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ORIGINAL ARTICLE

Salt-induced effects on growth and photosynthetic traits of *Orychophragmus violaceus* and its restoration through re-watering

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Abstract
Stressful environment such as drought and salinity affects the plant growth and development. This research was conducted to find out growth and photosynthetic threshold values in *Orychophragmus violaceus* (L.) O. E. Schulz for an appropriate regime to dilute the salted water. *O. violaceus* was subjected to different treatments of NaCl ($NC^{2.5}$: 2.5, NC^{5}: 5, NC^{10}: 10) g L^{-1}, Na_2SO_4 ($NS^{2.5}$: 2.5, NS^{5}: 5, NS^{10}: 10) g L^{-1}, and mixture of salts (MS^{1}: 2.5 NaCl + 10 Na_2SO_4; MS^{2}: 10 NaCl + 2.5 Na_2SO_4; MS^{3}: 5 NaCl + 5 Na_2SO_4) g L^{-1} and 0 as control followed by re-watering. Relative performance of stomatal conductivity and photosynthetic activity was found to have significantly affected plant growth under high-concentration salts levels at NC^{10}, NS^{10}, MS^{1}, and MS^{2}, respectively. Growth parameters were stable under slight ($NC^{2.5}$ and $NS^{2.5}$) to moderate stress (NC^{5}, NS^{5}, and MS^{3}) conditions due to fact that photosynthetic activities were partially maintained under stimulated carbonic anhydrase activity. Consequently, the increase in net photosynthetic rate was noted under moderate stress condition which was 44.13, 37.07, and 43.01%, respectively. However, *O. violaceus* was found as salt tolerant up to moderate stress level, and all growth and physiological parameters were recovered during re-watering phases. Relatively, better recovery noticed in net photosynthetic rate under moderate stress levels, and values were 55.62, 65.46, and 50.82%, respectively. Hence, it is suggested that salinity effect in plants could be reduced by re-watering, based on plant physiological characteristics.

Keywords Carbonic anhydrase activity · Physiological parameters · Salt stress · Water potential · Water-use efficiency

1 Introduction

The world is facing a great challenge of meeting food demand in the coming decades in the face of projected rapid population growth. In the midst of these, it has been estimated by United Nations Environment Program that approximately 20% of agricultural land in the world is salt-stressed (Flowers and Yeo 1995). Conversely, the output of crop's production is not increasing to match the requirement of food. It is therefore a serious concern for the scientists to put more salt-affected lands into cultivation. Soil salinity affects about one-third of the total irrigated crop land in North West region of China. On the other hand, the total available water for irrigation is about 5×10^5 m^3 km^{-2}, which fulfills only 18% of the total demand of irrigation (Chen et al. 2010). Moreover, this region is specifically characterized by high evaporation, soil salinization, and water shortage (Wang et al. 2011). So, to overcome stress due to salinity and water is a foremost issue in these areas to ensure agricultural sustainability and continued development of agriculture resources.

Plants are distinct in response to the mechanism of salts tolerance (Memon et al. 2010). This mechanism might be particularly important with concerns to survival of salt-tolerant plants such as species of Brassicaceae, which are considered to be moderately salt tolerant (Hayat et al.

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2007). *Brassicaceae* species are suitable for implementing the application of saline water irrigation. *Orychophragmus violaceus* (L.) O. E. Schulz from Brassicaceae family was used in this research work. *O. violaceus* is widely used for forage, health care, and gardening. However, it is an attractive plant that is used as food as well as oilseed crop in China (Nurfitri et al. 2013). In previous studies, *O. violaceus* is found as higher shade tolerant as compared to other Brassicaceae species because of its photosynthetic activity increased by regulation of higher CA activity (Wu et al. 2005, 2007, 2011). Therefore, *O. violaceus* could convert intracellular HCO_3^- into CO_2 and H_2O by carbonic anhydrase to change the cellular water status. Carbonic anhydrases (CA) is zinc-containing enzyme (Tavallali et al. 2009), which helps to prevent plants from water losses under stressful condition (Hu et al. 2011). CA played a significant role in photosynthetic process (Wu and Xing 2012; Xing and Wu 2012), and utilization of intracellular potential can reduce the external water consumption in case of water deficiency.

Salinity stress and water scarcity are the major concerns to be discussed to improve irrigation techniques with reducing irrigation cost. In order to improve irrigation water-use efficiency with water saving, mostly researchers' pay attention to resolving this issue through plant physiological characteristics (Zhang et al. 2011). Salt stress generates physiological instabilities in plants; as a result, plant growth characteristics are adversely effected (Munns and Tester 2008; Hajiaghaei-Kamrani and Hosseinniya 2013). The most important physiological factor affected by salinity is photosynthesis, which is the most essential and complex functional process in all plants (Munira et al. 2015). Exposure to salinity leads to changes in photosynthetic characteristics in plants (Nadeem et al. 2006), including transpiration rate (Karlberg et al. 2006) and relative leaf water content (Lee et al. 2005). As a result, photosynthesis is negatively influenced through stomatal limitation (Chaves et al. 2009), reduced transpiration rate, and water potential (Azevedo-Neto et al. 2004; Dudley et al. 2008). According to Kalapos (1994) and Bhatt et al. (2008), water potential tends to decrease as a result of reduction in the relative water content of plant leaves.

Reuse of diluted saline water for irrigation of plants becomes the readily available water when water resources are scarce. It would be a reasonable approach to use saline water as substitute resource for freshwater to irrigate the moderately salt-tolerant crops such as *O. violaceus*. To move forward in this research field, re-watering or dilution of saline water is a new index which could be helpful for regulation of saline water in order to sustain agricultural productivity and reduce irrigation cost. An appropriate dilution of salt water will save the water resource. *O. violaceus* is hypothesized as better restorability after re-watering or dilution of salted water. Thus, the objective of this study is to find out threshold values in *O. violaceus* for an appropriate regime to dilute the salted water through photosynthetic and growth characteristics in different salt stresses and subsequent re-watering conditions.

2 Materials and methods

Experiment site, treatments, and growth conditions – The experiment was carried out at the Institute of Agricultural Engineering, Jiangsu University, Zhenjiang, Jiangsu, China (32.20°N, 119.45°E). Seeds of *O. violaceus*, identical in size and color, homogeneous, and free from wrinkles, were chosen for this experiment. They were sown in 20-cell tray, containing equal quantities of vermiculite washed with distilled water. The seeds were left to grow inside the growth chamber under day/night temperature cycle of 25/20 °C, and 60% of relative humidity. Plants were irrigated daily with Hoagland solution (Hoagland and Arnon 1950). Full strength Hoagland solution containing the salts in the following proportions was used: macronutrient stock solution A ($g\ L^{-1}$): 101 KNO_3, 236.15 $Ca(NO_3)_2{\cdot}4H_2O$, 246.47 $MgSO_4{\cdot}7H_2O$, 115.03 $NH_4H_2PO_4$; macronutrient stock solution B ($g\ L^{-1}$): 5.56 $FeSO_4{\cdot}7H_2O$, 7.44 EDTA-Na_2; micronutrient supplement C ($g\ L^{-1}$): 0.075 KCl, 1.546 H_3BO_3, 0.446 $MnSO_4{\cdot}4H_2O$, 0.575 $ZnSO_4{\cdot}7H_2O$, 0.025 $CuSO_4{\cdot}5H_2O$, and 0.018 $(NH4)_6MO_7O_{24}{\cdot}4H_2O$ at pH 8.1 ± 0.5. After 21 days, plants were transferred into greenhouse under natural lighting with (25/18) ± 2 °C (day/night) temperature and 70% relative humidity. Homogenous seedlings which were showing the healthy growth selected for treatments. They were exposed to salt stress induced by NaCl, Na_2SO_4, and mixture of both salts at different four levels, in which one is control level. The concentrations of salts: NaCl ($NC^{2.5}$: 2.5, NC^5: 5, NC^{10}: 10 and 0 as control) $g\ L^{-1}$; Na_2SO_4 ($NS^{2.5}$: 2.5, NS^5: 5, NS^{10}: 10 and 0 as control) $g\ L^{-1}$, and mixture of salts (MS^1: 2.5 $g\ L^{-1}$ NaCl + 10 $g\ L^{-1}$ Na_2SO_4; MS^2: 10 $g\ L^{-1}$ NaCl + 2.5 $g\ L^{-1}$ Na_2SO_4; MS^3: 5 $g\ L^{-1}$ NaCl + 5 $g\ L^{-1}$ Na_2SO_4 and 0 as control) were selected and added into Hoagland solution for treatments and applied to plant. The control plants only received Hoagland solution.

