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RESEARCH ARTICLE



Effect of ellagic acid on growth and physiology of canola (*Brassica napus* L.) under saline conditions

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ABSTRACT

Salinity stress is limiting growth and productivity of plants in many areas of the world. Plants adopted different strategies to minimize the effect of salt stress. A pot experiment was conducted to investigate the morphological and physiological changes produced in Canola (*Brassica napus*) by exogenous application of ellagic acid (EA) under saline conditions. EA is an antioxidant, expected to reduce the effect of salinity stress. The seeds of two canola cultivars, Rainbow and Oscar, were soaked for 6 h with different concentrations of EA (0, 55 and 110 µg/ml). The soaked seeds were sown in small pots. Salt stress was imposed on the plants by applying NaCl solutions of different concentrations (0, 60 and 120 mM) and the duration of stress was for four weeks. Salinity stress reduced seed germination and disturbed the morphological and physiological attributes of *B. napus*. Application of EA as seed soaking reduced the effect of salinity and enhanced the growth of plants. Overall, we could confirm a significant role of EA by inducing salinity tolerance in *B. napus*.

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Antioxidant; ellagic acid;
salinity; seed soaking;
Brassic napus

Introduction

Salinity is the accumulation of excessive concentrations of soluble salts that reduce the growth of plants by osmotic stress (Mittal et al. 2015). High soil salinity is an important issue that greatly reduces plant productivity (Nounjan et al. 2012). The ratio of salt-affected soil is constantly increasing all over the world with estimate reaching up to 6% (Rabhi et al. 2010) and it is noticed that arable land may result into 50% land loss by 2050 (Latef and Chaoping 2011).

Salt stress is a complex matter resulting in a combination of osmotic and ion toxic effects as well as oxidative stress in plants (Nounjan et al. 2012; Porcel et al. 2012). Under stress condition, reactive oxygen species (ROS) are produced in living organisms and become the causative agents to damage important biomolecules (Weidinger and Andrey 2015). These amounts of intracellular oxidants are kept in balance by endogenous antioxidants (Poljsak et al. 2013). Under persistent stressful environment conditions, however, this balance is shifted towards elevated levels of oxidants, leading to oxidative stress. The efforts toward reduction in oxidative stress, the role of antioxidants is of great importance. Antioxidants can inhibit, prevent or delay the oxidation of biological compounds and scavenge free radicals thereby minimize oxidative stress (Sepúlveda et al. 2011).

Exogenous application of antioxidants has been proved to be useful in the amelioration of salt stress by scavenging the free radicals produced under stressful environment and protect the organism from different effects of stresses (Athar et al. 2008, 2009; El-Soud et al. 2013; Khan et al. 2013). Among different antioxidants, ellagic acid (EA) appeared one of the best to scavenge the ROS due to its polyphenolic characteristics. EA can be easily isolated from plants using different high performance liquid

chromatography (HPLC) methods (Braunberger et al. 2013) and it is also available commercially EA is a polyphenolic antioxidant and phytonutrient found in numerous vegetables and a variety of fruits such as raspberries, pomegranate, almonds, strawberries, walnuts, grapes, wide variety of berries and black currants (Malini et al. 2011).

EA is a good scavenger of hydroxyl radicals as these are considered to be one of the most dangerous to damage the important biomolecules (Halliwell and Gutteridge 1993). The antioxidant capability of EA is based, at least partly, upon its four hydroxyl groups present in its structure that scavenge both, hydroxyl and superoxide anion radicals (Pari and Sivasankari 2008). Through the structure–function relationship of EA, it has been suggested that both phenolic hydroxy groups and the lactone are necessary for its activity as a powerful antioxidant (Barch et al. 1996). Cozzi et al. (1995) showed a beneficial role of EA by scavenging free radicals of ROS in Chinese hamster ovary cells. However, EA has also other antioxidant roles such as binding to DNA, inhibition of the production of ROS and protection of DNA from alkylating injury (Cozzi et al. 1995). In plants, pretreatment of chickpea seeds with EA increased the resistance against osmotic stress (El-Soud et al. 2013).

