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Root Application Efficacy of Plant Biostimulants in a Tunisian *Portulaca Oleracea* l. Cultivar Grown under Salt Stress

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ABSTRACT

Plants biostimulants (PBs) have been shown to play multiple roles in plant growth and to improve crop tolerance to abiotic stresses such as salinity. The present investigation was undertaken for the first time to study the effect of PBs - a plant-derived protein hydrolysate (PH), a root activator (RA) and a root stimulator (RS) - on *Portulaca oleracea* L. tolerance to salt stress. For this purpose, a Tunisian *P. oleracea* cultivar was cultivated in pots under a greenhouse. Plants were treated with a factorial combination of three nutrient solutions (non-salt control, 50 and 100 mM NaCl) and three PBs were applied to roots. Growth and physiological parameters were then determined. Main results showed that salt stress decreased shoot and root dry biomass, chlorophyll and carotenoid contents while it increased the content in total soluble sugars, proline and relative water contents. However, root application of the three PBs induced some significant differences in the agronomical and physiological responses between PB treated and untreated plants when subjected to sodium chloride salinity from 50 and 100 mM NaCl. Overall, the present study proves that the root application of these PBs increases the performance of *P. oleracea* plants under salinity conditions. Therefore, PBs can be used to improve the salt-stress tolerance of vegetable crops by increasing their physiological responses to abiotic stress

Keywords: *Portulaca oleracea* L.; plant; salinity; biostimulant; growth; physiology.

ABBREVIATIONS: PBs = Plants biostimulants; PH = a plant-derived protein hydrolysate**;** RA = a root activator**;** RS = a root stimulator.

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INTRODUCTION

Salinity is considered as a major environmental stress that limits agricultural productivity and affects the quality of harvest (Zhang et al., 2019). Henceforth, alternative methods for improving the nutrient use efficiency of crops and consequently enhancing their performance as well as their tolerance to salt stress are of great interest in sustainable agriculture (Bulgari et al., 2015). Therefore, plant biostimulants (PBs) use can be a promising alternative to face salt stress in various vegetable crops and other plants (Ertani et al., 2013; Lucini et al., 2015; Rouphael et al, 2017a; Bulgari et al., 2017; Bulgari et al., 2019a). PBs represent an emerging class of agricultural inputs that can accelerate plant growth, protect plants against abiotic stresses and/or improve nutrient use efficiency by enhancing plant physiological processes such as nutrient uptake, growth and tolerance to abiotic stresses (Calvo et al., 2014; Colla et al., 2017; Posmyk and Szafrańska, 2016; Rouphael and Colla, 2018). In contrast to bioregulators and hormones, PBs improve plant metabolic processes without changing their natural pathway (Posmyk and Szafrańska, 2016) and their action could be synergistic (Rouphael et al., 2017b) multidirectional as demonstrated in tomato (Ertani et al., 2017) and maize plants (Santi et al., 2017).

The major categories of biostimulants are listed as: humic and fulvic acids, protein hydrolysates (PH) and other N-containing compounds, seaweed extract chitosan and other biopolymers, inorganic compounds, beneficial fungi and bacteria (Du Jardin, 2015). However, PH are among the main categories of PBs that have been given special attention hence, recent progress has been made in research and applications of this category of PBs in sustainable horticulture (Calvo et al., 2014; Bulgari et al., 2015; Parađiković et al., 2018; Zulfiqar et al., 2020). In brief, the PH enhances growth, with mechanisms based on improved key physiological, biochemical and molecular processes. Thus, their

positive effect on horticultural production is mostly due to plant growth-enhancing bioactive compounds such as phytohormones, amino acids and nutrients (Zulfiqar et al., 2020).

In summary, stress tolerance provided by PBs has been attributed to a number of mechanisms, most of them involving oxidative stress mitigation, an increase in osmolytes, changes in sterols and terpenes composition as well as an increase in glucosinolates. The degree of mitigation seems also to be related to the application way, being the root and foliar application the most effective treatment (Lucini et al., 2015).

Purslane (*Portulaca oleracea* L.) is an annual succulent plant belonging to the *Portulacaceae* family with wide geographic and environmental distribution. From ancient times, *P. oleracea* is typically regarded as a weed of vegetables as well as other crops. However, this plant is considered as a potential vegetable crop in many regions of the world since its identification as one of the best plant sources of ω-3 fatty acid, α-linolenic acid, as well as some antioxidants (α -tocopherol, β-carotene, ascorbic acid, and glutathione) (Gonnella et al., 2010). Moreover, *P. oleracea* has been used as a medicinal plant in folk medicine in many countries. In this context, several authors reviewed the phytochemistry and the pharmacological effects as well as the potential of *P. oleracea* (Gonnella et al., 2010; Zhou et al., 2015) for health and they have also described prospective research especially for genetic improvement of this crop (Amirul et al., 2014a).

Besides, purslane is highly adaptable to various stress conditions and could be considered as a moderately salt-tolerant plant (Yazici et al., 2007) with a salinity threshold value, given in terms of the electrical conductivity of saturated-soil extract (ECe) of 6.3 dS m^{-1} (Kumamoto et al., 1990 cited *in* Grieve and Suarez, 1997). Recently, the responses to salt stress of different species of *Portulaca* have been investigated in order to specify the mechanisms of salinity tolerance in this genus, promoting hence their use as a source of plant nutrients or ornamental species, in saline agriculture and sustainable development (Borsai et al., 2020).

Although many reports have been previously published on the effects of salt stress on *P. oleracea* (Yazici et al., 2007; Teixeira and Carvalho, 2009; Kafi and Rahimi, 2011; Sabir Ali et al., 2014; Amirul et al., 2014b; Amirul et al., 2015; Amirul et al., 2016), however to the best of our knowledge, no studies have been yet conducted on the influence of the plant biostimulants on purslane tolerance to salinity. Thus, the present study was undertaken for the first time to study the root application effects of three PBs - a plant-derived protein hydrolysate (PH), a root activator (RA) and a root stimulator (RS) - on the salt tolerance of a Tunisian cultivar of *P. oleracea* L. when subjected to NaCl stress.

MATERIALS AND METHODS

Plant material and growth conditions

Purslane (*Portulaca oleracea* L.) seeds were collected in June 2015 from plants of a Tunisian cultivar grown in the local region of Hiboun in Mahdia (Southern Tunisia, latitude.35°30' N; longitude 11°0,4' E; altitude 13 m). After harvest, purslane seeds were air-dried and stored at 4°C until use for further analysis.

After surface disinfection with sodium hypochloride 2% and washing with distilled water, seeds of purslane were germinated in peat on March, 11th 2016. After that, purslane seedlings were transplanted on April, 12th 2016 into 1.5 L volume pots filled with a mixture of 50:50 natural sand and brown peat as substrate. The pots were placed in a 68 m² polyethylene greenhouse at daylight (photoperiod varying from 13 to 16 hours) at the Higher Agronomic Institute of Chott-Mariem (latitude 35°49′31″ N; longitude 10°38′13″ E; altitude 24 m) in Sousse (East coast of Tunisia) and where the average temperature was 25°C and the relative humidity varied from 60 to 70%.

