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Comparative Growth and Ions Response to Phosphorus Application for Two *Brassica* Species under Salt Stress

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Authors' contributions

This work was carried out in collaboration with both the authors. Author BUZ designed the study, wrote the protocol and conducted study with author SN. Author SN managed the literature searches and performed the statistical analysis. Author BUZ wrote the final draft of the manuscript. Both the authors read and approved the final manuscript.

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ABSTRACT

The genetic level differences between *Brassica* species can have potential impact on their performance under salt stress conditions together with Phosphorus and Potassium applications. In this study we hypothesized that certain level of salt stress mitigation can be done with applications of Phosphorus and Potassium solutions. Germinated seeds of *Brassica juncea* (var. Khanpur raya) and *B. napus* (Faisal canola), raised to 10 days seedlings stage and transferred to continuously aerated nutrient solution For salt stress, applied NaCl @60 mM in the nutrient solution' also applied phosphorus as potassium di hydrogen phosphate (PDP) @ 0 and 10 mM in triplicates. Khanpur raya and Faisal canola responded significantly ($p < 0.01$) to the application of PDP for growth and ions relations under salt stress and non-stress. Under stress conditions, shoot fresh mass (SFM) of Khanpur raya increased 10 percent with 10 mM of applied PDP than its control whereas SFM of

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Faisal canola increased 8 percent than its control. Root fresh mass (RFM) of Khanpur raya increased 8 percent with 10 mM of applied PDP than its control whereas RFM of Faisal canola increase 10 percent than its control. Dry mass of Khanpur raya increased 11 percent with 10 mM of applied PDP than its control whereas SDM of Faisal canola increased 6 percent than its control. Root dry mass (RDM) of Khanpur raya increased 18 percent with 10 Mm of applied PDP than its control whereas RDM of Faisal canola increased 19 percent than its control. In Khanpur raya Na^{+} $/K^{+}$ ratio decreased 21 percent than the control, whereas this ratio decreased 24 percent in Faisal canola than its control. Under salt stress, physiological P-use in shoot and root of Khanpur raya increased 11 and 8 percent respectively than that of Faisal canola.

Keywords: Brassica juncea; B. napus; KH2PO4; growth; Na+ /K+ ratio; physiological P-use.

1. INTRODUCTION

Brassica juncea and *Brassica napus* are cultivated for vegetable and edible oil. These are utilized before flowering stage as eatable green veggie, otherwise, their growth and development continue until maturity to obtain eatable oil from the seeds. These are high valued crops for human consumption. Eatable green leaves with soft and fleshy shoots are cooked as favorite and delicious dish called *saag*, full of nutrients. Further development of the plants till flowering and seed formation depends on nutrient supply from the soil to the root system. All over the world favorable cooking oil is extracted from their seeds. These species are cultivated depending on the range of responses for growth under stress [1]. Salinity affects on the physiology of plant through change of water and ionic status in the cells [2]. Selectivity and accumulation of ions varied among *Brassica* species [3]. *Brassica* species usually show adaptability, better tolerance to salinity [4], but their response may be variable in varieties due to genetic composition and variable root environments.

Salinity as one of the most common environmental stresses in arid and semi-arid areas reduced crop yield severely [5], by affecting the availability, transport, and partitioning of nutrients [6]. This stress hampers plant growth patterns and physiological processes. Growth parameters are dependent on availability of water as water retention indicates its health and turgidity [7]. Rapeseed indicated negative effects of salinity on biomass of shoot and root [8]. *Brassica napus* and *Brassica campestris* are known as semi tolerant species [9]. In Pakistan 6.5 million hectares area is salt affected [10]. The degree of sensitivity for salt tolerance may be variable among varieties of a crop.

