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## Alleviation of salt stress in pearl millet (*Pennisetum glaucum* (L.) R. Br.) through seed treatments

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**Abstract** – The study was carried out to assess whether salt tolerance could be improved in pearl millet at the germination stage and vegetative stages by soaking the seeds of two cultivars, IC-8206 and 18-BY, for 8 h in distilled water, 150 mol·m<sup>-3</sup> NaCl, or polyethylene glycol (PEG-8000, -0.672 MPa), or by subjecting the seeds to chilling (5 °C) or heating (60 °C) for two days. Germination of both treated and non-treated seeds of both the cultivars was assessed for 8 days in Hoagland solution amended with 0 or 150 mol·m<sup>-3</sup> NaCl. Chilling, and to a lesser extent, PEG, increased the final germination percentage but not the germination rate of both the cultivars under both saline and non-saline conditions. Chilling alleviated the adverse effect of salt stress on IC-8206 in terms of fresh and dry weights of shoots and roots following 42 d in sand culture that received 150 mol NaCl·m<sup>-3</sup>. Chilling also reduced Cl<sup>-</sup> accumulation and, to a lesser extent, that of Na<sup>+</sup>, and enhanced K<sup>+</sup> and Ca<sup>2+</sup> accumulation in the shoots and roots of both cultivars under both saline and non-saline substrates. The reverse was true in plants raised from seeds treated with NaCl or PEG.

**germination / ion accumulation / ion content / pre-sowing treatment / pearl millet / salt tolerance**

**Résumé** – Réduction du stress salin du mil à chandelle (*Pennisetum glaucum* (L.) R. Br.) par le traitement des semences. L'étude a été conduite pour évaluer si la tolérance au sel peut être améliorée dans le mil à chandelle au stade germinatif et végétatif par trempage des semences de 2 variétés (IC-8206 et 18-BY) durant 8 h dans de l'eau distillée, avec 150 mol·m<sup>-3</sup> NaCl, ou du polyéthylène glycol (PEG-8000, -0.672 MPa), ou en soumettant les semences au froid (5 °C) ou à la chaleur (60 °C) pendant 2 jours. La germination des semences traitées ou non des 2 variétés a été testée pendant 8 jours dans une solution de Hoagland additionnée avec 0 ou 150 mol·m<sup>-3</sup> NaCl. Le passage au froid et, dans une moindre mesure, le PEG ont augmenté le pourcentage de germination finale mais n'ont pas affecté le taux de germination des 2 variétés soumises aux conditions salines et non salines. Le refroidissement a réduit l'effet néfaste du stress salin sur IC-8206 en terme de poids frais et sec des tiges et des racines après 42 jours de culture dans le sable qui avait reçu 150 mol·m<sup>-3</sup> NaCl. Le refroidissement a aussi réduit l'accumulation de Cl<sup>-</sup> et de manière moins importante celle de Na<sup>+</sup>, et a augmenté les accumulations de K<sup>+</sup> et de Ca<sup>2+</sup> dans les tiges et les racines des 2 variétés pour les substrats salins ou non. L'effet inverse fut vrai pour les plantes cultivées à partir de semences traitées avec NaCl ou PEG.

**germination / accumulation d'ions / teneur en ion / traitement avant semis / tolérance au sel**

### 1. INTRODUCTION

Soil salinity is known to suppress the growth of most crop species, but considerable differences in salinity tolerance exist between species [27]. The growth of pearl millet in saline medium is severely affected at different stages of the plant life cycle [4, 5]. For example, Ashraf and Idrees [4] noted that salinity stress caused reduction in the germination percentage and delayed germination of seeds of pearl millet. Similarly, Ashraf and McNeilly [5] found no consistent relationships

between salt tolerance of two-week-old plants and that of 7-week-old plants of 5 accessions of pearl millet.

