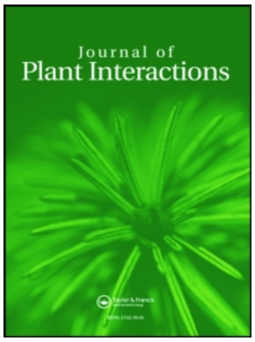

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RESEARCH ARTICLE

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Photosynthetic response of two okra cultivars under salt stress and re-watering

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ABSTRACT
Two cultivars of okra (Chinese green and Chinese red) were subjected to salt stress (0%, 6%, 12% and 18%) and equal proportions of NaCl and $CaCl_2$ in Hoagland's nutrient solution and re-watering. Salt stress significantly reduced growth parameters and photosynthetic attributes of both cultivars. Treatment subjected to 18% salt stress caused 90% redundancy in growth parameters of both cultivars compared to control. Re-watering gave a positive response for plant growth of both cultivars in different levels. Chinese green showed better recovery at 6–0% re-watering level and Chinese red showed 12–6% and 6–0%, due to its salt tolerance nature. Considering re-watering water use efficiency and net photosynthetic rate the optimum values of salt tolerance for Chinese green and Chinese red were 8.3% and 12.02%, respectively. The best re-watering degree found as salt stress level ranged from 12.02% to 6% for Chinese red and 8.3% to 2.3% for Chinese green. This study provided a new method for the determination of irrigation time and quantification in crops.

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KEYWORDS
Okra; photosynthetic rate; plant growth; salt stress; re-watering

Introduction

Water becomes saline water if it contains a high concentration of soluble salt. The cause of salinity could be man-made or due to historical processes (Jouyban 2012). Saline water or soil salinity is one of the most harmful environment problems in agriculture. It affects plant growth and crop production directly. About 37% of the world cultivated land is sodic and 23% is saline (Khan & Duke 2001). Salinity effects over 70 million hectares of agricultural land which is about 20% irrigated land and 2% dry land (FAO 2005). Currently, salinity affects nearly 33% of all irrigated land in the world (Munns 2005). In China, salinity impacts about one-third of the total irrigated crop land (Hou et al. 2007).

Salt stress affects plant growth in three different ways: reduction of soil water potential called osmotic stress, causing an ionic imbalance in cells and lastly ion toxicity. Reduction in plant growth is directly related to the soluble salt concentration and duration of stress (Tavakkoli et al. 2011). Salinity can influence several physiological processes including photosynthesis, transpiration, stomatal conductance and water potential, growth and yield production (Munns 2002; Lee et al. 2004; Akram et al. 2006; Kao et al. 2006; Munns et al. 2006; Moud & Maghsoudi 2008; Hayat et al. 2010; Yousif et al. 2010; Wang et al. 2012). The increase in salt stress causes a reduction in water uptake due to excess amount of salt present in the soil water solution due to decrease in water potential and water content (WC) (Romero-Aranda et al. 2001; Wang et al. 2012). During salt stress large amount of salt present in the leaf decreased water potential. Leaf stomata was closed due to presence of large amount of salts in the leaf that decreases transpiration and closed stomata reduced CO_2, due to this photosynthesis is decreased. (Parida et al. 2004; Sudhir & Murthy 2004; Yousif et al. 2010; Wani et al. 2013). Salt stress also reduced plant height, fresh weight (FW) and dry weight (DW) (Rui et al. 2009; Memon et al. 2010; Habib et al. 2012). Carbonic anhydrase (CA) is an enzyme whose activity transports carbon dioxide and protons across biological membranes and retains inorganic carbon within the cell (Tavallali et al. 2009). Some plants could convert intracellular HCO_3^- into CO_2 and H_2O by CA when they suffered from salt stress. The CA regulates photosynthesis in response to salt stress. CA activity enhances the conversion of bicarbonate to H_2O and CO_2, when the plant was suffering under salt stress (Hacisalihoglu et al. 2003; Clausen et al. 2005; Wu & Xing 2012).

A number of research works have been done with the ultimate aim of reducing the effect of salt stress on plant growth using different methods and techniques. A number of research works have been done on the removal of excess salt from the root zone by using different methods like scraping, flushing and leaching (Jouyban 2012). However, others have also worked on reducing the salinity effect using different irrigation methods (Alomran et al. 2012; Soomro et al. 2012; Patil et al. 2014). Others have also researched on growing halophyte crop using saline water (Khan et al. 2000; Khan & Duke 2001; Belkheiri & Mulas 2013). They have used different concentrations of saline water and checked the response of different crops in different concentration levels. On the basis of their results, they concluded that some species yield the best production under slight stress, whereas some give the best yield under moderate stress and some high salt stress (Fisarakis et al. 2001; Abid et al. 2002; Parida et al. 2004; Chen et al. 2010; Memon et al. 2010; Saleem et al. 2011; Riccardi et al. 2014). But previously nobody tried it to check the response of the crop by dilution of saline water or re-watering. Re-watering is like that if a crop-receiving water of high concentration of soluble salt gives that crop with a low concentration of soluble salt and checks its response on it. In this study, we will check the response of re-watering on okra cultivars.

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Okra (*Abelmoschus esculentus L.*) is recognized as an annual herbaceous plant grown in tropical and subtropical areas and serves as a source of carbohydrates, fats, vitamins and various minerals (Oyenuga 1968). Okra plant at earlier growth stages is more sensitive to salinity (Cerda et al. 1995), as it affects water relations and nutrient uptake of plants. The aim of the present study was to analyze the effects of salt stress and re-watering on some photosynthetic and growth parameters of two okra cultivars. Furthermore, finding the best re-watering time and best re-watering degree to dilute salt water could be helpful for saving water resources.

