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Full Length Research Paper

Impact of diatomite nutrition on two *Trifolium alexandrinum* cultivars differing in salinity tolerance

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Salt toxicity is one of the major problems in modern agriculture. Plants can employ silicon as a protective agent against stresses and the mechanisms of this process remain unknown. Two Egyptian clover (*Trifolium alexandrinum*) cultivars differing in salinity tolerance were used (Helaly, salt sensitive and Sarw1, salt tolerant). They were grown in pots filled with normal and saline soil (2000 and 3000 ppm) in the absence or presence of diatomites (0, 1.5, 3 and 4.5 g/kg soil). Results indicated that diatomite significantly offset the negative impacts of salinity and increased tolerance of sensitive cultivar of clover (Helaly) to salinity stress. Salinity decreased markedly all measured growth parameters (plant height and fresh and dry weight of fodder/pot), photosynthetic rate , the percentage of relative water content (%RWC), percentage of membrane stability index (%MSI), total photosynthetic pigment, and the contents of each of magnesium (Mg), potassium (K), phosphorus (P), and calcium (C) while it increased the levels of both amino acids, proline and sodium (Na) in both *T. alexandrinum* cultivars, however the effect was more profound on the sensitive line. Addition of diatomite at an upgraded rates solely or combined with both concentrations of NaCl significantly increased the above measured growth parameters, photosynthesis, %RWC, %MSI, total pigment and the accumulation of each of Mg, K, P and Ca. Moreover, it synergistically increased the content of total amino acids while, on the other hand, reduced the contents of proline and Na. Notably, the impact of diatomite in mitigating the deteriorative effect of salinity was clearly manifested more in sensitive lines of clover than in tolerant ones and under the higher dose of salinity (3000 ppm) as compared to the lower dose (2000 ppm). Moreover, diatomite fertilization either alone or interacting with salinity induced two distinctive protein electrophoratic bands (233 and 25 KD) which were absent in either the control or salinity stressed cultivars. In this respect, diatomite was most effective at 3 g/kg on Helaly and 4.5 g/kg on Sarw1 imposed to the higher dose of salinity (3000 ppm). Diatomite application either alone or combined with salinity induced several distinguished amplified DNA fragments in both clover cultivars using PCR- RAPD analysis , although the number of induced polymorphic DNA fragments were more in Helaly than in Sarw1. These results indicate that diatomite recovered and improved the morphologic, metabolic and biochemical status of both cultivars under salinity stress and especially the sensitive line (Helaly).

Key words: Diatomite, growth, proline, aminoacids, membrane stability index, relative water content, minerals, protein electrophorasis, RAPD-(DNA).

INTRODUCTION

Egyptian clover (*Trifolium alexandrinum L.*) is one of the most important economic cultivated crops in Egypt. It is used as a green fodder for graze and browse animal consumptions.

Excessive soil salinity, resulting from natural processes, crop irrigation with saline water or from poor subsurface drainage, occurs in many semi-arid to arid regions of the world where it inhibits plant growth and yield. Million hectares of agricultural areas as well as for the newly reclaimed lands is affected by varying degrees

Abbreviations: %RWC, % of relative water content; **%MSI,** % of membrane stability index **RAPD,** random amplified polymorphic DNA.

of salinity or sodicity (Lauchli and Epstein, 1990; Tahir et al., 2006).The major constraints for plant growth is the excessive uptake of mainly Cl^- and Na^+ as well as nutrients imbalance caused by disturbed uptake of nutrients (Tahir et al., 2006). To ensure food security and sustainable economy, there is dire need to find ways to improve salinity tolerance of various cultivated crops. Various chemical, physical and biological strategies are adopted for economic crop production on such soils (Tahir et al., 2006). Of all these strategies, exogenous application of nutrients has gained a considerable ground as a shotgun approach to ameliorate the adverse effects of salt stress (Tahir et al., 2006). Mineral nutrient applications that ameliorated the adverse effect of salt stress were either essential as K, Ca, N …etc. (Akram et al., 2007), or non-essential as Si (Tahir et al., 2006; Hanafy et al., 2008).

Diatomites de Mozambique (DDM) is a naturally occurring sedimentary rock primarily composed of fossilized remains of fresh water diatoms. It is chemically composed of SiO_2 (86 to 89%) in a soluble form available to plants and small amount of trace elements. It is considered as a complete, long lasting, recyclable, reusable and environmentally friendly soil fertilizer and enhancer by improving the physical structure of soil, aerating the plant's root zone, minimizing leaching and runoff thus increasing soil water retention and reducing watering. Subsequently, diatomite promotes stronger, healthier, higher-yielding plants that mature quickly and acquire self resistance against abiotic and biotic stresses (Kruger, 2006; Jessen, 2007; Abdalla, 2009; 2011).

Recent investigations showed Si efficiencies in mitigating salinity in various plants. For instance, the shoot and root growth was severely inhibited in rice grown at 100 mM NaCl but was significantly ameliorated by Si addition at 0.89 mM (Ahmad et al., 1992). Similar results were observed by Matichenkov and Kosobrukhov (2004) using wheat and barley. Another trial indicated that Si fertilization significantly improved dry matter and grain yield of two wheat genotypes differing in salinity tolerance (Tahir et al., 2006). Moreover, Si supply to roses imposed under different salinity levels significantly enhanced their vegetative growth, improved the overall plant appearance and resulted in a higher number of marketable flowers or plant (Ulmer, 2010). Si was also able to enhance the fresh and dry weights, plant height, girth, internode length, number of tillers and plants, number of fruits or plant and mean fruit weight (Ashraf, 2008; Savvas et al., 2009; Hashemi et al., 2010).

Silicon addition significantly correct the negative effects of salinity on chlorophyll a, b & total chlorophyll contents and on photosynthetic activity and leaf ultrastructural organelles of many tested plants which were badly damaged by salinity as it causes the disappearance of the plastid double membrane and the disintegration of the granae (Moussa, 2006; Liang et al., 2007; Savvas et al., 2009; Hashemi et al., 2010). Moreover, this element enhances salt tolerance in plants by enhancing the activity of antioxidant enzymes which, in turn, decreases the permeability of plasma membrane and in the mean time increases its integrity, stability and functioning (Liang et al., 2007; Savvas et al., 2009). Plants commonly respond to stress by increasing the production of amino acids and proline whereas Si treatment reduced them (Moussa, 2006; Kidane and Liang, 2008). A great benefit of Si application is that it can balance nutrient element in plant tissue through the suppression of Al, Mn and Na and by mediating the uptake of others, namely P, Mg, K, Fe, Cu and Zn (Chen et al., 2001). Tuna et al. (2008) found that the concentrations of Ca and K in both wheat cultivars (durum and bread wheat) were depressed under salinity but increased markedly in both shoots and roots after Si treatment. Na uptake was higher in plants grown under salinity, however Si application significantly reduced Na and Cl uptake, resulting in a significant increase in K: Na selectivity ratio in shoots (Tahir et al., 2006; Ulmer, 2010).

