β-carotene induced alteration in morphological and physiological aspects of fennel (*Foeniculum vulgare* Mill.) under salt stress

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Abstract: Two pot experiments were performed in Old Botanical Garden, University of Agriculture, Faisalabad to explore the role of exogenous application of β-carotene as foliar spray on fennel under saline conditions. Two fennel varieties Dulce and Indigenous were tested against salinity, i.e. 0 mM NaCl (Control) and 120 mM NaCl. Three levels of β-carotene 0 (water spray), 0.25 and 0.5 mM were applied through foliar spray. Salinity was maintained after 40 days of sowing. β-carotene treatment as foliar spray was applied after three weeks of salinity development. Salinity prominently reduced the growth and yield of fennel plant by reducing shoot fresh and dry weights, shoot length, photosynthetic pigments (Chlorophyll *a* and *b*, total chlorophyll and carotenoids). Shoot and root Na^+, Ca^{2+} and K^+ along with leaf MDA and H_2O_2 prominently increased. Imposition of salinity increased the level of antioxidants (catalase, peroxidase, superoxide dismutase, flavonoids and total phenolics), osmoprotectants (proline) and total soluble proteins. Exogenous spray of β-carotene improved the fresh and dry weight of shoot, length of shoot, antioxidants, osmoprotectants and also decreasing ROS production and shoot and root Na^+. Foliar application of β-carotene alleviate the destructive effects of salt stress in fennel plant. Of various β-carotene levels, 0.5 mM concentration showed more improvement in growth and yield of fennel plant as compared to others. Fennel variety Dulce proved to be more salt tolerant and showed better growth in all parameters as compared to Indigenous variety.

***Index-terms*:** Fennel, β-carotene, salt stress, antioxidants, osmoprotectants, photosynthetic pigments

I. INTRODUCTION

Anthropoid population came to be in intimacy with their surroundings which directly affect the plant growth and their development of various plant species including medicinal plants. In all cultures, medical resource is basically medicinal plant. Due to quality, safety and medical importance, medicinal plant resolve every issue related to health in developing and advance countries. Awareness of conventional awareness and remedial herbs take part in utilization and innovating of native plant reserves (Jamshidi-Kia *et al*., 2018).

Salt stress badly affect the areas where rainfall has no profound effect on plant growth, in this case salt stress show deleterious effect on plant morphology. Some areas especially arid and semi-arid, where there is high transpirational rate, evaporation and temperature is quite high; water quality and soil factors are highly affected (Mendes *et al*., 2019). High salt stress deteriorate structure of topsoil and restrict beneficial atmospheric-hydro equilibrium necessary for organic process take place in rhizome. As a consequence of each and every deleterious result of salinity, plant productivity reduced, at the same time cultivable area actuality destroyed inevitably (Hussain *et al.*, 2016).

Salinization raised the accumulation of sodium and chloride ions and reduced the absorption of Na^+, K^+, Mg^{2+} and Ca^{2+} ions in green part of plant (Mohammadi *et al*.,2022). The development of vegetative part is reduced by decrease in the intake of water, nutrient deficiency and deposition of harmful Na^+ and Cl^- ions. Na^+ is accountable for the distribution of sedimentary clay ore in the ground. Salt stress leads to reduced osmoprotectants, accumulation of toxic ions ion and ionic imbalance. The consequences of these include death of plant and yield losses (Hassan *et al*., 2021).

Photosynthetic pigments such as carotenoid protect plants from reactive oxygen species and also from several damages caused by photooxidation (Swapnil *et al*., 2021). Carotenoids have antioxidant property in such a way that they scavenge reactive oxygen species and also harmful radicals (Alam *et al*., 2021). Fruits and vegetables contains 90% carotenoids in all evolving nations (Meléndez-Martínez *et al.*, 2020). β-Carotene occurs in nature in orange-yellow vegetables and fruits, and also in leafy green vegetables in the form of hydrogen-carbon carotenoids (Celik *et al*., 2017). β-Carotene scavenge free oxygen radical, mainly at hypoxia. The interconnection of carotenoids interact with hydroperoxy radical might be act through an unbalanced β-carotene

radical substance. Moreover, they have the ability to protect the tissues and cells by acting as scavengers of reactive oxygen species (Young and Lowe, 2018).

β -carotene plays an important role in plants by acting as stress signal. β-carotene functions as strain sign to remodel expression of genes by activating cellular guard system in plants (Shumbe *et al*., 2014). Apocarotenoid signal production occurs through carotenoid cleavage dioxygenases (CCDs) during stress conditions. Apocarotenoids are accountable for managing root colonization in endomycorrhizae (EM) (Felemban *et al*., 2019). Carotenoids are probable alarming signals that allow plants to endorse some preventive measures in stressed condition. It is hypothesized that β-carotene may play potential role in fennel under saline conditions. Therefore research was aimed to study the role of β-carotene on morphology, physiology and biochemistry of fennel developed under saline condition.

II. MATERIALS AND METHODS

2.1 Site of sample collection

The experiment was performed to study the effect of foliarly applied β-carotene on morpho-physio and biochemical parameters of fennel under salt stress. The experiment was laid out in completely randomized design (CRD) with a three-factor factorial arrangement. Fennel seeds of two varieties (Dulce var. and Indigenous var.) were taken from Ayub Agricultural Research Institute (AARI), FSD, Pakistan and subjected to two levels of salt and three levels of beta-carotene according to following treatments:

2.2: Selected plant species: Two varieties of fennel, i.e. Dulce var and Indeginous var.

2.3 Levels of salt stress: control and 120 mM NaCl stress.

2.4 Levels of β-carotene: Three β-carotene levels (Control (water spray), 0.25 and 0.5 mM) were provided through foliar spray.

2.5 Pots arrangement and seeds sowing: Plastic pots containing 6 kg of sand were used for sowing purpose. In each pot, twelve seeds were sown while six plants were maintained after two weeks of germination.

2.6 Treatments application: Salinity was maintained in successive intervals after 45 days of sowing in the form of solution. Foliar mode of application of beta-Car was applied after 15 days of salinity application. Foliar spray of β-carotene was applied along with Tween-20 as surfactant.

2.7 Data recording: Data regarding different growth parameter, chlorophyll pigments,

biochemical aspects and antioxidant profiling were recorded following 75 days of seed implant while at crop maturity, yields were recorded

2.8 Morphological parameters:

Morphological parameters i.e. fresh and dry weight of root and shoot were recorded by electric balance. Length of shoot and root were measured by measure balance and recorded in (cm).

2.9 Biochemical parameters:

2.9.1 Photosynthetic pigments:

Photosynthetic pigments were measured by (Palta, 1990) protocol. Newly harvested leaf (0.1 g) were extracted in five ml 80 % acetone and incubated over night at room temperature. Spectrophotometer (Thermo Fisher) was used to read the absorbance was at 480, 663 and 645 nm.

2.9.2 Total soluble proteins:

Bradford method (Jones *et al*.,1989) was used to measure the total soluble protein. Leaf fresh material (0.25 g) was ground in 5 ml buffer. To 5 ml of Bradford reagent, the sample extract (100 µl) was added and vortexed. The absorbance was measured at 595 nm by using Thermo Fisher spectrophotometer.

2.9.3 Enzymatic antioxidants:

A pre chilled pestle and mortar was used to ground the newly harvested leaf (0.25 g) in five ml (pH 7.8) phosphate buffer. The extract was centrifuged at 12000 rpm for fifteen minutes in a centrifuge machine; supernatant was collected and stored at -20 °C to examine activeness of antioxidants.

2.9.4 Superoxide dismutase (SOD):

In cuvette 400 µl H_2O, potassium phosphate buffer 250 µl (pH 7.0), methionine (100 µl), triton X (100 µl), nitrobluetetrazolium (NBT) 50 µl, enzyme extract (50 µl) and (50 µl) riboflavin were added and placed under the lamplight for 15 minutes to procede the superoxide dismutase reaction. Absorbance was observed at 560 nm with a spectrophotometer. The SOD activity was examined by using Giannopolitis and Ries (1977) method.

2.9.5 Catalase (CAT)

Catalase activity was examined by Chance and Maehly (1955) method. Blend consist of cooled 1.9 ml potassium phosphate buffer pH 7.0, 0.1ml of enzyme extract and 1 ml (H_2O_2). At

the wavevector of 240 nm the saturation was noted after the interval of twenty seconds for two minutes by using spectrophotometer (IRMECO U2020).

2.9.6 Peroxidase (POD):

Chance and Maehly (1955) method was used to measure the activity of peroxidase. One ml of reaction mixture was maintained by using the 750 µl phosphate buffer (pH 7.8), 0.1 ml guaiacol, 100 µl H_2O_2 and 50 µl enzyme extract. Absorbance was noted at 470 nm by using spectrophotometer with an interval of 30 s for 120 s.

2.9.7 Total Free amino acids:

One ml of extract, 1 ml of pyridine (10%) and 1 ml ninhydrin (20%) were added in the test tubes. Test tubes were placed in water bath for 30 min at 100^o C. Then made the volume of reaction mixture up to 25 ml by the addition of distilled water. The specimens were vortexed and observed the absorbance at 570 nm on spectrophotometer by using model (Thermo-Fisher). Total free amino acids were measured by Van-Slyke (1912) technique.

2.10 Reactive oxygen species

2.10.1 Hydrogen peroxide (H_2O_2):

Fresh leaf samples (0.25 g) were ground in 5 ml TCA (0.1% W/V). Sample extract (500 µl), potassium phosphate buffer (0.5 ml) and one ml of potassium iodide (1 M) were vortexed for one minute. Spectrophotometer (IRMECO U2020) was used. Absorbance of the reaction mixture was observed at 390 nm. Junglee *et al*. (2014) method was used for the determination of Hydrogen peroxide.

