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Hydropriming with Moringa Leaf Extract Mitigates Salt Stress in Wheat Seedlings

Talaat Ahmed *, Ahmed Abou Elezz and Muhammad Fasih Khalid

Environmental Science Center, Qatar University, Doha 2713, Qatar; a.hassan@qu.edu.qa (A.A.E.); mfasih.khalid@qu.edu.qa (M.F.K.)
* Correspondence: author: t.alfattah@qu.edu.qa

Abstract: Salinity is the major constraint that decreases the yield and production of crops. Wheat has a significant value in agricultural food commodities. The germination and growth of wheat seedlings are a big challenge in salt-affected soils. The seed priming technique is used to mitigate salt stress and enhance the germination and growth of the crops. Therefore, the current study was conducted to evaluate the hydropriming of natural plant extract (moringa leaf extract) and water on wheat seeds and grown under different saline (0, 0.05, 0.1, 0.15, and 0.2 M NaCl) environments. The germination attributes (germination percentage, germination index, mean germination day, coefficient of variance, vigor index) and seedling growth (fresh weight, dry weight, root length, shoot length) were enhanced in the plants primed by moringa leaf extract. The germination percentage was observed 10% more at 0.2 M NaCl stress in seeds treated with moringa leaf extract than seeds treated with water. The nutrient (K, Ca, Mg, P, S, Fe, B, Mn, Zn, Cu) uptake was also observed more in the shoots and roots of wheat seedlings soaked in moringa leaf extract as compared to soaked in water. Controlled plants showed higher concentrations of toxic ions (Na) and reactive oxygen species (H_2O_2) in shoots and roots of wheat seedlings. The use of moringa leaf extract for priming wheat seeds will enhance their germination and growth by maintaining efficient nutrient uptake and restricting the toxic ions and reactive oxygen species accumulation.

Keywords: abiotic stress; germination; plant growth; reactive oxygen species; toxic ions

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1. Introduction

In arid and semiarid environments, due to climate change, abiotic stresses are a major threat to plants, specifically commercial crops. Among different abiotic stresses, salinity is one of the major factors that affect plant health and its production [1]. Wheat is one of the main food security crops in many countries around the world, including Qatar. However, Qatar depends mainly on imports to meet the domestic demand for wheat. Qatar's wheat production is deficient, and the productivity of the improved local bread wheat cultivar, Doha-88, is estimated at 2 tons/ha [2]. This low productivity is mainly due to the lack of well-adapted wheat varieties and suitable production technologies. Sustainable wheat production in Qatar can be obtained after developing new cultivars and advanced production technology. In Qatar, groundwater is the primary source of water irrigation that contains a significant amount of salt. Therefore, salt is accumulated in the surface layer of soil because of over-irrigation [3]. Moreover, the little rainfall and high rate of evaporation lead to salinity-related problems in these environments. When the seeds are exposed to abiotic stresses, crop germination capability and seed vigor are significantly affected, which further affects the crop growth, development, and yield [4].

To increase seed germination, different mechanisms have been analyzed by different scientists, and seed priming is most applicable among them [5–7]. Among different seed priming techniques, hydropriming is one of the most used techniques. Hydropriming allows the seeds to swiftly attain a sufficient amount of water and constant oxygen, which enhances germination by overcoming germination barriers specifically under unfavorable conditions, i.e., salinity, drought, etc. [7]. In hydropriming, different priming agents were used (i.e., salicylic acid, gibberellic acid, etc.), but the extract of leaves of many plants is found to be cheaper and more useful.

Moringa (*Moringa oleifera*) is well known as a miracle tree. It has been reported that moringa leaf extract has antioxidant properties [8]. In addition, the moringa leaf is also rich in many plant growth promoters such as cytokinin and zeatin [9,10]. Zeatin stimulates cell division and cell elongation. It boasts several enzymes' antioxidant properties and protects the plants' cells from the aging effects of the reactive oxygen species [11,12]. Therefore, the current study was aimed to investigate the potential effect of hydropriming on wheat seeds in moringa leaf extract and their responses on seed germination, growth, and nutrient uptake in leaves and roots of wheat seedlings under different salt (NaCl) stress conditions.

2. Material and Methods

2.1. Moringa Leaf Extract Preparation

Moringa leaves were collected from the Qatar University Agriculture Research Station located 60 km north of the State of Qatar in Rawdat Al-Faras. Fresh moringa leaves were collected, washed three times with distilled water, and dried in the air-dried oven at 45 °C for the next 48 h. The dried leaves were cut into small pieces before being ground into a fine powder. About 10 g of dried leaves powder was soaked into 50 mL polypropylene conical centrifuge tubes (Falcon 50 mL, Fisher Scientific, Pittsburgh, PA, USA) with distilled water and shacked for the next 48 h at lab conditions. The solution was centrifuged (SL 8 Benchtop Centrifuge, Thermo Fisher, Loughborough, UK) at 5000 rpm for 30 min. After that, the supernatant was filtered using a vacuum filter unit (1000 mL Buchner apparatus). The extract brownish and dark color solution was stored in the fridge at 4 °C until required.

2.2. Seed Germination and Seedling Growth

Sodium chloride (ACS reagent > 99%, Merck, Kenilworth, NJ, U.S.) was used to prepare saline solutions in distilled water. Five treatments, i.e., 0 M, 0.05 M, 0.1 M, 0.15 M and 0.2 M of NaCl were examined. Wheat seeds of *Triticum aestivum* (Cultivar Norin-*61*) were obtained from Tottori University-Japan. The healthy seeds with uniform size were sterilized with 20% (v/v) sodium hypochlorite for two min before washing three times with double distilled water. The wheat seeds were soaked in moringa leaf extract and water for 8 h and then placed in Petri dishes to germinate. A completely randomized design (CRD) with three replication was applied for this study.

