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# REINFORCING SALT TOLERANCE OF MARJORAM PLANTS BY FOLIAR APPLICATION OF PUTRESCINE

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## ABSTRACT

The aim of the present work was to study the effectiveness foliar application of putrescine [0.0, 10, 15 and 20 µM] in improving the salinity tolerance of marjoram plants. Marjoram plants grown under different levels of salinity [0.0, 10, 25 and 40% of sea water]. For this reason two pot experiments were conducted during 2001 and 2002 seasons. The obtained results clearly confirmed the absolute superiority of foliar application of 10 µ M putrescine treatment, which significantly increased plant height, number of branches/plant, herb fresh and dry weight /plant, essential oil percentage [ml/100 g F.W] and oil yield [ml/plant] under normal or saline conditions up to 25% sea water. The data also revealed that tolerance, which was much pronounced as a result of foliar application of 10 µM treatment, was more negative osmotic potentials (Osmotic Adjustment) through the accumolation of much more quantities of inorganic osmotica, i.e. N,P,$K^{+}$,$Mg^{+2}$, and the highest $K^{+}/Na^{+}$ ratio as well as the lowest $Na^{+}$ / $Ca^{+2}$ ratio in addition to considerable accumulation of organic protective osmolytes, i.e. sugars, proline, and free amino acid in their growing tissues which greatly exceeded the all other treatments ;especially untreated controls up to 25% Sea water level. This was accompanied with an endogenous hormonal status, in favor of increasing tolerance to salinity, i.e. the accumulation of IAA ,$GA_3$ and ABA in the stressed shoots. Such behavior seems to induce more ability for marjoram plants to continue their growth till maturity even under 25% sea water level of salinity. The obtained data suggested the successful possibility of foliar application of di-amine putrescine to improve salinity tolerance of the marjoram plants.

**Keyword:** Medicinal and aromatic plants, marjoram, salinity, sea water, diamin putrescine, nutrients, phytohormones.

## INTRODUCTION

Medicinal plants occupy a prominent position, because of the increasing demand of the local industry and export. In order to cover such increase, an increasing interest in the cultivation of medicinal and aromatic plants has been shown in Egypt. Recently, a considerable attention has been directed to the expansion of aromatic and medicinal plants in the newly reclaimed areas which represents the great hope in increasing our cultivated land and consequently the economic agricultural production. On the other hand, and due to the restricted resources of the fresh water from the River Nile, the use of saline water or even diluted sea water becomes one of the sources of irrigation water in such newly cultivated areas. Thus, it is requisite to improve the salinity tolerance of such medicinal and aromatic plants and consequently enhancing their ability to tolerate salinity which, in turn,

increasing the possibility of their successful cultivation in such newly reclaimed areas.

Marjoram plants [*Majorana hortensis* L.] is considered as an, important medicinal crop in Egypt and has great applications. The air dried leaves and flowering tops are used as condiment for seasoning soups dressing, stwes, poultry and sausage. It is also used as a stimulating in tooth pastes, whooping cough and larynx infections.

The diamine putrescine, the polyamines spermidine and spermine have been frequently described as endogenous plant growth regulators or intracellular second messengers mediating the effects of phytohormones (Bestford *et al.*, 1991). Moreover, the same auther added that the diamine putrescine and the polyamines spermidine and spermine are involved in important biological processes, e.g. ionic balance and DNA, RNA as well as protein stabilization. It is generally accepted that polyamines are associated with cell division and with active growth and metabolism (Kaur-Sawmey and Galston 1999).

Putrescine is very often a characteristic of a stress response. Although this accumulation could play a protective role in the cell (Krishnamurthy 1991). Furthermore, Torrigiani *et al.* (1993) concluded that two enzymes at least are responsible for the formation of putrescine; ornthine decarboxylase (ODC) and arginine decarboxylase (ADC). Willadino *et al.*. (1996) showed a significant increase in total polyamine content, especially caused by a rise in putrescine of maize embryogenic calluses at high-salt concentrations [1.2-2.0 % NaCl]. Recently, Hanafy *et al.*. (2002) and Fatouh (2003) found that the application of putrescine brought about a considerable alleviation of salinity stress injury and increased the shoot growth and yield of myrtle and wheat plants.

In this paper, we report the effectiveness of the treatment with foliar application of putrescine on improving salinity tolerance of the marjoram plants grown under different levels of salinity [% sea water].

