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Sunflower Seed-priming with Phosphate Salts and Seedling Growth under Salt Stress

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Authors' contributions

This work was carried out in collaboration between all authors. Author BUZ designed the study, wrote the protocol and conducted study with author MA. Author AA managed the literature searches. Authors IAM and MAU performed the statistical analysis. Author TT provided chemical and germplasm for the experiment. Author BUZ wrote the final draft of the manuscript that was approved by all the authors.

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ABSTRACT

Aims: It was assumed that halo-priming process under salinity stress may rely on phosphorus nutrient source. Nutrient priming of seeds of sunflower seeds may be beneficial for growth of seedlings under salt stress.

Study Design: Laid out the experiment in Complete Randomized Design, using NaCl @ 0, 15, 30, 45 and 60 mM on prime seeds from two sources of phosphorus salts in quadruplication.

Place and Duration of Study: The study was conducted in laboratory of Soil Salinity Research Programme of Land Resources Research Institute at National Agricultural research Centre, Islamabad, Pakistan during the period from July to September, 2016.

Methodology: Passed sunflower seeds through the priming process using two sources of phosphorus i.e. potassium dihydrogen phosphate (KDP) and ammonium dihydrogen phosphate (ADP). The seeds were then germinated and raised for seedlings establishment in hydroponics standard solution under salt stress (0, 15, 30, 45 and 60 mM sodium chloride).

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Results: The germination parameters, i.e. germination percentage, mean germination time, rate of germination index and coefficient of germination improved significantly (P< 0.01) with KDP priming than that of ADP under salinity stress. Seedlings vigor index enhanced with KDP than ADP. Fresh mass (18 to 36 percent) and dry mass (14 to 24 percent) of the seedlings were higher with KDP. With increasing Na^+, Ca^{2+} decreased. It was inferred that role of KDP source was prominent.

Conclusion: Consideration of KDP could be a valuable seed-priming approach for sunflower early germination and accelerative growth under salt stress.

Keywords: Germination; ionic relations; KH_2PO_4; $NH_4H_2PO_4$; sunflower.

1. INTRODUCTION

During germination process, seed imbibes a minute quantity of available water from soil. Salts in soil restrict the availability of water to a germinating seed. This process depends upon the nature and amount of salts besides soil properties and response of a plant species. The qualification of an ion of a salt to be nutrient is relative; as it depends on the nature of plant species. When the concentration of water-soluble ions exceeds a threshold value, the osmotic pressure of the solution increases resulting in building up of salinity. The responses of plants to high salinity may vary with different growth stages [1]. Crop species show a range of responses in the form of growth and yield reduction of salt stress [2] besides a change in ion ratio. Halo-priming of seed in an osmotic solution allows seed imbibe water to proceed to the first stage of germination, but prevents radicle protrusion through the seed coat. Seed priming has gained importance at the experimental and production level. Various seed priming treatments [3] can lessen the adverse and depressive effects of salinity on germination. During the osmotic effect of salinity, sodium ion causes shortage of water, may have indistinct links with other ions and this may restrict the entry of nutrients through the root system. Primed seeds, usually exhibit enhanced germination rate in many plant species [4]. Nutrient priming has been proposed as a novel technique that combines the positive effects of seed priming with an improved nutrient supply [5]. Among the macro-nutrients, phosphorus is imperative for meiosis and mitotic process and for tissue building up [6]. The current study was conducted as literature on nutrient priming using phosphorus salts is scarce. During halo-priming process, phosphorus salts may have variable effects on seed priming under salt stress and a positive effect on seedlings growth as well. With the objectives to differentiate, phosphorus-salt priming on sunflower seeds and then to record their post priming germination and seedling growth under salt stress, conducted a study.