2.10.2 Malondialdehyde (MDA):

Malondialdehyde was determined by Heath and Packer (1968). Fresh leaf sample (0.25 g) crushed in 3 ml of TCA (1% W/V), centrifuged at 12000 rpm for 15 min. One ml supernatant was added in four ml of thiobarbituric acid (TBA) (0.5% V/V) in test tubes and incubated the reaction mixture at 95 ^{o}C for one and half hour. Absorbance was observed with the help of spectrophotometer (IRMECO U2020) at 532 and 600 nm.

2.10.3 Total free proline:

Fresh leaf sample (0.25 g) was ground in five ml of sulphosalicylic acid (3%). One ml extract, 1 ml acid ninhydrin and one ml glacial acetic acid were added. Test tubes were placed in water bath at 100 ^{o}C for an hour, vortexed and then added two ml toluene. Two layers appeared

and absorbance of upper pinkish layer was recorded at 520 nm using spectrophotometer (IRMECO U2020). Free proline was determined by Bates *et al*. (1973) method

2.10.4 Flavonoids:

Fresh leaves (0.1 g) was ground in 2 ml of (80%) acetone. Flavonoid's concentration was examined by using the protocol of Kim *et al*. (1999). Centrifuge it. Distilled water (2 ml) and 0.5 ml of supernatant were poured in test tubes. After five minutes 0.6 ml of sodium nitrite (5%) and 0.5 ml of $AlCl_3$ (10%), 2 ml of NaOH (1M) and 2.4 ml distilled water were added in the test tubes. Spectrophotometer was used to observe the absorbance at 510 nm.

2.10.5 Anthocyanin:

Fresh leaf (0.1 g) was ground in 5 ml of acidified methanol with the help of pestle mortar. Stark and Wray (1989) method was used for anthocyanin determination. Centrifuged the reaction mixture. The absorbance was observed at 535 nm by using spectrophotometer (Thermo Fisher model).

2.10.6 Total Phenolics:

Total phenolics were determined by the Noreen and Ashraf (2009) methodology. Fresh leaf samples (0.25 g) ground in 5 ml acetone (80%) and centrifuged. Take 100 µl of supernatant in the test tube, add 1 ml distilled water and 500 µl of foliar cilatus reagent, vortexed and then added 2.5 ml of 20 percent sodium carbonate ($NaCO_3$). The volume of the samples was made up to 50 ml with the help of distilled water. Spectrophotometer Thermo Fisher was used to check the absorbance at 750 nm.

2.10.7 Total soluble sugars:

Total soluble sugars were measured by Yoshida *et al*. (1976) method. Fresh leaves (0.1 g) were extracted in 5 ml of distilled water. Water bath was used to boil the sample for an hour. After filtration, made the volume of extract up to 25 ml by the addition of distilled water. Anthrone reagent (5 ml) and 1 ml filtrate extract was added in test tubes, vortexed it and heated at 95 ^oC for 20 min. then cooled at room temperature. Observed The absorbance was recorded at 620 nm on spectrophotometer.

2.11 Inorganic ions determination:

Oven dried sample of root and shoot (0.1 g) were digested in 2 ml sulphuric acid (H_2SO_4). Allen *et al*. (1986) method was used for the measuring ionic contents The mixture was heated at 250 ^oC on hot plate. Hydrogen peroxide (H_2O_2) (1 ml) drop by drop with the help of glass pipette

until unless the flask material was colorless. Cooled the solution and made up to 50 ml volumes by the addition of distilled water. After that filtered the material. Digestion extract was used to measure sodium (Na^+), calcium (Ca^{2+}) and potassium (K^+) by using Sherwood flame photometer 410.

2.12 Yield Attributes:

After harvesting, number of umbels, number of seeds per plant and 1000 seed weight measured on electric balance separately.

2.13 Statistical analysis:

The data were analyzed statistically using analysis of variance (ANOVA) under completely randomized design (CRD), using STATISTICA 8.1 (Stat Soft Inc, Tulsa, Oklahoma, USA) software using three way.

III. Results

Salt stress prominently ($P \leq 0.001$) reduced growth parameters such as shoot fresh and dry weights, root fresh and dry weights, and shoot length in fennel. β-carotene showed significant ($P \leq 0.001$) increase in shoot fresh and dry weights, root fresh and dry weights, and shoot and root length (Table 1; Fig. 1), 0.5 mM of β-carotene showed better growth under control conditions in Indeginous variety. Overall, Indeginous variety performed better growth (Table 1; Fig. 1)

Photosynthetic pigments such as chlorophyll *a*, *b*, total chlorophyll content and carotenoids decreased under saline conditions. Exogenously applied β-carotene increased chlorophyll *a*, *b*, total chlorophyll and carotenoids. β-carotene (0.5 mM) showed better growth in photosynthetic pigments. Dulce variety showed better performance when foliarly applied with β-carotene (0.5mM) under control condition. In Indeginous variety, β-carotene (0.5 mM) showed better performance in total soluble proteins under control condition (Table 1; Fig. 2) Enzymatic antioxidants such as catalase, superoxide-dismutase and peroxidase activities increased under salt stress whereas foliarly applied beta-carotene increased activities of catalase and peroxidase. β-carotene (0.5 mM) showed better growth by increasing enzymatic antioxidants under stress condition in Dulce variety and overall Dulce variety showed better growth (Table 1;Fig 3)

Malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) increased under saline conditions. β-carotene decreased MDA and H_2O_2.. β-carotene (0.5 mM) better performed in Indeginous in MDA while (0.5mM) in Dulce variety in H_2O_2 under control and stressed condition (Table 4.1,4.2;Fig 4.3,4.4). Phenolics, total soluble proteins, total free proline, total free amino acids,

flavonoids, anthocyanin increased during salt stress while foliarly applied beta carotene increased total free amino acids, total free proline, total soluble sugar and total phenolics. β-carotene (0.5 mM) showed better performance in phenolics, total free proline in Indeginous while total soluble sugars and total free amino acid in Dulce under control and stress conditions. β-carotene (0.5 mM) performed better in Anthocyanin and Flavonoids in Dulce under salt and control (Table 1,2 ;Fig. 3, 4) Ionic contents such as shoot and root Na^+ ions increased during salt stress while shoot and root Ca^{+2}, K^+ ions decreased during salt stress. β-carotene decreased the shoot and root Na^+ ions. In Dulce variety, β-carotene (0.25 mM) showed better performance by decreasing Na^+ ions under stress condition while β-carotene (0.5 mM) performance better in shoot K^+ and Ca^{2+} under non-stress conditions in Dulce. In Indeginous variety, β-carotene (0.25 mM) was most effective in decreasing root Na^+ ions under stress condition while β-carotene (0.5 mM) was effective in root Ca^{2+} under control conditions. In Dulce variety, β-carotene (0.25 mM) was better for root K^+ under control conditions (Table 2; Fig. 4, 5). Yield parameters such as number of umbels per plant and seed weight decreased during salt stress while foliar application of β-carotene increased seed weight under salt stress. β-carotene (0.5 mM) performed better in yield in Indeginous variety while β-carotene (0.25 mM) in Dulce under control conditions (Table 2, 3 ;Fig. 4, 5,6)

Table 1. Mean squares from analysis of variance of data for growth attributes, photosynthetic pigments,total soluble protein, enzymatic antioxidants and MDA of fennel (*Foeniculum vulgare* Mill.) plant when beta -Carotene was applied as foliar spray under saline condition.

SOV	Df	Shoot fresh weight	Shoot dry weight	Root fresh weight	Root dry weight
Varieties (V)	1	0.138ns	0.0111 ns	0.017ns	0.0005ns
Salinity (S)	1	651.4***	14.421***	13.031***	0.653***
Beta-Carotene (β-Car)	2	18.56***	0.1038***	0.194***	0.004ns
V×S	1	2.935**	0.065***	0.318***	0.002ns
V×β-Car	2	0.613ns	0.043***	0.0200*	0.0008ns
S× β-Car	2	0.161ns	0.066***	0.0392***	0.008*
V×S× β-Car	2	0.328ns	0.007ns	0.0049ns	0.0003ns
Error	36	0.288	0.0030	0.0049	0.001
SOV	**Df**	**Shoot length**	**Root length**	**Chlorophyll *a***	**Chlorophyll *b***
Varieties (V)	1	196.4**	0.030ns	0.0216**	0.03206**
Salinity (S)	1	6327.3***	460.04***	12.3789***	1.49465***
Beta-Carotene (β-Car)	2	166.02***	4.676***	0.0039ns	0.07154***
V×S	1	38.70ns	19.508***	0.2537***	0.00053ns
V×β-Car	2	37.29ns	6.797***	0.0187***	0.009ns
S× β-Car	2	23.80ns	0.896ns	0.4671***	0.0008ns
V×S× β-Car	2	0.32ns	0.707ns	0.0586***	0.002ns
Error	36	21.26	0.695	0.0023	0.00483

SOV	Df	Total chlorophyll	Chl *a/b*	Carotenoid	Total soluble proteins
Varieties (V)	1	0.5280***	0.137ns	0.001***	0.222***
Salinity (S)	1	12.6003***	0.433ns	0.012***	0.009ns
Beta-Carotene (β-Car)	2	0.0319ns	0.513ns	0.001***	0.067***
V×S	1	0.0253ns	0.159ns	0.004***	0.036**
V×β-Car	2	0.0390ns	0.100ns	0.0001ns	0.002ns
S× β-Car	2	0.2193**	0.398ns	0.0003*	0.023ns
V×S× β-Car	2	0.2154**	0.522ns	0.00008ns	0.009ns
Error	36	0.0383	0.368	0.0001	0.008
SOV	**df**	**Catalase**	**Superoxide dismutase**	**Peroxidase**	**Malondialdehyde**
Varieties (V)	1	0.00009ns	8042.9***	8.184ns	0.9911ns
Salinity (S)	1	0.305***	21742.9***	47.322***	70.76***
Beta-Carotene (β-Car)	2	0.013**	163.1ns	1.536ns	0.714ns
V×S	1	0.001ns	15852.2***	0.396ns	0.108ns
V×β-Car	2	0.002ns	33.0ns	9.816*	19.37**
S× β-Car	2	0.016**	25.6ns	11.341*	4.952ns
V×S× β-Car	2	0.015**	54.6ns	9.191*	13.889**
Error	36	0.002	78.2	2.753	3.106

Table 2. Mean squares from analysis of variance of data for hydrogen peroxide,anthocyanin,flavonoid, total phenolics total soluble sugar, total free amino acid, total free proline, ionic contents and yield parameters of fennel (*Foeniculum vulgare* Mill) plant when beta -Carotene was applied as foliar spray under saline condition.