Several investigators reported that salinity reduced the synthesis of RNA, DNA and proteins which was correlated with increased amino acid contents (Youssef et al., 2003). Plants frequently produce a number of unique proteins either qualitatively or quantitatively as a part of their responses to environmental stresses (Orcutt and Nilsen, 2000). Some of these proteins were thought to belong to dehydrin group (25 to 60 KD), aquaporins (25 to 30 KD) or osmotin (25 KD). Dehydrins act as stabilizers that prevent or reduce the denaturation of cellular macromolecules under dehydrative conditions; aquaporins are membrane bound proteins that maintain cell turgor and water distribution within and among cells while osmotic play an important role in cell osmotic adjustment without perturbing its metabolic function (Campbell et al., 1998; Arora et al, 2000; Orcutt and Nilson, 2000). Salt stress enhanced the induction of a group of five proteins (24 to 26 KD) in rice tolerant supension culture (Shuguo et al., 2002). Si fertilization of stressed plants provide additional synthesis , under genetic control, of stress protein, antioxidant enzymes and phenols as protection molecules so as to ameliorate the deteriorative effect of stress on plants (Biel et al., 2008; Belanger, 2008). Cakir et al. (2005) used 184 SSR markers to make a genetic map, to identify tolerant lines of two wheat cultivars (Ducula-4 and Brookton) and to determine the genomic regions carrying abiotic stress tolerance genes within the wheat genome. They determined significant genetic regions for shoot and root growth under stress and normal conditions. For each trait four significant quantitative trait loci (QTLs) were identified. Notably, the most significant QTLs for stressed root and shoot growth were located on the long arm of chromosome 7B.

Recently, rapid progress has been made in cloning and characterization of the genes encoding Si-uptake and transporters in rice, maize, barley and cucumber

Table 1. Chemical constituents of diatomite.

SiO_2	89.00%
Al_2O_3	5.95%
Fe_2O_3	0.88%
CaO	0.10%
K_2O	0.63%
MgO	0.20%
Na_2O	0.32%
TiO_2	0.29%
H_2O	<3%

(Liang et al., 2005b; Mitani et al., 2008). This now makes it possible not only to study the complete transcriptomic responses of different plant species fed with Si under a variety of conditions, but also to compare this response among plants with different abilities to absorb Si. It is thus appeared that Si-treated plants responded much better to stresses by up-regulating a number of defense related genes and by displaying an overall better physiological activity than Si non-treated stressed plants (Belanger, 2008).

The present research was designed to test the appropriate concentration of diatomite which can mitigate the deletrious effect of salinity on growth, physiological and biochemical responses of two *T. alexandrinum* cultivars (Helaly and Sarw1) differing in salinity tolerance, to investigate whether stressed plants take up more diatomite than unstressed plants and lastly if the magnitude of alleviation is related to the severity of stress.

MATERIALS AND METHODS

Plant material and treatments

Seeds of *T. alexandrinum* Lymphocytic choriomeningitis virus (L.C.V). Helaly and cv. Sarw1 were obtained from the Agricultural Research Center, Giza, Egypt; examined for salinity tolerance in a preliminary experiment previously done and approved that the former cultivar is salt-sensitive while the later one is salt-tolerent. The seeds of both cultivars were sterilized with sodium hypochlorite (5%) for 5 min., and then washed thoroughly with distilled water. Afterwards, both seed cultivars were sown in 240 plastic pots (20 cm in diameter and 19 cm in depth) containing 2.5 Kg of garden soil per pot which were evenly mixed with 4 concentrations of DDM diatomite (0, 1.5, 3 and 4.5 g/kg soil). It's a natural diatomaceous earth originated from fossilized remains of fresh water diatoms with cell wall impregnated with silica. It had neutral pH and composed mainly of SiO_2 (86 to 89%) in a soluble form beneficial to plants (Table 1). 0.6 g/pot of seeds from both cultivars were sown in each pot and ten pots were specified for each concentration. Two weeks after sowing NaCl was added at three concentrations (0, 2000 and 3000 ppm) and accordingly the pots were arranged into 3 main groups. Each main group is subdivided into 4 subgroups for each cultivar (Helaly and Sarw1) as follows:

(1) The first main group constitutes 80 pots (40 pots for each cultivar) was kept as a control (NaCl-free) and was either containing or not containing diatomite (0, 1.5, 3 and 4.5 g/kg soil).
(2) NaCl at 2000 ppm was added to the second main group (40 pots for each cultivar) in the presence or absence of diatomite (0, 1.5, 3 and 4.5 g/kg soil).
(3) NaCl at 3000 ppm was added to third group (40 pots for each cultivar) in the presence or absence of diatomite (0, 1.5, 3 and 4.5 g/kg soil).

The experiment was conducted in greenhouse conditions at average day/night temperature 18/10° C ± 2, relative humidity 60 to 65% ± 2 and ambient light. The plants were harvested 90 days after sowing (DAS) at vegetative stage (first mowing). Ten plants were randomly selected for the measurement of growth criteria and gas exchange, another samples were taken (5 replicates/ treatment) and either oven-dried for determinations of amino acids, free proline and mineral elements or kept frozen for estimation of total chlorophyll, membrane stability index (MSI), total protein electrophorasis and RAPD analysis of genomic DNA.

Photosynthesis measurements

Photosynthetic rate was measured using an open gas portable photosynthesis system (LI-6400, LI-COR, BioSciences and USA). Measurements were performed on sunny days under natural light conditions and between 9 to 12 am on the uppermost fully expanded leaflets of 10 plants randomly chosen per treatment and expressed on a leaf area basis (Renault et al., 2001).

Biochemical analysis

The photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) were determined according to Metzner et al. (1965). Leaf relative water content (RWC) was estimated according at the method of Whetherely (1950). 0.5 g of fresh leaf material was weighed and placed in double distilled water for 4 h and then the turgid weight was recorded. It was finally dried in an oven at 65°C for 48 h and the dry weight was recorded. RWC was calculated as: $\%RWC = [(F.Wt - D.Wt) / (T.Wt - D.Wt)] \times 100$.

The membrane stability index (MSI) was determined according to Sairam et al. (2002). Two uniform sets of leaf discs were cut (0.1 g each) and placed in 10 ml of double distilled water. One set was kept at 40°C for 30 min and its conductivity recorded C_1 using a conductivity meter (HANNA HI 991300). The second set was kept in a boiling water bath (100°C) for 15 min. and its conductivity also recorded (C_2). Then, MSI was calculated as: $MSI = [1-C_1/C_2] \times 100$.

Free amino acids were extracted from leaf tissues and determined according to the method of Moore and Stein (1948), while free proline was determined according to Bates et al. (1973), A spectrophotometer of the Genway 6405 UV/Visible type was used for the determinations. Mineral elements were extracted from tissues similar to that of Chapman and Pratt (1961). Phosphorus was determined following the method described by Humphries (1956). Sodium and potassium were estimated photometrically according to Williams and Twine (1960). Calcium and magnesium were determined by atomic absorption spectrophotometer according to A.O.A.C. (1984).

Statistical analysis

Morphologic and gas exchange values are means ± standard error (SE) of 10 replicates while those of biochemical values are means ± standard error (SE) of 5 replicates. Significant differences were calculated using student's (t) test. SPSS was performed for multiple comparisons.

Table 2. The rapid primer names and sequences used in RAPD analysis.

RAPD primer name	Sequence (5' '3)
B05	5TGCGCCCTTC3
B09	5TGGGGGACTC3

Table 3. Changes in growth parameters in response to diatomite treatment of two *T. alexandrinum* cultivars subjected to salinity stress.