## MATERIALS AND METHODS

Two pot experiments were conducted in the plant physiology Devision, Faculty of Agric., Cairo Univ. and the Medicinal and Aromatic plant Research Dept., Horticulture Research Institute [HRI], Agricultural Research Center [ARC] during the two successive seasons 2001-2002. Marjoram seedlings [50 days after germination] were obtained from the experimental station of the Horticulture Research Institute in south AL-Tahrir.

In both experiments, marjoram seedling [12-15 cm in height, with 10-12 leaves] were transplanted on the of March, 18$^{th}$ 2001 in the first season and on the of March, 11$^{th}$ 2002 in the second one in pots of 30 cm. in diameter were filled with a mixture of 2:1 clay and fine sand. Only one vigorous seedling was left to grow in each pot. Four levels of salinity at the rate of 0.0, 10, 25 and 40% sea water, obtained from Mediterranean Sea, Gleem rigion, Alexandria [EC=51.56, ds/m=32998.4 ppm about 33000 ppm] were applied with each treatment of foliar application of putrescine, 4

treatments with 3 replictes each of which included 36 plants were used each as follows:

1- The control treatment [plants spraying with distilled water]
2- 10 µM 3- 15 µM 4- 20 µM

The pots were irrigated with water [7 days from planting] and then, the plants were equally irrigated either with tap water [in the control treatment] or with one of the different concentrations of sea water when needed. The plants were weekly sprayed with putrescine [started 2 weeks after planting]. Pots were fertilized with 2.1 g superphosphate [15.5% $P_2O_5$], 1.4g potassium sulphate [48% $K_2O$] and 2.8 g Ammonium sulphate [20.5% N].

Three cuts [samples] of 12 plants from each treatment were taken after 90, 150 and 210 days from planting by cutting the vegetative parts of all plants 5 cm above the soil surface. Plant height, number of branches/plant and herb fresh and dry weight/plant were recorded.

The mean values of growth determined in the two seasons were statistcally analyzed and compared using L.S.D. values at 5% level (Gomez and Gomez, 1984).

**Chemical analysis:**

The shoot was chemically analyzed in order to determine their chemical constituents.

For the detrmination of total nitrogen, the modified "Micro Kjeldahll" apparatus of Parnas and Wagner as described by Jones *et al.* (1991) were used. For total P, K, Ca, Na and Mg determinations the wet digestion of plant material was carried out as recommended Piper (1947). Phosphorus was estimated colorimetrically using the stannous chloride reduced molybdophosphoric blue color method in sulphoric acid system as described by Jackson (1973). K, Na, Ca and Mg were determined by the Atomic Absorption Spectrophotometer [GBC, 932 AA].

Hot ethanol extract was used for determination of total sugars using the phosphomolybdic acid method (A. O. A. C. 1975). Total free amino acids were determind using ninhydrin reagen (Moor and Stein, 1954). Free proline concentration in the fresh material was determined colorimetrically using sulphosaticylic acid ninhydrin method as described by Bates *et al.* (1973).

Extraction of plant hormones was carried out according to Sadeghian (1971). Methanolic extract of marjoram leaves collected in the 2$^{nd}$ cut were used for endogenous hormones estimation by Gas-Liquid Chromatography (GLC) (ATI-Unicam- 610 Series) according to the method described by Vogel (1975). The glass column (1.5m X 4mm) was packed with 1% OV-17. Temperature: injector 260°C, detector 300°C and column initially for 3 min. at 200°C then increased to 220°C (rate 20(C/min) for 4 min., then increased again to 240°C (rate 20°C/min) flow rates; carrier gas ($N_2$ special) 30 ml/min, hydrogen special 33ml/min. and synthetic air 330 ml/min. and the chart speed 1 cm/min.

The oil percentage was determined in the fresh herb using the method described by the Egyptian Pharmacopoeia (1984), and the essential

oil yield per plant was calculated in proportion to the herb fresh weight [oil yield/plant = plant fresh weight X oil percentage].

Chemical analysis for essential oil was conducted by using Ati-Unicum gas Liquid Chromatograph [GLC], 610 Series, to determine their main constituents as described by Gunther and Joseph (1978).

It is to be mentioned here that, only the second cut [150 days after planting] was taken for chemical analysis. Moreover the average values of the various characters determined in the two successive seasons were only tabulated and discussed in the present investigation.

