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Author for correspondence:
Hakimeh Mansouri, E-mail: soltani@csp.ir

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Effects of polyploidy on response of *Dunaliella salina* to salinity

Fatemeh Soltani Nezhad and Hakimeh Mansouri

Department of Biology, Faculty of Science, Shahid Bahonar University of Kerman, Kerman, Iran

Abstract

In this study, polyploidy level was determined by flow cytometry analysis. The effect of polyploidy by colchicine treatment was examined on the growth parameters, malondealdehyde (MDA), as well as activities of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) in response to different levels of salinity in *Dunaliella salina*. The results of algal growth indicated that 3 M NaCl was the optimal concentration of salt, since the highest enhancement in fresh and dry weight, chlorophyll and carotenoids, soluble sugar, glycerol, protein and starch content was observed in comparison to other concentrations. The amount of these metabolites declined in the concentrations under optimum salinity. The least and highest amounts of MDA were observed at 1 and 4 M NaCl respectively. Polyploidy in optimum concentration of salt, caused further increment of the above growth parameters. In relation to this, in most cases, treatment of 0.1% colchicine was most effective. The beneficial effects of polyploidy in non-optimal conditions were also found in some parameters such as biomass, chlorophyll, carotenoids, proteins and starch. Furthermore, the activity of antioxidant enzymes CAT, SOD and POD showed a positive significant correlation with salt stress and these were maximized at 4 M NaCl. Polyploidy (especially colchicine 0.1%) affected activity of these antioxidant enzymes in some concentrations of salt. Overall, our results suggest that the microalgae has significantly different responses to salt stress based on ploidy levels.

Introduction

Salinity stress is considered one of the most significant abiotic stresses, affecting various aspects of metabolism and physiology. To understand how algae respond and adapt to salinity changes, biochemical and physiological responses have been studied (Kirst, 1990). Information about the effects of salinity stress on induction of oxidative stress is limited in algae (Jahnke & White, 2003; Liu *et al*., 2007). Adapting to salinity in various ways, algae are divided into two groups according to their tolerance as halophytic (requiring salt for optimum growth) and halo-tolerant (having response mechanisms allowing them to live in saline environments) (Rao *et al*., 2007). As a unicellular photoautotroph halo-tolerant green algae, *Dunaliella* lacks a rigid cell wall. Furthermore, it can store many important organic compounds such as β-carotene, glycerol and others (Ghetti *et al*., 1999). Additionally, *Dunaliella* responds to salt stress by high accumulation of glycerol and certain proteins as well as enhanced elimination of Na^+ ions (Pick, 2002). However, the function and physiological role of glycerol may be different in each *Dunaliella* species. Moreover, it seems to function as an osmotic regulator such that cells without walls should maintain iso-osmotic conditions in their environment (Hadi *et al*., 2008). Following a change of external salinity, a basic biochemical response is observed at the internal glycerol level, changing the external salinity directly (Ben-Amotz, 1975, Chitlaru & Pick, 1991). Carotenoids (astaxanthin and lutein) act as complementary pigments in the harvesting complex and as protective agents against the active oxygen products (AOS) formed from photo-oxidation. They are also an integral part of the photosynthetic apparatus in the algae. These oxygen radicals can react to macromolecules and lead to cellular damage (Malanga *et al*., 1997). The algae have developed defensive systems against photo-oxidative damage and can remove these highly reactive oxygen species by antioxidative mechanisms. These antioxidant defensive systems consist of hydrophobic (carotenoids and α-tocopherol) and hydrophilic antioxidants (ascorbic acid and glutathione) and antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), ascorbic peroxidase (APX) and peroxidase (POD) (Malanga *et al*., 1997; Rijstenbil, 2002; Rao *et al*., 2007). In previous reports, it was mentioned that *D. salina* elevated the activities of the antioxidant enzymes and accumulated a large amount of carotenoids, α-tocopherol and ascorbic acid when grown in a culture medium containing high salt concentration and/or limiting nitrogen (El-Baz *et al*., 2002).