Salinity treatment	Plant cultivars	Diatomite treatment	Plant height	Weight of green fodder/pot	Drywt of fodder/pot
0	Helaly	0	40 ± 0.74	76.72 ± 0.67	8.67 ± 0.13
		1.5	49 ± 0.29	91.7 ± 0.53	9.34 ± 0.17
		3	53.4 ± 0.34	105.61 ± 0.71	11.66 ± 0.20
		4.5	56 ± 0.48	111.53 ± 0.93	12.8 ± 0.22
	Sarw 1	0	32.2 ± 0.31	66.28 ± 0.53	7.42 ± 0.14
		1.5	36 ± 0.35	69.28 ± 0.53	8.82 ± 0.19
		3	38 ± 0.47	84.0 ± 0.68	9.34 ± 0.22
		4.5	44.4 ± 0.53	87.25 ± 0.52	10.13 ± 0.16
2000 ppm	Helaly	0	28 ± 0.67	22.52 ± 0.36	4.23 ± 0.11
		1.5	30 ± 0.44	39.53 ± 0.42	5.63 ± 0.14
		3	37.8 ± 0.63	50.30 ± 0.51	6.31 ± 0.12
		4.5	43.2 ± 0.54	55.68 ± 0.54	7.14 ± 0.13
	Helaly	0	31.2 ± 0.46	33.45 ± 0.62	6.98 ± 0.16
		1.5	36 ± 0.49	58.83 ± 0.43	7.38 ± 0.19
		3	45 ± 0.51	61.87 ± 0.49	8.49 ± 0.18
		4.5	49 ± 0.52	68.97 ± 0.53	9.05 ± 0.20
3000 ppm	Helaly	0	22.6 ± 0.37	16.73 ± 0.57	2.93 ± 0.02
		1.5	25.4 ± 0.42	26.75 ± 0.63	3.36 ± 0.09
		3	31.8 ± 0.43	39.40 ± 0.54	4.08 ± 0.09
		4.5	35.0 ± 0.52	49.45 ± 0.34	5.53 ± 0.11
	Sarw 1	0	30 ± 0.27	30.20 ± 0.36	6.36 ± 0.12
		1.5	34.17 ±0.31	43.19 ± 0.48	7.05 ± 0.14
		3	40.6 ± 0.38	56.72 ± 0.55	8.04 ± 0.15
		4.5	46.2 ± 0.42	65.87 ± 0.68	8.93 ± 0.14

Electrophoratic analysis of total proteins

It was performed according to Laemmli (1970) using sodium dodecyl sulphate polyacrylamide gel electrophorasis (SDS PAGE). Staining of gel with silver nitrate was according the method of Goldberg and Warner (1997) and then scanning them using Gel doc 2000 system followed by analysation with software program.

Genomic DNA of egyptian clover leaves were extracted according to Graham and Henry (1997) and random amplified polymorphic DNA (RAPD) analysis was performed according to Williams et al. (1990) using 2 of 10-mer random primers obtained from Metabion AG Lena Christ Strasse, Martinstried, Deutschland as shown in Table 2.

Amplification reactions were performed in a final volume of 25 µl containing: 2.5 µl × PCR buffer, 125 µl 50 mM $MgCl_2$, 25 µl 2 mM dNTP, 0.625 µl 10 mM primer, 2 to 3 µl ng DNA, 1µg Tag polymerase and 16 µl Millipore H_2O. Reactions were performed using a Thermal cycler (Biometra, biomedizinische Analytic GmbH). The PCR was performed with the cyclic program of denaturation (one cycle) 94°C, 1 min at 37°C, 1min at 72°C and with a final extention of 10 min at 72°C PCR products were developed using ethidium bromide and visualized in UV using transilluminator.

RESULTS AND DISCUSSION

Growth responses

The data in Table 3 visualized a direct propotional relationship between diatomite treatment at all concentrations applied (1.5, 3 and 4.5 g/kg soil) and all measured growth parameters (fresh and dry weight of fodder/pot and plant height). The sensitive line of clover (Helaly) was more responsive to diatomite treatment than the tolerant one

(Sarw1). NaCl applied at both concentrations (2000 and 3000 ppm) progressivelydeclined these growth parameters in both lines of clover, although salinity was more effective on sensitive line (Helaly) than tolerant one (Sarw1). Supplementing diatomite at all concentrations (1.5, 3 and 4.5 g/kg soil) significantly increased plant height and fresh and dry weight of fodder/pot of both lines of clover grown under salinity stress. Its ameliorative effect was more pronounced on the sensitive line than the tolerant one. For instance, diatomite addition at 4.5 g/kg soil increased the dry wt. of fodder/pot by 147 and 195% (for Helaly) and 106 and 118% (for Sarw1) fewer than 2000 and 3000 ppm salinity, respectively. These results were in accordance with those of Tahir et al. (2006), Hanafy et al. (2008) and Savvas et al. (2009). It is proposed that diatomite lowered salinity stress by either coping with salinity in the rooting medium or inhibiting the mechanism of sodium transport to the leaves (Tuna et al., 2008). Moreover, Si could stimulate growth and yield under saline conditions by increasing plant water status, cell wall thickness, elasticity and strength thus preventing lodging and providing leaf erectness. It also increases the synthesis of RNA and DNA. This situation reduces transpiration and chlorophyll destruction, whereas it increases CO_2 assimilation rates which eventually resulted in an elevated rate of growth and yield (Hanafy et al., 2008; Abdalla, 2009; Ulmer, 2010).

Photosynthesis, relative water content, membrane stability index (msi) and some organic components

Raising salinity to 3000 ppm in the rooting medium of both *T. alexandrinum* cultivars reduced the photosynthetic rate, the % of relative water content and the membrane integrity as compared to untreated cultivars; however the effect was more profound on the sensitive lines. The inclusion of diatomite at upgraded rates (1.5, 3 and 4.5 g/kg soil) either alone or combined with salinity significantly raised the above measured increments. Notably, diatomite effect was more evident at high salinity levels (3000 ppm) and in the sensitive lines of clover (Table 4).

Similarly, there was a negative correlation between salinity stress and the content of total pigment, although a positive correlation was observed between salinity and the level of both amino acids and proline in both cultivars of clover. Salinity was much effective on the sensitive line (Helaly). Nonetheless, diatomite addition to the soil induced either significant increases in the values of total pigment and amino acids or decrease in free proline content as compared to unstressed or salinity stressed cultivars. Diatomite fertilization was beneficial in ameliorating the negative effect of salinity on sensitive line of clover as compared to either tolerant line (Sarw1) or non-salinized lines of both cultivars (Table 4). These results revealed that unstressed clover plants moderately respond to diatomite treatment. On the other hand, Si feeding caused stressed clover plants and specially the sensitive line to respond much better to salinity by increasing the physiological parameters and chemical components (photosynthesis, total pigment, %RWC, %MSI and amino acids) as compared to either salinity stressed (2000 and 3000 ppm) or unstressed clover plants of both cultivars. Similar results were obtained by Tuna et al. (2008) and Mukaram and Rahmatullah (2008) using two wheat varieties and Savvas et al. (2009) using zucchini squash. Accordingly, it appeals that the palliative effect of silicon on the inhibitory effect of salt stress can be induced through enhancing the activity of antioxidative enzymes for example, catalase, peroxidase and superoxidedismutase (Savvas et al, 2009; Abdalla, 2011) which results in a decrease in the permeability of plasma membrane thus maintaining its integrity, stability and functioning; an improvement in the ultrastructure of chloroplast which is accompanied by increasing chlorophyll content, photochemical efficiency and CO_2 assimilation rate thus increasing photosynthesis; and eventually a reduction in lipid peroxidation (Liang et al., 2007; Mukaram and Rahmatullah, 2008; Kosobryukhov et al., 2008; Savvas et al., 2009; Abdalla, 2009).

The increase in the percentage of leaf relative water content (%RWC) of both cultivars (Helaly and Sarw1) of clover in response to diatomite treatment under salinity stress was attributed to the deposition of Si as colloidal silica gel (SiO_2) in the xylem vessels and the cell wall of root, stem and leaves which thus restrict the bypass flow of transpired water that crosses the root cells towards the xylem vessels following an apoplastic pathway (Amador et al., 2005; Savvas et al., 2009) and also present a barrier to cuticular transpiration (Hull, 2004). Plants commonly respond to stress by increasing the production of amino acids and proline. Diatomite application caused a synergestic increase in the level of amino acids while it decreased proline accumulation. Diatomite has a beneficial role in increasing the contents of total soluble and insoluble proteins and induced new distinctive protein electrophoratic band (Abdalla, 2011). Proline has been considered as a carbon and nitrogen source for rapid recovery from stress and growth, a stabilizer for membranes and some macromolecules and also a free radical scavenger. Thus, it seems plausible that diatomite shows a protective role for clover plants to prevent them from being severely damaged by salinity stress (Moussa, 2006; Kidane and Liang, 2008).