The germination test was performed using Petri dishes (150 × 15 mm, Falcon) lined with Whatman filter paper No. 1 in a controlled growth chamber (day/night cycle of 16/8 h and R-humidity 50–60% at 25 ± 2 °C) for seven days. Twenty seeds were distributed on each dish with 30 mL of saline dilution treatments with three replicates. After maximum elongation, the germination parameters (germination percentage (*GP*), germination index (*GI*), mean germination rate (*MGR*), coefficient of variation (Cv_i), and vigor index (*VI*)) were calculated according to the work of [13].

2.3. Determination of Growth

Seedling growth under different salt and moringa extract treatments was assessed in terms of roots length and shoots in addition to fresh and dry weights. Seedlings were selected randomly from control and treated samples, divided into root and shoot before measuring the fresh weight and length. The length of seedlings was determined using a Digimizer software version 5.4.7 in mm. For estimation of dry weight, seedlings were freeze-dried (SP VirTis AdVantage Pro, Stone Ridge, NY, USA) for 48 h. The weight of samples was determined by analytical balance (AS 220.R2 PLUS, RADWAG, Radom, Poland).

2.4. Determination of Macro and Micronutrients

To prepare seedlings for nutrients and metal analysis, seedlings were washed three times with distilled water, and then samples were freeze-dried. Approximately 0.15 g of dried samples were weighed and digested with 5 mL HNO_3 and 2 mL H_2O_2 at 135 °C using a hot block for 2 h to determine the macronutrients; Ca, Mg, K, P, S, and micronutrients; B, Cu, Fe, Mn, Zn, and Na, in addition to Hg, a definite amount. The volume of digested sample was maintained up to 25 mL with deionized water. The elements were measured by inductively coupled plasma-optical emission spectroscopy (OPTIMA-5300DV, Perkin Elmar, Waltham, MA, USA), and contents of Hg were determined by cold vapor atomic absorption spectroscopy (AULA-254 Gold, Mercury Instruments, Karlsfeld, Germany).

2.5. Determination of Oxidative Stress Indices; Hydrogen Peroxide

According to Velikova et al. (2000), Hydrogen peroxide (H_2O_2) content was estimated in control and treated seedling samples [14]. Approximately 0.15 g of fresh tissue was homogenized in an ice bath with 2 mL 0.1% (*w/v*) trichloroacetic acid (TCA) before centrifugation at 12,000× *g* for 20 min at 4 °C. A total of 0.5 mL of the supernatant was added to 0.5 mL 10 mM potassium phosphate buffer (pH 7.0) and 1 mL 1 M KI. The absorbance of the reaction mixture was read at 390 nm using UV-VIS spectrometry. Hydrogen peroxide concentration was calculated by using a standard curve prepared with H_2O_2 [14,15].

2.6. Statistical Analysis

Data were examined for normality and homogeneity before starting the statistical analysis. One-way analysis of variance (ANOVA) followed by Tukey test to examine the significant differences between control and other treatments at 0.05 level. Linear regression was performed on excel. Principal component analysis (PCA) was constructed on SPSS by using a correlation matrix.

3. Results

3.1. Germination

Germination percentage (GP), germination index (GI), mean of the germination rate (MGR), coefficient of variation (CVt), and vigor index (VI) were decreased gradually through treatments in both soaking methods (MLE and water). Compared to the control, germination parameters (GP) (GI), (MGR), (CVt), and (VI) were dropped with a percentage difference 37%, 63%, 32%, 1%, and 93%, respectively, at 0.2 M NaCl under the effect of moringa soaking. While under the effect of water soaking, germination parameters were dropped more than the moringa soaking method. It was reduced up to 52%, 69%, 17%, 42%, and 97%, in (GP) (GI), (MGR), (CVt), and (VI) respectively, at 0.2 M NaCl (Table 1). Significant differences ($p < 0.05$) between treatments in GP, GI, VI, fresh weight, dry weight, root length, and shoot length under both soaking methods according to the Tukey test.

Table 1. Effect of salinity stress (NaCl) on the germination of wheat primed with moringa leaf extract (MLE) and water and grown under different saline conditions.

Treatments (NaCl)	Germination (Soaking in Water)					Germination (Soaking in MLE)				
	GP (%)	GI (Day)	MGR (Day^{-1})	CVt (%)	VI	GP (%)	GI (Day)	MGR (Day^{-1})	CVt (%)	VI
Control	92 ± 10	8.17 ± 0.66	0.42 ± 0.05	72.90 ± 10.39	2127.72 ± 463.95	87 ± 5	7.72 ± 0.51	0.44 ± 0.02	55.31 ± 4.11	2158.71 ± 260.38
0.05 M	84 ± 7	6.60 ± 0.53	0.42 ± 0.06	48.63 ± 10.91	1228.63 ± 177.21	77 ± 7	4.13 ± 0.70	0.30 ± 0.04	54.40 ± 9.09	1040.07 ± 60.39
0.15 M	49 ± 13	2.87 ± 0.65	0.37 ± 0.04	38.83 ± 7.61	465.05 ± 140.48	67 ± 5	3.98 ± 0.49	0.36 ± 0.03	35.91 ± 3.11	624.96 ± 112.75
0.1 M	58 ± 4	3.49 ± 0.55	0.36 ± 0.05	40.61 ± 12.17	306.18 ± 10.38	57 ± 12	3.20 ± 0.83	0.33 ± 0.03	49.88 ± 13.42	390.79 ± 104.77
0.2 M	44 ± 18	2.55 ± 1.06	0.35 ± 0.06	42.46 ± 20.77	59.83 ± 53.19	55 ± 10	2.86 ± 0.69	0.30 ± 0.05	54.61 ± 13.42	148.92 ± 81.19

GP = germination percentage; GI = germination index; MGR = mean germination day; CVt = coefficient of variance; VI = vigor index. ± standard deviation.

