American-Eurasian J. Agric. & Environ. Sci., 14 (11): 1304-1311, 2014
ISSN 1818-6769

DOI: 10.5829/idosi.aejaes.2014.14.11.748

Effect of NaCl Stress on *Pisum sativum* Germination and Seedling Growth with the Influence of Seed Priming with Potassium (KCL and KOH)

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Abstract: Abiotic stress is an important environmental problem limiting crop growth and productivity. Macronutrients such as K^+ have ability to overcome stress up to some extent. The study was carried out to investigate the effects of priming with macronutrients K^+ as KCl and KOH on *Pisum sativum* grown under salinity stress condition. Seeds were primed with 250 and 500ppm KCl and KOH and then treated with different concentrations (0mM, 40mM, 60mM and 80mM) of NaCl. Effects of NaCl, KCl and KOH were observed on germination percentage, seedling shoot length, seedling root length, seedling fresh and dry biomass. It was observed that germination and seedling growth (shoot length, root length, fresh and dry biomass) showed reduction in saline conditions. Applications of 250 and 500ppm KCl and KOH showed improvement in above mentioned parameters under saline as well as non-saline conditions.

Key words: Potassium chloride · Potassium hydroxide · *Pisum sativum* · Germination · Priming

INTRODUCTION

Biotic and abiotic environmental factors affect different growth parameters[1]. The non-living variable must influence the environment beyond its normal range of variation to adversely affect the population activities or individual physiology of the organism in a significant way [2]. Abiotic stress conditions also cause extensive losses to agricultural production worldwide, as they negatively affect plant development and productivity [3]. According to an estimate ofFAOSTAT [4] in all over the world over 6% of land is salt affected. Also in addition, out of 230 Mha of irrigated land, 45 Mha are salt affected. However, the intensity of salinity stress varies from place to place. Most of the crop plants are sensitive to salinity caused by high concentrations of salts in the soil [5, 6]. Salinity at higher levels causes both hyper ionic and hyper osmotic stress and can lead to plant damage. As a result of these effects may cause membrane damage, nutrient imbalance, altered levels of growth regulators, enzymatic inhibition and metabolic dysfunction, including photosynthesis which ultimately leads to plant death [7].

Seed Priming before germination caused the hydration in seed but roots are not appear [8]. Previous studies showed that seed priming successfully affect complex interactions. Priming factors are priming period, temperature, seed vigor, dehydration and primed seed storage [9]. Moreover hydro priming increased the germination and seedling growth under salt and drought stresses [10]. Also priming improves the rate and uniformity of seedling emergence and growth particularly under stress conditions [11], the effectiveness of different priming agents varies under different stresses and different crop species [8].

Potassium is one of the major nutrients, essential for plant growth and development. Actual soil concentrations of potassium vary widely, ranging from 0.04 to 3% [12]. Mostly plants require potassium ions (K^+) for protein synthesis and for the opening and closing of stomata, which is regulated a proton pumps to make the surrounding guard cells either turgid or flaccid. Potassium also functions in other physiological processes such as photosynthesis, protein synthesis, activation of some enzymes, phloem solute transport of photo assimilates into source organs and maintenance of cation anion

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balance in the cytoplasm and vacuole. Deficiency of potassium ions can impair a plant's ability to maintain these processes [13]. The main role of potassium is to provide the appropriate ionic environment for metabolic processes in the cytoplasm and as such functions as a regulator of various processes including growth regulation [14].

