Re-watering: An effective measure to recover growth and photosynthetic characteristics in salt-stressed *Brassica napus* L.

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ABSTRACT

Salinity is one of major environmental problem which is limiting the agricultural production. This research was conducted to evaluate the effect of re-watering on *Brassica napus* L., and determination of an appropriate regime for dilution of salted water by studying photosynthetic and growth response of *B. napus* to salt stress and subsequent re-watering. Plants were treated with NaCl (Nc_1: 2.5, Nc_2: 5, Nc_3: 10; g L^{-1}); Na_2SO_4 (Ns_1: 2.5, Ns_2: 5, Ns_3: 10; g L^{-1}) and mixed salts treatments (M_1: Nc_1+ Ns_3; M_2: Nc_3+ Ns_1; M_3: Nc_2+ Ns_2; g L^{-1}) and 0 as control, followed by re-watering. In salt stress phase, maximum reduction in net photosynthetic rate (P_N) was noted 79.54%, 80.72%, 84.54%, and 74.84% for Nc_3, Ns_3, M_1 and M_2, respectively, under high concentration levels. To maintain P_N, carbonic anhydrase (CA) activity was stimulated and kept water status stable under low (Nc_1 and Ns_1) to medium concentration levels (Nc_2, Ns_2 and M_3), and the decreases in P_N under Nc_2, Ns_2 and M_3 were 48.28%, 55.58% and 58.69%, respectively. However, during re-watering phase, growth and physiological parameters were recovered well due to regulation of CA activity under low to medium concentration levels. Relatively as compare to other stress levels more recovery in P_N was found after re-watering under medium concentration levels, which were 44.94%, 53.45% and 63.04%, respectively. Though aimed at consideration of high production in *B. napus*, the best re-watering time was found to be when plants undergo medium concentration levels. Therefore, this study provides a new method for dilution of saline irrigation based on plant physiology.

Key words: Carbonic anhydrase activity, growth, re-watering, salt stress, photosynthetic traits.

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INTRODUCTION

The stress due to salinity is a foremost environmental factor that severely affects the productivity of crop all over the world. Salinity is a major problem that affects 6% of the world's land and 20% of irrigated land (Chinnusamy et al., 2005). According to reports represented by United Nations, 50% of world crops lands are salt affected (Yokoi et al., 2002). Moreover, 40% of China which is equivalent to 8.1 Mha of the total cultivated land affected by salinity (Su et al., 2013) and water shortage (Wang et al., 2011). However, soil salinity affects about one third of the total irrigated crop land in North-West region of China (Chen et al., 2010). Overcoming stress due to water deficiency and salts accumulation is a foremost issue in these areas to ensure agricultural sustainability and continues production of food. *Brassica napus* L. is considered comparatively moderate salt tolerant crop grown under variant environmental conditions (Humaira and Rafiq, 2004) like temperature, salinity, and drought (Maggio et al., 2005; Hayat et al., 2007). Among all rapeseed crops, *B. napus* is one of the world's best and leading edible oil crops due to its improved oil content, high nutritious content, enrichment and stability in yield (Zum Felde et al., 2007). Furthermore, it contains less than 2% erucic acid and 5%-8% saturated fats which is lower than any other oil-seed crops (Raymer, 2002).

Salt accumulation in irrigated land from equally groundwater sources and irrigation increase the salinity to levels which creates physiological disturbances in plants, as a result, plant growth, plant quality, and plant yield are affected (Toorchi et al., 2011). The stress caused by salinity reduces overall photosynthetic capacity (Ashraf and Harris, 2013). From recent studies, it has been found that salt stress depresses the regulation of photosynthesis through limited stomatal opening (Chaves et al., 2009). On the other hand, the relative performance of stomatal conductivity and photosynthetic activity increases water-use efficiency (Vos and Groenwold, 1989) and leaf water potential. Leaf water potential, which is termed as energy status of water in leaves, tends to decrease as a result of decrease in relative water content (Arif et al., 2013). Consequently, some plants convert intracellular HCO_3^- into CO_2 and H_2O by carbonic anhydrase (CA) activity to maintain leaf water status, when they suffer water deficiency. As a result, C and water source are provided for the photosynthesis process itself by plant (Hu et al., 2011; Xing and Wu, 2012). Hence, CA activity works for survival of plant under stressed condition (Wu et al., 2006).

The salinity and scarcity of fresh water limits sustainable agricultural production and development (Wan et al., 2007). Meanwhile, the quality of irrigation water is also becoming low. As a result, saline water irrigation and lower quality-water, such as saline groundwater have been used more readily in agriculture to overwhelmed drought and sustain crop production (Verma et al., 2012; Li et al., 2015). Reuse of diluted saline water for irrigation of plants becomes the readily available water when water resources are scare. It would be a reasonable approach to use saline water as substitute resource for fresh water to irrigate the moderately salt tolerant crops such as *B. napus*. 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 economical irrigation. An appropriate dilution of salt water will save the water resource. Thus, the aim of this study was to find out threshold value in *B. napus* through physiological traits and growth status in different salt stresses and subsequent re-watering conditions. Afterwards, an appropriate regime for dilution of salted water was observed by studying the growth and photosynthetic response of *B. napus*.

MATERIALS AND METHODS

Plant material

The experiment was carried out at the Institute of Agricultural Engineering, Jiangsu University, Zhenjiang (32.20° N, 119.45° E), Jiangsu, China. Intact seeds of *B. napus*, identical in size and color, homogeneous and free from wrinkles, were chosen for this experiment. Seeds were cultivated 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% RH. Plants were daily irrigated with Hoagland solution (Hoagland and Arnon, 1950). After 21 d, plants were transferred into greenhouse under natural lighting with (25/18) ± 2 °C (day/night) temperature and 70% RH. Homogenous healthy seedlings were exposed to salt stress induced by NaCl, Na_2SO_4 and combination of both salts at four levels, in which one is control level. The treatments of salts were NaCl (Nc_1: 2.5, Nc_2: 5, Nc_3: 10, and 0 as control) g L^{-1}; Na_2SO_4 (Ns_1: 2.5, Ns_2: 5, Ns_3: 10, and 0 as control) g L^{-1} and in mixed salts (M_1: Nc_1 + Ns_3; M_2: Nc_3 + Ns_1; M_3: Nc_2 + Ns_2 and 0 as control) selected for treatment with Hoagland solution. The controlled treatment received full strength Hoagland solution.