SOV	Df	Hydrogenperoxide	Anthocyanin	Flavonoid	Total phenolics
Varieties (V)	1	0.0002**	0.00002ns	0.153ns	164.18***
Salinity (S)	1	0.004***	0.0006***	1.572***	220.91***
Beta-Carotene (β-Car)	2	0.00007ns	0.00001ns	0.117ns	122.50***
V×S	1	0.00001ns	0.00008**	0.366**	5.828ns
V×β-Car	2	0.00002ns	0.00002ns	0.0706ns	14.07ns
S× β-Car	2	0.000035***	0.00001ns	0.1806*	2.488ns
V×S× β-Car	2	0.00008ns	0.00002ns	0.1026ns	8.697ns
Error	36	0.00005	0.00001	0.047	5.350
SOV	**df**	**Total soluble sugars**	**Total free amino acid**	**Total free proline**	**Shoot Na^+**
Varieties (V)	1	0.424ns	0.118***	6.843***	44.08***
Salinity (S)	1	2.747ns	0.00001ns	8.984***	2730.08***
Beta-carotene (β-Car)	2	15.214***	0.0001ns	1.404**	7.11**
V×S	1	2.117ns	0.042**	0.012ns	1.08ns
V×β-Car	2	0.017ns	0.091***	0.0001ns	3.25ns

S× β-Car	2	2.237ns	0.005ns	0.535ns	6.53*
V×S× β-Car	2	1.379ns	0.022*	0.108ns	52.49***
Error	36	1.746	0.007	0.232	1.80
SOV	**df**	**Shoot K^+**	**Shoot Ca^{2+}**	**Root Na^+**	**Root K^+**
Varieties (V)	1	43.32**	1.171ns	0.047ns	27.755***
Salinity (S)	1	741.04***	29.29***	277.9 ***	563.7***
Beta-Carotene (β-Car)	2	0.550ns	0.328ns	9.536**	13.188***
V×S	1	34.003*	0.630ns	0.047ns	9.630**
V×β-Car	2	10.142ns	0.765ns	0.422ns	16.146***
S× β-Car	2	12.719ns	0.203ns	6.859**	10.146**
V×S× β-Car	2	16.519ns	0.365ns	9.891**	2.333ns
Error	36	6.244	1.0122	1.470	1.467
SOV	**df**	**Root Ca^{2+}**	**Number of umbels/plant**	**1000 seed weight**	**Seed yield/plant**
Varieties (V)	1	2.520ns	0.187ns	0.046ns	4779762***
Salinity (S)	1	15.187***	426.02***	9.991***	0.0000006***
Beta-Carotene (β-Car)	2	3.937**	3.812ns	0.140ns	209824ns
V×S	1	0.3333ns	1.688ns	0.001ns	1127215*
V×β-Car	2	0.270ns	4.188ns	0.188*	283643ns
S× β-Car	2	0.562ns	1.271ns	0.349**	29377.8ns

V×S× β-Car	2	0.395ns	0.813ns	0.004ns	234240ns
Error	36	0.684	1.799	0.049	223101

***,** and *=significant at 0.001, 0.01 and 0.05 levels, respectively; ns=non-significant; SOV=source of variance, df= degree of freedom, shoot Na^{+}=shoot sodium, shoot K^{+}=shoot potassium, shoot Ca^{+2}=shoot calcium, root Na^{+}=root sodium, root K^{+}=root potassium, root Ca^{+2}=root calcium

Table 3. Mean squares from analysis of variance of data for number of seed of fennel (*Foeniculum vulgare* Mill.) plant when beta-carotene was applied as foliar spray under saline condition.

SOV	df	Number of seed/plant
Varieties (V)	1	46613**
Salinity (S)	1	1073530***
Beta-Carotene (β-Car)	2	13504ns
V×S	1	26ns
V×β-Car	2	6374ns
S× β-Car	2	9659ns
V×S× β-Car	2	15141*
Error	36	4571

***,** and *=significant at 0.001,0.01 and 0.05 levels, respectively; ns=non-significant; SOV=source of variance, df=degree of freedom,

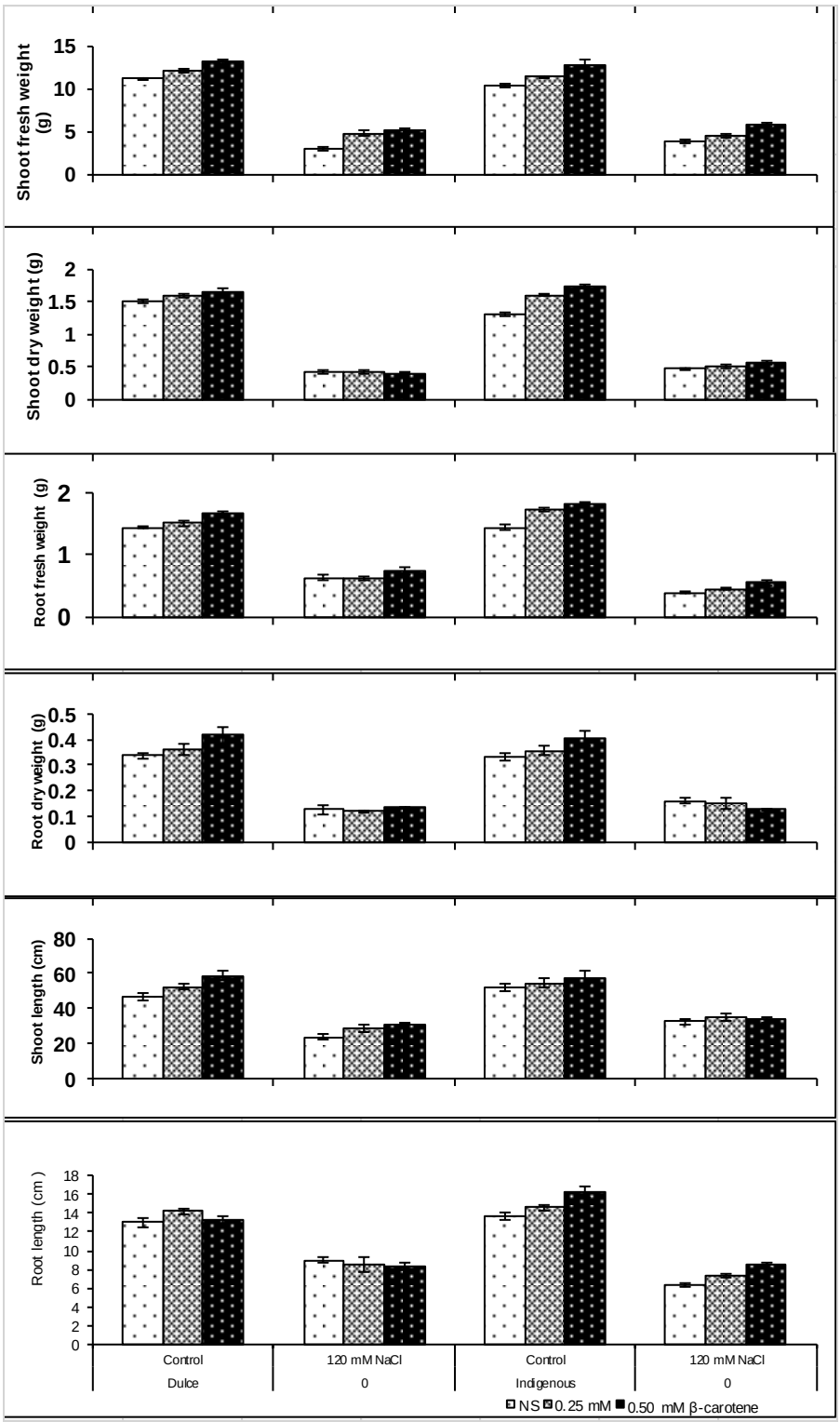
Shoot fresh weight (g)
15
10
5
0
Shoot dry weight (g)
2
1.5
1
0.5
0
Root fresh weight (g)
2
1
0
Root dry weight (g)
0.5
0.4
0.3
0.2
0.1
0
Shoot length (cm)
80
60
40
20
0
Root length (cm)
18
16
14
12
10
8
6
4
2
0
Control
120 mM NaCl
Control
120 mM NaCl
Dulce
0
Indigenous
0
NS
0.25 mM
0.50 mM β-carotene

Figure 1. Shoot fresh weight, shoot dry weight, root fresh weight, root dry weight,shoot length and root length of fennel (*Foeniculum vulgare* Mill.) plant when beta-carotene was applied as foliar spray under saline condition (NS=no spray =0 mM; 0.25 mM beta-carotene; 0.5 mM beta-carotene).