Phosphorus is an essential element for plant growth and development [11]. Its inconsistent supply/availability through the root system disintegrates metabolic processes in plants [12]. Plant takes phosphorus from the soil solution as phosphate ion. In soil P may be immobilized through precipitation with cat ions such as Ca^{2+}, Mg^{2+}, Fe^{3+} and Al^{3+}, depending on the particular properties of a soil [13]. Phosphorus is a component of the nucleic structure of plants that are used to regulate protein synthesis and transferring light energy into chemical energy. It is a vital component of ATP. Its inefficient availability disrupts metabolic processes in plants. This condition provoked that phosphorus absorption by plants from different sources of fertilizer has different effects on plant growth under stress conditions. [12]. Potassium and phosphorus are in synergistic relation in plants. Potassium ions deficiency can decrease the transport and utilization of assimilates [14]. It is used in protein synthesis, carbohydrate metabolism, and enzyme activation [15] besides in the cation-anion balance, osmo-regulation and water movement. Sodium ion competes with K^{+} for major binding sites during key metabolic processes in the cytoplasm. Maintaining the cellular K^{+} above a certain threshold and maintaining a low Na^{+}/K^{+} ratio is crucial for plant growth and salt tolerance. In plants, potassium ion is non-discretionary [16] and it activates a wide range of biochemical reactions. Salinity affects nutrient acquisition by interfering with K^{+} uptake [17]. Although phosphorus and potassium are in synergistic relations but their requirement in the *Brassica* species under study may be different due to genetic difference under salt stress. This study was conducted to record the impact of KH_2PO_4 (PDP) on growth parameters and ion relation in *Brassica* under saline conditions.

2. MATERIALS AND METHODS

Seeds of the two varieties viz Khanpur raya and Faisal canola of *Brassica juncea and B. napus* respectively were collected from Oilseeds

Program of Crop Sciences Institute at National Agricultural Research Centre, Islamabad, Pakistan. Treated the seeds with 1% sodium hypo-chlorite for 15 minutes [18] to get rid of seed borne pathogens. Germinated these seeds and raised seedlings using moist quartz sand with distilled water. Foam-plugged the seedlings after ten days of germination to the pots containing 2.5 L continuously aerated solution nutrient solution [19]. For salt stress, applied NaCl @60 mM in the nutrient solution also applied phosphorus as KH_2PO_4 (PDP) @ 0 and 10 mM. Applied the treatments in triplicates. Replaced the nutrient solution weekly. Conducted the study in the glass house under sun light. Adjusted pH of the solution to 6.0 with HCl or KOH and monitored regularly. The photoperiod was 12 ± 2 hours. Sampled the plants for 33 day of transplantation in pots. Recorded fresh mass of shoots and roots. Dried shoots and roots at 70°C. Ground dried mass of the plant material to pass a 40-mesh Wiley Mill. Digested ground samples of root and shoot separately in 1:2 perchloric-nitric di-acid mixture. Determined sodium and potassium ions by flame photometry. Measured phosphorus in the plant digested material as given by [20]. Calculated physiological P-use by shoot and root as under:

Physiological P-use (µg /mg) = dry mass/ P concentration

Analyzed the data statistically according to two factors complete randomized design and compared treatment means using LSD test by using [21].

3. RESULTS

The two varieties viz Khanpur raya and Faisal canola of *Brassica juncea and B. napus* respectively responded significantly ($p< 0.01$) to the application of potassium di hydrogen phosphate (PDP) for growth and ions relations under non-stress and stress condition of salts.

Under non-stress conditions, shoot fresh mass (SFM) of Khanpur raya increased 8 percent with 10 mM of applied PDP than its control whereas SFM of Faisal canola increased 6 percent than its control. At control and with 10 mM PDP application, SFM increased 13 and 15 percent respectively in Khanpur raya than that of Faisal canola. Under stress conditions, shoot fresh mass (SFM) of Khanpur raya increased 10 percent with 10 mM of applied PDP than its control whereas SFM of Faisal canola increased 8 percent under the above same conditions. At control and with 10 mM PDP application, SFM increased 11 and 14 percent respectively in Khanpur raya than that of Faisal canola (Table 1).

