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Full Length Article

Seed Priming with Extracts of two Seaweeds Alleviates the Physiological and Molecular Impacts of Salinity Stress on Radish (*Raphanus sativus*)

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Abstract

Seeds of radish were primed with the extract of either *Codium taylorii* or *Pterocladia capillacea* for 2 h, then sown in clay-sandy soil and left to grow under the effect of either 150 or 200 mM NaCl, however the control treatment was irrigated only with tap water, for 35 days. The results indicated that salinity decreased water content, shoot length, photosynthetic pigments and total lipids, while it increased total soluble proteins, proline, phenolic compounds and alkaloid contents. Priming with *C. taylorii* or *P. capillacea* alleviated the adverse effects of salinity on radish seedlings. Under 150 and 200 mM NaCl treatments, 5 bands with molecular weights 40, 70, 80, 100 and 171 kDa were newly synthesized as stress proteins, while that of the salt stressed seedlings primed with seaweed extracts showed appearance of four new bands with molecular weights 4, 15, 21 and 44 kDa with *C. taylorii* and 5, 6, 23 and 149 kDa with *P. capillacea*. The data of the ISSR analysis produced by five different primers reflected the appearance of seven new bands with the treatment 150 and 200 mM NaCl, and there was a disappearance of eight other bands. Priming of radish seeds with seaweed extract and treated with 150 and 200 mM NaCl resulted in the disappearance of five other bands. It might be concluded that the priming in seaweed extracts could represent a useful and powerful tool in alleviating salinity stress. © 2016 Friends Science Publishers

Keywords: Pigments; Leaf protein profile; Radish; Salinity; Seaweeds; Secondary metabolites

Introduction

Raphanus sativus L. (radish) is a member of family *Brassicaceae* and it is a cool-season plant which grows best in spring and autumn world-wide. Moreover, it is an important root vegetable that is rich in vitamin 'C' and minerals like sulphur, and it is used in the medical field as a treatment for liver disorders and respiratory illnesses (Kalantari *et al*., 2009).

Salinity is one of the major abiotic stresses in plant agriculture worldwide. Nearly 20% of the world's cultivated area and about half of the world's irrigated lands are affected by salinity (Kaya *et al*., 2002). The detrimental effects of salt on plants are a consequence of both; a water deficit that results from the relatively high solute concentrations in the soil and a specific Cl^- and Na^+ stress. The result is a wide variety of physiological and biochemical changes in plants that inhibit growth and development, reduce photosynthesis, photorespiration, protein and carbohydrate synthesis and disrupt amino and nucleic acids metabolism (Ahuja *et al*., 2010). Seed priming is an effective strategy to improve crops productivity under salinity stress. Afzal *et al*. (2013) reported that seed priming with ascorbate, salicylic acid and kinetin successfully alleviated the salinity-induced deleterious effects in wheat through decreasing the uptake of Na^+ and Cl^- and enhancing the uptake of K^+ in leaves under salinity stress.

A wide variety of seaweeds grows along the Egyptian Mediterranean coast, especially at Alexandria and has been widely used by coastal populations owing to their high nutritional values. The green alga, *Codium taylorii* and the red alga, *Pterocladia capillacea* are among the most abundant macro-algae on the Alexandria coast, particularly from spring to autumn (Aleem, 1993). They provide an excellent source of bioactive compounds such as essential fatty acids, vitamins, amino acids, minerals and growth promoting substances (Bhasker and Miyashita, 2005). Seaweeds have been reported to stimulate the growth and yield of plants via enhancing antioxidant properties, so it might develop tolerance to salinity stress (Zhang *et al*., 2003).

The aim of this study was to evaluate the influence of the seed priming by presoaking in the extract of two macro-algae, *C. taylorii* or *P. capillacea* on some physiological and molecular aspects of radish seedlings grown under salt stress through assessing the changes in photosynthetic pigments, total soluble protein, proline, phenolics, alkaloids, total lipids and glycinebetaine, as well as protein patterns and Inter-Simple-Sequence Repeat analysis (ISSR).

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Materials and Methods

Two marine macro-algal species, *C. taylorii* (green alga) and *P. capillacea* (red alga) were collected from the Mediterranean sea at El-Maamoura beach, Alexandria, Egypt, during summer season (May, 2014), (31° 12′ N, 29° 55′ E). Seaweed species were hand collected and washed with seawater to remove debris, shells, and sand. Seaweed species were hand collected and washed with seawater to remove debris, shells and sand. Samples were washed with tap water and oven-dried at 60°C for 72 h. The dried samples were ground in an electric mill and sieved through 2.0 mm sieve. The milled material is subjected to water extraction for 24 h. with continuous shaking (10 g/L) then filtered using Whatman No. 1 filter paper and stored at 4°C for further treatment.