3.2. Growth

Increasing the salinity (NaCl) stress levels declined the growth parameters of the wheat seedlings that were soaked in water and leaf extract of *Moringa oliefera*. Even though seeds were soaked in moringa leaf extract represented even better growth than those soaked in water. Compared to the control, fresh (FW) and dry weight (DW) was dropped steadily with percentage difference up to 77% and 72%, respectively, and length of roots and shoots by 96% and 86%, at 0.2 M NaCl under the effect of moringa soaking. Instead, Fresh (FW) and dry weight (DW) were dropped steadily up to 93% and 50%, respectively, and length of roots and shoots by 97% and 89%, at 0.2 M NaCl under the effect of water soaking (Table 2).

Table 2. Effect of salinity stress (NaCl) on the growth of wheat primed with moringa leaf extract (MLE) and water and grown under different saline conditions.

Treatments (NaCl)	**Growth (Soaking in Water)**				**Growth (Soaking in MLE)**			
	FW (g)	**DW (g)**	**RL (cm)**	**SL (cm)**	**FW (g)**	**DW (g)**	**RL (cm)**	**SL (cm)**
Control	1.74 ± 0.15	0.22 ± 0.02	63.92 ± 19.3	166.3 ± 19.0	2.31 ± 0.58	0.29 ± 0.03	95.8 ± 18.9	152.58 ± 11.9
0.05 M	0.92 ± 0.34	0.11 ± 0.04	21.4 ± 3.1	125.5 ± 10.8	1.32 ± 0.39	0.25 ± 0.06	18.01 ± 3.38	118.4 ± 12.4
0.15 M	0.36 ± 0.16	0.06 ± 0.02	3.9 ± 1.5	52.2 ± 5.7	0.70 ± 0.49	0.22 ± 0.17	6.53 ± 1.70	62.74 ± 13.3
0.1 M	0.22 ± 0.14	0.04 ± 0.02	10.8 ± 3.9	85.2 ± 14.1	0.81 ± 0.40	0.24 ± 0.13	10.0 ± 2.97	83.84 ± 13.2
0.2 M	0.12 ± 0.05	0.11 ± 0.08	2.23 ± 1.3	18.29 ± 11.9	0.53 ± 0.36	0.08 ± 0.06	4.1 ± 0.93	21.97 ± 11.9

FW = fresh weight; DW = dry weight; RL = root length; SL = shoot length; ± standard deviation.

3.3. Macronutrients

Seedlings treated with moringa leaf extract showed a reduction in K, Ca, Mg, P, and S by 76%, 81%, 45%, 38%, and 29% in roots and K, Mg, and S by 52%, 27%, and 19% in shoots, respectively compared to the control (Table 3). In contrast, Ca and P were enhanced by 40%, 37% in shoots. On the other hand, seedlings soaked in water only showed a decreased content in K, Mg, and S by 45%, 5%, 8% in roots and by 77%, 33%, 16% in shoots, while Ca and P have a stimulatory effect by 81%, 20%, and 113%, 42% in roots and shoots, respectively compared to the control (Table 3).

3.4. Micronutrients

The micronutrient's concentrations of Fe, B, Mn, and Zn in seedlings treated with MLE declined by 46%, 65%, 97%, and 109%, respectively, while Cu showed enhancement by 38% in roots, compared to the control values (Table 4). In contrast, Fe, B, and Cu concentrations improved gradually within treatments by 15%, 166%, and 31%, respectively, while Zn and Mn were dropped by 22%, 64% in shoots compared to the control (Table 4). The concentrations of Fe, Mn, and Zn in seedlings that soaked in water dropped by 7%, 52%, and 23% in roots, respectively, while B and Cu indicated improvement by 46%, 57% in roots, compared to the control (Table 4). Conversely, Fe, B, Zn, and Cu content enhanced steadily within treatments by 41%, 132%, 45%, and 120%, respectively. In comparison, Mn dropped by 66% in shoots compared to the control, although the maximum concentrations of the micronutrients were observed in shoots at 0.1 M NaCl level (Table 4).

Table 3. Mean values of macronutrients (mg/Kg) in wheat primed with water and moringa leaf extract (MLE) under different saline conditions.

Treatments (NaCl)		Macronutrients (Soaking in Water)					Macronutrients (Soaking in MLE)				
		K	Ca	Mg	P	S	K	Ca	Mg	P	S
Roots	Control	5936.30 ± 134.90	1149.25 ± 25.15	2338.48 ± 47.20	5879.25 ± 34.88	3284.83 ± 18.73	6698.21 ± 99.74	1185.17 ± 17.41	2312.42 ± 33.16	6109.07 ± 67.53	2703.44 ± 19.64
	0.05 M	5387.50 ± 195.64	849.33 ± 34.91	3055.41 ± 116.35	7224.52 ± 81.25	3516.45 ± 93.39	5200.03 ± 22.23	765.14 ± 4.54	2281.59 ± 8.65	6412.63 ± 60.94	2730.08 ± 8.56
	0.1 M	6550.47 ± 162.17	1944.82 ± 50.04	2527.56 ± 59.78	8184.88 ± 175.87	3784.04 ± 75.38	4224.73 ± 15.65	574.94 ± 1.26	2206.44 ± 4.95	6049.89 ± 116.07	2675.15 ± 42.18
	0.15 M	5160.74 ± 223.33	955.82 ± 37.89	2530.00 ± 94.07	7530.22 ± 148.24	3365.00 ± 35.31	4795.13 ± 19.91	598.58 ± 1.55	2011.18 ± 4.74	5781.52 ± 120.10	2597.09 ± 14.67
	0.2 M	3742.33 ± 77.69	2729.63 ± 59.54	2230.23 ± 38.03	7156.66 ± 157.11	3030.40 ± 70.09	3014.70 ± 58.15	501.11 ± 11.34	1466.79 ± 18.23	4163.86 ± 48.69	2013.21 ± 33.62
Shoots	Control	8484.92 ± 117.16	1607.88 ± 17.12	3104.31 ± 26.98	7450.38 ± 123.50	3985.20 ± 15.25	8222.75 ± 122.19	1418.96 ± 27.51	1760.37 ± 30.11	4944.41 ± 23.62	3005.07 ± 15.30
	0.05 M	7881.03 ± 136.07	757.86 ± 9.26	1468.28 ± 17.14	6812.66 ± 145.95	4202.46 ± 36.56	7945.67 ± 60.19	1046.09 ± 13.38	1447.22 ± 14.33	5777.16 ± 102.46	3273.34 ± 72.39
	0.1 M	10,654.71 ± 213.16	4031.75 ± 108.80	2143.13 ± 14.38	11,174.58 ± 281.59	5050.39 ± 229.14	5372.57 ± 22.30	561.76 ± 4.53	1139.84 ± 4.75	4940.49 ± 30.86	2390.87 ± 33.75
	0.15 M	7594.40 ± 100.63	1537.39 ± 21.95	1835.31 ± 27.25	8259.17 ± 173.49	3795.11 ± 76.60	6844.69 ± 100.74	1018.20 ± 21.59	1159.64 ± 20.19	5720.02 ± 49.76	3023.21 ± 16.21
	0.2 M	3749.04 ± 158.77	5798.88 ± 127.11	2220.99 ± 7.95	11,395.67 ± 314.41	3409.75 ± 158.12	4806.81 ± 135.61	2122.32 ± 7.84	1343.90 ± 27.44	7186.65 ± 132.31	2494.86 ± 69.27

