients from the seed when germination begins, which are provided by the cotyledons (Bewley and Black, 1985). The cotyledons dry biomass by various salts were in the order of $CaCl_2$ > NaCl > Na_2SO_4 > KCl > $CaCO_3$ (*Table 2*).

The higher tolerance of cotyledon to salt stress may be an adaptive strategy of this species to survive with adverse environmental factors (Ruffino *et al*., 2010). Studies carried out by Wang *et al*. (2012) also indicated that cotyledons of *Vicia cracca* were more tolerant to NaCl stress than shoots.

According to Bathellier *et al*. (2008), during imbibition of the seed of *P. vulgaris* plants, cotyledons mass decrease slowly but after three days it was found that accelerated markedly. Cotyledons reduced its dry weight because they are the only structures that provide nutrients to the seedling (Díaz-Ruiz, 2012).

According to *Fig. 3D*, the reduction in dry biomass of

cotyledons is proportional to germination rates and to the development of seedlings in dry biomass and in size. This establishes the relationships between growth of the shoot, root and provides nutrients from the cotyledons, which can be expressed by dry matter accumulation in the stem and root and to lose dry matter of cotyledons (Milberg and Lamont, 1997; Koyro *et al.*, 2008). In the germination process, the cotyledons lose metabolic dry matter and conserved structural dry matter during the development of the seedling so that they become the only structures that die (Díaz-Ruiz, 2012).

Table 2 - Analysis of variance for each treatment solution
(NaCl, Na$_2$SO$_4$, CaCl$_2$, CaCO$_3$ and KCl) used to investigate
the response of *Phaseolus vulgaris* var. *Djedida* to salinity stress

Treatment solution	Parameters	Source of variables	Degree of freedom	F de Fisher	*P*
NaCl	FGP	TRT	3	9.31	0.0019
	LTR	TRT	3	101.33	< 0,0001
	SECROOT	TRT	3	15.32	< 0,0001
	COTYDW	TRT	3	50.00	< 0,0001
	SLDW	TRT	3	84.97	< 0,0001
Na$_2$SO$_4$	FGP	TRT	3	18.86	< 0,0001
	LTR	TRT	3	290.04	< 0,0001
	SECROOT	TRT	3	36.15	< 0,0001
	COTYDW	TRT	3	50.14	< 0,0001
	SLDW	TRT	3	119.20	< 0,0001
CaCl$_2$	FGP	TRT	3	17.62	0.0001
	LTR	TRT	3	141.49	< 0,0001
	SECROOT	TRT	3	33.54	< 0,0001
	COTYDW	TRT	3	36.50	< 0,0001
	SLDW	TRT	3	82.16	< 0,0001
CaCO$_3$	FGP	TRT	3	6.97	0.0057
	LTR	TRT	3	12.03	< 0,0001
	SECROOT	TRT	3	1.15	0.3449
	COTYDW	TRT	3	4.54	0.0092
	SLDW	TRT	3	2.77	0.0578
KCl	FGP	TRT	3	15.11	0.0002
	LTR	TRT	3	129.21	< 0,0001
	SECROOT	TRT	3	23.18	< 0,0001
	COTYDW	TRT	3	77.80	< 0,0001
	SLDW	TRT	3	74.13	< 0,0001

TRT - Salt concentration, FGP - Final germination percentage (n=4*10), LTR - Length of the taproot (n=12), SECROOT - Number of secondary roots (n=12), COTYDW - Cotyledon dry weight (n=12), SLDW - Seedling dry weight (n=12)

Seedling dry weight

According to the *Fig. 3E*, the maximum seedling dry weight was related to 100 mM of $CaCO_3$ (89.94 mg), while the minimum was recorded in 100 mM of Na_2SO_4 (1.18 mg). Indeed, under a treatment solution of $CaCO_3$, the seedlings recorded the highest values under all the salinity levels and also compared to control. Then, the seedling dry biomass by various salts were in the order of $CaCO_3$ > KCl > NaCl > $CaCl_2$ > Na_2SO_4.

Generally, the decreases in seedling growth, due to the increase in the salt concentration, are caused by physicochemical effects or by osmotic-toxic salts, which exist in saline solutions (Sarker *et al*., 2014). Reductions in the biomass of *P. vulgaris* under saline condition were indicative of severe growth limitations under 300 mM (*Fig. 3E*), except for $CaCO_3$ treatment solution. Salinity had adverse effects not only on the biomass, but also on other morphological parameters.

In several legumes, such as faba bean (*Vicia faba*) (Tammam, 2003; Bulut and Akıncı, 2010), soybean (*Glycine max*) (Panneerselvam *et al*.,

1998), and chickpea (*Cicer arietinum*) (Akhter *et al*., 2004), salinity was reportedly found to reduce seedling dry biomass. The reduction in seedling development may be due to toxic effects of the salt used, as well as unbalanced nutrient uptake by the seedlings (Jamil *et al*., 2006).