Seeds of radish (*Raphanus sativus* L. cv. Baladey) were obtained from Agricultural Research Center (ARC), Giza, Egypt. Seeds were surface sterilized with 5% Clorox solution for 5 min, washed thoroughly with tap water three times then once with distilled water. Sterilized radish seeds were soaked for 2 h either in the extract of *C. taylorii* or *P. capillacea* or in distilled water. All of the soaked seeds were sown in plastic pots, each of 40 cm diameter and 20 cm length containing 8 kg of clay-sandy soil (2:1w/w) and irrigated with tap water. At full germination after six days, the pots were irrigated with 0, 150, 200 mM NaCl, while the pots of the control were irrigated with tap water till the end of growth.

The plants were left to grow under the natural day/night conditions (14 h light/10 h dark) at 26±2°C in the greenhouse and irrigated whenever needed to the age of 35 days. After that, plants were harvested for the determination of relative water content, shoot length and some physiological and molecular analyses.

Phytochemical Analysis

Photosynthetic pigments chlorophyll a (Chl *a*), chlorophyll b (Chl *b*) and carotenoids were determined in the fresh radish leaves quantitatively according to the method adopted by Metzner *et al*. (1965). Total soluble protein content of radish leaves was assayed quantitatively according to the method of Bradford (1976) using BSA as a standard protein. Total free proline content was measured according to Bates (1973) using a prepared calibration curve by proline. Total phenolic content in radish leaves was estimated quantitatively using the method described by Jindal and Singh (1975) using gallic acid as a standard phenol.

Total alkaloids content was determined spectrophotometrically according to the method of Fazel *et al*. (2008) by the reaction of alkaloids with bromocresol green (BCG) forming yellow complex which is extractable by chloroform. Alkaloids content was calculated using standard curve prepared from atropine. Total lipids were extracted from radish leaves using a mixture of chloroform and methanol (2:1) and the lipid content was determined quantitatively according to Bligh and Dyer (1959). Glycinebetaine (GB) was estimated in the dried leaf powders of radish using potassium iodide-iodine reagent and the solvent 1, 2-dichloro ethane as described by Grieve and Grattan (1983).

Molecular Analysis

Total leaf proteins were extracted from fresh leaves with Tris-HCl buffer (pH 8.0) supplemented with 0.2% SDS and 5 M urea. Leaf proteins were analyzed through the slab type SDS-PAGE using 12% polyacrylamide gel as described by Laemmli (1970). The gel was then photographed and the presence or absence of bands was scored as 1 or 0, respectively. Molecular weights of protein bands were calculated using Lab Image software version 2.7 produced by Kapelan GmbH, Germany.

The ISSR finger-printing procedures were based on the method described by Dogan *et al*. (2007). Five ISSR primers (Operon Nippon EGT CO. LTD) were screened for the production of polymorphic products from all samples of radish leaves. The sequences of the primers and their properties are mentioned in Table (1). The ISSR fingerprinting was visualized using a Gel Works 1D advanced gel documentation system (UVP, UK) and photographed under UV light. The size of each band was estimated using 100 bp DNA ladder (Fermentas) as a standard marker.

Results

The relative water content of radish leaves was decreased with the increment of NaCl concentration, which was more obvious at 200 mM NaCl (Fig. 1). Priming with extract of either *C. taylorii* or *P. capillacea* resulted in the enhancement of radish water content compared with that of the control treatment either with or without NaCl treatment. NaCl-induced salinity also reduced shoot length of radish plant compared with control treatment and the reduction was greater at higher NaCl level (200 mM). Priming with extract of two seaweeds; *C. taylorii* and *P. capillacea*, resulted in a significant increase of plant length of radish. Combination of algal treatments with the salt stressed plants resulted in alleviating the inhibitory effect of NaCl on the shoot length compared with the primed-salt stressed control (Fig. 2).