Re-watering was done on day 21 from the onset of salt stress treatment for 15 d. The order for re-watering was that plants were suffering in high stress level (10 g L^{-1} in both NaCl and Na_2SO_4) irrigated with medium stress level $Nc_{3\wedge2}$, $Ns_{3\wedge2}$ (5 g L^{-1} in both NaCl and Na_2SO_4, respectively), medium stress level (5 g L^{-1} in both NaCl and Na_2SO_4) irrigated with low stress level $Nc_{2\wedge1}$, $Ns_{2\wedge1}$ (2.5 g L^{-1} in both NaCl and Na_2SO_4, respectively) and low stress level (2.5 g L^{-1} in both NaCl and Na_2SO_4) irrigated with control level $Nc_{1\wedge0}$, $Ns_{1\wedge0}$ (0 g L^{-1} in both NaCl and Na_2SO_4, respectively). In mixed treatments, all levels re-watered with control ($M_{1\wedge0}$, $M_{2\wedge0}$, $M_{3\wedge0}$). This experiment was designed in a randomized block and five replicates were chosen for each physiological measurement.

Determination of growth parameters

Growth parameters were measured after treatment application and re-watering 3-times per 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: Plant length (P_H); stem diameter (S_D) and leaf area (L_A). The L_A was measured by a leaf area meter (Handheld Laser Leaf Area Meter, CI-203, CID Bio-Science, Camas, Washington, USA).

Determination of physiological characteristics

Leaves in salt stress phase and subsequently in re-watering phase were used for the determination of photosynthesis characteristics. Net photosynthetic rate (P_N), stomatal conductance (g_s) and water potential (Ψ) were measured at 09:00-11:00 h after every 3 d in both salt stress 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 µmol m^{-2} s^{-1}, 28 °C and 500 µmol mol^{-1}, respectively. A portable photosynthesis measurement system (LI-6400XT, LI-COR, Lincoln, Nebraska, 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 (Ψ) was measured with dew point microvolt meter in a C-52-SF universal sample room (Psypro; Wescor, Logan, Utah, USA).

Determination of carbonic anhydrase activity

The carbonic anhydrase (CA) activity was determined by using the pH method described by Wilbur and Anderson (1948) with modifications (Wu et al., 2011). The CA activity was expressed in Wilbur and Anderson (WA) units as WA [WAU g^{-1} (FW)] = $(t_0/t) - 1$, where t_0 and t were the time(s) measured for the pH change (8.2 to 7.2), with buffer alone (t_0) and with sample (t). Leaf tissues (weight select according to leaf size usually used 0.1 to 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 × g, 0 °C for 5 min and then placed on ice for 20 min. In brief, CA activity was examined at 0 °C to 2 °C in a mixture containing 4.5 mL 0.02 M barbitone buffer (5,5-diethylbarbituric acid pH 8.3), 0.4 mL of the sample and 3 mL CO_2 saturated H_2O.

Calculation of re-watering water use efficiency

Re-watering WUE was calculated by the increment of Ψ and P_N in leaves of *B. napus* 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 $P_N l$ (μmol m^{-2} s^{-1}) and Ψ_l (MPa) respectively (l was the osmotic 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 $P_N l^{(l-1)}$ and $\Psi l^{(l-1)}$ respectively ($l^{(l-1)}$ indicated that leaves were re-watered from salt stress level l to salt stress level l - 1. In other words, leaves re-watered to adjacent lower salt stress level $l > 1$, and l was positive integer).

Relationship between plant leaf water potential and cell sap solute concentration (Q) is:

$$\Psi_l = iQRT \quad [1]$$

where Ψ_l is plant leaf 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}); 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 expressed as:

$$P = \frac{MQ}{1000}\% \quad [2]$$

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

According to Equations [1] and [2], P could be rewritten as:

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

Proportion of water content in the total quality of leaf is 1 - P; *WC* (%) expressed as:

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

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 ($\Delta\Psi$) were calculated as:

$$\Delta P_{Nl}^{(l-1)} = P_{Nl}^{(l-1)} - P_{Nl} \quad [5]$$

$$\Delta\Psi_l^{(l-1)} = \Psi_l^{(l-1)} - \Psi_l \quad [6]$$

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

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

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

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 $C_{12}H_{22}O_{11}$, M is 342; Ψ is plant leaf water potential (MPa); i is dissociation coefficient (i = 1); R is gas constant (R = 0.0083 L MPa mol^{-1} K^{-1}); T is thermodynamic temperature (273+t °C) K.

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

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

where ΔWC^{*} $l^{(l-1)}$ 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 ($A = 6 \times 10^{-4}$).

According to Equations [5] and [8], re-watering WUE (WUE_R, mmol CO_2 mol^{-1} H_2O) is calculated as:

$$WUE_R l\text{^}(l-1) = \frac{\Delta PNl\text{^}(l-1)}{\Delta WC^{*}l\text{^}(l-1)} \quad [9]$$

Statistical analysis

All measurements were subjected to ANOVA to discriminate significant differences (defined as $P \leq 0.05$) between group means. Data are shown as the mean ± standard error (SE) (n = 5). These mean data were statistically analyzed under factorial design by using SPSS software version 13.0 (IBM Corporation, Armonk, New York, USA) and mean results were compared through LSD at 5% significance level ($P < 0.05$).

RESULTS

Photosynthetic traits in salt stress vs salt stress subsequently re-watering

The net photosynthetic rate (P_N) and stomatal conductance (g_s) expressed in two phases, salt stress phase and re-watering phase. The P_N and g_s significantly decreased with increasing salt concentration under different salt stress levels (Tables 1 and 2). Maximum reduction in P_N (79.54%, 80.72%, 84.54%, and 74.84%) and in g_s (81.74%, 87.79%, 90.20%, and 83.90%) was noted under high concentration (Nc_3, Ns_3, M_1 and M_2) of NaCl, Na_2SO_4 and mixed salts, respectively as compared to control. By comparing with control, P_N (100%), there was slight reduction in P_N (17.19% and 19.91%) and in g_s (17.76% and 22.46%) recorded under low (Nc_1 and Ns_1) concentration of NaCl and Na_2SO_4 while the highest reduction in P_N (84.54%) and in g_s (90.20%) was observed under M_1 concentration of mixed salts. In medium (Nc_2, Ns_2 and M_3) concentration of NaCl, Na_2SO_4 and mixed salts, the reduction in P_N was found 48.28%, 55.58% and 58.69%, while in g_s was noted 52.51%, 56.40% and 59.85%, respectively.