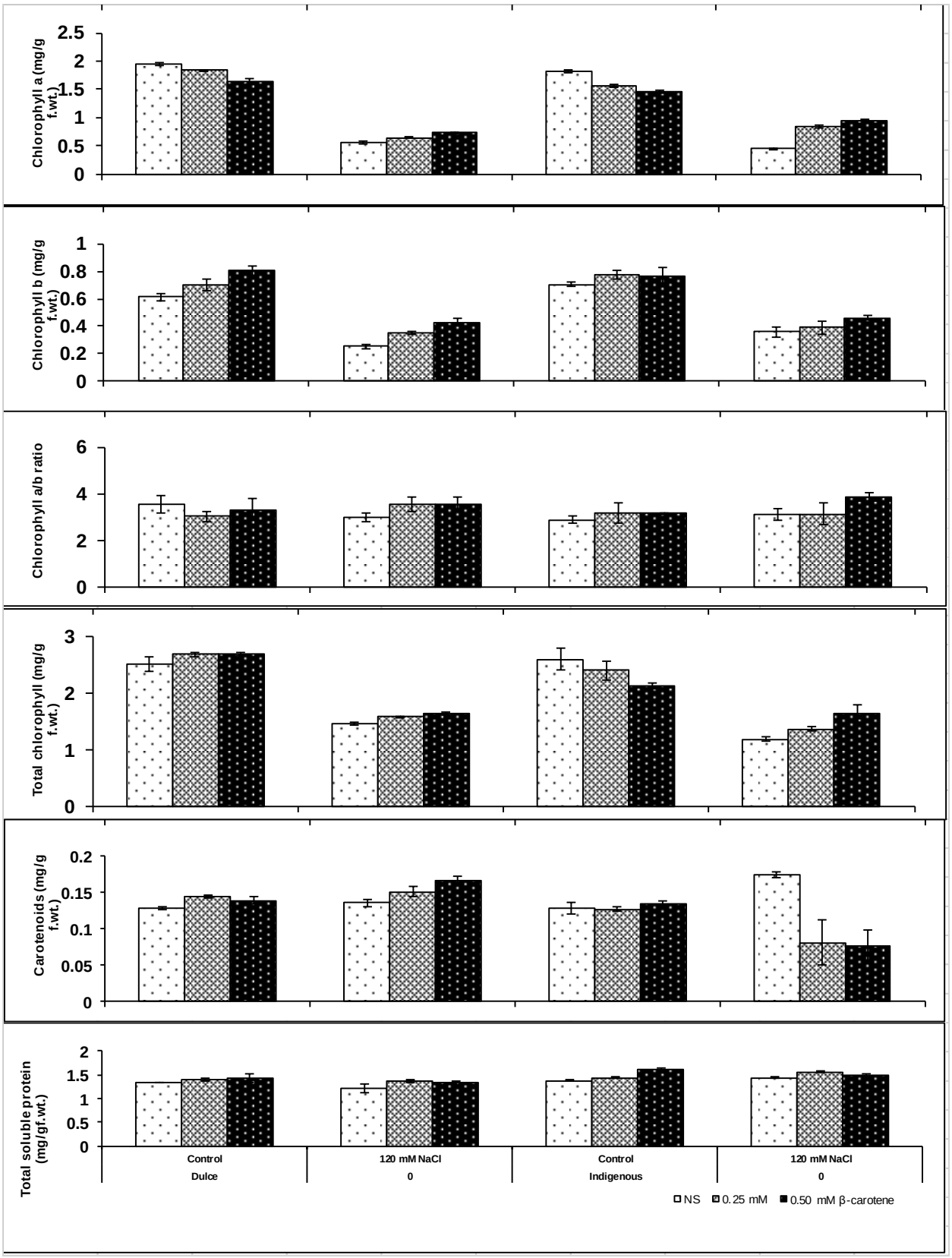

Figure 2. Chlorophyll a,chlorophyll b, chlorophyll a/b ratio, total chlorophyll, carotenoids and

total soluble proteins of fennel (*Foeniculum vulgare* Mill.) plant when beta-carotene was applied as foliar spray under saline condition.

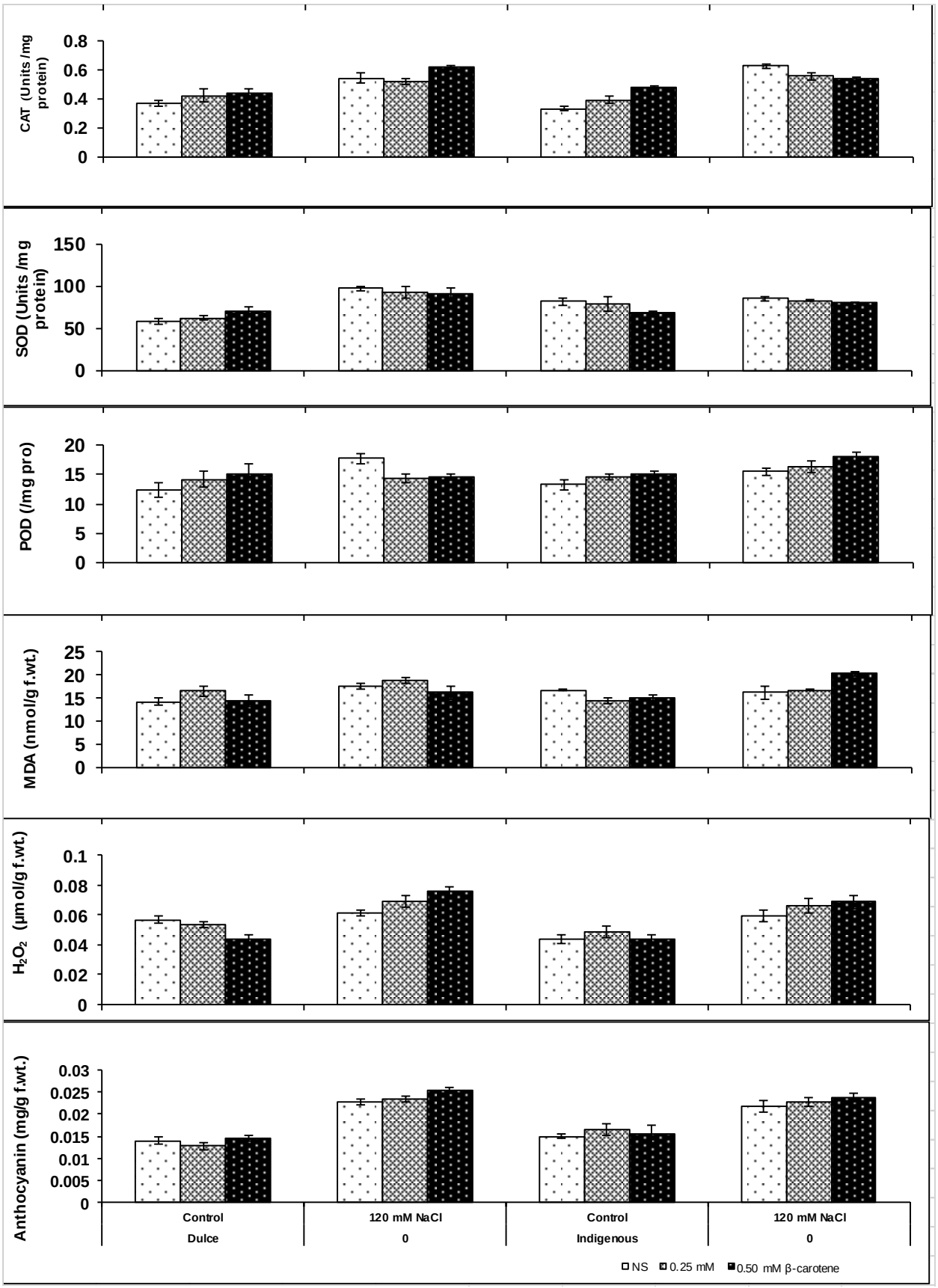
CAT (Units /mg protein)
0.8
0.6
0.4
0.2
0
SOD (Units /mg protein)
150
100
50
0
POD (/mg pro)
20
15
10
5
0
MDA (nmol/g f.wt.)
25
20
15
10
5
0
H2O2 (µmol/g f.wt.)
0.1
0.08
0.06
0.04
0.02
0
Anthocyanin (mg/g f.wt.)
0.03
0.025
0.02
0.015
0.01
0.005
0
Control
Dulce
120 mM NaCl
0
Control
Indigenous
120 mM NaCl
0
NS
0.25 mM
0.50 mM β-carotene

Figure 3 Catalase, superoxide-dismutase, peroxidase, melandialdehyde, hydrogen peroxide and anthocyanin of fennel (*Foeniculum vulgare* Mill.) plant when beta-carotene was applied as foliar spray under saline condition (NS= no-spray = 0 mM; 0.25 mM beta-carotene;0.5 mM beta-carotene)