Henckel and Strogonov [20] in 1961 reported that treating seeds with salt solutions of different salts before sowing (osmo-priming) increased salinity tolerance. Pre-soaking or priming seeds of a number of crops has improved germination, seedling establishment and, in some cases, stimulated vegetative growth and hence crop yield [1, 6, 19, 25]. For example, Idris and Aslam [20] found that osmo-priming of wheat seeds hastened germination under varying NaCl

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regimes. Recently, Ashraf and Rauf [6] conducted a study to determine whether salt tolerance could be induced in maize (*Zea mays*) at the germination stage by soaking seeds for 8 h in distilled water or 200 meq·L<sup>-1</sup> of NaCl, KCl, or CaCl<sub>2</sub>·2H<sub>2</sub>O. They subjected both primed and non-primed seeds to 0, 100 or 200 mol NaCl·m<sup>-3</sup> for 14 days. Although all priming agents were effective in alleviating the adverse effects of salt stress on maize germination, CaCl<sub>2</sub>·2H<sub>2</sub>O was the most effective.

Salinity stress perturbs the uptake and accumulation of essential nutrients [10, 16, 33, 40]. Generally, Ca<sup>2+</sup> and K<sup>+</sup> are decreased in plants under saline conditions [2, 24, 26]. In contrast, Ashraf and Rauf [6] reported that under saline conditions concentrations of Na<sup>+</sup>, K<sup>+</sup> and Ca<sup>2+</sup> increased significantly in all the parts of germinating maize seeds primed with NaCl, KCl, or CaCl<sub>2</sub>·2H<sub>2</sub>O, respectively. In addition, seeds primed with CaCl<sub>2</sub>·2H<sub>2</sub>O had the highest Cl<sup>-</sup> accumulation in all parts of the germinating seeds, followed by the seeds treated with NaCl and KCl. Most of the Ca<sup>2+</sup> was retained in the mesocotyl, because of which, transport of this ion to plumules and radicles was low. Increased Ca<sup>2+</sup> and K<sup>+</sup> concentrations in seeds of wheat [9, 21] and Wimmera ryegrass (*Lolium rigidum*) [28] were associated with increased seed germination under varying NaCl levels. Contrarily, Guerrier and Pinel [17] reported that seed treatments with calcium or potassium salts failed to increase the final germination percentage of pea (*Pisum sativum*) seeds under saline conditions.

The objective of the present study was, firstly, to assess the effects of different seed treatments (distilled water, NaCl, polyethylene glycol, or low or high temperatures) on salt tolerance of pearl millet at germination and subsequent vegetative growth. Secondly, we determined the effect of seed treatments on the uptake and accumulation of toxic ions such as Na<sup>+</sup> and Cl<sup>-</sup> in different plant parts.

## 2. MATERIALS AND METHODS

Seeds of two cultivars, 18-By and IC-8206, of pearl millet (*Pennisetum glaucum* (L.) R. Br.) were obtained from the Maize and Millet Research Station, Yousafwala, District Sahiwal, Pakistan. Seeds were surface sterilized in 10 percent sodium hypochlorite solution for 10 minutes, then rinsed with sterilized distilled water and air-dried at an ambient temperature of 32 °C in the laboratory. The sterilized seeds were soaked for 8 h in 150 mol NaCl·m<sup>-3</sup> (-0.672 MPa), polyethylene glycol (PEG mol. wt. 8000) at an osmotic potential of -0.672 MPa, (199.64 g·L<sup>-1</sup>) or distilled water. The osmotic potential values of both NaCl and PEG-8000 solutions were determined with a vapor pressure osmometer (Wescor 5520, USA). After the pre-sowing treatment, all seed samples were rinsed with distilled water, and dried in an oven at 40 °C for an hour to eliminate surface moisture. Seeds of both lines of pearl millet were exposed to 60 °C or 5 °C temperatures in an incubator for two days at 55% RH. All the

pre-treated seed samples were used in experiments within 24 h of treatment.

### 2.1. Germination experiment

Primed seeds, together with non-primed seeds, were germinated at 32 ± 4 °C in 11 cm glass Petri dishes on a layer of filter paper (Whatman # 41), moistened with 5 ml of distilled water or 150 mol NaCl·m<sup>-3</sup>. Fifty seeds were placed in each Petri dish.