Tables 1 and 2 also show the response of re-watering in P_N and g_s. It was observed that *B. napus* exhibited better results from stress phase to re-watering phase. The P_N and g_s increased significantly under low ($Nc_{1\text{^}0}$ and $Ns_{1\text{^}0}$) concentrations. Relatively, the maximum recovery were found under medium concentration ($Nc_{2\text{^}1}$, $Ns_{2\text{^}1}$ and $M_{3\text{^}0}$) which were 44.94%, 53.45% and 63.04% in P_N and 50.28%, 42.92% and 49.06% in g_s, respectively. However, salt stress at high concentration, affected P_N (18.93%, 18.54%, 14.37% and 18.57%) and g_s (20.11%, 16.76%, 13.42% and 13.87%) adversely followed the order as $Nc_{3\text{^}2}$, $Ns_{3\text{^}2}$, $M_{1\text{^}0}$ and $M_{2\text{^}0}$, respectively. However, additions of mixed salts at $M_{3\text{^}0}$ revealed the same effect both on P_N and g_s as compared to P_N and g_s under $Nc_{2\text{^}1}$, $Ns_{2\text{^}1}$ levels, respectively (Tables 1 and 2). It was also cleared from results that responses of P_N and g_s towards NaCl concentration was better than Na_2SO_4 and mixed salts concentrations during re-watering phase.

Table 1. Effect of salt stress and re-watering on net photosynthetic rate (P_N).

Salt stress phase	P_N	Reduction in P_N	Re-watering phase	P_N	Recovery in P_N
g L^{-1}	μmol CO_2 m^2 s^{-1}	%	g L^{-1}	μmol CO_2 m^2 s^{-1}	%
Control	20.58 ± 1.28a	100.00	Control	24.09 ± 1.06a	17.04
Nc_1	17.04 ± 1.03b	17.19	$Nc_{1\wedge0}$	21.20 ± 1.67b	24.41
Nc_2	10.64 ± 0.44c	48.28	$Nc_{2\wedge1}$	15.43 ± 2.49c	44.94
Nc_3	4.21 ± 1.73d	79.54	$Nc_{3\wedge2}$	5.01 ± 2.10d	18.93
Ns_1	16.48 ± 1.99b	19.91	$Ns_{1\wedge0}$	20.13 ± 0.49b	22.13
Ns_2	9.14 ± 0.77c	55.58	$Ns_{2\wedge1}$	14.03 ± 2.57c	53.45
Ns_3	3.97 ± 1.53d	80.72	$Ns_{3\wedge2}$	4.70 ± 0.70d	18.54
M_1	3.18 ± 0.30d	84.54	$M_{1\wedge0}$	3.64 ± 0.92d	14.34
M_2	5.17 ± 0.46d	74.87	$M_{2\wedge0}$	6.13 ± 2.04d	18.57
M_3	8.50 ± 0.19c	58.69	$M_{3\wedge0}$	13.86 ± 0.67c	63.04

The means ± SE (n = 5) in the table indicated the significant difference in net photosynthetic rate during salt stress phase and afterwards the recovery under re-watering phase at P ≤ 0.05, according to one way ANOVA and LSD. Nc_1: 2.5 g L^{-1} NaCl; Nc_2: 5 g L^{-1} NaCl; Nc_3:10 g L^{-1} NaCl; Ns_1: 2.5 g L^{-1} Na_2SO_4; Ns_2: 5 g L^{-1} Na_2SO_4; Ns_3:10 g L^{-1} Na_2SO_4; M_1: Nc_1+ Ns_3; M_2: Nc_3+ Ns_1; M_3: Nc_2+ Ns_2; $Nc_{1\wedge0}$: (2.5 → 0) g L^{-1} NaCl; $Nc_{2\wedge1}$: (5 → 2.5) g L^{-1} NaCl; $Nc_{3\wedge2}$: (10 → 5) g L^{-1} NaCl; $Ns_{1\wedge0}$: (2.5 → 0) g L^{-1} Na_2SO_4; $Ns_{2\wedge1}$: (5 → 2.5) g L^{-1} Na_2SO_4; $Ns_{3\wedge2}$: (10 → 5) g L^{-1} Na_2SO_4; $M_{1\wedge0}$: (12.5 → 0) g L^{-1} Hoagland solution; $M_{2\wedge0}$: (12.5 → 0) g L^{-1} Hoagland solution; $M_{3\wedge0}$: (10 → 0) g L^{-1} Hoagland solution.

Table 2. Effect salt stress and re-watering on stomatal conductance (g_s).

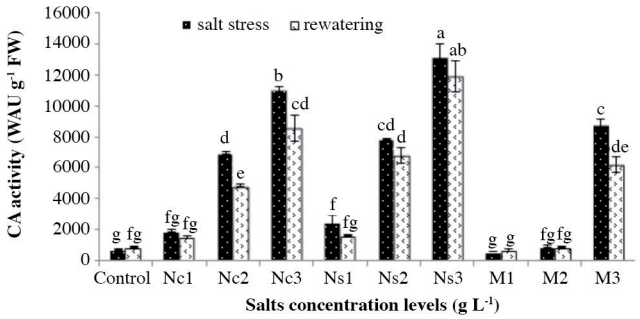
Salt stress phase	g_s	Reduction in g_s	Re-watering phase	g_s	Recovery in g_s
g L^{-1}	mol H_2O m^{-2} s^{-1}	%	g L^{-1}	mol H_2O m^{-2} s^{-1}	%
Control	0.46 ± 0.01a	100.00	Control	0.47 ± 0.01a	3.36
Nc_1	0.36 ± 0.02b	21.79	$Nc_{1\wedge0}$	0.42 ± 0.01b	17.77
Nc_2	0.22 ± 0.02c	52.51	$Nc_{2\wedge1}$	0.33 ± 0.01c	50.28
Nc_3	0.08 ± 0.01e	81.74	$Nc_{3\wedge2}$	0.10 ± 0.01e	20.11
Ns_1	0.32 ± 0.02b	29.92	$Ns_{1\wedge0}$	0.39 ± 0.01b	22.46
Ns_2	0.20 ± 0.01cd	56.40	$Ns_{2\wedge1}$	0.29 ± 0.01cd	42.92
Ns_3	0.06 ± 0.02e	87.79	$Ns_{3\wedge2}$	0.07 ± 0.03f	16.76
M_1	0.04 ± 0.01e	90.20	$M_{1\wedge0}$	0.05 ± 0.01g	13.42
M_2	0.07 ± 0.02e	83.90	$M_{2\wedge0}$	0.08 ± 0.02f	13.87
M_3	0.18 ± 0.02d	59.85	$M_{3\wedge0}$	0.27 ± 0.01d	49.06