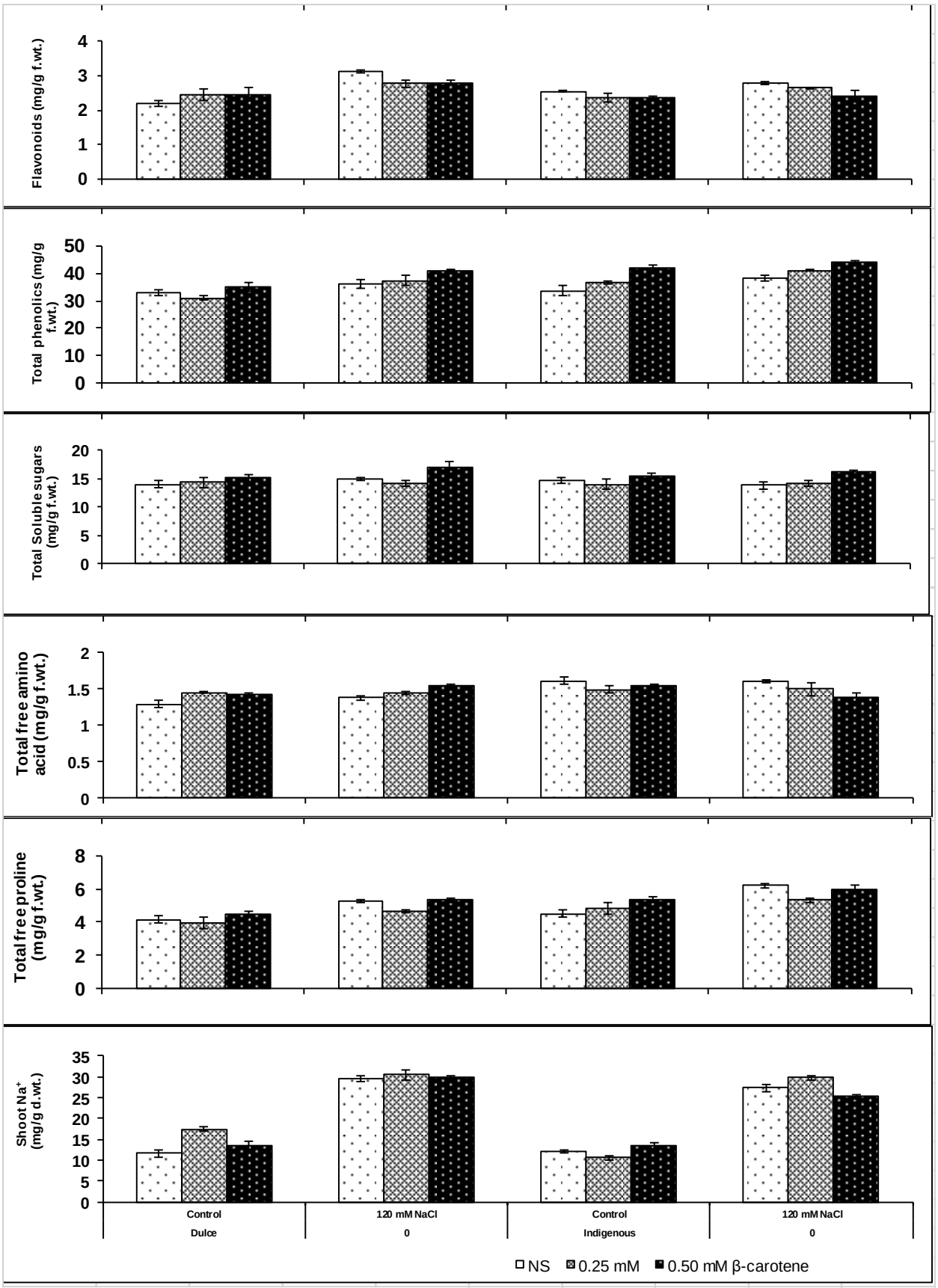
Flavonoids (mg/g f.wt.)
Total phenolics (mg/g f.wt.)
Total Soluble sugars (mg/g f.wt.)
Total free amino acid (mg/g f.wt.)
Total free proline (mg/g f.wt.)
Shoot Na+ (mg/g d.wt.)
Control
Dulce
120 mM NaCl
0
Control
Indigenous
120 mM NaCl
0
NS
0.25 mM
0.50 mM β-carotene

Figure 4. Flavonoids, total phenolics, total soluble sugars, free amino acid and shoot Na^+ of fennel (*Foeniculum vulgare* Mill.) plant when beta-carotene was applied as foliar spray under saline condition (NS=no spray=0mM; 0.25 mM beta-carotene; 0.5 mM beta-carotene).

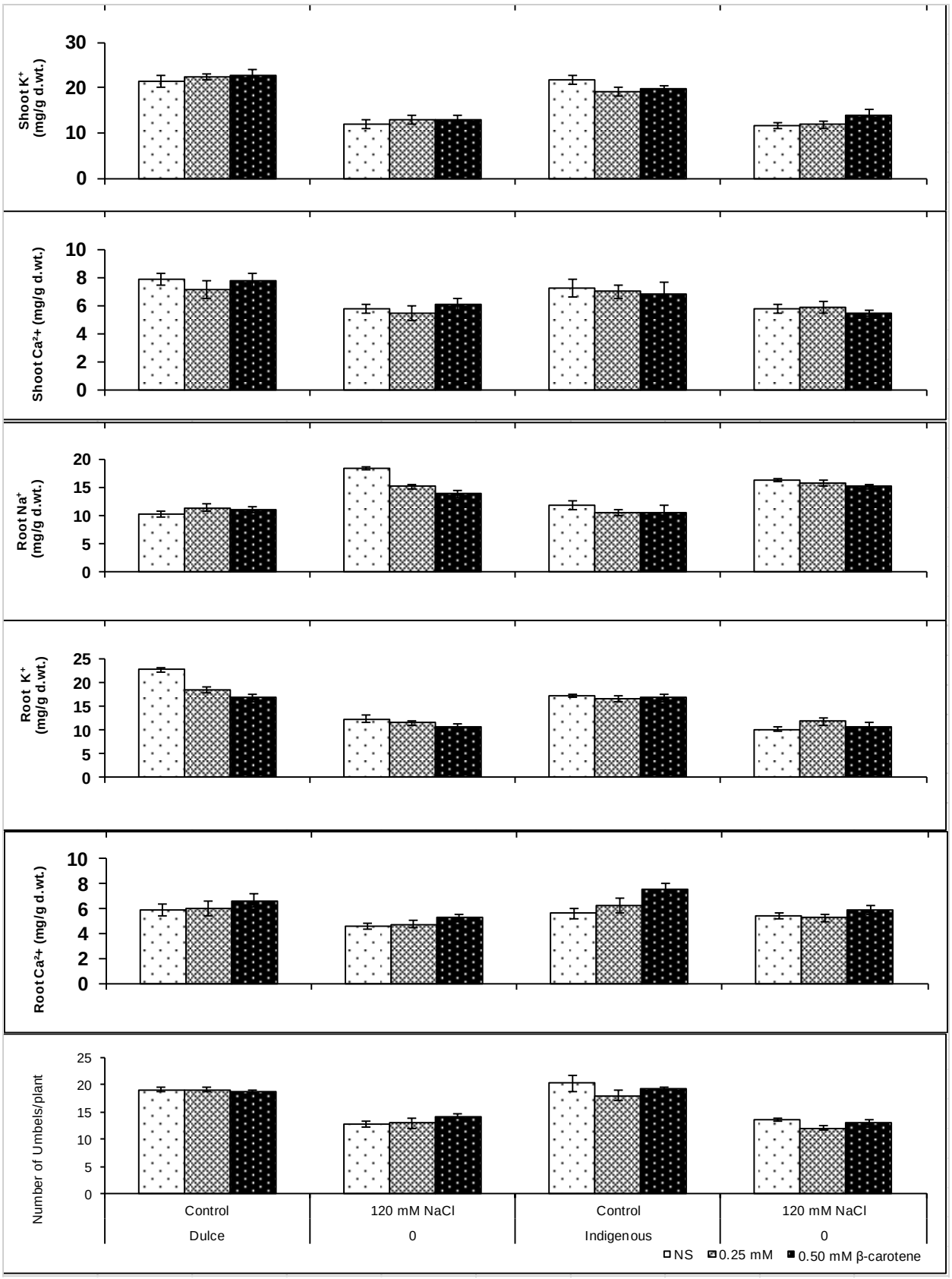
Shoot K+ (mg/g d.wt.)
30
20
10
0
Shoot Ca2+ (mg/g d.wt.)
10
8
6
4
2
0
Root Na+ (mg/g d.wt.)
20
15
10
5
0
Root K+ (mg/g d.wt.)
25
20
15
10
5
0
Root Ca2+ (mg/g d.wt.)
10
8
6
4
2
0
Number of Umbels/plant
25
20
15
10
5
0
Control
Dulce
120 mM NaCl
0
Control
Indigenous
120 mM NaCl
0
NS
0.25 mM
0.50 mM β-carotene

Figure 5. Shoot K^+, shoot Ca^{+2}, root Na, root K, root Ca and number of umbels of fennel (*Foeniculum vulgare* Mill.) plant when beta-carotene was applied as foliar spray under saline condition (NS=no spray=0 mM; 0.25mM beta-carotene; 0.5mM beta-carotene).

