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Effects of electromagnetic treated saline water on potatoes (*Solanum tuberosum* L.) physiological and nutritional characteristics

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A B S T R A C T

Purpose: This work was designed to assess the physiological response of three potatoes cultivars to saline water irrigation, and the role of the electromagnetic treatment on alleviating salinity impacts on potatoes crops. Research Method: The experiment was conducted under factorial RCBD with potato varieties Spunta, Bellini and Alaska under three irrigation treatments; ground water (C), saline water (SW) and electromagnetic saline water (EMSW). We analyzed soil proprieties, minerals and water usage efficiency, plant water status, chlorophyll pigments, absiscic acid (ABA) content, and expression of StNCED gene. Findings: Data showed that EMSW promote salt leaching from soil, decrease soil salinity and improve the efficiency of water and minerals use. Leaf area, leaf dry weight, stem thickness, tuber weight, water potential, water content and water use efficiency were more disturbed under SW. The ABA content in Alaska leaves was associated with StNCED expression level. The cultivar Alaska displayed highest leaf size, SPAD and minerals use efficiency. Spunta had upmost correlation between LA and WCap and highest water use efficiency. Contrarily, Bellini manifested less water potential and ABA content. Research limitations: No limitations were found. Originality/value: Thus, Spunta and Alaska revealed better physiological and metabolic capacity to tackle salinity. The results advance knowledge on potatoes response to salinity and could improve management of saline water.



INTRODUCTION

Saline water is becoming a foremost factor hampering the productivity of cultivated soils. About 20% (45 million ha) of irrigated lands, bringing one-third of the world's food, are salt-affected. Soil salinization results in more than US\$12billion annual losses in the world due to reduced crop productivity (Jägermeyr & Frieler, 2018). These proportions may be increased by climate change, saline irrigation and poor drainage. In Tunisia, a growing volume of saline water is being used in irrigation and plenty of irrigated areas showed a rising trend to salinization (Boughdiri et al., 2018). Scarcity of rainfall and high evaporation, principally during dry months of the year caused salts to move up and accumulate in the upper layers causing compact soil structure. This may offset root expansion, water absorption and numerous physiological and metabolic functions (Akrimi et al., 2021).

Potatoes, commonly cultivated in arid and semi-arid areas, are known for their low salt requirements (1.1 dS m⁻¹ EC of irrigation water and 1.7 dS m⁻¹ in the soil) (Machado & Serralheiro, 2017). Among the known implications of using saline water on potatoes are perturbations of ions homeostasis, photosynthesis and reduction in chlorophyll content (Bhargava et al., 1995). Phytohormone levels are also affected by changes in nutrient elements (Zhang et al., 2016). In this line, Marrush et al. (1998), observed a decrease of ABA concentrations in potassium-limited sorghum plants. In contrast, elevated ABA concentrations have been reported in potato (Bhargava et al., 1995). Plant water status regulation with ABA, is another fundamental physiological process induced by genes encoding enzymes and other proteins involved in cellular dehydration (Zhang et al., 2016).

The great challenge of producing more food crops with poor water quality resources like saline water, have focused many researches on assessment of the reliability of saline water treatment options. It is acknowledged that magnetic treatment of water is very favored at industrial level, specially, to avoid scale formation in pipes. However, magnetic water remains a contentious topic in agriculture. Effects of magnetic fields on water characteristics are linked to changes on dissolved ions, hydrogen bonds, clustering of water molecules, flow rate, viscosity etc. (Amor et al., 2017). It has been reported that magnetic water increases soil aeration and infiltration, which contribute to better soil moisture and reduction of salt buildup in the root zone (Hamza, 2019). The aptitude of electromagnetic water to improve soil structure and ions availability (Akrimi et al., 2021; Zhou et al., 2021), allows suggesting that electromagnetic saline water can enhance the efficiency of water and nutrient usage. In this work, we aim to establish the role of electromagnetic saline water (irrigation and drainage) on potatoes physiological behavior. We hypothesized that electromagnetic saline water may enhance soil properties, salt leaching and therefore plant water relations and ions usage. It is our aim to appraise the role of ABA signaling on physiological and metabolic modulations of potato varieties. The response patterns of leaf area, chlorophyll content, water potential and tuber weight, to water status of aerial parts were evaluated.