Re-watering was done on day 21 from the onset of salt stress treatment. The order of re-watering was that plants were suffering in high stress level (10 $g\ L^{-1}$ in both NaCl and Na_2SO_4) irrigated with moderate stress level $NC^{10\wedge5}$, $NS^{10\wedge5}$ (5 $g\ L^{-1}$ in both NaCl and Na_2SO_4, respectively), moderate stress level (5 $g\ L^{-1}$ in both NaCl and Na_2SO_4) irrigated with first stress level $NC^{5\wedge2.5}$, $NS^{5\wedge2.5}$ (2.5 $g\ L^{-1}$ in both NaCl and Na_2SO_4, respectively), and first stress level (2.5 $g\ L^{-1}$ in both NaCl and Na_2SO_4, respectively) irrigated with control level $NC^{2.5\wedge 0}$, $NS^{2.5\wedge 0}$ ($0\ g\ L^{-1}$ in both NaCl and Na_2SO_4, respectively). In the mixed treatments, all levels were re-watered with the control ($MS^{1\wedge 0}$, $MS^{2\wedge 0}$, $MS^{3\wedge 0}$). This experiment was designed in randomized block design, and a total of five replicates were chosen for each physiological measurement.

Photosynthetic rate and leaf water potential measurements – Leaves in salt stress phase and subsequently in re-watering phase were used for the determination of photosynthesis. Net photosynthetic (P_N), stomata conductance (g_S), transpiration rate (T_r), and water potential (Ψ) were measured at 9:00–11:00 a.m. after every 3 days in both salt stress phase and re-watering phase, respectively. Five plants from each treatment group were selected for the measurement. The photosynthetic active radiation (PAR), temperature, and CO_2 concentration during the measurements were $800\ \mu mol\ m^{-2}\ s^{-1}$, 28 °C, and $500\ \mu mol\ mol^{-1}$, respectively. A portable LI-6400XT photosynthesis measurement system (*LI-COR, Lincoln*, NE, USA) was used. Water-use efficiency (WUE) was calculated according to the following equation: $WUE = P_N/T_r$, where P_N is the net photosynthetic rate and T_r is the transpiration rate. Leaf water potential was measured with dew point microvolt meter in a C-52-SF universal sample room (Psypro, *Wescor*, USA), after every 3 days in both salt stress phase and re-watering phase, respectively. Five plants from each treatment group were selected for the measurement.

Determination of growth parameters – Growth measurements were considered during treatments and re-watering three times per each week in both cases, respectively. The five replicates were chosen for each treatment and also used to analyze the mean of each measurement. The measurements taken for growth analysis were: height of the plant (P^H); stem diameter (S^D), leaf area (L^A), and root length (R^L). The leaf area was measured by leaf area meter (handheld laser leaf area meter, CI, 203).

Measurement of carbonic anhydrase activity – The fourth and fifth youngest fully expanded leaves from the top were chosen for carbonic anhydrase (CA) activity measurement in both salt stress phase and re-watering phase, respectively. CA activity was determined by using the pH method described by Wilbur and Anderson (1948) with modifications (Wu et al. 2011). CA activity was expressed in Wilbur and Anderson as WA [$WAU\ g^{-1}$ (FW)] = (t_0 t^{-1}) − 1, where t_0 and t are the time (s) recorded for the pH change from 8.2 to 7.2 with buffer alone (t_0) and with sample (t). Five plants from each treatment group were used for the measurement. Leaf tissues (the weight select according to leaf size usually used 0.1–0.2 g) quickly freeze in liquid nitrogen and ground with 3 mL extraction buffer (0.01 M barbitone sodium with 0.05 M mercaptoethanol, pH 8.3). The homogenate centrifuged at $10{,}000\times g$, 0 °C for 5 min and then placed on ice for 20 min. In brief, CA activity was assayed at 0–2 °C in a mixture containing 4.5 mL of 0.02 M barbitone buffer (5,5-diethylbarbituric acid pH 8.3), 0.4 mL of the sample, and 3 mL of CO_2-saturated H_2O.

Determination of re-watering water-use efficiency – Re-watering water-use efficiency was calculated by the increment of leaf water potential (Ψ) and net photosynthetic rate (P_N) in the leaves of *O. violaceus* from salt stress to subsequent re-watering phase. In the experiment, four treatment levels, control, 2.5, 5, and 10% were marked as level 0, 1, 2, and 3, respectively. In stress phase, P_N and Ψ under level 0, 1, 2, and 3 were expressed as P_{N}-l ($\mu mol\ m^{-2}\ s^{-1}$) and Ψ_l (MPa), respectively (l was the salt stress level, $l = 0, 1, 2, 3$), while in re-watering phase, P_N and Ψ of leaves in salt stress levels 1, 2 and 3 after re-watering were expressed as $P_N l^\wedge(l-1)$ and $\Psi l^\wedge(l-1)$, respectively ($l^\wedge(l-1)$, indicated that leaves were re-watered from salt stress level l to salt stress level $l - 1$.

The relationship between plant tissue water potential (Ψ) and cell SAP solute concentration (Q) was:

$$\Psi = iQRT, \tag{1}$$

where Ψ is plant tissue water potential (MPa); i is dissociation coefficient ($i = 1$); Q is cell SAP solute concentration; R is gas constant ($R = 0.0083\ L\ MPa\ mol^{-1}\ K^{-1}$); and T is thermodynamic temperature (273 + t °C) K.

The relationship between proportion of solute quality in the total quality of leaf (P, %) and cell SAP solute concentration (Q) was:

$$P = \frac{MQ}{1000}\%, \tag{2}$$

M is the relative molecular mass of cell SAP solute, sugar $C_{12}H_{22}O_{11}$, and M is $342\ g\ mol^{-1}$.

According to Eqs. 1 and 2, P could be rewritten as:

$$P = \left(-\frac{\Psi M}{100iRT}\right)\% \tag{3}$$

Proportion of water content in the total quality of leaf was $1 - P$; it was expressed with WC (%).

$$WC = \left(1 + \frac{\Psi M}{100iRT}\right)\% \tag{4}$$

The leaves in salt stress levels 2, 3, and 4 were re-watered to adjacent lower salt stress levels, respectively. The increment of P_N (ΔP_N) and Ψ ($\Delta\Psi$) was calculated as:

$$\Delta P_N l^\wedge(l-1) = P_N l^\wedge(l-1) - P_N l, \tag{5}$$

$$\Delta\Psi l^\wedge(l-1) = \Psi l^\wedge(l-1) - \Psi l, \tag{6}$$

where l is the salt stress level, $l > 1$, and l is positive integer.