K = potassium; Ca = calcium; Mg = magnesium; P = phosphorous; S = sulfur.

Table 4. Mean values of micronutrients (mg/Kg) in wheat primed with water and moringa leaf extract (MLE) under different saline conditions.

Treatments (NaCl)		Micronutrients (Soaking in Water)					Micronutrients (Soaking in MLE)				
		Fe	B	Mn	Zn	Cu	Fe	B	Mn	Zn	Cu
Roots	Control	90.36 ± 1.66	17.06 ± 0.10	84.56 ± 2.06	158.94 ± 4.35	14.38 ± 0.28	76.19 ± 0.76	15.41 ± 0.62	77.71 ± 0.81	220.04 ± 1.93	12.12 ± 0.20
	0.05 M	76.05 ± 1.75	17.21 ± 0.32	88.57 ± 0.93	135.14 ± 1.43	13.63 ± 0.26	60.59 ± 1.00	6.94 ± 0.37	53.40 ± 0.64	104.73 ± 0.93	11.41 ± 0.22
	0.1 M	87.72 ± 2.00	54.78 ± 1.82	63.40 ± 1.20	148.55 ± 3.05	22.93 ± 0.16	60.08 ± 1.20	15.59 ± 0.18	47.83 ± 0.85	89.58 ± 0.88	11.47 ± 0.17
	0.15 M	98.13 ± 0.50	27.35 ± 1.42	56.98 ± 1.06	110.18 ± 1.74	16.65 ± 0.74	61.28 ± 0.60	23.80 ± 0.56	41.10 ± 0.69	85.47 ± 2.08	11.03 ± 0.05
	0.2 M	84.34 ± 2.81	27.18 ± 1.28	49.52 ± 0.90	125.47 ± 2.98	25.78 ± 1.38	47.61 ± 0.56	7.84 ± 0.24	27.04 ± 0.32	65.17 ± 0.74	8.79 ± 0.42
Shoots	Control	114.69 ± 1.47	14.56 ± 0.33	76.05 ± 0.94	109.42 ± 1.89	15.30 ± 0.17	99.44 ± 0.19	12.22 ± 0.33	42.55 ± 0.21	140.02 ± 1.05	13.00 ± 0.07
	0.05 M	93.68 ± 0.62	18.68 ± 0.22	34.61 ± 0.22	94.00 ± 0.71	17.91 ± 0.11	96.77 ± 2.82	21.35 ± 0.48	27.11 ± 0.37	125.06 ± 2.35	17.86 ± 0.10
	0.1 M	106.08 ± 1.74	51.3 ± 1.48	41.91 ± 1.13	189.63 ± 4.87	44.62 ± 1.79	75.07 ± 1.27	28.03 ± 0.50	20.92 ± 0.07	70.02 ± 0.57	12.45 ± 0.34
	0.15 M	118.03 ± 2.15	27.35 ± 1.42	29.00 ± 0.60	98.51 ± 1.61	15.63 ± 0.73	78.59 ± 0.52	81.13 ± 0.93	19.06 ± 0.25	73.41 ± 0.90	18.52 ± 0.46
	0.2 M	174.74 ± 4.21	71.40 ± 7.07	38.52 ± 0.79	173.48 ± 5.20	61.08 ± 5.01	115.32 ± 2.64	133.28 ± 3.66	21.82 ± 0.39	112.67 ± 2.01	15.51 ± 0.36

Fe = iron; B = boron; Mn = manganese; Zn = zinc; Cu = copper. ± standard deviation.

3.5. H_2O_2 and Na^+ Contents

The H_2O_2 content in shoots and roots of wheat seedlings was increased by increasing salt concentration (Figure 1). The H_2O_2 was observed more in the shoots and roots of wheat seedlings when treated with water as compared to when treated with moringa leaf extract (Figure 1).