MATERIALS AND METHODS

Field experiments and applied treatments

The experiment was conducted at the Regional Center of Agricultural Research of Sidi Bouzid (CRRA-Sidi Bouzid), Tunisia, during two growing seasons (October-February) 2015/2016 and 2016/2017. The mean annual precipitation was 200 mm. The mean evapotranspiration was 1200 mm. The soil texture was sandy-clay, with alkaline pH (7.7), 2.9 mS cm⁻¹ electrical conductivity (EC), 7% moisture content and 0.7 sodium absorption ratio (SAR). A factorial design was used with two factors: i) Varieties (Spunta, Bellini and



Alaska); ii) Irrigation treatments (C: ground water with 2.2 mS cm⁻¹ EC, EMSW: electromagnetic saline water with 8.5 mS cm⁻¹ EC and SW: saline water with 8.5 mS cm⁻¹EC), on the basis of a randomized complete block design (RCBD). Each plot consisted of 9 rows, 2.5 m long, with 100 cm space between rows and 40 cm distance between plants on the rows. Electromagnetic treatment of water was done with Aqua-4D[®] 60E series device, that serves to circulate water through an electro magnet tube 60E, designed for transmitting electromagnetic signals into water and connected to an electro magnet box (Command 60E Pro) pre-programmed to generate electromagnetic signals. The amount of irrigation water was calculated using crop evapotranspiration (ETc) that represent the reference evapotranspiration (ET0) multiplied by the potato crop coefficient (Kc). Values of Kc were 0.5; 1.15 and 0.75 for the beginning (Kcini), the middle (Kcmid) and the end (Kcend) of potatoes growth cycle.

Water and soil analyses

Samples of percolate water, originating from the three types of irrigation treatments (C, SW and EMSW), were taken monthly (Month 1: following one month of irrigation; Month 2: following two months of irrigation; Month 3: following three months of irrigation). Water and soil analysis were carried out according to Sparks (1996) protocols. Soil moisture content (Os) was determined by gravimetric method. Extractable ions were measured in three depths (D1: 0-30 cm, D2: 30-60 cm and D3: 60-90 cm). Sodium (Na⁺) and potassium (K⁺) contents were determined with flame photometer (Model No. PFP7 JENWAY). Bicarbonate (HCO₃-), calcium (Ca²⁺), magnesium (Mg²⁺) and chloride (Cl⁻) contents were quantified by titration method. Sulfate (SO₄²⁻) concentration was measured by colorimetric method. In fact, 5 g of air dried and sieved soil samples were added to 50 ml of distilled water. Then, the suspension was filtered and 50 ml of the filtrate was taken and added to the mixture (10 ml NaCl-HCl, 10 ml Glycerol-ethanol and 0.15 g barium chloride). Afterward, samples were stirred for about 1h and the absorbance was measured at 420 nm with spectrophotometer (Jenway 6300), using distilled water as blank. The calibration curve was drawn using standard sulphate solutions (Saxena, 2001). The pH and the electrical conductivity (EC) were measured using a pH meter "MP 22, Mettler Toledo, Switzerland" and conductivimeter "Hanna, HI8424, Canada" respectively.

Plant analysis

The efficiency of ions usage (UE) was calculated by dividing the plant dry weight (PDW) by the concentration of specific leaf element ([element]) (1):

$$UE = PDW/[element]$$
(1)

The content of Na⁺, K⁺ and Ca²⁺were measured using flame photometer (Model No. PFP7 JENWAY) and expressed in meq l^{-1} (Akrimi et al., 2021). Regarding chloride (Cl⁻) determination, powdered dry leaves were incubated overnight in a 0.1M HNO₃ and 10% glacial acetic acid solution. After centrifuging, Cl⁻ concentration was measured with chloridometer (SLAMED) (Gilliam, 1971). Nitrate content was determined using Nitrachek/Merkoquant® method. The stem thickness (ST) was calculated using the formula (2) adopted by Wang et al. (2017):

$$ST = (ST30 - ST0)/30$$
 (2)

Where ST30 and ST0 are respectively the stem thickness at day 30 after saline treatments and the stem thickness before saline treatments.

For leaf pigment determination, leaves were digested in acetone 80%, and the extracts were centrifuged for 5 min at $1.500 \times g$. The pellet was re-extracted and centrifuged again, until the supernatant become colorless. The obtained supernatants were pooled and the absorbance was recorded at 452, 644 and 663 nm. Concentrations of leaf pigments were calculated using the following equations (3-6) (Lin et al. 2006):

Chla (
$$\mu$$
g g - 1 FW) = (10.3 A663 - 0.918 A644) × V/1000 × FW (3)

Chlb (
$$\mu$$
g g - 1 FW) = (19.7 A644 - 3.87A663) × V/1000 × FW (4)

Carotenoids (µg g - 1 FW) = 4.2 A452 × $\frac{V}{1000}$ × FW - (0.026 chla + 0.42 chlb) (5)

Porphyrine ($\mu g g - 1 FW$) = [Chla] + [Chlb] + [Carotenoids] (6)

Were A is the absorbance, V: final volume of leaf extract, FW: leaf fresh weight. Leaf electrolyte leakage (EL) was calculated with the following formula (7):

$$EL = \left(\frac{EC1}{EC2}\right) \times 100 \tag{7}$$

Where EC1 and EC2 are the EC measured after incubation of leaves in a 32° C water bath for 2 h, and the EC after autoclaving at 121°C for 20 min respectively (Daneshmand et al., 2010).