According to Eqs. 4 and 6, the increment of WC (ΔWC) could be calculated as:

$$\Delta WCl^{\wedge}(l-1) = \Delta WCl^{\wedge}(l-1) - WCl = \frac{\Delta \Psi l^{\wedge}(l-1)M}{100iRT}, \quad (7)$$

where l is the salt stress level, $l > 1$, and l is positive integer; M is the relative molecular mass of cell SAP solute, sugar $C_{12}H_{22}O_{11}$,and M is 342; Ψ is plant tissue water potential (MPa); i is dissociation coefficient ($i = 1$); R is gas constant ($R = 0.0083$ L MPa mol^{-1} K^{-1}); and T is thermodynamic temperature (273 + t °C) K.

So, the increment of WC (ΔWC) could be calculated as:

$$\Delta WC^{*}l^{\wedge}(l-1) = \frac{\Delta WCl^{\wedge}(l-1) \times m}{18 \times 1814400A}, \quad (8)$$

where $\Delta WC^{*}l^{\wedge}(l-1)$ is increment of leaf water content per leaf area and per second, mmol m^{-2} s^{-1}, m (g) is leaf fresh weight, and A (cm^2) is the area of chamber.

According to Eqs. 5 and 8, re-watering water-use efficiency (WUE_R, mmol CO_2 mol^{-1} H_2O) was calculated as:

$$WUE_R l^{\wedge}(l-1) = \frac{\Delta P_N l^{\wedge}(l-1)}{\Delta WC^{*}l^{\wedge}(l-1)}. \quad (9)$$

Statistical analysis – All measurements were subjected to analysis of variance (ANOVA) to discriminate significant differences (defined as $P \leq 0.05$). Data are shown as the mean ± standard error (SE) ($n = 5$). These mean data were analyzed statistically using a factorial design through SPSS software (version 13.0, SPSS Inc.), and mean results were compared through LSD test at 5% significance level ($P < 0.05$).

3 Results

Net photosynthetic response in salt-stressed plants in comparison with re-watered plants – From the study, the net photosynthetic rate (P_N) significantly decreased with increasing salt stress in comparison with the control (Table 1). *O. violaceus* showed tolerance from slight ($NC^{2.5}$ and $NS^{2.5}$) to moderate (NC^5, NS^5, and MS^3) concentration levels of NaCl, Na_2SO_4, and mixture of salts. The increase in P_N (11.45, 11.08, 8.80, and 10.64%) was noted under high concentration (NC^{10}, NS^{10}, MS^1, and MS^2) of NaCl, Na_2SO_4, and mixture of salts, respectively, as compared to control. It was found that P_N (71.84 and 75.15%) was slightly affected under slight ($NC^{2.5}$ and $NS^{2.5}$) concentration of NaCl and Na_2SO_4 as compared to P_N (100%) under control condition. Upon comparing with other stress levels, *O. violaceus* exhibited minimum increase in P_N under MS^1 concentration of mixed salts. While in moderate (NC^5, NS^5, and MS^3) concentration of NaCl, Na_2SO_4, and mixture of salts, the increase in P_N was found as 44.13, 37.07, and 43.01%, respectively. It was also found from table that NaCl was nonsignificantly less toxic to P_N than Na_2SO_4.

Table 1 is also showing the response of P_N during salt stress subsequently re-watering. It was observed that *O. violaceus* exhibited better results significantly and nonsignificantly from stress phase to re-watering phase. During re-watering, P_N was increased by 39.20 and 35.87% under treatments with slight concentration levels of $NC^{2.5\wedge0}$ and $NS^{2.5\wedge0}$, respectively. However, salt stress at high concentration affected P_N adversely followed the order as $NC^{10\wedge5}$, $NS^{10\wedge5}$, $MS^{1\wedge0}$, and $MS^{2\wedge0}$, respectively. While in re-watering phase, the increment in P_N under severe salt stress treatments was found to be highest as 169.06, 118.66, 115.80, and 83.12%, respectively. By comparing with other stress levels after re-watering, relatively, more increments in P_N were found as 55.62, 65.46, and 50.82% under $NC^{5\wedge2.5}$, $NS^{5\wedge2.5}$, and $MS^{3\wedge0}$, moderate concentration levels, respectively. However, additions of mixture of salts at $MS^{3\wedge0}$ revealed the same effect on P_N as compared to P_N under $NC^{5\wedge2.5}$ and $NS^{5\wedge2.5}$ levels, respectively (Table 1).

The stomata conductance (g_s) significantly decreased with increasing salt stress as compared with the control (Table 2). The minimum values of g_s was noted as 20.00, 11.43, 8.57, and 8.57% under high concentration, NC^{10}, NS^{10}, MS^1, and MS^2 of NaCl, Na_2SO_4, and mixture of salts, respectively, as compared to control. While the maximum values of g_s was recorded 77.14 and 62.86% under $NC^{2.5}$ and $NS^{2.5}$, slight concentration of NaCl and Na_2SO_4 as compared to g_s (100%) was observed under control condition. The highest reduction was found in g_s under MS^1 concentration of mixture of salts, while in NC^5, NS^5, and MS^3, moderate concentration of NaCl, Na_2SO_4, and mixture of salts, the increments in g_s were found 51.42, 48.57, and 48.57% of that under control condition, respectively. It was also cleared from Table 2 that NaCl was nonsignificantly less toxic to g_s than Na_2SO_4.

Table 2 also shows the response of g_s during re-watering. It was observed that plants recover its status significantly and nonsignificantly during salt stress phase to re-watering phase. The g_s was increased by 81.58 and 80.00% under treatments with slight concentration levels of $NC^{2.5\wedge0}$ and $NS^{2.5\wedge0}$, respectively. The results showed that at moderate concentration levels of $NC^{5\wedge2.5}$, $NS^{5\wedge2.5}$, and $MS^{3\wedge0}$, *O. violaceus* showed better efficiency of g_s as 63.15, 62.86, and 55.26%, respectively, compared to high concentration levels. However, high concentrations of salt at $NC^{10\wedge5}$, $NS^{10\wedge5}$, $MS^{1\wedge0}$, and $MS^{2\wedge0}$ exerted the adverse

Table 1 Effect of salt stress subsequently re-watering on photosynthesis

Salt stress phase (g L^{-1})	Net photosynthetic rate (P_N) [μmol (CO_2) m^2 s^{-1}]	Reduction in P_N (%)	Re-watering phase (g L^{-1})	Net photosynthetic rate (P_N) [μmol (CO_2) m^2 s^{-1}]	Recovery in P_N (%)	Increment in P_N during re-watering phase (%)
Control	10.80 ± 0.21a	100.00	Control	14.06 ± 0.71a	100.00	30.29
$NC^{2.5}$	7.76 ± 0.34b	71.84	$NC^{2.5\wedge0}$	10.79 ± 0.65b	76.73	39.20
NC^{5}	4.76 ± 0.36c	44.13	$NC^{5\wedge2.5}$	7.41 ± 0.63c	52.72	55.62
NC^{10}	1.24 ± 0.17d	11.45	$NC^{10\wedge5}$	3.32 ± 0.32d	23.62	169.06
$NS^{2.5}$	8.11 ± 0.11b	75.15	$NS^{2.5\wedge0}$	11.03 ± 0.34b	78.39	35.87
NS^{5}	4.00 ± 0.08c	37.07	$NS^{5\wedge2.5}$	6.62 ± 0.47c	47.06	65.46
NS^{10}	1.20 ± 0.08d	11.08	$NS^{10\wedge5}$	2.62 ± 0.26de	18.64	118.66
MS^{1}	0.95 ± 0.05d	08.80	$MS^{1\wedge0}$	1.74 ± 0.04e	12.36	83.12
MS^{2}	1.15 ± 0.05d	10.64	$MS^{2\wedge0}$	2.48 ± 0.17de	17.61	115.80
MS^{3}	4.64 ± 0.36c	43.01	$MS^{3\wedge0}$	7.01 ± 0.30c	49.82	50.82