Under non stress conditions, root fresh mass (RFM) of Khanpur raya increase 9 percent with 10 mM applied PDP than its control whereas RFM of Faisal canola increases 17 percent under the above same conditions. At control and with 10 mM PDP application, RFM increased 20 and 17 percent respectively in Khanpur raya than that of Faisal canola. Under stress condition root fresh mass (RFM) of Khanpur raya increased 8 percent with 10 mM of applied PDP than its control whereas RFM of Faisal canola increase 10 percent in control under above same condition. At control and with 10 mM PDP application, RFM increased 20 and 18 percent respectively in Khanpur raya than that of Faisal canola (Table 2).

Under non stress condition shoot dry mass (SDM) of Khanpur raya increase 15 percent with 10 mM of applied PDP than its control whereas SDM of Faisal canola increase 11 percent increase with control under same above conditions. At control and with 10 mM PDP application, SDM increased 19 and 26 percent respectively in Khanpur raya than that of Faisal canola. Under stress conditions SDM of Khanpur raya increase 11 percent with 10 mM of applied PDP than its control whereas SDM of Faisal canola increased 6 percent under the same conditions. At control and with 10 mM PDP application, SDM increased 13 and 6 percent respectively in Khanpur raya than that of Faisal canola (Table 3).

Under non stress condition root dry mass (RDM) of Khanpur raya increase 18 percent with 10 mM of applied PDP than its control whereas RDM of Faisal canola increase 15 percent under above same conditions. At control and with 10 mM PDP application, RDM increased 19 and 23 percent respectively in Khanpur raya than that of Faisal canola. Under stress condition RDM of Khanpur raya increase 18 percent with 10 Mm of applied PDP than control whereas DMR of Faisal canola increase 19 percent under above same conditions. At control and with 10 mM PDP application, RDM increased 19 and 23 percent respectively in Khanpur raya than that of Faisal canola (Table 4).

Table 1. Shoot fresh mass of *Brassica juncea (*var. Khanpur raya) and *Brassica napus* (Var. Faisal canola) with KH_2PO_4 application under salt stress

Varieties	Na Cl applied (0 mM)		Means	Na Cl applied (60 mM)		Means	Mean of means
	KH_2PO_4 applied (mM)			KH_2PO_4 applied (mM)			
	Control	10		Control	10		
Khanpur raya	257. 1 b	277.83 a	267.4 A	231.3 e	255.0 c	243.15 B	255.27A
Faisal canola	228.5 f	242.27 d	235.3 C	208.3 h	224.2 g	216.25 D	225.77B
Means	242.8 B	260.05 A		219.8 D	239.6 C		
Mean of means	251.4 A			229.7 B			

Means sharing similar letter(s) in a column do not differ significantly at p < 0.01; CV (p < 0.01) =6.98 percent

Table 2. Root fresh mass of *Brassica juncea* (var. Khanpur raya) and *Brassica napus* (Var. Faisal canola) with KH_2PO_4 application under salt stress

Varieties	Na Cl applied (0 mM)		Means	Na Cl applied (60 mM)		Means	Mean of means
	KH_2PO_4 applied (mM)			KH_2PO_4 applied (mM)			
	Control	10		Control	10		
Khanpur raya	14.53 b	15.81 a	15.17 A	12.51 e	13.41 d	12.96 B	14.06 A
Faisal canola	12.21 f	13.51 c	12.71 C	10.07 h	11.020 g	10.54 D	11.62 B
Means	13.37 B	14.66 A		11.29 D	12.21 C	13.37 B	14.66 A
Mean of means	14.01 A			11.75 B			

Means sharing similar letter(s) in a column do not differ significantly at p < 0.01; CV (p < 0.01) =5.51 percent

Table 3. Shoot dry mass of *Brassica juncea* (var. Khanpur raya) and *Brassica napus* (Var. Faisal canola) with KH_2PO_4 application under salt stress