For ABA determination, powdered leaves were extracted in distilled autoclaved water with constant shaking at 4°C. The supernatant was collected after centrifugation $(10.000 \times \text{g}$ for 10 min) and diluted with TBS buffer. Then ABA was analyzed using Phytodetek ABA test kit (Agdia, Elkhart, IN, USA) by indirect enzyme-linked assay (ELISA). Color absorbance following reaction with substrate was determined at 405 nm using a plate autoreader (1420 Multilabel Counter Victor3TM, PerkinElmer) (Ruggiero et al., 2019). Leaf area (LA) was determined using leaf area meter (ADC-Biosciebtific Ltd, Hoddesdon, UK).

Total chlorophyll content (SPAD) was determined with chlorophyll meter (SPAD-502, Minolta, Japan). Water use efficiency (WUE) was calculated (8) as the ratio of potato yield (Y) to total water received (TWR):

WUE =
$$\left(\frac{Y}{TWR}\right)$$
 (Akrimi et al., 2021) (8)

Water content of aerial parts (Leaves and stems) (WC_{ap}) was calculated according to the following formula (9):

$$WCap(\%) = \left[(FW - DW) / FW \right] \times 100 \tag{9}$$

Where FW and DW are the fresh and dry weight of leaves and stems (Trifilò et al., 2022). Leaf water potential (Ψ_w), was measured with pressure chamber (*Scholander* model M-1000).

Tuber dry weight was determined after drying in an oven at 75 °C until weight stabilization.

The RNA was extracted from potato leaves using Trizol method and quantified by ND-1000 spectrophotometer (NanoDropTechnologies). The cDNA was synthesized from 1 μ g RNA using Superscript reverse transcriptase (invitrogen) in a 20 μ l reaction volume. The program for quantitative real-time PCR (qPCR) was set to 35 cycles of 5 minutes at 94 °C, 30



seconds at 94 °C, 30 seconds at 58 °C, 45s at 72 °C and 10 minutes at 72 °C with ABI 7900 HT (Applied Biosystems, Foster City, CA, USA). PCR reactions were carried out in 96-well optical reaction plates. Reaction included 4.5 μ l of 1:20 diluted cDNA, 4.28 μ M of primer (Forward primer: CATAATCGAAAACCCGGATG; Reverse primer: AACTTTTGGCCATGGTTCAG) of the *StNCED* gene and 6.25 μ l Sybr Green (Akrimi et al., 2021).

Statistics

Statistical analysis was performed using SPSS 20.0. Variables were subjected to two way analysis of variance (ANOVA). Means were compared using LSD's test at $p \le 0.05$. Linear regression and curve estimation were analyzed using a probability value of 0.05 as the benchmark of significance.

RESULTS

Soil characteristics

The soil EC in the three depths was set in the following decreasing trend Soil_{SW}>Soil_{EMSW}>Soil_C (Table 2). The ANOVA results revealed that changes in soil EC were significantly ($p \le .001$) dependent on irrigation treatments (IT) and soil depth (D), as least values were ascribed to C treatment and deep layer (D3: 60-90 cm). Given that, intermediate and highest values of EC were registered under EMSW and SW respectively. Meanwhile, pH was not influenced by D and IT (Table 1). Regarding soil moisture (Θ s), registered proportions were significantly higher under EMSW compared to SW, though C treatment maintains highest Θ level. The Θ s was always maintained above 5% within C and EMSW ensuring better soil moisture conditions. The ANOVA results depicted in table 1 revealed that soil Θ s was not affected by depth. Moreover, IT influenced significantly soil ions content except HCO₃⁻. The highest values of Na⁺, SO4²⁻ and Cl⁻ were recorded for soil irrigated with SW. Though, K⁺, Ca²⁺ and Mg²⁺ concentrations were higher in soil irrigated with EMSW. The ANOVA results revealed that all analyzed ions were significantly influenced by depth (Table 1).

Drained water characteristics

Results in Table 4 revealed that the EC and concentrations of Na⁺, Mg²⁺, SO₄²⁻ and Cl⁻were higher in water percolated from EMSW irrigated soil. Additionally, Na⁺, K⁺ and HCO₃⁻ concentrations were more important after the third month of irrigation. ANOVA results (Table 3) demonstrated that pH, Cl⁻, SO₄²⁻ and Mg²⁺ values were significantly affected by M×IT interaction. In effect, registered values were in the following order EMSW>SW>C for the three months of study. Meanwhile, K⁺ and HCO₃⁻ contents were not changed by IT.

Table 1. Analysis of variance for the effect of depth (D), irrigation treatment (IT) and their interactions on soil proprieties.