NaCl salinity stress negatively affected Chl *a* and Chl *b* contents in radish leaves (Fig. 3). The more pronounced decline in chlorophyll content was achieved by the higher NaCl level. Nevertheless, radish exposed to salinity stress showed a significant increment in carotenoids content, compared with the control treatment. Priming with seaweed extract improved Chl *a*, Chl *b* and carotenoids to levels higher than those of the control. Also, the combination of seaweeds and salinity treatments resulted in alleviating the

Table1: Primers ID, sequences, length in bp and annealing temperature **(**Tm**)** of the selected ISSR primers

Ser.	(ID)	Sequence 5`-3`	Tm (ºC)	Length (base pair)
01	CAG	CAGCAGCAGCAGCAGCAG	56	18
02	UBC811	GAGAGAGAGAGAGAGAC	48	17
03	UBC819	GTGTGTGTGTGTGTGTA	50	17
04	CGG	CGGCGGCGGCGGCGGCGG	68	18
05	829	TGTGTGTGTGTGTGTGC	62	17

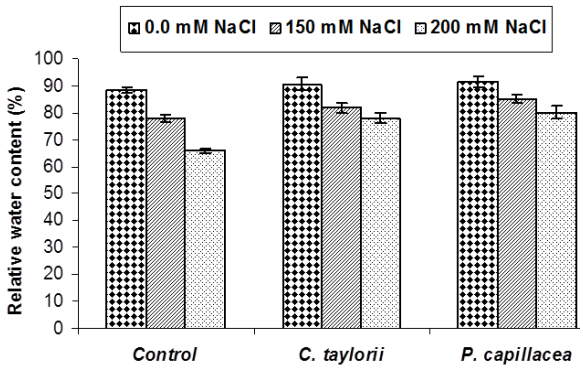

Fig. 1: Effect of seed priming with *C. taylorii* or *P. capillacea* extracts on the leaf relative water content (%) of radish plants (35-days-old) grown under 150 or 200 mM NaCl in clay-sandy soil (2:1 w/w). Value of each bar represents mean of three replicates with bars showing ±standard deviation

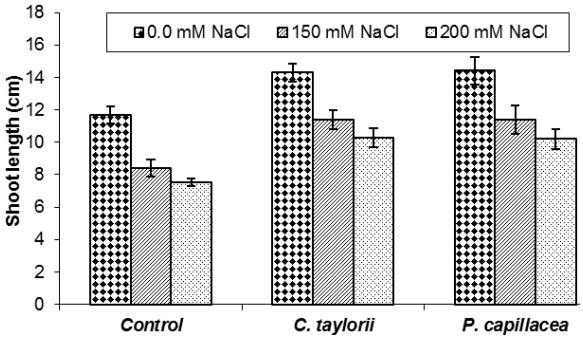

Fig. 2: Effect of seed priming with *C. taylorii* or *P. capillacea* extracts on the shoot length (cm) of radish plants (35-days-old) grown under 150 or 200 mM NaCl in clay-sandy soil (2:1 w/w). Each bar represents mean of three replicates with lines showing ± standard deviation

inhibitory effect of NaCl on Chl *a* and Chl *b* in comparison with the NaCl individual treatments.

The effect of NaCl and the priming with the extract of *C. taylorii* and *P. capillacea* treatments on the total lipid content of radish leaves. The amount of total lipids was decreased with the increase of NaCl level. However, priming with the extract of seaweeds resulted in the alleviation of this effect; especially in case of priming the seeds with *C. taylorii* extract (Fig. 4).

The total soluble protein content of radish leaves (Fig 4) was remarkably increased as a result of NaCl treatments and it was more pronounced with 200 mM NaCl (Fig. 5). Also, priming of the unstressed radish with the two seaweed extracts (*C. taylorii* and *P. capillacea*) relatively enhanced their accumulation of total soluble protein compared with the corresponding control (unprimed-unstressed control). The interaction of salinity and the priming with *P. capillacea* treatments resulted in increased protein contents in comparison with the NaCl application, but the interaction with *C. taylorii* treatments exerted negative effect on protein contents, where the protein contents were decreased compared with the NaCl stressed treatments.

Exposure of radish seedlings to NaCl salinity stress resulted in abundant accumulation of proline, where the higher NaCl concentration (200 mM) caused a two-fold increase in proline content in the unprimed-stressed control. The combination of seaweed treatments with NaCl caused a relative reduction in proline accumulation compared with this control (Fig. 6).

The results in (Fig. 7) show that the unprimed-stressed radish plants accumulated glycinebetaine (GB), which was increased with increasing NaCl level, while the interaction of priming with salinity resulted in a relative depression in this accumulation, compared with this control which was more pronounced with *C. taylorii*. However, in the unstressed plants, priming significantly lowered the level of GB, compared with their corresponding unprimed-unstressed control.