B. napus Linn. (Brassicaceae) stands third among oil seed crops of the world, after soybean and palm (Siddiqui et al. 2010) with oil yields of 33% (Chakrabarti et al. 2012). It is cultivated in 53 countries spreading all five continents (Siddiqui et al. 2010) and it is widely consumed as a vegetable (Yang et al. 2011). Keeping in mind the immense importance of *B. napus* as an oil-producing as well as fodder crop, its cultivation under saline conditions is on high demand. Therefore, we conducted the present study to determine the effect of exogenous application of EA on *B. napus* under saline conditions.

Materials and methods

A pot experiment was conducted to investigate the physiological changes in *B. napus* after exogenous application of EA under saline conditions. Two canola varieties namely Rainbow (V1) and Oscar (V2) were used under present study. The seeds of canola cultivars were obtained from Ayub Agriculture Research Institute (AARE), Faisalabad, Pakistan. Seeds were treated separately by soaking them for 6 h in solutions containing different concentrations of EA (0, 55 and 110 µg/ml). Pretreated seeds were sown in pots. One week after seed germination, different salt concentrations (0, 60 and 120 mM) were applied to the soil on the base of saturation percentage. Salt concentrations remained constant as the pots were closed at the bottom; evaporated water was refilled and the electrical conductivity of the medium was continuously checked by EC meter. The total time of salt treatment was for four weeks. After this period, plant material was harvested and morphological data, i.e. shoot length (cm), root length (cm), shoot fresh weight (g), root fresh weight (g), shoot dry weight (g), root dry weight (g), as well as physiological parameters like chlorophyll content and ion contents were collected. All studies have been performed with three individual samples.

Chlorophyll contents

Chlorophyll contents were determined according to the method (Arnon 1949) and Davies (1976). The fresh leaves of the *Brassica napus* plants were chopped into 0.5 cm segments and chlorophyll was extracted with 80% acetone by keeping the samples overnight at -10°C . The extract was centrifuged at $14,000\times g$ for 5 min and the absorbance of the supernatant was read at 480, 645 and 663 nm using a spectrophotometer (IRMECO U2020). The chlorophyll a and b contents were calculated by the following formulas:

$$\text{Chl.a} = [12.7(\text{OD}_{663}) - 2.69(\text{OD}_{645})] \times V/1000 \times W,$$

$$\text{Chl.b} = [22.9(\text{OD}_{645}) - 4.68(\text{OD}_{663})] \times V/1000 \times W,$$

$$V = \text{Volume of the extract (ml)},$$

$$W = \text{Weight of the fresh leaf tissue (g)}.$$

Digestion of plant material and ion determination

Dried and ground plant material was digested according to a reported method (Wolf 1982). In brief, plants were dried for 72 h at 70°C before grinding. 0.1 g was taken in each digestion tube and 2 ml of concentrated H_2SO_4 were added; it was then incubated overnight at room temperature. Thereafter, 0.5 ml

of H_2O_2 (35%) was poured down to the sides of the digestion tube, ported the tubes in a digestion block and heated at 350°C until fumes were produced and continued to heat for another 30 min. The digestion tubes were removed from the block and cooled. Then 0.5 ml of H_2O_2 were slowly added and tubes were placed back into the digestion block. The above step was repeated until the cold digested material was colorless. The volume of the extract was maintained up to 50 ml in volumetric flasks. The extract was filtered and used for the determination of ions.

The concentration of cations (K^+ , Na^+ , Ca^{2+} , Mg^{2+}) was analyzed on Atomic Absorption Spectrophotometer (AA 6300, Shimadzu, Japan).

Results

Salt stress severely affected both canola varieties although the effect of NaCl on shoot and root length varied among two varieties V1 and V2 (Table 1; Figure 1(a,b)). Salt stress particularly diminished shoot fresh and dry weight as well as root fresh weight (Figure 1(c–e)).

