

SALINITY STRESS TOLERANCE OF CAMELINA INVESTIGATED *IN VITRO*

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The ability of *Camelina sativa* to withstand salinity stress *in vitro* by adding NaCl (0, 25, 50, 75, 100, 125, 150, 175, 200mM) in Murashige and Skoog basal medium was studied. Performance of the plants was measured in terms of various growth parameters and physiological and biochemical tests performed on fully grown plants. The germination capacity, cotyledon unfolding and first true leaf emergence was reduced by 30.6, 17.3, and 28.8%, respectively in 200mM salt treatment with respect to control. The plant height, relative water content, and plant water content were decreased by 85.4, 10.8, and 9.8%, respectively, in stressed plants with respect to control. A decrease in chlorophyll *a* and *b* and total chlorophyll contents (by 81.3%), as well as of protein content was registered. Electrical conductivity increased by 52.8% in stressed plants over control, as expected. Other stress indicators like guaiacol peroxidase activity and malondialdehyde also increased with respect to control. At salt concentrations lower than 200mM, no clear cut retardation effects were seen. Thus, the present study opens up the scope of further assessment of survivability of camelina in salt contaminated soils.

growth; pigments; proteins; lipid peroxidation



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INTRODUCTION

Biotic and abiotic stresses affect crop yield and productivity all over the world. Among the abiotic stresses, salinity is one of the major factor impairing agricultural productivity worldwide (E p s t e i n et al., 1980). It causes the disruption of the homeostatic balance of water potential and ion distribution in plants resulting in decreased availability of water to root cells and the plants tend to accumulate high concentrations of Na⁺ and Cl⁻ in their vacuoles to protect their cytoplasmic water potential and metabolic imbalances. These metabolic imbalances cause oxidative stress (Z h u, 2002) and increased production of reactive oxygen species (ROS) – hydrogen peroxide (H₂O₂), hydroxyl radical (OH), and superoxide ions (S i m a e i et al., 2011). Scavenging of ROS in plant cells occurs by an endogenous protective mechanism involving antioxidant molecules and enzymes (J a l e e l et al., 2008; T u r k a n, D e m i r a l, 2009) like superoxide dismutase (SOD), glutathione reductase (GR), and peroxidases (ascorbate peroxidase (APX); guaiacol peroxidase (GPX)).

Many physiological processes of plants like seed germination, seedling growth, flowering, and fruit

set are adversely affected by high salt concentrations (A h m a d et al., 2002; A l i et al., 2004). In particular, salinity delays germination, reduces the shoot growth as expressed by reduced leaf area, and affects many physiological processes like electrical conductivity (EC) and relative water content (RWC). It also reduces photosynthetic activity by destruction of green pigments, lowering leaf area or by decreasing the activity of photosynthetic enzymes. Further, salinity affects the cell membranes and causes lipid peroxidation leading to higher accumulation of malondialdehyde (MDA) (L o k h a n d e et al., 2011).

Camelina (also popularly called Siberian mustard or false flax), belonging to the family Brassicaceae, is a native to Mediterranean region and grows in relatively colder climates. It has a unique oil profile, rich in omega-3 fatty acids, and seeds also accumulate Vitamin E and various antioxidants (Z u b r, 1997). Obviously, camelina seeds are important feedstocks both for cosmetics and biofuel industries. Despite its economic importance, there have hardly been any reports on the effect of salinity on camelina.

Limited studies on other members of family Brassicaceae suggest retardation in growth and impact on other physiological parameters. For example,

parameters like germination percentage, root/shoot ratio and dry matter content in *Brassica juncea* and *Brassica napus* were found severely affected upon exposure to salt stress (Qasim et al., 2004; Sharma et al., 2013).

The crop has been only recently introduced in India (Agarwal et al., 2010) and there is very little information on its ability to withstand different biotic and abiotic stress factors prevailing in India. It is a short duration crop and in most parts of India it grows only in a very short bracket of time, i.e. in winter months, when the temperature conditions are ideal for this crop. Thus, *in vitro* cultures provide an ideal alternative to characterize the crop at molecular and biochemical level and study its response to different climatic conditions. In the present study we have exposed camelina plants growing *in vitro* to different concentrations of sodium chloride (NaCl): non saline (0, 25, 50, and 75mM, corresponding to 0, 0.91, 1.82, and 2.73 g/l), slightly saline (100, 125, 150mM, corresponding to 3.65, 4.55, and 5.46 g/l), and medium saline (175, 200, and 300mM corresponding to 6.37, 7.30, and 10.95 g/l) as per Brouwer et al. (1985). The experiment was conducted *in vitro* with plants being grown on Murashige and Skoog (MS) medium. Although MS medium is composed of a cocktail of salts, it does not contain NaCl as its constituent. Thus, the control experiment was carried out without NaCl (0mM NaCl set), under the optimum conditions. Any additional NaCl provided was thus considered as a sub-optimal salinity environment. We assessed the impact of these concentrations on growth, physiological and biochemical parameters of *in vitro* grown *Camelina sativa* (cv. Calena) plants.

