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## Interactions between Halopriming and Hormopriming in Regulating the Vegetative Growth and Seed Quality of *Vicia faba* L.

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**Abstract:** High seed quality and growth performance is farmer's and agricultural enterprise's demand. However, increasing population increases food demand and due to global warming and limited natural resources, more attention has been paid to implementing different effective strategies. Halopriming and hormopriming have shown improvement in different growth and developmental stages in plants and under adverse environmental conditions. Therefore, this study was aimed to study the interaction impact of halopriming and hormopriming on growth and seed production of faba bean (*Vicia faba* L.). The interaction effect of seed priming was examined during germination and field growth performance of faba bean. Different salt substances NaCl (0, 25 and 50 mM) and CaSO<sub>4</sub> (0, 2.5 and 5 mM) were used as a halopriming. Salicylic acid (SA) (0, 0.5 and 1mM) was used as a hormopriming agent. Split-plot randomize complete block design was followed with three replicates. The results show a significant increase in seed germination when the seeds were haloprimed with CaSO<sub>4</sub> (2.5 and 5 mM) and compared with the control. NaCl (25 and 50 mM) increased the number of fruit set, brunches, pods and yield per plant significantly in comparison to the control. SA at 0.5 mM increased plant height significantly when compared with the control. These results may indicate that different varieties and species respond differently to seed priming. In addition, the interaction between the halopriming and hormopriming compounds may function as a buffer to modify the osmotic presser during imbibition, and may the seed coat of fabe bean, Aquadlge, variety prevent the transport of the compounds to the embryo. Therefore, it has been suggested and recommended to implement a combination between halopriming and hormopriming using different species and faba bean variety and study its effect at the molecular level during seed germination.

**Keywords:** Halopriming, Hormopriming, Seed quality, *Vicia faba* L.

### Introduction

The severity of salinity is threatening the agricultural ecosystem globally. The reduction of crop productivity is a consequence of the quality of irrigated water and poor agricultural

practices. It has been reported that a high level of dissolved salt was found in the Tigris and Euphrates rivers and soil salinity increases from north to south of Iraq (Qureshi & Al-Falahi,

2015; Jawad, 2020). Various techniques have been developed and targeted by plant scientists to maintain the yield under salinity challenges, globally. Seed priming is one of the techniques that has been used intensively lately to promote the pre-germination metabolic process and for better plant stress tolerance (Farooq *et al.*, 2019). Several methods of seed priming, including hydropriming, halopriming, osmopriming, solid matrix priming, biopriming, nutripriming, hormopriming, and other organic sources have been developed and applied (Sher *et al.*, 2019). Halopriming implies the use of specific salts such as potassium nitrate (KNO<sub>3</sub>), sodium chloride (NaCl), calcium sulfate (CaSO<sub>4</sub>), and calcium chloride (CaCl<sub>2</sub>) of variable concentrations to improve the germination, seedling growth, and productivity of diverse crop species under optimal and stressed conditions (Manonmani *et al.*, 2014). Hormopriming consists of the exogenous application of plant growth regulators or phytohormones that can improve crop performance under stressed environments. Salicylic acid (SA) and some other plant growth regulators have been used intensively as a seed priming agents to improve germination, growth and yield under various stress conditions (Mustafa *et al.*, 2019; Sher *et al.*, 2019).

*Vicia faba* L. common names faba bean, broad bean or fava bean belongs to the family Fabaceae. Faba bean is the cheapest and a major source of protein, dietary fiber, antioxidants and essential minerals and is used heavily in crop rotation systems, due to its ability to nitrogen fixation (Turco *et al.*, 2016). It has been demonstrated that faba bean is sensitive to salt stress (Nadeem *et al.*, 2019). The number of nods, which is responsible for nitrogen fixation, mineral nitrogen level, protein content and

nitrogenase was reduced significantly in the faba bean plants under salinity stress (Rabie & Almadini, 2005). Soil salinity causes osmotic potential, ion excess and toxicity, which leads to disturbance and limitation in seed germination and plant growth and development (Parihar *et al.*, 2015). High levels of Na<sup>+</sup>, Ca<sup>2+</sup>, and Cl<sup>-</sup> were found in faba bean plants exposed to salt stress and this was led to a decrease in the level of K<sup>+</sup> uptake, photosynthesis efficiency, stomatal conductance and generation of reactive oxygen species (ROS) (Gadallah, 1999, Semida *et al.*, 2021), thus the growth and yield components were negatively reduced. On the contrary, the low concentration of NaCl 60 mM was caused an increase in most vegetative growth parameters and protein, and a significant decrease at 240mM NaCl when compared with the control 0 mM NaCl treatment (Qados, 2011).

To avoid salinity stress, plant researchers introduced different mechanisms including salinity tolerance in salt sensitive crops. Seed priming is one of the techniques, which has been used for decades to improve germination and growth and also to identify the causes of salt tolerance in different cultivated crops. It has been reported that halopriming and hormopriming induce DNA repair, mRNA synthesis, cell cycle regulation, scavenging ROS, activation of stress-related signals and improve germination, seed vigor and tolerance to salt stress, however, this effect differs among different species and substance concentration and the duration of soaking (Majeed & Muhammad, 2019). Halopriming with NaCl 80 mM or CaSO<sub>4</sub> 80 mM proved to be effective methods and significantly increased germination and seedling growth components in *Vigna unguiculata* (L.) Walp. (Nabi *et al.*, 2020).

Different genotypes of faba bean responded differently to seed priming with different concentrations of salt (del Pilar Cordovilla *et al.*, 1995). Presoaking of faba bean seed in 140 mM NaCl solutions was resulted a significant increases in concentrations of certain compounds such as carotenoids, antioxidant enzymes activity, accumulation of osmotic solutes, and high selectivity of K<sup>+</sup>/Na<sup>+</sup> ratio in genotype 115 when compared to genotype 125 (Azooz, 2009). In spite of this, SA treatment was reduced the inhibitory effect of salt in genotype 125 and also improved the performance of both genotypes under control treatment, in the aforementioned study. Various studies were conducted to evaluate halopriming on germination and seedling growth including *Triticum aestivum* L.(Afzal *et al.*, 2008), *Vigna unguiculata* (Karim *et al.*, 2020), *Gerbera jamesonii* and *Zinnia elegans* (Ahmad *et al.*, 2017) and *Zea mays* (Kumari *et al.*, 2017). However, germination and growth of fabe bean seed haloprimed and hormoprime have not been examined under variable field conditions. Moreover, improving seed germination percentage and increasing yield has become one of the important goals of agriculturists and farmers.

Therefore, this work was amid to study the effect of halopriming, hormoprime and their interaction on faba bean germination and field performance in autumn sowing. For this purpose, laboratory and field trials were conducted.

## Materials & Methods

### Experimental site and treatments

Seeds of faba bean (*Vicia faba* L.) variety Aquadlge, producer BIOTEK seed ltd.com, Selcuklu, Konya, Turkey was obtained from a

local seed agent, Erbil, Kurdistan, Iraq. Seeds were treated with VITAVAX fungicide. Homogenized seeds of similar size were selected for laboratory and field trials. Different salt substances NaCl (0, 25 and 50 mM) and CaSO<sub>4</sub> (0, 2.5 and 5 mM) were used as a halopriming treatment (Seeds were soaked in the salt solution for 24 h). For hormoprime Salicylic acid (SA) (Scharlau, Barcelona, Spain) (0, 0.5 and 1mM) (Seeds were soaked in SA solution for 24 h) the solutions were prepared using purified water (Life Ahram company. Zakho, Iraq). The concentration of NaCl, CaSO<sub>4</sub> and SA were chosen according to the previous studies with some modification (Zaman *et al.*, 2005, Anaya *et al.*, 2018).

A laboratory experiment with three replicates following a completely randomised design was conducted. Ten seeds were placed onto two layers of paper towels on a plastic plate. At first, five mL of the same soaked solution was assigned for each experimental unit and then daily two mL was added for each plate. The plates were kept at a dark place with 22-24°C for seven days (Anaya *et al.*, 2018). The germination was monitored daily and recorded to calculate seed germination percentage (%G) and germination rate (GR) according to the following equations (Damalas *et al.*, 2019)

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

$$GR = \frac{\sum(\text{Number of germinated seeds at time})}{\text{Number of germinated seeds}}$$

A field experiment was carried out at the research centre farm belongs to the Directorate of Agriculture, Erbil, Kurdistan Region, Iraq (36.1°N, 44°E and 434 Meters above mean sea level) during the growing season (October 17<sup>th</sup>-April 20<sup>th</sup>) (2020–2021). Table (1) shows the soil structure and some physical and chemical

characteristics of the experimental site. The average temperature and humidity range between 5-28°C and 30-57%, respectively during the growing season (Anonymous, 2021). The land was plowed and then organic manure was applied evenly (100 gm.m<sup>-2</sup>), and land leveling was followed using a rotavator.

**Table (1): Soil structure, texture and some physical and chemical characteristics.**

Character	Value
<b>Soil structure g.kg<sup>-1</sup></b>	
Sand	90
Silt	569
Clay	341
Soil texture	Silty clay
Soil pH	8.46
EC (dS.m <sup>-1</sup> )	0.72
Organic matter g. kg <sup>-1</sup>	105
<b>Soil macronutrient availability mg.kg<sup>-1</sup></b>	
Nitrogen (N)	4.6
Phosphorus (P)	3.6
Potassium (K)	1.17

\*Soil analysis was conducted in a lab of Agricultural Engineering Sciences, University of Salahaddin.

A drip irrigation system was installed, the distance between the lines was 40 cm and 30 cm between the plants. On October 15<sup>th</sup> the treated seeds (haloprimed and hormoprimed) were planted by hand at a depth of 3-4 cm. Because of the huge number of treatments and limited land accessibility (Table 2), split-plot randomize complete block design with three replicates (3×3×3×3) was followed. Main-plot NaCl and sub-plot CaSO<sub>4</sub> and SA. Size of experimental unit (EU) 3.2 m<sup>2</sup> with ten plants. Fertigation was applied according to manufacture with water-soluble fertilizers, urea phosphate (17-55-0) (MENAFERT Straights, Amsterdam, Netherlands), 100 Kg.ha<sup>-1</sup> after a week of

planting seeds. And then the fertigation was continued every three weeks using NPK (20-20-20+TE) (NeuWeled® Series, Neufarm GmbH, Münster, Germany) until four weeks before harvesting. Weed and pest management was carried out as required.