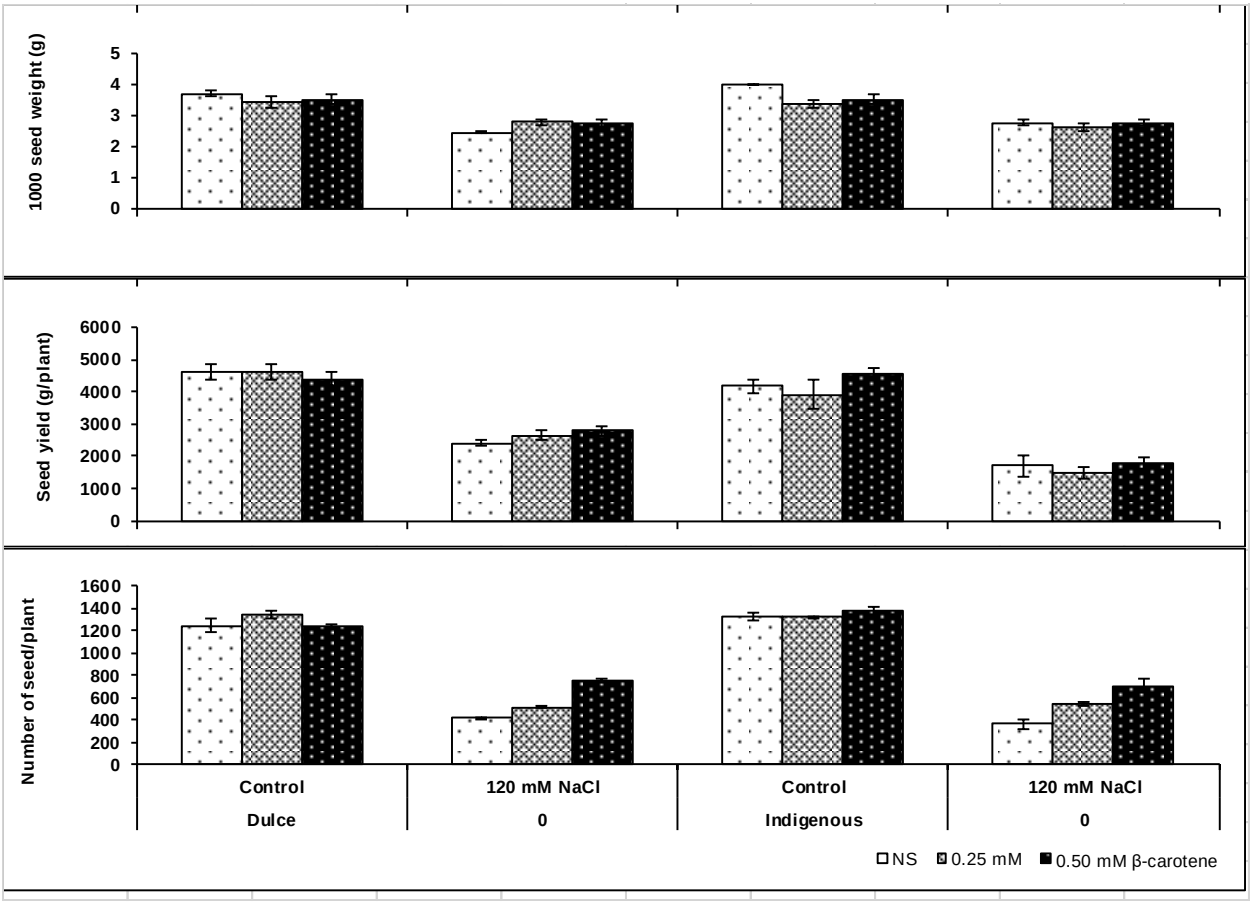

Figure 6. 1000 seed weight, seed yield and number of seed /plant of fennel (*Foeniculum vulgare* Mill.) plant when beta-carotene was applied as foliar spray under saline condition (NS=no spray=0 mM; 0.25mM beta-carotene; 0.5mM beta-carotene).

DISCUSSION

Salt stress is the major ecological aspect decreased plant cultivation and yield (Safdar *et al*., 2019). In recent study, salinity decreased the root and shoot fresh and dry matter content in fennel. Decrease in root and shoot matter content have been reported for desi chickpea (Dutta *et al*., 2018). In our study foliarly applied beta-CAR increased the weight of fresh and dry shoot, and plant height in fennel plant under saline conditions. Same outcomes have been observed in maize (EL Sabagh *et al*., 2021) golden rice (Das *et al*., 2020) and *Erwinia uredovora* (Simkin, 2021) where foliarly applied Beta-Carotene increased the growth under salt stress (Babaei *et al*., 2022).

Due to rising salinity levels i.e 0 to 6 dS/m, chlorophyll *a*, *b* and total chlorophyll in two Ocimum species reduced (Tanaka *et al*., 2018). The lack of photosynthetic pigments under saline condition due to reduced photosynthesis or reactive oxygen species production (Hernández, 2019.). Exogenously applied beta-CAR increased all photosynthetic pigments and carotenoids in fennel plants under salinity in our study. Enhanced concentrations of chlorophyll pigments under salinity by exogenous beta-CAR application were observed in maize (Satarova *et al*., 2019). In saline conditions, exogenous beta-CAR enhanced the photosynthetic capacity by lowering the sodium and chloride in *Solanum nigrum* (Ben Abdallah *et al*., 2016).

Imposition of 120 mM NaCl in our study increased the concentration of both MDA and H_2O_2 in both fennel varieties. Salinity raise the amount of hydrogenperoxide hence cause oxidative damage to phospholipid bilayer, hence disturb its impermeability or cause free radical damage in plant materials (AbdElgawad *et al*., 2016). Rising authentication subsist that membrane damage under salinity correlated with increasing synthesis of mainly harmful reactive oxygen containing species (Kapoor *et al*., 2019). Phospholipid bilayer oxidative damage defined as the amount of malondialdehyde (MDA), results in Omega-3 and Omega-6 fatty acids in the lipid membrane encounter oxidative damage due to accumulation of reactive oxygen chemicals (Gaschler and Stockwell, 2017). As lipid peroxidation is the symptom mostly ascribed to oxidative damage, it is often used as an indicator of increased damage (Rasool *et al*., 2013). In our study, exogenously applied beta-carotene lowered the contents of MDA and H_2O_2 under salinity. Same results were studied in cotton (Zafar *et al*., 2021) rice (Abdel Latef *et al*., 2019) and Canola (Bandehagh *et al*., 2021).

In our study salt application raised the SOD, CAT and POD activities in fennel. When plants were given salt application toleranting plants eventually synthesize increased concentration

of oxidation inhibitor enzymes to counteract the effect of reactive oxygen species. Superoxide dismutase provides crucial defensive system in case of reactive oxygen species and defend plants from drastic impairment produced by free radical oxide. Same results of relative activities of these enzymes were observed in wheat (Saddiq *et al*., 2021), arabidopsis (Shafi *et al*., 2015) pea (Berwal and Ram, 2018), cotton (Ibrahim *et al*., 2015), barley (Ahmed *et al*., 2013) etc. Following reactions are essential to change the hydrogenperoxide generated by superoxide dismutase to water as hydrogen peroxide is uptill now unsafe to plants and reactions including peroxide dismutase, catalase, and ascorbate peroxidase are essential (Kusvuran *et al*., 2016). Beta-CAR treatment as exogenous application improved the enzymatic antioxidants activity in fennel plants in recent study. Reduced MDA concentration and increased activeness of oxidation inhibitor with beta-CAR application was observed in wheat (Feki *et al*., 2017) and sesame (Koca *et al*., 2019). Higher peroxidase, catalase, and ascorbate peroxidase activeness correlate with superoxidase activity in accord with the unacceptable effect of free radical and hydrogen peroxide and the activity of these enzymes are greatly in accordance with resistance to oxidative damage caused by salt stress (Feki *et al*., 2017).

Beta-carotene stabilizes the ROS scavenging enzymes in oxidative stress (Guillory, 2004). Besides increase in salt concentration from 0 to 6 dS/m, proline quantity in leaves of sweet basil accumulate (Avdouli *et al*., 2021). In our study, beta-carotene improved the total proline, and soluble sugar in fennel under saline condition. These results collaborated with (Elhakem, 2020) in maize and Khan *et al*.(2017) in rice. Proline concentration frequently increased in grasses in salt stress, however proline contents are not adequate for osmolyte accumulation in grasses (Shafi *et al*., 2019). Srivastava and Kumar (2020) reported that in sugarcane further role suggest for proline apart from osmolyte accumulation in salt affected plants scavenge hydroxyl radical , stabilize membrane and also membrane proteins, as a drive for C and N for resilience, and mitigate oxidation reduction capacity in cells undergoing stressed condition (Shafi *et al*., 2019).

The sugar contents in our fennel plant was not affected during salinity stress and same results were shown in (Navarro *et al*., 2006). Boughalleb *et al*. (2017) revealed that table sugar, blood sugar and fruit sugars are the everyday solvable sugar store up in plants when given to non living environmental factors. The deposition of table sugar and blood sugar showed that their crucial role in the process of osmolyte accumulation in *Stipa lagascae* under saline conditions (Abdellaoui *et al*., 2017).

Foliarly applied beta-carotene in our study increased total soluble proteins during salinity stress. Increased quantity of total soluble proteins was observed in potato (Faried *et al*., 2016). Proteins also act as free radical scavengers that can confront salt stress causing substances at first hand and other protein groups such as regulatory enzyme in osmoprotectant confront salt stress secondarily (Kumari *et al*., 2019).

Regarding the numerous crucial solute substance contributes osmotic cathartic in plants expose to salt stress, indispensable amino acid are well known to perform a important role (Benzarti *et al*., 2014). Our results shows that free amino acids increased under saline condition in fennel when beta-carotene was applied exogenously . Same results were observed in rapeseed cultivars (El-Badri *et al*., 2021). Plants manufacture a lots of oxidation inhibitors to scavenge or detoxify reactive oxygen specie (Caverzan *et al*., 2016). Phenolics serve as oxidation inhibitors particularly defend plants in opposition to the harmful effects of rising reactive oxygen specie amount owing to salinity stress (Baskar *et al*., 2018). Phenolics are constitutive compounds in all higher plants (Naikoo *et al*., 2019). Exogenous beta-CAR application in recent study increased the total phenolics content under saline conditions in fennel. Our outcome is close to the particular observed in olive (Antonios *et al*., 2012), wheat (Rahdari and Hoseini, 2013), thyme (Bistgani, *et al*., 2019), sugarcane (Gupta *et al*., 2018) and broccoli (Kumar *et al*., 2022) etc.

The coenzyme method includes mostly carotenoid, and flavonoid. The purpose of this antioxidative system is to remove the harmful radicals generated during free radical damage and hence assist the plants to remain alive in process of such circumstances (Engwa, 2018). Ologundudu (2021) indicate a necessary function of β-carotene in the assemblage of (PS II) in the rejuvenation of injured tissue. Anyhow, foliar spray of this biomolecular compound give rise to substantial decrease in pigment molecules in salt affected fennel plants in the present inquiry, that unable to describe all in all favouring character of this compound in the living process report above. Moreover, Ashraf *et al*. (2019) describe a noticeable rise in carotenoids content in rice owing to foliar spray of β- carotene. In our study exogenous application of beta-Carotene increased the carotenoids and flavonoids in fennel plants.