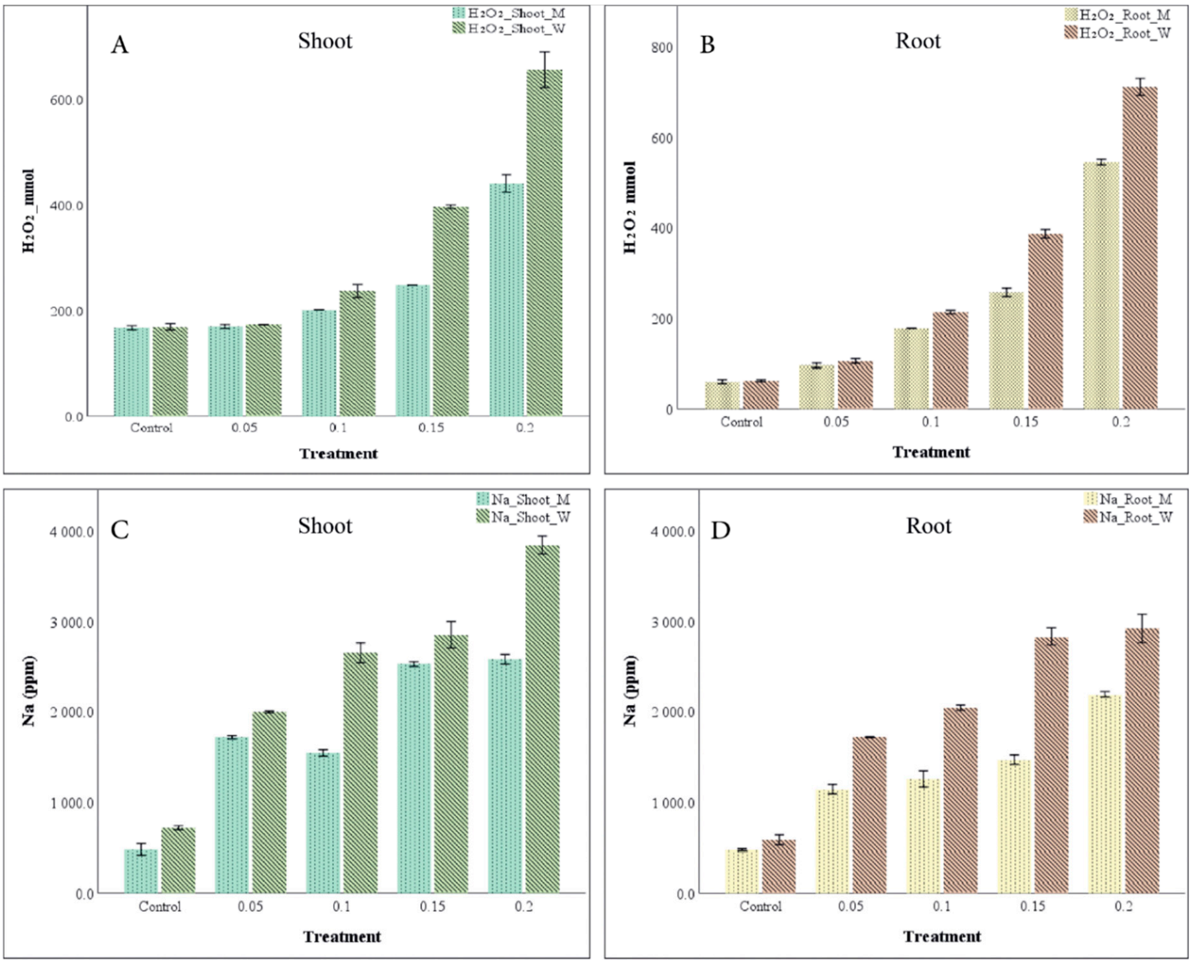

Figure 1. The effect of salt stress on wheat seedlings treated with moringa leaf extract and water on hydrogen peroxide and sodium content in leaves and roots. (**A**) Hydrogen peroxide in shoots; (**B**) hydrogen peroxide in roots; (**C**) Na in shoots; (**D**) Na in roots. Light color bar = moringa leaf extract; dark color bar = water.

Similarly, Na concentration was also increased under different saline conditions. The shoots and roots of wheat seedlings soaked in water exhibited more Na as compared to soaked in moringa leaf extract.

3.6. Linear Regression and PCA

The linear regression of Na and H_2O_2 was constructed and shown in Figure 2. The linear regression of Na and H_2O_2 indicated that by increasing the Na concentration, the production of H_2O_2 also increased, and the steep of the regression was higher in the shoots and roots of seeds treated with water as compared to moringa leaf extract (Figure 2).

PCA was applied on the 11 nutrient concentrations, in addition to H_2O_2, GP, and VI in to investigate the effect of factors on the wheat roots and shoots soaked in water and *Moringa oleifera* leaf extract individually, under the stress of saline (Figure 3, Table 5). The first two principal components (PCs) explained 46.95% (PC1), 31.88% (PC2) in roots, and 43.92% (PC1), 29.88% (PC2) in shoots (Figure 3a,b). PC1 in root treatments is robustly weighted in P, Cu, B, S, Fe, Ca, Na, and Mg, contributing 46.95% to the total variance, VI, Mn, Zn, GP, K, and H_2O_2 dominated PC2, contributing 31.88% to the total variance (Table 5). The data set showed, two independent components were extracted with a cumulative percent of 78.82% and 73.81% of the total variance in seedlings root and shoot, respectively, under different treatments. The first component in Table 5 demonstrated significantly strong positive loadings of P (0.92), Cu (0.91), B (0.87), S (0.84), Fe (0.77), and Ca (0.75), and moderate positive loadings of Na (0.67), and Mg (0.58). In contrast, the second component of the roots data set showed significantly strong positive loadings of VI (0.95), Mn (0.91), Zn (0.85), GP (0.82), K (0.77), and H_2O_2 (0.76). In the same manner, the shoots data set has two independent components (Table 5). The first component demonstrated significantly strong positive loadings of P (0.95), Fe (0.94), Ca (0.87), Zn (0.87), Cu (0.85), B (0.75), S (0.71), and moderate positive loading of Mg (0.58). In contrast, PC2 explained significantly strong positive loadings of VI (0.93), GP (0.82), Mn (0.79), K (0.72), although moderate negative loadings were characterized in Na (−0.76), H_2O_2 (−0.50).