The experiment was laid out in a completely randomized design with three replicates. The data for seed germination was recorded daily up to day 8 after the start of the experiment. A seed was considered germinated when the radicle or plumule were just visible. The inverse of the germination rate was calculated as days to 50% germination.

### 2.2. Glasshouse experiment

A pot experiment was conducted in the Botanic Garden of the University of Agriculture, Faisalabad, in a net-house supplied with natural sunlight during June-October 2001 to determine the influence of seed priming on vegetative growth. The average day and night temperatures were 37±3 °C and 24 ± 3 °C, respectively. The relative humidity ranged from 45.9–58.6 percent, the photoperiod from 11–12 h and the average PAR measured at noon ranged from 1020 to 1730 μmol·m<sup>-2</sup>·s<sup>-1</sup>. Ten seeds of each cultivar were sown equidistantly in each pot (28 cm diameter, 30 cm deep). Each pot contained 10 kg river sand (particle size 1.4–2.0 mm). The sand was washed thoroughly with tap water, with distilled water, and finally with Hoagland solution [13]. Two liters of 0 (control) or 150 mol·m<sup>-3</sup> NaCl in Hoagland's nutrient solution were applied weekly to each pot for 6 weeks. This volume was sufficient to flush through the salts already present in the sand. During the week distilled water (200–300 cm<sup>3</sup>) was applied to each pot daily to keep the sand moist and hence maintain the salt level. Soon after emergence, the seedlings were thinned to five per pot. The experiment was arranged in a completely randomized design with four replicates.

Plants were harvested from each pot 42 days after sowing. The plants were uprooted carefully, washed with distilled water, and the fresh mass of shoots and roots was recorded. Plant samples were dried in an oven at 65 °C to constant dry mass.

### 2.3. Determination of Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup> and Cl<sup>-</sup> in shoots and roots of plants

The dried plant material was ground to pass through a 2 mm sieve. The dried material (0.1 g) was digested in 2 mL of concentrated HNO<sub>3</sub> (specific gravity 1.42; assay 69–75.5% – BDH AnalaR). Na<sup>+</sup>, K<sup>+</sup> and Ca<sup>2+</sup> were determined with a flame photometer (Sherwood model 410, Japan). For Cl<sup>-</sup> determination, 0.1 g dry ground material of the plant parts mentioned earlier was heated at 80 °C for 4 h, and Cl<sup>-</sup> concentration in the extracts was determined with a chloride meter (Central Kagaku Corp., Japan).

**Table I.** Final germination percentage and days to 50% germination of two cultivars of pearl millet in response to pre-sowing seed treatments and 0 or 150 mol·m<sup>-3</sup> NaCl in Hoagland's nutrient solution.

Cultivars	NaCl in Hoagland solution (mol·m <sup>-3</sup> )	Pre-sowing treatments					
		Non-treated	Distilled water	NaCl (150 mol·m <sup>-3</sup> )	PEG-8000 (-0.672 MPa)	Low temp. (5 °C) for two days	High temp. (60 °C) for two days
Final germination percentage							
IC-8206	0	87.5	74.0	74.3	89.4	97.0	75.0
	150	66.1	64.7	41.5	70.2	76.6	65.6
18-BY	0	88.2	75.2	74.1	92.8	95.2	75.8
	150	72.0	62.6	51.1	78.4	86.1	71.1
LSD 5% (NaCl × Var. × Pre-sowing treat.) = NS							
Days to 50% germination							
IC-8206	0	1.9	2.0	3.4	2.1	1.7	3.8
	150	5.4	4.3	7.0	4.6	3.1	6.5
18-BY	0	1.7	1.8	2.3	1.6	1.4	2.1
	150	2.8	5.4	4.5	5.3	3.3	2.8
LSD 5% (NaCl × Var. × Pre-sowing treat.) = NS							
SV	df	Mean squares of germination %age			Mean squares of days to 50% germination		
Cultivars (Cv)	1	305.6NS			17.02NS		
Salinity (S)	1	3692.1***			129.4***		
Seed treatments (ST)	5	1096.1***			14.14NS		
Cv × S	1	70.9NS			0.95NS		
Cv × ST	5	27.9NS			4.06NS		
S × ST	5	154.3NS			7.28NS		
Cv × S × ST	5	199.5NS			4.22NS		

#### 2.4. Statistical analysis

All data were subjected to the analysis of variance technique [36]. Mean values were separated using the least significance difference test (LSD).