Data in (Fig. 8) indicate that NaCl stress significantly stimulated the accumulation of phenolic compounds in radish leaves and this was more pronounced by 200 mM NaCl. However, the combined treatment of salinity and priming with seaweeds resulted in a relative decline in its level compared with unprimed- stressed control.

NaCl stress stimulated the accumulation of total alkaloids in radish leaves which increased with the salinity level (Fig. 9). In combined treatment of salinity and priming resulted in a pronounced decline in alkaloids level relative to the stressed- unprimed control.

The SDS-PAGE is shown as an array of proteins with molecular weights ranging between 4 and 171 kDa (Table 2). During 150 and 200 mM NaCl levels, 5 bands with molecular weights 40, 70, 80, 100 and 171 kDa were expressed. Protein profiles of radish plants raised from seeds primed with seaweed extracts showed appearance of 4 new protein bands with molecular weights 4, 15, 21 and 44 kDa with *C. taylorii* and 5, 6, 23 and 149 kDa with *P. capillacea*, compared with the control.

Table 2: Effect of seed priming with *C. taylorii* or *P. capillacea* extracts on the leaf protein profile of radish seedlings grown under salinity stress

Ser.	M.W	Cont	150 mM NaCl	200 mM NaCl	*P. cap*	*C. tayl*	150 mM NaCl+*P. cap*	150 mM NaCl+ *C. tayl*	200 mM NaCl + *P. cap*	200 mM NaCl+ *C. tayl*	Type of bands
1	171	0	1	1	0	0	0	0	0	0	P
2	149	0	0	0	1	0	0	0	0	0	U
3	146	1	0	0	0	0	0	0	0	0	U
4	133	1	1	1	1	1	1	1	1	1	M
5	119	1	0	0	0	0	0	0	0	0	U
6	109	1	1	1	1	1	1	1	1	1	M
7	96	0	1	1	0	0	0	0	0	0	P
8	91	1	0	0	0	0	0	0	0	0	U
9	84	1	1	1	1	1	1	1	1	1	M
10	80	0	1	1	0	0	0	0	0	0	P
11	75	1	1	1	1	1	1	1	1	1	M
12	70	0	1	1	0	0	0	0	0	0	P
13	63	1	0	0	0	0	0	0	0	0	U
14	60	0	1	1	0	1	0	0	1	1	P
15	44	0	0	0	0	1	0	0	0	0	U
16	48	1	0	0	1	1	1	1	1	1	P
17	40	0	1	1	0	0	0	0	0	0	P
18	35	0	1	1	1	0	1	1	0	0	P
19	30	1	1	1	1	1	1	1	1	1	M
20	26	1	1	1	1	1	1	1	1	1	M
21	23	0	0	0	1	0	0	0	0	0	U
22	21	0	0	0	0	1	0	0	0	0	U
23	19	1	0	0	1	1	1	1	1	1	P
24	15	0	0	0	0	1	0	0	0	0	U
25	11	0	1	1	1	0	1	1	0	0	P
26	7	1	1	1	1	1	1	1	1	1	M
27	6	0	0	0	1	0	0	0	0	0	U
28	5	0	0	0	1	0	0	0	0	0	U
29	4	0	0	0	0	1	0	0	0	0	U

M.W. = molecular wight, Cont. = control, M = monomorphic, P = polymorphic, U = unique.

The ISSR analysis produced by the five primers (Fig. 10) revealed that radish leaves (35-days-old) produced 46 bands (20 polymorphic, 21 monomorphic and 5 unique). Under 150 and 200 mM NaCl, seven new bands were newly synthesized with molecular sizes of 597, 570 and 510 bp with CAG primer, 267 bp with UBC811 primer, 535 and 641 bp with UBC819, 410 bp with CGG and the last one 300 bp with 829 primer. Under the two levels of salinity, eight bands were disappeared with molecular sizes of 445 bp and 686 bp with primer CAG, 345 bp with primer UBC811, 355 bp with primer UBC819, 468 bp with primer CGG and 3183 bp, 235 bp and 357 bp with the primer 829.The combined treatment of any of the two salinity levels with priming resulted in the disappearance of five bands with molecular sizes of 532 bp with the CAG primer, two with molecular sizes of 668 and 264 bp with the UBC819 primer, fourth band with molecular size of 787 bp with the CGG primer and the fifth band 505 bp with 829 primer.