MATERIAL AND METHODS

Camelina sativa cv. Calena (EC-643910) was used in the present study. The plants were germinated under saline conditions, as described, and subsequent biochemical analyses were performed on one month old plants. Subsequently, the plants were allowed to complete their life cycle.

The chemicals used were of analytical grade procured from Sigma Aldrich, St. Louis, USA.

Seed sterilization and *in vitro* culture conditions

Camelina seeds were washed with distilled water under aseptic conditions in laminar air flow hood. To sterilize them, seeds were first treated with 0.1% HgCl₂ (mercuric chloride) for 5 min followed by a 30 s treatment with 70% ethanol and 3–4 washes with sterile distilled water. Seeds were blot dried and cultured for germination in MS media (Murashige, Skoog, 1962) containing different concentrations (0, 25, 50, 75, 100, 125, 150, 175, 200, 300mM) of NaCl. All the cultures were maintained at 24 ± 2°C

under a 16 h light and 8 h dark cycle (cool white fluorescent light, 30 μmol m⁻² s⁻¹) and 50–60% relative humidity (RH) in culture room.

Seed germination, growth, and moisture content

Data on the *in vitro* seed germination was recorded daily in terms of radicle emergence, cotyledon unfolding, and true leaf emergence. Each treatment had ten replicates of 20 seeds. The rate of germination was estimated in terms of Timson's index of germination velocity (IGV) as:

$$IGV = \Sigma (G/t) \quad (1)$$

where:

G = germination percentage

t = total period of germination (days)

Germination percentage and vigour was recorded for each treatment with 10 replications per treatment of 20 seeds each.

The plants growing under different treatments were uprooted carefully and washed for recording fresh and dry weight of the whole plant. The samples were oven dried at 94°C for 24 h and dry weight was recorded to compute plant water content:

$$\text{Plant water content} = (\text{fresh weight} - \text{dry weight}/\text{fresh weight}) \times 100 \quad (2)$$

Estimation of electrical conductivity and relative water content

Leaf discs of 1 cm² were washed with distilled water before placing them in a test tube containing 10 ml distilled water and incubated at 25°C on shaker (100 rpm) for 24 h. At the end of incubation the electrolytic conductivity I (EC I) of bathing solution was recorded by electrical conductivity meter WTW 315I/SET (WTW GmbH, Weilheim, Germany). The samples were autoclaved at 121°C for 20 min to completely decompose the tissues and release electrolytes. EC II was recorded after cooling the solution to room temperature (RT). The ionic concentration in the sap was determined by measuring the electrical conductivity using the formula:

$$EC (\%) = (EC I/EC II) \times 100 \quad (3)$$

Relative water content (RWC) was determined by recording the fresh weight (FW) of leaf first, and then turgid weight (TW) was taken by sinking the leaf in water for 24 h. Dry weight (DW) was determined after drying the leaves for 24 h in a hot air oven at 90°C (Barrs, Weatherly, 1962). The RWC was calculated as:

$$RWC (\%) = [(FW-DW)/(TW-DW)] \times 100 \quad (4)$$

Estimation of malondialdehyde level

The level of lipid peroxidation in plant tissues was measured by the determination of malondialdehyde (MDA). MDA content was determined with the thiobarbituric acid (TBA) reaction. A tissue sample was homogenized in 2 ml 10% trichloroacetic acid (TCA) and 0.25% TBA solution and centrifuged at 15 000 rpm for 15 min. The supernatant was heated at 95°C for 30 min and cooled immediately on ice bath. The non-specific absorbance of the supernatant at 600 nm was subtracted from the maximum absorbance at 532 nm for MDA measurement. The level of lipid peroxidation was expressed as mM/gm fresh weight of MDA formed using an extinction coefficient of $155 \text{ mM}^{-1} \text{ cm}^{-1}$ (Heath, Packer, 1968).

$$\text{Total MDA (mM/ml)} = (\text{absorbance at 532} - \text{absorbance at 600} \times \text{extinction coefficient} \times \text{path length}) \times \text{dilution factor} \quad (5)$$

Estimation of chlorophyll pigments and chlorophyll stability index

Chlorophyll (*a*, *b*, and total) concentrations were determined from leaf material ground in a pre-chilled mortar in 2 ml acetone (80% v/v). The homogenate was centrifuged at 3000 *g* for 10 min. After complete extraction, the mixture was filtered. The absorbance of the extract was measured at 663 and 645 nm using a spectrophotometer (Arnold, 1949).