Some mineral element contents

Application of salinity at both rates (2000 and 3000 ppm) induced a remarkable accumulation of sodium in both cultivars of *T. alexandrium* (Helaly and Sarw1), whereas it significantly reduced the amounts of each of potassium (K), calcium (Ca), phosphorus (P) and magnesium (Mg) above and below those of untreated cultivars respectively

Table 4. Changes in Photosynthetic rate, total pigment, amino acids, proline, of MSI and of RWC in response to diatomite treatment of two *T. alexandrinum* cultivars subjected to salinity stress.

Salinity treatment	Plant cultivars	Diatomite treatment	photosynthetic rate (u mole CO_2 m^{-2} S^{-1})	Chl (a+ b +c) $mg.g^{-1}$.fw	RWC	MSI	Amino acids (mg.g-1.DW)	Proline ($mg.g^{-1}$.DW)
0	Helaly	0	14.2 ± 0.63	0.68 ± 0.01	87.13 ± 0.31	86.3 ± 0.61	30.48 ± 0.24	4.95 ± 0.17
		1.5	14.8 ± 0.45	0.74 ± 0.013	88.20 ± 0.42	87.6 ± 0.77	31.78 ± 0.28	4.32 ± 0.26
		3	15.7 ± 0.25	0.79 ±0.020	89.13 ± 0.51	90.2 ± 0.76	32.96 ± 0.31	4.01 ± 0.13
		4.5	17.6 ± 0.55	0.85 ± 0.018	90.16 ± 0.63	91.9 ± 0.54	33.01 ± 0.38	3.85 ± 0.11
	Sarw 1	0	14.9 ± 0.38	0.85 ± 0.030	88.31 ± 0.64	85.4 ± 0.66	31.68 ± 0.23	5.15 ± 0.13
		1.5	15.6 ± 0.68	0.93 ± 0.043	89.11 ± 0.41	86.7 ± 0.62	32.74 ± 0.27	4.82 ± 0.21
		3	16.2 ± 0.73	0.96 ± 0.041	90.06 ± 0.37	88.9 ± 0.53	33.14 ± 0.38	4.33 ± 0.16
		4.5	18.3 ± 0.66	1.03 ± 0.038	91.64 ± 0.44	92.7 ± 0.48	34.69 ± 0.41	3.99 ± 0.26
2000 ppm	Helaly	0	11.3 ± 0.48	0.58 ± 0.031	76.9 ± 0.27	76.1 ± 0.49	31.44 ± 0.38	6.84 ± 0.24
		1.5	12.6 ± 0.33	0.62 ± 0.043	79.3 ± 0.36	78.8 ± 0.46	32.76 ± 0.42	6.03 ± 0.26
		3	13.8 ± 0.26	0.69 ± 0.027	81.6 ± 0.48	82.3 ± 0.37	34.94 ± 0.61	5.34 ± 0.30
		4.5	15.0 ± 0.38	0.71 ± 0.033	86.05 ± 0.55	85.9 ± 0.53	36.01 ± 0.56	4.83 ± 0.18
	Sarw 1	0	13.1 ± 0.42	0.76 ± 0.043	81.40 ± 0.68	79.3 ± 0.55	32.61 ± 0.78	7.89 ± 0.16
		1.5	14.9 ± 0.36	0.84 ± 0.039	83.05 ± 0.91	81.6 ± 0.62	35.96 ± 0.63	7.05 ± 0.14
		3	15.7 ± 0.34	0.89 ± 0.024	85.61 ± 0.74	83.8 ± 0.65	36.01 ± 0.54	6.73 ± 0.17
		4.5	16.8 ± 0.38	0.94 ± 0.033	87.91 ± 0.80	86.0 ± 0.49	38.94 ± 0.63	5.94 ± 0.10
3000 ppm	Helaly	0	8.8 ± 0.26	0.49 ± 0.041	62.40 ± 0.66	62.4 ± 0.38	36.84 ± 0.51	7.32 ± 0.13
		1.5	9.6 ± 0.23	0.53 ± 0.026	69.72 ± 0.99	67.9 ± 0.44	45.01 ± 0.54	7.01 ± 0.23
		3	11.9 ± 0.21	0.67 ± 0.037	73.45 ± 0.72	73.4 ± 0.58	47.94 ± 0.61	6.65 ± 0.24
		4.5	14.0 ± 0.28	0.70 ± 0.031	81.03 ± 0.73	80.9 ± 0.62	49.06 ± 0.63	5.42 ± 0.21
	Sarw 1	0	12.4 ± 0.31	0.66 ± 0.029	69.80 ± 0.81	74.3 ± 0.51	35.66 ± 0.62	8.41 ± 0.13
		1.5	13.8 ±0.25	0.71 ± 0.034	72.14 ± 0.46	77.8 ± 0.56	39.49 ± 0.59	7.84 ± 0.18
		3	14.6 ± 0.37	0.79 ± 0.036	76.31 ± 0.78	79.3 ± 0.36	42.06 ± 0.67	7.02 ± 0.19
		4.5	15.4 ± 0.42	0.88 ± 0.048	84.51 ± 0.53	82.6 ± 0.39	44.68 ± 0.78	6.19 ± 0.23

*The mean difference is significant at 0.05 level.

(Table 5). Addition of diatomite at all rates (1.5, 3 and 4.5 g/kg soil) either solely or combined with salinity induced a reverse situation. That is, it increased the accumulation of each of Ca, K, P and Mg while it reduced the content of Na significantly in both clover cultivars, although the effect was more pronounced on the sensitive line (Helaly) as compared to the tolerant one (Sarw 1) (Table 5). The increase in K: Na ratio in response to diatomite application to both clover cultivars grown under salinity stress was similarly reported by Matichenkov and Kosobrokhov (2004), Tahir et

Table 5. Changes in certain mineral element contents in response to diatomite treatment of two *T. alexandrinum* cultivars subjected to salinity stress values listed are expressed as mg.g^{-1} DW.