Material and method

Plant material and culture condition

This experiment was conducted at the School of agriculture equipment engineering, Jiangsu university, Jiangsu Province, China (32.20°N, 119.45°E), from May to August 2015. Okra (*Abelmoschus esculentus L.*) was used for this study. Seeds of two okra cultivars (Chinese green and Chinese red) were germinated in seedbeds with a mixture containing vermiculite, perlite and peat moss (2:1:1, v/v/v). Pots were kept under greenhouse conditions. During the germination stage, seeds were watered with Hoagland solution (Hoagland & Arnon 1950). This experiment was carried out with three replications. After 15 days, seedlings were transferred into a plastic pot of 9 cm diameter and 11 cm height containing vermiculite as a growing medium. Different concentrations of saline water were made in Hoagland nutrient solution, with an equal amount of NaCl and $CaCl_2$ added into Hoagland nutrient solution to make water of different concentrations such as control (0%), 6%, 12% and 18%, as shown in Table 1. When a plant had four fully expanded leaves saline water with different concentrations was applied. Plants were exposed to saline water for 21 days, after which plants that had received water of 6%, 12%, 18% were given water at 0%, 6%, 12%, respectively, which was regarded as re-watering and the re-watering phase continued for 28 days.

Physiological parameters

Photosynthetic rate (P_N), leaf intercellular CO_2 concentration (Ci), water use efficiency (WUE), transpiration rate (E) and stomatal conductance (g_s) were recorded using a portable LI-6400XT photosynthesis measurement system (LI-COR, Lincoln, NE, USA). A fully expanded youngest leaf from the top of each plant was used for all these measurements, which were performed during full sunshine from 9.00 am to 10.00 am. The following conditions were noted during data recording: ambient CO_2 concentration 400 μmol mol^{-1}, atmospheric pressure 99.9 kPa, photosynthetically active radiation at leaf surface was maximum up to 1000 μmol m^{-2} s^{-1}, molar flow of air/unit leaf area 403.3 mmol m^{-2} s^{-1}, water vapor pressure ranged from 7.0 to 8.8 mbar, temperature of leaf ranged from 25°C to 28°C and ambient temperature from 31.2°C to 35.9°C. WUE was calculated according to the following equation: $WUE = P_N/E$, where P_N is the net photosynthetic rate and E is the transpiration rate.

Water potential

Leaves in the salt stress and re-watering phase were used for the determination of water potential. Leaf water potential was measured with dew point micro voltmeter in a C-52-SF universal sample room (Psypro, *Wescor*, USA).

Growth parameters

Plant height, root length (RL), FW and DW were measured before treatment, after salt stress and after re-watering. Plant height and RL were measured with the help of a tape. Then the plant was removed from the growth medium, its FW was measured and then this plant was kept inside an oven at 82°C until it reached a constant weight and later the DW was measured with the help of a weighing scale.

Carbonic anhydrase activity

Leaves in the salt stress and re-watering phase were used for the determination of CA activity. Leaf sample (0.1–0.2 g) was quickly frozen 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 was centrifuged at 10,000 g, 0°C for 5 min and then placed inside the refrigerator for 20 min. The liquid was used to determine CA activity using the pH method described by Wilbur and Anderson (1948) with modifications (Wu et al. 2011). 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. CA activity was expressed in Wilbur and Anderson (WA) units as $WA = (t_0/t) - 1$, where t_0 and t were the time (s) measured for the pH change (8.2–7.2), with buffer alone (t_0) and with the sample (t).

Calculation of re-watering water use efficiency (WUE_R)

Re-watering water use efficiency (WUE_R) was calculated by the increment of water potential (Ψ) and net photosynthetic rate (P_N) in leaves of okra from salt stress to the re-watering phase. In the experiment, four treatment levels of 0, 6%, 12% and 18% were marked as levels 1, 2, 3 and 4, respectively. In the stress phase, P_N and Ψ under levels 1, 2, 3 and 4 were expressed as $P_N l$ (μmol m^{-2} s^{-1}) and Ψ_l (MPa) respectively, (l was the salt stress level, $l = 1, 2, 3, 4$). In the re-watering phase, P_N and Ψ of leaves in three salt stress levels 2, 3 and 4 after re-watering were expressed as $P_N l \wedge (l-1)$ and $\Psi l \wedge (l$

Table 1. Chemical characteristics of the treatment water.

Treatment	EC (ds/m)	pH	Ion concentration (mol/l) K^+	Na^+	Ca^{2+}	Mg^{2+}	Cl^-	SO_4^-
0	02.08	05.55	00.01	00.04	00.01	00.01	00.01	00.01
6%	10.33	04.31	00.08	00.17	00.08	00.12	00.08	00.03
12%	18.00	04.35	00.16	00.30	00.15	00.25	00.16	00.07
18%	25.70	04.40	00.24	00.43	00.23	00.37	00.25	00.09

−1), respectively ($l \wedge (l{-}1)$ indicated that leaves were re-watered from salt stress level l to salt stress level $l{-}1$. $l > 1$, and l was a positive integer).

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

$$\Psi = iQRT, \quad (1)$$

where Ψ was the plant leaf water potential (MPa), i was the dissociation coefficient ($i = 1$), Q was the cell SAP solute concentration (mol L^{-1}), R was the gas constant ($R = 0.0083$ L MPa mol^{-1} K^{-1}) and T was the 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:

$$P = MQ/1000\%, \quad (2)$$

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

According to Equations (1) and (2), P could be re-written as:

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

Proportion of WC in the total quality of leaf was *1−P*; it was expressed as WC (%).

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

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

$$\Delta P_N l \wedge (l-1) = P_N l \wedge (l-1) - P_N l, \quad (5)$$

$$\Delta\Psi l \wedge (l-1) = \Psi l \wedge (l-1) - \Psi l. \quad (6)$$

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

$$\begin{aligned}\Delta \mathrm{WC} l \wedge (l-1) &= \Delta \mathrm{WC} l \wedge (l-1) - \mathrm{WC} l \\ &= \Delta\Psi l \wedge (l-1) M/100iRT, \quad (7)\end{aligned}$$

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

Through the determination of leaf FW m (g) and leaf area A (cm^2), compared to 28 days re-watering time, increment of leaf WC per leaf area and per second could be calculated as:

$$\Delta \mathrm{WC}{*}l \wedge (l-1) = \Delta \mathrm{WC} l \wedge (l-1) \times m/18 \times 2419200A, \quad (8)$$

where $\Delta \mathrm{WC}^{*} l \wedge (l{-}1)$ was the increment of leaf WC per leaf area and per second, mmol m^{-2} s^{-1}.