$$\text{Chlorophyll } a = 12.25 A_{663} - 2.79 A_{645} \quad (6)$$

$$\text{Chlorophyll } b = 21.50 A_{645} - 5.10 A_{663} \quad (7)$$

$$\text{Total chlorophyll} = 7.15 A_{663} + 18.71 A_{645} \quad (8)$$

The chlorophyll stability indices (CSI) were measured (Kloeyer, 1958) using the formula

$$\text{CSI} = (\text{total chlorophyll content in stressed leaves} / \text{total chlorophyll content in control leaves}) \times 100 \quad (9)$$

Estimation of guaiacol peroxidase activity

Guaiacol peroxidase activity (GPX) was determined by monitoring the increase in absorbance at 470 nm as guaiacol was oxidized. The assay mixture contained 50mM phosphate buffer (pH 7.0), 0.1mM EDTA, 10mM guaiacol, 10mM H_2O_2 , and 50 μl of enzyme extract. Absorbance of reaction solution at 470 nm was determined in 15-s intervals with a spectrophotometer (Kar, Feierabend, 1984). GPX was measured using the formula:

$$\text{GPX} = \text{final absorbance} \times \text{extinction coefficient} \times 20 / \text{leaf weight} \quad (10)$$

where:

$$\text{Final absorbance} = (\text{first absorbance recorded} - \text{last absorbance recorded}) \text{ at } 470 \text{ nm} \quad (11)$$

Protein content

Protein content was determined by Bradford method using bovine serum albumin (BSA) as standard (Bradford, 1976).

Statistical analysis

The program Crop Stat (7.2.2007.2 module) for MS Windows (Biometrics unit, IRRI, Philippines) was used for the analysis of variance (ANOVA). The treatment means were compared using the Least Significant Difference test (LSD) at a significance level of $P \leq 0.05$.

RESULTS

Reduced growth and altered physiology under salt stress

Plant growth and vigour decreased proportionally to the increasing salt concentration. Though, the germination was 100% under all the conditions except at 300mM, but the rate of germination differed, as measured by Timson's index. Evidently, plants tolerated the turgor pressures induced by up to 200mM NaCl, but not 300mM, when 100% mortality was observed. Thus, the rest of the text below describes and discusses the results for salt concentrations up to 200mM. Expectedly, maximum germination capacity, cotyledon unfolding and true leaf were recorded in control (93.7, 78.8, and 78.5%, respectively), whereas minimum was recorded at a 200mM concentration (65.00, 65.1, and 55.9%, respectively). On the basis of ANOVA, there was a significant difference in different treatments at $P < 0.05$ significance level (Fig. 1A–C).

Height of the seedlings decreased with increasing concentrations (up to 200mM). Plant height increased significantly ($P < 0.05$) till day 28 in all the treatments except in the highest two concentrations (175 and 200mM). Among the latter, there was no significant difference in height between days 14–21 of treatment (Table 1). There was also a decline in fresh and dry weights of the plants. The maximum fresh (0.24 mg) and dry (0.016 mg) weight was achieved in control whereas minimum fresh (0.05 mg) and dry (0.0083 mg) weight was achieved in 200mM salt treatment. Increasing NaCl concentration in the medium caused a significant ($P < 0.05$) reduction in fresh weight of shoots as well as in dry weight (Table 2).

The RWC in leaves was significantly ($P < 0.05$) lower in 200mM salt treatment (80.3%) as compared to the control (90.1%). This co-related with a higher

Table 1. Effect of NaCl stress on plant height in *Camelina sativa* (in days 7–28 of treatment)

Treatment with NaCl (mM)	After 7 days	After 14 days	After 21 days	After 28 days
0 (control)	0.96 ^g	1.50 ^b	3.67 ^d	6.22 ^g
25	0.81 ^f	1.31 ^a	3.23 ^c	5.57 ^f
50	0.73 ^e	1.30 ^a	2.12 ^b	4.40 ^e
75	0.50 ^d	1.27 ^a	1.67 ^a	3.14 ^d
100	0.40 ^c	1.22 ^a	1.49 ^a	2.66 ^c
125	0.40 ^c	1.09 ^a	1.40 ^a	1.75 ^b
150	0.30 ^b	0.98 ^a	1.20 ^a	1.69 ^b
175	0.15 ^a	0.80 ^a	1.14 ^a	1.15 ^a
200	0.14 ^a	0.83 ^a	0.86 ^a	0.90 ^a
SE	0.02	0.04	0.10	0.14
LSD ($P \leq 0.05$)	0.06	0.15	0.29	0.40

SE = standard error, LSD = Least Squares Difference test; ^{a–g}values indicated by the same superscript are not significantly different at $P \leq 0.05$

Table 2. Effect of NaCl stress on fresh weight, dry weight biomass and plant water content in *Camelina sativa* (1 month old)