Values correspond to the mean ± SE ($n = 5$) photosynthesis during salt stress phase and afterward the relative percent increment under re-watering phase; distinct letters indicate significant differences at $P \leq 0.05$, according to one-way ANOVA and LSD

Table 2 Effect of salt stress subsequently re-watering on stomatal conductance

Salt stress phase (g L^{-1})	Stomata conductance (g_s) during salt stress [mol (H_2O) m^{-2} s^{-1}]	Reduction in g_s (%)	Re-watering phase (g L^{-1})	Stomata conductance (g_s) during re-watering [mol (H_2O) m^{-2} s^{-1}]	Recovery in g_s (%)
Control	0.35 ± 0.01a	100.00	Control	0.38 ± 0.02a	100.00
$NC^{2.5}$	0.27 ± 0.01b	77.14	$NC^{2.5\wedge0}$	0.31 ± 0.01b	81.58
NC^{5}	0.18 ± 0.02d	51.42	$NC^{5\wedge2.5}$	0.24 ± 0.02d	63.15
NC^{10}	0.07 ± 0.01e	20.00	$NC^{10\wedge5}$	0.10 ± 0.01e	26.32
$NS^{2.5}$	0.22 ± 0.01c	62.86	$NS^{2.5\wedge0}$	0.28 ± 0.01c	80.00
NS^{5}	0.17 ± 0.01d	48.57	$NS^{5\wedge2.5}$	0.22 ± 0.01d	62.86
NS^{10}	0.04 ± 0.01f	11.43	$NS^{10\wedge5}$	0.09 ± 0.01ef	23.68
MS^{1}	0.03 ± 0.00f	08.57	$MS^{1\wedge0}$	0.04 ± 0.00f	10.52
MS^{2}	0.03 ± 0.00f	08.57	$MS^{2\wedge0}$	0.06 ± 0.01f	15.79
MS^{3}	0.17 ± 0.01d	48.57	$MS^{3\wedge0}$	0.21 ± 0.01d	55.26

Values correspond to the mean ± SE ($n = 5$) stomatal conductance during salt stress phase and afterward the relative percent increment under re-watering phase; distinct letters indicate significant differences at $P \leq 0.05$, according to one-way ANOVA and LSD

effect on g_s and exhibited minimum increase, 26.32 23.68, 10.52, and 15.79%, respectively. It was also cleared from results that response of g_s toward NaCl concentration was better than Na_2SO_4 and mixed salts concentrations during re-watering phase (Table 2).

Transpiration (T_r) of *O. violaceus* was decreased significantly in the stress and recovered during re-watering phase as shown in Table 3. The results showed that T_r decreased during salt treatments at high concentration levels, NC^{10}, NS^{10}, MS^{1}, and MS^{2} as compared to control. In the re-watering phase, *O. violaceus* had showed better T_r at slight ($NC^{2.5\wedge0}$ and $NS^{2.5\wedge0}$) to moderate concentration levels ($NC^{5\wedge2.5}$, $NS^{5\wedge2.5}$, and $MS^{3\wedge0}$). Consequently, *O. violaceus* was found to be recovering well at these levels and using more water to decrease the transpiration rate and plant development. On the other hand, there was not found any recovery at $NC^{10\wedge5}$ and $NS^{10\wedge5}$ (in single salts) and at $MS^{1\wedge0}$ and $MS^{2\wedge0}$ levels (in mixture of salts) even during re-watering level.

Effect of salt stress on plant growth features – The application of stresses significantly affected the values of P^H, S^D, L^A, and R^L of *O. violaceus*. By following the results of P^H, S^D, and L^A under salt stress, it seemed that increase in salts concentration reduced the values of P^H, S^D, L^A, and R^L (Tables 4, 5, 6, 7). More precisely, the growth was slightly affected under slight ($NC^{2.5}$ and $NS^{2.5}$) to moderate levels (NC^{5}, NS^{5}, and MS^{3}). But the reduction in P^H was linked severely, and minimum increment was recorded as 9.78, 4.17, 1.75, and 3.10%, with high concentrations of single and mixture of salts under NC^{10},

Table 3 Effect of salt stress subsequently re-watering on transpiration

Salt stress phase (g L^{-1})	Transpiration (T_r) (mmol m^2 s^{-1})	Reduction in T_r (%)	Re-watering phase (g L^{-1})	Transpiration (T_r) (mmol m^2 s^{-1})	Recovery in T_r (%)	Increment in T_r during re-watering phase (mmol m^2 s^{-1})
Control	5.04 ± 0.24a	100.00	Control	4.13 ± 0.08a	100.00	− 0.91
$NC^{2.5}$	3.78 ± 0.11b	75.00	$NC^{2.5\wedge 0}$	3.12 ± 0.03b	75.70	− 0.66
NC^{5}	3.14 ± 0.05c	62.31	$NC^{5\wedge 2.5}$	2.32 ± 0.01c	56.11	− 0.83
NC^{10}	1.44 ± 0.14e	28.53	$NC^{10\wedge 5}$	1.36 ± 0.11d	32.99	− 0.08
$NS^{2.5}$	3.59 ± 0.24b	71.19	$NS^{2.5\wedge 0}$	3.08 ± 0.16b	74.63	− 0.51
NS^{5}	2.68 ± 0.05d	53.10	$NS^{5\wedge 2.5}$	2.28 ± 0.11c	55.32	− 0.39
NS^{10}	1.52 ± 0.03e	30.18	$NS^{10\wedge 5}$	1.28 ± 0.07d	31.09	− 0.24
MS^{1}	1.39 ± 0.06e	27.49	$MS^{1\wedge 0}$	1.14 ± 0.07d	27.52	− 0.25
MS^{2}	1.48 ± 0.02e	29.38	$MS^{2\wedge 0}$	1.25 ± 0.06d	30.22	− 0.23
MS^{3}	2.99 ± 0.20cd	59.20	$MS^{3\wedge 0}$	2.35 ± 0.01c	56.85	− 0.64

Values correspond to the mean ± SE ($n = 5$) transpiration during salt stress phase and afterward the relative percent increment under re-watering phase; distinct letters indicate significant differences at $P \leq 0.05$, according to one-way ANOVA and LSD

Table 4 Effect of salt stress subsequently re-watering on plant height

Salt stress phase (g L^{-1})	Plant height (P^H) (cm)	Increment in P^H during 21 days of salt stress phase (%)	Re-watering phase (g L^{-1})	Plant height (P^H) (cm)	Increment in P^H during 15 days of re-watering phase (%)
Cont.	39.9 ± 0.10a	66.25	Cont.	52.0 ± 1.00a	30.33
$NC^{2.5}$	35.5 ± 0.50b	34.90	$NC^{2.5\wedge 0}$	46.0 ± 0.50b	29.58
NC^{5}	31.5 ± 0.50c	22.91	$NC^{5\wedge 2.5}$	39.5 ± 0.15d	25.40
NC^{10}	24.7 ± 0.25d	09.78	$NC^{10\wedge 5}$	27.0 ± 0.10e	09.31
$NS^{2.5}$	34.7 ± 0.25b	33.39	$NS^{2.5\wedge 0}$	43.8 ± 0.05c	26.22
NS^{5}	30.8 ± 0.75c	23.91	$NS^{5\wedge 2.5}$	38.8 ± 0.45d	25.97
NS^{10}	22.5 ± 0.10e	04.17	$NS^{10\wedge 5}$	24.5 ± 0.05f	08.89
MS^{1}	23.2 ± 0.00e	01.75	$MS^{1\wedge 0}$	23.6 ± 0.15f	01.72
MS^{2}	23.3 ± 0.10e	03.10	$MS^{2\wedge 0}$	23.9 ± 0.20f	02.58
MS^{3}	30.7 ± 0.45c	20.64	$MS^{3\wedge 0}$	38.5 ± 0.35d	25.41