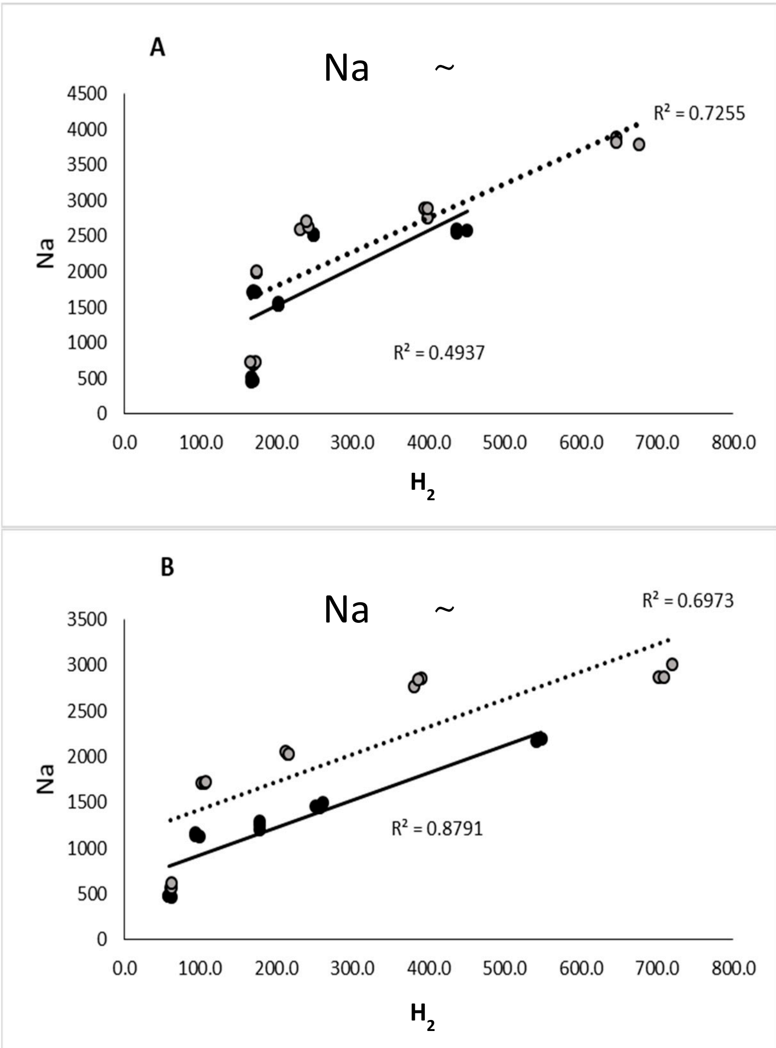

Figure 2. The linear regression shows the interaction between sodium (Na) and hydrogen peroxide (H_2O_2) in shoots (**A**) and roots (**B**) of wheat seedlings treated with moringa leaf extract (black filled, solid line) and water (gray filled, dotted line) under different saline conditions.

Table 5. PCA for germination, nutrients, toxic ions and reactive oxygen species in roots (a) and shoots (b) of wheat soaked in moringa leaf extract and water under NaCl stress.

Component	Total Variance Explained in Roots						Total Variance Explained in Shoots					
	Initial Eigenvalues			Rotated Component Matrix			Initial Eigenvalues			Rotated Component Matrix		
	Total	Variance %	Cumulative %	Variable	Loading PC1	Loading PC2	Total	Variance %	Cumulative %	Variable	Loading PC1	Loading PC2
1	6.572	46.946	46.946	P	0.918		6.150	43.929	43.929	P	0.945	
2	4.462	31.875	78.821	Cu	0.907		4.184	29.883	73.812	Fe	0.943	
3	1.500	10.717	89.538	B	0.876		1.917	13.693	87.505	Ca	0.874	
4	0.619	4.418	93.955	S	0.842		0.725	5.175	92.680	Zn	0.873	
5	0.340	2.430	96.385	Fe	0.768		0.527	3.766	96.446	Cu	0.850	
6	0.181	1.295	97.681	Ca	0.752		0.235	1.679	98.125	B	0.750	
7	0.165	1.175	98.856	Na	0.668		0.128	0.911	99.036	S	0.708	
8	0.105	0.752	99.608	Mg	0.586		0.083	0.594	99.630	Mg	0.588	
9	0.022	0.157	99.765	VI		0.956	0.021	0.151	99.780	VI		0.931
10	0.014	0.096	99.862	Mn		0.906	0.019	0.133	99.913	GP		0.821
11	0.011	0.076	99.938	Zn		0.846	0.007	0.051	99.964	Mn		0.792
12	0.005	0.037	99.974	GP		0.821	0.003	0.018	99.982	Na		−0.76
13	0.003	0.018	99.993	K		0.776	0.002	0.015	99.997	K		0.722
14	0.001	0.007	100.00	H_2O_2		0.757	0.00	0.003	100.00	H_2O_2		−0.50