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

$$\mathrm{WUE}_R l \wedge (l-1) = \Delta P_N l \wedge (l-1)/\Delta \mathrm{WC}{*}l \wedge (l-1). \quad (9)$$

Optimized value

Optimized value was calculated through the sum of total net photosynthesis rate (P_N) of every level during salt stress and re-watering and divided by 4 (4 is the total number of levels). This gives a mean value of P_N of all treatments during salt stress and re-watering. The mean value of P_N is:

$$21P_N + 28P_N/4 = ANSWER. \quad (10)$$

Adding all values of P_N during salt stress and re-watering at 0% and dividing by the *ANSWER* obtained through Equation (10) t gives $P_N{-}m$ means values at 0% during salt stress and the re-watering period.

In this situation Equation (11) becomes:

$$P_N - m(0\%) = 0\%P_N - m/ANSWER. \quad (11)$$

So using Equation (11), replacing $P_N{-}m$ of 0% with 6%, 12% and 18%, the $P_N{-}m$ of 6%, 12% and 18% can be calculated, respectively.

The mean value of re-watering use efficiency (WUE_R) was calculated by adding all values of WUE_R of all levels during re-watering and dividing by 4 (4 is the total number of levels). The mean value of WUE_R is

$$\begin{aligned}&\text{Adding all levels of re - water use efficiency } (\mathrm{WUE}_R)/4 \\ &= ANSWER.\end{aligned} \quad (12)$$

Adding all values of WUE_R at 6–0% and dividing by ANSWER obtained through Equation (12) gives WUE_R means values at 6–0% during the re-watering period.

$$\mathrm{WUE}_R(6-0\%) = 6 - 0\%\mathrm{WUE}_R/ANSWER. \quad (13)$$

So using Equation (13), replacing WUE_R of 6–0% with 12–6%, and 18–12%, the mean value of WUE_R of 6–0%, 12–6% and 18–12% can be calculated, respectively.

Using the values of $P_N{-}m$ of every level obtained through Equation (11) and WUE_R values of every level obtained through Equation (13), when a graph of these values is made, the point where these values intersect each other is the optimum value.

Statistical analysis

All measurements were subjected to analysis of variance (ANOVA) to discriminate significant differences (defined as $P \le .05$) between group means. Data were shown as the mean ± standard error (SE). These mean data were analyzed statistically using a factorial design through SPSS software (version 17.0, SPSS Inc.) and mean results were compared through the Tukey post hoc test at the 5% significance level ($P < .05$).

Results

Net photosynthetic

The net photosynthetic (P_N) rate of both okra cultivars in salt stress and the re-watering phase is shown in Figure 1(a,b). The net photosynthetic rate varies significantly within cultivars and salt stress level. Over the whole duration of the salt stress phase, the net photosynthetic of Chinese red was higher than Chinese green, but Chinese green had greater

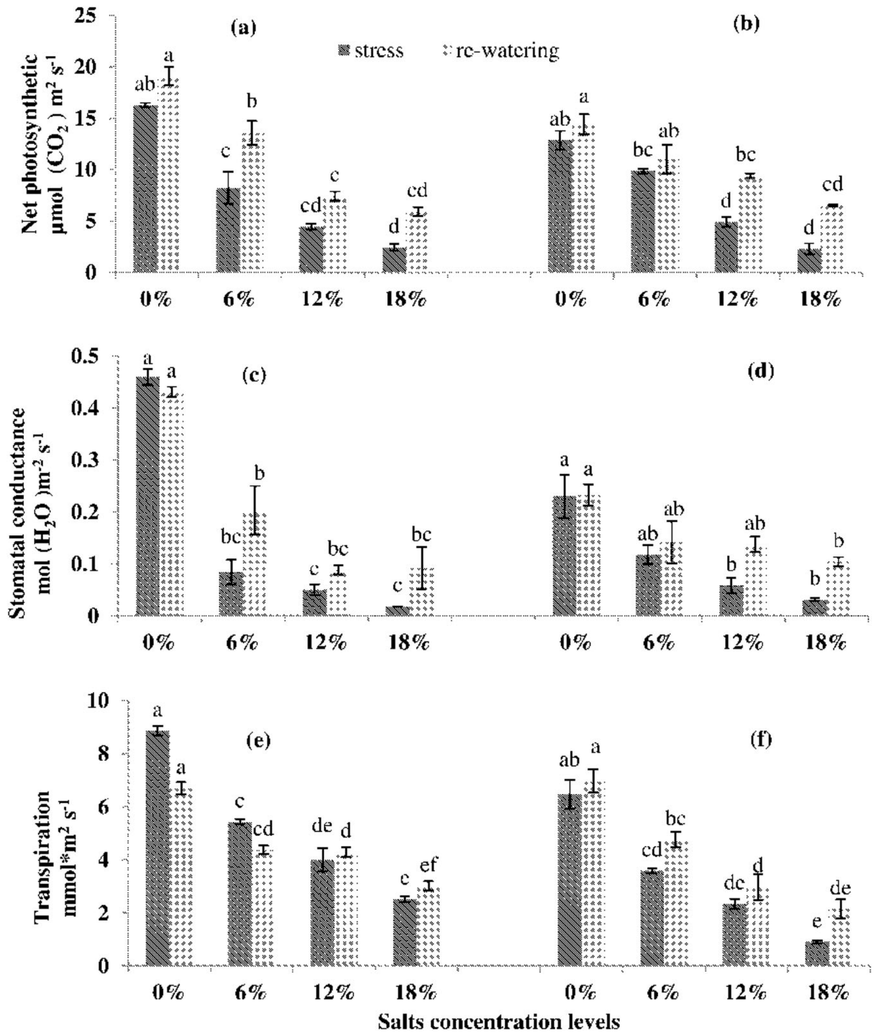

Figure 1. Effects of salt stress treatments on the photosynthetic (a, b), stomatal conductance (c, d), transpiration (e, f) of Chinese green and Chinese red; values represent means ± SE, followed by different letters in the same treatment process indicating a significant difference at $P \leq .05$, according to one-way ANOVA and Tukey tests.

net photosynthetic under control than Chinese red. The net photosynthetic of both cultivars under 18% was lower than under 12% and 6% salt stress levels. In the re-watering phase Chinese green and Chinese red showed significant results. Chinese green showed good recovery in all the levels but more recovery was found in the level 6–0%, wherein the photosynthetic rate increased by almost 60.5% compared to salt stress results. Chinese red also showed good recovery in the re-watering phase but more recovery was found in the 12–6% and 18–12% levels, wherein the photosynthetic rate increased by almost 50% and 35.3%. In between these two cultivars, Chinese red showed good recovery in the third and fourth levels compared to Chinese green; it showed that Chinese red had the ability to recover well from high salt stress.