Anthocyanins, a naturally occurring biochrome included in flavonoid class, present in flower, fruit, stem, root and leaves (Naing and Kim, 2018). Anthocyanin is generally present in vacuole found nearby reactive oxygen specie producing area (Naing *et al*., 2021). Following

abiotic stress, reactive oxygen specie such as hydrogen peroxide may be circulate promptly over membranes, various cellular chambers, and cell cavities (Gautam *et al*., 2017). Foliarly applied beta-carotene increased anthocyanin under salt stress. in fennel in our study. Same results were seen in sugarcane (Soares *et al*., 2019), rice (Chunthaburee *et al*., 2016).

Application of 120 mM NaCl disturbed the ionic balance in fennel in our research. Under the salt stress, crucial variation were found in shoot Na^+ and K^+ in two varieties coresponding to controls in fennel and also there was rise in sodium and reduction in K^+ and Ca^{+2} in our study. Same results were seen in maize (Gao *et al*., 2016), broad bean (Desouky *et al*., 2022), rice (Flower *et al*., 2015), canola (Kataria and Verma, 2018). Salt affected maize plants cumulate crucially reduced concentration of Na^+ and increased that of K^+, Ca^{2+} and P upon foliar spray of beta-carotene in comparison to those of salt affected plants receiving no exogenous treatment. Beta-carotene were found more helpful in ameliorate the concentration of Ca^{2+} and K^+ and decreasing Na^+ in the shoots of salt affected corn plants (Tuna *et al*., 2013). According to Akter and Oue (2018) addition of K^+ and Ca^{2+} in the greens and radicle of rice plants reduced mainly due to salinity. Anyhow, use of beta-caotene increased addition of K^+ in the radicle region of salt affected fennel plant in our study. The capability to control Na^+ movement into the shoots, and to decrease the Na^+ pile in the promptly developing shoot tissues, is particularly cardinal for maintaining higher production rates and defend the cellular activities in newly synthesizing cells from the drastic effects of Na^+ (Ishikawa and Shabala, 2019). In our study foliarly applied beta-carotene decreased the Na^+ ions under saline condition.

In our study maximum decrease in crop output was noticed at 120 mM NaCl. Such an unfavourable effect of salinity on the development and grain yield has been noticed in a number of crops, e.g., alfalfa (Bansal, 2016), carrot (Kielkowska *et al*.. 2019). In our study beta-carotene showed insignificant result on quantity of umbels and seed yield per plant. Similarly, beta carotene content expressed non significant effects on quantity of fertile tillers per plant, weight of 1000 grains and yield of grains according to plant *via* single earhead grain weight in pearl millet (Naveen *et al*., 2016).

Conclusions:

Salt stress at (120 mM NaCl) changed the physiological and biochemical parameters and caused reduction in growth and yield of fennel plants. However, β-carotene application i.e foliar spray and root growing medium application significantly increased the growth and yield in fennel plants. Salinity stress prominently increased both enzymatic and non-enzymatic antioxidants in fennel. Of different β-carotene levels (0, 0.25 and 0.5 mM), 0.5 mM prominently improved the physiological parameters under both saline and non-saline conditions. Application of β-carotene increased the endogenous level of carotenoids. Exogenously applied β-carotene lower the Na^+ ion content under saline conditions. β-carotene treated plants showed increased activities of catalase, peroxidase, superoxide dismutase, flavonoids and total phenolics in fennel plants under salt stress. Application of foliarly applied β-carotene prominently reduced H2O2 and MDA and increased in growth of fennel plants. Foliar spray of β-carotene showed increased in growth parameters under both saline and non-saline conditions. Out of two fennel varieties, Dulce variety proved to be more tolerant against salinity stress as compared to Indeginous variety.

***Conflict of interest*:**

The Authors declare that there is no conflict of interest

REFERENCES

AbdElgawad, H., Zinta, G., Hegab, M. M., Pandey, R., Asard, H., and Abuelsoud,W. 2016. High salinity induces different oxidative stress and antioxidant responses in maize seedlings organs. Frontiers in Plant Science. 7:276-280

Akter, M., and Oue, H. 2018. Effect of saline irrigation on accumulation of Na^+, K^+, Ca^{2+}, and Mg^{2+} ions in rice plants. Agriculture. 8:164-178.

Allen, S. E., Grimshaw, H. M., and Rowland, A.P. 1986. Chemical analysis. In: Methods in plant ecology, ed. by M.P.D. Chapman. Second edition. Blackwell Scientific Pub. Oxford, 285-344.

Ashraf, M.A., Riaz, M., Arif, M. S., Rasheed, R., Iqbal, M., Hussain, I., and Mubarik, M. S. 2019. The role of non-enzymatic antioxidants in improving abiotic stress tolerance in plants. In Plant tolerance to environmental stress. CRC Press. 129-144.

Avdouli, D., Max, J. F., Katsoulas, N., and Levizou, E. 2021. Basil as secondary crop in cascade hydroponics: exploring salinity tolerance limits in terms of growth, amino acid profile, and nutrient composition. Horticulturae. 7:203-220.

Babaei, M., Shabani, L., and Hashemi-Shahraki, S. 2022. Improving the effects of salt stress by β-carotene and gallic acid using increasing antioxidant activity and regulating ion uptake in (*Lepidium sativum* L.) Botanical Studies. 63:1-10.

Bandehagh, A., Dehghanian, Z., Henry, R., and Hossain, M. A. 2021. Salinity tolerance in Canola: insights from proteomic studies. In Brassica Breeding and Biotechnology. IntechOpen.

Bansal, S. 2016. Effect of salinity on biological nitrogen fixation in alfalfa (*Medicago sativa*) and its response to applied mineral nitrogen.

Baskar, V., Venkatesh, R., and Ramalingam, S. 2018. Flavonoids (antioxidants systems) in higher plants and their response to stresses. *In* : Gupta, D. K., Palma, J. M., and Corpas, F. J. Antioxidants and antioxidant enzymes in higher plants. Springer. 253-268.

Bates, L. S., Waldren, R. P., and Teare, I. D. 1973. Rapid determination of free proline for water stress studies. Plant and Soil. 39:205–207.

Benzarti, M., Rejeb, K. B., Messedi, D., Mna, A.B., Hessini, K., Ksontini, M., Abdelly, C., and Debez, A. 2014. Effect of high salinity on *Atriplex portulacoides*: Growth, leaf water relations and solute accumulation in relation with osmotic adjustment. South African Journal of Botany. 95:70-77.

Berwal, M. and C. Ram. 2018. Superoxide dismutase: A stable biochemical marker for abiotic stress tolerance in higher plants. Abiotic and biotic stress in plants 1:1-10.

Bistgania, Z. E., Hashemi, M., DaCosta, M., Craker, L., Maggic, F., and Morshedlood, M.R .2019. Effect of salinity stress on the physiological characteristics, phenolic compounds and antioxidant activity of (*Thymus vulgaris* L.) and (*Thymus daenensis*) Celak. Industrial Crops and Products. 135:311–320.

Caverzan, A., Casassola, A., and Brammer, S. P. 2016. Reactive oxygen species and antioxidant enzymes involved in plant tolerance to stress. *In*: Shanker, A.K., and C. Shanker,C Abiotic and Biotic Stress in Plants; Recent Advances and Future Perspectives. Intech Croatia 17:463-480.

Celik, S. E., Bekdeser, B., Tufan, A. N., and Apak,R. 2017. Modified radical scavenging and antioxidant activity measurement of β-carotene with β-Cyclodextrins complexation in aqueous medium. J. Anal. Sci. Technol. 33:299-305.

Chance, B., and Maehly, A.C. 1955. Assay of catalases and peroxidases.

Das, K., and Roychoudhury, A. 2014. Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. Frontiers in Environmental Science. 2:53.

Desouky, A.F., Ahmed, A.H.H., Stutzel, H., and Hanafy, M. S. 2022. Physiological and biochemical responses of two faba bean (*Vicia faba* L.) varieties grown in vitro to salt stress. J. Crop Sci. Biotech. 1:1-10.

Dutta, T., Neelapu, N. R. R., Wani, S. H., and Challa, S. 2018. Response of pulses to drought and salinity stress response. A physiological perspective:Wani, S. H., and Jain, M. 2018. Pulse improvement. Springer. 77-98.

El-Badri, A. M., Batool, M., Mohamed, I. A. A., Wang, Z., Khatab, A., Sherif, A., Ahmad, H., Khan, M. N., Hassan, H. M., Elrewainy, I. M., and Kuai, J. 2021. Antioxidative and metabolic contribution to salinity stress responses in two rapeseed cultivars during the early seedling stage. Antioxidants. 10:12-27.

Engwa, G. A. 2018. Free radicals and the role of plant phytochemicals as antioxidants against oxidative stress-related diseases. Phytochemicals: Source of antioxidants and role in disease prevention (eds) Asao,T., and Asaduzzaman, M. IntechOpen. 7:49-74.

Faried, H. N., Ayyub, C. M., Amjad, M., and Ahmed, R. 2016. Salinity impairs ionic, physiological and biochemical attributes in potato. Pakistan Journal of Agriculture Science. 53:17-25.

Feki, K., Tounsi, S., and Brini, F. 2017. Comparison of an antioxidant system in tolerant and susceptible wheat seedlings in response to salt stress. Spainish Journal of Agricultural Research. 15:805-812.

Gao, Y., Lu, Y., Wu, M., Liang, E., Li,Y., Zhang, D., Yin, Z., Ren, X., Dai,Y., Deng, D., and Chen, J. 2016. Ability to remove Na^+ and retain K^+ correlates with salt tolerance in two maize inbred lines seedlings. Frontiers in Plant Science. 7:1716-1751.

Gautam, V., Kaur, R., Kohli, S., Verma,V., Kaur, P., Singh, R., Saini, P., Arora, S., Thukral, A. K., Karpets,Y.V., and Kolupaev, Y.E. 2017. ROS compartmentalization in plant cells under abiotic stress condition. In: Khan, M. I. R., and Khan, N.A. Reactive oxygen species and antioxidant systems in plants: role and regulation under abiotic stress Springer, Singapore. 89-114.

