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24-EPIBRASSINOLIDE MODULATES BIOMASS PRODUCTION, GAS EXCHANGE CHARACTERISTICS AND INORGANIC NUTRIENTS IN CANOLA (*BRASSICA NAPUS* L.) UNDER SALT STRESS

SHAHID MEHMOOD, EJAZ HUSSAIN SIDDIQI*, IZAH NAWAZ AND NOREEN NASIR

Department of Botany, University of Gujrat, Pakistan
Corresponding author's email: ejaz.hussain@uog.edu.pk

Abstract

Present research work was designed to appraise the impact of exogenously applied 24-Epibrassinolide (24-EpiBL) in two canola varieties (*Brassica napus* L.) under salt stress. Seed of both varieties (Cyclone and Dunkled) were acquired from Ayub Agriculture Research Institute (AARI), Faislabad, Pakistan. Ten healthy seeds of each variety were sowed in plastic pots and after 15 days of seed germination, salinity stress was applied at (0 mM and 100 mM NaCl) in the solution of Hoagland nutrients. After salt treatment, 20 days old plants were foliar sprayed with 24-EpiBL at different levels (0, 0.5 and 1 µmol 24-EpiBL). Salinity stress remarkably declined biomass production, gas exchange parameters, WUE, RWC, root and shoot mineral nutrients (K^+ and Ca^{2+}) and their ratios while conc. of Na^+ and Cl^- ions were improved in root and shoots of each variety. Foliar applied 24-EpiBL lessened the adversities of salinity stress and upgraded the values of the root, shoot length and dry mass, intercellular CO_2 conc., rate of photosynthetic activity, rate of transpiration, stomatal conductance, water-use efficiency, leaf relative water content, mineral contents (Ca^{2+}, K^+) and nutrients ratios in root and shoots under control and salt-affected situations.

Key words: Brassinolide, Photosynthetic rate, Transpiration rate, Nutrients, Canola, Salt regime.

Abberevations: 24-EpiBL: 24-epibrassinolide, AARI: Ayub Agriculture Research Institute, WUE: Water use efficiency, RWC: Relative water content, BRs: Brassinosteroids, CRD: Complete Randomized Design, IRGA: infra-red gas analyser, PGR: Plant growth regulator.

Introduction

Salinization is the main problem that spreads rapidly in the whole world and greatly inhibits plant growth, development, and production (Ahmad *et al*., 2015; Negrao *et al*., 2017). It is assessed that about 7 percent of total land area and 20 percent of the cultivated land area is severely affected through a higher concentration of salts worldwide (Hussain *et al*., 2016). Salinity stress causes water stress inside plant cells as a result water loss from the cells and agricultural productivity is affected adversely (Bose *et al*., 2014). Different levels of salinity brutally affect the various plants' physio-chemical functions, such as a remarkable decrease in the rate of photosynthetic activity and translocation of ions from roots towards shoots (Goltsev *et al*., 2012; Abdallah *et al*., 2018). It reduces the root, shoot length and dry biomass by inhibiting the hydrolysis of reserve food and their transportation towards growing tissues (Farhoudi, 2010; Kandil *et al*., 2012). Salinity reduces the water potential of soil, as a result, water and nutrients become unavailable for the plant (Omami & Hammes, 2006). Salts accumulation produces ion toxicity, osmotic stress that induces destructive effects on normal plant functioning and inhibits plant growth. Photosynthesis is the most important physiological function of the plant body and salinity affect its all stages. Water stress under salinity decrease the leaf turgor pressure and rate of transpiration; additionally stomata close as a result stomatal conductance and substomatal CO_2 concentration reduce that inhibit the rate of photosynthetic activity (Chaves *et al*., 2009). Further, more salts in the growing medium affect the uptake of vital nutrients like calcium and potassium through various transporters located at the plasma membrane and induce ions imbalance (Tzortzakis, 2010; Hashem *et al*., 2016). Accretion of sodium chloride ions in the soil causes the ion imbalance by reducing the conc. of other ions like calcium and potassium that leads to cell death (Abd-Allah *et al*., 2015; Sarwat *et al*., 2016). Accumulation of salts at higher rates also leads a decline in mineral nutrients ratios like K^+/Na^+ and Ca^{2+}/Na^+ which are necessary for normal development and production by causing osmotic stress and specific ion toxicity (Babu *et al*., 2012). Brassinosteroids (BRs) are the most vital plant growth regulators that improve plant growth, development and production. These steroidal hormones belong to a class of polyhydroxylated plant growth stimulators that resist the plant against several environmental stresses including drought, cold, heavy metal, pesticides and salts stress (Sharma *et al*., 2016; Ahmad *et al*., 2017). Wani *et al*., (2017) exogenously applied 24-epibrassinolide (EBR) improved the growth rates, nutrients uptake, rate of gas exchange parameters, amount of relative water content, and ionic balance in crop plants during stress conditions. It shows a vital function in the amelioration of salinity induced harmful effects on the growth rate, ions concentration, relative water content, and stomatal conductance (Derevyanchuk *et al*., 2015). Brassinolides also improved the nutrients uptake, assimilation rates, and plasma membrane functioning during saline conditions. All these investigations showed that epibrassinolide is more effective against the adverse effects of salinity.

Canola (*Brassica napus* L.) of the Brassicaceae family also known as rapeseed is one of the third most momentous crops after soybean and palm. It is extensively cultivated in the whole world for the production of oil and is also considered a big source of biodiesel production (Milazzo *et al*., 2013). Salinity reduced the productivity of canola crops like other plant crops. Several canola cultivars showed resistance against salt stress, while some cultivars were defenceless (Gharelo-Shokri *et al.*, 2016).