Varieties	Na Cl applied (0 mM)		Means	Na Cl applied (60 mM)		Means	Mean of means
	KH_2PO_4 applied (mM)			KH_2PO_4 applied (mM)			
	Control	10		Control	10		
Khanpur raya	20.56 c	23.62 a	22.09 A	18.51 e	21.16 b	19.83 B	20.73 A
Faisal canola	18.28 g	20.35 d	19.31 C	16.87 h	18.38 f	17.62 D	18.45 B
Means	19.42 C	21.98 A		17.69 D	19.77 B	19.42 C	
Mean of means	20.7 A			18.73 B		20.7 A	

Means sharing similar letter(s) in a column do not differ significantly at p < 0.01; CV (p < 0.01) =7.22 percent

Table 4. Root dry mass of *Brassica juncea* (var. Khanpur raya) and *Brassica napus* (Var. Faisal canola) with KH_2PO_4 application under salt stress

Varieties	Na Cl applied (0 mM) KH_2PO_4 applied (mM)		Means	Na Cl applied (60 mM) KH_2PO_4 applied (mM)		Means	Mean of means
	Control	10		Control	10		
Khanpur raya	0.87 b	1.03 a	0.95 A	0.75 d	0.88 b	0.81 B	0.88 A
Faisal canola	0.73 e	0.84 c	0.78 C	0.63 g	0.69 f	0.66 D	0.72 B
Means	0.8 B	0.93 A		0.69 D	0.78 C	0.8 B	
Mean of means	0.86 A			0.73 B			

Means sharing similar letter(s) in a column do not differ significantly at $p < 0.01$; CV ($p < 0.01$) =4.78 percent

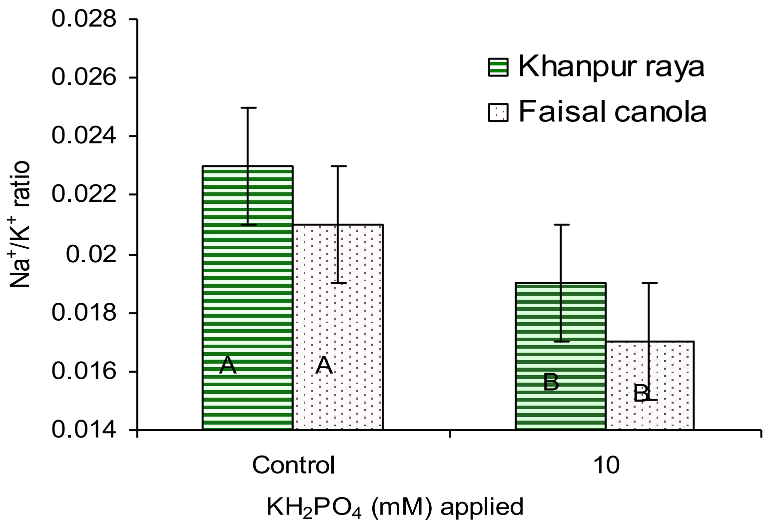

Fig. 1. Sodium potassium ratio in *Brassica juncea* (var. Khanpur raya) and *Brassica napus* (var. Faisal canola) with KH_2PO_4 application under salt stress as NaCl

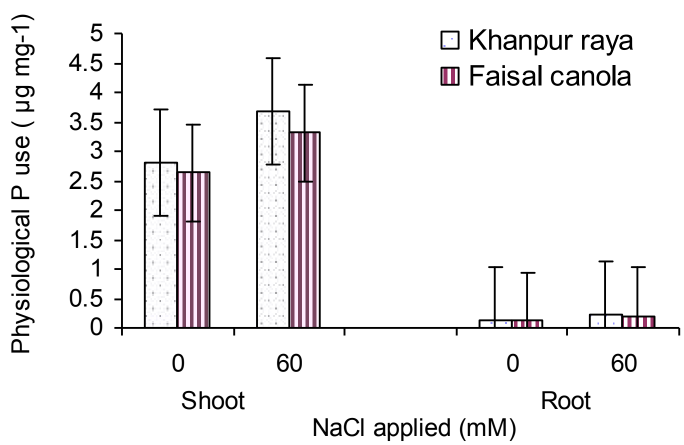

Fig. 2. Physiological P-use by *Brassica juncea* (var. Khanpur raya) and *Brassica napus* (var. Faisal canola) with KH_2PO_4 application under salt stress

Under saline conditions with the application of PDP, in Khanpur raya Na^+ /K^+ ratio decreased 21 percent than the control, whereas this ratio decreased 24 percent in Faisal canola than its control (Fig. 1). Under salt stress, physiological P-use in shoot and root of Khanpur raya increased 11 and 8 percent respectively than that of Faisal canola (Fig. 2).