Experimental design, biostimulant and NaCl treatments

The experimental design was the split-plot with three replications for each treatment and each experimental unit consisted of 7 plants. The NaCl treatments (control, 50 and 100 mM NaCl) were considered as the main factor while the sub-factor was the root application of three commercial PBs: a plant-derived protein hydrolysate (PH; Trainer®; Italpollina S.p.A., Rivoli Veronese, Italy), a root activator (RA; Acrecio ®; Agronutrition, Carbonne, France) and a root stimulator (RS; Osiryl®; Frayssinet, France). These lasts were applied at concentrations complying with the recommendations from the manufacturers (PH = 3 mL L^{-1}; RA = 10 mL L^{-1}; RS= 1 mL L^{-1}; The detailed composition of each PB was the following: PH contained 41% of organic matter, 5% of nitrogen and 31% of free amino acids and peptides; RA is made up of 4 active ingredients (humic acids, pure L-Tryptophan, pure L-Methionine and Acreciactiv = a stimulator for roots); RS consisted of 50% ‘of Osyr’ = a component which is effective to protect the auxins against enzymatic degradation). A control (CT) consisting of no application of biostimulants was also considered in this experiment.

Before initiation of treatments, purslane plants were fertigated daily with a slightly modified Coïc and Lesaint (1973) nutrient solution (pH 5.0) which is composed of 12.2 meq L^{-1} NO_3^-; 2.2 meq L^{-1} NH_4^+; 3.05 meq L^{-1} $H_2PO_4^-$; 5.7 meq L^{-1} K^+; 6.2 meq L-1 Ca^{2+}; 1.5 meq L^{-1} Mg^{2+}; 1.4 meq L^{-1} $SO4^{2-}$ and 2 mg L^{-1} Fe^{2+}. The three NaCl treatments (0, 50, and 100 mM NaCl) were obtained by adding 25 mM NaCl gradually (to avoid salinity shock) to nutrient solutions during a 4 days period. Finally, the NaCl × PB treatments started on April, 26th 2016 and were applied to plant roots using a volume of 30 mL pot^{-1} at weekly intervals during the experiment.

Growth measurements

Plant growth determination was performed on 7 treated and untreated (control) plants. Measurements of plant

height (cm), total root length (cm) and fresh as well as dry matter weights of shoot and root plant parts were evaluated by destructive harvests. Besides, the leaf area ($cm^2.cm^{-1}$) of treated plants was also determined using a planimeter (Li-Cor area meter, model 3100, Li-Cor USA). Thus, plants were separated firstly into shoot and root parts. Each part was immediately weighed (fresh matter weight) then wrapped in clean paper bags, labeled before oven-dried at 65°C for 48 h to constant weight and reweighted (dry matter weight).
Finally, the dry matter contents of shoots and roots were determined according to the following formula:

$$\text{DMW } (\%) = \frac{\text{DMW}}{\text{FMW}} \times 100$$

Where DM: dry matter weight (g), FMW: fresh matter weight (g) and DMW: dry matter weight.

Chlorophyll and carotenoid analyses

Chlorophyll and carotenoids pigments in fresh leaves of treated and untreated (control) plants were extracted according to the standard method of Torrecilas et al. (1984). The extraction took place in darkness at 4 °C for 72 h by adding 5 milliliters of 80% pure acetone to fresh leaf samples cut into discs of approximately 100 mg each. The absorbance of the extract was measured at 460 nm, 645 nm and 665 nm.
Chlorophyll and carotenoids contents were calculated using the formulas described by Mackinney (1941) and Arnon (1949) and then expressed as $mg.g^{-1}$ fresh matter weight.

Determination of total soluble sugars

Total soluble sugar contents were determined from fresh leaves following the phenol-sulphuric acid method of Robyt and White (1987). The absorbance of the solution was measured at 640 nm and compared with a standard curve to determine total soluble sugar contents before their expression as $mg.g^{-1}$ fresh matter weight.

Determination of proline content

Proline content was determined using a ninhydrin colorimetric method of Troll and Lindsay (1955) as modified by Dreier and Göring (1974). The proline content was calculated from a standard curve (absorbance = 528 nm) and then expressed as µg $proline.g^{-1}$ fresh matter weight.

Determination of relative water content

The relative water content (RWC) of leaves was calculated from the equation of Schonfeld et al. (1988):

$$\text{RWC } (\%) = \left(\frac{\text{FW} - \text{DW}}{\text{TW} - \text{DW}}\right) \times 100$$

Where FW = fresh weight of leaves (mg); DW= dry weight of leaves (mg) and TW = turgid weight of leaves (mg).

Statistical analyses

All experiments were conducted in triplicates and the results were expressed as mean values ± standard deviation (SD). Data were statistically analyzed using the two-way analysis of variance (ANOVA) procedure by the statistical package SAS 8.00 version (SAS Institute, 1999) for estimating the effects of factors and their interactions. The differences between treatment means were compared by using the Duncan's multiple range tests at the probability level of 5%.

RESULTS

Effects of plant biostimulant application and NaCl treatments on plant growth

Table 1 summarizes the effects of the application of the three PBs (PH, RAo and RS) and NaCl treatments (0, 50, 100 mM NaCl) on some growth parameters (plant height, leaf area and total root length) of *P. oleracea*.
As shown in Table 1, plant height was significantly affected by NaCl treatments and was decreased by 8.40 and 16.03% compared with the control under 50 and 100 mM NaCl, respectively. On the contrary, no significant reduction in plant height has been recorded when the biostimulant RS was applied under increasing levels of salinity although a slight decrease of 0.92 and 2.95% was noted under moderate (50 mM NaCl) and severe (100 mM NaCl) salt stress, respectively. However, the application of the biostimulant PH did not affect significantly the plant height under moderate (50 mM NaCl) salt stress. In contrast, we noted that severe salt stress (100 mM NaCl) led to a substantial decline in plant height estimated by 7.78%. Additionally, the same trend is observed with the biostimulant RA which didn't induce a significant difference in the height between treated and untreated (CT) plants under moderate salt stress (50 mM NaCl) although a slight decrease of 7.54% in comparison to the control was observed. However, this decline by about 13.67% was rather significant under severe salt stress (100 mM NaCl) (Table 1).
In addition, salt stress caused a significant decrease of leaf area of *P. oleracea* in control plants and this decrease is more pronounced with the increasing of NaCl concentrations (Table 1). However, no significant difference was observed between the leaf area of control and treated plants when the biostimulant PH was applied, whereas this parameter decreased slightly under moderate (50 mM NaCl) and severe salt stress (100 mM NaCl) by 2.01 and 6.77%, respectively, compared to the control (Table 1). Besides, the leaf area of the plants treated by the two biostimulants RA and RS and when subjected to moderate salt stress (50 mM NaCl) decreased but not significantly by 16.71 and 6.31%, respectively, in comparison to the control. Under severe salt stress (100 mM NaCl), the leaf area of the plants treated by the biostimulants RA and RS was significantly reduced by 24.55 and 14.91%, compared with the control (Table 1).
Indeed, increasing NaCl concentration from 50 to 100 mM induced a significant increase of the total root length

Table 1. Effects of plant biostimulant application (CT, PH, RA and RS) and NaCl treatments (control, 50 and 100 mM NaCl) on some growth parameters (plant height, leaf area and total root length) of a Tunisian *Portulaca oleracea* L. cultivar.