Due to interest in a homogenous genetic background, some studies conducted in recent decades compared polyploids and their diploid relatives for tolerance to environmental stressors. Polyploidy is caused by doubling chromosomes of a single species (autopolyploidy) or the hybrids between two species (allopolyploidy). Polyploids clearly show higher resistance to drought, heat, cold, salt, viruses, fungi and pest stressors compared with their diploid relatives (Zhang *et al*., 2010). Therefore, polyploidy has been considered an effective way to

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increase the resistance to environmental stresses in plants, playing important roles in agriculture and forestry (Xiong *et al.*, 2006). Polyploids also show variation in physiological functions or gene expressions. These alterations might affect stress responses and phenotypess (Wang *et al.*, 2013). *Ectocarpus siliculosus* has an alternation of haploid gametophytes and diploid heterozygous sporophyte, showing a moderate tendency toward heteromorphism (Muller, 1975). Differences in responses to salt stress between gametophytes and sporophytes originating from the same parent plants were confirmed. The sporophytes are osmotically more robust than gametophytes, having higher salt tolerance than their corresponding gametophytes. These differences are the result of differing physiological tolerance to environmental conditions between diploid and haploid plants. There is some evidence that the variation in the salt tolerances between *E. siliculosus* gametophytes and sporophytes may not result from obvious differences in ploidy level alone (Thomas & Kirst, 1991).

Comparative cytochemical studies of untreated and colchicine-treated cells of algae (*Chlamydomonas*) illustrated an enhancement in the content of starch, protein and lipid materials. The data also suggest that the enlarged walls of treated cells are composed of a complex polysaccharide (Walne, 1966). The aim of this work was to study the effect of polyploidy on many important metabolites, increasing of carotenoids under polyploidy and also the antioxidant enzymatic system of *D. salina* when grown in a medium containing different levels of NaCl.

Materials and methods

Growth conditions

Dunaliella salina was isolated (Sharma *et al.*, 2012) from the Salt River of Shahdad (30°24′16.164″N 57°40′57.828″E) in Kerman, Iran and identified based on physiological and morphological descriptions in the references cited in Massyuk (1973c) and Borowitzka & Siva (2007). After transferring the samples to the laboratory, EC (33.5 ds cm^{-1}) and pH (7.75) of water were measured. Algae were cultured on the agar plate. After 2–3 weeks, each colony was transferred to 20 ml liquid growth medium (Artificial Seawater, ASW) with NaCl added to obtain the required salinity medium at 2 M (pH 7.5) (Raja *et al.*, 2007). The cultures were incubated in a growth chamber under 16/8 h light-dark provided by cool white fluorescent lamps at an intensity of 49 μmol photons m^{-2} s^{-1} at 25 ± 2°C, and were shaken manually twice a day to ensure a uniform illumination of the cells. The stock cultures were sub-cultured at least once per month.

Polyploidy induction by colchicine

Haploid vegetative cells of *D. salina* were exposed to concentrations of 0.1 and 0.5% colchicine (Sigma-Aldrich), made in culture solution for 36 h. After passing these times, algae cells were washed completely two or three times with culture solution to free algae from alkaloid traces (Sarma, 1957) and then were finally transferred to a sterile fresh medium for 21 days. Afterward, the treated cells (0.5×10^3 cells per μl) with colchicine were counted by cell counter. The fresh cultures containing 1, 2, 3 and 4 molar NaCl with 3 replications per treatment were provided and inoculated with 10 ml (5×10^6 cells) stock cultures of every colchicine treatment (0, 0.1 and 0.5%). The various treated samples were allowed to grow under the same conditions as control samples. At the end of 3 weeks, all samples were centrifuged and the pellets obtained were then frozen and stored at −70°C prior to analysis.

Flow cytometry analysis

Ploidy level of the cells of *D. salina* treated with different colchicine concentrations at different time of exposure was determined by BD FACS Calibur cell sorting system flow cytometry (FCM) (USA) equipped with two lasers. First, the cells were harvested by centrifuging at 3000 rpm for 5 min. After discarding the supernatant, the obtained pellet was resuspended in a specific lysis buffer (300 μl) of kit connected to a flow cytometry device with high resolution DNA analysis of nuclei optimal for some plant species and microorganisms. This kit has two steps: disaggregation and propidium iodide staining. Nuclear suspensions were filtered through a 50 μm nylon filter and 5 μl of propidium idoid 0.1 mg ml^{-1} (PI, Sigma, USA) added to each sample. A maximum cell concentration of 500,000 cells ml^{-1} was accepted for each culture for staining. All processes must operate on ice and samples must also be maintained on ice until analysis by FCM. Histograms were analysed using the internal software of FCM (BD FAC Station data processing system), determining peak position and the relative ploidy index of the samples (Galbraith *et al.*, 1983).

Analytical methods

Biomass was determined by filtering 20 ml of algal culture through a pre-weighed Whatman GF/C filter. The filter with algae was dried overnight at 60°C in a hot air oven and weighed again to estimate the final dry weight. To obtain fresh weight, the Whatman filter was wetted with culture medium then weighed. After filtering 20 ml algal culture by vacuum pump, the wetted filter with fresh biomass was weighed again.