The foremost objective of the current study was to observe the level of salinity stress which could change physiology of gas exchange characteristics, accumulation of nutrients in the plant tissues and foliar applied 24-epibrassinolide ameliorate the salt-induced toxic effect on two canola varieties.

Materials and Methods

This research work was conducted at the Botanical Garden, University of Gujrat, Gujrat, Pakistan, to appraise the noxious effects of salinity stress through exogenously applied 24-Epibrassinolide (24-EpiBL) on two canola (*Brassica napus* L.) varieties. Seeds of two canola varieties (Cyclone, Dunkled) were acquired from Ayub Agriculture Research Institute (AARI), Faisalabd, Pakistan. The experimental work was performed in plastic pots under normal condition during 2017-2018. River sand was used as a growth medium. Ten healthy seeds of both varieties were sowed in every pot. Then after fifteen (15) days of seed propagation thinning was done with hand and the remaining five plants of each replicate were treated with two levels of salinity (0mM and 100mM NaCl) along with Hoagland solution through roots. Then after 20 days of salt treatment, 24-EpiBL was applied exogenously in spray form at three different concentrations (0, 0.5, 1.0μM 24-EpiBL) in the morning time. The research work was performed in Complete Randomized Design (CRD) with four replicates.

Collection of Data

Biomass production: To measure growth parameters, one plant from each replicate was uprooted and washed with tap water then separate the root from the shoot. After separation measures the root and shoots length in (cm) by using a measuring scale. After that, all replicate samples were placed for 7 days in the oven at 65°C and measure the both shoot and root dry mass by using an electric balance.

Gas exchange attributes: To measure the gaseous exchange parameters including the net CO_2 assimiltion rate, transpiration, stomatal conductance and sub-stomatal CO_2 conc. infra-red gas analyser (IRGA) LCA-4 ACD was used. All these measurements were done on the intact fourth leaf from the upper in full bright sunlight (10 am - 2 pm) with some modifications: surface area of a leaf was 11.35 cm^2, the temperature of the leaf chamber differed from 36.2-42.9°C, atmospheric CO_2 342.13 μmol/mol, inside leaf chamber molar gas flow rate 251μmol/s, molar airflow rate per unit leaf area 221.06 mol/m^2s, gas flow rate inside leaf chamber 396 ml/min, and atmospheric pressure 99.95 kPa.

Water use efficiency: Water use efficiency was measured by given formula:

$$\text{Water use efficiency} = A/E$$

where,
A =Photosynthetic rate, and E = Rate of transpiration

Relative water content: To examine the leaf relative water content (RWC), an intact leaf was taken from all replicates, labelled with a permanent marker, and fresh weight was recorded. After that, all samples were soaked for 2 hours in distilled water and the turgid weight of each sample was noted. After this leaf samples were kept in an oven for the period 48 hrs at 70°C and the dry biomass was recorded. Then the RWC values using the following formula were calculated:

$$\text{RWC (\%)} = \frac{\text{(Leaf fresh weight – Leaf dry weight)}}{\text{(Leaf turgid weight – Leaf dry weight)]}} \times 100$$

Nutrients analysis: For nutrients determination like (Na^+, K^+, and Ca^{2+}) in root and shoot the protocol of Wolf, (1982) was used. Oven-dried ground sample of root and shoot (1.0 g), was placed it into digestion tubes then 2.5 ml conc. sulphuric acid (H_2SO_4) was added and the sample at room temperature was incubated for overnight. After incubation add 1 mL of hydrogen peroxide (H_2O_2) and after few mints, the tubes were kept in the digestion chamber, heat the sample at 350°C until fumes were formed. The heat was sustained for 30 minutes then all tubes were taken out from the digestion chamber and cool the sample. After that tubes were heated again at the same temperature in the digestion chamber until fumes were produced again for 20 minutes. At a point, the sample became completely colourless and the process of digestion became complete. Then add the 50 mL distilled water in the digested material and filtered it with Whatman filter paper. Sodium, potassium, and calcium ions were done by using the flame photometer (Jenway, PEP). A series of standard values for each cation (Na^+, K^+, and Ca^{2+}) was prepared that varies from 5-25 mg L^{-1}. After detection from the flame photometer compared the value of each cation with standard curves and notes the concentration of the nutrient.

Chloride determination: The procedure of Cl^- determination is quite different, for this take 1.0g dried ground sample of root and shoot and placed it into a test tube then makes it volume 10 mL by adding deionized distilled water. Then heat the sample until its volume decreased and become half of its original volume. Then again make the sample volume 10 mL by adding more distilled water and determined the conc. of Cl^- ions in all samples using chloride analyzer with specification i.e. Model No-926, Sherwood Scientific Limited Cambridge, UK.

Experiment design and statistical data analysis: Designed of the present experiment was completely randomized design (CRD) having four (04) replicates. The COSTAT computer package (*CoHort software*, Berkeley, USA) was used for working out analyses of variance of all parameters.

Results

Accumulation of salt in the growth medium significantly reduced the biomass production such as root and shoot length and their dry weight in each canola variety (Cyclone, Dunkled), but exogenously applied 24-EpiBL at the level of 0.5 and 1 μmol ameliorated the effect of salinity and improved the biomass production. On a comparison basis, it was observed that the variety "Dunkled" showed better performance than the "Cyclone" in response to salinity (Fig. 1).