4. DISCUSSION

Under salt stress and non stress conditions applied KH_2PO_4 (PDP) at the rate of 2 and 10 mM increased bio mass of shoot and root in Khanpur raya than Faisal canola. However, the quantification of biomass of these organs varied.

The fresh mass of shoot and root is made up of water and tissues that require different nutrients. All nutrients dissolved in the water are absorbed by the roots and transported in the form of nutrient solution to the different organs of the plant. Under salt stress condition roots are not adhere to absorbs water from soil due to water potential difference. In this case, there is deficiency of water and nutrients in the plant resulting in a decrease in biomass. Salinity limits the growth and development of plant besides water retention, an important property of a plant tissue that indicates its health and turgidity [7]. Nutrient interdependence and availability to plants under salt stress is the main focal point of nutrient management under such conditions. Under non-stress conditions, all the required nutrients remain in adequate amounts and in accessible forms for timely utilization by plants. Among the plant nutrients, phosphorus is an essential element for plant growth and its development [11]. Increase in fresh mass was a function of a differential release of phosphorus from fertilizer in response to plants growth [12]. Dry mass of plants is the net result of the metabolic processes. Phosphate is utilized in cell synthesis and biochemical linkages. A minute quantity of sodium ion remains beneficial for growth, depending upon the germplasm genetics. A particular amount of sodium ion may be, utilized in place of potassium ion and maybe, sodium and potassium ions cause balance up to a certain level of salt stress conditions to support growth processes. Phosphorus is in synergistic relation with potassium ion under the presence of sodium ion [10]. In this study, with the application of PDP, Na^{+}/K^{+} ratio decreased in shoot and root. In crop plants low Na^{+}/K^{+} ratio is desirable. Potassium ion has synergistic behavior with phosphorus and antagonistic relation with sodium ion. Sodium ion caused deficiency of water in root and shoot, besides disorder in nutrients resulting in decrease in biomass of both the varieties. However, despite applying PDP, Na^{+}/K remained higher in Khanpur raya than Faisal canola, but biomass of Khanpur raya was higher than that of Faisal canola. Khanpur raya was more salt tolerant than Faisal canola. Potassium ion is recognized as a rate-limiting factor for crop yield and quality [22]. Under salinity stress, NaCl-induced K^{+} loss is more in salt sensitive than tolerant plant varieties [23]. In Khanpur raya Na^{+}/K^{+} ratio was higher, inverse to its bio mass magnitude showing its tolerance for salt stress. Physiological P-use in shoot of Khanpur raya was higher than that of Faisal canola. This genetic stability in Khanpur raya made it possible to build up biomas under salt stress as compare to Faisal canola. Phosphorus uptake and utilization by plants plays a vital role in the determination of final crop yield and in addition, phosphorus dynamics in the soil plant system is a function of the consolidated effects of its transformation, availability, and utilization caused by soil rhizosphere and plant processes [24]. Plant processes in the form of metabolism were higher in Khanpur raya and this trait was not supported by Faisal canola genetically. Compared to other crops, *Brassicas* are generally considered to grow well in soils with low P availability [25], but when P availability is obstructed by salt stress, only genetically efficient varieties grow well, as was Khanpur raya.

5. CONCLUSION

Under salt stress, the enhanced application of potassium di hydrogen phosphate increased biomass of *Brassica juncea* (Cv. Khanpur raya) than that of *B. napus* (Cv. Faisal canola). Application of potassium ion along with phosphorus is beneficial for nutrient utilization by plants in saline environments.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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