The amounts of chlorophyll a, chlorophyll b, total chlorophyll and carotenoids were measured spectrophotometrically as described by Lichtenthaler (1987).

Total soluble carbohydrates and starch were analysed according to the methods employed by Roe (1955) and Thayumanavan & Sadasivam (1984). Proteins were extracted according to Bradford (1976) spectrophotometrically.

To determine glycerol content, 4 ml of the growth culture of *D. salina* was centrifuged at 1500 rpm for 10 min at room temperature, and then the precipitated pellets were washed twice in a solution of 1.5 M NaCl and 5 mM phosphate buffer at pH 7.5. One ml of periodate reagent and 2.5 ml of acetyl acetone reagent was added to 200 μl of precipitated pellets. The mixture was incubated at 45°C for 20 min. After that, optical density was determined at 410 nm. Results were compared to the prepared standard curve using the known amount of glycerol (Chitlaru & Pick, 1991).

The level of lipid peroxidation was determined by estimating malondialdehyde (MDA) and other aldehydes content using thiobarbituric acid (TBA) as the reactive material following the method employed by Heath & Packer (1968).

Enzyme assay

Enzymes assayed in this work were extracted by grinding 0.15 g of fresh algae in a porcelain mortar contain 1.5 ml phosphate buffer containing 50 mM (pH 7.5) ethylene diamine tetraacetic acid (EDTA), 1 mM phenyl methanesulfonyl fluoride (PMSF) and polyvinylpyrrolidone (PVP) 1%. The extract was centrifuged for 15 min at 4°C at 14,000***g*** and the supernatant was assayed for enzymatic activity and quantification of protein by the Bradford method (1976). All operations were performed at 4°C. The activity of superoxide dismutase (SOD) (EC 1.15.1.1), catalase (CAT) (EC 1.11.1.6) and Guaiacol peroxidase (GPX) (EC 1.11.1.7) was determined according to the methods employed by Giannopolitis &

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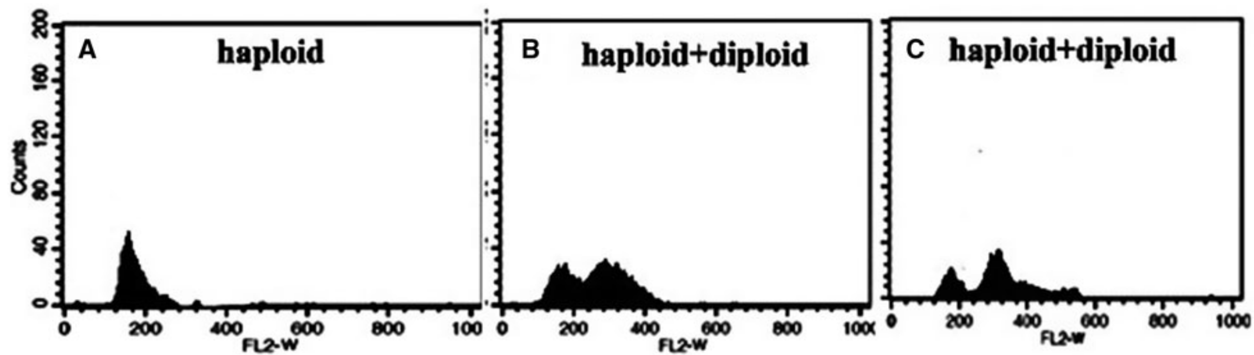

Fig. 1. Histograms of relative DNA content of *D. salina.* (A) Histogram of relative fluorescence intensity of nuclei isolated from the control cells, (B) the treated cells with 0.1% colchicine and (C) the treated cells with 0.5% colchicine.

Ries (1977), Azevedo *et al.* (1998) and Urbanek *et al.* (1991), respectively.

Statistical analysis

The experiment was arranged in a completely randomized design with three replicates. SPSS software was employed for statistical analysis, and graphs were plotted by Excel software. Means were compared using Duncan's multiple range tests at $P < 0.05$.

Results

In Figure 1, in histogram (a) related to control cells (without colchicine treatment), the peak in channel 160 indicates the haploid cells. In the two other histograms, channel 320 indicates diploid cells and both histograms (histogram (b), treated cells with 0.1% colchicine and histogram (c), 0.5% colchicine) also show that there are still haploid cells, but with increase of colchicine concentration, the percentage of haploid cells is lower.

First, it should be mentioned that according to our previous results (unpublished), the cultures treated with 0.1 and 0.5% colchicine contained 58 and 74% polyploid cells, respectively.