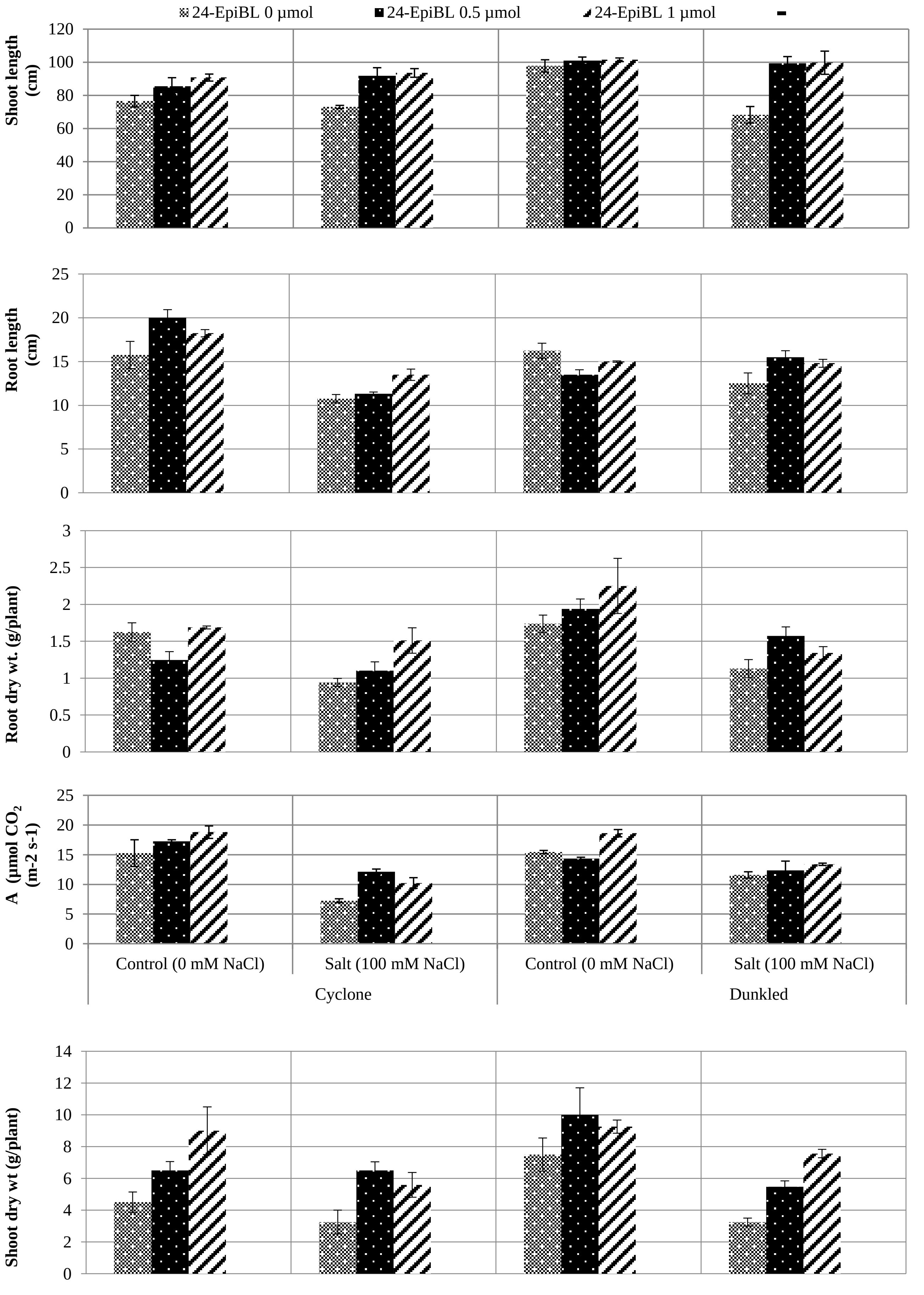

Fig. 1. Length of root, length of shoot, dry weight of shoot, dry weight of root, photosynthetic rate (A) of two canola varieties when subjected to foliar use of 24-epibrassinolide during saline and control environments.

Gas exchange parameters such as net CO_2 assimlation rate, transpiration rate, stomatal conductance and intercellular substomatal CO_2 conc. also remarkably decreased due to accumulation of salt in root zone and foliar use of 24-EpiBL at levels of 0.5 & 1µmol had overcome the salt-induced toxicity and enhanced the values of these attributes under saline environment (Figs. 1,2). Whereas significant result was noted in both varieties for salinity and foliar-applied 24-EpiBL improved the values of all these parameters (Table 1).

In addition, water-use efficiency and amount of relative water content showed decline under salt stress (100 mM NaCl) and foliar spray of 24-EpiBL at the levels of 0.5 & 1 µmol has improved WUE and RWC in each variety under control and saline environment (Fig. 2). Comparatively, the canola variety "Dunkled" remained better than "Cyclone". However, statistical analysis showed significant result with the application of 24-EpiBL and salinity stress (Table 1).

Root and shoot Na^+ and Cl^- remarkably increased in each variety under salinity and foliar spray of 24-EpiBL (0.5 & 1µmol) decreased the conc. of these ions and has eliminated the destructive effects of salt stress (Fig. 3). Data of variance for conc. of sodium and chloride ions showed significant result during salinity stress (Table 2) while interaction among all factors (Salinity, variety and 24-EpiBL) was significant in case of shoot Na^+, shoot Cl^- and root Cl^- ions but root Na^+ showed non-significant result (Table 2).