Stomatal conductance

Stomatal conductance of the two cultivars was decreased significantly in salt stress and increased significantly in re-watering as shown in Figure 1(c,d). Stomatal conductance of Chinese green was higher in the control phase than that of Chinese red. Chinese green showed a significant decrease in stomatal conductance within salt stress level, wherein there was 82.2% decrease in stomatal conductance of Chinese green in 6% salt stress level than control. But the reduction was non-significant under salt stress level of 12% and 18%, respectively. However, in Chinese red, stomatal conductance also decreased in every salt stress level, but more decease was found in 18% salt stress level, wherein almost 86.3% decreased than control. In between these two cultivars in every salt stress level Chinese red had a higher value than Chinese green. Chinese green showed good recovery at 6–0% in the re-watering phase, wherein almost 40% increased. On the other hand, Chinese red showed good recovery at 12–6% and 18–12% in the re-watering phase, almost 38% and 30% increase in stomatal conductance. In between these two cultivars, Chinese red showed good results in the third and fourth levels and Chinese green showed a good result only in the second level.

Transpiration

Transpiration of the two cultivars was decreased significantly in salt stress and increased significantly in re-watering as shown in Figure 1(e,f). Chinese green showed a sudden decrease in transpiration at 6% salt stress level than control but within salt stress level, a gentle reduction was observed. Chinese green had a higher value of transpiration in every stress level as compared to Chinese red. Chinese red also showed decrease in transpiration than control but more decrease was found under 18% stress level. In the re-watering

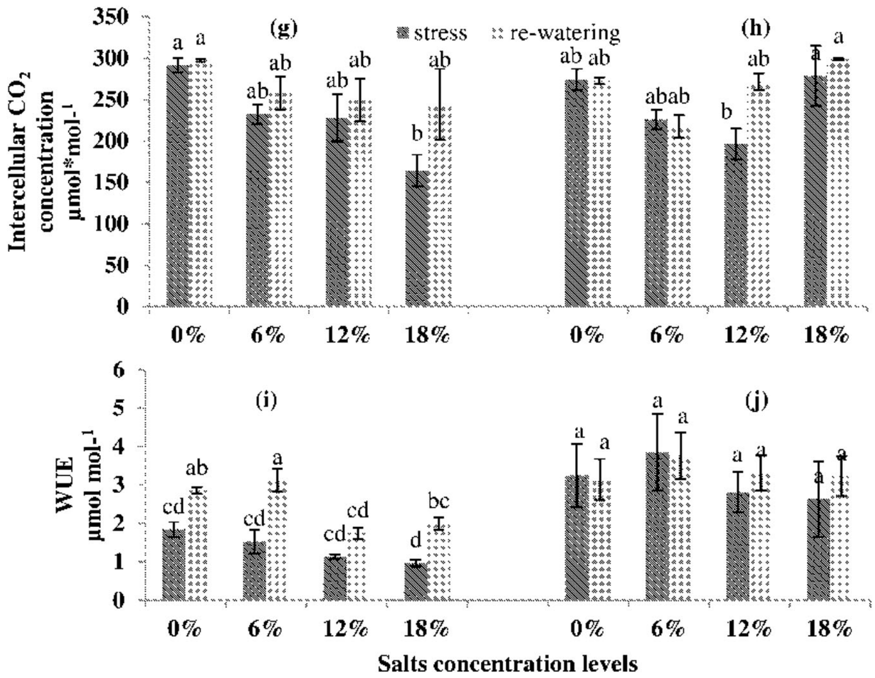

Figure 2. Effects of salt stress treatments on the intercellular CO_2 concentration (g, h) and WUE (i,j) of Chinese green and Chinese red; values represent means ± SE, followed by different letters in the same treatment process indicating a significant difference at $P \leq .05$, according to one-way ANOVA and Tukey tests.

phase, Chinese green had less transpiration value at level 6–0% as compared to the value of salt stress; these results could be due to the Chinese green recovering well in this level and that is likely the reason the crop utilized more water resulting in decreased transpiration. Chinese red showed an increase in transpiration at every re-watering level. In between these two cultivars, Chinese green had more transpiration compared to Chinese red likely because Chinese red uses water for plant development or recovery, hence the decrease in transpiration rate.

Intercellular CO_2 concentration and WUE

Intercellular CO_2 concentration of both cultivars showed significant results (Figure 2(g,h)). Chinese green showed more than 50% reduction in 18% salt stress level could be due to the closure of stomata. There was no significant difference between 6% and 12% salt stress levels in Chinese green. Chinese red showed quite interesting results in the salt stress phase, wherein the value was decreased by 6% and 12% but in the 18% salt stress level intercellular CO_2 concentration was increased, which could be due to stomatal limitation that helps plants to increase their intercellular CO_2 concentration. In comparison between Chinese green and Chinese red in the salt stress phase, Chinese green had more value in 6% and 12% but Chinese red had more value at 18% salt stress phase due to stomatal limitation. In the re-watering phase, the increase in intercellular CO_2 of both cultivars was almost the same. Chinese green showed good recovery in 18–12% and Chinese red showed good recovery in 12–6%.

WUE of both okra cultivars varied under salt stress and re-watering phase. Chinese green showed significant results in salt stress and re-watering phase but Chinese red showed non-significant results in both stress and re-watering (Figure 2(i,j)). Chinese green had lower WUE in every stress because of the high value of transpiration. Chinese red had slightly higher WUE at 6% stress level but this value was non-significant compared to the other levels; thus Chinese red had stable WUE and it could be due to decrease in transpiration in every stress level. In between these two cultivars, Chinese red had higher WUE than Chinese green in the salt stress phase. In the re-watering phase, Chinese green had more increase in 6–0%, almost 48% WUE increase. This increase was due to a decrease in transpiration. Chinese red maintained the same WUE in the re-watering phase as in the stress phase. Chinese green had a higher value at the 6–0% level and in the other levels Chinese red had more.