The results related to algal growth indicated that the highest fresh and dry weight of *D. salina* was observed at concentration of 3 M NaCl (Table 1). Algae exposed to 4 M NaCl contained the least amount of fresh and dry weight. In concentrations ranging from 2 to 4 M, it was found that 0.1% colchicine had a positive effect on fresh and dry weight, respectively in the treated algae. Algae exposed to 0.5% colchicine only showed an increase in dry weight in 3 M NaCl.

Table 1 demonstrates the impact of colchicine and salinity on chlorophyll and carotenoid content in *Dunaliella*. At higher and lower concentrations of 3 M NaCl, a significant reduction in the amount of chlorophyll was observed. The lowest amount of chlorophyll was observed in 1 M salinity. In this respect, chlorophyll b was more sensitive than chlorophyll a. A concentration of 0.1% colchicine caused an enhancement in chlorophyll a and b content compared with control samples (without colchicine) at 2 and 3 M NaCl, and treatment of 0.5% colchicine had no significant change compared with controls to salinity 3 M, but at 4 M NaCl caused an increase in content of chlorophyll a and b.

Carotenoid content was enhanced with increasing of salinity in culture. The highest cellular carotenoid level was recorded at 4 M NaCl. Algae exposed to 0.1% colchicine showed enhancement of carotenoids accumulation at all salinities (1–4 M) (Table 1). Treatment of 0.5% colchicine increased the carotenoid content in the concentration range of 1–3 M NaCl.

The decrease in carbohydrate content (Figure 2A) was observed under 1 and 4 M salinity. Treatment of 0.1% colchicine positively affected soluble sugar content under 3 and 4 M salinity. In addition, a significant increase in sugar content was noticed in the algae treated with 0.5% colchicine and 3 M NaCl.

After exposure to different salinity levels, a considerable change in the glycerol concentrations was evident in untreated-colchicine samples (Figure 2B). The maximum production of glycerol value was achieved in 4 M salinity. Induced, polyploidy created by 0.1 and 0.5% colchicine considerably affected increasing of glycerol content at optimum salinity level (3 M NaCl).

Ranges of 1–3 M NaCl stimulated protein accumulation (Figure 2C). However, algae exposed to 4 M NaCl had the least amount of protein. The algae treated by 0.1% colchicine showed an increase in protein content at all salinity levels. The highest increase was observed in the algae treated with 0.1% colchicine and 3 M NaCl. Higher concentration of colchicine caused an increase in protein content under 1 and 4 M salinity.

The amount of starch was higher in the algae grown with 3 and 4 M NaCl (Figure 2D). The algae treated by both concentrations of colchicine had more starch content at all salinity levels in comparison to untreated-colchicine algae. The highest increase was observed at 3 M NaCl with ~1.5-fold of untreated cells at this concentration (Figure 2D).

The present results showed a correlation between increasing of salinity level and that of MDA and other aldehydes content (Figure 3). Algal cultures exposed to 0.1 and 0.5% colchicine had less MDA and other aldehydes content under 2, 3 and 4 M NaCl. In this regard, concentration of 0.1% colchicine was more effective. Furthermore, the results showed that lipid peroxidation was stimulated in the algae treated by 0.5% colchicine under 1 M salinity.

Salinity significantly affected the activity of ROS scavenging enzymes (Figure 4A–C). Catalase activity in the algae grown under optimum salinity (1, 2 and 4 M) increased in comparison to cultures containing 3 M NaCl (Figure 4A). Induced polyploidy by 0.1 and 0.5% colchicine stimulated catalase activity at cultures containing 3 and 4 M NaCl. Polyploidy had no significant changes at two other salinity concentrations in catalase activity.

The results revealed that the activity of peroxidase (PX) increased ~2-fold in the cultures treated by 4 M NaCl in comparison to other salinity treatments (1, 2 and 3 M NaCl) (Figure 4B). Specific peroxidase activity of treated algae by colchicine in salinities 3 and 4 M was lower than that of their controls. Like catalase, there was no significant change in the PX between control and colchicine treatments in the concentrations of 1 and 2 M NaCl.

The specific activity of SOD was almost doubled at 4 M NaCl in *D. salina.* Samples containing polyploidy cells showed a remarkable increase compared with the control at the same salinity concentration (Figure 4C).