The means ± SE (n = 5) in the table indicated the significant difference in stomatal conductance during salt stress phase and afterwards the recovery under re-watering phase at P ≤ 0.05, according to one way ANOVA and LSD. Nc_1: 2.5 g L^{-1} NaCl; Nc_2: 5 g L^{-1} NaCl; Nc_3:10 g L^{-1} NaCl; Ns_1: 2.5 g L^{-1} Na_2SO_4; Ns_2: 5 g L^{-1} Na_2SO_4; Ns_3:10 g L^{-1} Na_2SO_4; M_1: Nc_1+ Ns_3; M_2: Nc_3+ Ns_1; M_3: Nc_2+ Ns_2; $Nc_{1\wedge0}$: (2.5 → 0) g L^{-1} NaCl; $Nc_{2\wedge1}$: (5 → 2.5) g L^{-1} NaCl; $Nc_{3\wedge2}$: (10 → 5) g L^{-1} NaCl; $Ns_{1\wedge0}$: (2.5 → 0) g L^{-1} Na_2SO_4; $Ns_{2\wedge1}$: (5 → 2.5) g L^{-1} Na_2SO_4; $Ns_{3\wedge2}$: (10 → 5) g L^{-1} Na_2SO_4; $M_{1\wedge0}$: (12.5 → 0) g L^{-1} Hoagland solution; $M_{2\wedge0}$: (12.5 → 0) g L^{-1} Hoagland solution; $M_{3\wedge0}$: (10 → 0) g L^{-1} Hoagland solution.

CA activity and water potential in salt stress and subsequently in re-watering

The CA activity of *B. napus* under salt stress condition showed its regulation which varied with stress level (Figure 1). It activated significantly in low (Nc_1, Ns_1) to medium concentration levels (Nc_2, Ns_2 and M_3) as compared to control. It had maximum values under Nc_3 and Ns_3 concentration levels. But at M_1 and M_2 concentration levels, CA activity was not activated due to under high stress condition especially under M_1 concentration level. 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 mixed salts treatments.

Figure 1. Effect of salt stress and subsequent re-watering on the regulation of carbonic anhydrase (CA) activity.



The means ± SE (n = 5) in the figure indicate significant differences between different stress and re-watering levels at P ≤ 0.05, according to one-way ANOVA and LSD.
WAU is Wilbur and Anderson Unit which expresses the CA activity in WA units as WA [WAU g^{-1} (FW)] = $(t_0/t) - 1$, where t_0 and t were the time(s) measured for the pH change (8.2 to 7.2), with buffer alone (t_0) and with sample (t) and FW was the fresh weight of leaves.

In re-watering phase, CA activity showed better performance. The CA activity was successfully activated under $Nc_{2\wedge1}$, $Ns_{2\wedge1}$ and $M_{3\wedge0}$, concentrations respectively. However, salt stress subsequent re-watering resulted in an adverse effect under $M_{1\wedge0}$ and $M_{2\wedge0}$ concentrations (Figure 1). The CA activity of *B. napus* was the lowest at $M_{1\wedge0}$ and $M_{2\wedge0}$ concentration levels and nearly undetectable even after re-watering.

According to our results, Ψ significantly decreased going towards more negative with increasing salt stress (Table 3). The minimum decrease in Ψ was noted in low concentration levels (Nc_1, Ns_1) as compared with the control. However, the maximum decrease in Ψ was noted at high concentration levels (Nc_3, Ns_3, M_1 and M_2), respectively.

Table 3. Effect salt stress vs. salt stress subsequently re-watering on water potential (Ψ).

Salt stress phase	Ψ	Re-watering phase	Ψ	Increment in Ψ during re-watering phase
g L^{-1}	MPa	g L^{-1}	MPa	
Control	-0.86 ± 0.03a	Control	-0.73 ± 0.10a	0.13
Nc_1	-1.42 ± 0.01a	$Nc_{1\wedge0}$	-0.99 ± 0.24a	0.44
Nc_2	-1.91 ± 0.09ab	$Nc_{2\wedge1}$	-1.54 ± 0.37ab	0.36
Nc_3	-3.01 ± 0.28b	$Nc_{3\wedge2}$	-2.79 ± 0.11b	0.22
Ns_1	-1.43 ± 0.50a	$Ns_{1\wedge0}$	-1.03 ± 0.05ab	0.40
Ns_2	-2.24 ± 0.20b	$Ns_{2\wedge1}$	-1.76 ± 0.15ab	0.47
Ns_3	-3.44 ± 1.33bc	$Ns_{3\wedge2}$	-3.07 ± 1.15c	0.37
M_1	-4.08 ± 0.85c	$M_{1\wedge0}$	-3.79 ± 0.80c	0.28
M_2	-2.64 ± 0.20b	$M_{2\wedge0}$	-2.31 ± 0.17bc	0.33
M_3	-1.74 ± 0.24ab	$M_{3\wedge0}$	-1.29 ± 0.25ab	0.45

The means ± SE (n = 5) in the table indicated the significant difference in water potential during salt stress phase and afterwards the increment under re-watering phase at P ≤ 0.05, according to one way ANOVA and LSD. Nc_1: 2.5 g L^{-1} NaCl; Nc_2: 5 g L^{-1} NaCl; Nc_3:10 g L^{-1} NaCl; Ns_1: 2.5 g L^{-1} Na_2SO_4; Ns_2: 5 g L^{-1} Na_2SO_4; Ns_3:10 g L^{-1} Na_2SO_4; M_1: Nc_1+ Ns_3; M_2: Nc_3+ Ns_1; M_3: Nc_2+ Ns_2; $Nc_{1\wedge0}$: (2.5 → 0) g L^{-1} NaCl; $Nc_{2\wedge1}$: (5 → 2.5) g L^{-1} NaCl; $Nc_{3\wedge2}$: (10 → 5) g L^{-1} NaCl; $Ns_{1\wedge0}$: (2.5 → 0) g L^{-1} Na_2SO_4; $Ns_{2\wedge1}$: (5 → 2.5) g L^{-1} Na_2SO_4; $Ns_{3\wedge2}$: (10 → 5) g L^{-1} Na_2SO_4; $M_{1\wedge0}$: (12.5 → 0) g L^{-1} Hoagland solution; $M_{2\wedge0}$: (12.5 → 0) g L^{-1} Hoagland solution; $M_{3\wedge0}$: (10 → 0) g L^{-1} Hoagland solution.