## RESULTS AND DISCUSSION

**1-Growth characters:**

The results in Table (1) generally indicated that all growth characters; plant height, No. of branches/plant, herb fresh and dry weights (g/plant) in all cuts (samples) dates [90, 150 and 210 days after transplanted (seedling)] significantly decreased as the salinity level [% sea water] increased. These results are in agreement with those previously stated by Yossef and Rady (2000) on rosemary plants and Abdel-Hafiz Ahmed (2001) on marjoram plants. These results might be ascribed to the high osmotic pressure of soil solution which restricted the absorption of water by plant root and/ or to the toxic effects of certain ions in soil solution. Besides, salinity has been shown to reduce the synthesis of DNA, RNA and protein in many plants which might lead to disturbance in metabolic activities, cell division and elongation and the activities of the mitochondria and chloroplasts were reduced. This explanation is in agreement with those stated by Gallab and Nesiema (1999), EL-Fouly *et al.* (2001) and Salem *et al.* (2002).

On the other hand, in all cuts, as the rate of putrescine increased, all growth characters significantly decreased when compared with control treatment [0.0 putrescine] except for the lower level [10μM] which induced an opposite trend. At 10 and 25% sea water, in all cuts, treatment with 10 μM produced significantly higher values of the studied growth parameters when compared with the control untreated plants, while at 40% sea water a negative trend was elicited. These increments, at 25% sea water, attained 24.1, 29.9 and 29.2% for herb fresh weight [g/plant] and 23.7, 30.8 and 25.2% for herb dry weight [g/plant] in first, second and third cuts, respectively, as compared to the control. Also, at the higher rates [15 and 20 μM] of putrescine under all salinity levels, all growth characters of marjoram plants were not significantly changed as compared with the control treatment. This clearly indicates that the highest levels of putrescine have an inhibitory effects on the growth of marjoram plants.

**Table (1): Effect of putrescine foliar application treatments on the growth characters of marjoram plants grown under different levels of salinity [% sea water] combined analysis for two season.**

| Putrescine (µM) | Cuts | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1st | | | | 2nd | | | | 3rd | | | |
| | % Sea Water | | | | | | | | | | | |
| | Plant height (Cm.) | No. of branches /plant | Herb fresh weight (g/plant) | Herb dry weight (g/plant) | Plant height (Cm.) | No. of branches /plant | Herb fresh weight (g/plant) | Herb dry weight (g/plant) | Plant height (Cm.) | No. of branches /plant | Herb fresh weight (g/plant) | Herb dry weight (g/plant) |
| | Control | | | | | | | | | | | |
| Control | 27.79 | 15.11 | 24.83 | 8.19 | 33.15 | 21.16 | 49.25 | 22.32 | 31.12 | 24.13 | 65.61 | 27.81 |
| 10 | 33.85 | 19.23 | 32.46 | 10.89 | 40.92 | 26.72 | 66.53 | 28.38 | 36.19 | 31.11 | 87.34 | 35.74 |
| 15 | 23.33 | 12.23 | 21.64 | 6.21 | 28.64 | 18.83 | 41.82 | 19.72 | 25.92 | 21.45 | 56.31 | 23.63 |
| 20 | 21.69 | 10.17 | 19.79 | 5.42 | 26.79 | 15.54 | 37.45 | 15.11 | 22.75 | 17.61 | 49.25 | 18.79 |
| | 10% | | | | | | | | | | | |
| Control | 24.65 | 13.85 | 18.75 | 6.34 | 29.29 | 18.29 | 41.85 | 19.45 | 26.72 | 20.69 | 56.39 | 22.61 |
| 10 | 29.42 | 16.91 | 23.81 | 7.99 | 36.62 | 22.54 | 52.11 | 23.82 | 31.64 | 25.77 | 68.11 | 27.14 |
| 15 | 19.35 | 10.84 | 16.29 | 5.11 | 25.62 | 14.32 | 34.43 | 15.62 | 21.43 | 17.22 | 44.31 | 17.83 |
| 20 | 16.79 | 8.79 | 12.81 | 4.22 | 22.43 | 12.61 | 30.81 | 12.75 | 16.71 | 14.91 | 36.72 | 12.65 |
| | 25% | | | | | | | | | | | |
| Control | 22.14 | 11.95 | 15.96 | 5.11 | 26.89 | 16.95 | 35.66 | 15.57 | 21.92 | 18.65 | 49.19 | 18.89 |
| 10 | 27.26 | 14.92 | 19.81 | 6.32 | 31.62 | 19.44 | 46.31 | 20.36 | 27.31 | 23.14 | 63.53 | 23.64 |
| 15 | 16.73 | 7.62 | 13.45 | 4.02 | 20.81 | 10.61 | 27.52 | 13.83 | 17.83 | 14.83 | 32.11 | 13.69 |
| 20 | 12.69 | 5.33 | 10.53 | 3.13 | 18.65 | 9.01 | 23.84 | 8.65 | 14.11 | 10.02 | 28.53 | 10.49 |
| | 40% | | | | | | | | | | | |
| Control | 19.95 | 8.14 | 10.89 | 3.95 | 22.95 | 12.25 | 27.54 | 11.92 | 17.75 | 15.98 | 39.42 | 13.91 |
| 10 | 15.69 | 6.45 | 8.63 | 2.81 | 18.42 | 9.52 | 23.11 | 9.11 | 15.39 | 11.72 | 31.25 | 11.73 |
| 15 | 13.46 | 5.33 | 6.94 | 2.02 | 16.61 | 8.45 | 21.79 | 8.01 | 11.65 | 9.45 | 26.19 | 8.92 |
| 20 | 10.55 | 4.29 | 5.89 | 1.75 | 13.52 | 6.89 | 18.81 | 6.74 | 10.01 | 8.11 | 24.85 | 6.75 |