### 3. RESULTS

Salt stress reduced the final germination percentage of both primed and non-primed seeds of both the cultivars (Tab. I). However, there was an alleviating effect of two priming treatments, chilling and PEG, on final germination of both the cultivars under saline conditions. The lowest germination was recorded under both saline and non-saline conditions of the seeds primed with NaCl, and the second lowest germination was found in seeds treated with distilled water.

Although the rate of germination (days to 50% germination) of both primed and un-primed seeds of both cultivars decreased significantly (the more days to 50% germination the lower the rate of germination would be) due to salt stress (Tab. I), the chilled seeds of IC-8206 took fewer days to reach 50 percent germination under both saline and non-saline conditions compared with the untreated seeds.

However, none of the other priming treatments was found effective in accelerating germination.

The fresh and dry weights of shoots and roots of 42-day-old plants of both pearl millet cultivars decreased under saline conditions (Tab. II). However, plants of both cultivars, raised from chilled seeds, showed significantly higher shoot and root biomass as compared with those raised from seeds treated with other priming agents, particularly under non-saline conditions; but in contrast, in saline conditions chilling was found effective in alleviating the adverse effect of salt on only IC-8206, if the growth is compared with plants raised from untreated seeds or seeds treated with other priming agents. Pre-soaking of seeds of both the cultivars in distilled water failed to increase biomass production above that achieved with non-treated seeds under both saline and non-saline conditions. Plants raised from seeds treated with NaCl had much lower fresh and dry weights of shoots and roots than those raised from non-treated seeds (Tab. II), irrespective of salinity regime.

Shoot Na<sup>+</sup> concentration generally increased in the plants of both cultivars under salt stress, irrespective of seed treatment (Tab. III). Chilling of seeds proved to be very effective in reducing the concentrations of Na<sup>+</sup> in the shoots of both the cultivars, but a more marked reduction in this cation

**Table II.** Fresh and dry weights of shoots and roots of 42-day-old plants of two cultivars of pearl millet in response to pre-sowing seed treatments and 0 or 150 mol·m<sup>-3</sup> NaCl in Hoagland's nutrient solution.