2. MATERIALS AND METHODS

Treated seeds of *Helianthus annus* (cv. AAS-501) with 1 % sodium hypochlorite for 15 minutes [7] and washed three times with distilled. Placed seeds separately in 5 mM of potassium dihydrogen phosphate (KDP) and ammonium dihydrogen phosphate (ADP) solutions for five hours. For surface dehydration air-dried the seeds. In petridishes placed ten seeds on filter paper in quadruplicates. Germinated seeds in dark germination at 25℃ under salinity stress containing 15, 30, 45 and 60 mM sodium chloride with nutrient solutions [8]. Recorded data from 3^{rd} to 8^{th} day of germination. Calculated mean germination time (MGT) according to the equation of [9]. Converted observed germination percentage into ASIN form for statistical analysis purpose as worked by [10]. According to [11] calculated rate of germination index (RGI). Calculated sseedling vigor index (VIG) as given by [12]. Coefficient of germination (CG) estimated as given by [13]. After recording fresh mass (FM), the seedlings were rinsed with deionised water and then dried at 65℃. After recording dry mass (DM), each sample was cut into small pieces, mixed and separately digested in a perchloric-nitric (1:2) di-acid mixture [14]. In the digested material recorded concentrations of sodium, potassium and calcium ions using atomic absorption spectroscopy. Analyzed statistically the data according to two factors CRD and compared treatment means using LSD test [15].

3. RESULTS AND DISCUSSION

Pre-germination treatment of sunflower seeds with osmo-primers, i.e. KH_2PO_4 (KDP) and $NH_4H_2PO_4$ (ADP) and then imposing salt stress with NaCl up to 60 mM affected significantly (*P* = 0.01) seed germination, seedling growth and ionic concentrations.

3.1 Mean Germination Time and Germination Percentage

Mean germination time (MGT) was the same at control and 15 mM NaCl stress. At 30, 45 and 60 mM NaCl stress, it was lower with KDP than ADP primer in concordance with the stress level (Table 1). With KDP priming at control and up to 45 mM NaCl, germination percentage (GP) was 90 percent and was 80 percent with 60 mM NaCl stress. With ADP primers at control and 15 mM NaCl, GP was 90 percent and was 80 percent with 30 to 60 mM NaCl stress. Germination percentage under salt stress improved when the seeds were primed with KDP than ADP. The lower the MGT, the rapid the germination [16] during which radicle appeared. Priming causes reduction in seed coat adherence due to imbibitions, which may permit to emerge out radicle without resistance as reported by [17]. In this study, MGT was lower with KDP than ADP. In KDP, there might be positive role of K^+ as a conjugate of phosphate ion to enhance GP and to decrease MGT. Generally, increasing salinity level decreased germination percentage [18]. Previous work on different crops has indicated that measurements of MGT are a possible alternative assessment of seed vigor [19]. It can be inferred that using KDP, seed vigor was improved leading to early germination.

3.2 Rate of Germination Index and Coefficient of Germination

Increasing salt stress affected rate of germination index (RGI) and coefficient of germination (CG) using both the osmo- primers (Table 2). In seeds priming with KDP, RGI decreased 2, 12, 16 and 25 percent at 15, 30, 45 and 60 mM NaCl respectively than the control, whereas with ADP it decreased 12, 24, 36 and 62 percent respectively. In seeds primed with KDP, RGI was 12 to 50 percent higher than that of ADP under salt stress. Coefficient of germination (CG) also decreased with the increasing level of salinity. In seeds primed with KDP, it decreased 2, 4, 5 and 7 percent at 15, 30, 45 and 60 mM NaCl respectively than the control, whereas in seeds primed with ADP it decreased 2, 4, 8, and 12 percent respectively. In seeds primed with KDP, the increase in CG was 11, 24, 36 and 62 percent at 15, 30, 45 and 60 mM NaCl stress respectively as compared to priming with ADP. The germination index expresses the speed of the germination and allows a higher differentiation among treatments. With increasing level of salinity, RGI was decreased [20]. Higher GRI values indicated higher and faster germination. Under salt stress, RGI improved with KDP than with ADP. Here also, the composition of the salt was functioning. Co-efficient of germination is the sum of ratios of the number of seeds germinated to the product of time specific number of seeds germinated. [21] reported that coefficient of germination is an indicator of the level of the germination variability. It measures germinated variability of seed according to salinity stress. In this study, salinity impeded germination, but CG indicated variability in germination with increasing salinity stress after treatment of different osmo-primers and then applying salt stress conditions. Seeds primed with KDP improved CG than with ADP at increment level of salt stress.