	θs	pН	EC	Na^+	\mathbf{K}^+	Ca ²⁺ and	SO_4^{2-}	HCO3 ⁻	Cl-
		_				Mg^{2+}			
D	0.89 ^{ns}	0.22 ^{ns}	32.73***	33.14***	11.86***	14.48***	17.75***	8.34***	26.56***
IT	5.78**	0.15 ^{ns}	220.43***	54.92***	63.32***	1.80^{***}	9.97***	2.34 ^{ns}	28.41***
D×IT	0.78 ^{ns}	0.049 ^{ns}	1.66 ^{ns}	0.84 ^{ns}	1.69 ^{ns}	0.70 ^{ns}	3*	0.26 ^{ns}	4.12**

ns: not significant, $p \le 0.05$, $p \le 0.01$, $p \le 0.01$.



• *				· · · · · · · · · · · · · · · · · · ·
		Soilc	Soilsw	Soilemsw
Os (%)	D1	7.61±0.33 ^a	4.08±0.44°	5.53±0.08 ^b
	D2	5.59±0.41 ^a	4.08±0.63 ^b	5.81±0.21 ^a
	D3	5.64±0.33 ^a	4.09±0.45 ^b	5.06±0.08 ^a
EC (dSm ⁻¹)	D1	4.57±0.00°	8.45±0.02 ^a	7.22±0.41 ^b
	D2	3.51±0.28°	7.64±0.15 ^a	6.44±0.01 ^b
	D3	3.49±0.28°	7.31±0.15 ^a	6.24±0.01 ^b
pH	D1	8.14 ± 0.10^{a}	8.29±0.01 ^a	8.14±0.00 ^a
	D2	8.30±0.02 ^a	8.35±0.02 ^a	8.25±0.00 ^a
	D3	8.33±0.02 ^b	8.35±0.02 ^a	8.35±0.00 ^a
Na ⁺ (meql ⁻¹)	D1	3.03±0.10°	4.50±0.21 ^a	3.36±0.22 ^b
	D2	1.93±0.10°	3.63±0.24 ^a	2.70±0.30 ^b
	D3	1.80 ± 0.07^{b}	3.40±0.06 ^a	1.94 ± 0.46^{b}
K^{+} (meq l ⁻¹)	D1	2.53±0.14 ^a	1.30±0.07°	2.03±0.10 ^{ab}
	D2	1.96±0.09 ^a	1.10 ± 0.18^{b}	1.73±0.04 ^a
	D3	1.87 ± 0.04^{a}	1.13±0.07 ^b	1.77±0.04 ^a
Ca^{2+} and Mg^{2+} (meq l ⁻¹)	D1	11.07±0.81ª	8.53±0.91 ^b	9.73±0.59 ^b
	D2	10.27±0.65 ^a	7.60±0.28°	10.40±0.37 ^b
	D3	7.60±0.37 ^a	6.00±0.49 ^b	7.87 ± 0.39^{a}
SO_4^{2-} (meq l^{-1})	D1	12.06±0.94°	15.77±1.20 ^a	13.10±0.55 ^b
	D2	7.69±1.29°	13.68±0.47 ^a	8.17±0.54 ^b
	D3	8.32 ± 0.76^{b}	10.00±0.60 ^a	9.98±0.15 ^a
HCO_3^{-} (meq l^{-1})	D1	10.66±0.81 ^a	9.33±1.16 ^a	10.66±1.14 ^a
	D2	8.66±0.81 ^a	6.00±0.70 ^a	7.33±0.81 ^a
	D3	8.00 ± 0.70^{a}	6.00±0.70 ^a	6.10 ±0.81 ^a
$Cl^{-}(meq l^{-1})$	D1	53.33±4.08°	76.66±4.08 ^a	63.44 ±3.22 ^b
	D2	33.33±4.08°	63.33±4.08 ^a	53.33±3.33 ^b
	D3	33.33±5.40°	53.33±3.33ª	36.66±3.33 ^b

Table 2. Physico-chemical proprieties of soil irrigated with ground water (Soil_C), saline water (Soil_{SW}) and electromagnetic saline water (Soil_{EMSW}) after three months of irrigation in three depths (D1: 0-30 cm, D2: 30-60 cm and D3: 60-90 cm).

Means \pm SE (n = 4). Different letters indicate significant differences between irrigation treatments, in the same depth, according LSD's test.

Table 3. Analysis of variance of the effect of month of irrigation (M), irrigation treatment (IT) and their interactions on percolating water characteristics.

	pН	EC	Na ⁺	K^+	Ca ²⁺	Mg ²⁺	SO4 ²⁻	HCO3 ⁻	Cl
М	7.17^{***}	18.02***	3.20 ^{ns}	48.10^{***}	17.33***	18.79***	32.96***	16.88^{***}	7.81***
IT	1.51 ^{ns}	99.26***	161.63***	3.24 ^{ns}	15.99^{***}	15.30***	314.77***	0.55 ^{ns}	51.26***
M×IT	9.03***	1.86 ^{ns}	0.53 ^{ns}	2.15 ^{ns}	2.28 ^{ns}	3.59^{*}	27.95***	0.55 ^{ns}	8.40^{***}
	· C* · · * ·	0 05 ** <0	01 **** < 01	0.1					

ns: not significant, $p \le 0.05$, $p \le 0.01$, $p \le 0.01$.