Treatment with NaCl (mM)	Fresh weight (mg)	Dry weight (mg)	Plant water content (%)
0 (control)	0.24 ⁱ	0.016 ^a	93.4 ^b
25	0.21 ^h	0.015 ^a	92.9 ^b
50	0.19 ^g	0.014 ^a	92.4 ^b
75	0.17 ^f	0.013 ^a	92.0 ^b
100	0.14 ^e	0.012 ^a	91.4 ^b
125	0.11 ^d	0.011 ^a	90.8 ^b
150	0.08 ^c	0.010 ^a	88.1 ^a
175	0.07 ^b	0.0087 ^a	86.8 ^a
200	0.05 ^a	0.0083 ^a	84.2 ^a
SE	0.0021	0.00071	0.8838
LSD ($P \leq 0.05$)	0.00628	0.00215	2.6

SE = standard error, LSD = Least Squares Difference test; ^{a–h}values indicated by the same superscript are not significantly different at $P \leq 0.05$

EC of the leaf sap (by 52.8%) found in 200mM NaCl treatment compared to the control (Fig. 2).

Alterations in membrane functions

Statistically significantly ($P < 0.05$) higher MDA accumulation was observed at salt concentrations of 150–200mM (Fig. 3A).

Changes in antioxidant enzyme activities

Guaiacol peroxidase showed a gradual increase in response to NaCl stress. GPX activity was increased by 68.88% in 200mM treatment with respect to control (Fig. 3B). Notably, the protein content was found to decrease significantly ($P < 0.05$) with the increasing concentration of salt (Fig. 3C). In control, the protein content was found to be the highest, while in the 200mM concentration it was reduced by 28.45% with respect to control.

Reduced content of photosynthetic pigments

Photosynthetic pigments in leaves of the plant were reduced with increasing salinity in the media, and this in itself is a stress symptom. The increase of NaCl in the medium was accompanied by a gradual decrease in chlorophyll *a*, chlorophyll *b*, and total chlorophyll content by 84.74, 69.1, and 81.29%, respectively, compared to the control (Table 3). The salt stress treatments resulted also in a significant ($P < 0.05$) reduction in chlorophyll stability index (by 81.3%) in 200mM salt treatment with respect to control.

DISCUSSION

Salinity resulting from inherent higher salt concentrations in soil or irrigation water represents one of major environmental stresses. It limits crop pro-

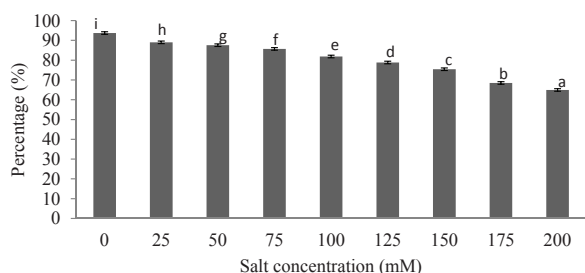


Fig. 1A. Effect of NaCl stress in *Camelina sativa* (1 month old) on the rate of germination
values indicated by the same letter are not significantly different at $P \leq 0.05$

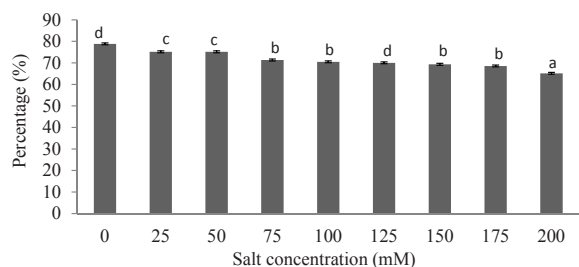


Fig. 1B. Effect of NaCl stress in *Camelina sativa* (1 month old) on cotyledon unfolding
values indicated by the same letter are not significantly different at $P \leq 0.05$

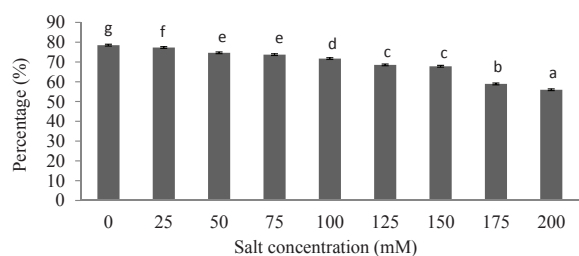


Fig. 1C. Effect of NaCl stress in *Camelina sativa* (1 month old) on true leaf emergence
values indicated by the same letter are not significantly different at $P \leq 0.05$

ductivity in a multifold manner, causing disruption of membranes, nutrient imbalances, changes in the levels of growth regulators, enzyme inhibitions, and various metabolic dysfunctions (salinity may lead to disruption of photosynthesis, cellular homeostasis, changes in water relations and ionic balance, and generation of