Pea (*Pisum sativum*) is a member of family Fabaceae. It is a rich source of protein, amino acids, sugars, carbohydrate, vitamins A and C, calcium and phosphorus, also having a small quantity of iron. *Pisum sativum* is an annual plant, its life cycle consist of one year. It is a cool season crop grown in many parts of the world in winter to early summer depending on location. The average pea weighs in between 0.1 and 0.36 grams [15]. Peas are nutritionally important because peas are starchy and high in fibers, protein, vitamins, minerals and lutein. Its dry weight is about one-quarter protein and one-quarter sugar [16]. Peas are special benefit to managing blood sugar disorders since it stabilize the blood sugar levels. It is found that legumes were associated with an 82% reduction in risk of Heart attack. Also contain isoflavones. Isoflavones are phytonutrients that can act like weak estrogens in the body whose dietary consumption reduced the risk of certain health conditions, including breast and prostate cancer. This study was conducted to investigate the effect of NaCl, KCl and KOH on germination and seedling growth of *Pisum sativum*.

MATERIAL AND METHODS

In order to determine the effects of different salinity levels and osmo priming on germination and seedling growth, experiment was carried out in Plant Physiology Laboratory, Department of Botany, Abdul Wali Khan University, Mardan, Pakistan.*Pisum sativum* seeds were collected from Agricultural Research Institute, Tarnab Farm, Peshawar.

Pisum sativum seeds were surface sterilized with 70% ethanol for 30 seconds and then washed with D.H_2O. Seeds were primed with KCl (250 and 500ppm) and KOH (250 and 500ppm) for 1 hour and dried. 60 petri-plates were lined with filter paper, autoclaved and 5 seeds were arranged /plate. Thenthese plates were moistened with 3ml water/NaCl solutions. Germination response was studied on different levels of NaCl salinity 0, 40mM (0.61 dS/m), 60mM (0.77 dS/m) and 80mM (0.87 dS/m), replicating each concentration three times. All plates were kept in incubator at 26ºCfor germination (seeds were considered to be germinated with the emergence of the radicle). The germinated seeds were counted after every 24hrs, a continuous increase was observed in germination percentage. After 7 days of seed germination, germination percentage, shoot and root lengths, fresh and dry biomass of seedlings were recorded. Statistical analysis of the data was carried out as outlined by Little and Hills [17] and Gomez and Gomez [18]. Data was analyzed using computer program Costat 3.03. Mean separation of data was carried out using Duncan Multiple Range test [19].

RESULTS AND DISCUSSION

Germination Percentage: Results showed that increase in salinity cause non- significantly reduction in the germination percentage of *Pisum sativum* seeds (Table 1). This reduction in germination percentage is in agreement of earlier findings on cereals [20], sunflower [21], spinach [22], chickpea [23] and barley [24]. Ayers and Hayward [25] reported that higher salinity levels aggravate delay in emergence of seedlings and finally reduced germination percentage. Similar results were also studied in mustard, by Das *et al.,* [26].

In present study seeds primed with KCl and KOH showed increase in germination percentage in non saline as well as in saline medium. Liu *et al.,* [27] found similar results in maize when seeds primed with KCl showed improved germination percentage due to physicochemical activities. Seed Priming reduced the adherence of seed coat due to imbibition, which allows the emergence of radicle without resistance [28]. Farhoudi *et al*., [29] studied canola for seed priming and it showed increased seed germination under salinity stress because seed priming reduced cell damage and harmful ion absorption of canola seedlings. When papaya seeds primed with potassium salt its germination was increased because they increased water uptake which is important for germinating seed [30, 31]. Farhoudi, [32] observed that when sun flower seeds treated with KNO_3 concentrations showed the highest seed germination.

Seedling Shoot Length: Shoot and root lengths are the most important parameters for salt stress because roots absorb water due to direct contact with soil and then shoots enable its supply in whole plant. For this reason, shoot and root lengths provide important indications of a plant's response to salt stress [33]. Different

Table 1: Effect of seed priming with different concentrations of KCl and KOH on germination percentage of *Pisum sativum* germinated and grown under different NaCl concentrations.