Ions and water usage efficiency

From Table 5 and 7, ions usage efficiency, in response to irrigation treatments, differed among assessed varieties, as indicates the significant effect of irrigation treatment, variety and their interactions. Alaska had highest ions UE. The UE of Na⁺, Ca²⁺, Cl⁻ and NO₃⁻ were least under SW. However, K⁺UE was enhanced with EMSW only in Alaska. There is also a clear variation in ions UE between studied elements. In fact, Na⁺ UE was higher than other studied elements; NO₃⁻ UE was higher than Cl⁻UE while K⁺, Cl⁻, and Ca²⁺ had comparable values. The antagonism between Cl⁻ and NO₃⁻ accumulation may reflect the osmoregulatory role of Cl⁻ in potato leaves, whereas NO₃⁻ may be allocated to plant organs. ANOVA analysis revealed that, WUE was significantly (p≤0.05) least under SW and in Bellini variety. Indeed, Spunta had highest WUE.



		С	SW	EMSW
EC (dSm ⁻¹)	Month 1	$5.41 \pm 0.47^{\circ}$	9.66 ± 0.34^{b}	13.07 ± 1.50^{a}
	Month 2	6.78 ± 0.62^{b}	10.89 ± 1.12^{a}	13.27 ± 1.95^{a}
	Month 3	$7.05 \pm 1.24^{\circ}$	14.57 ± 0.89^{b}	17.59 ± 0.07^{a}
pH	Month 1	7.56 ± 0.00^{a}	7.46 ± 0.00^{b}	$7.05 \pm 0.00^{\circ}$
	Month 2	7.11 ± 0.08^{a}	6.99 ± 0.19^{a}	7.18 ± 0.02^{a}
	Month 3	6.67 ± 0.27^{b}	7.31 ± 0.13^{a}	7.23 ± 0.01^{a}
Na ⁺ (meql ⁻¹)	Month 1	8.46 ± 0.21^{c}	39.33 ± 0.81^{b}	49.66 ± 4.72^{a}
	Month 2	$12.23\pm3.43^{\rm c}$	39.23 ± 5.04^{b}	48.66 ± 1.72^{a}
	Month 3	13.56 ± 3.45^{b}	48.66 ± 2.04^{a}	53.00 ± 3.53^{a}
K ⁺ (meql ⁻¹)	Month 1	1.55 ± 0.21^{b}	2.94 ± 0.07^a	3.00 ± 0.07^{b}
	Month 2	7.14 ± 0.28^{a}	6.23 ± 0.37^a	6.86 ± 0.80^{a}
	Month 3	9.00±0.70 ^b	8.66±1.35 ^b	13.15 ± 1.08^a
$Ca^{2+}(meql^{-1})$	Month 1	20.13 ± 2.47^{a}	15.20 ± 3.18^{a}	22.13 ± 4.71^{a}
	Month 2	37.33 ± 4.32^a	22.26 ± 3.11^{b}	39.20 ± 5.16^a
	Month 3	38.80 ± 4.82^{a}	24.26 ± 3.46^{b}	32.93 ± 3.40^a
$Mg^{2+}(meql^{-1})$	Month 1	15.40 ± 3.20^{b}	15.33 ± 1.77^{a}	18.53 ± 2.16^a
	Month 2	10.80 ± 2.31^{b}	23.13 ± 1.75^{a}	28.53 ± 1.31^{a}
	Month 3	$6.06 \pm 1.68^{\circ}$	22.00 ± 5.65^{b}	38.00 ± 4.67^{a}
$SO_4^{2-}(meql^{-1})$	Month 1	4.45±0.55°	25.83±0.52 ^b	39.96±4.20 ^a
	Month 2	4.14±0.75°	25.53±1.65 ^b	43.94±0.29 ^a
	Month 3	5.20±0.35°	23.23±2.21 ^b	22.31 ±1.59 ^a
HCO ₃ ⁻ (meql ⁻¹)	Month 1	8.66 ± 0.81^{a}	8.00 ± 1.41^{a}	8.00 ± 1.41^{a}
	Month 2	9.33 ± 0.81^{a}	8.00 ± 1.41^{a}	10.66 ± 1.60^{a}
	Month 3	12.66 ± 1.63^{a}	13.33 ± 0.81^a	14.00 ± 1.41^{a}
Cl ⁻ (meql ⁻¹)	Month 1	$17.33\pm0.81^{\circ}$	62.00 ± 1.41^{b}	115.33 ± 2.94^{a}
	Month 2	36.66 ± 10.61^{c}	86.00 ± 11.37^{b}	94.66 ± 2.94^{a}
	Month 3	$59.33 \pm 13.06^{\circ}$	105.33 ± 9.14^{b}	134.66 ± 2.16^{a}

Table 4. Characteristics of drained water after three months of irrigation (Month 1, 2, and 3).