**Table (2): A representative and organized factors (main plot and sub-plot) within a replicate in the experiment.**

0 mM NaCl		25 mM NaCl		50 mM NaCl	
0 mM CaSO <sub>4</sub>	0 mM SA	0 mM CaSO <sub>4</sub>	0 mM SA	0 mM CaSO <sub>4</sub>	0 mM SA
	0.5 mM SA		0.5 mM SA		0.5 mM SA
	1mM SA		1mM SA		1mM SA
2.5 mM CaSO <sub>4</sub>	0 mM SA	2.5 mM CaSO <sub>4</sub>	0 mM SA	2.5 mM CaSO <sub>4</sub>	0 mM SA
	0.5 mM SA		0.5 mM SA		0.5 mM SA
	1mM SA		1mM SA		1mM SA
5 mM CaSO <sub>4</sub>	0 mM SA	5 mM CaSO <sub>4</sub>	0 mM SA	5 mM CaSO <sub>4</sub>	0 mM SA
	0.5 mM SA		0.5 mM SA		0.5 mM SA
	1mM SA		1mM SA		1mM SA

**Growth morphology and phenotype**

Faba beans seeds were emerged 10 days after planting and the data was recorded daily until all the seeds emerged. The total number of sown and emerged seeds 810. The growth was monitored continuously almost every day. Plants were initiated flowering six weeks after the planting date (APD) and continued. For seed quality and quantity, the fruit set was then determined at 22 weeks APD, by counting the number of fruit sets per plant, which was counted for three randomly chosen plants per EU. At the same time the total number of branches was counted manually for three plants

per EU. At the harvesting stage of 26 weeks APD when 90% of the pods became brown in color, the growth and seed related parameters were recorded including; plant height, which was measured from the soil surface to the highest point for three plants per EU. The total number of pods per plant was counted manually for three randomly chosen plants per EU. Total yield per plant was measured for individual plants and also for EU, which was then converted to t. ha<sup>-1</sup>. Length of a pod (cm) and the number of seeds per pod were measured for ten randomly chosen pods per plant. The fresh and dry weight of pods and seeds was done for five randomly chosen pods per plant and three plants per EU. The total number of tested plants was 3-5 per EU, and almost 243 plants for the whole experiment.

#### Data analysis

Data from laboratory and field experiments were analyzed using SPSS version 25. The data was subjected to one-way analysis of variance (ANOVA) with three replications. Differences between means were tested using Duncan test at  $p < 0.05$ .

#### Results

Halopriming and hormoprining treatments show no effect on seed germination of faba bean variety Aquadlge. No statistical difference was found when seeds were treated with 25 and 50 mM NaCl and compared to the control treatment (Fig. 1A). CaSO<sub>4</sub> treatments improved seed germination significantly (Fig. 1B). Germination percentage was not affected by SA treatments (Fig. 1C). The interaction between halopriming and hormoprining showed that seeds treated with 25mM NaCl, 0mM CaSO<sub>4</sub> & 0mMSA and 50mM NaCl, 5mM CaSO<sub>4</sub> & 0mMSA had the highest germination percentage respectively (Fig. 1D). The germination

percentage was decreased significantly when seeds were treated with 50mM NaCl 0mM CaSO<sub>4</sub> and 0mMSA and 0 mM NaCl 0mM CaSO<sub>4</sub> and 1mMSA (Fig. 1D). No significant and statistical differences were found between the other treatments on germination percentage as shown in fig. (1D).

The germination rate of faba bean seeds was not affected by the halopriming and hormoprining treatments. The NaCl, CaSO<sub>4</sub> and SA treatments had no effect on germination rate, respectively (Figs. 2A, B and C). The fastest germination rate was recorded for seeds treated with (0 mM NaCl, 0mM CaSO<sub>4</sub> and 0 mM SA), (0 mM NaCl, 2.5 mM CaSO<sub>4</sub>, 0 and 0.5 mM SA) and 25 mM NaCl, 5mM CaSO<sub>4</sub> and 0.5 mM SA (Fig. 2D). The lowest germination rate was recorded for seeds treated with (50 mM NaCl, 0mM CaSO<sub>4</sub> and 0 mM SA), and no significant differences were found in between the other interaction treatments (Fig. 2D).

The number of fruit set was recorded for faba bean plants from randomly selected plants in EU. It was found that NaCl treatments increased the number of fruits set per plant significantly and statistically when compared to the control plants (Fig. 3A). The main effect of CaSO<sub>4</sub> and SA treatments were not affected on the number of fruits set per plant (Figs. 3B and C). The interaction effect of the halopriming and hormoprining treatments were showed the highest number of fruits set when seeds were treated with 50 mM NaCl, 2.5, 5mM CaSO<sub>4</sub>, 0.5mM and 1mM SA, respectively (Fig. 3D). The lowest value of fruit set was recorded in the interaction treatments 0 mM NaCl, 2.5, 5mM CaSO<sub>4</sub> and 0mM SA. The other interaction treatment did not show any differences (Fig. 3D).

Similarly, the average number of branches per plant was increased significantly when the seeds were treated with 25mM and 50 mM NaCl when compared to the control treatment of 0mM NaCl (Fig. 4A). CaSO<sub>4</sub> and SA seed treatments did not show any statistical effect on the number of branches per plant (Figs. 4B and C). The interaction effect of halopriming and hormoprining showed that 25mM NaCl, 0mM CaSO<sub>4</sub>, 0.5mM SA and 50 mM NaCl, 2.5mM CaSO<sub>4</sub>, 0.5mM SA seed interaction treatments recorded the highest number of branches per plant, and 0 mM NaCl, 2.5mM CaSO<sub>4</sub>, 0 mM SA recorded the lowest value of the number of branches per plant (Fig. 4D). The rest of the treatments showed no significant differences (Fig. 4D).

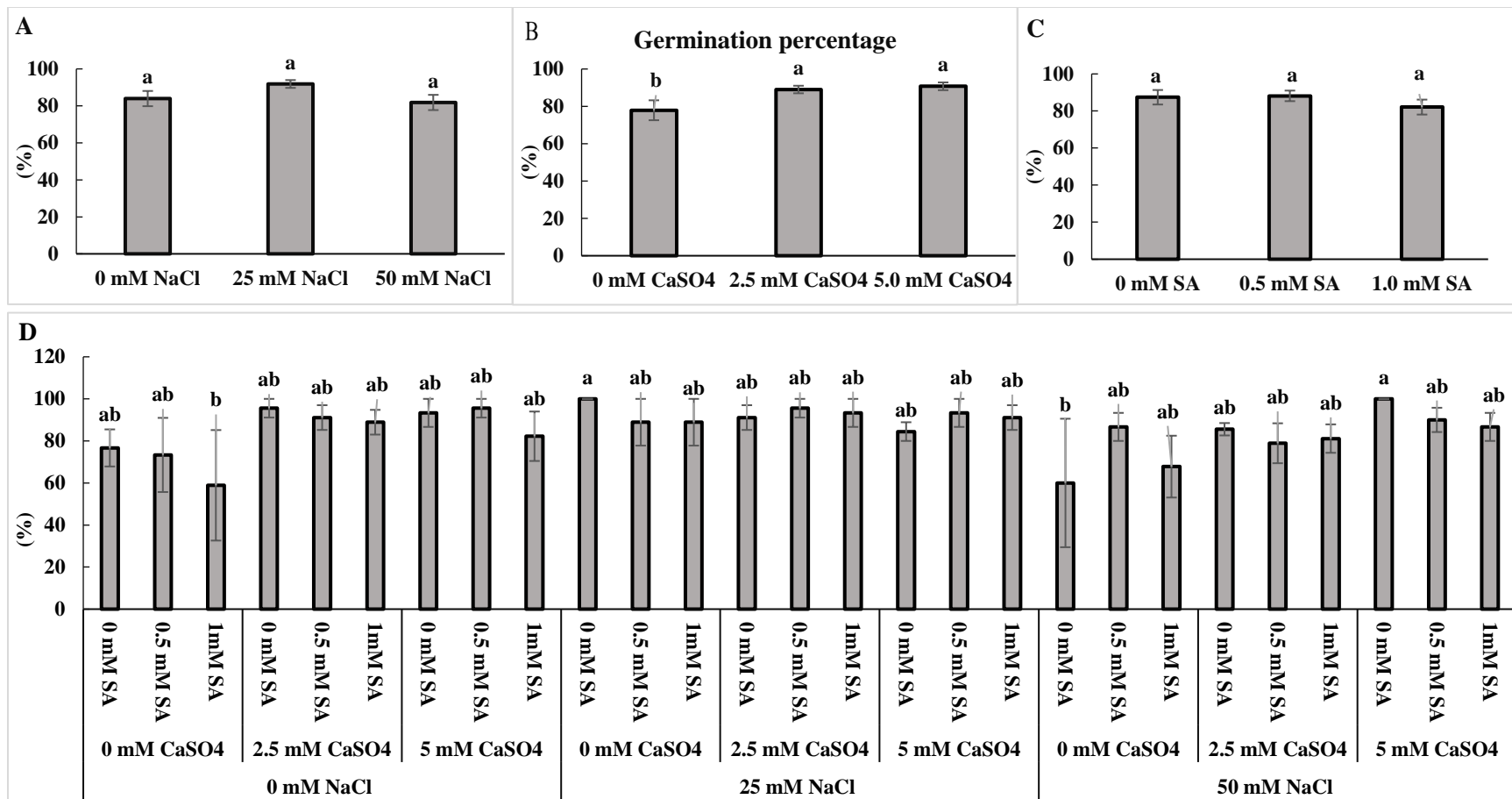
Plant height of faba bean plants was recorded 26 weeks APD. Halopriming seeds of faba bean with NaCl and CaSO<sub>4</sub> had no effect on plant height when compared to the control treatment (Figs. 5A, and B). However, hormoprining with SA 0.5 and 1mM SA were recorded as the highest plant in comparison to the control (Fig. 5C); 50 mM NaCl, 2.5 mM CaSO<sub>4</sub> and 0.5 mM SA interaction treatment was recorded as the highest significant plant height (Fig. 5D). The lowest plant height was recorded when the seed of faba bean was treated with 50 mM NaCl, 5 mM CaSO<sub>4</sub> and 0 and 1mM SA, and there were no significant differences between the last treatment and the control (0 mM NaCl, 0 mM CaSO<sub>4</sub> and 0 mM SA) and (0 mM NaCl, 2.5 mM CaSO<sub>4</sub> and 0 mM SA) (Fig. 5D).