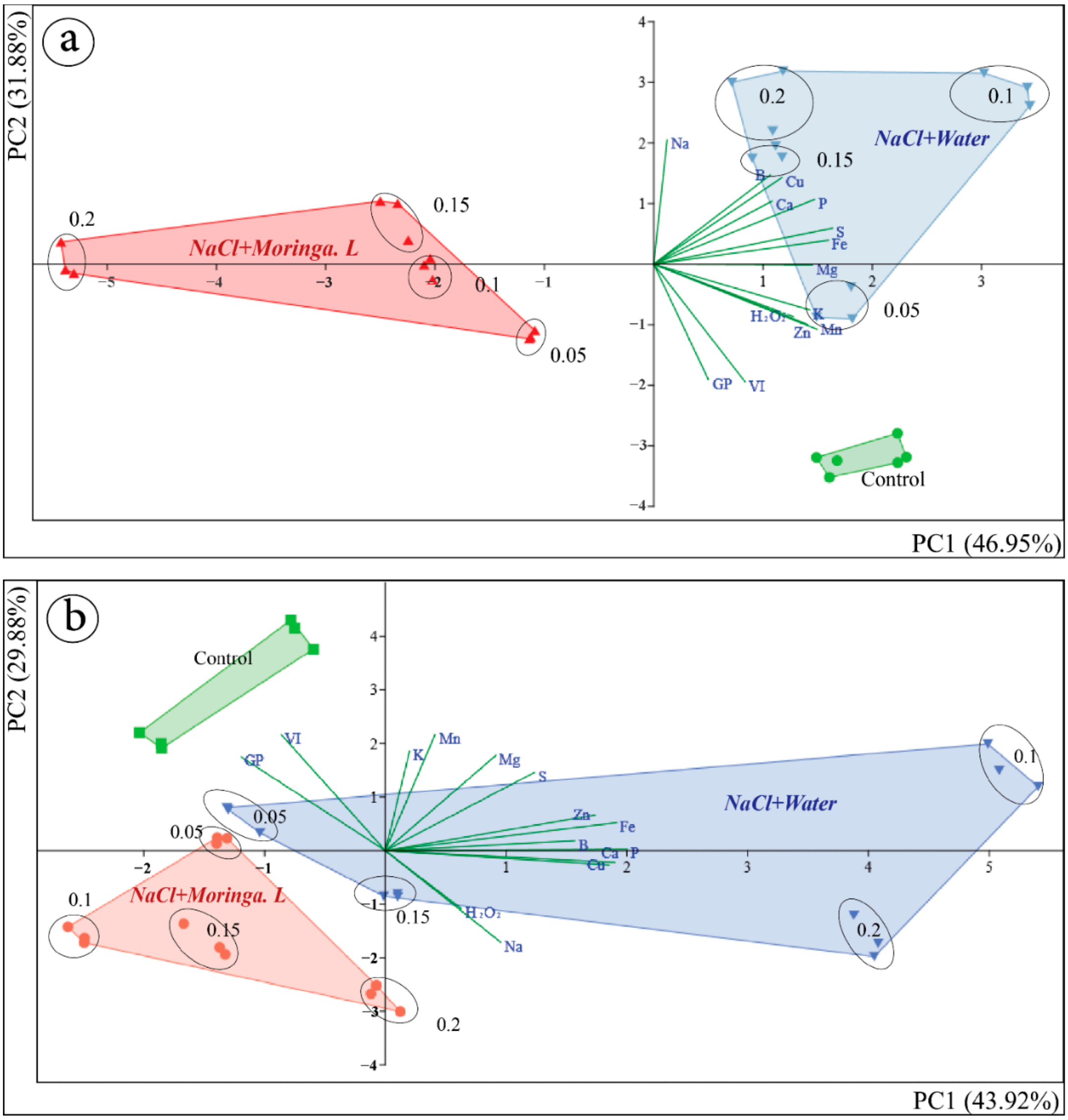

Figure 3. PCA of germination, nutrients, toxic ions, and reactive oxygen species in roots (**a**) and shoots (**b**) of wheat soaked in moringa leaf extract and water under NaCl stress.

4. Discussion

Salinity is one of the major factors that affect plant growth and development [16]. In our study, germination attributes of wheat were strictly affected by increasing the salt concentration. However, the seeds hydroprimed with moringa leaf extract showed more germination percentage, germination index, and vigor index as compared to seeds hydroprimed by water under different saline conditions (Table 1). Cytokinins have a great impact on plant germination and growth under stress conditions [17]. Moringa leaf extract has the cheapest and valuable source of cytokinins in the form of zeatin [10]. Hydropriming mainly changes physiological attributes, and accumulated ions with an ample amount of water help the plant to germinate under unfavorable conditions [18]. Hydropriming mainly increases the adenosine triphosphate (ATP) production and induced RNA activity, which helps the plant to germinate [19]. Demir and Ermis (2003) and Yagmur and Kaydan (2008) also observed similar findings on sunflower, potato seeds, and pyrethrum [20,21]. It was observed that after germination, the plant growth and development also enhanced in primed seeds as compared to non-primed seeds (Khalid et al., 2019b). The hydropriming enhances the cell division process in the apical meristem of seedlings which ultimately increases the plant growth and development [22]. In our study, the seedling growth and development (fresh weight, dry weight, root length, and shoot length) of wheat was decreased by increasing salt concentration (Table 2), but the decrease was more in plants primed with water as compared to plants primed with moringa leaf extract. Our findings are in line with Meriem et al. (2014) and Mirabi and Hasanabadi (2012), which found that primed seeds showed more growth in coriander and tomato seedlings under salinity as compared to non-primed [23,24]. Yasmeen et al. (2013) reported that by application of moringa leaf extract, wheat plants showed more growth by modulating their antioxidant defense mechanism under saline conditions [12].

Salinity affects the nutrient uptake in plants [25]. The decrease in nutrient uptake is associated with the accumulation of toxic ions. The nutrient uptake was affected in shoots and roots of wheat seedlings under different saline conditions (Table 3, 4). Seeds primed with moringa leaf extract cope with salt stress more efficiently and maintain their nutrients somehow as compared to seeds with water. The nutrients play an important role in plant different mechanisms.