Discussion

The imposition of salinity stress inhibited shoot length and relative water contents of radish leaves. However, presoaking of seeds in seaweed extracts significantly improved these parameters under salinity stress (Fig. 2 and 3). The recorded decline in the growth and water content of radish seedling as a result of salinity stress has also been reported for many crop species (Aymen *et al*., 2014). This resultant decrease in water content with salinity might be because that salinity has both osmotic and specific ionic effects followed by disturbance in the water relations of plants, reduction of turgor potential and decline in growth due to stomatal closure and reduced photosynthesis (Saffan, 2008). According to Munns (1993) these reductions in plant length under salinity may be due to the accumulation of toxic ions which adversely affect cell division and expansion. Nasim *et al*. (2008) reported also that the suppression of the uptake of essential nutrients like P and K which result in the reducing metabolism rate. However, Stirk *et al*. (2004) found that seaweeds contain macro and micro-nutrients, amino acids, vitamins, cytokinins, auxins and abscisic acid thus affect cellular metabolism in treated seeds, leading to enhanced seedlings growth. In addition, seaweeds contain polysaccharides that are known to improve seedling growth in a similar way to hormones (Rolland *et al*., 2002).

The recorded reduction of chlorophyll content under salinity stress might be attributed to increased chlorophyll degrading enzyme (chlorophyllase) activity (Iyengar and Reddy, 1996), increased inhibition of

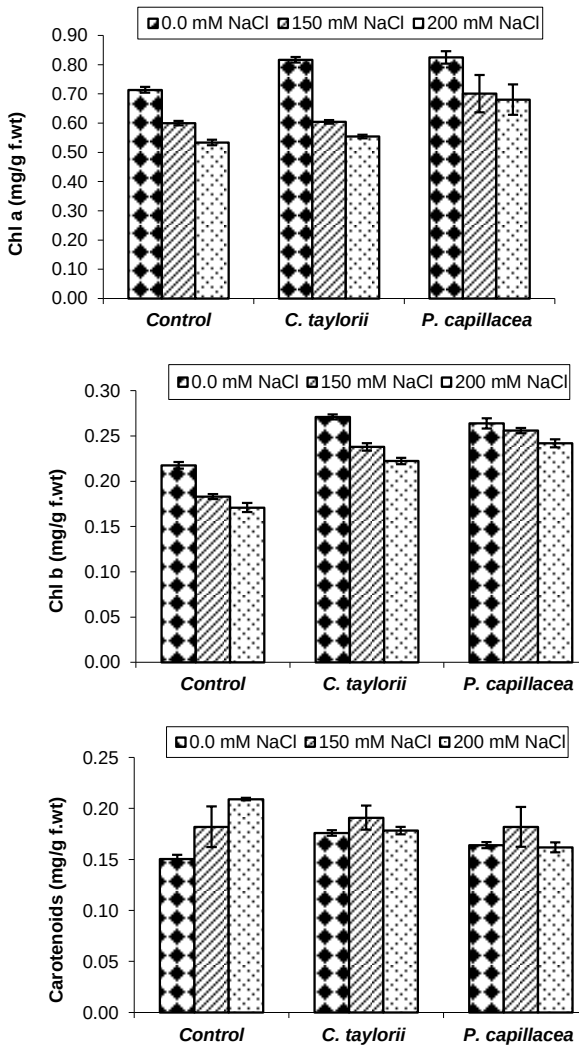

Fig. 3: Effect of seed priming with *C. taylorii* or *P. capillacea* extracts on the chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*) and carotenoids of radish plants (35-days-old) grown under 150 or 200 mM NaCl in clay-sandy soil (2:1 w/w). Value of each bar represents mean of three replicates with bars showing ± standard deviation

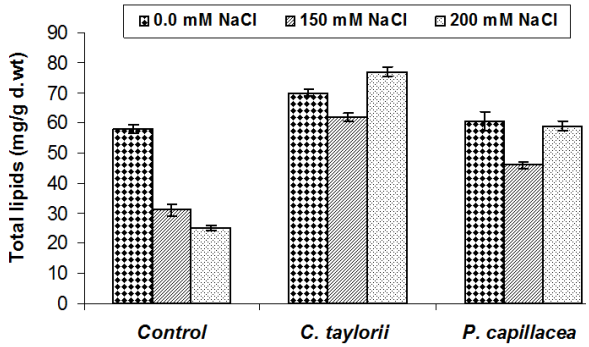

Fig. 4: Effect of seed priming with *C. taylorii* or *P. capillacea* extracts on the total lipids of radish leaves (35-days-old) grown under 150 or 200 mM NaCl in clay-sandy soil (2:1 w/w). Value of each bar represents mean of three replicates with bars showing ± standard deviation