Soaking seeds in EA caused a dose-dependent reaction that differed in the two canola varieties. The highest concentration of 110 µg/ml EA reduced salinity effects at the low NaCl treatments in V1, resulting in longer shoots (Figure 1(a)) whereas medium concentrations of 55 µg/ml EA were more effective in V2, particularly a high salt concentrations of 120 mM NaCl (Figure 1(a)). Root length was generally variable in all salt treatments and little influenced by the addition of EA in both canola varieties (Figure 1(b)).

Medium concentration of EA (55 µg/ml) was most effectively reducing high salinity stress of 60 and 120 mM NaCl in both varieties when shoot fresh weight (Figure 1(c)), shoot dry weight (Figure 1(d)) and root fresh weight (Figure 1(g)) were analyzed. EA (55 µg/ml) also positively affected root dry weight (Figure 1(f)) in both canola varieties. However, at the highest NaCl treatments, root dry weight was higher without the addition of EA; this trend was also observed at 60 mM NaCl in V2.

Analysis of variance of data has indicated that the saline medium reduced the growth of plants (Table 2). Application of EA alleviated the effect of salinity and enhanced the growth of the plants. Among different concentration of EA, 110 µg/ml of EA increased the chlorophyll a under saline conditions at V1 and in V2 at 120 mM of salinity, 110 µg/ml of EA increased the chlorophyll a content (Figure 2(a)). With regard to chlorophyll b, 55 µg/ml of EA increased the content in V1 at 60 mM NaCl but not at 120 mM NaCl while in V2, 110 ppm of EA was more effective at both salinity treatments (Figure 2(b)). Salinity stress also reduced the total chlorophyll amounts

Table 1. Analysis of variance of data of shoot and root length and shoot and root fresh and dry weight of *B. napus* under saline and non-saline conditions by exogenous application of EA.

Source	DF	Shoot length	Root length	Shoot fresh weight	Root fresh weight	Shoot dry weight	Root dry weight
EA	2	0.18	0.78	1.15	3.10	0.18	2.52
NaCl	2	1.74**	3.41**	0.94	9.86**	1.21	9.38**
Variety	1	9.24**	3.38	2.56	15.75**	4.11	4.90**
EA \times NaCl	4	5.95**	1.47	5.11**	3.17**	5.69**	2.84**
EA \times Variety	2	1.04	2.38	1.07	1.12	2.55	0.65
NaCl \times Variety	2	1.13	2.97	0.30	6.60**	0.29	2.07
EA \times NaCl \times Variety	4	0.92	0.55	2.98**	2.68**	1.67	1.46
Error	36						
Total	53						