Plant biostimulant	Salinity level (mM NaCl)	Plant height (cm. plant^{-1})	Leaf area (cm^2.plant^{-1})	Total root length (mm plant^{-1})
	Control	24.64 ± 1.83 c	17.35 ± 0.08 b	11.11 ± 2.93 c
	50	22.57 ± 2.31 d (- 8.40 %)	15.50 ± 0.70 c (- 10.66 %)	13.11 ± 1.76 ab (+18.00 %)
CT	**100**	20.69 ± 0.45 e (- 16.03 %)	15.31 ± 0.92 c (- 11.75 %)	14.11 ± 4.04 a (+ 27.00 %)
	Control	28.15 ± 2.42 a	17.87 ± 2.31 b	17.33 ± 2.74 a
	50	27.72 ± 1.24 a (- 1.52%)	17.51 ± 1.70 b (- 2. 01 %)	15.11 ± 2.67 a (- 12.81 %)
PH	**100**	25.96 ± 1.25 bc (- 7.78 %)	16.66 ± 0.97 bc (- 6.77 %)	14.22 ± 3.11 a (- 17.94 %)
	Control	25.97 ± 1.36 bc	21.54 ± 2.34 a	13.67 ± 2.18 ab
	50	24.01 ± 1.51 c (- 7.54 %)	17.94 ± 2.77 b (- 16.71 %)	13.11 ± 3.86 ab (- 4.09 %)
RA	**100**	22.42 ± 1.40 d (- 13.67 %)	16.25 ± 1.05 bc (- 24.55 %)	13.00 ± 3.39 ab (- 4.90 %)
	Control	27.05 ± 3.29 a	20.07 ± 2.85 a	14.33 ± 3.12 a
	50	26,80 ± 2,25 a (- 0.92 %)	18.80 ± 1.90 ab (-6.32%)	14.00 ± 3.28 a (- 2.30%)
RS	**100**	26.25 ± 1.80 ab (- 2.95 %)	17.06 ± 0.98 b (- 14.91%)	13.89 ± 2.20 ab (- 3.07%)

*Values followed by different superscripts (a-e) in the same row are significantly different at probability level p < 0.05 (Duncan test).
**Values in parentheses represent reduction or increase percentages compared to the control.
CT: Control (no biostimulants added); PH: Plant-derived protein hydrolysate; RA = Root activator; RS = Root stimulator.

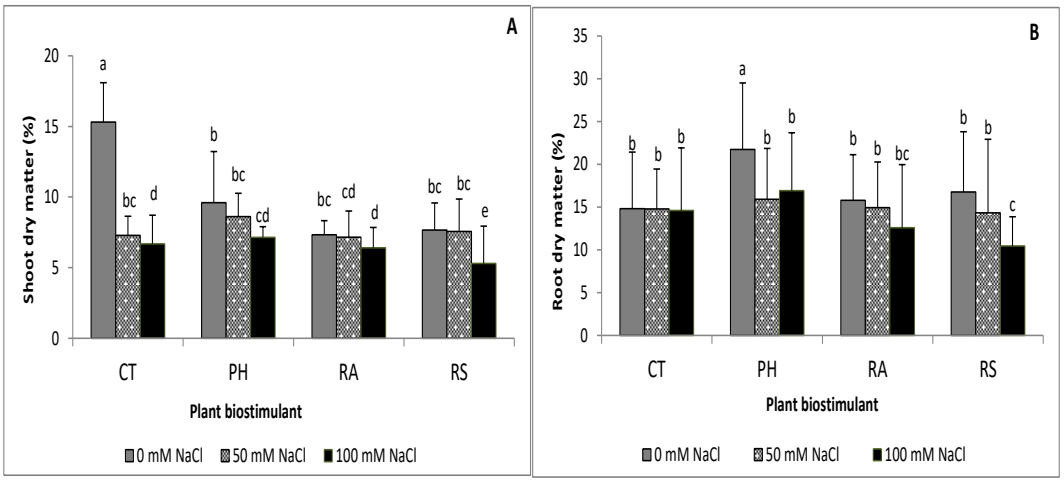

Figure 1. Effects of plant biostimulant application (CT, PH, RA and RS) and NaCl treatments (0, 50 and 100 mM NaCl) on shoot (A) and root dry matter (B) of a Tunisian *Portulaca oleracea* L. cultivar.
*Values followed by different superscripts (a-c) are significantly different at probability level p < 0.05 (Duncan test).
CT: Control (no biostimulants added); PH: Plant-derived protein hydrolysate; RA = Root activator; RS = Root stimulator.

of *P. oleracea* plants by 18 and 27%; respectively in comparison to the control (Table 1). However, this decrease in the root length is not significant in plants treated with the three PBs and subjected to moderate (50 mM NaCl) and severe (100 mM NaCl) salt stress. Indeed, the highest values are, on one hand, observed when plants have been treated by the biostimulant PH and on the other hand, the root length of these treated plants decreased but not significantly under moderate (50 mM NaCl) and severe (100 mM NaCl) salt stress by 17.9% and 12.8%, respectively, in comparison to the control. The same tendency was recorded when the two others biostimulants RA and RS have been applied to the plants whose root lengths decreased under moderate (50 mM NaCl) salt stress by 4.0 and 2.3%, respectively when compared to untreated plants. This decrease is almost the same under severe (100 mM NaCl) salt stress in plants treated by the biostimulants RA and RS and whose root length decreased by 4.90 and 3.07%, respectively, compared to the control (Table 1).

Effects of plant biostimulant application and NaCl treatments on shoot and root biomass

The dry matter of *P. oleracea* shoots and roots as a function of plant biostimulant application and NaCl treatments are presented in Figure 1.

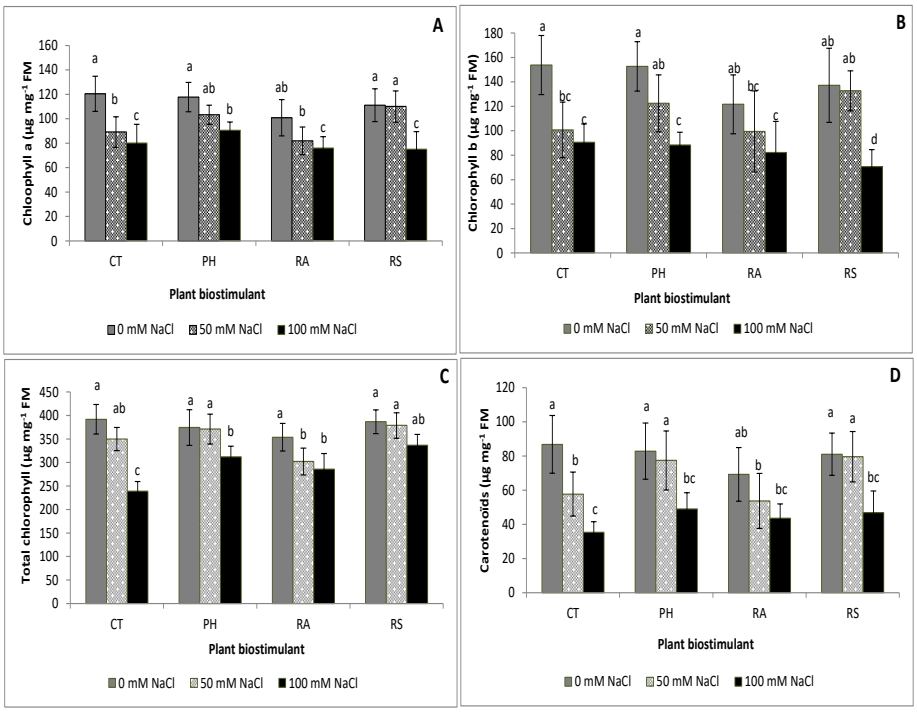

Figure 2. Effects of plant biostimulant application (CT, PH, RA and RS) and NaCl treatments (0, 50 and 100 mM NaCl) on chlorophyll a (A), chlorophyll b (B), total chlorophyll (C) and carotenoid contents (D) of a Tunisian *Portulaca oleracea* L. cultivar.
*Values followed by different superscripts (a-c) are significantly different at probability level $p < 0.05$ (Duncan test).
CT: Control (no biostimulants added); PH: Plant-derived protein hydrolysate; RA = Root activator; RS = Root stimulator.