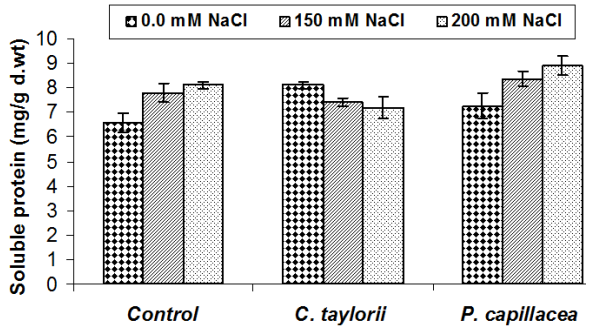

Fig. 5: Effect of seed priming with *C. taylorii* or *P. capillacea* extracts on the soluble protein of radish leaves (35-days-old) grown under 150 or 200 mM NaCl in clay-sandy soil (2:1 w/w). Value of each bar represents mean of three replicates with bars showing ± standard deviation

pigment synthesis as a result of breakdown of chlorophyll precursors, less accumulation of Mg ions and/or weakening of protein-pigment-lipid complexes (Sivasankaramoorthy, 2013). It has been reported that carotenoids level increased under salinity stress in wheat (Hamada, 1996) and lemon grass (Mane *et al*., 2010). The recorded increased level of carotenoids in radish plants under salt stress might be due to the protective role of carotenoids for chloroplasts from photo-oxidative damage by scavenging ROS (De Pascale *et al*., 2001).

The present results indicate that the photosynthetic pigments of the salt stressed and unstressed radish leaves were enhanced by seed priming with the extract of the two seaweeds (*C. taylorii* and *P. capillacea*). Similar results were recorded by Gharib *et al.* (2014) who attributed the increase in photosynthetic pigments and activity under salinity in rosemary primed with *Sargassum* to the role of seaweeds in the increase of cell metabolic rate and retarding of senescence by protecting chloroplasts from senescing and retarding chlorophyll destruction and/or increase chlorophyll biosynthesis due to high content of N and Fe in seaweeds (structural components of chlorophyll). However, Buchanan *et al*. (2000) claimed that seaweeds increase pigment content by maintaining membrane integrity through reducing lipase and lipoxygenase activities, which are involved in membrane breakdown.

The increased lipid production due to seaweed treatments in the present study (Fig. 4) may be due to the more stabilizing effect of the extract on membrane lipids and the enhanced activity of lipids synthesizing enzymes. Also, the increased lipid production as a result of the combination of the priming with seaweed extract and salt stress might be due to the decreased generation of ROS which cause lipid peroxidation and disrupts

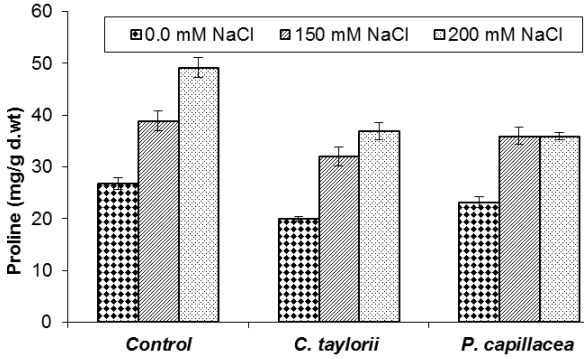

Fig. 6: Effect of seed priming with *C. taylorii* or *P. capillacea* extracts on the proline of radish leaves (35-days-old) grown under 150 or 200 mM NaCl in clay-sandy soil (2:1 w/w). Value of each bar represents mean of three replicates with bars showing ± standard deviation

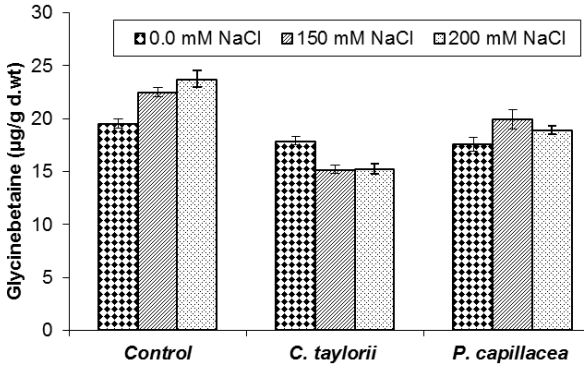

Fig. 7: Effect of seed priming with *C. taylorii* or *P. capillacea* extracts on the glycinebetaine of radish leaves (35-days-old) grown under 150 or 200 mM NaCl in clay-sandy soil (2:1 w/w). Value of each bar represents mean of three replicates with bars showing ± standard deviation