**Significant at .05 levels; DF = Degree of freedom.

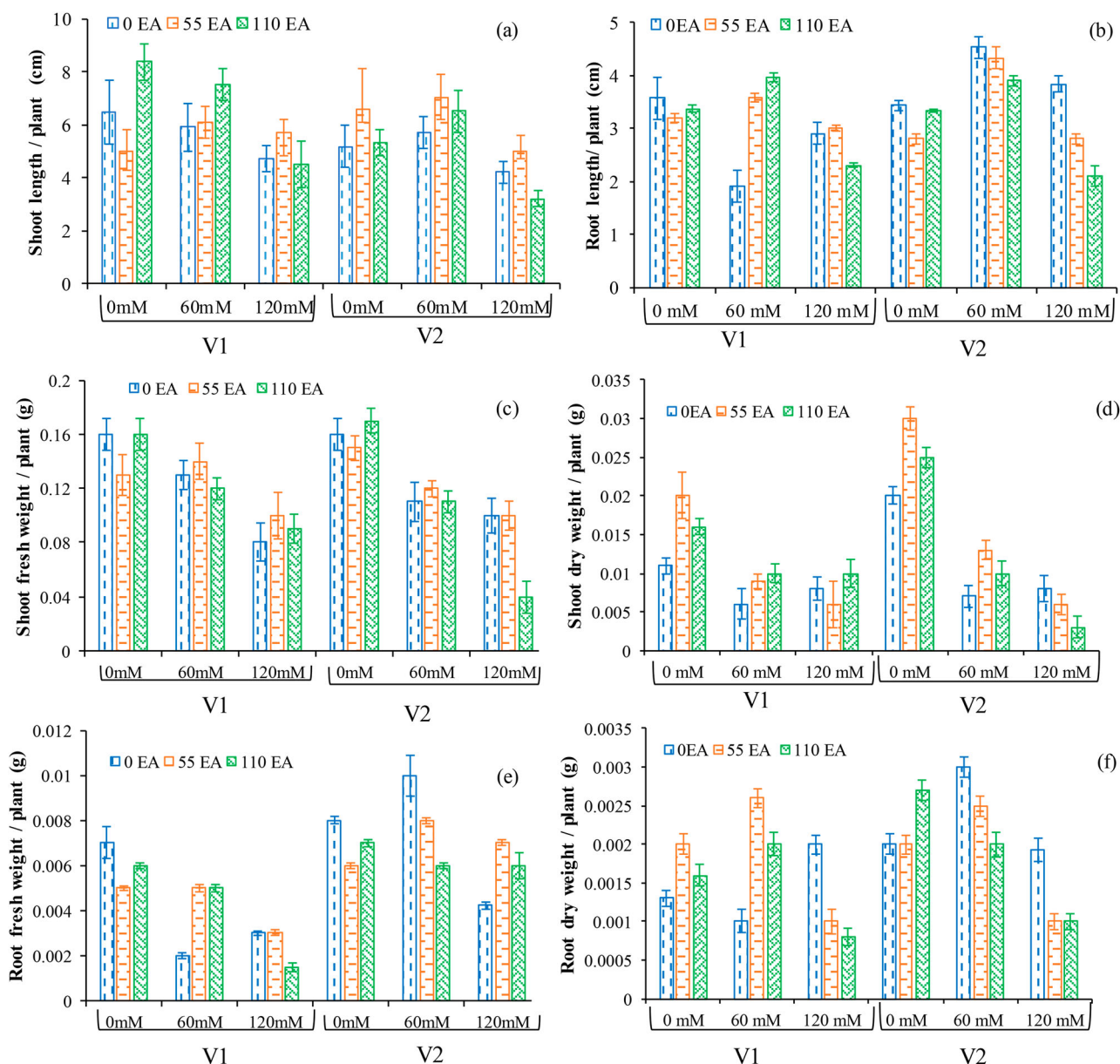


Figure 1. Effect of seed priming with EA on shoot (a) and root length (b), shoot fresh (c) and dry weight (d), root fresh (e) and dry weight (f) of *B. napus* under non-saline (0 mM) and saline (60 mM and 120 mM NaCl) conditions; V1 = variety 1; V2 = variety 2.

and the application of EA alleviated the effect of salinity when applied at 110 µg/ml at V1. In V2, an increase of the total chlorophyll was also observed after EA addition, particularly at high concentration, i.e. 120 mM NaCl (Figure 2(c)). Little surprisingly, sodium concentration increased under saline conditions (Figure 2(d)). In regard to potassium, application of 110 µg/ml EA enhanced the concentration of potassium, particularly in V1, but also in V2 at the highest salt treatment

(Figure 2(e)). Magnesium and calcium concentration was also affected by salinity. Application of EA ameliorated the effect of salinity stress on these ions. For magnesium, this was evident with 110 µg/ml of EA that reduced the effect of salinity at 60 mM NaCl (Figure 2(f)). Regarding calcium, 110 µg/ml of EA was effective at 120 mM NaCl in V1 and 55 µg/ml of EA reduced the effect of salinity in V2 at the highest salt treatment (Figure 2(g)).

Table 2. Analysis of variance of the data of chlorophyll a, b and total, sodium, potassium, magnesium and calcium concentration of *B. napus* under saline and non-saline conditions by exogenous application of EA.