Cultivars	Pre-sowing treatments						
	NaCl in Hoagland solution (mol·m <sup>-3</sup> )	Non-treated	Distilled water	NaCl (150 mol·m <sup>-3</sup> )	PEG-8000 (-0.672 MPa)	Low temp. (5 °C) for two days	High temp. (60 °C) for two days
Fresh weights of shoots (g/plant)							
IC-8206	0	25.5	24.2	15.4	11.2	47.9	43.2
	150	7.7	5.6	4.8	3.1	14.6	7.9
18-BY	0	29.6	27.6	22.9	7.2	46.6	27.6
	150	5.4	4.9	3.7	3.1	6.5	4.5
Dry weights of shoots (g/plant)							
IC-8206	0	3.8	3.6	2.2	1.6	6.2	6.1
	150	0.9	0.7	0.6	0.4	1.9	1.1
18-BY	0	3.1	3.0	2.3	1.0	5.0	2.9
	150	0.8	0.8	0.5	0.3	1.0	0.6
Fresh weights of roots (g/plant)							
IC-8206	0	2.1	1.8	1.4	0.40	4.0	3.4
	150	0.6	0.5	0.3	0.20	1.2	0.9
18-BY	0	2.6	2.5	1.9	0.50	5.6	2.0
	150	0.6	0.3	0.2	0.20	0.7	0.3
Dry weights of roots (g/plant)							
IC-8206	0	0.40	0.20	0.20	0.10	0.60	0.50
	150	0.10	0.07	0.05	0.03	0.10	0.07
18-BY	0	0.30	0.30	0.30	0.10	0.70	0.30
	150	0.10	0.10	0.05	0.03	0.10	0.05
LSD 5% (NaCl × Var. × Pre-sowing treat.) for fresh and dry weights of shoots and roots = NS							
SV	df	Mean squares of shoot F.wt	Mean squares of shoot D.wt	Mean squares of root F.wt	Mean squares of root D.wt	Mean squares of root F.wt	Mean squares of root D.wt
Cultivars (Cv)	1	80.8NS	10.39***	0.096NS	0.000NS	0.096NS	0.000NS
Salinity (S)	1	11020.2***	162.5***	99.9***	1.92***	99.9***	1.92***
Seed treatments (ST)	5	974.0***	14.01***	14.3***	0.196***	14.3***	0.196***
Cv × S	1	14.6NS	3.30*	2.38NS	0.000NS	2.38NS	0.000NS
Cv × ST	5	86.4NS	1.97NS	1.58NS	0.016NS	1.58NS	0.016NS
S × ST	5	456.8***	6.02***	5.40***	0.091***	5.40***	0.091***
Cv × S × ST	5	65.4NS	0.99NS	0.93NS	0.016NS	0.93NS	0.016NS

was observed in 18-BY. Similarly, chilling also caused reduction in shoot Cl<sup>-</sup> in both cultivars under saline substrate, but this effect was more pronounced in IC-8206 than in 18-BY. However, plants of IC-8206, raised from seeds treated with distilled water, NaCl, or PEG accumulated higher Na<sup>+</sup> in the shoots than those from non-treated seeds or seeds treated with low or high temperatures. Plants of 18-BY raised from seeds exposed to high temperature accumulated considerably more Na<sup>+</sup> in the shoots than plants raised from seeds given other seed treatments (Tab. III).

Shoot K<sup>+</sup> and Ca<sup>2+</sup> concentrations were considerably higher in the plants from chilled seeds than from other seed treatments, irrespective of cultivar or salinity regime (Tab. III). In contrast, treating seeds with NaCl led to lower K<sup>+</sup> and Ca<sup>2+</sup> in the shoots than those raised from non-treated seeds or all other seed treatments. Chilling of seeds caused a significant reduction in accumulation of Cl<sup>-</sup> in the shoots of both cultivars in both saline and non-saline conditions (Tab. III). In contrast, plants raised from seeds primed with NaCl had a relatively higher Cl<sup>-</sup> concentration in the shoots

**Table III.** Ion concentrations ( $\text{mg} \cdot \text{g}^{-1}$  dry wt.) in shoots of 42-day-old plants of two cultivars of pearl millet in response to pre-sowing seed treatments and 0 or  $150 \text{ mol} \cdot \text{m}^{-3}$  NaCl in Hoagland's nutrient solution.