**Valus labelled with (*) are significant according to L.S.D. valus at 0.05%.**

The beneficial effects of diamine putrescine in the plant growth and metabolism and consequently the positive effect of its exogenous application on the salt-stressed plants due to the considerable alleviation of stress. Injury induced by sea water irrigation was strongly obtained by several authors Walter (1995), Lima *et al.* (1999) and Lefevre and Lutts (2000). In this concern, David (1996) mentioned that, the diamine and polyamines are involved in important biological processes, e.g. ionic balance and DNA, RNA and protein synthesis, also polyamines are currently considered to be regulators of plant growth and development owing to their effects on cell division and differentiation. Moreover, Willadino *et al.* (1996) who reported that stimulation of shoot growth under saline conditions induced by putrescine may be due to its effects of the synthesis of macromolecules for polyamines which are known to increase nucleic acids synthesis and stimulate various processes associated with the synthesis of protein as well as promotion of cell division. Recently Fatouh (2003) found that weekly spraying either 10 µM putrescine or 2ppm ABA and 15 KR gamma rays significantly incresed all growth characters of wheat plants under normal or saline conditions up to 30% sea water.

The produced marjoram plants from 10 µM putrescine treatment were tolerant up to 25% salinity and were able to continue their growth till maturity.

**2- Chemical analysis:**

**a-Essential oil percentage and oil yield:**

The obtained data in Table (2) clearly indicated that, the essential oil percentage and oil yield [ml/plant] gradually increased in the untreated plants as salinity increased. The highest essential oil percentage and oil yield in leaves reached 0.89% and 0.32 ml/plant respectively, at 25% sea water followed by 0.61% and 0.26 mL/plant at 10% sea water followed by 0.87% and 0.24 mL/plant at 40% sea water compared to the lowest value 0.45% and 0.22 mL/plant at the control treatment. The increase in essential oil percentage and oil yield under salinity stress may be attributed to increase in the number of oil glands per unit area. Higher specialized structures containing secretory and accumulatory elements [oil cells, glandular trichomes and glandular epidermis] were probably stimulated as a method of protection against salinity hazard. This suggestion is in agreement with those reported by Youssef and Rady (2000) and Hanafy *et al.* (2002).

Also, the results presented in Table (2) clearly showed that treatment with 10 µM putrescine considerably increased the essential oil percentage and oil yield over control under normal or saline conditions up to 25% sea water followed by a sharp decrease under 40% sea water and all other putrescine treatments. This may be due to an increase in metabolic activities leading to the enhancement of sugars content, which may be considered as antisenescence effect of putrescine, as sugars are considered the precursor for the formation of essential oil.