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Table 1. Effect of different concentrations of colchicine on the growth expressed as fresh and dry weight and photosynthetic pigments of *D. salina* growing in media containing a range of salt concentrations (1 to 4 M NaCl)

Parameters	NaCl (M)	Colchicine (%) 0	0.1	0.5
Fresh weight (mg ml^{-1})	1	4.366 ± 0.351 ef	4.66 ± 0.737 ef	4.033 ± 0.737 fg
	2	5.466 ± 0.305 d	6.433 ± 0.057 c	5.566 ± 0.404 d
	3	6.833 ± 0.305 bc	7.933 ± 0.378 a	7.333 ± 0.378 ab
	4	4.066 ± 0.057 fg	4.9 ± 0.3 de	3.566 ± 0.503 g
Dry weight (mg ml^{-1})	1	0.433 ± 0.057 ef	0.466 ± 0.115 def	0.366 ± 0.057 f
	2	0.533 ± 0.057 de	0.766 ± 0.057 c	0.6 ± 0.1 d
	3	1.066 ± 0.115 b	1.433 ± 0.057 a	1.33 ± 0.057 a
	4	0.366 ± 0.057 f	0.566 ± 0.115 de	0.333 ± 0.057 f
Chl a (mg g^{-1} FW)	1	0.617 ± 0.018 f	0.791 ± 0.047 f	0.804 ± 0.017 f
	2	1.466 ± 0.17 de	2 ± 0.3 c	1.69 ± 0.225 d
	3	2.236 ± 0.11 bc	3.124 ± 0.052 a	2.235 ± 0.209 b
	4	1.293 ± 0.008 e	1.555 ± 0.138 de	2.163 ± 0.131 bc
Chl b (mg g^{-1} FW)	1	0.026 ± 0.001 f	0.043 ± 0.002 f	0.064 ± 0.015 f
	2	0.257 ± 0.02 de	0.407 ± 0.08 c	0.333 ± 0.1 cd
	3	0.597 ± 0.066 b	0.769 ± 0.03 a	0.68 ± 0.047 b
	4	0.229 ± 0.028 e	0.254 ± 0.018 de	0.411 ± 0.059 c
Toltal chlorophyll (mg g^{-1} FW)	1	0.643 ± 0.022 f	0.834 ± 0.045 f	0.868 ± 0.028 f
	2	1.724 ± 0.22 de	2.407 ± 0.267 d	2.0239 ± 0.324 d
	3	2.834 ± 0.056 bc	3.893 ± 0.037 a	2.967 ± 0.321 b
	4	1.522 ± 0.024 e	1.810 ± 0.128 de	2.574 ± 0.189 cd
Total carotenoids (mg g^{-1} FW)	1	0.303 ± 0.029 h	0.450 ± 0.016 g	0.511 ± 0.011 fg
	2	0.575 ± 0.017 f	0.645 ± 0.025 e	0.676 ± 0.092 e
	3	0.861 ± 0.026 d	1.150 ± 0.013 b	1.058 ± 0.076 c
	4	1.125 ± 0.085 bc	1.285 ± 0.012 a	0.932 ± 0.034 d

Means of three replications ± SD. Means followed by different letters in a column are significantly different ($P < 0.05$) according to Duncan's test.

Discussion

Optimal concentration of NaCl for growth of *D. salina* was 3 M, therefore the maximum fresh and dry weight content was gained at this concentration, but the fresh weight decreased with higher and lower concentrations of salinity. A negative trend in the growth rate of the microalga *Chlorella* sp. was observed when the NaCl concentration was further increased. This decrement in growth is due to accumulation of reactive oxygen species (Kalita *et al.*, 2011). Cultures with 58% polyploidy level (0.1% colchicine) had more fresh and dry weight at most salinity levels. However, higher percentage of polyploidy (74%) induced by 0.5% colchicine treatment increased dry weight only in the optimum condition (3 M NaCl). This suggests that the presence of an equal percentage from haploid and polyploid cells had an improvement effect on the algae growth. In one experiment on diatoms, a correlation between 4N polyploidy class (with 21% percentage of ploidy) and increased growth rate was shown. Vonshak & Richmond (1981) provided evidence that increase in the cellular DNA content in blue-green algae occurred in association with an increase in growth rate, indicating an increase in the number of genomes in the cell. D'Amato & Durante (2003) reported that the increased availability of nuclear templates was reflected in the growth of protoplast and its functional activity. He also indicated the correlation between the rate of RNA synthesis and the cell ploidy level. Thus, increased availability of RNA templates allows for higher level of protein translation and ultimately higher production of structural and functional proteins increasing the growth rate. Higher growth rate is desirable for commercialization as it allows more biomass to be produced within a shorter period of time. Bagheri & Mansouri (2014) reported that mixoploidy *Cannabis sativa* induced by colchicine treatment showed better growth compared with tetraploid plants. Despite the importance of polyploidy, there are few studies on induction of polyploidy or its effect on algae, but in plants, it is well known that polyploidy leads to an increase in organ size, which may be caused by changes in activities of cell division and expansion as the result of the duplication of gene loci and increase in nuclear DNA content (Sugiyama, 2005). Furthermore, in other studies, plants with higher yield were observed by colchicine treatment compared with untreated plants (Mensah *et al.*, 2007). Some varieties of potato responded significantly and positively in plant height, number of leaves and fresh weight of plant after colchicine treatment (Alam *et al.*, 2011).