Values correspond to the mean ± SE ($n = 5$) plant height during salt stress phase and afterward the relative percent increment under re-watering phase; distinct letters indicate significant differences at $P \leq 0.05$, according to one-way ANOVA and LSD

NS^{10}, MS^{1}, and MS^{2}, respectively. Afterward during salt treatments, *O. violaceus* exhibited lowest increment in P^H, 1.75% under MS^{1} concentration of mixture of salts. While in moderate concentration of NaCl, Na_2SO_4, and mixture of salts, NC^{5}, NS^{5}, and MS^{3}, the increments in P^H during salt stress phase were found as 22.91, 23.91, and 20.64%, respectively (Table 4). Reduction in the increment of L^A during salt stress phase was observed continuously from control to high concentration levels (NC^{10}, NS^{10}, MS^{1}, and MS^{2}) of NaCl, Na_2SO_4, and mixture of salts (Table 5).

The control exposed the highest (100%) L^A followed by slight concentrations, moderate concentrations, and high concentrations. Similarly, control had the highest (100%) S^D and R^L followed by slight concentrations and moderate concentrations. But, salt stress at high concentrations (NC^{10}, NS^{10}, MS^{1}, and MS^{2}) exerted a severe effect on S^D and R^L (Tables 6, 7).

Afterward, Tables 4, 5, 6, and 7 also show the effect of salt stress and subsequent re-watering on P^H, S^D, L^A, and R^L of *O. violaceus*. The results showed that an increment in growth of plants from salt stress phase to re-watering phase. A statistical analysis specified that the increments which were observed during re-watering were significant, except for the concentration at high levels, $NC^{10\wedge 5}$, $NS^{10\wedge 5}$, $MS^{1\wedge 0}$, and $MS^{2\wedge 0}$. Relatively during 15 days application of re-watering, the maximum percent increments in P^H, S^D, L^A, and R^L were found under moderate concentration levels, $NC^{5\wedge 2.5}$, $NS^{5\wedge 2.5}$, and $MS^{3\wedge 0}$, respectively. However, at high concentration, the degree of salt levels exhibited the adverse effect of salt stress on P^H, S^D, L^A, and R^L (Tables 4, 5, 6, 7). Overall, increment was higher in single

Table 5 Effect of salt stress subsequently re-watering on plant leaf area

Salt stress phase (g L^{-1})	Leaf area (L^A) (cm^2)	Increment in L^A during 21 days of salt stress phase (%)	Re-watering phase (g L^{-1})	Leaf area (L^A) (cm^2)	Increment in L^A during 15 days of re-watering phase (%)
Cont.	50.3 ± 0.25a	62.26	Cont.	63.2 ± 0.10a	25.65
$NC^{2.5}$	45.3 ± 0.10b	31.56	$NC^{2.5\wedge0}$	57.2 ± 0.15b	26.27
NC^{5}	38.2 ± 0.10d	17.33	$NC^{5\wedge2.5}$	45.5 ± 0.15d	19.11
NC^{10}	29.3 ± 0.15f	08.52	$NC^{10\wedge5}$	31.5 ± 0.15e	07.51
$NS^{2.5}$	43.7 ± 0.10c	30.97	$NS^{2.5\wedge0}$	54.6 ± 0.05b	24.94
NS^{5}	37.0 ± 0.40e	17.59	$NS^{5\wedge2.5}$	44.4 ± 0.15d	20.00
NS^{10}	27.2 ± 0.10gh	04.62	$NS^{10\wedge5}$	28.0 ± 0.15f	02.94
MS^{1}	28.3 ± 0.05 g	01.80	$MS^{1\wedge0}$	28.6 ± 0.05f	01.06
MS^{2}	26.7 ± 0.10 h	02.69	$MS^{2\wedge0}$	27.3 ± 0.05g	02.25
MS^{3}	37.1 ± 0.65de	17.93	$MS^{3\wedge0}$	44.4 ± 0.05d	19.68

Values correspond to the mean ± SE ($n = 5$) leaf area during salt stress phase and afterward the relative percent increment under re-watering phase; distinct letters indicate significant differences at $P \leq 0.05$, according to one-way ANOVA and LSD

Table 6 Effect of salt stress subsequently re-watering on plant stem diameter

Salt stress phase (g L^{-1})	Stem diameter (S^D) (cm)	Increment in S^D during 21 days of salt stress phase (%)	Re-watering phase (g L^{-1})	Stem diameter (S^D) (%)	Increment in S^D during 15 days of re-watering phase (%)
Cont.	0.39 ± 0.01a	56.00	Cont.	0.52 ± 0.01a	33.33
$NC^{2.5}$	0.34 ± 0.01b	47.83	$NC^{2.5\wedge0}$	0.43 ± 0.01b	26.47
NC^{5}	0.29 ± 0.00cd	31.82	$NC^{5\wedge2.5}$	0.36 ± 0.01d	24.14
NC^{10}	0.22 ± 0.01e	15.79	$NC^{10\wedge5}$	0.25 ± 0.01e	13.64
$NS^{2.5}$	0.31 ± 0.01c	47.62	$NS^{2.5\wedge0}$	0.41 ± 0.01c	32.26
NS^{5}	0.28 ± 0.02d	27.27	$NS^{5\wedge2.5}$	0.34 ± 0.01d	21.43
NS^{10}	0.19 ± 0.01f	11.76	$NS^{10\wedge5}$	0.22 ± 0.01f	15.79
MS^{1}	0.17 ± 0.01g	06.25	$MS^{1\wedge0}$	0.18 ± 0.01g	05.88
MS^{2}	0.19 ± 0.01f	11.76	$MS^{2\wedge0}$	0.21 ± 0.01f	10.53
MS^{3}	0.28 ± 0.01d	33.33	$MS^{3\wedge0}$	0.34 ± 0.01d	21.43

Values correspond to the mean ± SE ($n = 5$) stem diameter during salt stress phase and afterward the relative percent increment under re-watering phase; distinct letters indicate significant differences at $P \leq 0.05$, according to one-way ANOVA and LSD

NaCl than single Na_2SO_4, although the concentration of salts was not varied.

Effects of salt stresses and subsequently re-watering on CA activity and water potential – The CA activity of *O. violaceus* under salt stress condition showed its regulation which varied with stress level (Fig. 1). It activated significantly in slight, $NC^{2.5}$ and $NS^{2.5}$, to moderate concentration levels, NC^5, NS^5, and MS^3, as compared to control. It had maximum values under moderate concentration levels, NC^5, NS^5, and MS^3, respectively. Nevertheless, at NC^{10} and NS^{10} (in single salts) and at MS^1 and MS^2 levels (in mixture of salts), CA showed no activity due to high stress condition especially at MS^1 level (Fig. 1) and nearly undetectable. CA activity in NaCl treatment was significantly activated than Na_2SO_4 treatments. Also, CA activity was significantly activated in both single NaCl and Na_2SO_4 concentrations than treatments of mixture of salts. In re-watering phase, CA activity showed better performance. The CA activity was successfully activated and higher under $NC^{5\wedge2.5}$, $NS^{5\wedge2.5}$, and $MS^{3\wedge0}$ concentrations. However, salt stress subsequently re-watering resulted in an adverse effect under $MS^{1\wedge0}$ and $MS^{2\wedge0}$ concentrations. CA activity of *O. violaceus* was the lowest at $MS^{1\wedge0}$ and $MS^{2\wedge0}$ concentration levels and nearly undetectable even after re-watering (Fig. 1).