Cultivars	NaCl in Hoagland solution ( $\text{mol} \cdot \text{m}^{-3}$ )	Pre-sowing treatments					
		Non-treated	Distilled water	NaCl ( $150 \text{ mol} \cdot \text{m}^{-3}$ )	PEG-8000 ( $-0.672 \text{ MPa}$ )	Low temp. ( $5 \text{ }^\circ\text{C}$ ) for 2 days	High temp. ( $60 \text{ }^\circ\text{C}$ ) for 2 days
Shoot $\text{Na}^+$ ( $\text{mg} \cdot \text{g}^{-1}$ dry wt.)							
IC-8206	0	7.6	9.4	7.0	8.4	7.5	7.6
	150	10.0	14.5	12.6	13.7	9.7	11.4
18-BY	0	5.5	8.1	5.7	7.9	5.0	9.4
	150	8.3	12.5	9.5	10.2	7.5	15.3
Shoot $\text{K}^+$ ( $\text{mg} \cdot \text{g}^{-1}$ dry wt.)							
IC-8206	0	36.8	33.9	22.8	31.3	45.1	36.0
	150	31.0	24.9	18.3	23.3	33.9	29.4
18-BY	0	45.1	44.0	30.0	33.8	58.5	36.3
	150	29.3	27.1	20.6	23.2	40.4	26.8
Shoot $\text{Ca}^{2+}$ ( $\text{mg} \cdot \text{g}^{-1}$ dry wt.)							
IC-8206	0	6.6	6.5	4.5	4.8	9.3	6.3
	150	6.2	5.6	2.8	4.3	8.0	4.8
18-BY	0	4.3	3.3	2.9	3.5	8.0	5.0
	150	3.3	3.0	2.0	3.0	7.5	3.5
Shoot $\text{Cl}^-$ ( $\text{mg} \cdot \text{g}^{-1}$ dry wt.)							
IC-8206	0	14.8	22.5	24.0	16.3	9.8	19.5
	150	18.1	29.3	32.5	19.3	14.3	26.8
18-BY	0	25.8	30.0	33.3	26.5	18.0	27.3
	150	27.0	34.3	35.3	28.8	22.5	30.5
LSD 5% (NaCl $\times$ Var. $\times$ Pre-sowing treat.) for $\text{Na}^+$ , $\text{K}^+$ , $\text{Ca}^{2+}$ and $\text{Cl}^-$ = NS							
SV	df	Mean squares of shoot		Mean squares of shoot	Mean squares of shoot	Mean squares of shoot	
		$\text{Na}^+$		$\text{K}^+$	$\text{Ca}^{2+}$	$\text{Cl}^-$	
Cultivars (Cv)	1	34.6*		388.4*	65.5***	1345.1***	
Salinity (S)	1	353.9***		2642.1***	22.9***	489.8***	
Seed treatments (ST)	5	39.6***		856.1***	45.6***	500.6***	
Cv $\times$ S	1	1.46NS		210.4NS	0.71NS	26.6NS	
Cv $\times$ ST	5	14.9*		58.1NS	3.52*	0.59NS	
S $\times$ ST	5	4.86NS		32.8NS	0.77NS	0.37NS	
Cv $\times$ S $\times$ ST	5	3.08NS		9.11NS	0.57NS	0.43NS	

compared with all other seed treatments in both saline and non-saline conditions.

The pattern of accumulation of  $\text{Na}^+$  in the roots in response to different seed treatments was different from that in the shoots. Plants of IC-8206 raised from the seeds primed with distilled water, NaCl, or PEG had more  $\text{Na}^+$  in the roots than those raised from seeds given other treatments (Tab. IV). In

contrast, plants of 18-BY raised from chilled seeds had less  $\text{Na}^+$  in the roots than those from other seed treatments, irrespective of salinity regime. Seed chilling caused increased accumulation of  $\text{K}^+$  and  $\text{Ca}^{2+}$  in roots in both control and saline conditions. Root  $\text{Cl}^-$  was higher and root  $\text{K}^+$  and  $\text{Ca}^{2+}$  were lower in plants from seeds treated with NaCl or water than from other seed treatments, irrespective of cultivar or salinity regime (Tab. IV).

**Table IV.** Ion concentrations ( $\text{mg}\cdot\text{g}^{-1}$  dry wt.) in roots of 42-day-old plants of two pearl millet cultivars in response to pre-sowing seed treatments and 0 or  $150\text{ mol}\cdot\text{m}^{-3}$  NaCl in Hoagland's nutrient solution.