Whereas, salt addition in rooting medium has reduced the concentration of root & shoot (K^+ and Ca^{2+}) ion in each canola variety (Figs. 3,4). Exogenous use of 24-EpiBL at the levels of 0.5 and 1 µmol improved the values of these ions in each variety and eliminated the negativity of salt stress (Figs. 3,4). "Dunkled" variety showed better performance than the "Cyclone" and showed more tolerance against salinity. In addition, a significant result was obtained by analysis of variance regarding shoot K^+, root K^+ and root Ca^{2+} while, shoot Ca^{2+} showed the non-significant result in salt and 24-EpiBL treated plants (Table 2).

Accumulation of salt through rooting medium greatly reduced the shoot K^+/Na^+ ratio in both varieties of canola under saline conditions (Fig. 4). On comparing both varieties, "Dunkled" showed better performance than the "Cyclone" at a salinity level of 100 mM NaCl (Fig. 4). Whereas exogenous use of 24-EpiBL (0.5 and 1 µmol) lessened the divergent effect of salt stress in each variety and improved the status of the K^+/Na^+ ratio (Fig. 4). Moreover, values of the K^+/Na^+ ratio showed the non-significant result in salt-treated and 24-EpiBL treated plants of both canola varieties (Table 2). Similarly, salt at 100 mM NaCl also declined the values of root K^+/Na^+ ratio in each canola variety and foliar use of 1 µmol 24-EpiBL improved the values of root K^+/Na^+ ratio by mitigating the injurious effects of salinity stress (Fig. 4). Variety "Dunkled" showed better resistance against salinity at 1 µmol 24-EpiBL than "Cyclone" (Fig. 4). Values of root K^+/Na^+ ratio showed significant result when plants of each variety was treated with 24-EpiBL under salinity stress (Table 2). Values of shoot Ca^{2+}/Na^+ ratio also declined in the existence of more salt in the growth medium of both varieties of canola (Fig. 4). Whereas, foliar spray of 0.5 and 1 µmol of 24-EpiBL has increased the values of shoot Ca^{2+}/Na^+ ratio in "Cyclone" but "Dunkled" did not show any response to 24-EpiBL and remained unchanged (Fig. 4). By analysing the data of variance, it was observed the values of shoot.

Table 1. Growth attributes, gas exchange characteristics, water-use efficiency and relative water content of two canola (*Brassica napus* L.) varieties treated with exogenously applied PGR 24-EpiBL during saline and control environments.

Source of variance	**df**	**Length of shoot**	**Length of root**	**Shoot dry wt.**
PGR 24-epiBL	2	0.000***	0.023*	0.000***
Salinity	1	0.067 ns	0.000***	0.000***
Variety	1	0.000***	0.483 ns	0.006**
PGR 24-epiBL x Salinity	2	0.005**	0.267 ns	0.885 ns
PGR 24-epiBL x Variety	2	0.832 ns	0.104 ns	0.729 ns
Salinity x Variety	1	0.012*	0.000***	0.239 ns
PGR 24-epiBL x Salinity x Variety	2	0.168 ns	0.000***	0.161 ns
Error	36	71.069	2.673	3.615
Source of variation	df	Root dry wt.	A	E
PGR 24-epiBL	2	0.025*	0.001**	0.012*
Salinity	1	0.000***	0.000***	0.000***
Variety	1	0.003**	0.174 ns	0.057 ns
PGR 24-epiBL x Salinity	2	0.260 ns	0.065 ns	0.565 ns
PGR 24-epiBL x Variety	2	0.164 ns	0.038*	0.015*
Salinity x Variety	1	0.147 ns	0.004**	0.840 ns
PGR 24-epiBL x Salinity x Variety	2	0.256 ns	0.934 ns	0.283 ns
Error	36	0.117	4.039	0.277

Source of variation	**df**	**g_s**	**Ci**	**WUE**	**RWC**
PGR 24-epiBL	2	0.000***	0.040*	0.296 ns	0.075 ns
Salinity	1	0.000***	0.001**	0.191 ns	0.000***
Variety	1	0.000***	620 ns	0.906 ns	0.000***
PGR 24-epiBL x Salinity	2	0.001***	0.349 ns	0.023*	0.001**
PGR 24-epiBL x Variety	2	0.000***	0.105 ns	0.073 ns	0.509 ns
Salinity x Variety	1	0.491 ns	0.671 ns	0.006**	0.357 ns
PGR 24-epiBL x Salinity x Variety	2	0.350 ns	0.504 ns	0.855 ns	0.085 ns
Error	36	798.083	1046.888	0.493	47.888