3.3 Seedling Vigor Index

Seedling Vigor Index (SVI) decreased 9, 10, 13 and 19 percent at 15, 30, 45 and 60 mM NaCl, respectively than the control when the seeds were primed with KDP (Fig. 1). With the same increasing level of salt stress SVI decreased 5, 8, 19 and 40 percent respectively than the control where the seeds were primed with ADP. With KDP, SVI was higher than ADP up to 30 percent with NaCl stress. Seedling vigor measures emerged shoot through the stressed growth medium. It is a function of seed quality and genetics. This function is lost when a seed is exposed to intolerant level of salt stress, may be due to harming the seed coat or anxious metabolism. In this study, SVI improved when the seeds were primed with KDP as compared to ADP. The effect of KDP on SVI was prominent up to 30 mM NaCl application. It could be due to threshold magnitude of available K^+ ion.

3.4 Fresh and Dry Mass of Seedlings

Fresh mass (FM) of the seedlings decreased 10, 15, 20 and 25 percent than the control using KDP primed seeds at 15, 30, 45 and 60 mM NaCl (Fig. 2). Also, using ADP primed seeds at 15, 30, 45 and 60 mM NaCl, FM decreased 14, 21, 28 and 35 percent respectively than the control. Conversely, in seeds primed with KDP, FM was higher (18 to 36 percent) than the seeds primed with ADP under NaCl application up to 60 mM. Dry mass (DM) of the seedlings decreased 9, 12, 17 and 20 percent than the control using KDP primed seeds at 15, 30, 45 and 60 mM NaCl (Fig. 2). Using ADP primed seeds at 15, 30, 45 and 60 mM NaCl, DM decreased 10, 14, 21 and 26 percent respectively than the control.

Conversely, with KDP primer, DM was higher (14 to 24 percent) than using ADP primer at 15 to 60 mM NaCl stress. Fresh mass of plant material consisted of tissue based on water and other chemicals. Included salts in a plant affect water potential. Water retention is an important property of a plant tissue that indicates its health and turgidity [22]. Antagonistic relations between fresh mass of plant to increasing salinity stress has already been reported [23]. Adverse effect of salt stress on dry mass has also been observed by increasing 'salinity crossing tolerance level' decreased dry mass of plants. Dry mass of plant material is the net out come of the resultant metabolic activities [22]. Here sodium ion interferes with beneficial biochemical activities, and valuable pathways are broken down resulting in dry mass. This indicates senescence leading to reduced biomass and yield. Reduction in seedling growth under saline conditions is due to increase in sodium chloride toxicity [24]. In many species, salt sensitivity is associated with the accumulation of sodium ion in photosynthetic tissues [25]. External osmotic potential created by NaCl might have affected fresh mass by preventing water uptake.

Table 1. Impact of NaCl stress on mean germination time and germination percentage (ASIN) of sunflower after seed priming with potassium dihydrogen phosphate (KDP) and ammonium dihydrogen phosphate (ADP)

NaCl applied (mM)	Mean germination time (MGT) (hours)		ASIN	
	KDP	ADP	KDP	ADP
0	38.4 a	38.4 a	71.6 a	71.6 a
15	36.5 b	36.5 b	71.6 a	71.6 a
30	36.5 b	35.5 c	63.4 b	71.6 a
45	35.5 c	33.6 d	63.4 b	71.6 a
60	33.6 d	27.8 e	63.4 b	63.4 b

Means sharing similar letter(s) for a parameter do not differ significantly at p < 0.01

Table 2. Impact of NaCl stress on rate of germination index and coefficient of germination of sunflower after seed priming with potassium dihydrogen phosphate (KDP) and ammonium dihydrogen phosphate (ADP)