The results showed a significant reduction in the amount of chlorophyll at higher and lower concentrations of 3 M NaCl, but in the colchicine treated cultures of *D. salina*, the amount of chlorophyll a and b significantly increased (especially in 0.1% colchicine). The total chlorophyll content of *Sesame indicum* L. treated with

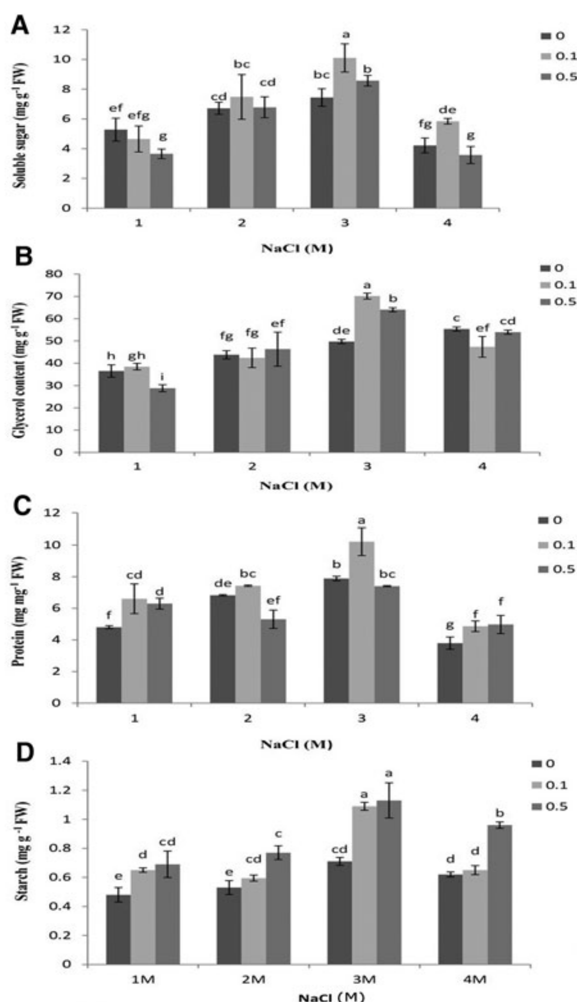

Fig. 2. Effect of different salinity concentrations on (A) soluble carbohydrate, (B) glycerol synthesis, (C) total protein and starch content (D) of *D. salina.* Cultures were grown in medium containing a wide range of salinity (1 to 4.0 M NaCl). The vertical bars represent the standard deviation; different letters above the bars indicate significance level at $P < 0.05$.

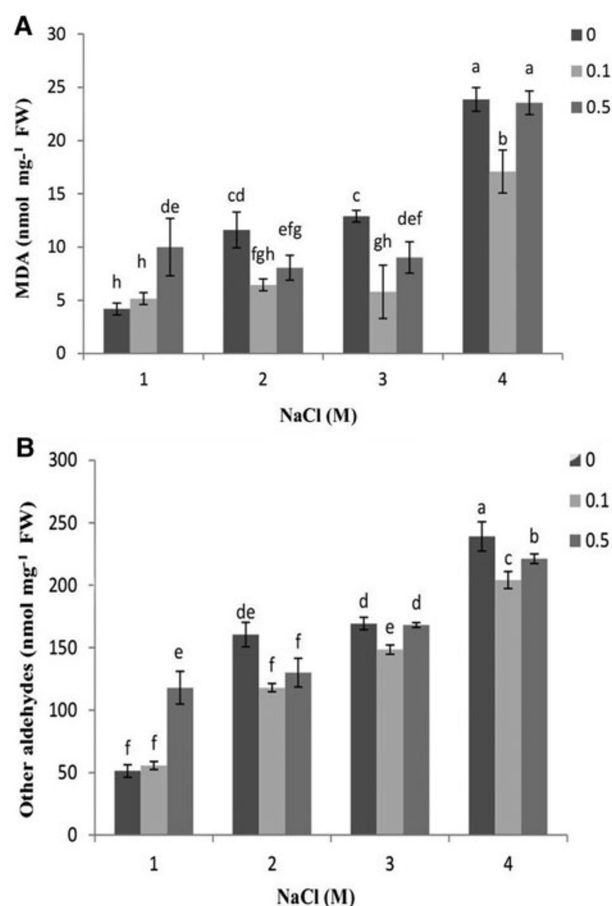

Fig. 3. Changes in the content of (A) MDA and other aldehydes (B) in *D. salina* in response to different concentrations of colchicine under salinity. Different letters above the vertical bars indicate significance level at $P < 0.05$.

colchicine was enhanced at higher concentrations. Contrary to these findings, with increasing ploidy level in *Juncus effusus*, chlorophyll content decreased significantly (Xu *et al.*, 2010).