As shown in Figure 1a, the highest shoot dry matter (15.30 %) was recorded in the control. However, results showed that salinity severely affected shoot dry matter in untreated plants by PBs and this effect is more pronounced with the salt constraint sharpness. However, no significant difference was observed between the shoot matter in control plants and those treated with PBs under moderate salt stress (50 mM NaCl). On the other hand, the shoot dry matter of plants grown under severe salt stress (100 mM NaCl) decreased but not significantly in control plants (6.69%) as well as in treated plants by PH (7.14%) and RA (6.40%). However, this reduction was significantly more remarkable in plants treated by RS (5.30%) (Figure 1a).

As shown in Figure 1b, root dry matter of control *P. oleracea* plants was not significantly different between untreated plants (0 mM NaCl) and those subjected to moderate (50 mM NaCl) and severe salt stress (100 mM NaCl).

Despite that the highest significant root dry matter (21.75%) was noted in control plants treated with the plant biostimulant PH, the application of the two plant biostimulants RA and RS didn't improve significantly the root dry matter in control (0 mM NaCl). Furthermore, root dry matter of plants treated with the three PBs was not significantly different between plants subjected to moderate (50 mM NaCl) and severe salt stress (100 mM NaCl); with the exception of those treated with the plant biostimulant RS and grown under severe salinity constraint (Figure 1b). It is important to note that under severe salt stress conditions, the lowest root dry matter (10.47 %) was observed in plants treated with the plant biostimulant RS although this value was not significantly different from that recorded in plants treated with RA (Figure 1b).

Effects of plant biostimulant application and NaCl treatments on chlorophyll and carotenoid contents

The effects of plant biostimulant application and NaCl treatments on chlorophyll a, chlorophyll b and total chlorophyll as well as carotenoids contents in the leaves of *P. oleraceae* plants are shown in Figure 2. As shown in figure 2, salt stress-induced decreases, as well as chlorophyll as in carotenoid contents and this decrease, is more pronounced with the salinity constraint sharpness.

Nevertheless, the application of the three PBs does not significantly affect chlorophyll and carotenoid pigment contents in plants grown under moderate salt stress (50 mM NaCl) compared to control plants (Figure 2). On the contrary, the chlorophyll and carotenoid contents in plants subjected to severe salt stress (100 mM NaCl) were significantly lower in comparison with the control and even lower when the PBs have been applied (Figure 2).

Effects of plant biostimulant application and NaCl treatments on total soluble sugar, proline and relative water contents

Table 2 showed the effects of the plant biostimulant

Table 2. Effects of plant biostimulant application (CT, PH, RA and RS) and NaCl treatments (control, 50 and 100 mM NaCl) on total soluble sugars, proline and relative water contents (RWC) of a Tunisian *Portulaca oleracea* L. cultivar.

Plant biostimulant	Salinity level (mM NaCl)	Total soluble sucres (mg g^{-1} FW)	Proline (µg g^{-1} FW)	RWC (%)
	Control	3.05 ± 1.13 c	3.429 ± 0.004 a	88.28 ± 1.35 ab
		4.34 ± 1.40 bc	3.434 ± 0.001 a	87.33 ± 0.73 bc
	50	(+ 42.29 %)	(+ 0.146 %)	(- 1.09 %)
		4.04 ± 1.49 bc	3.430 ± 0.003 a	82.91 ± 1.85 d
CT	**100**	(+ 32.45 %)	(+ 0.029 %)	(- 6.48 %)
	Control	4.19 ± 0.57 bc	3.426 ± 0.003 a	84.68 ± 0.50 cd
		5.14 ± 1.00 ab	3.434 ± 0.011 a	84.73 ± 0.35cd
	50	(+ 22.67 %)	(+ 0.234 %)	(+ 0.06 %)
		6.80 ± 1.70 a	3.426 ± 0.007 a	88.34 ± 0.87 ab
PH	**100**	(+ 62.29 %)	(+ 0.00%)	(+ 4.32 %)
	Control	4.97 ± 1.43 b	3.430 ± 0.008 a	88.62 ± 0.69 ab
		5.10 ± 1.60 ab	3.432 ± 0.006 a	89.38 ± 0.57 ab
	50	(+ 2.61 %)	(+0.058 %)	(+ 0.86 %)
		5.62 ± 1.47 ab	3.473 ±0.069 a	91.23 ± 0.47 a
RA	**100**	(+ 13.07 %)	(+ 1.195 %)	(+ 2.95 %)
	Control	3.91 ± 1.18 c	3.429 ± 0.004 a	83.00 ± 0.96 d
		4.42 ± 1.25 bc	3.441 ± 0.004 a	88.11 ± 1.33 ab
	50	(+ 13.04 %)	(+ 0.350 %)	(+ 6.15%)
		5.39 ± 0.32 ab	3.430 ± 0.006 a	88.68 ± 0.77 ab
RS	**100**	(+ 37.85 %)	(+ 0.029 %)	(+ 6.84 %)

*Values followed by different superscripts (a-c) in the same row are significantly different at probability level $p < 0.05$ (Duncan test).
**Values in parentheses represent reduction or increase percentages compared to the control.
CT: Control (no biostimulants added); PH: Plant-derived protein hydrolysate; RA = Root activator; RS = Root stimulator.

application and NaCl treatments on total soluble sugars, proline and relative water contents in the leaves of *P. oleraceae* plants.

Salt stress increased the total soluble sugar contents in control as well as in plants treated with the three PBs. Thus, the highest significant soluble sugar content (6.80 mg.g^{-1} FW) was recorded within plants treated by the plant biostimulant PH and subjected to 100 mM NaCl, whereas the lowest one (3.05 mg.g^{-1} FW) was noted in the control without application of salt stress nor plant biostimulant (Table 2).

As shown in Table 2, we noted a slight but not significant increase of the proline content in the control as well as in plants treated by the three PBs, respectively under moderate (50 mM NaCl) and severe (100 mM NaCl) salt stress. Thus, the proline content in *P. oleraceae* leaves increased in response to NaCl stress.

The relative water content (RWC) in purslane leaves was significantly affected by salinity and biostimulant application (Table 2). Irrespective of the biostimulant treatment, increasing levels of NaCl reduced the RWC by 1.09 and 6.48%, respectively under moderate (50 mM NaCl) and severe (100 mM NaCl) salt stress. Although the highest significant RWC was noted when the biostimulant RA was applied to plants subjected to 100 mM NaCl, the application of the two plant biostimulants PH and RA does not significantly affect the RWC in leaves of plants grown under both moderate (50 mM NaCl) and severe (100 mM NaCl) salt stress. However, when plants were treated with the biostimulant RS, a significant increase was observed in RWC by 6.15 and 6.84%, respectively under moderate (50 mM NaCl) and severe (100 mM NaCl) salt stress, in comparison to control plants (Table 2).