Bayuelo-Jiménez *et al*. (2002) indicate that the term 'salt tolerance during seed germination' is used only to refer to situations where the seed germinates rapidly under salt stress conditions. No distinction is made between osmotic and ionic effects of the salinity stress. Likewise, salt tolerance during early seedling growth is assessed on the absolute growth at a given salt concentration, as well as the percentage of growth under salt stress relative to growth under non-stress conditions. The same authors demonstrate that tolerance to salinity in *Phaseolus* species might also vary with developmental stages. Salt tolerance at germination and at the seedling stage appears to be controlled by more than one gene and is highly influenced by salt type and concentration (Yamaguchi and Blumwald, 2005; Munns, 2005).

(A)

(B)

(C)

(D)

(E)
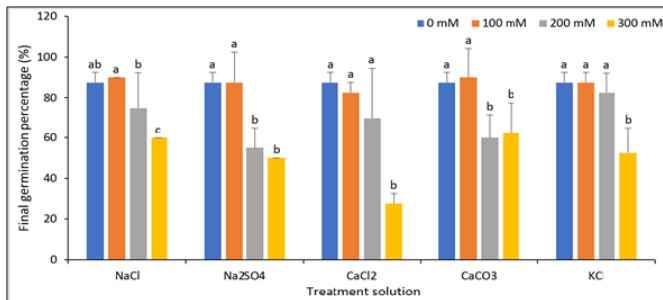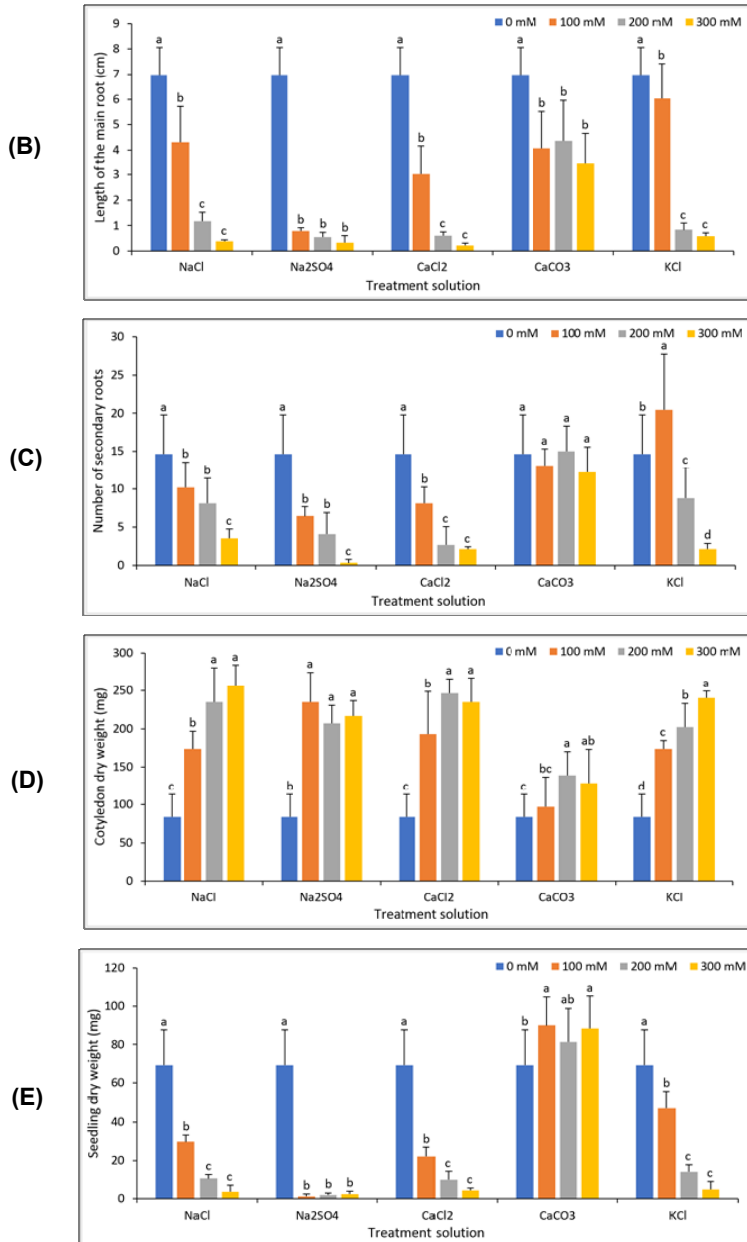
Figure 3 - Interaction effect of salt types and concentrations on: (A) Final germination percentage, (B) Length of the taproot, (C) Number of secondary roots, (D) Cotyledon dry weight and (E) Seedling dry weight in *Phaseolus vulgaris* var. *Djedida* after 10 days of incubation in Petri dish (For each treatment solution, means followed by the same letter are not significantly different at 5% probability level).

CONCLUSIONS

During germination stage, the radicle emergence would be controlled by the environment osmolarity, while the later growth of the seedling would be limited by the reserve mobilization. It can be concluded that salt stress decreased seed germination and seedling length at early growth stage. As the results demonstrated, the inhibitory effects of the five salt types studied in the germination phase of common bean differed substantially, especially in the case of NaCl, $CaCl_2$ and Na_2SO_4. Inhibitory effects of these salts on common bean were very strong, compared to those of $CaCO_3$ and KCl. These differential behaviors of *P. vulgaris* var. *Djedida* seeds according to the salt types are presumably due to the fact that the same concentration of salt generates different osmotic potentials and the osmotic effect may have a greater influence on germination.

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