The number of pods per plant was recorded 26 weeks APD. The number of pods per plant of faba bean was increased significantly when the seeds were soaked with 25 mM and 50 mM NaCl when compared to the control treatment, respectively (Fig. 6A). CaSO<sub>4</sub> and SA soaking

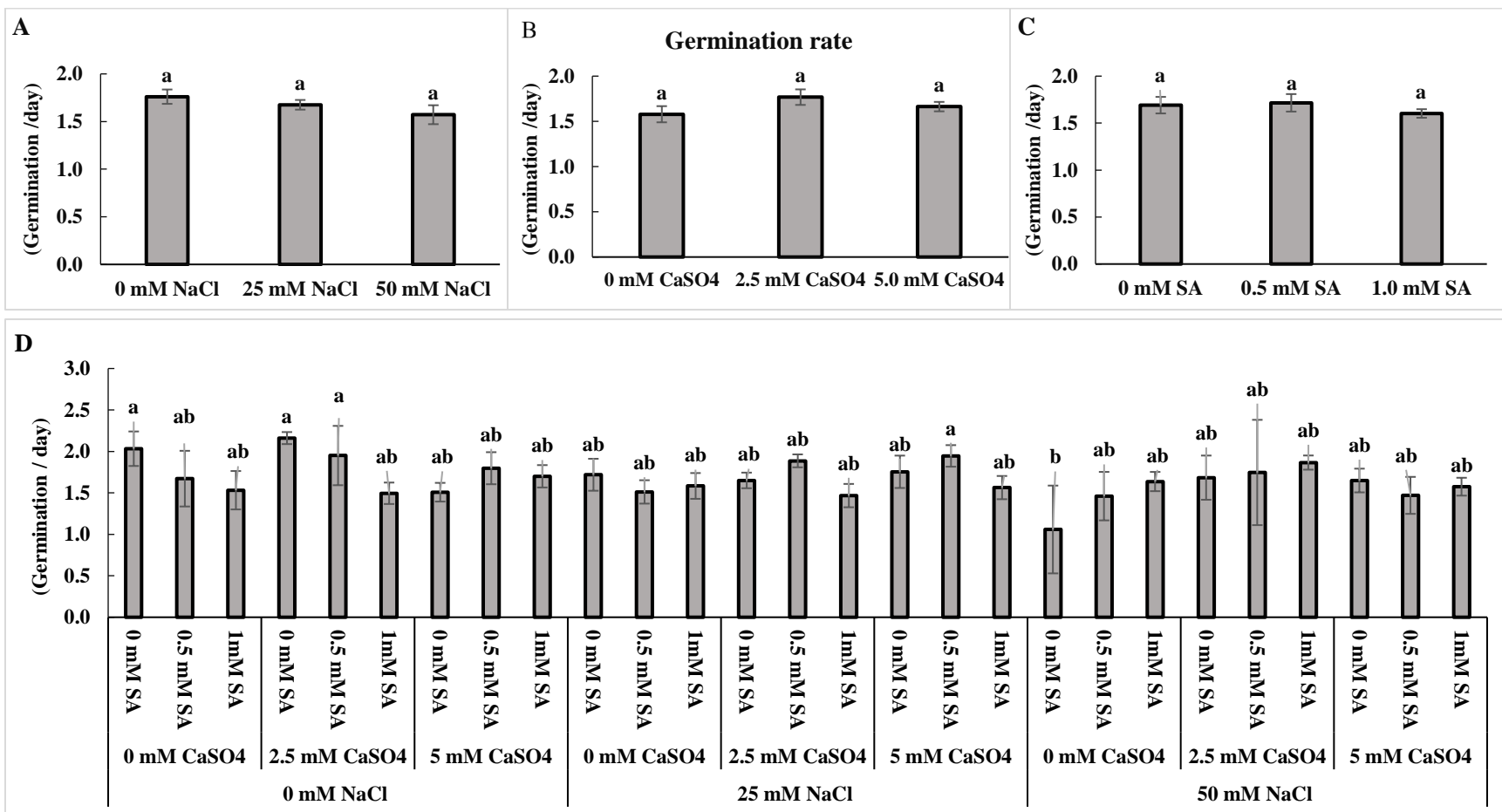
treatment did not show any effect on the number of pods per plant (Figs. 6B and C). Similarly, the interaction graph showed that 25mM NaCl, 0mM CaSO<sub>4</sub> and 0 mM SA produced the highest number of pods per plant, and the lowest was recorded in plants treated with 0 mM NaCl, 2.5mM CaSO<sub>4</sub> and 0mM SA (Fig. 6D). In general, no significant difference between the interaction effect of 0, 25, 50 mM NaCl except for some of the treatments with 0 mM NaCl (Fig. 6D).

The yield per plant was increased significantly when seeds were treated with NaCl and compared with the control treatment (Fig. 7A). On the contrary, CaSO<sub>4</sub> and SA treatment did not show any statistical effect on yield per plant (Figs. 7B, and C). A similar effect was also shown in the interaction effect of halopriming and hormoprining treatment (Fig. 7D). The result showed that (25mM NaCl, 0mM CaSO<sub>4</sub>, 0mM SA) and (50mM NaCl, 2.5mM CaSO<sub>4</sub>, 0.5 mM SA) produced the highest yield when compared to the lowest produced plant treated with 0 NaCl, 5mM CaSO<sub>4</sub> and 0.5 SA treatment (Fig. 7D).

Total pod yield and seed yield (t. ha<sup>-1</sup>) was recorded similar values when plants were treated with NaCl, CaSO<sub>4</sub> and SA, separately, (Figs. 8A, B, and C). Seed yield was decreased significantly when seeds were soaked with 5 mM CaSO<sub>4</sub> (Fig. 8B).

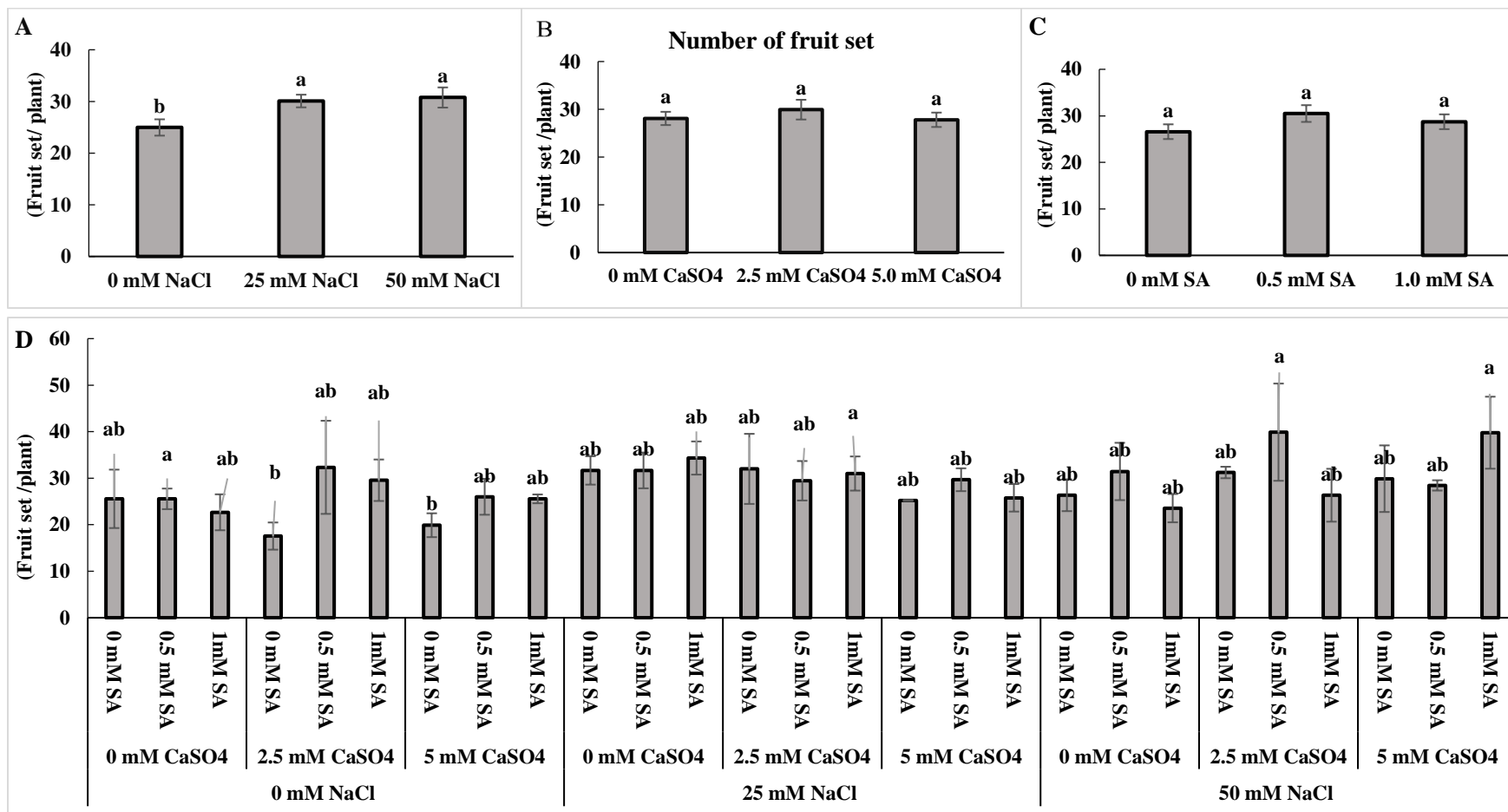


**Fig. (1):** Halopriming and hormoprping impact on seed germination percentage (%) of faba bean. (A) The main effect of NaCl on %G. (B) The main effect of CaSO<sub>4</sub> on %G. (C) The main effect of SA on %G. (D) The interaction effect of NaCl, CaSO<sub>4</sub> & SA on %G. Values with different letters indicate significant difference at P < 0.05 according to Duncan multiple range test, n = 3, error bars represent ± SE.