**Table(2): Effect of putrescine foliar application treatments on essential oil percentage; oil yield; concentrations of total sugars (TS) as mg glucose/g. F.W.; free amino acids (AA) and proline (P) as mg /g. F.W.; indol-3-acetic acid (IAA), gibberlic acid ($GA_3$) and abscisic acid (ABA) as µg /g F.W.; N, P, K, Na, Ca and Mg as mg/g. D.W. as well as K/Na and Na/Ca ratios in the shoots of marjoram plants grown under different levels of salinity (% sea water) [combained analysis for two seasons].**

| Putrescine (µM) | % Sea Water | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Oil % mL/100 F.w. | Oil yield ml/plant | TS | P | AA | IAA | $GA_3$ | ABA | N | P | K | Na | Ca | Mg | K/Na | Na/Ca |
| | Control | | | | | | | | | | | | | | | |
| Control | 0.45 | 0.22 | 4.49 | 0.25 | 2.56 | 11.71 | 9.91 | 2.81 | 33.41 | 4.62 | 35.31 | 2.65 | 4.52 | 2.95 | 13.32 | 0.59 |
| 10 | 0.57 | 0.38 | 4.85 | 0.40 | 2.78 | 12.23 | 10.66 | 2.93 | 35.63 | 4.83 | 38.40 | 2.91 | 4.85 | 3.25 | 13.19 | 0.60 |
| 15 | 0.43 | 0.18 | 5.67 | 0.59 | 2.95 | 12.45 | 10.81 | 3.11 | 36.15 | 4.95 | 40.55 | 3.51 | 5.21 | 3.61 | 11.55 | 0.67 |
| 20 | 0.37 | 0.14 | 5.81 | 0.71 | 3.11 | 12.56 | 10.94 | 3.33 | 38.42 | 5.21 | 43.72 | 4.99 | 5.63 | 3.85 | 9.61 | 0.89 |
| | 10% | | | | | | | | | | | | | | | |
| Control | 0.61 | 0.26 | 5.53 | 0.39 | 3.75 | 10.21 | 8.79 | 2.94 | 30.11 | 4.31 | 32.32 | 5.11 | 5.94 | 3.52 | 6.32 | 0.86 |
| 10 | 0.83 | 0.48 | 7.81 | 0.55 | 5.61 | 14.25 | 11.31 | 4.56 | 38.41 | 5.89 | 45.61 | 3.75 | 7.62 | 5.11 | 12.16 | 0.49 |
| 15 | 0.55 | 0.19 | 5.11 | 0.26 | 3.51 | 9.73 | 7.82 | 2.81 | 27.32 | 3.91 | 28.43 | 5.41 | 4.77 | 3.31 | 5.26 | 1.13 |
| 20 | 0.49 | 0.15 | 4.95 | 0.21 | 3.32 | 9.11 | 7.45 | 2.53 | 25.11 | 3.54 | 25.31 | 6.92 | 4.46 | 3.11 | 3.66 | 1.55 |
| | 25% | | | | | | | | | | | | | | | |
| Control | 0.89 | 0.32 | 5.89 | 0.58 | 5.44 | 9.34 | 7.65 | 3.31 | 27.32 | 2.96 | 26.49 | 6.81 | 7.11 | 3.81 | 3.89 | 0.96 |
| 10 | 0.99 | 0.45 | 9.11 | 0.75 | 7.81 | 16.51 | 12.29 | 5.62 | 34.61 | 6.11 | 49.11 | 8.53 | 10.31 | 8.31 | 5.76 | 0.83 |
| 15 | 0.73 | 0.20 | 4.71 | 0.43 | 4.73 | 8.75 | 6.93 | 2.64 | 23.56 | 2.81 | 23.45 | 8.91 | 6.63 | 3.82 | 2.63 | 1.34 |
| 20 | 0.67 | 0.16 | 4.53 | 0.35 | 4.52 | 7.44 | 6.71 | 2.45 | 22.14 | 2.53 | 20.61 | 9.22 | 6.29 | 3.55 | 2.24 | 1.47 |
| | 40% | | | | | | | | | | | | | | | |
| Control | 0.87 | 0.24 | 6.73 | 0.83 | 8.11 | 7.68 | 5.44 | 3.82 | 25.51 | 2.65 | 19.79 | 9.59 | 8.33 | 3.95 | 2.06 | 1.15 |
| 10 | 0.45 | 0.10 | 5.95 | 0.61 | 6.89 | 6.85 | 4.93 | 2.94 | 23.32 | 2.41 | 17.31 | 10.21 | 6.91 | 4.31 | 1.69 | 1.48 |
| 15 | 0.32 | 0.07 | 5.11 | 0.33 | 5.66 | 6.53 | 4.75 | 2.41 | 21.11 | 2.23 | 15.72 | 10.83 | 5.64 | 3.51 | 1.45 | 1.92 |
| 20 | 0.27 | 0.05 | 4.21 | 0.25 | 4.33 | 6.45 | 4.49 | 2.27 | 18.71 | 2.11 | 11.81 | 11.52 | 4.41 | 3.33 | 1.03 | 2.61 |
| L.S.D. at 5% | 0.07 | 0.11 | | | | | | | | | | | | | | |