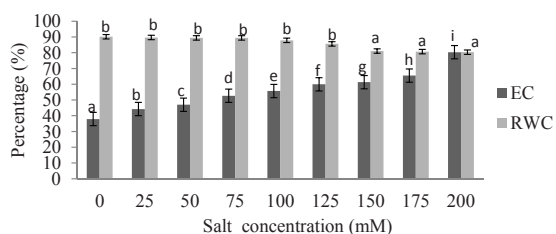


Fig. 2. Effect of NaCl stress in *Camelina sativa* (1 month old) on electrical conductivity and relative water content
values indicated by the same letter are not significantly different at $P \leq 0.05$

ROS). Summarily, the wholesome changes from the level of cellular biochemistry to plant physiology and growth may often be lethal. Nevertheless, the levels of turgor pressure induced by salinity, the plant in question and the stage of its growth at the time of exposure to salinity are few factors that may affect the levels of mortality and retardation in growth of plants (H a s a n u z z a m a n et al. 2013).

The mechanism of inhibition of germination and seedling growth by NaCl may be related to radicle emergence due to insufficient water absorption, or may be ascribed to toxic effects on the embryo (A z z a et al., 2007). We have found that in camelina, salinity postponed the germination, but did not inhibit it, which is a long standing view on germination under saline condition (A y e r s , W e s t c o t , 1985). Similar observations have earlier been made on cotton (A s h r a f , A h m a d , 2000), lentil (M i s r a , S a x e n a , 2009), and *Salvinia natans* (J a m p e e t o n g , B r i x , 2009). In alfalfa plants, U h v i t s (1964) observed that seeds emerged slowly and at higher concentrations could not germinate. The effects of salinity are clearly manifested during the further growth of the plants as well. In the present experiment, this impact was visible in terms of the reduced height and weight of the plants. Evidently in R a f i q et al. (2008), fresh and dry weights of local mung bean calli decreased as the concentration of NaCl increased in the medium. Reduction in growth attributes has also been observed in other crops like wheat (K a u s a r , S h a h b a z , 2013), rice (S h a h b a z , Z i a , 2011), eggplant (S h a h e e n et al., 2012), and mung bean (H a l e e m , M o h a m m e d , 2007).

Salt stress is the major factor that enhances the peroxidation of macromolecules through oxidative damage of membrane components. Conceptually, at higher concentrations of salt, accumulation of ions occurs and these ions interfere the membrane functions and affect the internal solute balance and nutrient absorption, causing deficiency of nutrients (G r a t t a n , G r i e v e , 1998). The rise in MDA content under higher NaCl concentrations, as seen in the present experiment, may be due to the lack of salt dependent up regulation of its antioxidant enzyme system under salinity stress. Similar rise in MDA level was also found in *Catharanthus roseus* (G a r g , 2010), *Withania somnifera* Dunal (S a b i r et al., 2012), and buckwheat (A g h a l e h et al., 2009). Salinity causes the removal of potassium ions from plant roots, which causes physiological imbalance because potassium is necessary for protein synthesis. Potassium loss causes diminished plant growth and development. Osmotic stress causes a significant reduction (by 9.85%) in water uptake resulting in low water content in plant indicating that the plant was under stress. Such a stress is induced after initial diminution or a complete halt in plant growth, which causes reduced osmotic potential inhibiting the absorption of water and nutrients by roots.

Table 3. Effect of NaCl stress on chlorophyll content (Chlorophyll a, Chlorophyll b, and total Chlorophyll) in *Camelina sativa* (1 month old)

Treatment with NaCl (mM)	Chlorophyll a	Chlorophyll b	Total chlorophyll
0 (control)	245.16 ^e	88.66 ^e	333.83 ^g
25	208.44 ^d	84.41 ^e	292.85 ^f
50	133.93 ^c	82.82 ^e	216.75 ^e
75	87.84 ^b	76.57 ^d	164.41 ^d
100	82.78 ^b	73.1 ^d	155.87 ^c
125	80.20 ^b	46.41 ^c	126.61 ^c
150	68.44 ^b	34.57 ^b	103.01 ^b
175	48.92 ^a	23.19 ^a	72.12 ^a
200	37.40 ^a	25.05 ^a	62.46 ^a
SE	5.78	1.92	6.08
LSD ($P \leq 0.05$)	17.34	5.76	18.23

SE = standard error, LSD = Least Squares Difference test; ^{a-g}values indicated by the same superscript are not significantly different at $P \leq 0.05$

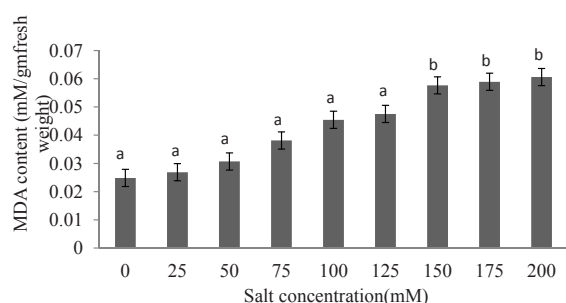


Fig. 3A. Effect of NaCl stress on malondialdehyde content in *Camelina sativa* (1 month old) values indicated by the same letter are not significantly different at $P \leq 0.05$