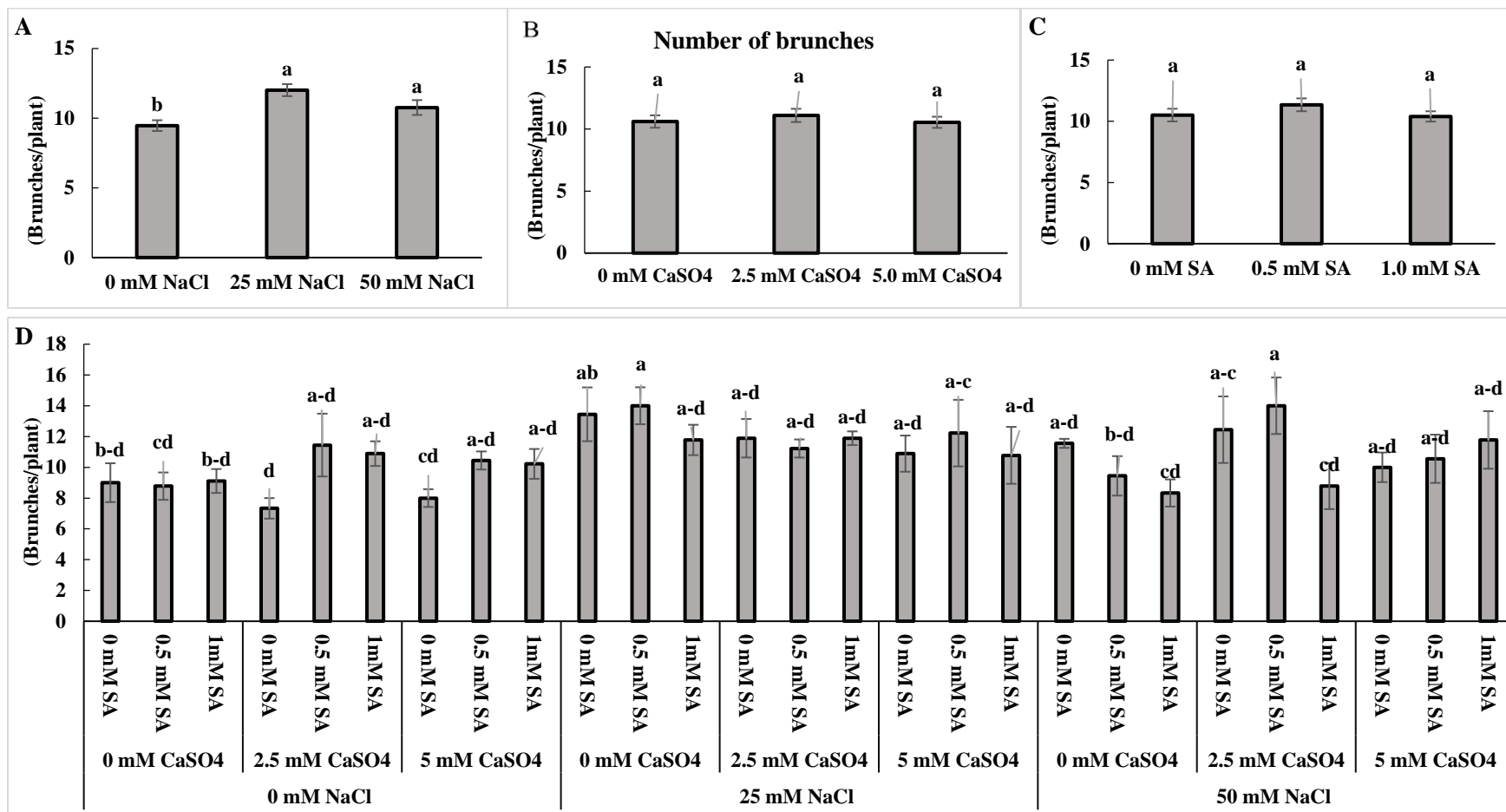


**Fig. (2):** Halopriming and hormoprming impact on seed germination rate (GR) (Germination per day) of faba bean.(A) The main effect of NaCl on GR (B) The main effect of CaSO<sub>4</sub> on GR. (C) The main effect of SA on GR (D) The interaction effect of NaCl, CaSO<sub>4</sub> &SA on GR. Values with different letters indicate significant difference at P < 0.05 according to Duncan multiple range test, n = 3, error bars represent ± SE.