According to our results, water potential (Ψ) significantly decreased going toward more negative with increasing salt stress compared with control (Table 8). The minimum decrease in Ψ was noted at $NC^{2.5}$ and $NS^{2.5}$, slight concentration levels as compared to other levels. But, the maximum decrease in Ψ was noted at NC^{10} and NS^{10}, in single salts. However, in the mixture of salts, it was

Table 7 Effect of salt stress subsequently re-watering on plant root length

Salt stress phase (g L^{-1})	Root length (R^L) cm	Increment in R^L during 21 days of salt stress phase (%)	Re-watering phase (g L^{-1})	Root length (R^L) cm	Increment in R^L during 15 days of re-watering phase (%)
Control	15.60 ± 0.50a	65.15	Control	20.84 ± 0.78a	33.60
$NC^{2.5}$	13.49 ± 0.42b	32.41	$NC^{2.5\wedge 0}$	18.40 ± 0.65b	36.39
NC^{5}	11.02 ± 0.23c	19.14	$NC^{5\wedge 2.5}$	14.20 ± 0.30c	28.90
NC^{10}	8.64 ± 0.35d	6.12	$NC^{10\wedge 5}$	9.45 ± 0.74d	9.30
$NS^{2.5}$	12.90 ± 0.52b	30.58	$NS^{2.5\wedge 0}$	16.80 ± 0.10b	30.23
NS^{5}	10.78 ± 0.29c	18.85	$NS^{5\wedge 2.5}$	13.50 ± 0.30c	25.23
NS^{10}	7.87 ± 0.87e	4.57	$NS^{10\wedge 5}$	8.20 ± 0.25e	4.25
MS^{1}	6.12 ± 0.10e	1.50	$MS^{1\wedge 0}$	6.20 ± 0.30e	1.20
MS^{2}	6.38 ± 0.76e	1.35	$MS^{2\wedge 0}$	6.39 ± 0.20e	0.12
MS^{3}	10.54 ± 0.36c	18.58	$MS^{3\wedge 0}$	13.18 ± 0.1c	25.05

Values correspond to the mean ± SE ($n = 5$) plant root length during salt stress phase and afterward the relative percent increment under re-watering phase; distinct letters indicate significant differences at $P \leq 0.05$, according to one-way ANOVA and LSD

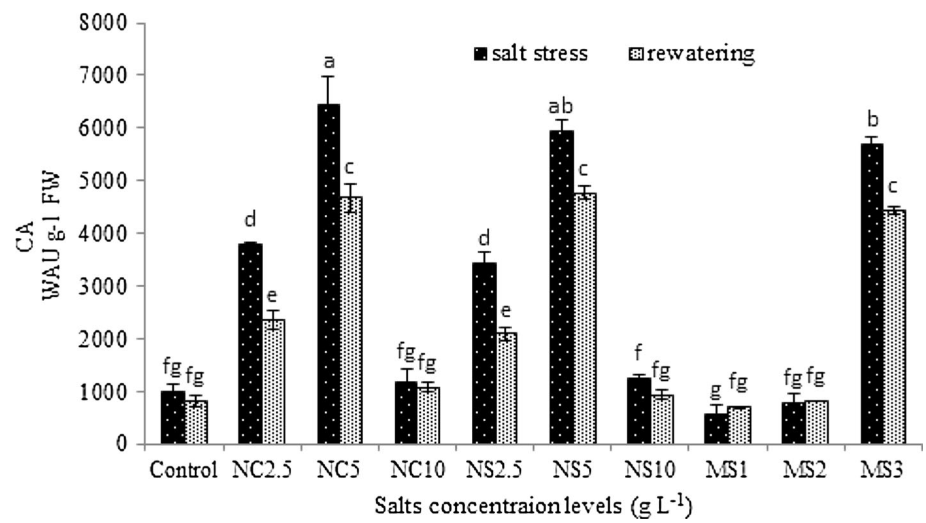


Fig. 1 Effects of salts stress subsequently re-watering on carbonic anhydrase activity (CA) in *O. violaceus*. Values correspond to the mean ± SE ($n = 5$) carbonic anhydrase activity during salt stress phase and afterward the relative percent increment under re-watering phase; distinct letters indicate significant differences at $P \leq 0.05$, according to one-way ANOVA and LSD

Table 8 Effect of salt stress subsequently re-watering on leaf water potential

Salt stress phase (g L^{-1})	Leaf water potential (Ψ) (MPa)	Re-watering phase (g L^{-1})	Increment in Ψ during re-watering phase (MPa)
Control	− 0.98 ± 0.05a	Control	0.11
$NC^{2.5}$	− 1.80 ± 0.13b	$NC^{2.5\wedge 0}$	0.70
NC^{5}	− 2.33 ± 0.13c	$NC^{5\wedge 2.5}$	0.59
NC^{10}	− 3.24 ± 0.05d	$NC^{10\wedge 5}$	0.42
$NS^{2.5}$	− 1.88 ± 0.03b	$NS^{2.5\wedge 0}$	0.71
NS^{5}	− 2.61 ± 0.11d	$NS^{5\wedge 2.5}$	0.69
NS^{10}	− 3.38 ± 0.05d	$NS^{10\wedge 5}$	0.31
MS^{1}	− 4.28 ± 0.19e	$MS^{1\wedge 0}$	0.39
MS^{2}	− 3.25 ± 0.04d	$MS^{2\wedge 0}$	0.38
MS^{3}	− 2.35 ± 0.13cd	$MS^{3\wedge 0}$	0.53

Values correspond to the mean ± SE ($n = 5$) leaf water potential during salt stress phase and afterward the relative percent increment under re-watering phase; distinct letters indicate significant differences at $P \leq 0.05$, according to one-way ANOVA and LSD severely decreased at MS^1 and MS^2 levels. Afterward, minimum decrease in Ψ was observed under control condition and maximum decrease was found in Ψ under MS^1 concentration of mixed salts. But at MS^3 level, Ψ was significantly decreased as compared to control but the decrease was equivalent to the values at NC^5 and NS^5 levels (Table 8). As a comparison between single salts and mixture of salt, it was observed that Ψ was significantly less affected in NaCl concentrations than Na_2SO_4 and mixed salts concentrations.

While in re-watering phase, the outcomes of the results showed that Ψ of *O. violaceus* was recovered during stress phase to re-watering phase (Table 8). The increment in Ψ also increased under slight levels ($NC^{2.5\wedge 0}$ and $NS^{2.5\wedge 0}$) to moderate levels ($NC^{5\wedge 2.5}$, $NS^{5\wedge 2.5}$ and $MS^{3\wedge 0}$) but it decreased at high concentration levels ($NC^{10\wedge 5}$, $NS^{10\wedge 5}$, $MS^{1\wedge 0}$, and $MS^{2\wedge 0}$), respectively. As in salts comparison with mixture of salts, Ψ was significantly increased from salt stress phase to re-watering phase in NaCl and Na_2SO_4 concentrations than mixed salts concentrations. However, the degree of salts were still showed the adverse effect on increment of Ψ even during re-watering under high levels, $NC^{10\wedge 5}$, $NS^{10\wedge 5}$, $MS^{1\wedge 0}$, and $MS^{2\wedge 0}$, respectively.