Salinity treatment	Plant Cultivars	Diatomite Treatment	Na	K	Ca	Mg	P
0	Helaly	0	6.59 ± 0.02	7.14 ± 0.06	3.60 ± 0.03	1.76 ± 0.01	2.93 ± 0.07
		1.5	6.14 ± 0.01	7.88 ± 0.09	4.38 ± 0.06	1.93 ± 0.03	3.06 ± 0.06
		3	5.86 ± 0.03	8.65 ± 0.07	5.28 ± 0.09	2.65 ± 0.04	4.28 ± 0.05
		4.5	5.21 ± 0.03	9.2 ± 0.05	6.75 ± 0.10	3.78 ± 0.06	5.16 ± 0.04
	Sarw 1	0	5.48 ± 0.04	6.95 ± 0.05	3.08 ± 0.06	1.98 ± 0.02	3.07 ± 0.03
		1.5	5.01 ± 0.02	7.31 ± 0.02	3.95 ± 0.08	2.08 ± 0.03	3.88 ± 0.02
		3	4.76 ± 0.01	8.02 ± 0.06	4.76 ± 0.11	2.99 ± 0.04	4.69 ± 0.05
		4.5	4.42 ± 0.03	9.00 ± 0.09	5.98 ± 0.13	4.06 ± 0.01	5.76 ± 0.07
2000 ppm	Helaly	0	9.44 ± 0.05	5.91 ± 0.11	2.64 ± 0.07	1.16 ± 0.01	2.68 ± 0.04
		1.5	8.93 ± 0.03	6.33 ± 0.09	3.05 ± 0.08	1.52 ± 0.03	2.92 ± 0.08
		3	8.02 ± 0.04	7.92 ± 0.07	3.82 ± 0.13	1.93 ± 0.05	3.86 ± 0.06
		4.5	7.10 ± 0.06	9.80 ± 0.12	4.32 ± 0.09	2.45 ± 0.01	4.98 ± 0.09
	Sarw 1	0	8.14 ± 0.02	6.23 ± 0.14	2.83 ± 0.11	1.02 ± 0.02	2.17 ± 0.01
		1.5	7.83 ± 0.09	7.03 ± 0.09	3.14 ± 0.14	1.83 ± 0.03	2.69 ± 0.03
		3	7.22 ± 0.10	8.93 ± 0.03	4.31 ± 0.12	2.46 ± 0.05	3.14 ± 0.04
		4.5	6.24 ± 0.09	10.21 ± 0.15	5.06 ± 0.03	3.12 ± 0.04	3.96 ± 0.05
3000 ppm	Helaly	0	11.85 ± 0.08	4.70 ± 0.06	1.94 ± 0.08	0.89 ± 0.01	1.03 ± 0.03
		1.5	9.94 ± 0.09	5.84 ± 0.18	2.28 ± 0.13	1.18 ± 0.03	1.88 ± 0.06
		3	8.15 ± 0.08	6.45 ± 0.12	2.91 ± 0.02	1.69 ± 0.02	2.67 ± 0.08
		4.5	7.72 ± 0.07	8.60 ± 0.14	3.68 ± 0.04	2.01 ± 0.01	3.84 ± 0.09
	Sarw 1	0	9.83 ± 0.08	4.90 ± 0.12	2.08 ± 0.06	0.74 ± 0.04	0.97 ± 0.02
		1.5	8.35 ± 0.03	6.03 ± 0.05	2.89 ± 0.08	0.86 ± 0.05	1.58 ± 0.05
		3	7.98 ± 0.06	7.23 ± 0.08	3.41 ± 0.09	0.93 ± 0.08	2.05 ± 0.06
		4.5	6.98 ± 0.05	9.06 ± 0.07	4.68 ± 0.07	1.35 ± 0.09	3.19 ± 0.09

*The mean difference is significant at 0.05 level

al. (2006), Liang et al. (2007) and Ashraf. (2008).

Low K uptake under saline and sodic conditions hampers crop production on these soils. K has a signify-cant role in improving plant water status and mitigating the toxic effects of Na. Si application enhanced K/Na selectivity ratio, causes the compartmentation of Na ions into vacuoles, alters ion microdistribution in roots and leaves and decreases the uptake and transport of Na while increases the uptake and transport of K thus even-tually mitigating Na ion toxicity and enhancing growth and dry matter accumulation (Tahir et al, 2006; Liang et al, 2007).

Chen et al (2001) claimed that a great benefit of Si application is that it can balance nutrient element in plant tissue through the suppression of Na, Al and Mn and by mediating the uptake of P, Mg, K, Fe, Cu and Zn. Moreover, Amador et al. (2005) realized that Si inclusion in salinized nutrient solution in which cowpea and kidney bean were grown reduced the accumulation of Na and Cl while increased the contents of both Ca and K in the roots and shoots of both plants. They concluded that Si inclusion in the nutrient solution is beneficial as it improves the growth, the physiological characteristics and may contribute to a more balanced nutrition by enhancing root activity which, in turn, enhances nutrient uptake under NaCl stress (Tesfagiorgis et al., 2008; Abdalla, 2009).

Biochemical genetic markers (SDS-protein electrophorasis)

SDS-electrophoratic patterns of leaf total protein of diatomite treated and untreated clover genotypes subjected to two levels of salinity (2000 and 3000 ppm) were shown in Table 6 and Figure 1. The total number of bands represented for all treatments were 27, although they were only 25 bands in salinity-treated cultivars. They have molecular weight ranged from 345 to 14 KD. Among such bands either 19 (due to diatomite treatment of both

Table 6. SDS - PAGE analysis of protein bands of leaves in response to diatomite treatment of two *Trifolium alexandrinum* cultivars subjected to salinity stress.

Band no.	M.W. (KD)	Band																							
		Helaly												Sarw 1											
		0				2000 ppm				3000 ppm				0				2000 ppm				3000 ppm			
		0	1.5	3	4.5	0	1.5	3	4.5	0	1.5	3	4.5	0	1.5	3	4.5	0	1.5	3	4.5	0	1.5	3	4.5
1	345	1.8	1.9	2	2.1	1.9	2	2.2	2.4	2.1	2.2	2.3	2.6	1.9	2.1	2.3	2.4	2.1	2.4	2.6	2.8	2.2	2.4	2.7	2.9
2	308	1.7	1.9	2	2.2	—	—	2.3	2.6	1.9	2.4	2.7	—	2	2.2	2.4	2.6	—	—	2.9	3	2.4	2.6	—	—
3	292	0.6	0.7	0.9	1	0.7	0.9	1	1.2	0.9	1.1	1.3	1.5	0.8	1	1.2	1.4	1	1.2	1.6	1.7	1	1.2	1.3	1.5
4	284	0.5	0.6	0.7	0.9	—	0.7	0.9	1.1	0.8	1	1.3	1.4	0.7	0.8	0.9	1.1	0.8	—	1.1	1.3	1	—	—	1.5
5	272	2	2.2	2.3	2.4	2.2	2.4	2.6	2.8	2.3	2.6	2.9	3	2.4	2.5	2.6	2.7	2.6	2.7	2.8	3	3.1	3.2	3.3	3.5
6	253	2.1	2.2	2.4	2.5	2.3	2.5	2.7	2.9	2.5	2.7	2.8	3	2.2	2.4	2.6	2.9	2.4	2.6	2.8	3.2	2.6	2.9	3.1	3.5
7	233	—	—	—	5.2	—	—	5.4	6.6	—	—	5.9	7	—	—	3.2	—	—	—	—	—	—	—	—	6.5
8	218	4.3	—	—	—	—	—	—	—	—	—	—	—	3.1	3.9	—	—	—	—	—	—	—	—	—	—
9	204	3.6	3.9	4.4	4.9	—	—	5	5.2	4.4	4.6	5.8	—	2.8	3.1	3.6	4.3	—	—	4.2	4.6	3.4	—	—	5
10	186	4.5	4.7	4.9	5.3	4.8	4.9	5.2	5.4	5.2	5.5	5.9	6.3	3	3.3	3.4	3.6	3.4	3.8	3.9	4.2	4.3	4.6	4.8	4.9
11	173	4.8	4.9	5.3	5.6	5.1	5.3	5.6	6.1	5.6	6.3	6.8	7	3.9	4.2	4.6	5.8	4.1	4.3	4.9	6.4	5	5.3	5.6	6.4
12	162	3.3	3.7	—	—	4		4.9	5.4	—	—	—	—	2.9	—	—	—	—	—	4.6	5	3.8	4.2	—	—
13	148	0.9	1.3	1.4	1.9	1.2	1.6	1.7	2	1.4	1.7	1.9	2.3	0.7	0.9	1.2	1.3	0.9	1.2	1.4	2	1.2	1.4	2.1	2.3
14	137	—	—	—	2.1	1.8	—	2.2	2.4	1.8	2	2.6	2.9	—	—	3	3.8	—	—	—	—	3.2	3.8	4.6	4.9
15	126	0.7	0.9	1.3	1.4	0.9	1.2	1.4	1.5	1.2	1.4	1.5	1.7	0.9	1.1	1.3	1.5	1.3	1.6	1.8	2.3	1.6	1.9	2.3	2.7
16	118	0.9	1.3	1.6	1.9	1.3	1.4	1.6	2.3	1.6	1.8	1.9	2.3	0.7	0.9	1.4	1.7	1	1.2	1.5	1.7	1.3	1.5	1.7	2.2
17	109	1.6	1.9	2.1	2.3	2.2	2.5	2.8	—	2.5	2.7	3	4.2	1	1.4	1.6	1.9	1.6	1.7	—	—	2.1	2.4	3	3.5
18	97	1.1	1.6	1.7	2	1.6	1.7	1.8	2.1	1.9	2.2	2.4	2.9	0.8	0.7	0.9	1.6	1	1.4	1.6	1.9	1.4	1.7	1.9	2.2
19	91	0.9	—	1.3	1.6	—	—	1.8	2.4	1.4	1.6	2.2	—	0.5	—	1.1	1.4	—	—	—	—	1.1	—	—	2
20	83	1	1.3	1.6	1.9	1.3	1.5	1.7	2.2	1.7	1.9	2.2	2.5	0.6	0.8	0.9	1.2	1.3	1.6	1.8	2.2	1.5	1.8	2.3	2.6
21	76	1.1	1.5	1.7	2	1.4	1.7	1.9	2.3	1.6	2.2	2.7	2.9	0.7	0.9	1	1.3	1	1.3	1.4	1.6	1.3	1.5	1.7	2.3
22	68	—	—	2	2.4	—	—	2.6	3	2.1	2.7	2.9	—	—	—	1.4	—	—	—	1.7	2.1	2	2.2	2.6	2.8
23	54	—	2.3	2.4	3.1	—	—	3	3.9	2.3	3	3.6	—	—	1.7	2	—	—	—	2.6	3.1	1.9	—	—	3.3
24	43	0.8	1.1	1.3	1.4	—	—	1.6	1.8	1.2	1.8	1.9	—	0.5	0.7	1	1.4	—	—	1.3	1.4	1	—	—	1.8
25	25	—	2.6	2.9	3.6	—	—	3.7	4.3	—	3.9	4.6	5.4	—	1.7	2.1	—	—	—	2.6	3.5	—	2.9	3	3.4
26	19	2.8	3.6	3.9	4.5	3.1	—	4.6	5.2	3.8	4.4	4.9	5.4	2	2.3	2.6	3	—	—	3.1	3.4	2.8	—	—	4.3
27	14	1.4	1.7	1.9	2.1	1.7	1.9	2.2	2.6	2	2.2	2.4	2.8	0.9	1.1	1.3	1.6	1.2	1.3	1.6	1.9	1.8	1.9	2.1	2.4
f bands		22	22	23	25	17	15	26	25	23	24	25	19	22	22	25	21	15	14	22	22	24	19	17	24
Peared		5	5	4	2	10	12	1	2	4	3	2	8	5	5	2	6	12	13	5	5	3	8	10	3
Bands		—	2	3	5	1	1	9	9	3	1	2	2	—	2	4	1	—	—	8	8	9	1	1	2