NaCl applied (mM)	Rate of germination index (RGI)		Coefficient of germination (CG)	
	KDP	ADP	KDP	ADP
0	88.9 a	87.5 a	33.3 a	32.5 b
15	87.5 a	77.8 ab	32.5 b	31.7 d
30	77.8 ab	66.7 bc	31.9 c	31.1 f
45	75.0 ab	55.6 c	31.5 e	29.9 g
60	66.7 bc	33.3 d	31.1 f	27.9 h

Means sharing similar letter(s) for a parameter do not differ significantly at p < 0.01

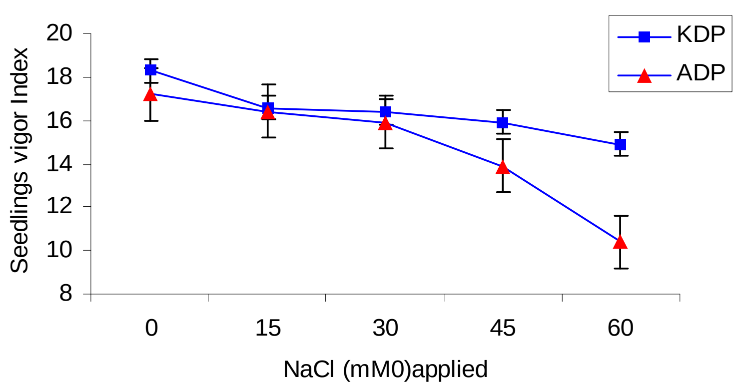

Fig. 1. Impact of NaCl stress on SVI of sunflower seedling after seed priming with potassium dihydrogen phosphate (KDP) and ammonium dihydrogen phosphate (ADP)

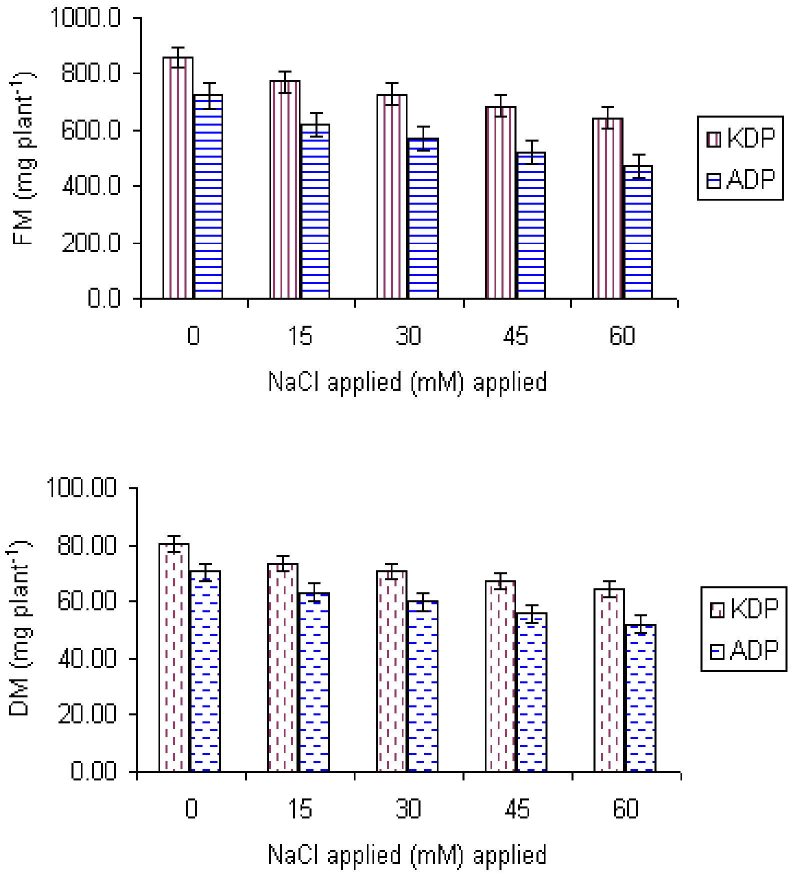

Fig. 2. Impact of NaCl stress on fresh mass (FM) and dry mass (DM) of sunflower seedling after seed priming with potassium dihydrogen phosphate (KDP) and ammonium dihydrogen phosphate (ADP)