Water potential

Water potential of both okra cultivars varied in stress and re-watering phase, showing significant result in both phases (Figure 3(i,k)). Chinese green showed a significant decrease in water potential in every stress level, but more decrease in the 18% stress level, almost a decrease of 63% than control. Chinese red also showed the same results as found with Chinese green and a decrease of almost 60% was observed under 18% stress level. Chinese green had more decrease in 12% salt stress level as compared to red and the remaining levels had almost the same results. In the re-watering phase, the water potential of Chinese green increased in every level but there was more increase in 6–0% and 12–6% levels, almost 18% and 21% increase. Chinese red recovered well in every re-watering level but more increase was found in 12–6% and 18–12% levels, almost 32% and 38% increase, respectively.

WC of both cultivars showed significant results with a decrease in stress and increase in the re-watering phase (Figure 3(m,n)). Chinese green and Chinese red had lower WC at 18% than control in salt stress. In between salt stress levels, both cultivars had non-significant values at 6% and 12%. In the re-watering phase, both cultivars showed increase in WC at every level. Chinese green showed more increase in 12–6% and Chinese red showed more increase in 18–12%. This increase in WC showed that dilution of solutions has a positive effect on plant growth.

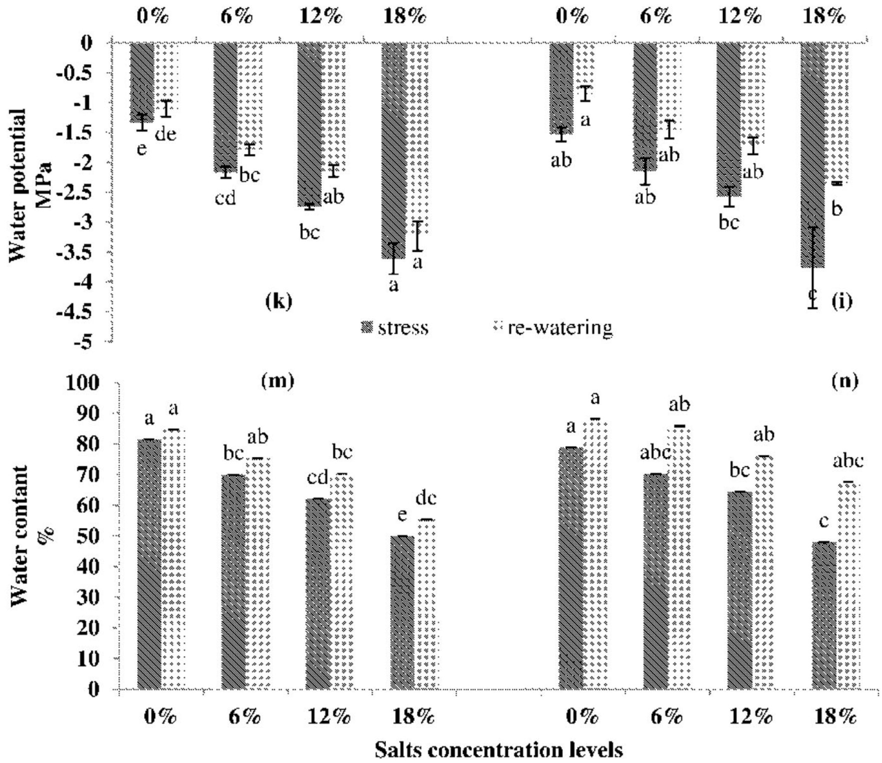

Figure 3. Effects of salt stress and re-watering treatments on the water potential (k, i) and WC (m, n) of Chinese green and Chinese red; values represent means ± SE, followed by different letters in the same treatment process indicating a significant difference at $P \leq .05$, according to one-way ANOVA and Tukey tests.

Growth parameters

Salt stress significantly reduced the plant height, RL, FW and DW of both okra cultivars. Decrease in plant height at 6% and 18% salt stress level was same in both cultivars but at 12%, plant height of Chinese green was decreased more as compared to Chinese red as shown in Table 2. Re-watering has a positive effect on both okra cultivars in different levels. Chinese green showed a better result at all levels but more increment was found at 6–0%. Chinese red showed good result at 12–6% and 6–0% levels; in these levels increments were more than 60%.

RL of both cultivars decreased significantly in salt stress as shown in Table 3. The RL of Chinese green was more sensitive to salt stress than that of Chinese red. The effects started to be significant at the 6% level and continued up to the last level in both cultivars. At the 18% level, the effect was almost the same for both cultivars. Re-watering gives good results in both cultivars. Chinese green showed better recovery at 6–0% and Chinese red at 12–6% and 6–0%. In the last level, at 18–12% both cultivars did not recover probably due to irrigation water still having a high amount of salts. This most likely is the reason for the plant not being able to recover.

The effect of salt stress treatments on plant FW of both okra cultivars was significant as shown in Table 4. A significant difference began at the 6% level compared to control in both cultivars. More decrease was found at 12% and 18% levels within salt stress in both cultivars. Re-watering at the 6–0% level gives maximum recovery of both cultivars and more than 80% increment was found in this level. Chinese red at 12–6% level showed 25% increment in plant FW and it showed good recovery at a higher level of stress. In 18–12%, both cultivars did not recover because the high amount of salt had already damaged the plant so badly that re-watering did not affect it.

The effect of salt stress treatment on the DW of both cultivars was significant as shown in Table 5. In both cultivars, at 6% salt stress level almost 40% reduction was found and at 18% salt stress level almost 100% reduction was found. This result showed that the plant of both cultivars almost died in 18% stress level. Within the second and third salt stress level, Chinese green had more DW reduction than Chinese red. Re-watering only gives better recovery at 6–0% in both cultivars.

Carbonic anhydrase

CA activity varied within cultivars, salt stress level and duration. CA activity was higher in Chinese red than Chinese green as shown in Figure 4(o,p). In Chinese green, CA activity

Table 2. Plant height of both okra cultivars after salt stress and re-watering.