Cultivars	NaCl in Hoagland solution ( $\text{mol}\cdot\text{m}^{-3}$ )	Pre-sowing treatments					
		Non-treated	Distilled water	NaCl ( $150\text{ mol}\cdot\text{m}^{-3}$ )	PEG-8000 ( $-0.672\text{ MPa}$ )	Low temp. ( $5\text{ }^{\circ}\text{C}$ ) for 2 days	High temp. ( $60\text{ }^{\circ}\text{C}$ ) for 2 days
Root $\text{Na}^+$ ( $\text{mg}\cdot\text{g}^{-1}$ dry wt.)							
IC-8206	0	22.5	27.5	24.5	26.7	21.5	21.7
	150	30.5	43.3	36.5	39.3	27.7	33.5
18-BY	0	15.3	24.1	15.7	21.9	15.8	27.6
	150	24.5	36.8	27.2	30.2	21.3	45.8
Root $\text{K}^+$ ( $\text{mg}\cdot\text{g}^{-1}$ dry wt.)							
IC-8206	0	19.5	15.9	11.6	15.0	22.5	18.0
	150	16.0	13.0	9.5	12.0	18.5	14.5
18-BY	0	22.3	22.0	16.0	16.3	29.5	19.0
	150	15.9	14.0	10.7	13.0	20.5	13.0
Root $\text{Ca}^{2+}$ ( $\text{mg}\cdot\text{g}^{-1}$ dry wt.)							
IC-8206	0	6.3 a	9.8 a	7.3a	8.8ac	10.5a	5.3 a
	150	5.1 a	7.6 a	5.8a	6.3a	8.7a	4.1 a
18-BY	0	9.0 b	7.0 a	12.5b	16.7b	23.5b	5.0 a
	150	15.0c	6.0 a	8.6a	9.5c	22.5b	4.5 a
Root $\text{Cl}^-$ ( $\text{mg}\cdot\text{g}^{-1}$ dry wt.)							
IC-8206	0	13.5	15.3	19.4	14.0	9.8	14.5
	150	15.5	17.5	21.5	16.3	13.5	16.8
18-BY	0	9.5	11.9	13.5	10.0	9.0	10.5
	150	13.8	16.8	18.5	15.5	11.5	16.3

LSD 5% (NaCl  $\times$  Var.  $\times$  Pre-sowing treat.) for  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Cl}^-$  = NS

Means with the same letters in each column do not differ significantly at 5% level.

SV	df	Mean squares of root $\text{Na}^+$	Mean squares of root $\text{K}^+$	Mean squares of root $\text{Ca}^{2+}$	Mean squares of root $\text{Cl}^-$
Cultivars (Cv)	1	392.4***	111.1***	506.7***	221.1***
Salinity (S)	1	2925.4***	540.2***	56.9***	226.1***
Seed treatments (ST)	5	358.1***	221.9***	229.9***	102.6***
Cv $\times$ S	1	0.135NS	62.6***	1.761NS	10.4NS
Cv $\times$ ST	5	176.1***	12.14***	124.2***	8.96NS
S $\times$ ST	5	47.0***	6.48*	22.6***	4.21NS
Cv $\times$ S $\times$ ST	5	13.2NS	3.65NS	16.3**	5.67NS

#### 4. DISCUSSION – CONCLUSION

It is now evident that priming of seeds of different crops can alleviate the adverse effect of salinity stress on germination and seedling establishment and, in some cases, it can enhance crop yield [1, 18, 19, 30, 31]. The presoaking of seeds allows the hydration of membranes and proteins, and the initiation of various metabolic systems. These are arrested when the seeds

are dried or moisture is withheld, but recommence when the seeds imbibe water for the second time [7]. The pre-sowing treatments cause initiation of the early metabolic processes and the re-drying of seeds arrest, but do not reverse, the initial stages of germination so that on the availability of suitable conditions, the time taken to germinate is reduced [7]. However, from the results of the present study, it is evident that pre-sowing chilling of seeds improved the growth of both

cultivars under normal non-saline conditions. It means chilling was effective in improving plant vigor under non-saline conditions, and this effect had been found to be carried over to saline conditions, particularly in IC-8206, but not in 18-BY. The alleviation of adverse effects of salt stress by pre-chilling in the IC-8206 line of pearl millet has also been observed to some extent, e.g., Indiangrass (*Sorghastrum nutans*) [12, 37], brown mustard (*Brassica juncea*) [34] and parsnip (*Pastinaca sativa*) [14].