ns = Non-significant; df= Degrees of freedom

It is significant *, **, *** at the level 0.05, 0.01, 0.001 repectivitly

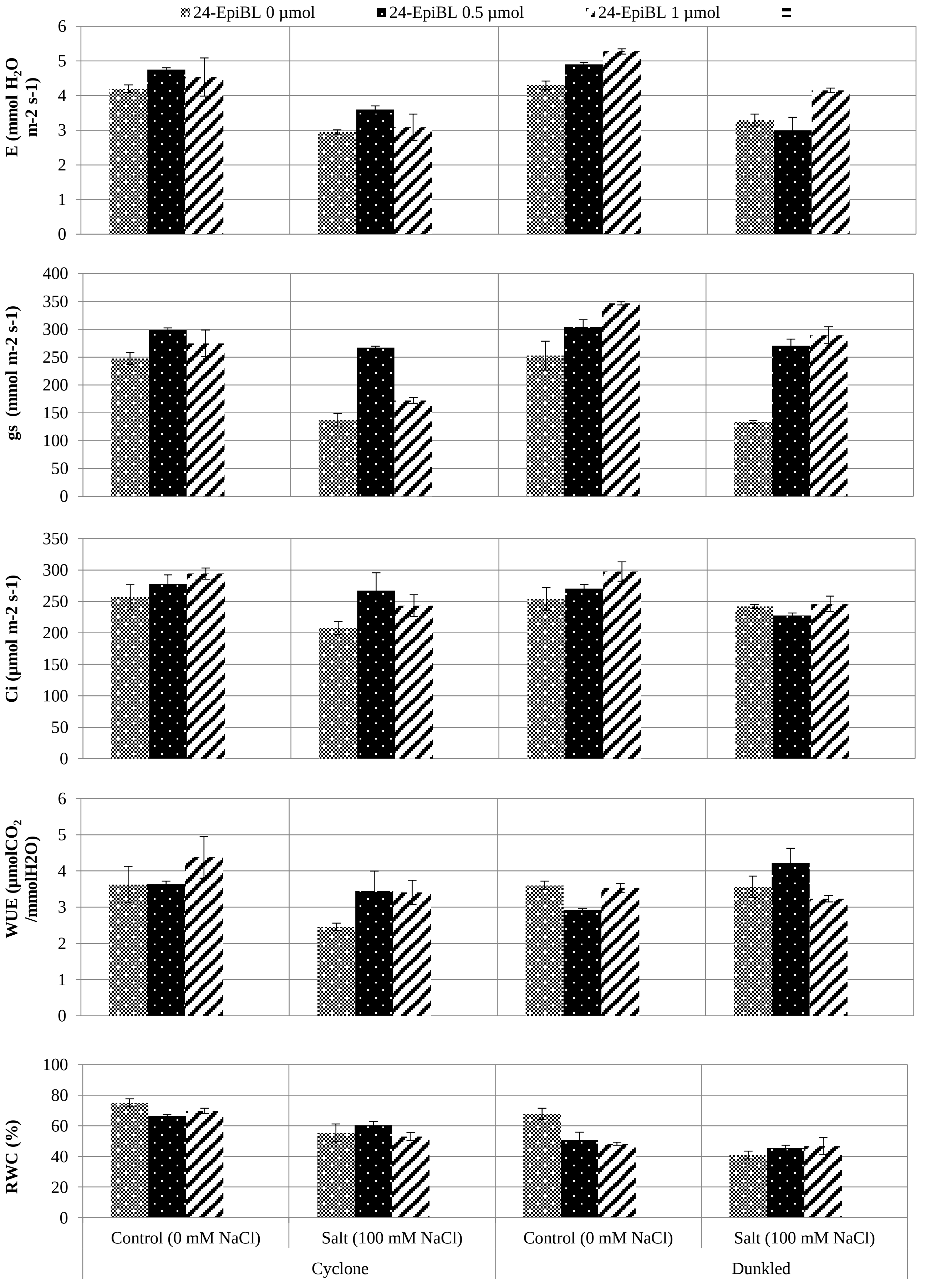

Fig. 2. Transpiration rate (E), stomatal conductance (gs), sub-stomatal CO_2 conc., water-use efficiency (WUE), relative water content (RWC) of two canola varieties when subjected to foliar use of 24-epibrassinolide during saline and control environments.