There is a direct relationship between carotenoids content and increased levels of salinity. High salinity caused an increment in carotenoids content and inversely low salinity caused a decrease in carotenoids content. The photosynthetic apparatus does not use light energy sufficiently, and the excess energy leads to formation of free radicals rather than active oxygen molecules (singlet oxygen) under high salinity. These radicals are responsible for peroxidation reactions destroying diverse compounds of photosynthesis apparatus. Thus, the algae like *Dunaliella* and *Chlorella* accumulate large amounts of pigments to scavenge or omit and reduce the radicals (El-Baz *et al.*, 2002). Induction of polyploidy in *D. salina* increased the carotenoids content, especially in 0.1% colchicine in all salinities. Comparative results of chlorophyll a, chlorophyll b, total chlorophyll and carotenoids between tetraploid and diploid plants of cannabis demonstrated no significant changes (Bagheri & Mansouri, 2014).

The amounts of protein and sugar in *Dunaliella* increased to 3 M NaCl, but in high salinity, their contents declined. However, Rao *et al.* (2007) reported a rise in carbohydrate content when the algae were cultured at different salinities. However, polyploidy raised protein and sugar content in some salt concentrations compared with their controls. Levine *et al.* (1990) in accordance with the present result, reported that salt stress inhibited growth and development, decline in photosynthesis, respiration and protein synthesis, and disturbed nucleic acid metabolism. Bagheri & Mansouri (2014) reported that tetraploid cannabis had higher values of total proteins compared with control plants (Bagheri & Mansouri, 2014). In the mentioned study, the highest amount of protein and sugar was found in mixoploid plants. Jaskani *et al.* (2005) reported that the total sugar content was comparable in diploid and tetraploid fruits of watermelon line. Like our results, Grange *et al.* (2003) observed higher total sugar content in triploid than in diploid fruit of watermelon, but in tetraploid cannabis, a reduction in carbohydrate content was observed due to decrease of CO_2 fixation (Bagheri & Mansouri, 2014). It can be illustrated that in polyploid cells with higher DNA content, protein and sugar biosynthesis pathways were stimulated.

With increasing of salinity, the content of glycerol in this alga rose and reached its maximum in 4 M NaCl. The main role of glycerol in *Dunaliella* seems to be as an osmotic regulator so that it is a compatible solute at high concentrations of salt, protecting enzyme activity. According to Avron & Ben-Amotz (1992), glycerol is an important compound for osmotic regulation in *Dunaliella*. Polyploidy increased the amount of glycerol only in the optimum growth condition. These results showed that in spite of an increase in DNA content, the genes related to glycerol biosynthesis at a high salinity level (4 M) were inactive.

In this study, the amount of starch increased at 3 and 4 M NaCl, but polyploidy induced starch accumulation at all concentrations of salinity. For *D. parva*, with higher concentrations of salt in the medium a higher rate of glycerol synthesis was