Water-use efficiency and re-watering water-use efficiency – Table 9 shows the WUE of *O. violaceus* at different salts concentration levels. WUE represents response of the salt stress level and the plants tolerance against stress. The WUE showed the nonsignificant reduction under $NC^{2.5}$ and $NS^{2.5}$, slight concentration levels, 100% and 106.04%, as compared to WUE under control (100%), respectively. As salts concentration increased at NC^{10}, NS^{10} MS^1, and MS^2, values of WUE significantly decreased as compared to the control. The minimum WUE was recorded as 32.09% at MS^1 in mixture of salts. But then again WUE was also the same at NC^5, NS^5, and MS^3 levels, respectively. However, the stress-persuaded maximum decrease was recorded at NC^{10} and NS^{10} (in single salts) and subsequently at MS^1 and MS^2 levels (in mixture of salts).

Table 9 below also shows the response of percent increment during re-watering in WUE of *O. violaceus*. It was observed that plants recovered its WUE during salt stress subsequently re-watering. The re-watering of plants lessened the effect of salt stresses considerably and showed the substantial increase under $NC^{5\wedge 2.5}$, $NS^{5\wedge 2.5}$, and $MS^{3\wedge 0}$ concentration levels. The results showed the significant increment in values at all levels in the single as well as in the mixture of salts. It showed the needs and also the requirements of plants for water during stress to re-watering phase, especially at $NC^{10\wedge 5}$, $NS^{10\wedge 5}$, $MS^{1\wedge 0}$, and $MS^{2\wedge 0}$ levels, respectively, although the WUE was also the same at NC^5, NS^5, and MS^3 levels, respectively, during re-watering.

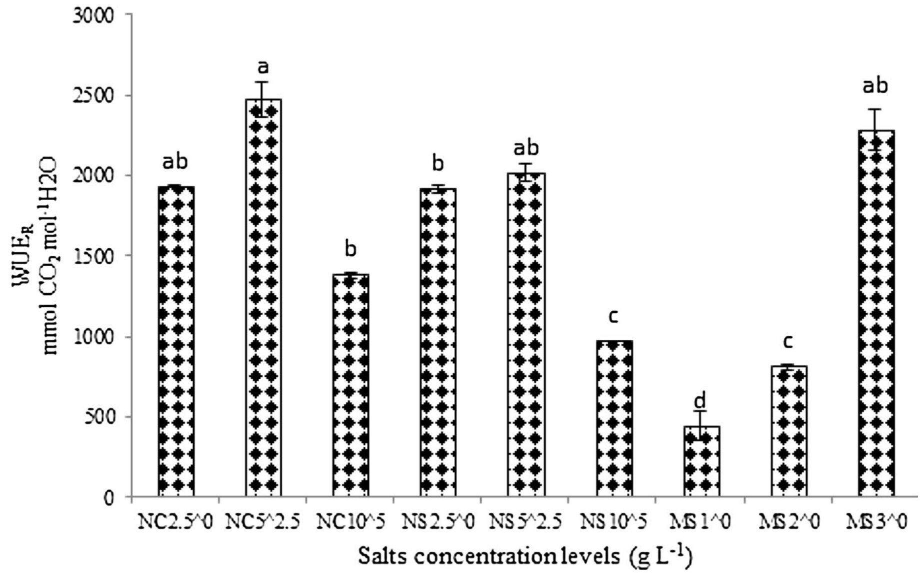
Re-watering water-use efficiency of *O. violaceus* in each stress level is shown in (Fig. 2). The $WUE_R^{10\wedge 5}$ of plants (under NC^{10} and NS^{10} level in both single salt) was lower than $WUE_R^{5\wedge 2.5}$ (at NC^5 and NS^5 levels, respectively). Although, WUE_R under mixed treatment, $WUE_R^{1\wedge 0}$ and $WUE_R^{2\wedge 0}$ (at MS^1 and MS^2 levels) was decreased significantly lower than the value at MS^3, this trend was showing no improvement in plant growth even after re-watering. Among all the WUE_R values (at NC^5, NS^5, and subsequently at MS^3), $WUE_R^{5\wedge 2.5}$, $WUE_R^{3\wedge 0}$ had maximum values and also showed better recovery. Comparatively, the plants treated with Na_2SO_4 (at NS^{10} level) affected more and showing lower WUE_R than the plants treated with NaCl (at

Table 9 Effect of salt stress subsequently re-watering on water-use efficiency

Salt stress phase ($g\ L^{-1}$)	Water-use efficiency (WUE) ($\mu mol\ mol^{-1}$)	Reduction in WUE (%)	Re-watering phase ($g\ L^{-1}$)	Water-use efficiency (WUE) ($\mu mol\ mol^{-1}$)	Recovery in WUE (%)	Increment in WUE during re-watering phase (%)
Control	2.15 ± 0.06a	100.00	Control	3.40 ± 0.12b	100.00	46.04
$NC^{2.5}$	2.05 ± 0.08a	100.00	$NC^{2.5\wedge 0}$	3.45 ± 0.20b	100.00	68.29
NC^5	1.51 ± 0.19b	73.17	$NC^{5\wedge 2.5}$	3.19 ± 0.17b	93.82	111.25
NC^{10}	0.87 ± 0.12c	40.46	$NC^{10\wedge 5}$	2.44 ± 0.16c	71.76	180.45
$NS^{2.5}$	2.28 ± 0.16a	106.04	$NS^{2.5\wedge 0}$	3.61 ± 0.28a	106.17	58.33
NS^5	1.50 ± 0.04b	69.76	$NS^{5\wedge 2.5}$	2.90 ± 0.17bc	85.29	93.33
NS^{10}	0.79 ± 0.07c	36.74	$NS^{10\wedge 5}$	2.05 ± 0.23cd	60.29	159.49
MS^1	0.69 ± 0.06c	32.09	$MS^{1\wedge 0}$	1.54 ± 0.07d	45.29	123.19
MS^2	0.76 ± 0.04c	35.34	$MS^{2\wedge 0}$	1.88 ± 0.22cd	55.29	147.36
MS^3	1.60 ± 0.15b	74.41	$MS^{3\wedge 0}$	3.01 ± 0.23b	88.52	88.12

Values correspond to the mean ± SE ($n = 5$) water-use efficiency during salt stress phase and afterward the relative percent increment under re-watering phase; distinct letters indicate significant differences at $P \leq 0.05$, according to one-way ANOVA and LSD

Fig. 2 Effects of salts stress subsequently re-watering on re-watering water-use efficiency in *O. violaceus*. Values correspond to the mean ± SE ($n = 5$) re-watering water-use efficiency during salt stress phase and afterward the relative percent increment under re-watering phase; distinct letters indicate significant differences at $P \leq 0.05$, according to one-way ANOVA and LSD



NC[10] level) though it was significantly lower in mixture of salts (at MS[1] and MS[2] levels) than single NaCl and Na_2SO_4.

4 Discussion

Effect of salt stress on growth and photosynthetic traits – Salt stress slowed the growth rate of plants (Abbas et al. 2014). The decline of water limited photosynthetic rate could likely be as a result of stomata closure (Hu et al. 2009). Increased salt stress caused closing of g_s of *O. violaceus*. The *O. violaceus* responded differently in photosynthetic to different salt stress levels. During the whole treatment period, plants kept a lower g_s at high concentration levels (NC^{10}, NS^{10}, MS^1, MS^2) (Table 2) and impacted an adverse effect on P_N (Table 1), directly influencing the performance of P^H, S^D, L^A, and R^L becoming the reason for the reduction in plant growth and development (Tables 4, 5, 6, 7). It may be because the water status of leaves disturbed by increasing salt stress through stomatal limitations. Similar results were reported by Siddiqui et al. (2008) in *Brassica juncea* L. and Shahid et al. (2011) in okra. Consequently, *O. violaceus* showed photosynthetic tolerance under slight levels ($NC^{2.5}$ and $NS^{2.5}$) to moderate concentration levels (NC^5, NS^5, and MS^3) of NaCl, Na_2SO_4, and mixed salts. Thus, this situation demonstrated the best threshold photosynthetic adaptability and tolerance of *O. violaceus* under medium concentration levels.