DISCUSSION

Drought and salinity are the most serious consequence of anthropogenic climate change on agricultural systems, which can be reduced in some way by using PBs. The advantage of using these substances is due to their effectiveness in improving crop productivity and quality (Del Buono, 2021). Indeed, it has been reported that application of PBs improved in one hand, the plant growth and yield of several horticultural crops by stimulating carbon and nitrogen metabolism and increasing nitrogen assimilation. On the other hand, PBs have been reported to enhance the tolerance to biotic and abiotic stresses of many crops (Paul et al., 2019; Rouphael and Colla, 2020). Interestingly, the application of the PBs as biostimulants imposed a marked remodulation of the metabolic pathways of amino acids by an accumulation of secondary metabolites which are involved in plant stress responses (Rouphael et al., 2020).

In the present study, as expected, salinity decreased the growth and cause alterations in the physiological process of purslane plants as recently reported by Zaman et al. (2020). Thus, it can be seen in Table 1 that salinity limited the plant height of *P. oleracea*. This result is in agreement with those of Amirul et al. (2016) who noted that at varying salinity levels, there are consecutive and significant decreases in plant height of

12 purslane accessions collected from different locations in western peninsular Malaysia. In a previous study, Amirul et al. (2014b) noted that plant heights of salt-treated purslane accessions were significantly reduced in comparison to the control and this decrease is more pronounced with increasing salt stress. Additionally, the plant height of purslane from Sudan was adversely affected by increasing concentrations of NaCl salts (Sabir et al., 2014). Reductions in plant height caused by salt stress have also been observed in fennel (*Foeniculum vulgare* Mill.) (Abou El Maged et al., 2008). In contrast, the three PBs tested did not affect significantly the plant height of *P. oleracea* under moderate salt stress (50 mM NaCl). Indeed, it has been reported that the application of a plant-derived protein hydrolysate increases lettuce (*Lactuca sativa* L.) performance when plants are grown under salinity conditions (Lucini et al., 2015). However, the positive effect of the plant biostimulant application depends on the plant species, the cultivar, the climatic conditions, the concentration, the origin and the period of their application (Lisiecka et al., 2011).

Based on our experimental data, it was shown that the leaf area of *P. oleracea* decreased in the control with the increasing of NaCl concentrations (Table 1). Indeed, it has been shown that the immediate response to salt stress is the reduction of leaf area expansion rate and this expansion is inhibited with extending NaCl severity (Wang and Nii, 2000). However, the application of the PBs to *P. oleracea* plants treated with moderate salt stress (50 mM NaCl) didn't induce a significant reduction of their leaf area whereas this reduction is significantly more pronounced under severe salt stress (100 mM NaCl). In contrast, Lucini et al. (2015) showed that the total root surface of lettuce was significantly affected by salinity and biostimulant application, with no significant salinity × biostimulant interaction. Thus, increasing the NaCl concentration from 1 to 25 mM in the nutrient solution decreased the total root surface by 6.7%. However, the root surface was significantly higher when leaf and root plants were treated with the biostimulant PH in comparison to the control. Indeed, the application of biostimulant can alter the morphology of the lettuce root system and thereby increasing the root surface, which could be considered as a salt tolerance mechanism (Tuteja, 2007).

Despite the decrease of leaf area, our experiment showed an increase in the total root length of *P. oleracea* plants grown only under salt stress (Table 1). Indeed, Kafi and Rahimi (2011) demonstrated that purslane root characteristics are effective at salt tolerance and help to absorb water and essential elements under salt conditions. In the same context, Neamatollahi et al. (2009) have shown that increasing NaCl concentrations induced an increase of the root length in fennel (*Foeniculum vulgare* Mill.) which could be considered as a mechanism to tolerate salt stress. In contrast, results of Lucini et al. (2015) showed that salinity didn't affect significantly the total root length of plants in lettuce. These study noted that total root length was highly influenced by bio stimulant application, but not by salinity, while there was no salinity × biostimulant interaction. They also reported that the highest value of the total shoot length was recorded in the root-foliar application treatment, followed by the root application treatment, whereas the lowest value was observed in the untreated lettuce plants.

Salinity severely affected shoot dry matter of *P. oleracea* in untreated plants by PBs and this effect is more pronounced with increasing NaCl levels (Figure 1a). This result is in accordance with that previously reported by Yazici et al. (2007) who found that shoot fresh and dry weight of purslane seedlings decreased by 35 and 62%, respectively under 70 and 140 mM NaCl after 30 days treatments. Similarly, Amirul et al. (2014b) observed a significant decrease of the dry matter contents in salt treated purslane accessions when compared to control and this reduction is more pronounced increasing salinity stress. Our results are also in agreement with those of Lucini et al. (2015) who observed a greater shoot dry weight of lettuce plants treated with the biostimulant PH in comparison to the control, which indicate that both foliar application and foliar-root application of this bio stimulant can mitigate the deleterious effects of salinity. Indeed, the application of the plant-derived protein hydrolysate (PH) containing amino acids and small peptides elicited a hormone-like activity and hence, increased the total dry biomass of plants as demonstrated by Colla et al. (2014). In a previous study, Ertani et al. (2013) also showed that the application of a PH increased the plant biomass in *Zea mays* and this effect is due to the content of this bio stimulant in plant growth regulators, such as triacontanol and indole-3-acetic acid. More recently, Bulgari et al. (2019b) reported that the bio stimulant tested increased significantly the fresh weight of lettuce grown under salinity conditions. Moreover, our results indicated that in case of severe salt stress, the plant-derived bio stimulant is inefficient to improve the shoot dry biomass of *P. oleracea* plants while in the absence of salinity conditions, several beneficial effects of the plant-derived protein hydrolysate bio stimulants on plant growth including biomass production as well as nutrient uptake, especially nitrogen and iron have been reported (Cerdán et al., 2009; Colla et al., 2014).

On the other hand, root dry matter of control *P. oleracea* plants was not significantly different between control plants and those subjected to salt stress treatments (Figure1b). In the same context, Kafi and Rahimi (2011) noted that salinity induced a negative effect on both shoot and root dry weights of purslane, but shoot tissues received more stress than roots. Although, growth of purslane was disturbed in high salinity, this plant could produce enough biomass at the level of 120 mM NaCl (Kafi and Rahimi, 2011). Results of this study showed that the root dry weight of *P. oleracea* plants was significantly affected by salinity and bio stimulant application (Figure1b). In the same context, Lucini et al. (2015) demonstrated that the root dry weight of lettuce plants was significantly higher when leaf and root were treated with the bio stimulant PH in comparison to the control. Previously, Ertani et al. (2009) showed that the use of two types of PH as bio stimulants increased significantly root dry weight in maize plants grown under salt stress.

Overall, the findings concerning the effect of salt-stress

on plant growth of *P. oleracea* indicate that increasing NaCl concentration didn't affect the root dry matter plants. This confirmed the idea that this plant is a salt-tolerant plant (Kafi and Rahimi, 2011; Sabir Ali et al., 2014) such as several vegetable crops (Shannon and Grieve, 1999) and barley (Katerji et al., 2006) but unlike other species such as fennel (*Foeniculum vulgare* Mill.) (Ashraf and Akhtar, 2004; Abou El Maged et al., 2008). Indeed, the *P. oleracea* root absorbing area increased when the plant-derived bio stimulants were applied and hence, could be considered as a salt tolerance mechanism (Tuteja, 2007).