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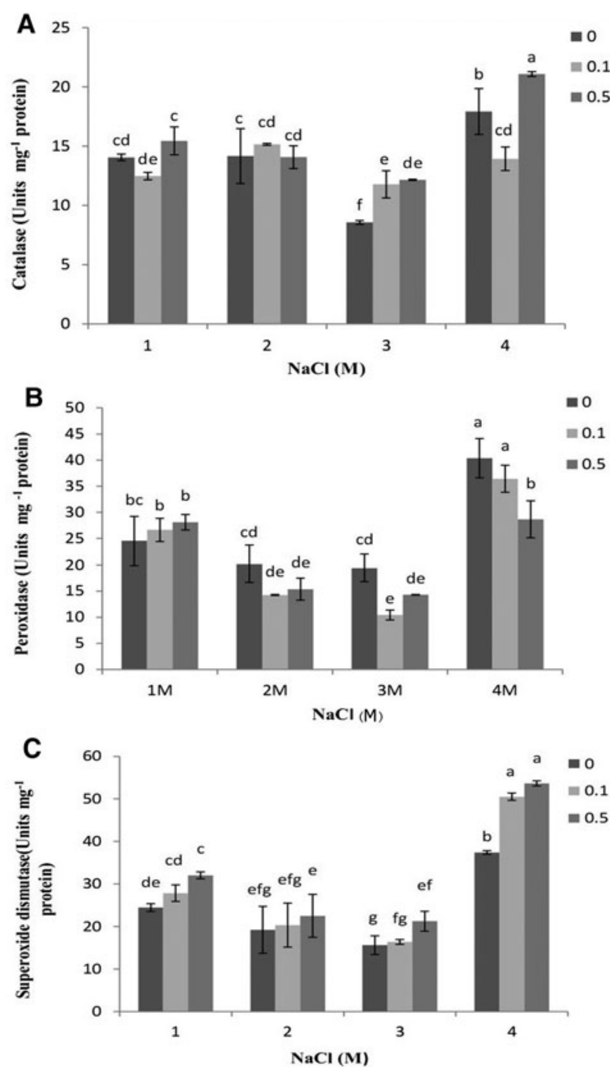

Fig. 4. The activities of (A), CAT (Catalase), (B), GPX (Guaiacol peroxidase), (C), SOD (superoxide dismutase) in *D. salina* in response to different salinities. Letters above vertical bars indicate significance level at $P < 0.05$.

observed whereas this relation does not exist between starch content and the medium salinity (Gimmler & Molle, 1981). In polyploid races of *Atriplex confertifolia*, content of DNA, enzyme activity, photosynthesis per cell and cell volume increased (Warner & Edwards, 1989). Higher photosynthetic rate in polyploidy samples can be explained by inducing starch accumulation. The leaves of mixoploid and tetraploid plants of *Cannabis sativa* L. compared with control plants contained higher amounts of starch (Bagheri & Mansouri, 2014).

Raja *et al.* (2007) mentioned that part of the main physiological responses was caused by stress conditions, including the flux of carbon between starch production in the chloroplast, synthesis of glycerol in the cytoplasm and accumulation of carotenoids. Goyal (2007) also observed that increased salt stress caused increments in the contribution of products of starch breakdown to glycerol synthesis. However, in the present study, we did not find a special relationship between these metabolites.

Results show that salinity significantly increased MDA and other aldehydes content in controls. However, in treated samples with 0.1% colchicines, the amount of MDA declined considerably. MDA is an important indicator of the damage caused by salt stress (Verslues *et al.*, 2006). Lower lipid peroxidation in polyploid algae can be attributed to the effect of non-enzymatic antioxidant compounds (such as carotenoids) protecting the photosynthetic apparatus from damage by quenching triplet chlorophyll and singlet oxygen, in addition to the different strategies in the enzymatic system (Young & Frank, 1996). Salinity increased O_2^- and H_2O_2 content in both diploid (2X) and tetraploid (4X) of *Robinia pseudoacacia*. However, 2X plants showed higher $O2^-$ and H_2O_2 content compared with 4X plants at the end of 10 days of experiment. Additionally, MDA content increased in 2X and 4X after salt treatment, and 2X showed much higher levels than those of 4X, indicating more damage to membranes in 2X plants (Wang *et al.*, 2013).

Gossett *et al.* (1996) suggested that protection from oxidative damage was induced under salt stress by more active ascorbate-glutathione cycles and a higher level of antioxidant enzymes such as CAT, SOD and POD. Accordingly, *D. salina* exposed to the high level of salinity (4 M NaCl) showed a significant increase in SOD, CAT and GPX activity, indicating that these enzymes were utilized for enzymatic scavenging of AOS. Polyploidy had no remarkable impact on increase of antioxidant enzymes activity, except its impact on the SOD activity in the algae treated by 4 M NaCl.

Interestingly, the activities of the antioxidant enzymes (POD and APX) in 4X plants increased under salt stress. Similar to the present results, tetraploid *Robinia pseudoacacia* showed higher SOD activity than that of diploid plants, indicating that 4X had a more efficient enzymatic antioxidant system against salt stress and dealing with ROS compared with 2X plants (Wang *et al.*, 2013).

In conclusion, we investigated the existence of different percentages of polyploidy cells in *D. salina* cultures and its response to salinity. As expected, all analysed parameters showed variation in each of the three ploid populations. In general, mixoploid cultures showed better characterization than haploid cultures, especially under optimum condition (3 M NaCl). Thus, populations with equal percentages of haploid and polyploid cells (0.1% colchicine treatment) had a higher function. We argue that polyploidy can be used to increase algal productivity.

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