This mechanism includes the induction of metabolic activities that lead to the increase of acetyl CoA production, which in turn converts into pyruvic acid, that gives mevalonic acid as a result of its metabolism. Mevalonic acid is considered essential for the formation of isopropene, that forms terpens which is considered as the essential oil component of aromatic oils. Similar suggestions were reported by Youssef and Rady (2000, Hanafy *et al.* (2002) and Pessarakli (2002).

**B- Sugars, proline and total free amino acids:**

Total sugars, proline and free amino acids concentration increased dramatically by treatment with different concentration of sea water Table (2). The treated marjoram plants with low concentration [10 µM] of putrescine were characterized by clear positive correlation between salinity levels up to 25% sea water and accumulation of total sugar, proline and amino acids. As with herb fresh and dry weight in marjoram plants Table (1), under 25% sea water, the treated plants with 10 µM accumulated nearly 2-folds of the protective compounds; sugars and proline as well as amino acids compared with untreated plants. On the contrary all other putrescine treatments under all salinity levels and 40% sea water for 10 µM caused noticeable reduction in the accumulation of the protective compounds when compared with the control. These findings are in agreement with those reported by Ibrahim and Shehata (2000), Ahmed (2002), Essa (2002) and Nuttall *et al.* (2003). In this connection, Binzel and Reuveni (1994) reported that under saline conditions the accumulation of non toxic substances such as sucrose, proline, organic acids, pigments, nucleic acids and protein is considered to be protective adaptation and that the survival of plants under saline conditions depends upon the regulation of metabolic processes and the quantitative ratio between the protective and the toxic metabolic intermediate.

Moreover, it has been suggested that the high concentration of organic solutions in the cytoplasm could have the following roles: a- a contribution to the osmotic balance when electrolytes are lower in the cytoplasm than the vacuole, b- a protective effect of enzymes in the presence of high electrolytes in the cytoplasm (Marschner, 1995). The sugars as osmolytes can enable plants to keep better water relations under stress conditions (Ghallab and Nesiem, 1999). More recently, Pessarakli (2002) concluded that plant use soluble sugars as an osmoticum under saline conditions. Hence, the plants that fail to increased soluble sugars biosynthesis could not tolerate salt stress.

The endogenous concentration of free proline in plants can be used as an indicator of salt tolerance. For each plant, it appears that there is an external salt concentration above which the plant's proline level sharply rises. This critical point is directly to the ability of plant to tolerate salt. Thus, measurements of concentration can be used to determine salt resistance of plants (Ghalab and Nesiem, 1999 and Pessarakli, 2002). Moreover, proline and other compatible solutes are believed to case the minimal inhibition of metabolism. Also, proline is organs osmolytes solute with an amphiphilic molecule protects the hydrophobic parts of proteins which suffer first when water potential is lowered. By forming association with the hydrophobic

proteins of macromolecules, proline converts them into hydrophilic parts (Binzel and Reuveni, 1994). Recently, Eid-Noha (2001). Showed that, free proline content increased in the leaves of banana cultivars in response to salt stress. Salem *et al.* (2002) with faba bean found that, proline, sugars and free amino acids increased with increasing salinity level.

New class of genes, called "Osm"[osmotic tolerance] genes that is used for protection against osmotic stress and may work in a similar manner in plants, bacteria and animals now attracted the attention of physiologysts, through their action following salinity. The over produced proline may be explained on the basis that osmogenes govern the production of a class of molecules such as betaine and proline that protect the cell and its constituents against "dehydrationosm" Ghallab and Nesiem, (1999) and Pessarakli, (2002).

Also, many reports proved the rapid increase in synthesis and accumulation of sugars under saline conditions. Nasir *et al.* (2000) reported that leaves of salt-tolerant line of sugarcane showed high degree of osmotic adjustment by the accumulation of more $K^+$ , free proline and sugar contents. Cordoba *et al.* (2001) found that roots from salt-treated plants accumulated higher concentrations of soluble sugars. Concerning accumulation of amino acid with increasing salinity levels, El-Shafey *et al.* (1998) and Fatouh Youssef (2003) reported that amino acid concentration increased in wheat callus with increasing salinity levels.