Means \pm SE (n = 4). Different letters indicate significant differences between irrigation treatments in the same month according to LSD's test (p ≤ 0.05).

C: Ground water; EMSW: Electromagnetic saline water; SW: Saline water.

Table 5. Sodium usage efficiency (Na⁺UE), potassium usage efficiency (K⁺UE), calcium usage efficiency (Ca²⁺UE), chloride usage efficiency (Cl⁻UE) and water use efficiency (WUE) of potato varieties.

^		Spunta	Bellini	Alaska
Na ⁺ UE (g meq l ⁻¹)	С	261.00 ± 29.04^{a}	77.97 ± 25.09^{a}	900.71 ± 250.59^{a}
	SW	$12.78 \pm 1.01^{\circ}$	$9.97 \pm 2.54^{\circ}$	$36.42 \pm 5.94^{\circ}$
	EMSW	42.44 ± 0.20^{b}	28.74 ± 0.31^{b}	126.97 ± 0.54^{b}
K+UE (g meq l-1)	С	17.35 ± 0.69^a	6.86 ± 1.11^a	20.57 ± 2.03^a
	SW	8.99 ± 0.84^{b}	$3.28 \pm 1.01^{\text{b}}$	$12.25 \pm 0.62^{\circ}$
	EMSW	11.44 ± 0.36^{b}	3.86 ± 6.58^b	14.06 ± 0.80^b
Ca ²⁺ UE (g meq l ⁻¹)	С	$9.70\pm0.51^{\rm a}$	7.31 ± 0.93^{a}	16.69 ± 1.64^{a}
	SW	4.33 ± 0.28^{c}	3.48 ± 0.70^{c}	$7.93 \pm 0.63^{\circ}$
	EMSW	5.90 ± 0.29^{b}	5.03 ± 0.71^{b}	10.77 ± 0.40^{b}
Cl ⁻ UE (g mmoll ⁻¹)	С	12.07 ± 0.63^a	9.16 ± 0.35^a	24.02 ± 1.55^a
	SW	$5.42 \pm 0.32^{\circ}$	$5.65 \pm 1.72^{\circ}$	14.23 ± 1.27^{b}
	EMSW	9.13 ± 0.47^{b}	7.73 ± 0.71^{b}	20.82 ± 0.40^a
	С	197.68 ± 18.17^{a}	69.08 ± 26.17^{a}	639.53 ± 139.82^{a}
NO_3^{-} (g ppm ⁻¹)	SW	$12.24 \pm 3.21^{\circ}$	$7.28 \pm 1.70^{\circ}$	$33.36 \pm 6.88^{\circ}$
	EMSW	38.61 ± 1.44^{b}	22.72 ± 2.41^{b}	113.91 ± 6.03^{b}
	С	5.31 ± 0.16^a	3.82 ± 0.15^a	4.99 ± 0.18^{a}
WUE (Kg m ⁻³)	SW	4.20 ± 0.19^{b}	2.63 ± 0.35^{b}	3.08 ± 0.15^{ab}
	EMSW	$4.94\pm0.14^{\rm c}$	2.81 ± 0.23^{b}	3.51 ± 0.16^{b}

Means \pm SE (n = 4). Different letters in the same row indicate significant differences between irrigation treatments according to LSD's test at (p <0.05).



Stem thickness, leaf pigment content and electrolyte leakage

From Table 6 and ANOVA results summarized in Table 7, the ST did not show any significant differences among saline treatments (SW and EMSW) in Spunta and Bellini. However, Alaska had its ST 63%higher under EMSW than SW. Concentrations of chlorophyll b, carotenoids and porphyrin did not show any significant difference between water treatments. In opposition, lowest chlorophyll a concentration was registered under SW. Already; SPAD behaved similar response to IT, as least values were recorded for plants grown under SW (Figure 1). The ANOVA results on Chla showed significant effect of water treatments and variety. Thus highest concentrations were recorded for C treatment and Alaska variety. Leaf EL varied substantially among irrigation treatments in Spunta and Alaska with highest and least values were recorded for C and SW respectively. Comparable level of EL was found in Bellini plants irrigated with SW and EMSW.