	Treatments	PH	PH (%)	Re-watering	ΔPH	ΔPH (%)
Green	0%	49.34 ± 0.43a	100.00	0%→0%	28.77 ± 0.60a	100.00
	6%	35.77 ± 0.31b	027.50	6%→0%	23.70 ± 0.63b	082.30
	12%	18.99 ± 0.25c	061.50	12%→6%	08.43 ± 0.29c	029.30
	18%	08.19 ± 0.31d	083.40	18%→12%	02.70 ± 0.27d	009.38
Red	0%	54.35 ± 1.07a	100.00	0%→0%	31.70 ± 1.19a	100.00
	6%	40.46 ± 0.56b	025.60	6%→0%	26.03 ± 0.86b	082.20
	12%	26.50 ± 0.51c	051.25	12%→6%	20.36 ± 1.18c	064.30
	18%	06.76 ± 0.26d	087.70	18%→12%	03.00 ± 0.28d	009.40

Note: Effects of salt stress treatments on the plant height of Chinese green and Chinese red; values represent means ± SE, followed by different letters in the same treatment process indicating a significant difference at $P \leq .05$, according to one-way ANOVA and Tukey tests.

Table 3. RLs of both okra cultivars after salt stress and re-watering.

	Treatments	RL (cm)	RL (%)	Re-watering	ΔRL (cm)	ΔRL (%)
Green	0%	12.86 ± 0.31a	100.00	0%→0%	11.48 ± 0.52a	100.00
	6%	08.80 ± 0.15b	031.50	6%→0%	07.42 ± 0.27b	064.63
	12%	04.04 ± 0.15c	068.58	12%→6%	01.99 ± 0.27c	017.30
	18%	01.94 ± 0.70d	084.90	18%→12%	00.65 ± 0.03c	005.60
Red	0%	13.69 ± 0.47a	100.00	0%→0%	10.50 ± 0.59a	100.00
	6%	10.46 ± 0.51b	023.50	6%→0%	09.40 ± 0.21a	089.50
	12%	06.09 ± 0.33c	055.50	12%→6%	04.07 ± 0.29b	038.70
	18%	01.82 ± 0.27d	086.70	18%→12%	00.88 ± 0.00c	008.30

Note: Effects of salt stress treatments on the RL of Chinese green and Chinese red; values represent means ± SE, followed by different letters in the same treatment process indicating a significant difference at $P \leq .05$, according to one-way ANOVA and Tukey tests.

Table 4. Fresh weights of both okra cultivars after salt stress and re-watering.

	Treatments	FW (g)	FW (%)	Re-watering	ΔFW (g)	ΔFW (%)
Green	0%	29.63 ± 0.60a	100.00	0%→0%	39.20 ± 0.28a	100.00
	6%	18.57 ± 0.51b	037.32	6%→0%	31.38 ± 0.60b	080.00
	12%	13.03 ± 0.31c	056.00	12%→6%	06.40 ± 0.15c	016.32
	18%	02.56 ± 0.13d	091.30	18%→12%	02.10 ± 0.11d	005.30
Red	0%	33.46 ± 0.66a	100.00	0%→0%	46.38 ± 0.31a	100.00
	6%	21.31 ± 0.46b	036.31	6%→0%	40.68 ± 1.00b	087.70
	12%	17.86 ± 0.16c	046.60	12%→6%	12.40 ± 0.34c	026.70
	18%	03.73 ± 0.19d	088.80	18%→12%	01.33 ± 0.08d	002.86

Note: Effects of salt stress treatments on the FW of Chinese green and Chinese red; values represent means ± SE, followed by different letters in the same treatment process indicating a significant difference at $P \leq .05$, according to one-way ANOVA and Tukey tests.

Table 5. DWs of both okra cultivars after salt stress and re-watering.

	Treatments	Dry weight (DW) (g)	DW (%)	Re-watering	ΔDW (g)	ΔDW (%)
Green	0%	4.10 ± 0.15a	100.00	0%→0%	8.53 ± 0.11a	100.00
	6%	2.41 ± 0.11b	041.20	6%→0%	6.28 ± 0.13b	073.60
	12%	1.19 ± 0.11c	070.90	12%→6%	0.27 ± 0.02c	003.16
	18%	0.17 ± 0.00d	095.80	18%→12%	0.12 ± 0.00c	001.40
Red	0%	4.56 ± 0.23a	100.00	0%→0%	9.57 ± 0.20a	100.00
	6%	2.90 ± 0.17b	036.40	6%→0%	7.28 ± 0.13b	076.00
	12%	1.98 ± 0.04c	056.50	12%→6%	0.54 ± 0.03c	005.64
	18%	0.04 ± 0.00d	099.10	18%→12%	0.15 ± 0.01c	001.56

Note: Effects of salt stress treatments on the DW of Chinese green and Chinese red; values represent means ± SE, followed by different letters in the same treatment process indicating a significant difference at $P \leq .05$, according to one-way ANOVA and Tukey tests.

at 18% salt stress level was 42 times higher than control and Chinese red at 18% salt level had 58 times more CA activity than control. In the re-watering phase, Chinese green had more decrease in 6–0% and 12–6% salt stress levels. Chinese red had more reduction at 12–6% re-watering. Decreases in CA activity in both cultivars were almost the same.

Correlation between different parameters of the two cultivars

The Pearson correlation coefficients for the relationship between the different parameters of Chinese green and Chinese red are shown in Table 6. P_N of Chinese green had a strong positive significant correlation with g_s, Ψ, WC, RL, FW and DW. g_s found a strong significant correlation with *E*, RL and FW. *E* found a positive strong significant correlation with Ψ, WC and RL. Ψ found a strong significant relationship with WC. PH found a positive significant strong relation with RL.

Chinese red P_N had strong correlation with g_s, *E*, FW and DW. g_s had a strong positive relationship with *E*. *E* had a strong significant relation with FW. Ψ had a strong relation with WC, same as in Chinese green. PH had a strong significant correlation with RL and FW had a strong significant correlation with DW.

Re-watering water use efficiency

Re-watering water use efficiencies (WUE_R) of both okra cultivars in each stress level are shown (Figure 5). WUE_R of Chinese green and Chinese red were significantly different and they all were positive no matter the degree of re-watering. The results show that both cultivars had the highest value of WUE_R at the 18% level. This shows that both cultivars recovered well at the last level.

Optimized value

Draw a graph between net photosynthetic rate and re-watering water use efficiency (WUE_R) as shown in Figure 6. To solve these equations, get the value of Chinese green, $X = 8.3\%$, and Chinese red, $X = 12.02\%$. With the help of these values, the best re-watering times for Chinese green when they suffered from 12% salt stress and best re-watering degree, 8.3–2.3%, as well as of Chinese red when they suffered from 12% salt stress level and best re-watering degree, 12.02–6%, can be found out.