The super positive effects of low concentration [10 µM] of putrescine treatment on the sugars, proline and amino acids in the shoots under saline conditions would be explained as due to the major role of di-amine putrescine as well as polyamines in the plant metabolism and the physiological processes as well as the positive effects of their exogenous application on the plants grown under saline conditions. In this regard, Fatouh Youssef (2003) mentioned that, weekly spraying either with 10 µM putrescine or 2 ppm ABA and 15 KR gamma rays accelerated the accumulate of $K^+$, $Ca^{+2}$, $Mg^{+2}$, sugars, proline and amino acids in the shoots of salt-stressed wheat plants.

**C- Phytohormones:**

Salinity caused reduction in indole-3-acitic acid [IAA] and gibberellic acid [GA3] concentrations Table (2). On the other hand, abscisic acid [ABA] increased. Similar results were reported by EL-Antably *et al.* (1994), Amer *et al.* (1995), Ghallab and Nesiem (1999) and Ibrahim and Shehata (2000).

Also, the results presented in Table (2) clearly showed that treatment with 10 µM putrescine considerably increased IAA, GA3 and ABA concentrations over control under normal or saline conditions up to 25% sea water followed by a sharp decrease under 40% sea water and all other putrescine treatments. In accordance with these results, Zeinab and Sallam (1996) reported that with increasing Na+ concentration, the tryptophan synthesis β-monomers were gradually dissociated from the oligomers producing less active isoenzyme. This reduced the biosynthesis of L-tryptophan and consequently that of IAA, so that wheat growth was retarded or even stopped. Also, at higher salinity there was an accumulation of

gibberllin inhibitors and no gibberellin activity was found in wheat plants. EL-Desoky and Atwai (1998) stated that in sour orange the biological activities of cytokinins, gibberellins and auxins were significantly reduced by excess salinity [5000 ppm] in the irrigation water. Recently, Wang-Yongyin *et al.* (2001) considered ABA is the primary hormone that mediates plant responses to stresses such as cold, drought and salinity; thus its endogenous level increased with salinity stress.

The beneficial effects of exogenous application of putrescine in improving salinity tolerance through minimizing the detrimental effects of salinity were previously evidenced by Bestford *et al.* (1991) who stated that putrescine and polyamines have been frequently described as endogenous plant growth regulators or intracellular second messengers mediating the effects of phytohormones. Also, the same auther added that putrescine and polyamine involved in the ionic balance and DNA, RNA and protein stabilization. Kaur-Sawhmey and Galston (1991) reported that polyamines are associated with cell division and with active growth and metabolism. Recently, Fatouh Youssef (2003) reported that salt-tolerance which more pronounced as a result of weekly spraying either with 10 µM putrescine or 2 ppm ABA and 15 KR gamma rays were positively associated with increased IAA, GA3 and ABA by increasing salinity up to 30% sea water. Therefor, the same author suggested the possibilty of successful application gamma rays, ABA and putrescine to improve salt tolerance of the sensitive wheat cultivars.

**D-Minerals:**

The obtained data in Table 2 clearly showed that concentrations of $Na^{+}$, $Ca^{+2}$ and $Mg^{+2}$ gradually increased in the shoots of the untreated plants as salinity increased, the concentrations of N, P and K exhibited an opposite trend. On the other hand, the accumulation of N, P, $K^{+}$, $Mg^{+2}$ and $Ca^{+2}$ uptake into marjoram plants treated with 10 µM putrescine was observed as the salt level increased up to 25% water, while all other putrescine treatments under all salinity level and 40% sea water for 10 µM putrescine treatment greatly decreased such uptake and accumulation when compared to the respective values of the untreated control plants. This strongly emphasizied the superiority of 10 µM putriscine in enhancing the uptake and accumulation of N, P, $K^{+}$, $Ca^{+2}$and $Mg^{+2}$, while inhibited $Na^{+}$ uptake as previously reported for sugars, proline, total free amino acids and phytohormones Table (2) either in the growing plants under salinity levels up to 25% sea water. The favourable effects of lower level of putriscine [10 µM] were reflected on the growth Table (1), protective compounds and nutrient uptake Table (2). These effects may be as a result of plant adaptation to stress conditions.