Source	DF	Chlorophyll a	Chlorophyll b	Total chlorophyll	Na ⁺	K ⁺	Mg ²⁺	Ca ²⁺
EA	2	0.42	2.02	2.12	17.44**	19.65**	22.42**	3.48**
NaCl	2	3.58**	0.15	3.86**	17.01**	14.03**	6.86**	2.69
Variety	1	0.72	0.55	3.21**	12.11**	45.28**	6.14**	1.68
EA × NaCl	4	4.42**	2.58	7.39**	63.98**	34.04**	56.29**	23.93**
EA × Variety	2	6.83**	1.76	9.76**	38.32**	29.79**	41.13**	18.95**
NaCl × Variety	2	14.58**	5.41**	22.58**	3.23	42.93**	7.65**	1.73
EA × NaCl × Variety	4	2.92	0.55	6.15**	56.11**	8.19**	41.49**	13.31**
Error	36							
Total	53							

**Significant at .05 levels; DF = [Degree of freedom].

Discussion

Salinity is one of the major issues responsible for reduction in agricultural yield (Flowers and Muscolo 2015). Salt affects the growth of crop plants by reducing the uptake of water by roots (Sakina et al. 2016). The most critical phases of plant life are seed germination and growth of seedlings and these are adversely affected by various environmental changes especially salt stress (El-Soud et al. 2013). Plants exposed to salinity stress undergo numerous physiological and

biochemical changes because salt stress involves oxidative stress (El-Soud et al. 2013). EA has the ability to protect the plants against stresses because of its antioxidant activity (Valdés et al. 2011). It was observed during the current study that the fresh and dry weight of plants is strongly affected by salt stress. This reduction in weight under salinity can be attributed to an increase in Na^+ concentration that disturbs the ionic and osmotic balances in plants (Aghamir et al. 2015; Forieria et al. 2016) resulted in decreased biomass.