In this study, salt stress causes a decrease in photosynthetic activity in leaves of *P. oleraceae* plants by reducing the content in chlorophyll pigments (Figure 2) as demonstrated by Leblebici et al. (2009) in *Spirodela polyrrhiza*. In fact, the older leaves begin to develop chlorosis and eventually fall with the extending salt stress severity (Agastian et al., 2000). Furthermore, this decrease could be attributed to an increasing of the chlorophyllase activity or changes in the lipid-protein ratio of protein pigment complexes (Iyengar and Reddy, 1996). On the contrary, Kafi and Rahimi (2011) noted that relative chlorophyll content value (SPAD) in purslane leaves significantly increased with increasing salt concentration. Besides, Amirul et al. (2015) reported that total carotenoid contents of 12 purslane accessions were significantly reduced when plants were subjected to 8 $dS.m^{-1}$ of salinity. However, plant responses to salt stress were variables in comparison to control because the carotenoid content has increased in some accessions while it has decreased in other ones with increasing salinity levels. Recently, it has been demonstrated that chlorophyll levels decreased in some of the studied genotypes of *Portulaca* under salt stress notably *P. oleracea* L. subsp. *oleracea* and to a lesser extent in *P. grandiflora*. Furthermore, an increase in these pigments was registered in *P. oleracea* "Toucan Scarlet Shades" and *P. halimoides*. On the contrary, carotenoids contents increased at the highest salt stress level (400 mM NaCl) in all genotypes tested except *P. oleracea* L. subsp. *oleracea* where it nearly remained equal to that determined in its control plants (Borsai et al., 2020). In a recent study, Xing et al. (2020) studied the molecular mechanisms underlying tolerance of purslane to saline stress through transcriptome and metabolome profiles. Results of their investigation showed that expression levels of genes for photosynthesis and aquaporins were depressed at 200 mM NaCl, suggesting that saline stress might inhibit photosynthesis and water uptake.

However, finding from this study showed that chlorophyll and carotenoid pigment contents were not significantly different between the control and treated-plants with the three PBs under moderate salt stress (50 mM NaCl) (Figure 2). In contrast, chlorophyll and carotenoid contents in plants subjected to severe salt stress (100 mM NaCl) were significantly lower in comparison with the control and even lower when the PBs have been applied (Figure. 2). In the same context, Lucini et al. (2015) reported that the SPAD index which is indicative of chlorophyll contents was highly influenced by salinity application, but not by bio stimulant; and there was also no salinity × biostimulant interaction. Irrespective of the biostimulant treatment, increasing the NaCl concentration from 1 to 25 mM decreased by 14.0% the SPAD index in lettuce leaves. On the contrary, Bulgari et al. (2019b) showed that biostimulant treatments positively affected the chlorophyll and carotenoid contents of lettuce grown under increasing NaCl concentrations. Although that biostimulant treatments caused a slightly increment of the considered pigments, the effect was not statistically relevant in comparison to controls. Additionally, Di Mola et al. (2019) found that the foliar application of vegetal-based bio stimulants induced a higher SPAD index as well as chlorophyll and carotenoids content in leaves of baby rocket.

Furthermore, salt stress increased the total soluble sugar contents in control as well as in plants treated with the three plant-derived biostimulants (Table 2). As expected, plants subjected to salt stress and without application of biostimulants increased their content in total soluble sugars which is consistent with previous studies conducted on others crops grown under salt constraint such as common wheat (*Triticum aestivum*) (Sairam et al., 2002), barley (*Hordeum vulgare*) (Hassani et al., 2008), and cucumber (*Cucumis sativus*) (Dong et al., 2011). Nonetheless, a slight increase or a decline of total soluble sugar contents in response to salinity was noted in different species and cultivars of *Portulaca*. Consequently, it is difficult to assess the role of total soluble sugars in salt tolerance of the analyzed *Portulaca* plants (Borsai et al., 2020). In contrast, the total sugars levels were not affected by biostimulant treatments in greenhouse lettuce grown under salt stress as reported by Bulgari et al. (2019b).

In our case and to the best of our knowledge, no earlier studies were reported in the literature about the effect of PBs on the total soluble sugars of purslane grown under salt stress conditions. Thus, it was reported for the first time that the total soluble sugar contents in leaves of *P. oleraceae* increased in response to increasing salt stress and even more when PBs were applied in comparison to the control.

Besides, the proline content in *P. oleraceae* leaves increased in response to NaCl stress (Table 2). The results of this study are in agreement with those of Yazici et al. (2007) who noted an accumulation of this osmoprotectant in purslane seedlings grown under salinity conditions. Indeed, free proline content in leaves of purslane reached the highest level when the plants were exposed to 140 mM NaCl for 30 days and showed three times higher proline accumulation when compared to control plants (Yazici et al., 2007). Additionally, proline levels significantly increased in different species and cultivars of *Portulaca* cultivated under salt stress. However, proline accumulation was not related to the degree of salt tolerance. Indeed, the lower content was observed in the most least damaged genotype, *P. grandiflora* (Borsai et al., 2020). In fact, accumulation of the proline in leaves of purslane grown under salt-stressed conditions might be an adaptive feature to improve its succulence and maintain water balance against salinity-induced osmotic stress (Kafi and Rahimi, 2011). Nevertheless, the osmotic potential couldn't be adjusted because the proline contents are not always

high in leaves of some plants grown under stress (Hoque et al., 2007). However, the proline content in the leaves of *P. oleraceae* plants treated with the three PBs didn't increased significantly under salinity conditions (Table 2). A similar result has also been reported by Lucini et al. (2015) in lettuce plants treated with the bio stimulant PH and subjected to 1 and 25 mM NaCl. These authors suggested that the mechanism underlying the alleviating effects of the bio stimulant on salt stress is not similar to that inducing the stimulation of the proline accumulation. Additionally, Bulgari et al. (2019b) found that increasing levels of salinity caused an increase in proline concentration in control plants of greenhouse lettuce while the bio stimulant treatments at 0.2 mL.plant^{-1} dose kept lower the proline levels.

On the other hand, the relative water content (RWC) in purslane leaves was significantly affected by salinity as well as by bio stimulant treatments (Table 2). In the same context, Yazici et al. (2007) noted that RWC in purslane leaves increased by 26% after 18 days of salinity treatments while a reduction by 17% in this parameter was observed under both 70 mM and 140 mM NaCl exposures after 30 days. Similarly, Kafi and Rahimi (2011) reported that the leaf RWC of purslane decreased partly up to 120 mM salinity and then remained unchanged. In contrast, Teixeira and Carvalho (2008) reported that salinity treatments did not significantly affect the RWC of purslane leaves when the experiment was conducted in spring. These studies suggested that, although the plants were exposed to salt stress, they were able to maintain the water levels in their leaves. It is noteworthy to mention that results obtained herein are the first report studying the effect of PBs on RWC in leaves of purslane. Therefore, it could be suggested that the root application of the plant biostimulant RS under salinity conditions increased significantly the RWC in leaves of purslane when compared to control plants and hence, enhanced it salt-stress tolerance. In another case and when the microorganisms or microorganism-based biostimulants are applied to enhance salt tolerance of plants, it has been reported that RWC was 5% higher in root inoculated tomato plants with *Azotobacter chroococcum* 76A, in comparison to the control (Van Oosten et al., 2018).