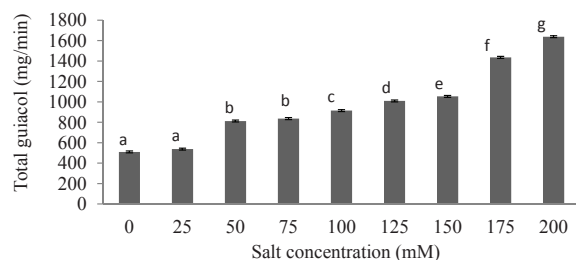


Fig. 3B. Effect of NaCl stress on guaiacol peroxidase activity in *Camelina sativa* (1 month old) values indicated by the same letter are not significantly different at $P \leq 0.05$

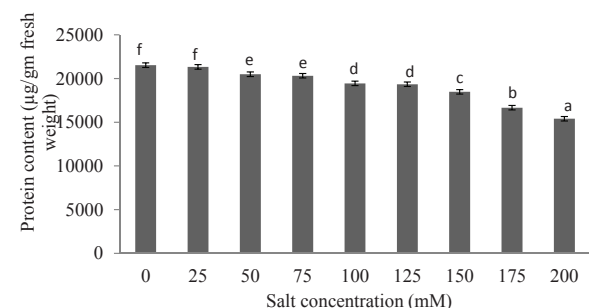


Fig. 3C. Effect of NaCl stress on protein content in *Camelina sativa* (1 month old) values indicated by the same letter are not significantly different at $P \leq 0.05$

ROS increase with the increment in salt stress (Ashraf, Harris, 2004) and lead to overproduction of O_2 and H_2O_2 by the impairment of cellular electron transport within different subcellular compartments. Antioxidative enzymes play a central protective role in the detoxification of O_2 and H_2O_2 by a scavenging process. Accordingly, a significant increase of peroxidase activity was found in response to the increasing concentration of NaCl in this study. Similarly, *Withania somnifera* Dunal (Sabir et al., 2012) and *Brassica juncea* L. also showed peroxidase activity increasing with the rising concentration of NaCl. The reduction in photosynthetic rates under salt stress is due to the reduction in water potential as a result of higher concentrations of Na^+ and/or Cl^- accumulated in chloroplasts. Thus, the availability of CO_2 for carboxylation reactions is restricted, which in turn affects carbon metabolism or photophosphorylation. Further, salinity minimizes the loss of water through transpiration and affects light-harvesting and energy-conversion systems leading to alteration in chloroplast activity, and hence reduced photosynthetic rates. In other words, plants are exposed to excess light energy which is harmful to photosystem II (PSII) if not dissipated. Salt stress can predispose plants to photoinhibition and photodamage of PSII causing a progressive loss of chlorophyll content. This would help in reducing the absorption of light by leaves (Ahmad, Prasad, 2012). The decrease in the chlorophyll content under salt stress is a commonly reported phenomenon in various studies. Our study suggests that the chlorophyll content lowers with the increasing salt concentration. The findings are in agreement with those of Tort, Turkeyilmaz (2004) on barley (*Hordeum vulgare* L.), Siler et al. (2007) on *Centaurium erythraea* (L.), Turan et al. (2007) on bean plant *Phaseolus vulgaris* (L.), Taffuo et al. (2010) on *Vigna subterranean* (L.), and Garg (2010) on *Catharanthus roseus*.

CONCLUSION

Reduction of plant growth by salinity differs between species and even between varieties and cultivars due to variability of salt tolerance among domestic and wild germplasms (Bolarian et al., 1991; Ghoulam et al., 2002). The present study has demonstrated that there were considerable differences among the morphological, physiological, and antioxidative responses of *Camelina sativa* to salinity exposure. With the elucidation of physiological and biochemical responses, there was a clear demarcation of sensitivity of this plant to higher salt concentration by having more electrical conductivity, higher lipid peroxidation, rapid loss of chlorophyll, increased guaiacol peroxidase activity, and reduced biomass. The growth reduction under NaCl stress could be due to osmotic as well as ionic pressure. Changes in growth parameters, non-enzymatic and enzymatic antioxidant activities under *in vitro* conditions can be considered as factors of adaptive value which led to differential responses in plants especially with respect to growth, osmotic adjustment, and antioxidant enzyme activities against salt stress.