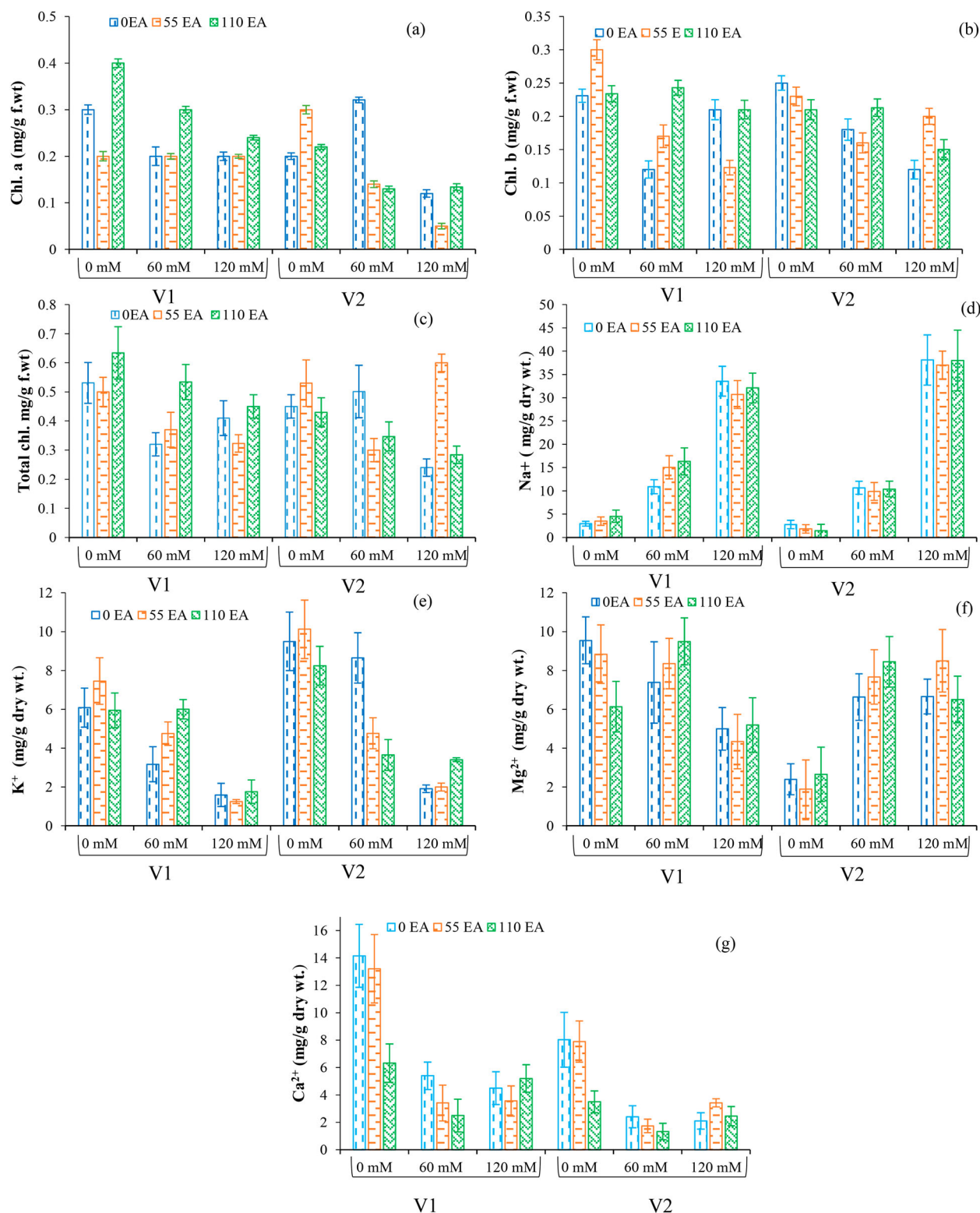


Figure 2. Effect of seed priming with EA on chlorophyll a (a) chlorophyll b (b) total chlorophyll (c) Na^+ (d) K^+ (e), Ca^{2+} (f) and Mg^{2+} (g) of *B. napus* under saline and non-saline conditions.

Salinity stunted the shoot length in plants and it was found to change the mechanism of photosynthetic machinery (Aghamir et al. 2015). As shown in the present study, the chlorophyll a and b contents remarkably decreased by the application of salinity stress. It is a well-known fact that K^+ , Mg^{2+} and Ca^{2+} are essentially required for plant growth (Wang et al. 2013) but an increase in the salt level of soil results in decreasing absorption of essential mineral nutrients by the plants (Shabani et al. 2015).

It has been observed previously that Potential antioxidants produced within plants and applied exogenously reduced the effects of stresses in plants (Athar et al. 2008, 2009; Khan et al. 2013; Alhasnawi et al. 2015; Agada 2016). Ascorbic acid is a strong antioxidant, and it involves in cell wall expansion, cell division, stimulate total leaf area, promote photosynthetic pigments and improve the tolerance of plants against multifarious stresses by scavenging ROS (Gallie 2013; Hameed et al. 2015; Dey et al. 2016). Salicylic acid has been involved in regulation of important physiological processes such as nitrogen metabolism, photosynthesis and osmolyte regulation (Khan et al. 2014). It has been reported that exogenous application of salicylic acid reduces the effects stress toxicity (Palma et al. 2013; Khan et al. 2015). Brassinolide has strong antioxidant activities to scavenge the ROS. The exogenous application of brassinolide is a very effective to minimize the effects of stresses. Numerous reports are available in the literature that brassinolide is an important plant hormone and antioxidants that reduce the effects of stresses in plants (Ashraf et al. 2010; Fariduddin et al. 2014; Gill et al. 2017). It has been reported in the literature that Tocochromanols are also the most effective and beneficial group of lipophilic phenolic antioxidants (Housam et al. 2014). EA is a natural polyphenolic antioxidant found in various vegetables and fruits (Malini et al. 2011). The pretreatment of seeds with EA enhanced the germination rate and fresh weight of the seedling (El-Soud et al. 2013). Here, we show that EA is one of the best antioxidants to protect the investigated plants against salinity stress. Due to its antioxidant properties, EA can enhance the growth and yield of the crop.

We thus conclude that exogenous application of EA reduced the effect of salinity stress and enhanced the growth of the canola plants.

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AK contributed to experimental design and planning. SN conducted the experiments. IL participated in preparing the manuscript and idea sharing collaboration. HN conducted the data analysis. MAH prepared discussion of results and was involved in the preparation of the manuscript.

Disclosure statement

No potential conflict of interest was reported by the authors.

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