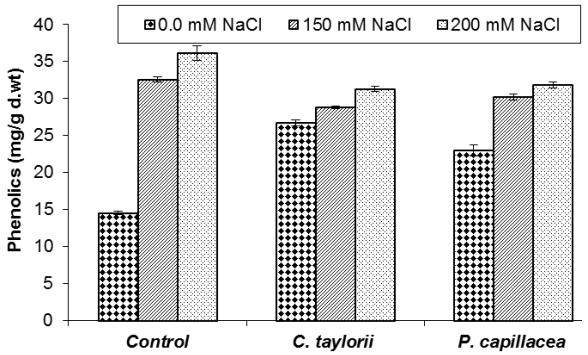

Fig. 8: Effect of seed priming with *C. taylorii* or *P. capillacea* extracts on the phenolic compounds of radish leaves (35-days-old) grown under 150 or 200 mM NaCl in clay-sandy soil (2:1 w/w). Value of each bar represents mean of three replicates with bars showing ± standard deviation

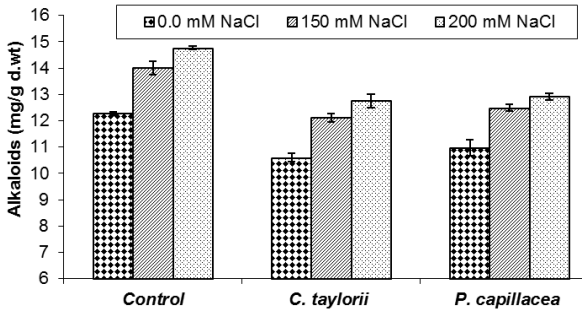

Fig. 9: Effect of seed priming with *C. taylorii* or *P. capillacea* extracts on the alkaloids of radish leaves (35-days-old) grown under 150 or 200 mM NaCl in clay-sandy soil (2:1 w/w). Value of each bar represents mean of three replicates with bars showing ± standard deviation

enzyme activity. Coinciding with these results, Rani and Usha (2013) reported that the seaweed treatment increased the accumulation of total lipids content in *Cassia angustifolia*.

The resultant increment in protein content of radish with salt treatments (Fig. 5) is in accordance with the findings of Kapoor and Srivastava (2010) in black lentil and Gomathi *et al.* (2013) in sugarcane. Such a rise in protein contents may be attributed to the increased availability and absorption of necessary elements (Ca, Na, K, Mg, N and Zn) present in the seaweed extracts (Sivasankari *et al*., 2011). Proteins that accumulate in plants under saline conditions may provide a storage form of nitrogen that is re-utilized later (Singh *et al*. 1987) and may play a role in osmotic adjustment. It has been concluded that a number of proteins induced by salinity are cytoplasmic which can cause alterations in cytoplasmic viscosity of the cells (Hasegawa *et al*., 2000). According to Elennany (1997), the increase in protein content by salt stress was due to reductions in proteolysis caused by salinity leading to slower depletion of reserve protein rather than enhancement in protein synthesis.

The resultant depression in proline content of radish by seaweed priming (Fig. 6) was associated with the resultant increase in the total soluble protein, which may mean that there was an integration of proline in protein synthesis. This result is in accordance with the results of Abdel-Aziz *et al*. (2011) and Latique *et al.* (2013) for *Amaranthus tricolor* and *Phaesolus vulgaris*, respectively. This decrease might be due to the reduction in proline precursor, glutamic acid, levels (Naidu *et al*., 1991).

The accumulated GB in the salt stressed radish plants was decreased by the priming with seaweed extracts (Fig. 7) which may be due the improvement effect of priming in the alleviation of the oxidative stress under salt stress. GB is known for its defensive effects in higher plants against salt/osmotic stress by maintaining osmotic potential adjustment (Ashraf and Foolad, 2007). Meanwhile, GB