Salt stresses severely affect the photosynthetic rate because of variations in water potential within tissues (Ashraf and Foolad 2007). The variation in water potential affected the opening and closing of stomata, causing an imbalance in gaseous exchange and disturbance of photosynthetic activities (Chartzoulakis et al. 2002a). To overcome this condition, CA activity becomes activated in leaves providing partially carbon and water source to plants when they were suffering from deficiency of water (Wu and Xing 2012). The CA activity in *O. violaceus* showed good water regulatory under different levels of salt stresses. CA activity was initially activated, when the leaves were under slight levels ($NC^{2.5}$ and $NS^{2.5}$). A considerable and significant increase of CA activity occurred under moderate stress levels (NC^5, NS^5, and MS^3) (Fig. 1). There was substantial increase occurred in P^H, S^D, and L^A even under slight to moderate stress levels, respectively. It was an indication of development of growth parameters due to increase in P_N and performance of photosynthetic activity in result of regulation of CA activity. The obtained outcomes suggest that *O. violaceus* is found to use the same strategy as it adopted higher shade tolerant for survival because of its higher CA activity (Wu et al. 2011) and prevent plants from water losses (Hu et al. 2011). This condition exposed the threshold for adaptability and tolerance of *O. violaceus* under slight to moderate levels. *O. violaceus* established stress adaptability under slight to moderate salts stress conditions. The WUE of leaves was enhanced through regulation of CA and by maintaining the variations in leaf water potential. It was reported by Jaleel et al. (2008) that WUE is an essential attribute to utilize in the development of two varieties of *Catharanthus roseus* (L.) G. Don under water scarcity environments.

Re-watering influence on the development of growth and physiological parameters – The application of re-watering could result in stress relief and better plant development (Fig. 2). Since production part of photosynthesis might be used for the regeneration of ribulose-1,5-disphosphate, plant growth parameters did not correlated with P_N significantly when plant suffered under high stress conditions. So, at that point, an increase in P_N (169.06, 118.66, 115.80 and 83.12%) was not helpful in recovery of plant under $NC^{10\wedge5}$, $NS^{10\wedge5}$, $MS^{1\wedge0}$, and $MS^{2\wedge0}$, respectively. Though *O. violaceus* recovered and maintained its growth development response to photosynthetic traits successfully under slight levels ($NC^{2.5\wedge0}$ and $NS^{2.5\wedge0}$), its subsequent application was found at moderate levels ($NC^{5\wedge2.5}$, $NS^{5\wedge2.5}$ and $MS^{3\wedge0}$) as compared to high levels. Even in the re-watering phase, stomatal conductance was still inhibited at high concentration levels ($NC^{10\wedge5}$, $NS^{10\wedge5}$, $MS^{1\wedge0}$, and $MS^{2\wedge0}$) (Table 2). The obtained results supported by findings of Yousfi et al. (2016), which suggested that reduction in g_s by re-watering after severe drought stress resulted in decrease of P_N in all species of *Medicago laciniata* (L.) Mill. Thus, unhealthy growth status of *O. violaceus* was observed due to limited water uptake, which resulted in abruptly decreasing rate of T_r (Table 3). It was shown that the CA activity was still inhibited under high concentration (Fig. 1) and water regulation caused by CA could not work. The need of supply of H_2O and CO_2 for photosynthesis was not enough to stable water content, which becomes the reason of reduction in leaf water potential. So it was hard for *O. violaceus* to be recovered from the excessive salt stresses. *O. violaceus* growth could be maintained and recovered successfully under NaCl than Na_2SO_4 when there are no variations in amount.

Re-watering water-use efficiency (WUE_R) is an important index of *O. violaceus* to adapt different behaviors to different salt stresses following re-watering. Better effect of re-watering was found in plants due to higher WUE_R (Fig. 2). A little contribution was found in this research direction by Galle et al. (2009) and Flexas et al. (2009), focusing on photosynthetic acclimation followed by re-watering to induce recovery. Though the WUE is an instantaneous value determined by instrument, it cannot reflect the effect of re-watering on plant. WUE_R is a new and important index which specifies better effect of re-watering on plant. Because the WUE_R intended that the addition in water content directed to the increment of P_N in leaves of *O. violaceus* from stress phase to re-watering phase. The massive decrease of WUE under high stress exposed the wilting point and high demand of water for plants (Table 8). For that reason, it was necessary for plants to be re-watered before wilting stage. The level at which net photosynthetic rate is to be maintained during periods of drought and water stress, and the capability of fast recovery of photosynthesis after re-watering is an important point in plant tolerance adaptation to drought stress environment (Chartzoulakis et al. 2002b). Therefore, *O. violaceus* was to be re-watered under moderate concentration levels at $WUE_R^{5\wedge2.5}$ and $WUE_R^{3\wedge0}$ levels under NC^5, NS^5, and also consequently at MS^3, respectively, because P_N works more effectively and increased by 55.62, 65.46, and 50.82%, respectively. Thus, moderate concentration levels were considered the best zone for threshold tolerance and production of *O. violaceus* under saline condition. The relative effect of mixture of salt at $MS^{3\wedge0}$ was same with single salts under $NC^{5\wedge2.5}$ and $NS^{5\wedge2.5}$, respectively. It is concluded that single salt was more toxic to plant growth than mixture of salts. It is also demonstrated that *O. violaceus* has stress bearing adaptability under only slight to moderate salt stress conditions.

A considerable reduction was observed even during re-watering in WUE_R under $WUE_R^{10\wedge5}$, $WUE_R^{1\wedge0}$, and $WUE_R^{2\wedge0}$ (at NC^{10}, NS^{10}, MS^1, and MS^2). During the whole treatments of single salt and mixture of salts, plants growth was inhibited at high concentration levels and showed inactivated effect of application of re-watering to plants. According to Yousfi et al. (2016), after application of re-watering, there is partial recovery found in some species of *Medicago laciniata* (L.) Mill., due to severe drought stress. Upon re-watering of plants which were suffered from high water stress condition under NC^{10}, NS^{10}, MS^1, and MS^2, indicated that it is difficult for plants to be recovered from rapid increase in the assimilation rate. The basic mechanism of photosynthetic biochemistry adopted by plant under stress condition is not regulated due to deficiency of water. However, the decrease in net CO_2 uptake is not only the reason of stomatal closure to decrease photosynthetic rate (Cornic 2000). Therefore, plants suffering from high salt stress stayed stunt. It also indicated the inactiveness of P_N when plant suffered from stress conditions. It is most likely due to the production of photosynthesis, which was used for regeneration of other parts like ribulose-1,5-disphosphate. Thus, this situation demonstrated an inadaptability of *O. violaceus* under high stress levels. Therefore, *O. violaceus* was thought to be the species with single and mixture of salts tolerance adaptability under slight to moderate stress conditions.

In conclusion, *O. violaceus* is tolerant to salt stress under low to medium concentration levels and WUE_R left positive effects on the growth and developments of plants at $NC^{5\wedge2.5}$, $NS^{5\wedge2.5}$, and $MS^{3\wedge0}$ levels. Though aimed at consideration of high production in *O. violaceus*, the best re-watering time was found when plants undergo moderate concentration levels. This regime was considered as best for growth of *O. violaceus* under saline conditions and dilution of saline water. Therefore, the effect of salinity on plant could be reduced by diluting the saline irrigation (re-watering) and also by mixing of salts response to physiological behaviors. Application of dilution of saline irrigation could be helpful to maintain crop productivity, reduce irrigation cost, and save water resources.

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