Treatment	Germination (%) Control	Germination (%) 250 ppm KCl	Germination (%) 500 ppm KCl	Germination (%) 250 ppm KOH	Germination (%) 500 ppm KOH
Control					
Mean	80.000 a	86.666 a	100.000 a	53.333 a	60.000 a
SE	±11.547	±6.666	±0.000	±17.638	±23.094
40 mM NaCl					
Mean	73.333 a	93.333 a	93.333 a	53.333 a	53.333 a
SE	±17.638	±6.666	±6.666	±6.666	±13.333
	(-8.333)	(+7.692)	(-6.666)	(+0.000)	(-11.111)
60 mM NaCl					
Mean	66.666 a	86.666 a	93.333 a	53.333 a	60.000 a
SE	±6.666	±13.333	±6.666	±17.638	±11.547
	(-16.666)	(+0.000)	(-6.666)	(+0.000)	(+0.000)
80 mM NaCl					
Mean	66.666 a	73.333 a	86.666 a	46.666 a	73.333 a
SE	±13.333	±6.666	±6.666	±6.666	±17.638
	(-16.666)	(-15.384)	(-13.333)	(-12.500)	(+22.222)
$LSD_{0.05}$	42.102	28.761	18.828	43.482	55.429

Means followed by different letters in the same column differ significantly at 95% probability level according to New Duncan's Multiple Range Test.
Figures in parentheses indicate % promotion (+) and reduction (-) over control.

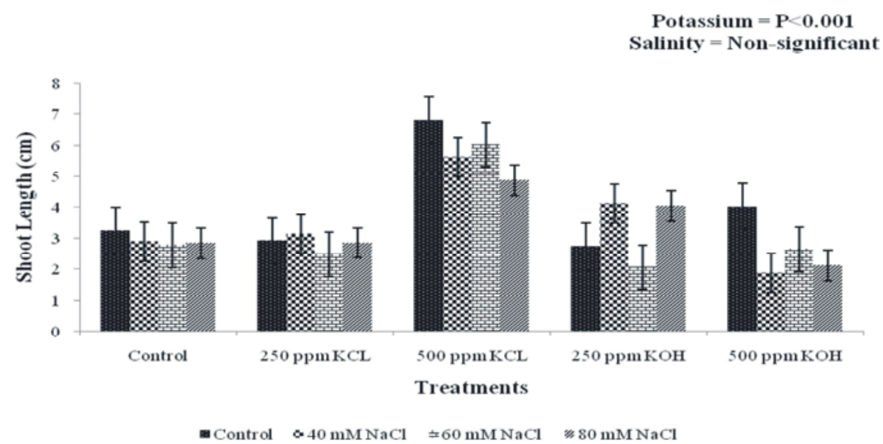

Fig 1: Effect of seed priming with different concentrations of KCl and KOH on seedling shoot length of *Pisum sativum* grown under different NaCl concentrations.

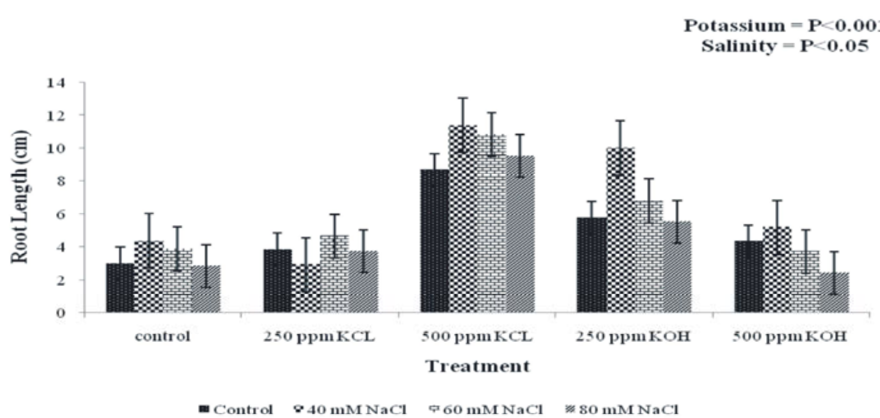

Fig 2: Effect of seed priming with different concentrations of KCl and KOH on seedling root length of *Pisum sativum* grown under different NaCl concentrations.