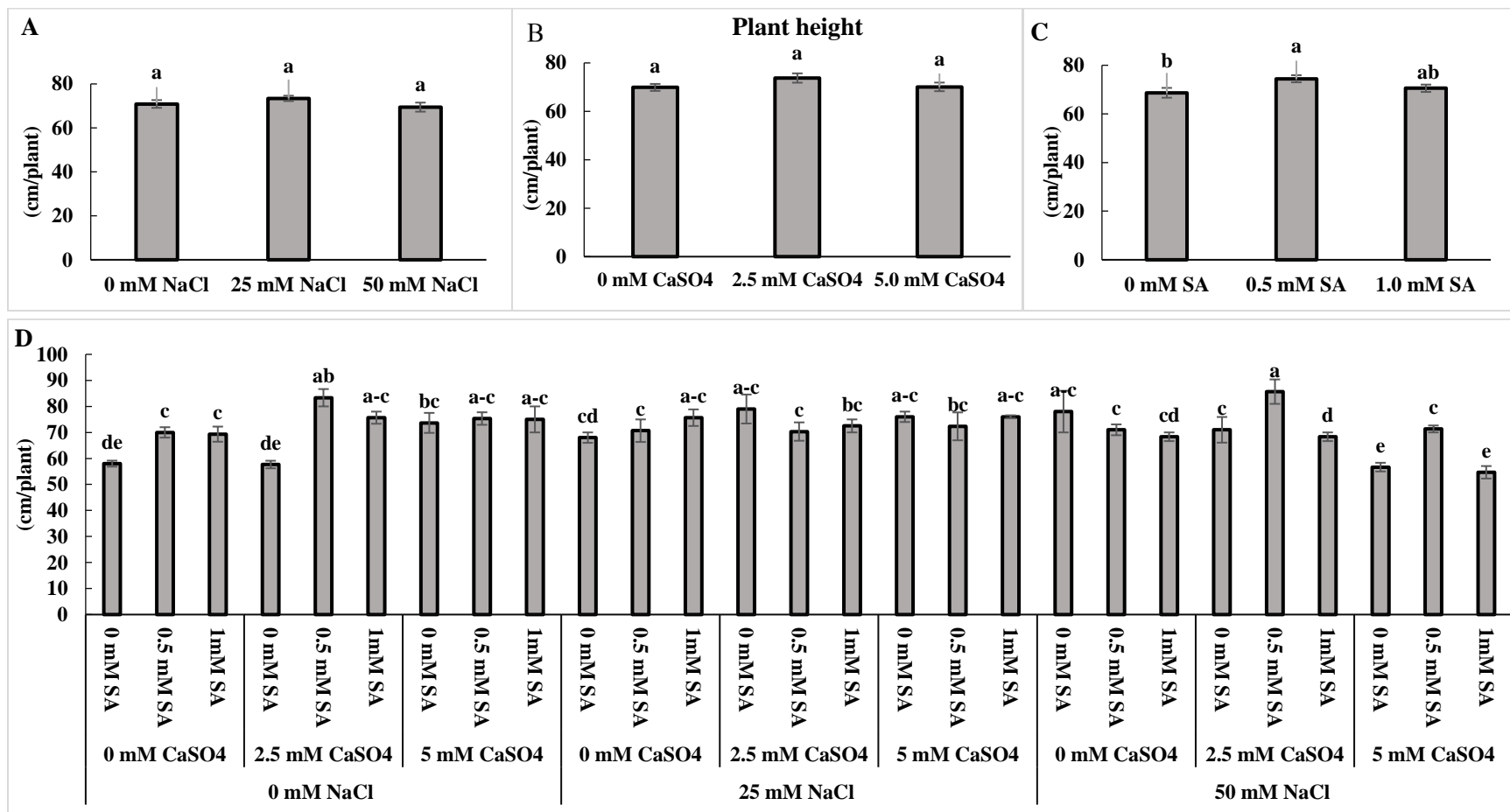




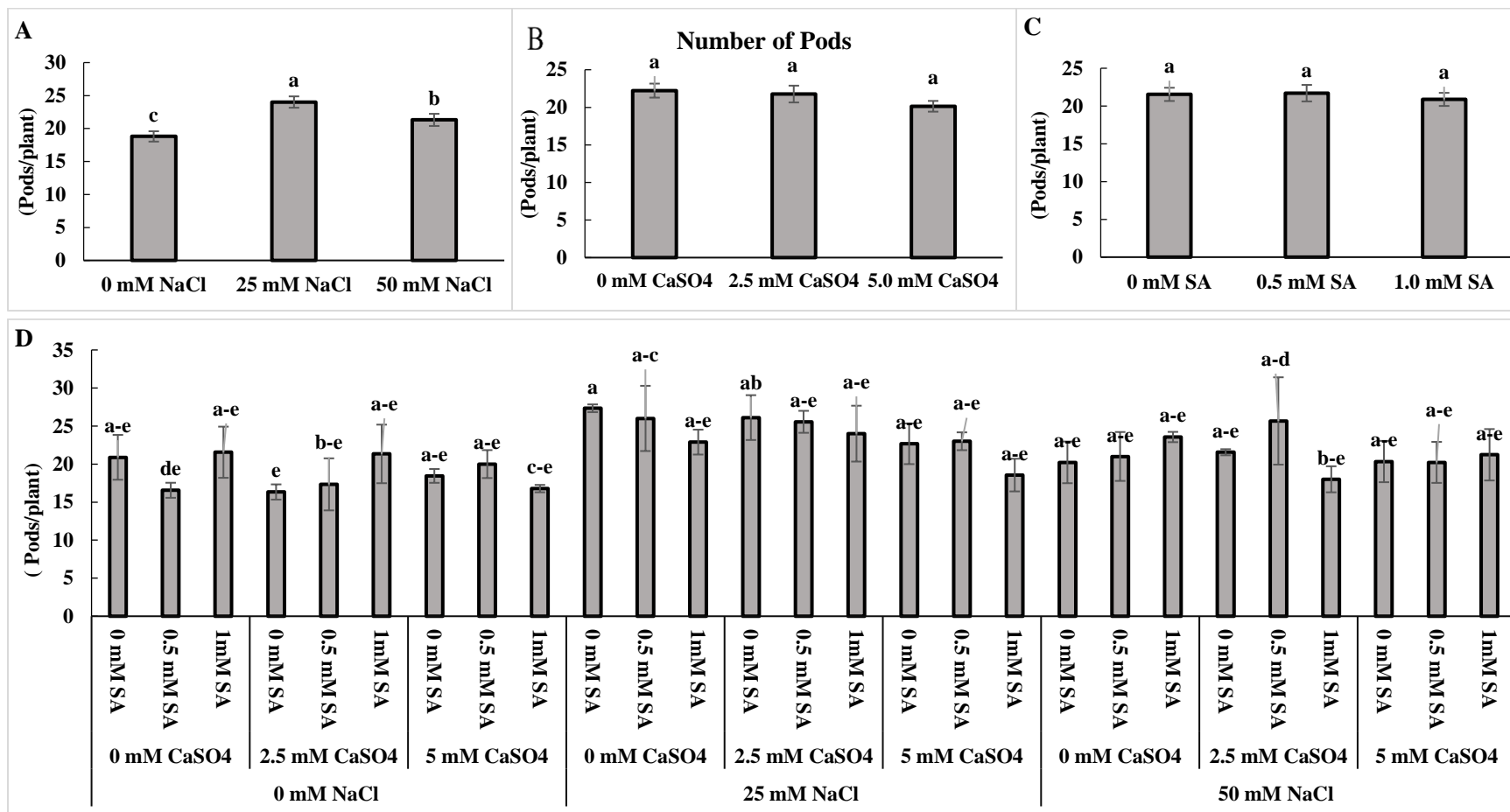
**Fig. (3):** Halopriming and hormoprming impact on the number of fruits set 22 weeks APD of faba bean. (A) The main effect of NaCl on the number of fruits set. (B) The main effect of CaSO<sub>4</sub> on the number of fruits set. (C) The main effect of SA on the number of fruits set. (D) The interaction effect of NaCl, CaSO<sub>4</sub> &SA on the number of fruits set. Values with different letters indicate significant difference at P < 0.05 according to Duncan multiple range test, n = 3, error bars represent ± SE.



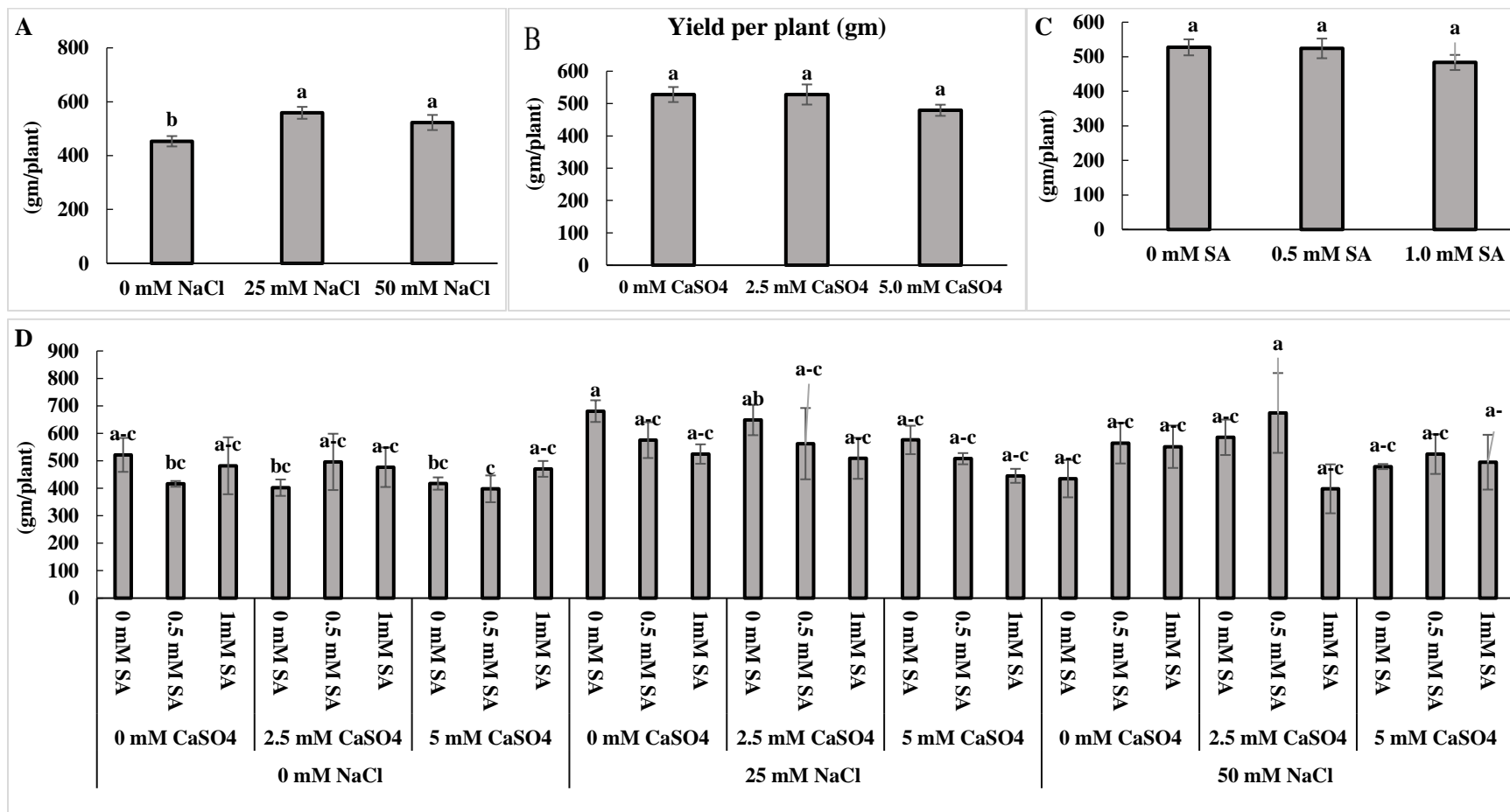
**Fig. (4):** Halopriming and hormoprming impact on the number of brunches per plant of faba bean. (A) The main effect of NaCl on the number of brunches per plant. (B) The main effect of CaSO<sub>4</sub> on the number of brunches per plant. (C) The main effect of SA on the number of brunches per plant. (D) The interaction effect of NaCl, CaSO<sub>4</sub> &SA on the number of brunches per plant. Values with different letters indicate significant difference at P < 0.05 according to Duncan multiple range test, n = 3, error bars represent ± SE.



**Fig. (5):** Halopriming and hormoprining impact on plant height (cm) of faba bean. (A) The main effect of NaCl on plant height. (B) The main effect of CaSO<sub>4</sub> on plant height. (C) The main effect of SA on plant height. (D) The interaction effect of NaCl, CaSO<sub>4</sub> &SA on plant height. Values with different letters indicate significant difference at P < 0.05 according to Duncan multiple range test, n = 3, error bars represent ± SE.



**Fig. (6):** Halopriming and hormopriming impact on the number of pods per plant of faba bean. (A) The main effect of NaCl on the number of pods per plant. (B) The main effect of CaSO<sub>4</sub> on the number of pods per plant. (C) The main effect of SA on the number of pods per plant. (D) The interaction effect of NaCl, CaSO<sub>4</sub> & SA on the number of pods per plant. Values with different letters indicate significant difference at P < 0.05 according to Duncan multiple range test, n = 3, error bars represent ± SE.



**Fig. (7):** Halopriming and hormoprimg impact on yield per plant (gm) of faba bean. (A) The main effect of NaCl on yield per plant (gm). (B) The main effect of CaSO4 on yield per plant (gm). (C) The main effect of SA on yield per plant (gm). (D) The interaction effect of NaCl, CaSO4 &SA on yield per plant (gm). Values with different letters indicate significant difference at  $P < 0.05$  according to Duncan multiple range test,  $n = 3$ , error bars represent  $\pm$  SE.