While in medium (Nc_2, Ns_2 and M_3) concentration of NaCl, Na_2SO_4 and mixed salts, the decrease in Ψ was found to almost same. As a comparison between single salts and mixture of salts, it was observed that Ψ was significantly less affected in NaCl concentrations than Na_2SO_4 and mixed salts concentrations.

In re-watering phase, the outcome of the results showed that Ψ of *B. napus* was recovered. The increment in Ψ also increased under low ($Nc_{1\wedge0}$ and $Ns_{1\wedge0}$) to medium concentration levels ($Nc_{2\wedge1}$, $Ns_{2\wedge1}$ and $M_{3\wedge0}$) and it decreased at high concentration levels ($Nc_{3\wedge2}$, $Ns_{3\wedge2}$, $M_{1\wedge0}$ and $M_{2\wedge0}$), respectively. However, the degree of salts were still showed the adverse effect on increment of Ψ even during re-watering under high levels ($Nc_{3\wedge2}$, $Ns_{3\wedge2}$, $M_{1\wedge0}$ and $M_{2\wedge0}$) (Table 3).

Effect of salt stress on plant growth

The application of stress significantly affected P_H, S_D and L_A of *B. napus*. By following the results of P_H, S_D and L_A under salt stress, it appeared that increase in salts concentration decreased the values of P_H, S_D and L_A (Table 4). Comparing to control, the P_H decreased by 70.58%, 78.76%, 96.73% and 87.91% for Nc_3, Ns_3, M_1 and M_2, respectively, under high concentration of NaCl, Na_2SO_4 and mixed salts. It was found that P_H (14.15 and 12.40 cm) under low (Nc_1 and Ns_1) concentration levels of NaCl and Na_2SO_4 is slightly affected as compared to P_H (15.30 cm) under control. Upon comparing with other salts levels, *B. napus* exhibited maximum decrease in P_H (96.73%) under mixed salts concentration (M_1). While in medium (Nc_2, Ns_2 and M_3) concentration of NaCl, Na_2SO_4 and mixed salts, the decrease in P_H were recorded 43.79%, 49.67% and 49%, respectively. Reduction in S_D was observed continuously from control to high concentrations levels (Nc_3, Ns_3, M_1 and M_2) of NaCl, Na_2SO_4 and mixed salts (Table 4). The control treatment showed the maximum S_D (0.195 cm) followed by low concentrations (0.145 and 0.135 cm) with decrease of 25.37% and 30.77%, medium concentrations (0.095, 0.085 and 0.085 cm) with percent decrease of 51.28%, 56.41% and 56.41% and high concentrations 0.035, 0.015, 0.005, and 0.010 cm with decrease of 82.06%, 92.31%, 97.44% and 94.76%, respectively. Similarly, control had the highest L_A (19.07 cm^2) followed by low concentrations with decrease of 21.82% and 33.28 % and medium concentrations with decrease of 51.34%, 54.06% and 54.12%. But, salt stress at high concentrations (Nc_3, Ns_3, M_1 and M_2) exerted a severe influence on L_A and reductions found were 81.91%, 89.52%, 97.91% and 94.76%, respectively.

Table 4. Effect of salts stress on plant height (P_H), stem diameter (S_D) and leaf area (L_A) in *Brassica napus*.

Treatments NaCl/Na_2SO_4	Variables					
	P_H	P_H	S_D	S_D	L_A	L_A
	cm	%	cm	%	cm^2	%
Control	15.30 ± 0.30a	100.00	0.195 ± 0.02a	100.00	19.07 ± 0.48a	100.00
N_{C1}	14.15 ± 0.25ab	92.48	0.145 ± 0.01b	74.36	14.92 ± 0.65b	78.18
N_{C2}	8.60 ± 0.60c	56.21	0.095 ± 0.01cd	48.72	9.28 ± 0.71c	48.66
N_{C3}	4.50 ± 0.30d	29.42	0.035 ± 0.01e	17.94	3.45 ± 0.45d	18.09
N_{S1}	12.40 ± 0.40b	81.04	0.135 ± 0.01bc	69.23	12.72 ± 0.27b	66.72
N_{S2}	7.70 ± 0.20c	50.33	0.085 ± 0.01d	43.59	8.75 ± 0.83c	45.94
N_{S3}	3.25 ± 0.25de	21.24	0.015 ± 0.01e	7.69	2.00 ± 0.20de	10.48
M_1	1.00 ± 0.50f	3.27	0.005 ± 0.01e	2.56	0.40 ± 0.10e	2.09
M_2	1.85 ± 0.25e	12.09	0.010 ± 0.01e	5.12	1.00 ± 0.00de	5.24
M_3	7.80 ± 0.30c	51.00	0.085 ± 0.01d	43.59	8.75 ± 0.75c	45.88

The means ± SE (n = 5) in the table indicated the significant difference in plant height, stem diameter and leaf area during salt stress phase at $P \leq 0.05$, according to one way ANOVA and LSD.
Nc_1: 2.5 g L^{-1} NaCl; Nc_2: 5 g L^{-1} NaCl; Nc_3:10 g L^{-1} NaCl; Ns_1: 2.5 g L^{-1} Na_2SO_4; Ns_2: 5 g L^{-1} Na_2SO_4; Ns_3:10 g L^{-1} Na_2SO_4; M_1: Nc_1+ Ns_3; M_2: Nc_3+ Ns_1; M_3: Nc_2+ Ns_2; $Nc_{1\wedge0}$: (2.5 → 0) g L^{-1} NaCl; $Nc_{2\wedge1}$: (5 → 2.5) g L^{-1} NaCl; $Nc_{3\wedge2}$: (10 → 5) g L^{-1} NaCl; $Ns_{1\wedge0}$: (2.5 → 0) g L^{-1} Na_2SO_4; $Ns_{2\wedge1}$: (5 → 2.5) g L^{-1} Na_2SO_4; $Ns_{3\wedge2}$: (10 → 5) g L^{-1} Na_2SO_4; $M_{1\wedge0}$: (12.5 → 0) g L^{-1} Hoagland solution; $M_{2\wedge0}$: (12.5 → 0) g L^{-1} Hoagland solution; $M_{3\wedge0}$: (10 → 0) g L^{-1} Hoagland solution.