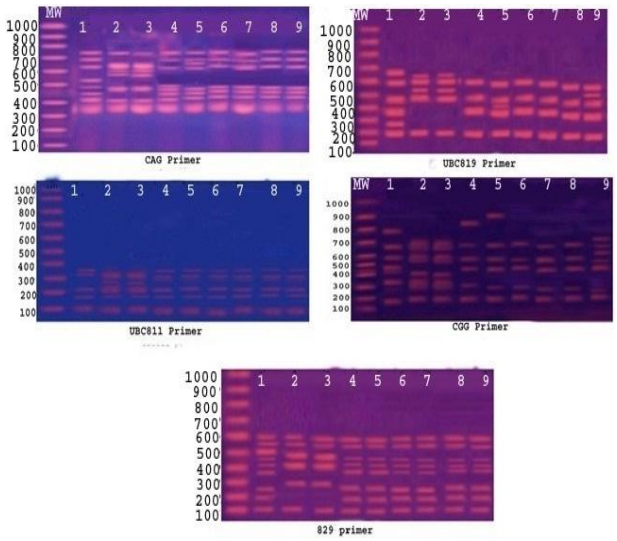

Fig. 10: ISSR fingerprinting profile produced by five primers showing the effect of seed priming with *C. taylorii* or *P. capillacea* extracts on the DNA patterns of radish leaves (35-days-old) grown under 150 or 200 mM NaCl in clay-sandy soil (2:1 w/w)

1= control, **2**= 150 mM NaCl, **3**= 200 mM NaCl, **4**= priming with *P. capillacea* extract, 5= priming with *C. taylorii* extract, **6**= priming with *P. capillacea* and grown at 150 mM NaCl, **7**= priming with *P. capillacea* and grown at 200 mM NaCl, **8**= priming with *C. taylorii* and grown at 150 mM NaCl and **9**= priming with *C. taylorii* and grown at 200 mM NaCl

could stabilize macromolecular activity and membrane integrity from the denaturing effects of high salt concentrations, and had a protective rather than osmotic effect (Ahmad *et al*., 2013). In addition, GB can scavenge ROS and induce expression of salt stress responsive genes (Liang *et al*., 2013).

The recorded increase in the total phenolic compounds in the seaweed primed-stressed radish plants is in harmony with the results of Lola-Luz *et al.* (2014), who reported that seaweed extract resulted in increasing of total phenolic compounds in broccoli. This increase could be due to osmotic stress or an increase in plant hormone activities. Thus, the induction of phenolic accumulation under salt stress, with and without priming, demonstrates that the induction of secondary metabolism is one of the defense mechanisms adapted by the plants to face saline environment (Radi *et al*., 2013). The increase in total alkaloid contents under salinity is parallel to the accumulation of proline. This could be due to the fact that ornithine, the precursor of alkaloids, and proline have the same precursor namely, glutamic acid (Ahmed and Leete, 1970). Therefore, salinity could inhibit the transamination reactions and hence the glutamic acid is accumulated and transformed to other nitrogenous compounds such as proline and ornithine. Proline was accumulated and ornithine was further transformed to alkaloid. However, the decline in alkaloids as a result of seaweeds application might be due to the high antioxidant capacity of the seaweeds which protect the plant from the harmful effects of ROS mediated during the metabolic activities of the plant.

The new proteins synthesized in response to salt stress in radish are referred to as salt shock proteins, which are suggested to protect the cell against the adverse effect of salinity (Hameed *et al*., 2010) and may have a role in providing tolerance or adaptation of plants to salt stress via assisting the cells to carry out their metabolic activities during adverse conditions (Elennany, 1997). The appearance of new bands in the seaweed primed radish plants (Table 2) may be due to the presence of a large number of bioactive compounds in seaweed extracts such as growth regulators (auxins, cytokinins and gibberellins), fatty acids, amino acids and mineral nutrients, that accordingly affect plant growth and cell division positively and be effective in the protein polypeptide expression (Sridhar and Rengasamy, 2011).

The results of ISSR patterns in the present study revealed the appearance of new bands in the salt stressed radish plants which may be due to the effect of the ion toxicity by NaCl, which enters the nucleus and induces signaling events leading to changes in gene expression and alterations in DNA remodeling (Zorb *et al*., 2009). Also, the disappearance of specific bands by salinity may be due the production of ROS that induces genotoxic damage and induce structural changes in DNA, such as chromosomal rearrangement, strand breaks, base deletions, pyrimidine dimers, mutations, cross-links and base modifications (Gill and Tuteja, 2010). However, priming of radish seeds with seaweed extract resulted in disappearance of some bands and this may be an outcome of a series of biochemical and molecular changes in the primed-stressed plants to adapt themselves to an altered gene expression (Wang *et al*., 2003).

Conclusion

Finally, it can be concluded that the seaweeds are potential excellent sources of highly bioactive materials that could be a useful method which alleviates salinity stress by induction of several secondary metabolites, synthesis of new proteins, DNA, changes in the number and percentage of bands in the protein profile and ISSR patterns in radish leaves.

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