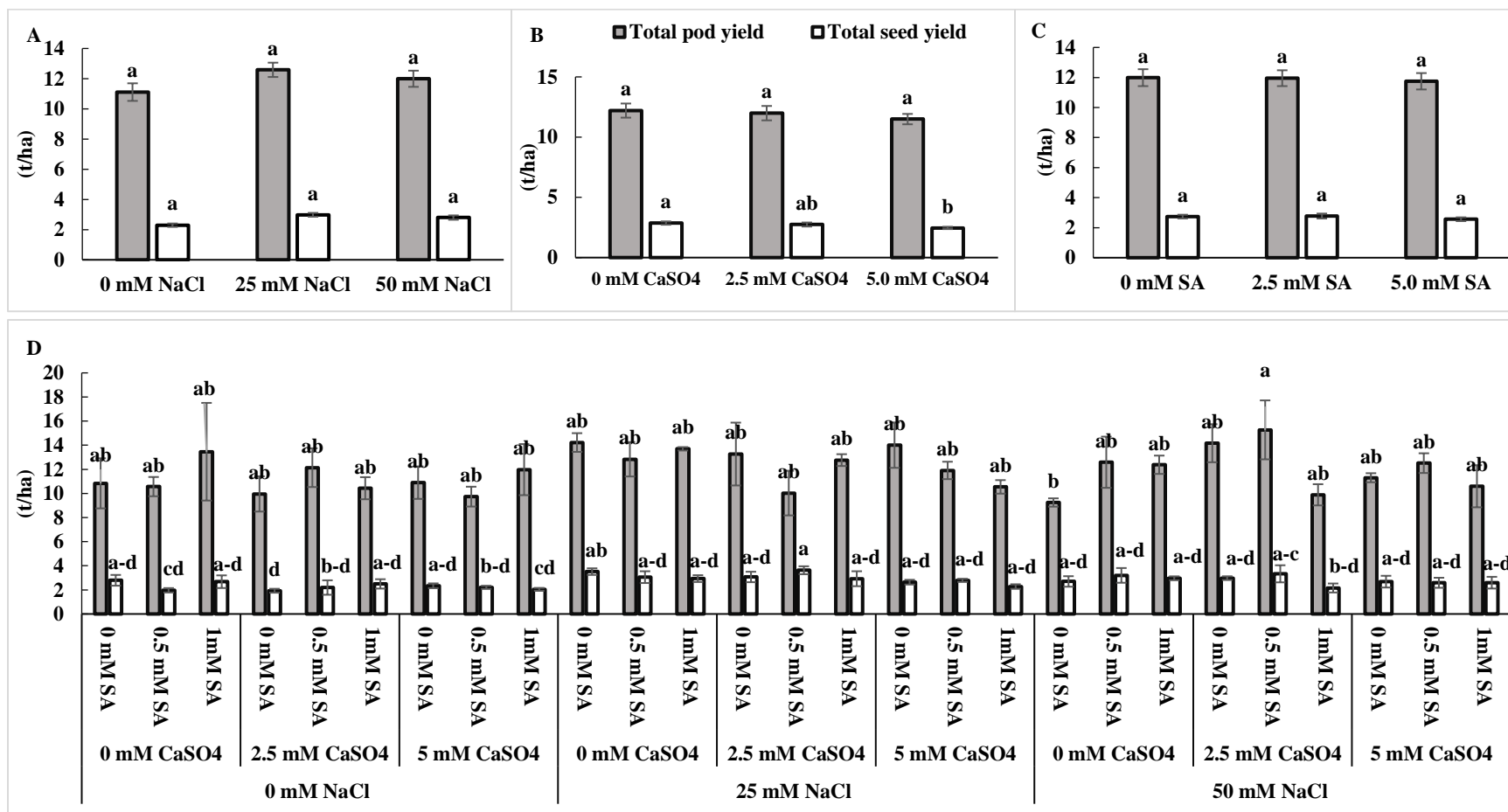


Fig. (8): Halopriming and hormopriming impact on total pod yield and seeds yield (t.h<sup>-1</sup>) of faba bean. (A) The main effect of NaCl on total pod yield and seeds yield (t.h<sup>-1</sup>). (B) The main effect of CaSO<sub>4</sub> on total pod yield and seeds yield (t.h<sup>-1</sup>). (C) The main effect of SA on total pod yield and seeds yield (t.h<sup>-1</sup>). (D) The interaction effect of NaCl, CaSO<sub>4</sub> & SA on total pod yield and seeds yield (t.h<sup>-1</sup>). Values with different letters indicate significant difference at P < 0.05 according to Duncan multiple range test, n = 3, error bars represent ± SE.

The highest value of plant pod production was recorded when seeds were treated with 50mM NaCl, 2.5mM CaSO<sub>4</sub> and 0.5 mM SA, and the lowest in 50mM NaCl, 0mM CaSO<sub>4</sub> and 0 mM SA (Fig. 8D). No significant differences were found among the rest of the other interaction treatments. Besides pod yield, seed production increased significantly with 25mM NaCl, 2.5mM CaSO<sub>4</sub>, 0.5mM SA treatment in comparison 0mM NaCl, 2.5mM CaSO<sub>4</sub>, 0mM SA (Fig. 8D).

Halopriming and hormopriming and their interaction had no effect on the length of a pod of faba bean (Figs. 9A, B, C and D). Similarly, the main effect of NaCl, CaSO<sub>4</sub> and SA showed no significant differences in the number of seeds per pod when compared to the control treatment, respectively (Figs. 10A, B and C). However, the interaction effect showed that 25mM NaCl had increased and decreased the number of seeds per pod significantly when interacted with 2.5, 5mM CaSO<sub>4</sub> and 0.5 mM SA, respectively (Fig. 10D). (25mM NaCl, 2.5mM CaSO<sub>4</sub>, 0.5 mM SA) treatment enhanced the number of seeds per pod significantly when compared with (25mM NaCl, 5mM CaSO<sub>4</sub>, 0.5 mM SA), which showed the lowest number of seeds per pod. However, there were no significant differences between the aforementioned treatments and control (Fig. 10D).

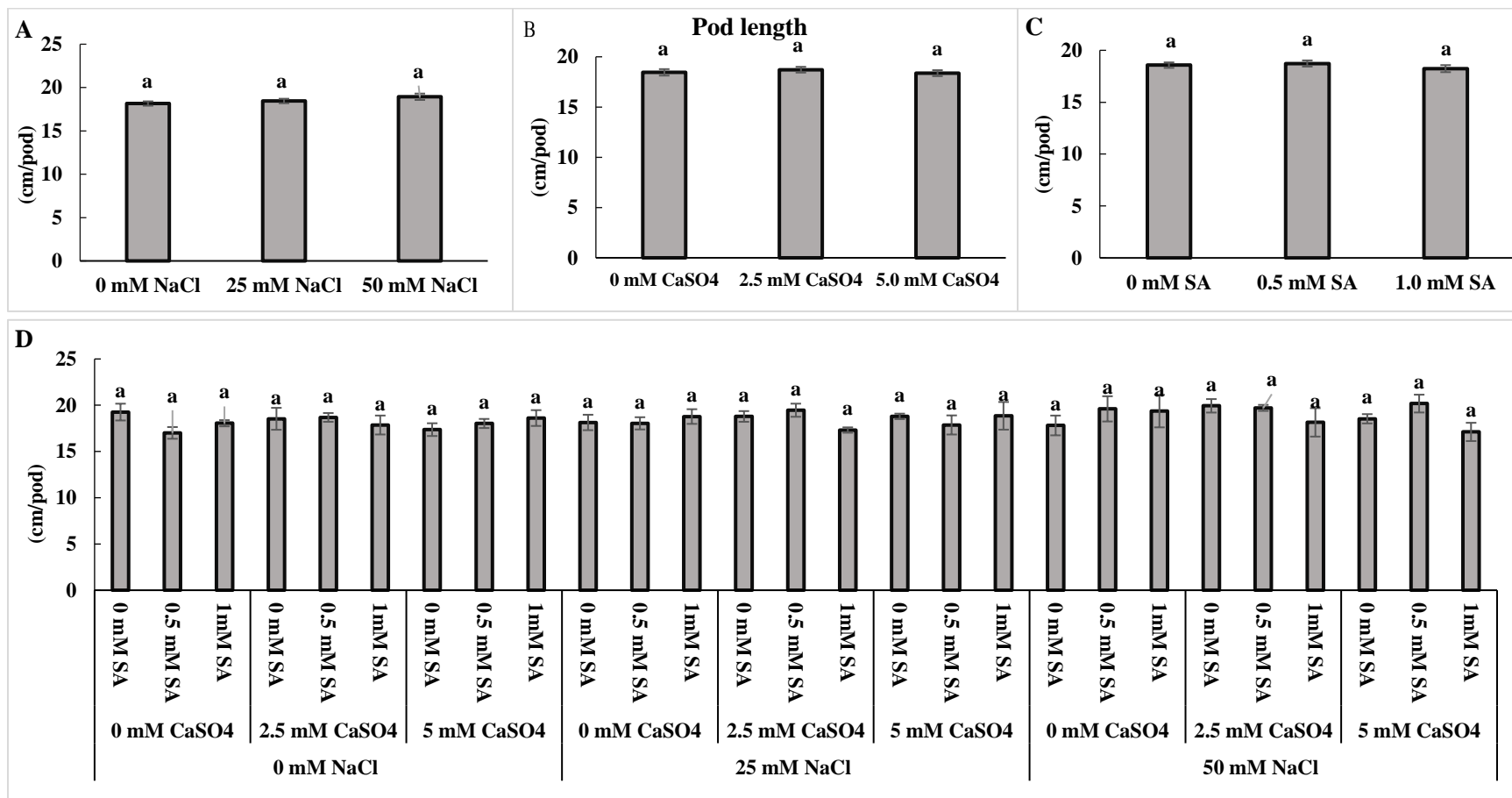
At the end of the experiment, fresh and dry weights of pods and seeds were recorded. It was found that the main effect of NaCl, CaSO<sub>4</sub> and SA did not show any changes in the fresh and dry weight of a pod (Figs. 11A, B, and C). NaCl at a concentration of 50 mM when was interacted with 2.5mM CaSO<sub>4</sub> and 0, 0.5mM SA increased the fresh and dry weight of the pod, but there were no significant differences when was compared to the control treatment (0 mM NaCl,

0mM CaSO<sub>4</sub>, 0mM SA), respectively (Fig. 11D). Almost the same results were found on the fresh and dry weight of the seed (Figs. 12A, B, and C). The fresh and dry weight of the seed was increased by using (50mM NaCl, 0mM CaSO<sub>4</sub>, 0mM SA) and (50mM NaCl, 0mM CaSO<sub>4</sub>, 0.5 mM SA), but no significant differences were found when was compared to the control, respectively (Fig. 12D).