Discussion

Photosynthetic rate and plant growth under salt stress

In this study, salt stress significantly reduces plant fresh weight and DW of both okra cultivars as shown in Tables 4 and 5. These results are in conformity with mulberry (Ahmad et al. 2010), sunflower (Akram & Ashraf 2011), mustard (Hayat et al. 2011) and okra (Saleem et al. 2011). Chinese red was found to be salt tolerant as

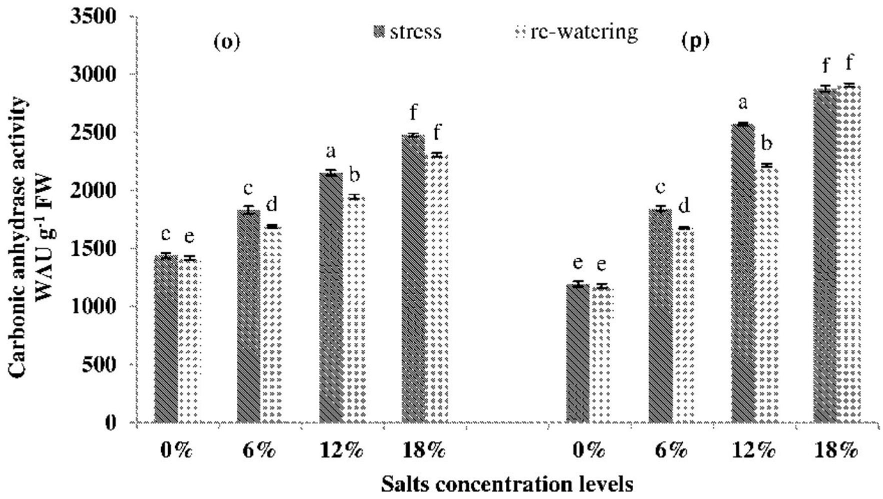

Figure 4. Effects of salt stress treatments on the CA activity of Chinese green (o) and Chinese red (p); values represent means ± SE, followed by different letters in the same treatment process indicating a significant difference at $P \leq .05$, according to one-way ANOVA and Tukey tests.

compared to Chinese green. This partial growth of these two cultivars may have been due to the partial regulation of various physiological parameters involved in the growth processes of the cultivars. Physiological parameters like photosynthetic capacity have a strong relationship with plant growth (Fisarakis et al. 2001; Sudhir & Murthy 2004; Saleem et al. 2011). In this study, salt stress reduced the net photosynthetic rate, stomatal conduction, transpiration, intercellular CO_2 concentration and WUE of both cultivars but WUE of Chinese red remain non-significant. The decrease in P_N may be due to a large amount of salt concentration present in the water, which increases the osmotic potential of soil and the plant cannot uptake water easily as in the case of non-saline water. Deficiency of water decreased the water potential and WC of the plant leaves that were found in this study (Figure 3). Due to the excess amount of salt present in the leaf and water deficiency stomatal closure occurs which reduces the transpiration rate (Redondo-Gómez et al. 2007) and decreases intercellular CO_2 concentration (Wani et al. 2013), and finally decreases plant height, RL, FW and DW (Saleem et al. 2011; Wani et al. 2013). CA is a type of enzyme that catalyzes the rapid conversion of HCO_3^- and gives H_2O and CO_2 when the plant was under salt stress and maintains the photosynthetic rate (Wu & Xing 2012; Xing & Wu 2015). In this study, CA increased in every stress level of both okra cultivars (Figure 4). The high CA activity in Chinese red showed good flexibility and better regulatory ability under salt stress. The net photosynthetic rate in Chinese red with the high CA activity was reduced less by salt stress compared to Chinese green (Figure 1(a,b)).

Re-watering effect on plant growth

In this study, both cultivars showed the positive response of re-watering and showed good recovery in different levels. Chinese green showed a better effect of re-watering under 6–0% and 12–6% levels. Water potential, WC and photosynthetic parameters were increased at 6–0% and 12–6%

Table 6. Correlation of different parameters of the two okra cultivars.

		g_s	E	WUE	Ci	Ψ	WC	CA	PH	RL	FW	DW
Green	P_N	0.91**	0.78**	0.78**	0.64**	0.89**	0.89**	−0.11	0.63**	0.80**	0.89**	0.88**
	g_s		0.86**	0.55**	0.72**	0.81**	0.81**	−0.21	0.70**	0.82**	0.81**	0.77**
	E			0.27	0.57**	0.81**	0.81**	0.06	0.89**	0.89**	0.74**	0.60**
	WUE				0.47*	0.63**	0.63**	−0.13	0.20	0.42*	0.67**	0.75**
	Ci					0.68**	0.68**	0.08	0.44*	0.52**	0.57**	0.53**
	Ψ						1.00**	0.23	0.71**	0.83**	0.87**	0.79**
	WC							0.23	0.71**	0.83**	0.87**	0.79**
	CA								0.14	0.05	0.09	−0.03
	PH									0.93**	0.74**	0.57**
	RL										0.90**	0.79**
	FW											0.96**
Red	P_N	0.89**	0.88**	0.07	0.16	0.84**	0.84**	−0.12	0.59**	0.71**	0.79**	0.77**
	g_s		0.84**	−0.02	0.29	0.76**	0.76**	−0.17	0.58**	0.67**	0.68**	0.64**
	E			0.03	0.03	0.77**	0.75**	−0.12	0.72**	0.83**	0.88**	0.85**
	WUE				−0.27	0.37	0.31	0.07	0.15	0.13	0.13	0.09
	Ci					−0.09	−0.10	−0.62**	−0.26	−0.25	−0.24	−0.17
	Ψ						0.96**	0.08	0.50*	0.59**	0.72**	0.69**
	WC							0.09	0.46*	0.57**	0.73**	0.70**
	CA								0.22	0.10	0.05	−0.09
	PH									0.94**	0.67**	0.51**
	RL										0.84**	0.73**
	FW											0.96**

Note: P_N: net photosynthetic; g_s: stomatal conductance; E: transpiration; WUE: water use efficiency; Ci: intercellular CO_2 concentration; Ψ: water potential; WC: water constant; CA: carbonic anhydrase; PH: plant height; RL: root length; FW: fresh weight; DW: dry weight.