Hydropriming significantly enhances the antioxidant defense mechanism under salt stress conditions [25]. The accumulation of toxic ions Na significantly decreases the uptake of K and Ca ions, which ultimately affects the physiological attributes, i.e., photosynthesis, stomatal conductance, etc. [16]. However, the different mechanisms, including maintaining the uptake of toxic ions and compartmentalization of toxic ions, are the indication of tolerance mechanisms in plants. So, in our study, seeds primed with moringa leaf extract showed less uptake of Na ions in shoots and roots of wheat seedlings. The results indicated that the tolerance mechanism of wheat seedlings primed with moringa leaf extract compartmentalizes the Na ions and restricts their uptake more efficiently as compared to the seeds primed with water. Khalid et al. (2020) also reported that restricted accumulation of toxic ions enhances the accumulation of other minerals, which maintains the plant metabolism and anchors the plant to fight more efficiently against salt stress [7]. The accumulation of toxic ions causes an imbalance in the plant nutrients, which affects its physiological attributes and production of different reactive oxygen species (ROS) [16]. H_2O_2 is one of the major ROS. Its accumulation in the cell results in disturbed cellular metabolism in mitochondria and chloroplast [26]. The degree of tolerance is identified by the H_2O_2 content in leaves and roots [12,27].

Wheat seeds treated with moringa leaf extract cope with the saline environment more efficiently by reducing the production of H_2O_2 as compared to those treated with water under different saline conditions. Similar findings were observed by the authors of [28]. Similar findings were observed that moringa leaf extract enhanced the seed germination of maize [29] and common bean [30] under salt stress condition

PCA illustrated that increasing the NaCl level (from 0.05 to 0.2 M) is often associated with decreasing germination parameters of wheat seedlings (Figure 3; Table 5). A positive relationship between nutrients and roots elongation of seeds soaked in water. However, the higher levels of NaCl (0.2 M) are associated with root inhibition due to the accumulation of Na, while roots of seeds are soaked in moringa leaf extract represents lower inhibition, especially at 0.05 and 0.1 M treatments. The contribution of shoots elongation of seeds soaked in moringa leaf extract and water. Germination parameters (GP and VI) were correlated with low levels (0.05, 0.1 M) in both treatments while increasing the level of Na associated with shoots inhibition. However, seeds treated with water showed the highest level of inhibition due to the strong accumulation of Na in shoot cells during germination of wheat seedlings.

5. Conclusions

It is concluded from the results that seed priming of wheat is very much useful to cope with the adverse saline condition (Figure 4). The right choice of primed solution will also play an important role under unfavorable conditions. Wheat seeds treated with moringa leaf extract showed more strong response by maintaining their germination and growth attributes under different saline conditions as compared to the seeds primed with water. Moringa leaf extract can restrict the accumulation of toxic ions more efficiently and decrease the production of ROS, which ultimately makes the plant tolerate adverse saline conditions. Therefore, the hydropriming of wheat seeds with moringa leaf extract will enhance their growth and development under saline conditions. Further studies on wheat seeds will be performed by hydropriming with moringa leaf extract in the open field to assess the response of mature wheat plants and the yield and quality of the grains.

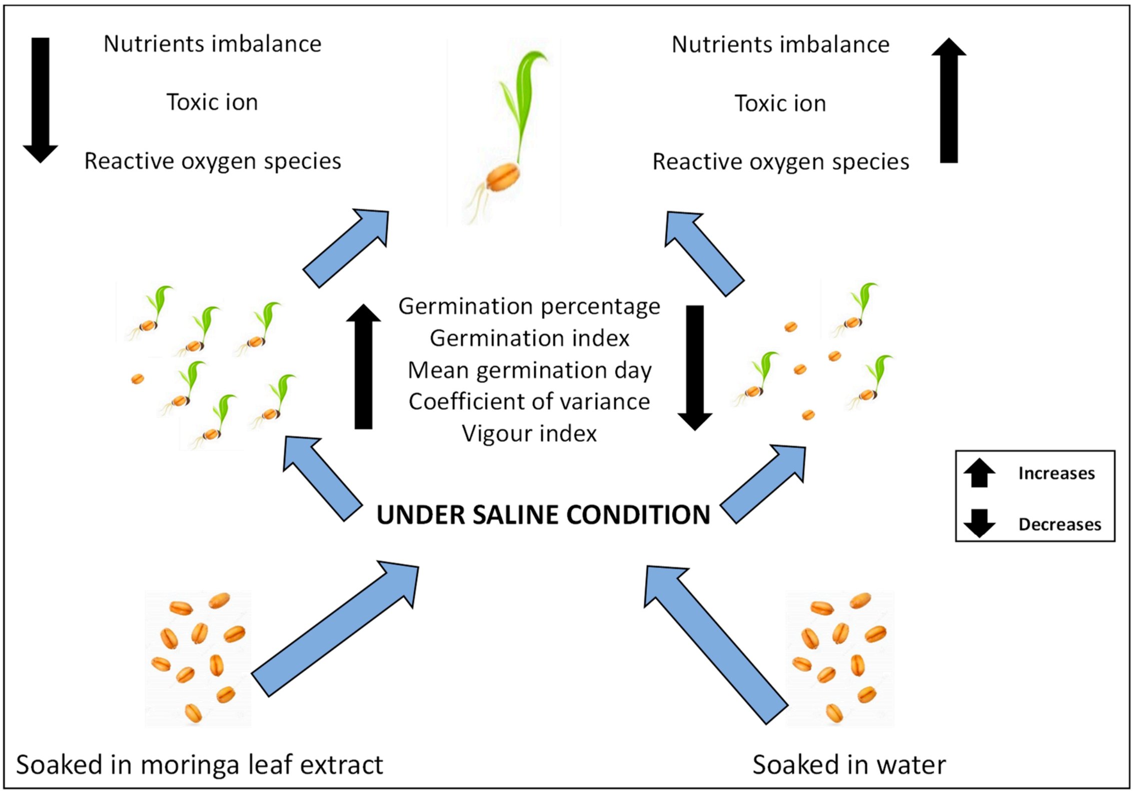

Figure 4. Schematic diagram showed the hydropriming effect of moringa leaf extract and water on wheat seeds under saline condition.

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