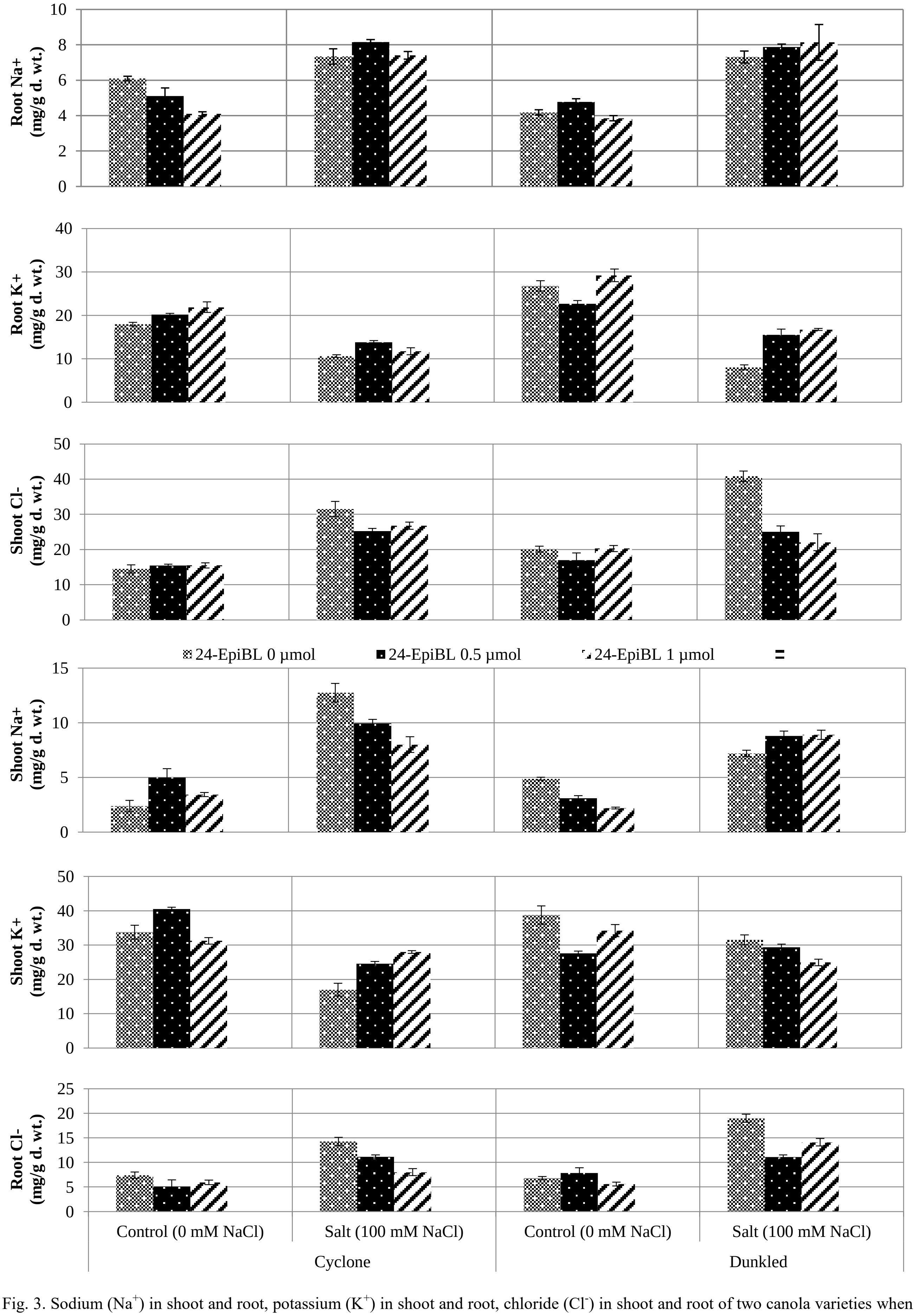

Fig. 3. Sodium (Na^+) in shoot and root, potassium (K^+) in shoot and root, chloride (Cl^-) in shoot and root of two canola varieties when subjected to foliar use of 24-epibrassinolide during saline and control environments.

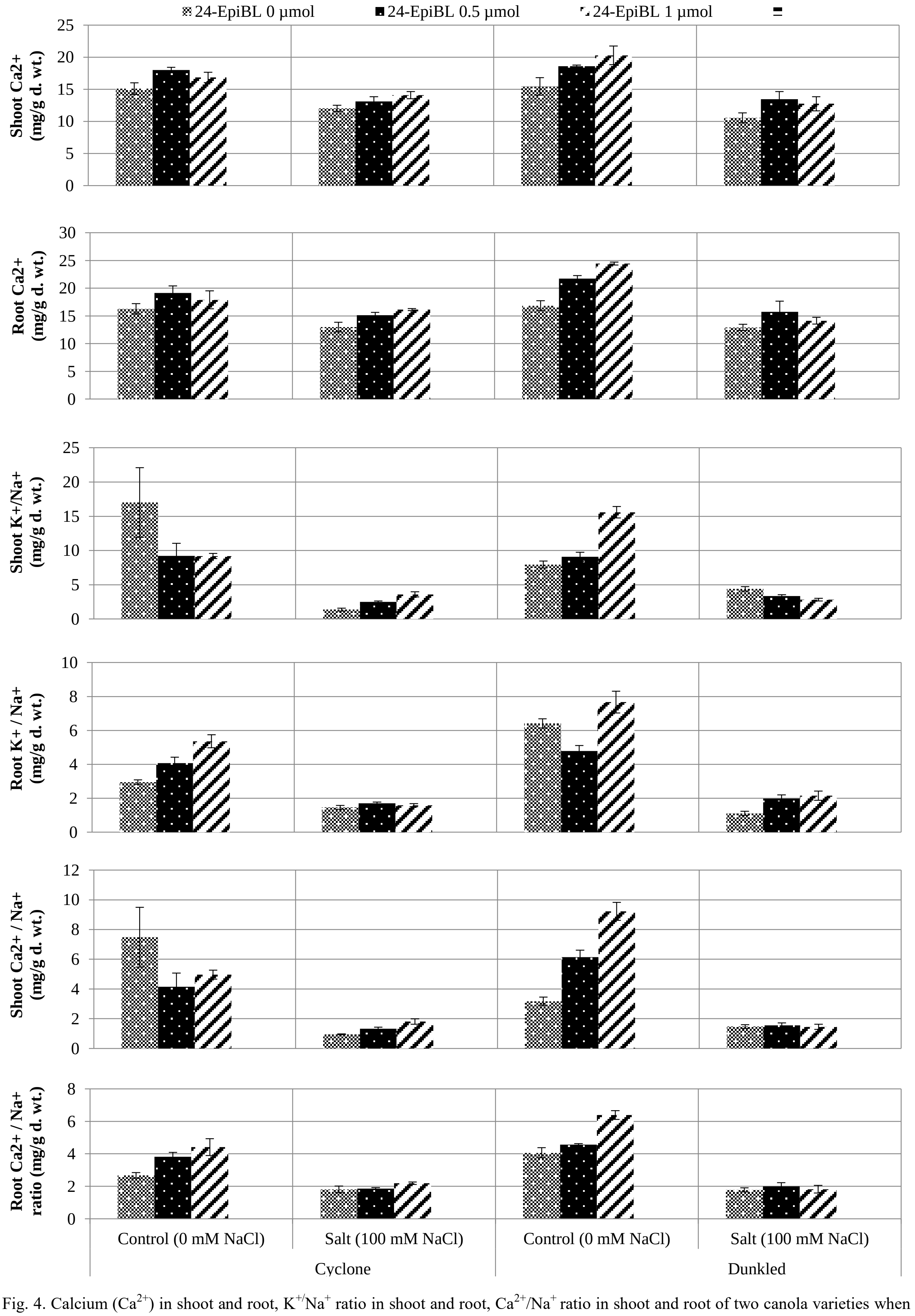

Fig. 4. Calcium (Ca^{2+}) in shoot and root, K^{+}/Na^{+} ratio in shoot and root, Ca^{2+}/Na^{+} ratio in shoot and root of two canola varieties when subjected to foliar use of 24-epibrassinolide during saline and control environments.