* Correlation is significant at the .05 level (2-tailed).

** Correlation is significant at the .01 level (2-tailed).

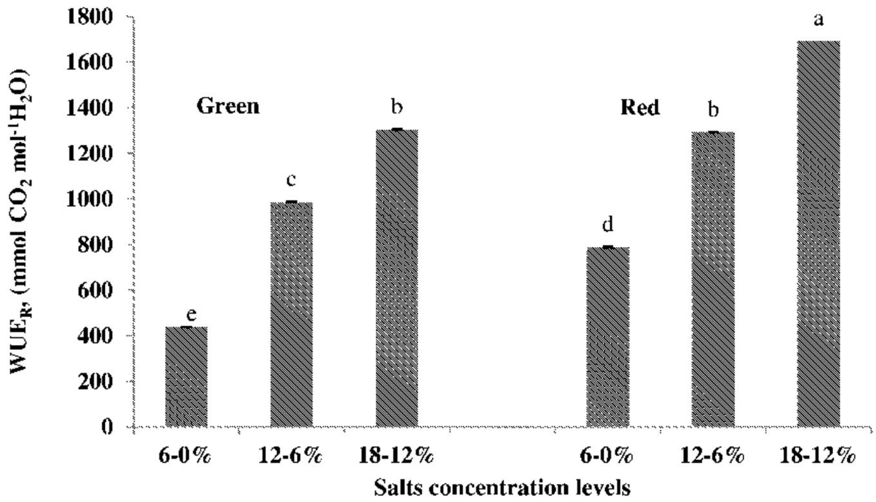

Figure 5. WUE_R of Chinese green and Chinese red; values represent means ± SE, followed by different letters in the same treatment process indicating a significant difference at $P \leq .05$, according to one-way ANOVA and Tukey tests.

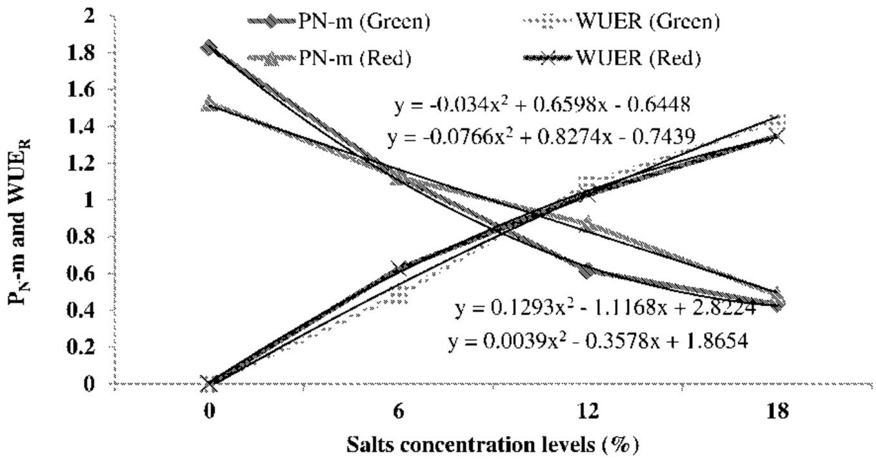

Figure 6. Optimized values' graph between P_N–m and WUE_R of Chinese green and Chinese red.

levels, but growth parameters were increased only under the 6–0% level, respectively. Chinese red also showed a better effect of re-watering in all levels; photosynthetic attributes, water potential, WC increased in all levels but plant growth was increased in 12–6% and 6–0%. The increase in the photosynthetic attribute is due to the negative relationship of salt concentration and water potential (Maksimović et al. 2010; Saleem et al. 2011; Shahid et al. 2011). Due to decreased salt concentration into the water solution helps plant to increase water potential in the leaf, due to decreased salt concentration into leaf helps stomata to open and get CO_2, thus photosynthetic rate was increased. According to the results, Chinese green was found tolerant to salt stress at 6% but Chinese red was found tolerant at 12%, because crop tolerance is related to plant growth (Shrivastava & Kumar 2015). At 18%, salt stress level re-watering was not more effective and it may be due to high salt stress plant being not be able to regulate ion concentration, since they may have had severe physiological dysfunction causes to leading to a decrease in growth rate and then leading to the death of the whole plant. More than 50% callseped of the plant during salt stress was not recovered in re-watering (Brodribb & Holbrook 2005), which was found in both cultivars in 12–18%.

Re-watering water use efficiency (WUE_R)

During re-watering, the photosynthetic rate and WC of both okra cultivars increased. Re-watering water use efficiency (WUE_R) is the new technique that was used to check the water status of the plant leaf and its impact on photosynthetic activities. WUE_R means increase in P_N per increase in WC from salt stress to re-watering. WUE_R of both okra cultivars increased at every re-watering level (Figure 5). But, growth parameters of Chinese green increased at 6–0% and Chinese red increased at 12–6% and 6–0% levels. According to the optimized value that was found between P_N and WUE_R (Figure 6), Chinese green should be re-watered when it is subjected to between 6% and 12% salt stress and re-watering degree should be 8.3% to 2.3% and Chinese red should be re-watered when it is subjected to 12% salt stress and re-watering degree should be 12% to 6%. Finally, according to the optimized value and results of plant growth, it should be concluded that Chinese green should give maximum production at 8.3–2.3% saline water and Chinese red at 12–6%.

Conclusion

Salt stress caused a marked reduction in growth parameters and physiological processes like photosynthetic rate, stomatal conductance, transpiration, WUE, water potential, WC and intercellular CO_2 concentration. Re-watering gives a positive response in both okra cultivars. Chinese green showed better growth recovery at 6–0% and Chinese red at 12–6% and 6–0% re-watering levels. Thus, Chinese red could be considered as more salt tolerant than Chinese green. In 18–12%, the plant could not recover because the higher stress damaged the whole plant, which was not able to recover even after dilution of the salt concentration solution. The optimized value of both cultivars would be helpful for these cultivars to use saline water resources and get maximum production.

Disclosure statement

No potential conflict of interest was reported by the authors.

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