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REFERENCES

- Agarwal A, Pant T, Ahmed Z (2010): *Camelina sativa*: a new crop with bio-fuel potential introduced in India. *Current Science*, 99, 1195.
- Aghaleh M, Niknam V, Ebrahimzadeh H, Razavi K (2009): Salt stress effects on growth, pigments, proteins and lipid peroxidation in *Salicornia persica* and *S. euro-paea*. *Biologia Plantarum*, 53, 243–248.
- Ahmad P, Prasad MNV (2012): *Abiotic stress responses in plants: metabolism, productivity and sustainability*. Springer-Verlag, New York.
- Ahmad S, Khan NI, Iqbal MZ, Hussain A, Hassan M (2002): Salt tolerance of cotton (*Gossypium hirsutum* L.). *Asian Journal of Plant Sciences*, 1, 715–719.
- Ali Y, Aslam Z, Ashraf MY, Tahir GR (2004): Effect of salinity on chlorophyll concentration, leaf area, yield and yield components of rice genotypes grown under saline environment. *International Journal of Engineering Science and Technology*, 1, 221–225.
- Arnon DI (1949): Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiology*, 24, 1–15.
- Ashraf M, Ahmad S (2000): Influence of sodium chloride on ion accumulation, yield components and fibre characteristics in salt-tolerant and salt-sensitive line of cotton (*Gossypium hirsutum* L.). *Field Crops Research*, 66, 115–127.
- Ashraf M, Harris PJC (2004): Potential biochemical indicators of salinity tolerance in plants. *Plant Science*, 166, 3–16.
- Ayers R, Westcot W (1985): *Water quality for agriculture*. FAO Irrigation and Drainage Paper No. 29, FAO, Rome.
- Azza M, Fatma AM, EL-Quensi EM, Farahat MM (2007): Responses of ornamental plants and woody trees to salinity. *World Journal of Agricultural Sciences*, 3(3), 386–395.
- Barrs HD, Weatherley PE (1962): A re-examination of the relative turgidity technique for estimating water deficits in leaves. *Australian Journal of Biological Sciences*, 15, 413–428.
- Batool A, Ashraf M, Akram NA, Al-Qurainy F (2013): Salt-induced changes in growth, some key physio-biochemical attributes, activities of enzymatic and levels of non-enzymatic antioxidants in cauliflower (*Brassica oleracea* L.). *The Journal of Horticultural Science and Biotechnology*, 88, 231–241.
- Bolarian M, Fernandez F, Cruz V, Cuartero J (1991): Salinity tolerance in four wild tomato species using vegetative yield-salinity response curves. *The Journal of American Society of Horticultural Sciences*, 116, 286–290.
- Bradford MM (1976): A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. *Analytical Biochemistry*, 72, 248–254.
- Brouwer C, Goffeau A, Heibloem M (1985): *Irrigation water management: Training manual No. 1 – Introduction to irrigation*. FAO Corporate Document Repository, www.fao.org/docrep/. 13/03/2001.
- Epstein E (1980): Responses of plants to saline environments. In: DW Rains, RC Valentine, A Hollaender, eds: *Genetic Engineering of Osmoregulation*. Plenum Press, New York, 7–21.
- Garg G (2010): *In vitro* screening of *Catharanthus Roseus* L. cultivars for salt tolerance using physiological parameters. *International Journal of Environmental Science and Development*, 1, 24–30.
- Ghoulam C, Foursy A, Fares K (2002): Effects of salt stress on growth, inorganic ions and proline accumulation in relation to osmotic adjustment in five sugar beet cultivars. *Environmental and Experimental Botany*, 47, 39–50.
- Grattan S, Grieve C (1998): Salinity–mineral nutrient relations in horticultural crops. *Scientia Horticulturae*, 78, 127–157.
- Haleem A, Mohammed MA (2007): Physiological aspects of mungbean plant (*Vigna radiata* L. Wilczek) in response to salt stress and gibberellic acid treatment. *Research Journal of Agricultural and Biological Sciences*, 3, 200–213.
- Hasanuzzaman M, Nahar K, Fujita M (2013): Plant response to salt stress and role of exogenous protectants to mitigate salt-induced damages. In: Ahmad P, Azooz MM, Prasad MNV (eds): *Ecophysiology and responses of plants under salt stress*. Springer-Verlag, New York, 25–87.