Giannopolitis, C. N., and Ries, S. K. 1977. Superoxide dismutases: I. Occurrence in higher plants. Plant Physiology. 59:309-314.

Gupta, D. K., Palma, J. M., and Corpas, F. J. 2018. Antioxidants and antioxidant enzymes in higher plants. Berlin : Springer International Publishing.1-11

Heath, R. L., and Packer, L.1968. Photoperoxidation in isolated chloroplast. I. Kinetics and stoichiometry of fatty acid peroxidation. Archives of Biochemistry and Biophysics. 125:189–198.

Hernandez, J. A., 2019. Salinity tolerance in plants: trends and perspectives. International Journal of Molecular Science. 20:1-8.

Hussain, M. I., Lyra, D. A., Farooq, M., Nikoloudakis, N., and Khalid, N. 2016. Salt and drought stresses in safflower: a review. Agronomy for Sustainable Development. 36:1-31.

Ibrahim, W., Ahmed, I. M., Chen, X., and Wu, F. 2017. Genotype-dependent alleviation effects of exogenous GSH on salinity stress in cotton is related to improvement in chlorophyll content, photosynthetic performance, and leaf/root ultrastructure. Environmental Science and Pollution Research. 24:9417-9427.

Ishikawa, T., and Shabala, S. 2019. Control of xylem Na^+ loading and transport to the shoot in rice and barley as a determinant of differential salinity stress tolerance. Plant Physiology. 165:619-631.

Jamshidi-Kia, F., Lorigooini, Z., and Amini-Khoei, H. 2018. Medicinal plants: Past history and future perspective. Journal of Herbmed Pharmacology. 7:1-7.

Jones, C. G., Hare, J. D., and Compton, S. J. 1989. Measuring plant protein with the Bradford assay. Journal of Chemical Ecology. 15:979-992.

Junglee, S., Urban, L., Sallanon, H., and Lopez-Lauri, F. 2014. Optimized assay for hydrogen peroxide determination in plant tissue using potassium iodide. American Journal of Analytical Chemistry. 5: 730.

Kapoor, D., Singh,S., Kumar,V., Romero, R., Prasad, R., and Singh, J. 2019. Antioxidant enzymes regulation in plants in reference to reactive oxygen species (ROS) and reactive nitrogen species (RNS). Plant Gene 19:100182.

Kataria, S., and Verma, S. K. 2018. Salinity stress responses and adaptive mechanisms in major glycophytic crops: the story so far. *In*: Kumar, V., Wani, S.H., Suprasanna, P., and Tran, L.P. Salinity responses and tolerance in plants. Springer. 1-39.

Kiełkowska, A., Grzebelus, E., Lis-Krzyscin, A., and Mackowska, K. 2019. Application of the salt stress to the protoplast cultures of the carrot (*Daucus carota* L.) and evaluation of the response of regenerants to soil salinity. Plant Cell Tissue and Organ Cell Culture. 137:379-395.

Kim, J. H., Jeon, S. M., Park, Y. A., Choi, M. S., and Moon, K. D. 1999. Effects of safflower seed (*Carthamus tinctorious* L.) powder on lipid metabolism in high fat and high cholesterol-fed rats. Journal of the Korean Society of Food Science and Nutrition. 28: 625-631

Kumar, S., Sharma, S., Kumar, V., Sharma, R., Minhas, A., and Boddu, R. 2022. Cruciferous vegetables: A mine of phytonutrients for functional and nutraceutical enrichment. *In*: Hernandez-Ledesma,B., and Martinez-Villaluenga, C. Current advances for development of functional foods modulating inflammation and oxidative stress Academic Press. 401-426.

Kumari, N., Malik, K., Rani, B., Jattan, M., Avtar, R., Devi, S., and Arya, S.S. 2019. Insights in the physiological, biochemical and molecular basis of salt stress tolerance in plants. *In*: Giri, B., and Varma, A. Microorganisms in saline environments: strategies and functions. Springer. 353-374.

Kusvuran, S., Kiran, S., and Ellialtioglu, S.S. 2016. Antioxidant enzyme activities and abiotic stress tolerance relationship in vegetable crops. *In*: Shanker, A. K., and Shanker, C. (Eds.), Abiotic and Biotic Stress in Plants. Recent Advances and Future Perspectives. IntechOpen, Croatia 1:481-506.

Meléndez-Martínez, A. J., Mandic, A. I., Bantis, F., Bohm,V., Borge, G. I. A., Brncic, M., Bysted, A., Cano, M. P., Dias, M.G., Elgersma, A., and Fikselova, M. 2020. A comprehensive review on carotenoids in foods and feeds: Status quo, applications, patents, and research needs. Critical Reviews in Food Science and Nutrition. 1-51.

Mendes, W. R., Araujo, F. M. U., and Er-Raki, S. 2019. Integrating Remote Sensing Data into Fuzzy Control System for Variable Rate Irrigation Estimates. Irrigation-Water Productivity and Operation, Sustainability and Climate Change.

Naikoo, M. I., Dar, M. I., Raghib, F., Jaleel, H., Ahmad, B., Raina, A., Khan, F.A., and Naushin, F. 2019. Role and regulation of plants phenolics in abiotic stress tolerance: An overview. Plant Signaling Molecules. 157-168.

Naing, A.H., and Kim,C. K. 2021. Abiotic stress-induced anthocyanins in plants: Their role in tolerance to abiotic stresses. Plant Physiology. 172:1711-1723.

Naveen, R., Sumathi, P., and Manivannan, N. 2016. Trait association among beta carotene content and yield attributes in recombinant inbred lines of pearl millet [*Pennisetum glaucum* (L.) R. Br.]. Agricultural Science Digest 36:202-206.

Noreen, Z., and Ashraf, M. 2009. Changes in antioxidant enzymes and some key metabolites in some genetically diverse cultivars of radish (*Raphanus sativus* L.) Environmental and Experimental Botany. 67:395-402.

Ologundudu, F. 2021. Antioxidant enzymes and non-enzymatic antioxidants as defense mechanism of salinity stress in cowpea (*Vigna unguiculata* L. Walp)-Ife brown and Ife bpc. Bulletin of the National Research Centre. 45:1-6.

Palta, J. P. 1990. Leaf chlorophyll content. Remote Sensing Reviews. 5:207-213.

Rasool, S., Ahmad, A., Siddiqi, T.O., and Ahmad, P. 2013. Changes in growth, lipid peroxidation and some key antioxidant enzymes in chickpea genotypes under salt stress. Acta Physiologiae Plantarum. 35:1039-1050.

Saddiq, M.S., Iqbal, S., Hafeez, M.B., Ibrahim, A. M., Raza, A., Fatima, E. M., Baloch, H., Woodrow, P., and Ciarmiello, L. F. 2021. Effect of salinity stress on physiological changes in winter and spring wheat. Agronomy. 11:1193-1199

Safdar, H., Amin, A., Shafiq, Y., Ali, A., Yasin, R., Shoukat, A., Hussan, M. U., and Sarwar, M.I. 2019. A review: Impact of salinity on plant growth. Natural Science. 17 :34-40.

Shafi, A., Zahoor, I., and Mushtaq,U. 2019. Proline accumulation and oxidative stress: Diverse roles and mechanism of tolerance and adaptation under salinity stress. In Salt Stress, Microbes, and Plant Interactions: mechanisms and molecular approaches Springer, Singapore. 269-300.

Shafi, A., Chauhan, R., Gill, T., Swarnkar, M. K., Sreenivasulu,Y., Kumar,S., Kumar, N., Shankar, R., Ahuja, P. S., and Singh, A.K. 2015. Expression of SOD and APX genes positively regulates secondary cell wall biosynthesis and promotes plant growth and yield in Arabidopsis under salt stress. Plant Molecular Biology. 87:615-631.

Simkin, A. J. 2021. Carotenoids and apocarotenoids in planta: Their role in plant development, contribution to the flavour and aroma of fruits and flowers, and their nutraceutical benefits. Plants 10:2321-2341.

Soares, C., Carvalho, M. E., Azevedo, R. A., and Fidalgo, F. 2019. Plants facing oxidative challenges-A little help from the antioxidant networks. Environmental and Experimental Botany. 161:4-25.

Srivastava, S., and Kumar, P. 2020. Abiotic stress responses and tolerance mechanisms for sustaining crop productivity in sugarcane. *In*: Hasanuzzaman, M. Agronomic Crops: Stress responses and tolerance. Springer, Singapore. 3:29-47.

Stark, D., and Wray,V. 1989. Anthocyanins. In: Harborne JB (ed) Methods in plant biology, vol I. Plant Phenol. Academic Press/Harcourt Brace Jovanovich, London, 325-356

Tanaka, H., Yamada, S., Masunaga, T., Yamamoto, S., Tsuji, W., and Murillo-Amador, B. 2018. Comparison of nutrient uptake and antioxidative response among four Labiatae herb species under salt stress condition. Journal of Soil Science and Plant Nutrition. 64:589-597

Tuna, A.L., Kaya, C., Altunlu, H., and Ashraf, M. 2013. Mitigation effects of non-enzymatic antioxidants in maize (*Zea mays* L.) Plants under salinity stress. Australian Journal of Crop Science. 7:1181-1188.

Yoshida , S., Forno, D.A., Cook, J. H., and Gomez, K. A. 1976. Laboratory manual for physiological studies of rice, 3rd edn. International Rice Research Institute. Manila

Young, A. J., and Lowe, G. L. 2018. Carotenoids—antioxidant properties. Antioxidants 7:p.28

Zafar, M. M., Shakeel, A., Haroon, M., Manan, A., Sahar, A., Shoukat, A., Mo, H., Farooq, M.A., and Ren, M. 2021. Effects of salinity stress on some growth, physiological, and biochemical parameters in cotton (*Gossypium hirsutum* L.) Germplasm. Journal of Natural Fibers. 19:8854-8886.

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