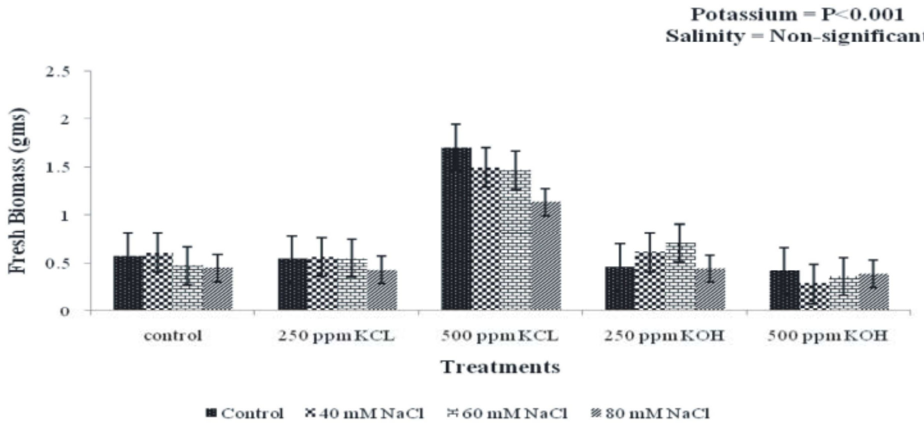

Fig 3: Effect of seed priming with different concentrations of KCl and KOH on seedling fresh biomass of *Pisum sativum* grown under different NaCl concentrations.

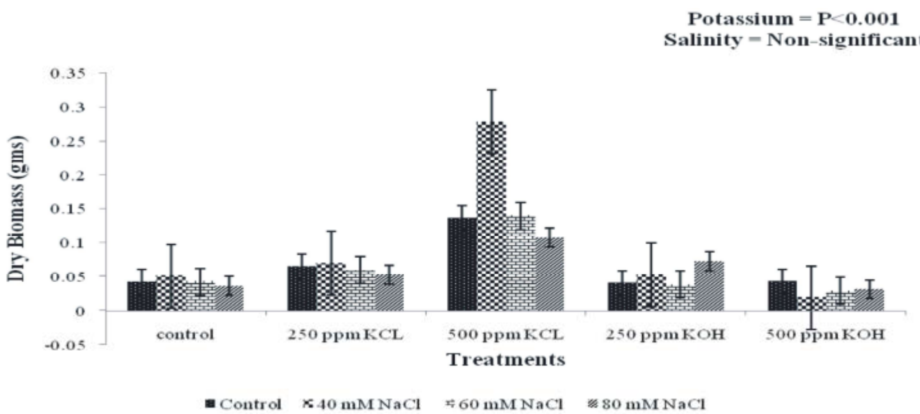

Fig 4: Effect of seed priming with different concentrations of KCl and KOH on seedling dry biomass of *Pisum sativum* grown under different NaCl concentrations.

concentrations of NaCl treatments were applied on *Pisum sativum* plants cause non-significant reduction in seedling shoot length at high salinity level. Shoot and root growth was restrained by salinity stress. In most cultivars the amount of decrease under higher salinity levels was more or less equal. Xiong and Zhu [34] reported that salt stress inhibited the translocation efficiency and assimilation of stored materials due to this it's caused a reduction in shoot growth. Naseer *et al.,* [35] reported that shoot length reduction is due to the excessive accumulation of salt in cell wall which limits the cell wall elasticity and modifies the metabolic activities. Further, cell wall becomes rigid, due to secondary cell appears sooner as a consequence the turgor pressure efficiency decrease the cell enlargement. Shoot remain small due to thess processes. Other scientists have similar results such as Rahman., *et al.,* [36] on wheat and Bybordi and Tabatabaei [37] and Jamil *et al.,* [38] on vegetables species. In present study seeds primed with K (KCl and KOH) showed decrease in seedling shoot length as compare to their respective controls in *Pisum sativum* plants. The present study also demonstrated and recorded that the plant height of primed seeds were different from plants derived from non-primed treatments when exposed to different salinity levels. Similar results in melon were reported by Sivritepe *et al.,* [39].