## Discussion

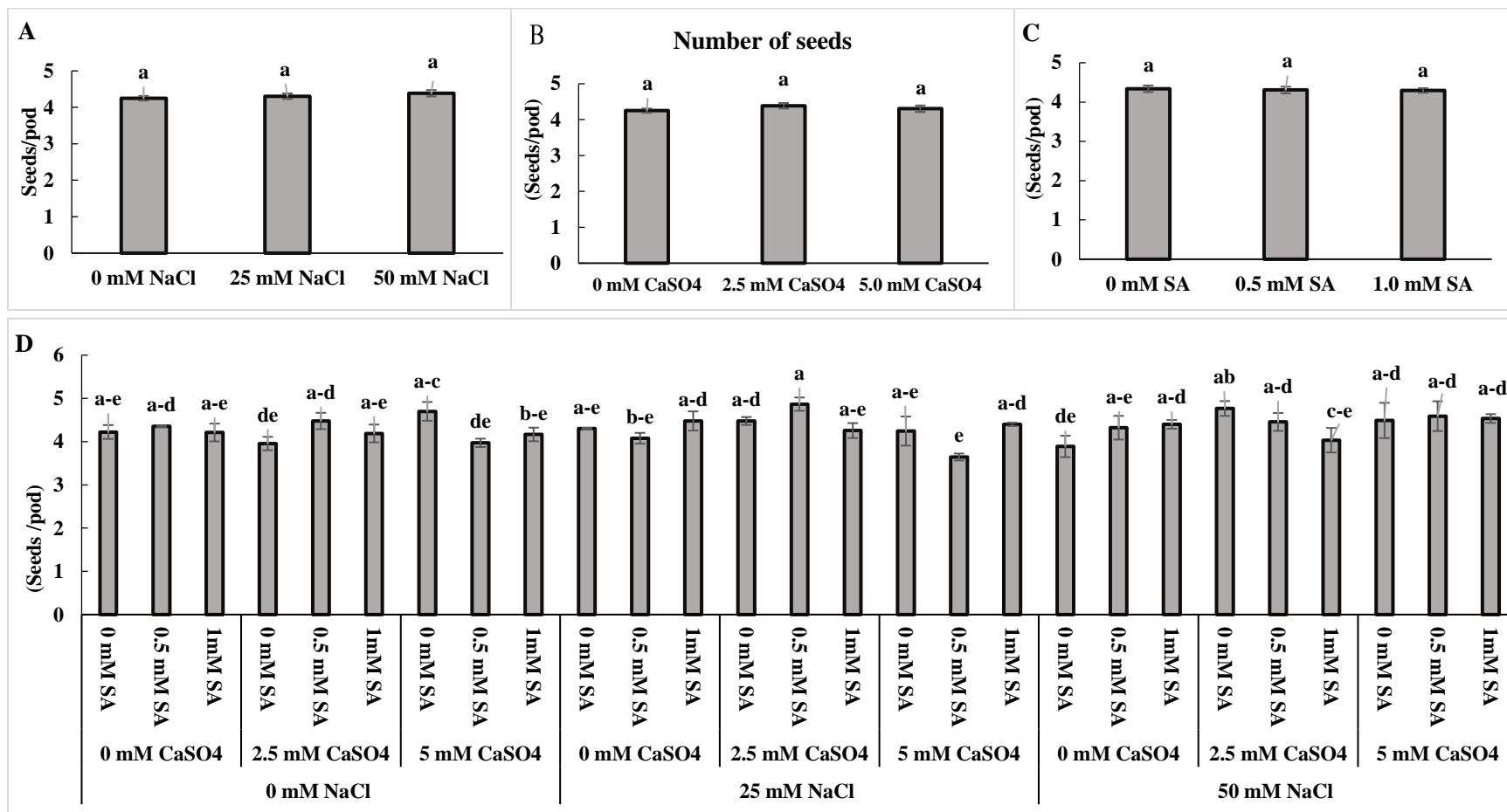
Halopriming with NaCl and CaSO<sub>4</sub> significantly improved some of the vegetative, flowering and yield and seed germination parameters significantly. NaCl and CaSO<sub>4</sub> halopriming increased statistically and significantly fruit set per plant, the number of branches, the number of bods, yield per plant and germination percentage of faba bean, respectively. In agreement with this result, previous studies demonstrated that halopriming improves germination metabolism, and productivity of many crops under optimal and suboptimal conditions (Qados, 2011; Sher *et al.*, 2019; Nabi *et al.*, 2020). However, different species and genotypes can develop different mechanisms of adaptation to seed priming, specifically halopriming. The result of this study showed that NaCl priming had no significant differences in germination percentage, germination rate, plant height, seed and pod yield (t.h<sup>-1</sup>), pod length, the number of seeds per pod, pod and seed fresh and dry weight when compared to the control treatment.

It has been demonstrated that different seed structures may vary as to water content. Seeds with prevailing endosperm reserves have a higher water content in the embryonic axis in relation to the endosperm, under a given environment (Bradford, 1990). Therefore, findings of this study may indicate that the seed



**Fig. (9): Halopriming and hormoprining impact on pod length of faba bean. (A) The main effect of NaCl pod length. (B) The main effect of CaSO<sub>4</sub> on pod length. (C) The main effect of SA on pod length. (D) The interaction effect of NaCl, CaSO<sub>4</sub> &SA on pod length. Values with different letters indicate significant difference at P < 0.05 according to Duncan multiple range test, n = 3, error bars represent ± SE.**





**Fig. (10):** Halopriming and hormoprming impact on number of seeds per pod of faba bean. (A) The main effect of NaCl on number of seeds per pod. (B) The main effect of CaSO<sub>4</sub> on number of seeds per pod. (C) The main effect of SA on number of seeds per pod. (D) The interaction effect of NaCl, CaSO<sub>4</sub> & SA on number of seeds per pod. Values with different letters indicate significant difference at P < 0.05 according to Duncan multiple range test, n = 3, error bars represent ± SE.

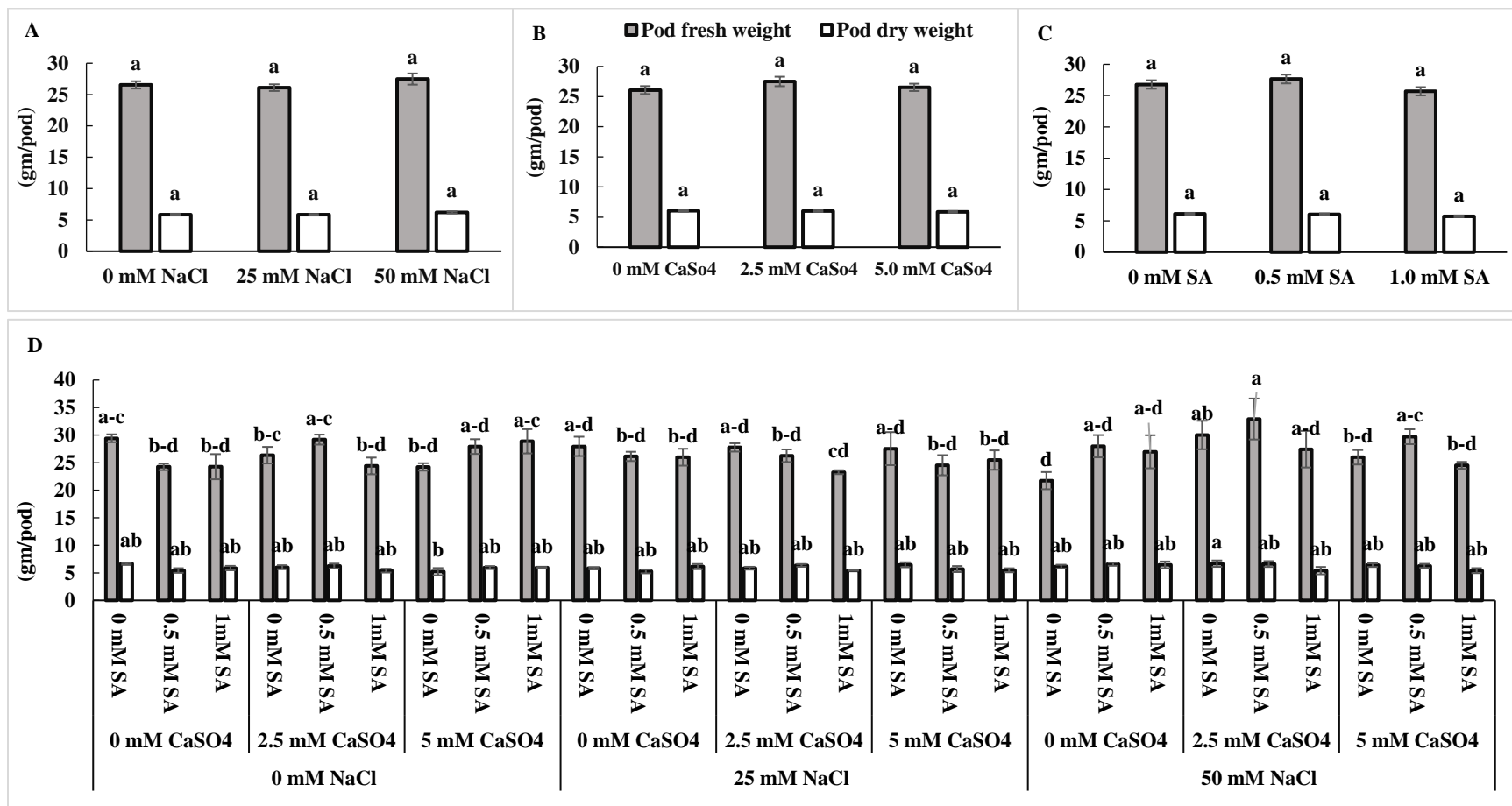
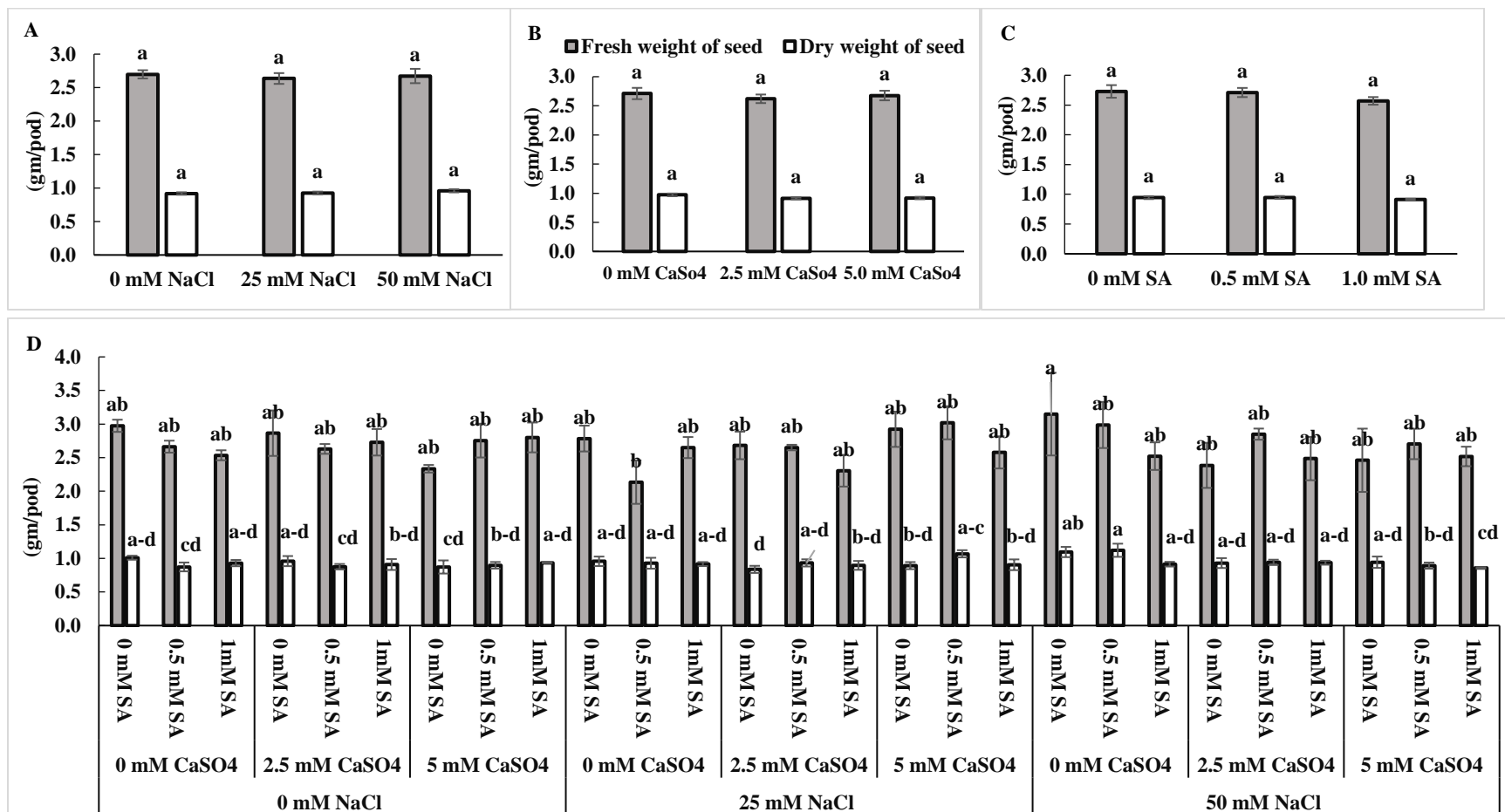