CONCLUSION

In conclusion, the results obtained herein on the effects of three PBs namely PH, RA and RS on a Tunisian *P. oleraceae* cultivar grown under salt stress indicated some significant differences concerning all agronomic and physiological parameters considered. On one hand, *P. oleraceae* was shown to be significantly affected by increasing sodium chloride salinity from 50 and 100 mM NaCl and the effect was even more pronounced with the severity of salt stress. On the other hand, the root application of the three PBs acted in general, positively on the most parameters studied. Interestingly, the biostimulant PH was selected as the best compared to the two other ones for the majority of the growth and physiological parameters determined under salt constraint. It could be also emphasized that this study confirmed the idea that *P. oleraceae* is a moderate salt-tolerance species but it is recommended to apply PBs as effective means to improve its crop performance when it is cultivated under saline environment.

Therefore, PBs may represent a potentially interesting tool, especially within the framework of organic farming, to improve in one hand, the tolerance to abiotic stresses and on the other hand, to enhance the productivity and quality of crops. Finally, it would be interesting to characterize the bioactive components of PBs and overall, to elucidate the physiological stimulation and molecular mechanisms since the mode of action of these PBs is still largely unknown. In the future, researches should focus on the development of a second generation of PBs where synergies and complementary mechanisms can be functionally designed for more sustainable agriculture.

REFERENCES

Abou El-Maged, MM Zaki, MF, Abou-Hussein SD (2008). Effect of organic manure and different levels of saline irrigation water on growth, green yield and chemical content of sweet fennel. Aust. J. Appl. Sci., 2: 90-98.

Agastian P, Kingsley SJ, Vivekanandan M (2000). Effect of salinity on photosynthesis and biochemical characteristics in mulberry genotypes. Photosynthetica 38: 287-290.

Amirul AM, Juraimi AS, Rafii MY, Hamid AA, Kamal Uddin M, Alam MZ, Latif MA (2014a). Genetic improvement of purslane (*Portulaca oleracea* L.) and its future prospects. Mol. Biol. Rep., 41: 7395-7411.

Amirul AM, Juraimi AS, Rafii MY, Abdul Hamid A, Aslani F (2014b). Screening of purslane (*Portulaca oleracea* L.) accessions for high salt tolerance. Sci. World J. 12p.

Amirul AM, Juraimi AS, Rafii MY, Hamid AA, Aslani F, Alam MZ (2015). Effects of salinity and salinity-induced augmented bioactive compounds in purslane (*Portulaca oleracea* L.) for possible economical use. 45Food Chem., 169: 439-447.

Amirul AM, Juraimi AS, Rafii MY, Abdul Hamid A, Aslani F, Hakim MA (2016). Salinity-induced changes in the morphology and major mineral nutrient composition of purslane (*Portulaca oleracea* L.) accessions. Biol. Res., 49: 24.

Arnon D (1949). Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiol., 24: 1-15.

Ashraf M, Akhtar N (2004). Influence of salt stress on growth, ion accumulation and seed oil content in sweet fennel. Biol Plantarum 48 (3) : 461-464.

Borsai O, Al Hassan M, Negrus C, Raigón MD, Boscaiu M, Sestras RE, Vicente O (2020). Responses to salt stress in *Portulaca:* Insight into its tolerance mechanisms. Plants, 2020, 9: 1660.

Bulgari R, Cocetta G, Trivellini A, Vernieri P, Ferrante A (2015). Biostimulants and crop responses: a review. Biol. Agric. Hortic. 31(1) : 1-17.

Bulgari R, Morgutti S, Cocetta G, Negrini N, Farris S, Calcante A, Spinardi A, Ferrari E, Mignani I, Oberti R, Ferrante A (2017). Evaluation of borage extracts as potential biostimulant using a phenomic, agronomic, physiological, and biochemical approach. Front. Plant Sci., 8 : 935.

Bulgari R, Franzoni G, Ferrante A (2019a). Biostimulants application in horticultural crops under abiotic stress conditions. Agronomy, 9: 306.

Bulgari R, Trivellini A, Ferrante A (2019b). Effects of two doses of organic extract-based biostimulant on greenhouse lettuce grown under increasing NaCl concentrations. Front. Plant Sci., 9:1870.

Calvo P, Nelson L, Kloepper JW (2014). Agricultural uses of plant biostimulants. Plant Soil 383(1-2) : 3-41.

Cerdán M, Sánchez-Sánchez A, Oliver M, Juárez M, Sánchez-Andreu JJ (2009). Effect of foliar and root applications of amino acids on iron uptake by tomato plants. Acta Hortic. 830 : 481-488.

Coïc Y, Lesaint C (1973). La nutrition minérale et en eau des plantes en horticulture avancée. Rev. Hortic., 2316: 29-34.

Colla G, Hoagland L, Ruzzi M, Cardarelli M, Bonini P, Canaguier R, Rouphael Y (2017). Biostimulant action of protein hydrolysates: unraveling their effects on plant physiology and microbiome. Front.