cultivars), 14 (due to salinized plants of both cultivars and sensitive cultivar (Helaly) imposed under salinity and remediated by different rates of

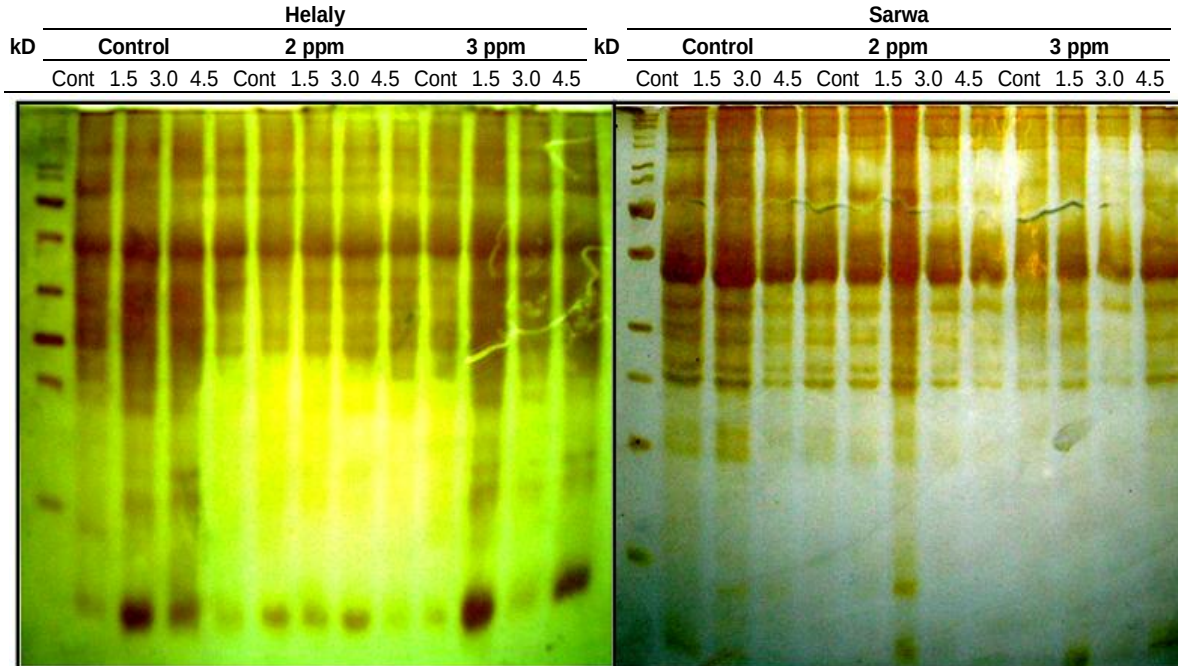

Figure 1. SDS-PAGE analysis of protein bands of leaves in response to diatomite treatment of two *Trifolium alexandrinum* cultivars subjected to salinity stress.

diatomite) or 13 bands (due to tolerant salinized cultivar treated with diatomite) were commonly detected while the other bands showed distinguishable variability in response to various treatments. Three newly synthesized protein bands having molecular weights 137, 68 and 54 KD were detected (in Helaly and Sarw1 grown under 3000 ppm NaCl and in Helaly (137 KD) under 2000 ppm, while 8 bands with 308, 284, 218, 204, 162, 91, 43 and 19 KD have dis-appeared (6 and 7 in Helaly and Sarw1 subjected to 2000 ppm while only 2 and 1 in Helaly and Sarw1 subjected to 3000 ppm, respectively) due to salinity treatment. Treating Helaly and Sarw1 by diatomite at 4.5 and 3 g/kg soil respectively induced two additional new protein bands having molecular weight 233 and 25 KD while less number of synthesized bands appeared due to diatomite treatment of both cultivars at 1.5 g/kg. On the other hand, 3 bands only disappeared with 218, 162 and 91 KD in both cultivars in response to diatomite application at the 3 rates as comparable to untreated control cultivars. When diatomite at 3 rates were added to salinity stressed cultivars of clover at both rates (2000 and 3000 ppm) the same 5 newly synthesized protein bands occurred (233, 137, 68, 54 and 25 KD) in both cultivars as those in diatomite treated unstressed cultivars. Diatomite was most effective at 3 g/kg on Helaly and 4.5 g/kg on Sarw1 imposed to the higher dose of salinity (3000 ppm).Reversibly, the greatest number of disappeared bands 7(308, 218, 204, 162, 91, 43 and 19 KD)occurred in Helaly treated with the lowest concentration of diatomite (1.5 g/kg) subjected to 2000 ppm salinity , while in Sarw1 the maximum number of disappeared bands were 8(308, 284, 218, 204, 162, 91, 43 and 19 KD) in response to the interactive effect of diatomite and salinity (1.5 g/kg + 2000 ppm and 3 g/kg + 3000 ppm). These results clearly manifest that diatomite fertilization either alone (at 4.5 g/kg in Helaly and 3 g/kg in Sarw1) or combined to salinity (3 g/kg and 4.5 g/kg + 2000 ppm and 4.5 g/kg + 3000 ppm in Helaly while 4.5 g/kg + 3000 ppm in Sarw1) recovered and improved the morphologic , metabolic and biochemical status of both cultivars of clover and especially the sensitive one (Helaly) by restoring and maintaining the protein bands and also re-establishing the amino acids that constitute the protein. The obtained induced and restored protein bands confirmed the significance of diatomite as a distinctive fertilizer in mitigating the deteriorative effect of salinity by accelerating the gene function to perform and encourage, in an elevated rate, new silence genes to operate which was determined by the new gene expression obtained using SDS-PAGE analysis (Table 6 and Figure 1).