Table 2. Shoot and root mineral content and their ratios of two canola (*Brassica napus* L.) varieties treated with exogenously applied PGR 24-EpiBL during saline and control environments.

Source of variation	**df**	**Na^+ in shoot**	**Na^+ in root**	**K^+ in shoot**	**K^+ in root**
PGR 24-epiBL	2	0.003**	0.105 ns	0.671 ns	0.000***
Salinity	1	0.000***	0.000***	0.000***	0.000***
Variety	1	0.000***	0.137 ns	0.033*	0.000***
PGR 24-epiBL x Salinity	2	0.364 ns	0.022*	0.018*	0.000***
PGR 24-epiBL x Variety	2	0.106 ns	0.102 ns	0.000***	0.011*
Salinity x Variety	1	0.006**	0.037*	0.000***	0.0001***
PGR 24-epiBL x Salinity x Variety	2	0.000***	0.265 ns	0.000***	0.0005***
Error	36	1.045	0.614	8.645	3.552
Source of variation	df	Cl^- in shoot	Cl^- in root	Ca^{2+} in shoot	Ca^{2+} in root
PGR 24-epiBL	2	0.000***	0.000***	0.0005***	0.0001***
Salinity	1	0.000***	0.000***	0.000***	0.000***
Variety	1	0.003**	0.0001***	0.575 ns	0.035*
PGR 24-epiBL x Salinity	2	0.000***	0.0002***	0.661 ns	0.301 ns
PGR 24-epiBL x Variety	2	0.002**	0.408	0.506 ns	0.421 ns
Salinity x Variety	1	0.157 ns	0.002**	0.052 ns	0.005**
PGR 24-epiBL x Salinity x Variety	2	0.014*	0.0004***	0.273 ns	0.033*
Error	36	9.156	2.555	3.842	4.765
Source of variation	df	K^+/ Na^+ in shoot	K^+ /Na^+ in root	Ca^{2+} /Na^+ in shoot	Ca^{2+}/Na^+ in root
PGR 24-epiBL	2	0.251 ns	0.0005***	0.438 ns	0.000***
Salinity	1	0.000***	0.000***	0.000***	0.000***
Variety	1	0.954 ns	0.000***	0.185 ns	0.0002***
PGR 24-epiBL x Salinity	2	0.297 ns	0.0003**	0.222 ns	0.0001***
PGR 24-epiBL x Variety	0.054 ns	2	0.055 ns	0.460 ns	0.621 ns
Salinity x Variety	1	0.307 ns	0.000***	0.663**	0.000***
PGR 24-epiBL x Salinity x Variety	2	0.0009***	0.003**	001**	0.083 ns
Error	36	10.864	0.428	5.941	0.286

ns=non-significant; df= degrees of freedom

It is significant *, **, *** at the level 0.05, 0.01, 0.001 repectivitly

Ca^{2+}/Na^+ did't show significant result under salt stress and 24-EpiBL treated plants in each variety (Table 2)

Values of root Ca^{2+}/Na^+ ratio also deteriorated under salinity (100 mM NaCl) in each canola variety but the use of 24-EpiBL improved the values of root Ca^{2+}/Na^+ ratio at a higher level (1µmol) in variety "Cyclone" and at lower level (0.5 µmol) in "Dunkled" by releasing the effects of salinity (Fig. 4). Also, the values of root Ca^{2+}/Na^+ showed significant result in response to 24-EpiBL under salinity (Table 2).