Table 5. Effect of re-watering on plant height (P_H), stem diameter (S_D) and leaf area (L_A) in *Brassica napus*.

Re-watering NaCl/Na_2SO_4	Variables					
	Δ P_H (cm)	Δ P_H (%)	Δ S_D (cm)	Δ S_D (%)	Δ L_A (cm^2)	Δ L_A (%)
Control	14.20 ± 0.30a	100.00	0.160 ± 0.01a	100.00	22.11 ± 0.12a	100.00
$Nc_{1\wedge0}$	11.50 ± 0.50b	80.98	0.135 ± 0.01ab	84.37	19.34 ± 0.60b	87.47
$Nc_{2\wedge1}$	7.70 ± 0.20cd	54.22	0.085 ± 0.00c	53.13	12.84 ± 0.34d	58.07
$Nc_{3\wedge2}$	4.80 ± 0.30e	33.80	0.045 ± 0.01d	28.13	4.20 ± 0.70de	19.00
$Ns_{1\wedge0}$	9.20 ± 0.30c	64.79	0.125 ± 0.01b	78.13	16.94 ± 0.48c	76.88
$Ns_{2\wedge1}$	6.85 ± 0.35d	48.24	0.070 ± 0.0cd	43.75	11.03 ± 0.27d	49.84
$Ns_{3\wedge2}$	3.90 ± 0.10f	27.46	0.035 ± 0.01de	21.87	2.00 ± 0.20f	9.04
$M_{1\wedge0}$	1.25 ± 0.25g	8.80	0.015 ± 0.01e	9.38	1.25 ± 0.25f	5.65
$M_{2\wedge0}$	2.45 ± 0.05fg	17.25	0.020 ± 0.01e	12.50	1.75 ± 0.25f	7.68
$M_{3\wedge0}$	7.15 ± 0.15d	50.35	0.075 ± 0.01cd	46.87	10.92 ± 0.07d	49.39

The means ± SE (n = 5) in the table indicate significant difference in plant height, stem diameter and leaf area during re-watering phase at $P \leq 0.05$, according to one way ANOVA and LSD.
Nc_1: 2.5 g L^{-1} NaCl; Nc_2: 5 g L^{-1} NaCl; Nc_3:10 g L^{-1} NaCl; Ns_1: 2.5 g L^{-1} Na_2SO_4; Ns_2: 5 g L^{-1} Na_2SO_4; Ns_3:10 g L^{-1} Na_2SO_4; M_1: Nc_1+ Ns_3; M_2: Nc_3+ Ns_1; M_3: Nc_2+ Ns_2; $Nc_{1\wedge0}$: (2.5 → 0) g L^{-1} NaCl; $Nc_{2\wedge1}$: (5 → 2.5) g L^{-1} NaCl; $Nc_{3\wedge2}$: (10 → 5) g L^{-1} NaCl; $Ns_{1\wedge0}$: (2.5 → 0) g L^{-1} Na_2SO_4; $Ns_{2\wedge1}$: (5 → 2.5) g L^{-1} Na_2SO_4; $Ns_{3\wedge2}$: (10 → 5) g L^{-1} Na_2SO_4; $M_{1\wedge0}$: (12.5 → 0) g L^{-1} Hoagland solution; $M_{2\wedge0}$: (12.5 → 0) g L^{-1} Hoagland solution; $M_{3\wedge0}$: (10 → 0) g L^{-1} Hoagland solution.

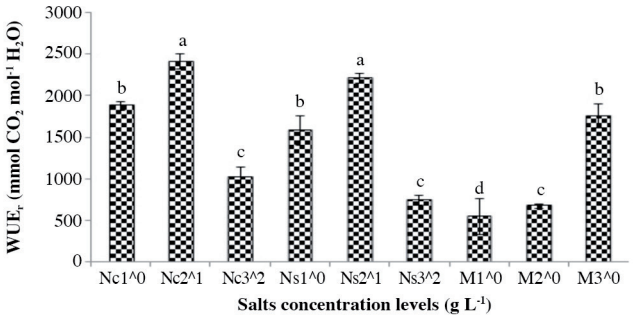
Afterwards, Table 5 shows the effect of salt stress and subsequent re-watering on P_H, S_D and L_A of *B. napus*. A statistical analysis specified that the increments observed during re-watering were significant, except for the concentration at high levels ($Nc_{3\wedge2}$, $Ns_{3\wedge2}$, $M_{1\wedge0}$ and $M_{2\wedge0}$). The recovery in ΔP_H (80.98% and 64.79%), ΔS_D (84.37% and 78.13) and ΔL_A (87.47% and 76.88%) were significantly higher under low concentrations ($Nc_{1\wedge0}$ and $Ns_{1\wedge0}$), respectively. Moreover by comparing with other stress levels after re-watering, relatively, the recovery in ΔP_H (54.22%, 48.24% and 50.35%), ΔS_D (53.13%, 43.75% and 46.87%) and ΔL_A (58.07%, 49.84% and 49.39%) found under medium concentration ($Nc_{2\wedge1}$, $Ns_{2\wedge1}$ and $M_{3\wedge0}$). However, at high concentration, the degree of salt levels exhibited the adverse effect of salt stress on ΔP_H, ΔS_D and ΔL_A. However, additions of mixed salts at $M_{3\wedge0}$ revealed the same effect on ΔP_H, ΔS_D and ΔL_A as compared to ΔP_H, ΔS_D and ΔL_A under $Nc_{2\wedge1}$, $Ns_{2\wedge1}$ levels, respectively (Table 5).

Water use efficiency and re-watering water-use efficiency

The WUE showed non-significant reduction from control (100%) to low concentrations (92.26% and 87%) under Nc_1 and Ns_1 followed by medium concentrations (80.80%, 75.23% and 73.06%) under Nc_2, Ns_2, and M_3). The maximum WUE was recorded under high concentration (108.66%, 117.03%, 126.93% and 118.57%) at Nc_3, Ns_3, M_1 and M_2. However, the stress-persuaded maximum increase was recorded at M_1 levels (Table 6). While, re-watering of plants reduced the effect of salts stresses significantly, and showed the significant increase in WUE. The results showed that values increased significantly at control, $Nc_{1\wedge0}$, $Nc_{2\wedge1}$, $Nc_{3\wedge2}$, $Ns_{1\wedge0}$ and $Ns_{2\wedge1}$ and $Ns_{3\wedge2}$, respectively in single salt but the increment was reduced in comparison with WUE under salt stress phase. WUE was the same at moderate levels ($Nc_{2\wedge1}$, $Ns_{2\wedge1}$ and $M_{3\wedge0}$), during re-watering. WUE had the maximum values under high levels ($Nc_{3\wedge2}$, $Ns_{3\wedge2}$, $M_{1\wedge0}$ and $M_{2\wedge0}$).