Table 6. Leaf pigment content, electrolyte leakage (EL) and stem thickness (S	ST) of potato varieties.	
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		Spunta	Bellini	Alaska
	С	0.07±0.01 ^a	0.04±0.01 ^a	0.17±0.01 ^a
ST (cm)	SW	0.04 ± 0.00^{b}	0.05 ± 0.02^{a}	0.11±0.01 ^b
	EMSW	0.04 ± 0.00^{b}	0.04±0.01 ^a	0.18±0.03 ^a
	C	2154.98±213.84 ^a	1564.08±253.63 ^a	1810.73±604.21ª
Chl a (µg g ⁻¹ FW)	SW	1261.90±154.47°	862.53±32.00°	1348.13±256.83°
	EMSW	1917.30±253.75 ^b	1112.18±161.43 ^b	1541.57±170.69 ^b
Chl b (µg g ⁻¹ FW)	C	1101.09±195.63 ^a	1405.23±176.29 ^a	1499.50±72.80 ^a
	SW	953.61±167.80 ^a	792.49±56.90°	1121.44±203.58 ^{ab}
	EMSW	1013.55±204.04ª	1164.88±172.72 ^b	1343.16±210.99ª
	C	762.64±75.27 ^a	303.26±456.90 ^a	1111.82±469.45 ^a
Carotenoid (µg g ⁻¹ FW)	SW	558.39±126.49 ^a	240.64±163.63 ^a	409.93±172.34°
	EMSW	424.75±104.89 ^a	362.74±0.00 ^a	740.06±240.45 ^b
	с	3680.83±504.28ª	3580.33±681.41ª	4422.06±61.85ª
Porphyrin (µg g ⁻¹ FW)	SW	3429.30±538.48ª	3209.97±591.05 ^a	2879.51±567.30 ^b
	EMSW	3038.09±261.10 ^a	2017.79±20.37b	3624.79±621.54ª
	с	63.91±4.78 ^a	69.17±9.23 ^a	83.93±4.33ª
EL (%)	SW	60.31±6.60 ^b	70.44±3.25 ^a	72.57±4.12 ^b
	EMSW	64.42±5.16 ^a	65.13±6.27 ^a	80.07±3.61 ^a

Means \pm SE (n = 4). Different letters in the same row indicate significant differences between irrigation treatments according to LSD's test (p <0.05).

Table 7. Analysis of variance of the effect of irrigation of treatment (IT), variety (V) and their interactions on stem thickness (ST), leaf pigments, electrolyte leakage (EL), water (WUE) and ions use efficiency (UE).

	ST	Chla	Chlb	Carotenoid	Porphyrin	EL
V	56.65**	5.45*	3.60*	0.54 ^{ns}	2.41 ^{ns}	12.43***
IT	3.04 ^{ns}	4.38^{*}	2.25 ^{ns}	0.16 ^{ns}	3.03 ^{ns}	7.50^{**}
IT×V	3.19 ^{ns}	1.69 ^{ns}	2.42 ^{ns}	5.66**	2.79^{*}	2.17 ^{ns}

ns: not significant, $p \le 0.05$, $p \le 0.01$, $p \le 0.01$, $p \le 0.01$.

Table 7	7. (Continue	ed).					
	WUE	Na+UE	K+UE	Ca ²⁺ UE	Cl ⁻ UE	NO ₃ ⁻ UE	
V	81.40^{***}	17.43**	142^{**}	89.09**	211.77**	28.48**	
IT	55.44***	29.09**	57.24**	69.93**	53.58**	46.36**	
IT×V	3.51*	11.16**	2.82^{*}	4.27^{*}	4.22*	15.83**	

ns: not significant, * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq .001$.



ABA content and StNCED expression level

From Figure 1 A, ABA level in potato leaves was significantly increased under SW in Bellini and Alaska. However, ABA synthesis remained nearly constant in Spunta, under the three types of irrigation treatments. The least values of ABA content in Bellini leaves suggested a less in ABA metabolism. The increase in ABA synthesis of Alaska under saline treatments was associated to *StNCED* over expression. In Spunta, the marked increase of *StNCED* expression (2 fold) under EMSW was not consistent with ABA level.



Fig. 1. Changes of leaf absiscic acid (ABA) content (A) and relative expression pattern of *StNCED* gene (B) in potatoes.



Fig. 2. Relationship between leaf area (LA) and water content of aerial part (WCap); SPAD and water content of aerial part (WCap); leaf water potential (Ψ w) and water content of aerial part (WCap).





Fig. 3. Relationship between tuber dry weight and water content of aerial part (WCap); tuber dry weight and leaf dry weight.

Leaf area, SPAD and water status

Leaf area (LA) of Spunta have globally an increased trend as WCap increased, reaching 2245 cm² by 87% WCap (R²=0.39) in plants grown under C treatment; 1132 cm² by 75% WCap (R²=0.56) under SW and 1750 cm² by 88% WCap (R²=0.47) under EMSW (Fig. 2). Low correlation coefficients (R²) were found between LA and WCap in Bellini and Alaska. The linear relationship between SPAD and WCap was clearer and showed higher R² coefficients. Concomitantly to WCap, SPAD was more elevated under C and EMSW. In spite, Bellini showed a similarity in SPAD values between SW and EMSW. Nevertheless, Alaska had the highest SPAD and LA. In the other hand, it seems that Bellini irrigated with SW was less efficient in water usage due to the inability to sustain high Ψ w, that ranged from -9.5 to -18 under SW facing values within the range -8 to -17 in Spunta and -7 to -14 in Alaska.