Plant Sci. 8: 2202.
Del Buono D (2021). Can biostimulants be used tomitigate the effect of anthropogenic climate change on agriculture? It is time to respond. Sci. Total Environ., 751: 141763.
Di Mola I, Ottaiano L, Cozzolino E, Senatore M, Giordano M, El-Nakhel C, Sacco A, Rouphael Y, Giuseppe Colla G, Mori M (2019). Plant-based biostimulants influence the agronomical, physiological, and qualitative responses of baby rocket leaves under diverse nitrogen conditions. Plants, 8: 522.
Dong CJ, Wang XL, Shang QM (2011). Salicylic acid regulates sugar metabolism that confers tolerance to salinity stress in cucumber seedlings. Sci. Horticult., 129(4): 629-636.
Dreier W, Göring M (1974). Der Einfluss hoher Salzkonzentrationen auf verschiedene physiologische parameter von Maiswurzeln [The influence of high salt concentrations on various physiological parameters of corn roots] Wiss Z. Der H.U. Berlin, Nath. Naturwiss. R. 23: 641-644.
Du Jardin P (2015). Plant biostimulants: Definition, concept, main categories and regulation. Sci. Hortic. 196: 3-14.
Ertani A, Schiavon M, Nardi S (2017). Transcriptome-wide identification of differentially expressed genes in *Solanum lycopersicon* L. in response to an alfalfa-protein hydrolysate using microarrays, Front. Plant Sci. 8 : 1159.
Gonnella M, Charfeddine M, Conversa G, Santamaria P (2010). Purslane: A review of its potential for health and agricultural aspects. Eur. J. Plant Sci. Biotechnol., 4 (Special Issue 1): 131-136.
Grieve C, Suarez D (1997). Purslane (*Portulaca oleracea* L.): a halophytic crop for drainage water reuse systems. Plant and Soil. 192: 277-283.
Hassani A, Dellal A, Belkhodja M, Kaid-Harche M (2008). Effet de la salinité sur l'eau et certains osmolytes chez l'orge (*Hordeum vulgare*). Eur. J. Sci. Res., 23 (1): 61-69.
Hoque MA, Okuma E, Banu MNA, Nakamura Y, Shimoishi Y, Murata Y (2007). Exogenous proline mitigates the detrimental effects of salt stress more than the betaine by increasing antioxidant enzyme activities. J. Plant Physiol., 164: 553-561.
Kafi M, Rahimi Z (2011). Effect of salinity and silicon on root characteristics, growth, water status, proline content and ion accumulation of purslane (*Portulaca oleracea* L.). Soil Sci. Plant Nut., 57(2): 341-347.
Katerji N, Van Hoorn JW, Hamdy A, Mastrorilli M, Fares C, Ceccarelli S, Grando S, Oweis T (2006). Classification and salt tolerance analysis of barley varieties. Agric. Water Manag. 85: 184-192.
Iyengar ERR, Reddy MP (1996). Photosynthesis in high salt tolerant plants. In: Pesserkali, M. (Ed.). Hand Book of Photosynthesis. Marshal Deker. Baten Rose, USA, pp: 56-65.
Leblebici Z, Aksoy A, Duman F (2009). Influence of salinity on the growth and heavy metal accumulation capacity of *Spirodela polyrrhiza* (*Lemnaceae*). Turk. J. Biol., 35 : 215-220.
Lisiecka J, Knaflewski M, Spizewski T, Fraszczak B, Kaluzewicz A, Krzesinski W (2011). The effect of animal protein hydrolysate on quantity and quality of strawberry daughter plants cv. 'Elsanta'. Acta Sci. Pol.- Hortoru., 10(1): 31-40.
Lucini L, Rouphael Y, Cardarelli M, Canaguier R, Kumar P, Colla G (2015). The effect of a plant-derived biostimulant on metabolic profiling and crop performance of lettuce grown under saline condition. Sci. Hortic., 182: 124-133.
Mackinney G (1941). Absorption light by chlorophyll solutions. J. Biol. Chem. 140: 315-322.
Neamatollahi E, Bannayan M, Ghanbari A, Haydari M, Ahmadian A (2009). Does hydro and osmo-Priming improve fennel (*Foeniculum vulgare*) seeds germination and seedlings growth? Not. Bot. Hort. Agrobot. Cluj. 37 (2): 190-194.
Paul K, Sorrentino M, Lucini L, Rouphael Y, Cardarelli M, Bonini P, Bonini P, Reynaud H, Canaguier R, Trtilek M, Panzarova K, Colla G (2019). Understanding the biostimulant action of vegetal-derived protein hydrolysates by high-throughput plant phenotyping and metabolomics: a case study on tomato. Front. Plant Sci., 10:47.
Parađiković N, Teklić T, Zeljković S, Lisjak M, Špoljarević M (2018). Biostimulants research in some horticultural plant species - A review. Food Energy Secur., e00162.
Posmyk MM, Szafrańska K (2016). Biostimulators: A new trend towards solving an old problem. Front. Plant Sci., 7: 7-48.
Robyt JR, White BJ (1987). Biochemical Techniques: Theory and Practice, Wave land Press, Prospect Heights, IL. pp: 330-332.
Rouphael Y, Cardarelli M, Bonini P, Colla G (2017a). Synergistic action of a microbial-based biostimulant and a plant derived-protein hydrolysate enhances lettuce tolerance to alkalinity and salinity. Front. Plant Sci., 8 : 131.
Rouphael Y, Colla G, Giordano M, El-Nakhel C, Kyriacou MC, De Pascale S (2017b). Foliar applications of a legume-derived protein hydrolysate elicit dose-dependent increases of growth, leaf mineral composition, yield and fruit quality in two greenhouse tomato cultivars. Sci. Hortic., 226: 353-360.
Rouphael Y, Colla G (2018). Synergistic biostimulatory action: designing the next generation of plant biostimulants for sustainable agriculture. Front. Plant Sci., 9:1655.
Rouphael Y, Colla G (2020). Editorial: Biostimulants in agriculture. Front. Plant Sci. 11: 40.
Rouphael Y, Lucini L, Miras-Moreno B, Colla G, Bonini P, Cardarelli M (2020). Metabolomic responses of maize shoots and roots elicited by combinatorial seed treatments with microbial and non-microbial biostimulants. Front. Microbiol., 11:664.
Sabir Ali AK, Mohamed BF, Dreyling G (2014). Salt tolerance and effects of salinity on some agricultural crops in the Sudan. Journal of Forest Products & Industries, 3(2): 56-65.
SAS Institute (1999). SAS/STAT User's Guide, version 8. SAS Institute Inc., Cary, N.C.
Sairam RK, Veerabhadra Rao K, Srivastava GC (2002). Differential response of wheat genotypes to long term salinity stress in relation to oxidative stress, antioxidant activity and osmolyte concentration. Plant Sci., 163: 1037-1046.
Santi C, Zamboni A, Varanini Z, Pandolfini T (2017). Growth stimulatory effects and genome-wide transcriptional changes produced by protein hydrolysates in maize seedlings. Front. Plant Sci., 8: 433.
Schonfeld MA, Johnson RC, Carver BF, Mornhinweg DW (1988). Water relations in winter wheat as drought resistance indicators. Crop Sci., 28: 526-531.
Shannon MC, Grieve CM (1999). Tolerance of vegetable crops to salinity. Sci. Hortic., 78:5-38.
Teixeira M, Carvalho IS (2009). Effects of salt stress on purslane (*Portulaca oleracea*) nutrition. Ann. Appl. Biol., 154: 77-86.
Torrecilas A, Leon A, Del Amor F, Martinez-Mompean MC (1984). Determinación rápida de clorofila en discos foliares de limonero [Rapid determination of chlorophyll in the leaf discs of lemon trees]. Fruits 39: 617-622.
Troll W, Lindsley J (1955). A photometric method for the determination of proline. J. Biochem., 655-660.
Tuteja N (2007). Links mechanisms of high salinity tolerance in plants. Meth. Enzymol., 428: 419-438.
Van Oosten MJ, Di Stasio E, Cirillo V, Silletti S, Ventorino V, Pepe O, Raimondi G, Maggio A (2018). Root inoculation with *Azotobacter chroococcum* 76A enhances tomato plants adaptation to salt stress under low N conditions. BMC Plant Biol., 18, 205: 12p.
Wang Y, Nii N (2000). Changes in chlorophyll, ribulose biphosphate carboxylase-oxygenase, glycine betaine content, photosynthesis and transpiration in *Amaranthus tricolor* leaves during salt stress. J. Hortic. Sci. Biotech., 75: 623-627.
Yazici I, Türkan I, Hediye Sekmen A, Demiral T (2007). Salinity tolerance of purslane (*Portulaca oleracea* L.) is achieved by enhanced antioxidative system, lower level of lipid peroxidation and proline accumulation. Environ. Exp. Bot., 61: 49-57.
Zaman S, Bilal M, Du H, Che S (2020). Morphophysiological and comparative metabolic profiling of purslane genotypes (*Portulaca oleracea* L.) under salt stress. BioMed Res. Int. 2020: 1-17.
Zhou Y-X, Xin H-L, Rahman K, Wang S-J, Peng C, Zhang H (2015). *Portulaca oleracea* L.: A review of phytochemistry and pharmacological effects. BioMed Res. Int., pp.1-11.
Zulfiqar F, Casadesús A, Brockman H, Munné-Bosch S (2020). An overview of plant-based natural biostimulants for sustainable horticulture with a particular focus on moringa leaf extracts. Plant Sci., 295: 110194.
Zhang H, Li Y, Meng YL, Cao N, Li DS, Zhou ZG, Chen BL, Dou FG (2019). The effects of soil moisture and salinity as functions of groundwater depth on wheat growth and yield in coastal saline soils. J. Integr. Agric., 18: 2472-2482.