Re-watering water-use efficiency (WUE_R) of *B. napus* at each stress level is shown in Figure 2. The $WUE_{R3\wedge2}$ of

Figure 2. Effect of salts stress and subsequent re-watering on re-watering water use efficiency (WUE).



The means ± SE (n = 5) in the figure indicate significant differences between different stress and re-watering levels at P ≤ 0.05, according to one-way ANOVA and LSD.

B. napus under $Nc_{3\wedge2}$ and $Ns_{3\wedge2}$ concentration levels in both single salt was lower than control. The $WUE_{R2\wedge1}$ of *B. napus* had maximum values under medium concentration levels at $Nc_{2\wedge1}$ and $Ns_{2\wedge1}$ followed by low concentrations levels $WUE_{R1\wedge0}$ at $Nc_{1\wedge0}$ and $Ns_{1\wedge0}$, respectively. Comparatively, the plants treated with Na_2SO_4 concentration at $Ns_{3\wedge2}$ level effected more and showed lower WUE_R than the plants treated with NaCl concentration at $Nc_{3\wedge2}$ level. Although, WUE_R under mixed treatment $WUE_{RM1\wedge0}$, $WUE_{RM1\wedge0}$ and $WUE_{RM1\wedge0}$ at $M_{1\wedge0}$ and $M_{2\wedge0}$ concentration levels was decreased badly. Among all the concentrations levels, WUE_R showed its better effects on *B. napus* performance at medium concentrations ($Nc_{2\wedge1}$, $Ns_{2\wedge1}$ and $M_{3\wedge0}$) of NaCl, Na_2SO_4 and mixed salts.

Relationship between P_N, g_s, WUE, Ψ and CA activity

The Pearson correlation coefficients for the relationship between the different physiological properties *B. napus* are shown in Table 7. Correlations between P_N and physiological parameters were observed and P_N was found to be positively correlated with g_s and Ψ, but had no correlation with WUE and CA activity, which revealed the opposite trend during stress condition. The negative

Table 6. Effect salt stress vs. salt stress subsequently re-watering on water use efficiency (WUE).

Salt stress phase	WUE	Variation in WUE during salt stress phase	Re-watering phase	WUE	Variation in WUE during re-watering phase
g L^{-1}	μmol mol^{-1}	%	g L^{-1}	μmol mol^{-1}	%
Control	3.23 ± 0.11c	100.00	Control	6.95 ± 0.00d	100.00
N_{C1}	2.98 ± 0.03d	92.26	$Nc_{1\wedge0}$	6.15 ± 0.27d	88.48
N_{C2}	2.61 ± 0.09e	80.80	$Nc_{2\wedge1}$	4.84 ± 0.18ef	69.64
N_{C3}	3.51 ± 0.02c	108.66	$Nc_{3\wedge2}$	7.93 ± 0.04c	114.10
N_{S1}	2.81 ± 0.11e	87.00	$Ns_{1\wedge0}$	5.44 ± 0.04e	78.27
N_{S2}	2.43 ± 0.03f	75.23	$Ns_{2\wedge1}$	4.42 ± 0.26g	63.60
N_{S3}	3.78 ± 0.10b	117.03	$Ns_{3\wedge2}$	8.44 ± 0.10bc	121.43
M_1	4.10 ± 0.11a	126.93	$M_{1\wedge0}$	8.96 ± 0.14a	128.92
M_2	3.83 ± 0.03b	118.57	$M_{2\wedge0}$	8.52 ± 0.12bc	122.59
M_3	2.36 ± 0.01f	73.06	$M_{3\wedge0}$	4.75 ± 0.04f	68.34

The means ± SE (n = 5) in the table indicate significant difference in water use efficiency during salt stress phase and afterwards under re-watering phase at P ≤ 0.05, according to one way ANOVA and LSD.

Nc_1: 2.5 g L^{-1} NaCl; Nc_2: 5 g L^{-1} NaCl; Nc_3:10 g L^{-1} NaCl; Ns_1: 2.5 g L^{-1} Na_2SO_4; Ns_2: 5 g L^{-1} Na_2SO_4; Ns_3:10 g L^{-1} Na_2SO_4; M_1: Nc_1+ Ns_3; M_2: Nc_3+ Ns_1; M_3: Nc_2+ Ns_2; $Nc_{1\wedge0}$: (2.5 → 0) g L^{-1} NaCl; $Nc_{2\wedge1}$: (5 → 2.5) g L^{-1} NaCl; $Nc_{3\wedge2}$: (10 → 5) g L^{-1} NaCl; $Ns_{1\wedge0}$: (2.5 → 0) g L^{-1} Na_2SO_4; $Ns_{2\wedge1}$: (5 → 2.5) g L^{-1} Na_2SO_4; $Ns_{3\wedge2}$: (10 → 5) g L^{-1} Na_2SO_4; $M_{1\wedge0}$: (12.5 → 0) g L^{-1} Hoagland solution; $M_{2\wedge0}$: (12.5 → 0) g L^{-1} Hoagland solution; $M_{3\wedge0}$: (10 → 0) g L^{-1} Hoagland solution.

Table 7. Pearson correlation coefficients among different physiological parameters of *Brassica napus* (n = 5).

		g_s	WUE	Ψ	CA
Salt stress	P_N	0.96*	-0.46	0.90*	-0.47
	g_s		-0.46	0.85*	-0.45
	WUE			-0.70*	-0.25
	Ψ				-0.24
		g_s	WUE	Ψ	CA
Re-watering	P_N	0.98*	-0.61	0.95*	-0.45
	g_s		-0.64*	0.91*	-0.40
	WUE			-0.72*	-0.11
	Ψ				-0.24

*Correlation is significant at the 0.05 level. 2-tailed significance is used. g_s: Stomatal conductance; WUE: water use efficiency; Ψ: water potential; CA: carbonic anhydrase activity; P_N: net photosynthetic rate.

relationship between P_N and WUE and CA activity suggested that the presence of salts could inhibit the growth of *B. napus*.