Tuber weight and water status of aerial part

Tuber weight of Spunta was increased favorably with the increase of WCap. Similar relationship between tuber weight and WCap was found in Alaska plants grown under C and EMSW (Fig. 3). This was most probably due to the increased production and mobilization of photoassimilates to tubers. Additionally, tuber biomass was positively correlated to leaf dry biomass, in Spunta (under C, SW and EMSW) and Alaska (under C and EMSW). The positive relationship between leaf dry biomass and tuber weight seems to consent to better underground biomass accumulation.

DISCUSSION

Avoidance of soil salinization and ionic metabolism disturbance are of prime importance under saline irrigation. We expected that EMSW can improve nutrient availability and uptake. Our assumption was based on early findings that EMSW enhanced soil proprieties and plant physiological behavior (Akrimi et al., 2021). These results are referred to changes on soil structure, making easier for water to flow and to be absorbed by plants. The enhancement of soil moisture under EMSW is likely linked to better macro-aggregate structure (Zhou et al., 2021). One of the reasons of reduction of sodium and sulfate, in soil watered with EMSW, is



probably the higher leaching rate that occurs due to higher water flow rate (Mostafazadeh-Fardet al., 2012).

The less K⁺UE in Alaska plants grown under SW, suggest that K⁺ was used as an osmoticum in leaf tissues, under SW, and allocated to plant organs under EMSW. This implies that Alaska was more efficient user of potassium than Spunta and Bellini. Therefore, greater leaf expansion of Alaska can be attributed to potassium nutrition. Data also revealed that highest ions UE in Alaska, along with salt stress, can be one of main reasons for enhanced resistance to salinity. Likewise, elevated Ca²⁺UE of Alaska may be a key criterion for cell membrane integrity maintains, thereby reducing Na⁺ and Cl⁻ toxicity (Grattan & Grieve, 1999). Moreover, enhanced NO₃⁻ UE, in plants grown under EMSW, may diminish its use as osmolyte, facilitating its assimilation by the plant and therefore increasing the dry biomass of leaves and tubers. In this way, Juan et al. (2015) proposed that NO₃⁻ usage may be increased when Cl⁻ is not sufficiently available in the soil. This assumption also supports the high Cl⁻ leaching in soil irrigated with EMSW.

The less ABA content in Bellini leaves may be attributed to diminished stress signals. Like so, Ma et al. (2017) stated that when stress signals are diminished ABA is metabolized into inactive products. Decreased ABA concentrations have been also attributed to limited potassium absorption (Marrush et al., 1998). This in part agrees our results, since Bellini was less efficient in mineral use. Further, although *StNCED* over expression mirrored changes in ABA content in Alaska, the high abundance of this transcript in Spunta, watered with EMSW, confuses assessment of its role in ABA biosynthesis. Similar explanation was devoted by Destefano-Beltran et al. (2006) where *StNCED* genes have been identified to exhibit either tissue-specific or developmentally regulated expression in potato tubers. Meanwhile, in the case of Alaska the constitutive over expression of *StNCED* may be a key feature in minimizing water loss and therefore increasing growth and production.

The prominent decrease in SPAD under SW may be credited to high Na⁺ accumulation in leaf tissues. In fact, the plant absorb high amounts of sodium instead of potassium and calcium, and this leads to calcium and potassium deficiency, decrease and even acceleration of pigment degradation and early senescence (Naheed et al., 2021). The decline in SPAD and chlorophyll pigments is also another reason of low photosynthetic activity and therefore growth weakening in salt stressed plants. In the other hand, it appears that Alaska was more salt tolerant as it had high SPAD and LA. Meanwhile, Bellini presents a resemblance in SPAD between SW and EMSW treatments. The difference between varieties was possibly owed to differential regulation of growth at metabolic level. Furthermore, the higher correlation between SPAD and WCap in Spunta may illustrate that this variety possibly, prevented early senescence through an adequate water status (Dahal et al., 2019). Positive correlations between i) LA and WCap, ii) SPAD and WCap suggest that leaf size and chlorophyll content may be enhanced by adequate water content of aboveground part. Overall, the reduction of soil salinity under EMSW contributes mainly to an increase in Ψ w. In this concern, it seems that Bellini was more susceptible to salinity as it sustains least Ψw and WUE.

CONCLUSION

Results showed an enhancement of salt leaching from soil, reduction of soil salinity and decline of Na⁺ accumulation in potato leaves under EMSW. The increased ability to minerals use and ABA metabolism promoted water status and growth especially in Alaska. In view of the less correlation between tuber weight and WCap, less efficiency in ions usage and ABA content, we suggested that Bellini was more sensitive to salinity. Inversely, high correlation



between tuber weight-WCap and leaf area-WCap in Spunta extended the ability to maintain growth and yield under EMSW. These results suggest that EMSW may provide opportunity for alleviation of salinity in potatoes, while depending in variety.

Conflict of interest

Authors declare no conflict of interest.

Author Contribution Statement

R.A. performed the experiments, analyzed data and wrote the first draft of the manuscript. H.H. conceived the research and revised the manuscript.

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