Seedling Root Length: Different concentrations of NaCl treatments applied on *Pisum sativum* plants showed significant reduction in seedling root length as compare to non-saline medium. In root and shoot development reduction may be due to the toxic effects of NaCl used as well as the uptake of unbalanced nutrient of seedlings. It may be due to the ability of root system to the control entry of ions to shoot in the presence of NaCl and it has the crucial importance for plant survival [40]. In earlier studies, Hussain and Rehman [41, 42] and Ghorashy *et al.,* [43] found that seedlings roots were more sensitive than shoots. Werner and Finkelstein [44] reported that elevated salinity slowed down water uptake by seeds, therefore germination and root elongation were inhibited. Werner and Finkelstein [44] also reported that salinity decreases absorption of water and growth of root and shoot. It's reported that, nutrient absorption and root growth speed is significantly decreases by salinity [45]. Munns and Termaat [46] suggested that salinity decrease radicle and plumule growth and if we increase salinity level, the amount of reduction will increase.

In this study seeds primed with K (KCl and KOH) showed increase in seedling root length in non saline as well as in saline medium in *Pisum sativum* plants. Farhoudi, [32] reported that seed priming with potassium (KNO_3) increase radicle length at highest salinity level as compared to control but did not show any significant difference between priming level. Nascimento [47] reported that muskmelon seed priming with KNO_3 increase root and shoot length as compared to other priming treatments.

Seedling Fresh and Dry Biomass: Different concentrations of NaCl treatments were applied on *Pisum sativum* showed non-significant reduction in seedling fresh and dry biomass in both unprimed and primed sets. For primed seeds, fresh and dry biomass was higher than for unprimed ones at different levels of NaCl. Fresh and dry biomass showed decline with increasing salt stress, which was observed by Badr-uz-Zaman *et al.,* [48, 49] and it is due to salt effects on metabolic processes, by reducing water potential. Okçu *et al*., [50] reported that under saline conditions reduction in seedling growth is due to increase in sodium chloride toxicity and the accumulation of sodium ions in the photosynthetic tissues [51]. Kaya and Day [52] reported that salt stress decrease the weight of safflower seedling. They suggested that decrease in growth of safflower seedling is due to ionic stress and osmotic stress of salts. By increasing salinity, fresh and dry biomass of plumule decreased, which might be attributed to decrease in remobilization of the seed reserves from cotyledons to the embryonic axis. The factors that affected the growth rate of embryonic axis also had affect on transfer from cotyledons to the embryonic axis and reserve remobilization [53]. Shoot fresh and dry biomass was extensively affected by seed priming and salinity levels in coriander plant [54]. Shoot weight progressively decreased with the rise of stress level as compared with control. Fortmeier and Schubert [55] found same results in barley.

In present study seeds primed with K (KCl and KOH) showed significant improvement in seedling fresh and dry biomass at high salinity level as compare to their respective control. Seed priming by KNO_3 solution showed beneficial effects on seed germination under salt stress condition have been yet observed in other crop research such as muskmelon Demir [56] and Pea Okçu *et al.,* [50]. Singh and Rao [57] stress that KNO_3 effectively improved germination, seedling growth and seedling vigour index of the seeds of sunflower varieties. Sunflower seed priming by KNO_3 under salinity condition increase seedling fresh weight and seedling growth as compared non priming seeds [32].

CONCLUSION

In the present study it is concluded that salinity showed adverse effect on seeds germination and seedling growth of *Pisum sativum* plants. These adverse effects of salinity were overcome by the application of potassium nutrient through seed priming with KCl and KOH. K^+ application through seed priming lead to a substantial improvement in germination percentage, seedling shoot length, seedling root length, seedling fresh and dry biomass.

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