DISCUSSION

Photosynthetic response traits and growth

Photosynthesis characteristics were different in their response to different salt stress levels (Table 1). The variations in P_N were found to be dependent on water status of leaves. The deficiency of water limited P_N occurred due to through stomatal closure (Hu et al., 2009). Stomatal opening and closing are considered as response of drought stress for short term duration (Rouhi et al., 2007). *Brassica napus* was found as different in their P_N response to different salt stress levels. Salt stress severely affects the P_N of *B. napus* under high concentration levels (Nc_3, Ns_3, M_1, and M_2) (Table 1). It might be because of the water status of leaves disturbed by increasing salt stress through stomatal limitations. Similar results were reported by (Qasim et al., 2003) in canola and *Brassica juncea* L. (Siddiqui et al., 2008). Consequently, *B. napus* showed photosynthetic tolerance under low (Nc_1, Ns_1) to medium (Nc_2, Ns_2 and M_3) concentration of NaCl, Na_2SO_4 and mixed salts. Thus, this situation demonstrated the threshold photosynthetic adaptability and tolerance of *B. napus* under medium concentration levels.

Salt stresses exerted a toxic effect on the P_N due variations in Ψ within the tissues (Ashraf and Foolad, 2005). As the salts within the plant tissues increase, Ψ decreases and affects the opening and closing of stomata. This is in return lastly causes imbalance in gas exchange and disturbs the photosynthetic activity (Chartzoulakis et al., 2002a) and affected the plant growth development. At that point, C and water source provide by CA which enhance the activity of photosynthetic process because CA in leaves convert intracellular HCO_3^- into CO_2 and H_2O (Xing and Wu, 2012). The CA activity in *B. napus* was activated under low concentration (Nc_1, Ns_1) and showed good regulatory under medium concentration of NaCl (Nc_2, Ns_2 and M_3), Na_2SO_4 and mixed salts (Figure 1). Therefore, WUE of leaves was enhanced in *B. napus* through regulation of CA and by maintaining the variations in leaf Ψ.

High salts concentrations caused a clear reduction in growth of *B. napus* (Table 4). The reduction in growth was due to decrease in P_N. It is well documented by Parida and Das (2005), salt stress distresses leaf Ψ, g_s and growth rate. A considerable increase occurred even during stress conditions in growth attributes under moderate concentration levels of NaCl, Na_2SO_4 and mixed salts (Nc_2, Ns_2 and M_3) (Table 4). It indicated the growth performance of *B. napus* respond to P_N and threshold adoptability under medium salts concentrations. Therefore, *B. napus* was thought to be species with single and mixed salts tolerance adaptability under medium stress conditions.

Re-watering effects

The application of re-watering had better effect on plants growth development. Re-watering had a positive impression on P_N of leaves in *B. napus*. After application of re-watering, P_N rate was recovered and maintained its status successfully under low ($Nc_{1\wedge0}$ and $Ns_{1\wedge0}$) concentration levels followed by moderate concentration ($Nc_{2\wedge1}$, $Ns_{2\wedge1}$ and $M_{3\wedge0}$) levels, as compared to high levels. But under high concentration ($Nc_{3\wedge2}$, $Ns_{3\wedge2}$, $M_{1\wedge0}$ and $M_{2\wedge0}$) levels even in the re-watering phase, photosynthetic activity was restricted due to g_s inhibition (Tables 1 and 2). It reflected that CA activity was also inhibited under high concentration (Figure 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 replenish leaf water status which became the reason of reduction in Ψ. Consequently, plant growth attributes like P_H, S_D and L_A showed the unhealthy status due to inhibited water uptake movements. It is also may be due to the toxic effect of salt on growth (Silveira et al., 2009).

Re-watering water-use efficiency (WUE_R) is an important index of *B. napus* to adapt different behaviors to different salts stresses subsequent re-watering. It meant that increment in water content directed to the increment of P_N in leaves of *B. napus*. Better effect of re-watering found in plants due to higher WUE_R (Figure 2). Water regulation caused by CA in plant can change the variation of Ψ or g_s to some extent concentration levels of salts and the water regulation effect is hysteretic. However, the WUE is an instantaneous value and cannot reflect the re-watering effect on plant. As a result, WUE_R is a new index tends to indicate the better of re-watering effect on plant. A considerable decrease was noticed even during re-watering conditions in WUE_R at high levels ($Nc_{3\wedge2}$, $Ns_{3\wedge2}$, $M_{1\wedge0}$ and $M_{2\wedge0}$). According to Yousfi et al. (2016), after application of re-watering, there is partially recovery found in some species of *Medicago laciniata* (L.) Mill., due to severe drought stress. Upon re-watering of plants which suffered from high water stress condition under $Nc_{3\wedge2}$, $Ns_{3\wedge2}$, $M_{1\wedge0}$ and $M_{2\wedge0}$, indicated that it was 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 impaired 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 stunted.

The variation in osmotic potential and water potential occurred because of lower water content. For that reason, it was necessary for plants to be re-watered prior to their wilting stages. The regime is considered very important in plant tolerance adaptation to drought stress environment in which net photosynthetic rate is maintained and recovered during periods of drought and water-stress (Chartzoulakis et al., 2002b). Accordingly, salt tolerance adaptability of *B. napus* was better under low to moderate salt stress conditions. Therefore, dilution of salted water or re-watering of salted water could be done at Ns_2, Nc_2 regime, by considering the best level for threshold tolerance and production of *B. napus* under saline condition. However, if salinity is caused by mixture of salts then M_3 regime is better to consider for dilution of saline irrigation because the relative effect of mixture of salt at M_1 was the same with single salts under Nc_2 and Ns_2, respectively. It reflected that single salt might be more toxic to plant growth than mixture of salts.

CONCLUSION

In conclusion, according to this work, *Brassica napus* is able to tolerate salt stress under low to medium concentration levels. In this aspect, at regime of medium concentration, WUE_R left positive effects on the growth and developments of plants and also show better restorability. Thus, the effect of salinity in *B. napus* could be reduced by diluting the saline irrigation water and also by mixing of salts in response to physiological behaviors. Application of dilution of saline irrigation could be helpful to maintain crops productivity, reduce irrigation cost and save water resources.

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