Discussion

Salt stress is a well-known problem that influences agricultural crops by causing osmotic and ionic stresses on growth, development and metabolic activities (Siddiqui *et al*., 2017; Estaji *et al*., 2018). Salinity reduced the plant growth by altering various physiological and biochemical processes like gaseous exchange attributes, water relations and ions imbalance (Sabir *et al*., 2009; Mehta *et al*., 2010). Plant hormones play a vital role under saline conditions by mitigating the inhibiting effects of salt (Raja *et al*., 2017; Verma *et al*., 2017). Plant growth regulator, 24-epibrassinolide (24-EpiBL) is a class of polyhydroxy steroidal plant hormone with remarkable growth-stimulating impact (Vardhini & Anjum, 2015). Brassinosteroids provide resistance in the plant against salinity stress by mitigating the adverse effects on morphological, physiological and biochemical processes and improve the growth and development of plant (Ahammed *et al*., 2020; Fariduddin *et al*., 2014). In current research work, salt-induced growth medium declined the root, shoot length and dry biomass by causing ion toxicity and osmotic stress that enables the plant to take up water and transport toward growing tissues. These inhibiting effects of salinity were overcome by exogenous application of 24-EpiBL at different levels. The result given by Lu *et al*., (2013) was also consistent with our finding where inhibitory effects of salinity on the shoot, root length and dry mass mitigated by foliar spray of 24-EpiBL. Another study reported by Ekinci *et al*., (2012) where he described that growth of lettuce seedling was improved by using 24-EpiBL in spray form under higher salt concentration. The same results related to root and shoot dry biomass given by Badran *et al*., (2015) while working on wheat plants under salinity; they stated that foliar use of GB alleviates the salinity effects and improved the dry biomass. In the present research salinity greatly diminished the activities of gas exchange attributes by altering the enzymes functioning in canola variety and such inhibitory effects of salinity improved by treating the plants with 24-EpiBL exogenously. In literature parallel findings were given by Hu *et al*., (2013) in chilli (*Capsicum annuum*) plants and Lima & Lubato, (2017) in cowpea plants. Hayat *et al*., (2010) findings on maize are corroborated with our findings where the injurious effect of salinity on gas exchange attributes ameliorated by foliar spray of 24-EpiBL at different levels. Alyemeni *et al*., (2013) demonstrated the results regarding the photosynthetic rate where the use of 24-EpiBL lessened the salt-induced inhibitory effects on *Brassica juncea* in saline growth medium. In literature result revealed by Perveen *et al*., (2010) corroborated with our findings where they reported that in wheat plants destructive effect of salinity on transpiration rate was released with 24-EpiBL in spray form. Ding *et al*., (2012) stated that in eggplant stomatal conductance was improved by exogenously applied 24-EpiBL under higher levels of salt stress. Finding of Ishaq *et al*., (2021) related to substomatal CO_2 conc. are matched with present research work where they stated that exogenous use of ascorbic acid abolished the salinity effects on wheat plants. In our findings, the values of water use efficiency reduced due to a reduction in water potential under saline conditions and such inhibition improved by treating canola plants with a spray of 24-EpiBL. Huang *et al*., (2009) recorded the same result on tomato plants where WUE increased by foliar use of SA under the salt regime. Karlidag *et al*., (2011) also found the same result related to relative water content in strawberry plants where the use of 24-EpiBL overcome salt-inducing inhibitory effects. Saline growth medium also decreased the RWC in safflower varieties (Erdal & Cakirlar, 2014) and this reduction in relative water content improved by treating the plant with foliar spray of glycine betaine. Salt accretion in the growth medium affects the concentration of necessary mineral ions like K^+ and Ca^{2+}, which are important for normal plant functioning (Ferguson & Grattan, 2005). In our research work, salinity decreased the content of K^+ and Ca^{2+} ions. Imbalance among nutrients due to ion toxicity was overcome by exogenous use of 24-EpiBL. Saline growth medium at higher concentration directly affects the root ion operating canal system (Parida & Das, 2005).

Restriction in the uptake of the vital nutrient is the basic reason for a decline in plant growth, development and production due to induction of oxidative damage and osmotic stress. The concentration of Na^+ ion increased in rice (*Oryza sativa*) (De-Leon *et al*., 2015) and canola (*Brassica oleracea*) (Gu *et al*., 2016) that was overcome by treating the plants with 24-EpiBL. Rahman *et al*., (2002) reported that GB enhanced the content of K^+ ion in rice plant under salinity. A remarkable rise in the conc. of Na^+ & Cl^- and decline in K^+ & Ca^{2+} ions under salinity stress were observed in green beans where 24-EpiBL improved mineral nutrients (Yasar *et al*., 2006). Salt containing growth medium has a great impact on ions stability by causing ionic toxicity (Azhar *et al*., 2017). In the cell cytosol, the K^+/Na^+ ratio produces resistance against salinity (Shabala *et al*., 2016) but in the present study salt at 100 mM NaCl lowered the root and shoot K^+/Na^+ ratio in each variety. A decrease in both canola verities was overcome by exogenous use of 24-EpiBL at different levels. In literature, the study by Shu *et al*., (2015) matched with our findings where foliar use of 24-EpiBL improved the K^+/Na^+ ratio in cotton (*Gossypium hirsutum*) in control and saline conditions. Findings by Yasar *et al*., (2006) in green beans and Babu *et al*., (2012), in tomato are also consistent with the above results where the K^+/Na^+ ratio improved after 24-EpiBL supplementation under salinity. In the above study, the ratio of Ca^{2+}/Na^+ decreased in presence of salt and the use of 24-EpiBL eliminated the inhibitory effects of salinity in both canola varieties. The 24-EpiBL improved the values of Ca^{2+}/Na^+ ratio in shoots and roots of the various crop including barley, wheat, rice etc. by releasing the inconsistent effects of salinity (Dong *et al*., 2017).

Generally, salinity induced adverse effects on biomass production, gaseous exchange parameters like photosynthetic activity, transpiration, stomatal conductance and intercellular CO_2 conc., amount of WUE, leaf relative water content and necessary root and shoots minerals nutrients like Na^+, Cl^-, K^+ and Ca^{2+} in both varieties of canola crop plants. Whereas, exogenous use of 24-EpiBL in spray form ameliorates the destructive effects salinity and improve values of growth-related parameters, gaseous exchange parameters, WUE, RWC and vital minerals during saline and control conditions.

Conclusion

Salinity-induced reduction in the growth, gas exchange characters and mineral nutrients (shoot and root K^+ and Ca^{2+}) of two canola varieties was overcome by the foliar application of plant growth regulator 24-epibrassinolide. In the future study, when gene-induced salt tolerance is recognized in two canola varieties using suitable molecular techniques.

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