Fig. (11): Haloprimer and hormoprimer impact on fresh and dry weight of faba bean pods. (A) The main effect of NaCl on fresh and dry weight of pods. (B) The main effect of CaSO<sub>4</sub> on fresh and dry weight of pods. (C) The main effect of SA on fresh and dry weight of pods. (D) The interaction effect of NaCl, CaSO<sub>4</sub> & SA on fresh and dry weight of pods. Values with different letters indicate significant difference at P < 0.05 according to Duncan multiple range test, n = 3, error bars represent ± SE.



**Fig. (12):** Halopriming and hormoprming impact on fresh and dry weight of faba bean seeds. (A) The main effect of NaCl on fresh and dry weight of seeds. (B) The main effect of CaSO<sub>4</sub> on fresh and dry weight of seeds. (C) The main effect of SA on fresh and dry weight of seeds. (D) The interaction effect of NaCl, CaSO & SA on fresh and dry weight of seeds. Values with different letters indicate significant difference at P < 0.05 according to Duncan multiple range test, n = 3, error bars represent ± SE.

water content in the embryo and endosperm modify the NaCl concentration absorbed by the embryo axis in the process of imbibition and balanced the osmotic pressure, and the seed coat prevented salt to reach the embryo, that is why the NaCl halopriming treatment did not improve the germination in faba bean. However, in agreement with the results of Qados (2011) and Sher *et al.* (2019) who reported that NaCl halopriming improve the growth and development of various plant species, the number of fruit set, the number of branches, the number of bolls and yield per plant was increased significantly by the use of NaCl in faba bean in this study.

Halopriming with CaSO<sub>4</sub> has enhanced the germination percentage of faba bean seeds when was compared with the control treatment, and this result agrees with the previous finding by Nabi *et al.* (2020), who demonstrated that germination and seedlings components were improved in *Vigna unguiculata* (L.). However, the number of fruits set, the number of branches, plant height, yield and seed parameters did not show any improvement when treated with CaSO<sub>4</sub> and compared with the control. A study was shown that calcium salt seed coating was more effective to enhance germination and growth than seed priming, and also shown that CaSO<sub>4</sub> in the seed coat were higher than embryo axis and cotyledon in *Phaseolus vulgaris* L. (Mazibuko & Modi, 2005). Therefore, the main and interaction effects of CaSO<sub>4</sub> on the aforementioned germination and growth parameters were not recognizable when compared to the control treatment, maybe due to the restriction of the compound in the seed coat of faba bean.

Hormopriming using different phytohormones has been widely demonstrated

as an effective agent that can alleviate the negative impact of many abiotic stresses and improve the germination and overall performance of crops (Rhaman *et al.*, 2020; Garcia *et al.*, 2021). The results of this study displayed a non-significant difference between SA treatments and control in all the studied parameters of faba bean. However, previous studies showed increasing germination performance and enhanced morphological and physiological attributes in rice (Hussain *et al.*, 2016; Wang *et al.*, 2016). There is no clear evidence about faba bean seed hormopriming, and our result may indicate that due to hard seed coat of faba bean prevented the transport of the compound to the embryo during the imbibition. In addition, the seeds obtained from the Aquadlge variety of faba took a long time to cook (data not shown) which may indicate that this variety performs differently to seed priming.

## Conclusion

Halopriming and hormopriming have been used by farmers and seed companies to improve seed germination and improve the abiotic stress resistance of plants. In this study different techniques were used, halopriming and hormopriming and their interaction were examined in faba bean. Seed germination and growth and developmental were measured. In the field, NaCl halopriming increased significantly fruit set per plant, the number of branches, the number of bolls and yield per plant of faba bean. SA at 0.5mM increased plant height of faba bean in the field. The laboratory germination test demonstrated improvement in germination percentage when seeds were haloprimed with CaSO<sub>4</sub>. However, the most of the findings of this study show no clear differences between halopriming and

hormoprining treatment when compared to the control, in general. The non-significant effect of haloprining and hormoprining may be due to the hard seed coat of the examined variety, and could be improved by extending the time of seed soaking or increasing the concentration of haloperimed and hormoprined compounds and using different varieties or species. In addition, further study is required to provide evidence of the effectiveness of seed priming on the molecular level during imbibition, pre-germination and emergence.

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### Conflict of interest

As for the requirements of the publishing policy, there is no potential conflict of interest for the authors.

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### Contributions of Authors

**N. M.E.K.:** Constructed the idea and hypothesis for research; planned the methodology, Analysed the data, Wrote the manuscript.

**K.N.S.:** Collection the data.

**S. F. M.:** Provided financial support.

**K. A. A.:** Took responsibility for land preparation and provided tools and equipment that were vital for the project.

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## التداخل بين هالوبرايميك وهورموبرايميك في تنظيم النمو الخضري ونوعية البذور لنبات الباقلاء *Vicia faba* L.

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**المستخلص:** من أهم مطالب الفلاحين والمؤسسات الزراعية هي الجودة العالية للبذور واداء النمو، ولكن الزيادة السكانية تزيد الحاجة الى الغذاء وبسبب الانحباس الحراري ومحدودية المصادر الطبيعية، الاهتمام ازداد بتطبيق استراتيجيات مختلفة وفعالة. هالوبرايميك و هورموبرايميك قد تبين انها تحسن من نمو وتطور النبات في مراحل مختلفة وبعد تعرضها اي ظروف مناخية مختلفة. ولهذا هدفت هذه الدراسة الى تقييم تأثير التداخل بين هالوبرايميك و هورموبرايميك على نمو وانتاج البذور لنبات الباقلاء (*Vicia faba* L.). تم اختبار التأثير المتبادل بين هالوبرايميك وهورموبرايميك خلال انبات البذور والنمو والإداء الحقل لنبات الباقلاء. تراكيز مختلفة من ملح NaCl (0، 25 و 50) ملي مولار و CaSO<sub>4</sub> (0، 2.5 و 5) ملي مولار استخدمت ك هالوبرايميك و حامض ساليسيليك (0، 0.5 و 1) ملي مولار استخدم كهورموبرايميك. تم تنفيذ التجربة بتطبيق القطع المنشفة بقطاعات العشوائية الكاملة وبثلاث مكررات. النتائج بينت اختلافات معنوية وزيادة في عقد الثمار و عدد الافرع وعدد القرينات والحاصل للنبات الواحد عندما عاملت البذور بـ NaCl (25 و 50) ملي مولار. CaSO<sub>4</sub> (2.5 و 5) ملي مولار ادى الى زيادة في النسبة المئوية للبذور. ارتفاع النبات ازداد باستخدام حامض ساليسيليك بتركيز 0.5 ملي مولار. هذه النتائج تشير الى ان انواع واصناف مختلفة من النباتات تستجيب بشكل مختلف لمعاملة البذور قبل الانبات. بالإضافة الى هذه، التداخل بين هالوبرايميك وهورموبرايميك لربما يعمل كعامل منظم لتحويل الضغط الازموزي خلال عملية التشرب، أو لربما غلاف البذرة لنبات الباقلاء صنف Aquadlge قد منع من دخول وانتقال المركبات من والى الجنين. ولهذا تم الاقتراح والتوصية بتطبيق التداخل بين هالوبرايميك وهورموبرايميك على اصناف مختلفة من الباقلاء او انواع اخرى من النباتات ودراسة على المستوى الجزيئي خلال انبات البذور.

**الكلمات المفتاحية:** هالوبرايميك، هورموبرايميك، نوعية البذور، نبات الباقلاء *Vicia faba* L.