Similar results were obtained by House et al. (2003). They found that five new protein bands (135, 70, 44, 36 and 24 KD) appeared in *Nitraria retusa* plants during summer. These proteins include dehydrins (25 to 60 KD) or osmotin (25 KD) which are important in plant adaptation to desiccation and ionic effects of saline conditions. Induction of dehydrin was effective so as to stabilize, prevent or reduce the denaturation of other cellular micromolecules under dehydrative conditions

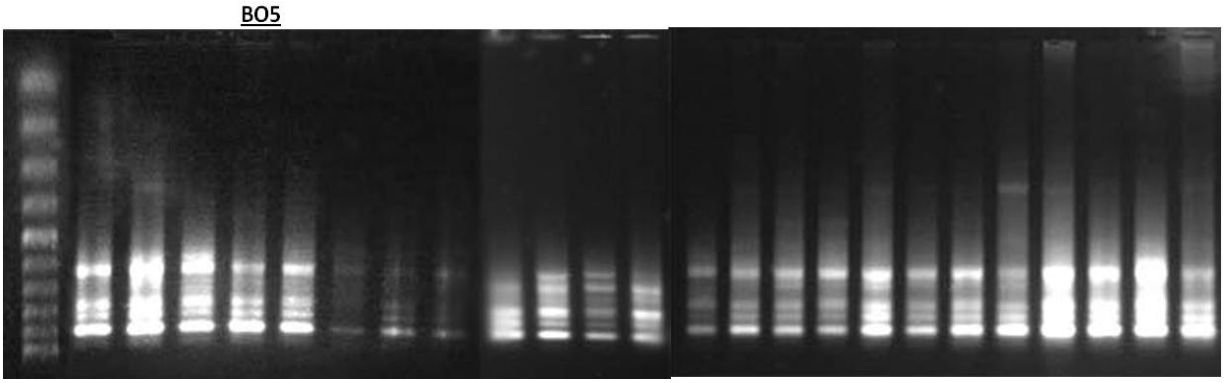

Figure 2. RAPD analysis of the polymorphic amplified bands in response to diatomite treatment of two *Trifolium alexandrinum* cultivars subjected to salinity stress using primer BO-5.

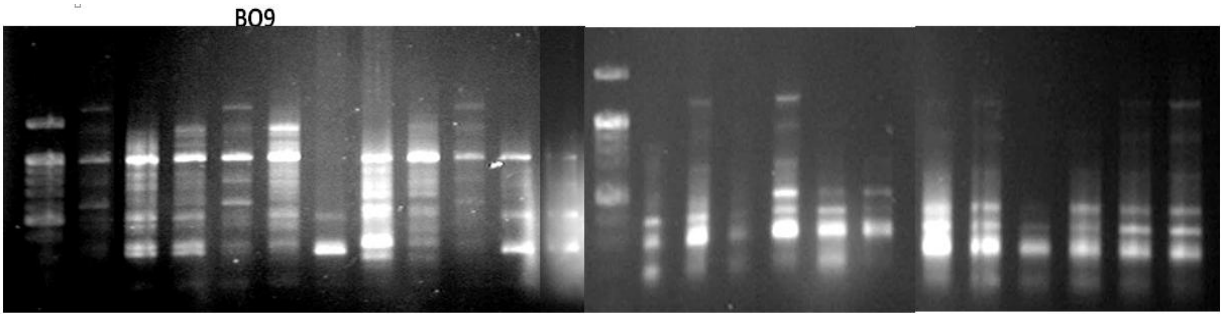

Figure 3. RAPD analysis of the polymorphic amplified bands in response to diatomite treatment of two *Trifolium alexandrinum* cultivars subjected to salinity stress using primer BO-9.

(Campbell et al., 1998). Osmotin is a type of responsive protein that plays an important role in osmotic adjustment to the cells and adaptive mechanism for salt and drought stresses either by facilitating rapid accumulation of proline and glycine betaine localized in the cytoplasm and acts as a non toxic osmaticum following osmotic adjustment to occur without perturbing metabolic function or by providing certain metabolic alterations in the cells which may be helpful in osmotic adjustment (Orcutt and Nilson, 2000; Youssef et al., 2003). Concerning diatomite, comparable results were reached by Abdalla, (2009) using Vicia faba plants. She detected three distinctive protein bands having molecular weights 289, 45 and 34 KD due to diatomite application of faba beans at 3 rates (2.5, 5 and 10 g/kg soil). In another trial using Lupinus albus plants, diatomite either alone or combined by water stress induced 6 new protein bands with 190, 152, 102, 92, 47 and 34 KD or a unique band with 92 KD successively (Abdalla, 2011). Collectively, these results revealed that diatomite addition to drought or salinity stressed plants not only recovered but even encouraged stressed plants to operate new silence genes so as to express new protein as a natural defense response to stress. Thus, in the present work, it appeals that dehydrin (25 to 60 KD) was induced under salinity stress of clover plants whereas both dehydrin and osmotin (25 KD) were induced in response to diatomite application either alone or combined by salinity which emphasizes the role of diatomite in remediating the negative effect of salinity.

Molecular genetic markers (PCR-RAPD analysis)

Two arbitrary random amplified polymorphic DNA (RAPD) primers; B05 and B09 were used to amplify the genotypes of untreated and diatomite treated *T. alexandrinum* cultivars (sensitive and tolerant) subjected to two levels of salinity (Figures 2 and 3; Tables 7and 8).

Using primer B05, a total of 13 amplified DNA fragments ranging in size from 970 to 136 bp were detected, among which 11 fragments were polymorphic while 2 were commonly detected in both untreated and variously treated samples. The number of amplified fragments differs from one cultivar to another indicating that not all clover cultivars are always identical in their DNA ability to be amplified. For instance, 4 amplified

Table 7. RAPD analysis of the polymorphic amplified bands in response to diatomite treatment of two *Trifolium alexandrinum* cultivars subjected to salinity stress using primer Bo5.