- Heath RL, Packer L (1968): Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. *Archives of Biochemistry and Biophysics*, 125, 189–198.
- Jaleel CA, Manivannan P, Murali PV, Gomathinayagam M, Panneerselvam R (2008): Antioxidant potential and indole alkaloid profile variations with water deficits along different parts of two varieties of *Catharanthus roseus*. *Colloids and Surfaces B: Biointerfaces*, 62, 312–318.
- Jampeetong A, Brix H (2009): Effects of NaCl salinity on growth, morphology, photosynthesis and proline accumulation of *Salvinia natans*. *Aquatic Botany*, 91, 181–186.
- Kaloyereas SA (1958): A new method for determining drought resistance. *Plant Physiology*, 33, 232–233.
- Kar M, Feierabend J (1984): Metabolism of activated oxygen in detached wheat and rye leaves and its relevance to the initiation of senescence. *Planta*, 160, 385–393.
- Kausar F, Shahbaz M (2013): Interactive effect of foliar application of nitric oxide (NO) and salinity on wheat (*Triticum aestivum* L.). *Pakistan Journal of Botany*, 45, 7–73.
- Lokhande VH, Srivastava S, Patade VY, Dwivedi S, Tripathi RD, Nikam TD, Suprasanna P (2011): Investigation of arsenic accumulation and tolerance potential of *Sesuvium portulacastrum* (L.) L. *Chemosphere*, 82, 529–534.
- Misra N, Saxena P (2009): Effect of salicylic acid on proline metabolism in lentil grown under salinity stress. *Plant Science*, 177, 181–189.
- Murashige T, Skoog F (1962): A revised medium for rapid growth and bioassay with tobacco tissue culture. *Physiologia Plantarum*, 15, 473–497.
- Qasim M, Ashraf M, Ashraf Y, Ahmad R, Nazli S (2004): Some growth related characteristics in canola (*Brassica napus* L.) under salinity stress. *International Journal of Agriculture and Biology*, 4, 665–668.
- Rafiq M, Mali M, Khatri A, Dahot MU (2008): Callus induction and regeneration in local mungbean (*Vigna radiate* L. Wilczek) under salt stress. *Journal of Biotechnology*, 136, 147–169.
- Sabir F, Sangwan RS, Kumar R, Neelam S (2012): Salt stress-induced responses in growth and metabolism in callus cultures and differentiating *in vitro* shoots of Indian ginseng (*Withania somnifera* Dunal). *Journal of Plant Growth Regulation*, 31, 537–548.
- Shahbaz M, Zia B (2011): Does exogenous application of glycine betaine through rooting medium alter rice (*Oryza sativa* L.) mineral nutrient status under saline conditions? *Journal of Applied Botany and Food Quality*, 84, 54–60.
- Shaheen S, Naseer S, Ashraf M, Akram N (2012): Salt stress affects water relations, photosynthesis and oxidative defense mechanisms in *Solanum melongena* L. *Journal of Plant Interaction*, 8, 85–96.
- Sharma P, Sardana V, Banga SS (2013): Salt tolerance of Indian mustard (*Brassica juncea*) at germination and early seedling growth. *Environmental and Experimental Biology*, 11, 39–46.
- Siler B, Mistic D, Filipovic B, Popovic Z, Cvetic T, Mijovic A (2007): Effects of salinity on *in vitro* growth and photosynthesis of common centaury (*Centaureum erythraea* Rafn.). *Archives of Biological Sciences*, 59, 129–134.
- Simaei M, Khavarinejad A, Saadatmand S, Bernard F, Fahimi H (2011): Interactive effects of salicylic acid and nitric oxide on soybean plants under NaCl salinity. *Russian Journal of Plant Physiology*, 58, 783–790.
- Taffouo VD, Wamba OF, Yombi E, Nono GV, Akoe A (2010): Growth, yield, water status and ionic distribution response of three bambara groundnut (*Vigna subterranean* (L.) Verdc.) landraces grown under saline conditions. *International Journal of Botany*, 6, 53–58.
- Tort N, Turkyilmaz B (2004): A physiological investigation on the mechanisms of salinity tolerance in some barley culture forms. *Journal of Forest Science*, 27, 1–16.
- Turan MA, Kalkat V, Taban S (2007): Salinity-induced stomatal resistance, proline, chlorophyll and ion concentrations of bean. *International Journal of Agricultural Research*, 2, 483–488.
- Turkan I, Demiral T (2009): Recent developments in understanding salinity tolerance. *Environmental and Experimental Botany*, 67, 2–9.
- Uhvits R (1964): Effects of osmotic pressure on water absorption and germination of alfalfa seeds. *American Journal of Botany*, 33, 278–285.
- Zhu JK (2002): Salt and drought stress signal transduction in plants. *Annual Review of Plant Biology*, 53, 247–273.
- Zubr J (1997): Oil-seed crop: *Camelina sativa*. *Industrial Crop Production*, 6, 113–119.

ABBREVIATIONS: APX = ascorbate peroxidase, CSI = chlorophyll stability index, DW = dry weight, EC = electrical conductivity, FW = fresh weight, G = germination percentage, GPX = guaiacol peroxidase, H₂O₂ = hydrogen peroxide, MDA = malondialdehyde, MS = Murashige and Skoog, OH = hydroxyl radical, PSII = photosystem II, RH = relative humidity, ROS = reactive oxygen species, RWC = relative water content, SOD = superoxide dismutase, TBA = thiobarbituric acid, TCA = trichloroacetic acid, TW = turgid weight.

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