3.5 Relation of Potassium and Calcium with Sodium

The relation of sodium ion with calcium and potassium ions in the seedlings is shown in the Fig. 3. With KDP, the level of Na^+ in the whole plant was lower than that of ADP priming. Relative mean ratio of Ca^{2+} to Na^+, and K^+ to Na^+ (0.72 and 0.16 respectively) was higher with KDP than with ADP. There was 0.1 to 5 % difference in concentration of Na^+ in seedlings from seeds primed with KDP and ADP. Sodium ion concentration was at low profile in seedlings grown from KDP-seed priming. Using KDP, germination of sunflower seed was improved by the presence of potassium ion in the priming medium. It was observed by [22] Badr et al. 2010 that intake of K^+ in rice was highest with application of KDP than the other potassium sources. Calcium concentration in sunflower seedlings increased when its seeds were primed with potassium sulphate [26]. In this study, the presence of phosphorus also helped improved the cell propagation process for possible germination, since for root and shoot development phosphorus is essential [27]. Priming is responsible to repair the age related cellular and subcellular damage of low vigor seeds that may accumulate during seed development [28]. Priming of seed promotes germination by repair of the damaged proteins, RNA and DNA [29].

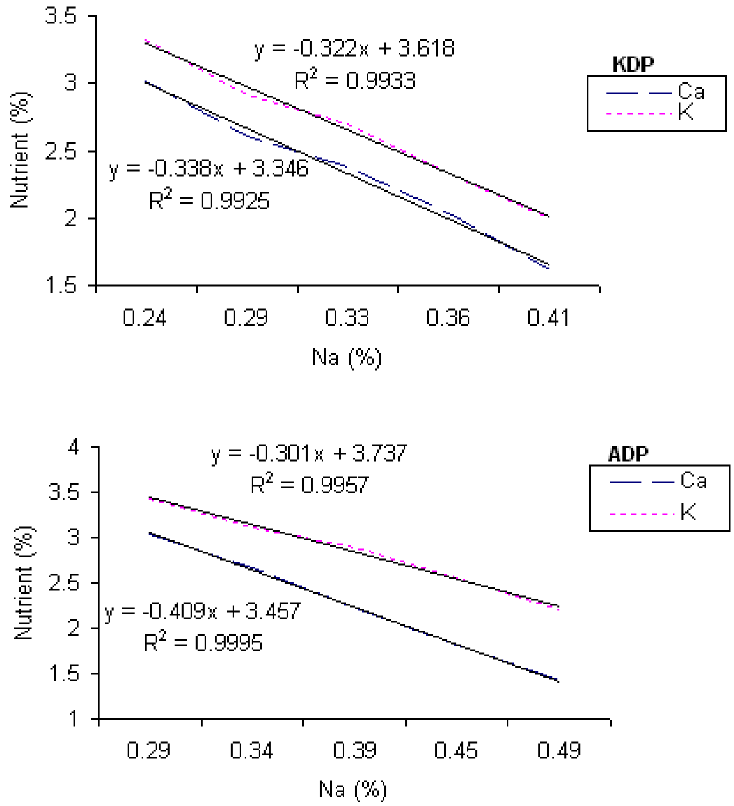

Fig. 3. Relation of sodium with calcium and potassium in sunflower seedling under NaCl stress after seed priming with potassium dihydrogen phosphate (KDP) and ammonium dihydrogen phosphate (ADP)

4. CONCLUSIONS

Salt stress exposed the differential response of sunflower seeds, initially when primed with KH_2PO_4 (KDP) and $NH_4H_2PO_4$ (ADP). The role of KDP was prominent over ADP as seed germination parameters indicated prominent difference of change of the seed primer sources. KDP proved to be better source having potassium and phosphate ions simultaneously for priming of sunflower seeds under salt stress. KDP- primed seeds also produce healthy seedlings under salt stress having lower level of sodium ion than the other ions. Using phosphorus salts as priming agent for seeds of sunflower is promising and this can be tried on other crops as well, for rapid germination of seeds under salt stress.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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