Band no.	Band size (bp)	Helaly												Sarw 1											
		0				2000 ppm				3000 ppm				0				2000 ppm				3000 ppm			
		0	1.5	3	4.5	0	1.5	3	4.5	0	1.5	3	4.5	0	1.5	3	4.5	0	1.5	3	4.5	0	1.5	3	4.5
1	970														6	13									
2	832																17								
3	700	13													9	16			11	18	20		14	21	27
4	413	9																							
5	324	10															46			69					
6	263		18	24	23	27	36	49	54																
7	245	11	16	18	23	14	22	29	34	15	23	31	39	14	18	23	29	17	28	37	45	21	29	34	56
8	227		9	11	16	10		17	21		18	21	27	10	14				22	26	28		28	39	44
9	207			14	22	17																			
10	192		8	13	17	29	19	26	34																
11	178	13									22	28	31	19	24	26	33	23	27	34	39		32	37	46
12	165	18									34	41	48	22	36	47	52	27	31	44	57		38	45	60
13	136	21	27	36	48	24	28	31	37	28	37	43	54	26	32	39	42	32	39	45	55	47	56	61	73
Total no. of bands		7	5	6	6	6	4	5	5	2	5	5	5	5	5	4	4	4	6	7	6	2	6	6	6

DNA segments of 263, 227, 207 and 192 bp were produced in the sensitive cultivar Helaly but not in the tolerant one in response to salinity treatment at the lower dose only (2000 ppm); the same 4 polymorphic DNA fragments (263, 227, 207 and 192 bp) were produced in Helaly in response to the different doses applied of diatomite while a total of another 4 amplified DNA fragments, (263, 227, 207 and 192 bp) were produced in Helaly in response to the different doses applied of diatomite while a total of another 4 amplified DNA fragments , (970 and 700 bp due to 1.5 and 3 g/kg while 832 and 324 bp due to the highest rate, 4.5 g/kg) were induced in Sarw1. When diatomite treated at all rates to salinized Helaly cultivar imposed to 2000 ppm , the amplified DNA fragments decreased to three (263, 227 and 192 bp) or one (227 bp) at 3000 ppm salinity. On the other hand, one distinctive fragment (700 bp) was induced in response to diatomite treatment of Sarw1 imposed to both grades of salinity (Figure 2 and Table7). When the oligonucleotide primer B09 was used, it produced a total of 12 amplified DNA fragments ranging in size from 1380 to 80 bp, among which 11 fragments were polymorphic while one was commonly detected in both untreated and differently treated samples. Two polymorphic DNA fragments (550 and 80 bp) were induced in response to the highest rate of diatomite while only (550 bp) was detected in response to the medium dose (3 g/kg) in Helaly. The same 2 DNA fragments were determined in Helaly in response to the interactive effect of the 2 higher rates of diatomite and salinity (4.5 g/kg + 2000 or 3000 ppm; 3 g/kg + 3000 ppm), while only one fragment was amplified (550 bp) in response to the lowest rate of diatomite and higher rate of salinity (1.5 g/kg + 3000 ppm) and another one (80 bp) in response to 1.5 g/kg + 2000 ppm. Concerning Sarw1, a unique DNA fragment (1380 bp) was amplified in response to diatomite treatment at 3 and 4.5 g/kg which did not exist in the control. Another two amplified DNA fragments were detected (900 and 300 bp), the first one was in response to diatomite treatment at all rates to salinized cultivars imposed to both grades (2000 and 3000 ppm) whereas the second DNA fragment was due to the highest rate of diatomite and both rates of salinity(4.5 g/kg+ 2000 or 3000 ppm). One distinctive polymorphic DNA (1380 bp in Sarw1 and 80 bp in Helaly) was induced due to salinity treatment at the low dose (2000 ppm). (Figure 3 and Table 8). It was manifested that diatomite fertilization either alone or combined with salinity induced the highest number of DNA amplified segments (especially in the sensitive cultivar Helaly) as compared to either untreated or

Table 8. RAPD analysis of the polymorphic amplified bands in response to diatomite treatment of two Trifolium alexandrinum cultivars subjected to salinity stress using primer Bo9.

Band no.	Band size(bp)	Helaly												Sarw 1											
		0				2000 ppm				3000 ppm				0				2000 ppm				3000 ppm			
		0	1.5	3	4.5	0	1.5	3	4.5	0	1.5	3	4.5	0	1.5	3	4.5	0	1.5	3	4.5	0	1.5	3	4.5
1	####	7	11		15	10	16	21								18	26	17							
2	####	10	16			13							22	17			32	23	28		36				
3	900	16	21	26	31	18	22	26	36	21	24	31	36						26	31	39	-	30	38	46
4	800	14	20	28	33	17	24	32	39	23	27	34	42	23	29	36		25							
5	700	8	12	17	23	11	19	26	34	17	22	34	46	13	19	23	27	16	22	27	39			33	41
6	550			12	20				24		16	22	28	10			19				25	18			
7	500	9	13	16	21	12	16	23	32	14	19	25	29	14	19	26		18	26	34				39	47
8	400	13	18	21	25	16	19	26		19		31	46	19	25	31	37	21	27	33	42	24	26	31	36
9	300	7	13		19	14	18	22	27	21	28	32	39												
10	250	11	16	19	22	16	19	25	29	22	28	34	44	12	17	27	31	19	26	33	39	23	34	46	54
11	180	6	10	13	17	10	14	18	22	13	18	26	31	9			23		18				26		
12	80				14	9	18	24	29				28	34	8		19			26	34				
Total no. of bands		10	10	8	11	11	10	10	9	8	8	9	11	9	6	6	8	7	7	6	7	3	4	5	5

salinity stressed cultivars which reflected the role of diatomite as a positive genetic marker for tolerance to stress conditions. In addition, the presence of different amplified DNA segments in the salt tolerant cultivar (Sarw 1) and their absence in the sensitive one (Helaly) using primers B05 and B09 might be related to salt tolerance and can be used as a genetic marker for salt tolerance in clover.

Similar suggestion was indicated by Abdel-Tawab et al. (1997) who were able to differentiate between salt-tolerance and salt sensitive sorghum genotypes by using RAPD markers. On the other hand, Si treatment to both abiotic and biotic stressed plantsresponded much better than unstressed plants by up-regulating a number of defense-related genes and by displaying an overall better physiological activity than non-treated plants which would indicate that the benefits of Si feeding are manifested primarily, if not exclusively under conditions of stress (Belanger, 2008). Accordingly, recent advances in genomics makes it possible not only to study the complete transcriptomic responses of different plant species fed with Si under a variety of conditions, but also to compare this response among plants with different ability to absorb Si (Belanger, 2008). Therefore, Si is instrumental in alleviating stress by:

(1) The formation of various organic defense compounds (protein, proline, soluble sugars, antioxidant enzymes, phenols) through alteration of gene expression (Epstein, 2008; Abdalla, 2009).
(2) Causing partial blockage of the transpirational bypass flow which reduces transpiration rate. This condition reduces osmotic stress and salt toxicity (Liang et al., 2004).
(3) Stimulating root plasma membrane and tonoplast and H^+-ATP activity which increases K and decreases N uptake and transport from roots to leaves. This situation increases K: Na ratio, alters the microdistribution of ions in roots and leaves and causes the compartmentation of salt ions into vacuoles thus reducing Na toxicity (Liang et al., 2007).
(4) Enhancing antioxidant enzyme and non-enzymatic activity which decreases lipid peroxidation and membrane permeability and, in turn, maintain the fluidity, structure, integrity and function of the plasma membrane (Hashemi et al., 2010).
(5) Complexation, co-precipitation or immobilezation of toxic metal ions with Si in the root cell vacuoles thus reducing Na toxicity in shoots (Liang, 2005).
(6) Deposition in leaf cell wall which enhances its rigidity, strength and extensibility especially in the growing region. These effects keep leaf erect thus increasing light interception and thereby increasing photosynthesis (Ma and Yamaji, 2006).

In the mean time, Si improved the ultra-structure of chloroplast which were badly damaged by salinity and enhanced photochemical efficiency thus improving photosynthetic activity (Kosobryukhov et al., 2008).
(7) Enhancing root elongation and activity and balancing the nutrient element in plant tissues through the suppression of the uptake of Al, Mn and Na toxicity while mediating the uptake of other nutritive elements as P, Mg, K, Fe, Cu and Zn, thus decreasing salt toxicity (Chen et al., 2001).
(8) Optimizing soil fertility through improving water, physical and chemical soil properties and maintaining nutrients in plant available forms thus reducing salt toxicity (Matichenkov and Kosobrukhov, 2004).
(9) Acting as a potentiator of plant defense responses or as an activator of strategic signaling proteins which may therefore interact with several key compounds of plant stress signaling systems, thus ultimately leading to induced resistance against biotic and abiotic stresses (Fauteux et al., 2005).

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