While osmo-conditioning of pearl millet seeds with NaCl did not alleviate the adverse effect of salt stress on the vegetative growth or germination of both cultivars, priming with PEG improved germination of both cultivars under salt stress. The effectiveness of PEG as an osmo-priming agent has been reported in a number of crops, e.g., parsley (*Petroselinum crispum*) [29, 30], sunflower (*Helianthus annuus*) [11], pea (*Pisum sativum*) seeds [35], carrot (*Daucus carota*), lettuce (*Lactuca sativa*), onion (*Allium cepa*) and Welsh onion (*Allium fistulosum*) [39]. In addition, osmo-priming of seeds with NaCl exacerbated the adverse effect of salt stress on the germination and later vegetative growth stage of both pearl millet lines. However, Cayuela et al. [8] observed that under salt stress the seedlings of tomato emerged earlier from seeds primed with NaCl than from non-primed seeds. Similarly, Katembe et al. [23] investigated the effect of NaCl as priming agent on germination and seedling growth of two *Atriplex* species under salt stress. They found that priming with NaCl improved the seed performance more than iso-osmotic PEG solution.

Hydro-priming also did not increase the germination or vegetative growth of two pearl millet cultivars compared with non-treated seeds under both saline and non-saline conditions. Similar results were reported by Chaudhri and Wiebe [9] in wheat; no germination took place in NaCl when wheat seeds were primed only with water, but a considerable improvement in germination resulted from seeds primed with  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ . Likewise, Pill and Necker [31] reported that hydro-priming failed to improve germination in Kentucky bluegrass (*Poa pratensis*) under normal non-saline conditions. In contrast, there are numerous studies in the literature which exhibit the considerable effectiveness of hydro-priming on germination and later growth in different plant species under both saline and non-saline conditions, e.g., wheat [21], *Acacia tortilis* and *A. coriacea* [32], and only under normal non-saline conditions e.g., maize (*Zea mays*), rice (*Oryza sativa*), chickpea (*Cicer arietinum*) [19] and white-flowered gourd (*Lagenaria siceraria*) [22].

Pre-sowing chilling of seeds of pearl millet lowered the accumulation of  $\text{Cl}^-$  and, to a lesser extent,  $\text{Na}^+$ , and enhanced the accumulation of  $\text{K}^+$  and  $\text{Ca}^{2+}$  in the plant tissues of both cultivars, but these effects were greater in 18-BY, the line which did not show any significant improvement under saline conditions. However, the lower uptake of toxic mineral elements and higher uptake of beneficial mineral elements is generally associated with greater salt tolerance of most crop species [3, 10, 16, 33, 38]. For example, pre-sowing chilling of *Brassica juncea* seeds alleviated NaCl stress on germination and seedling growth, decreased  $\text{Na}^+$  and slightly increased  $\text{K}^+$  and  $\text{Ca}^{2+}$  concentrations in seedlings [34].

Plants subjected to low temperature show many morphological, biochemical and physiological changes [25], which suggests that low temperature response is controlled by a large number of genes with complex interaction and/or there is a cascade of changes controlling gene expression during acclimation that involves common genetic pathways with divergent links [15]. It is thus possible that during pre-chilling some factors/genes may have been stimulated in seeds of IC-8206 whose effect lasted until the vegetative growth of the plants.

In conclusion, chilling and PEG were effective pre-sowing seed treatments in alleviating the adverse effect of salt stress in both cultivars of pearl millet during germination. At the vegetative stage, chilling was found to be effective in improving plant vigor under normal non-saline conditions, and this effect was carried over to saline conditions in only IC-8206. This treatment lowered  $\text{Cl}^-$  accumulation and enhanced  $\text{K}^+$  and  $\text{Ca}^{2+}$  accumulation in plants under saline substrate. Hydropriming did not alleviate the adverse effect of salt stress, and priming with NaCl exacerbated the adverse effect of salt stress on pearl millet. Further research is needed to optimize the effectiveness of chilling treatment at different time intervals, using a number of cultivars of pearl millet, or in combination with other agents.

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