The ratio of K/Na was gradually decreased as the salinity increased. Contrary Na/Ca ratio exhibited an opposite trend, because the rate of $Na^{+}$ increases was higher than that of $K^{+}$ or $Ca^{+2}$ as the salt level increased.
Pessarakli (2002) stated that reduction in N under saline conditions may be due to reduction in water absorbed and a decrease in root permeability. The high concentration of Na in the high salt level suggested that under saline conditions sodium influx across the plasmalemma to the vacuole may play a major role in permitting turgor maintenance. Some crops show marked

benficial effects of $Na^+$ specially if the $K^+$ supply is limited. These crops take up large amounts of Na+ which contribute to the osmotic potential of the leaves and increases resistance to water stress. In the wheat plants, the yield response to $Na^+$ often exceeds that of $K^+$ (Ghallab and Nesiem, 1999). Also, under saline conditions $Na^+$ has important specific effects. Both $Na^+$ and $K^+$ will move along the electrochemical gradients of tissue but because of either discrimination of the cell membranes or $Na^+$ extrusion, the ultimate concentration ratio may be 20 $K^+$ to $N^+$ (Marschner, 1995). The decreased P concentration associated with salinity treatment may be ascribed to the higher pH values of $Na^+$ affected soils in which might hinder P availability to plants (Rengel, 1999) or due to the harmful effects of salinity that cause release of some membrane proteins required for uptake and active transport of this element in roots (Pessarakli, 2002). Moreover, the decrease in $K^+$ concentration under salinity levels may be attributed to the antagonism between $K^+$ and $Na^+$ cations which increased considerably as salinity increased (Salem *et al.*, 2002).

Taize and Zeiger (1991) postulated that $Mg^{+2}$ concentration in chloroplasts may influence photosynthesis during water stress through its role in coupling electron transport to ATP production. The plants with the lower $Mg^{+2}$ concentration maintained higher photosynthetic rates as leaves became hydrate. Pessarakli (1994) indicated that $Ca^{+2}$ is strongly competitive with $Mg^{+2}$ and binding sites on the root plasma membrane appear to have less affinity for the highly hydrated $Mg^{+2}$ than for $Ca^{+2}$. Thus, high concentration of substrate $Ca^{+2}$ usually result in increased leaf $Ca^{+2}$ along with a marked reduction leaf $Mg^{+2}$. Moreover, the presence of sufficient Ca in saline solution is essential to prevent the accumulation of toxic levels of Na, because Na uptake by plants is strongly regulated by Ca in the growth medium (Ghallab and Nesiem, 1999).

Specific effects of high $Na^+/Ca^{+2}$ were recorded in relation to beans (Wilkinson, 1994). Growth of beans was markedly influenced by the external $Na^+/Ca^{+2}$; at high external NaCl. Growth of beans decreased and $Na^+$ increased in the levels only when $Na^+/Ca^{+2}$ exceeded 17. Increase in membrane permeability due to the high Na+/Ca++ is the primary cause of these response. Nuttall *et al.* (2003) found that in wheat plants when the $K^+/Na^+$ ratio was 2.5, adverse effects of salinity could be expected. $K^+/Na^+$ ratio 1.5 is corresponding to 50% reduction in growth.

The positive effect of exdogenous application of putrescine on the nutritional salts of the salt stress marjoram plants might be ascribed to overcoming the subsitution occurred betwwen Na and K by the putrescine application for enhanceing K uptake that is required for the normal metabolic processes and its role as an osmotic component. In accord with the obtained results in the prresent work Krishnamurthy (1991) reported that, foliar application with 10 µM putrescine inhibited $Na^+$ and $Cl^-$ uptake and accelerated the accumulation of $K^+$, $Ca^{+2}$, $Mg^{+2}$, proline and endogenous putrescine in the leaves of salt-stressed rice plants. More recently, Fatouh Youssef (2003) reported that, weekly spraying either with 10 µM putrescine or 2 ppm ABA and 15 KR gamma rays inhibited $Na^+$ and accelerated the

accumulation of K, Ca, Mg, N, P, proline, total free amino acids and sugars in roots and shoots of salt-stressed wheat plants.

**E- Essential oil components:**

Salinity caused increases in the percentage of α-Terpineol [the main component], cineole, terpinene-4-ol, linalyl-acetate and α-terpenolene Table (3). On the other hand, the percentage of α-pinene, â-pinene, limonene, linalool, citronellol, αTerpineol acetate, geraniol and unidentified decreased as the salinity level [% sea water] increased. This could be ascribed to the enhancing effect of salinity stress to synthesize and accumulated some essential oil constituents while inhibiting the others as a pattern of defence against salinity stress. Similar results were reported by Hanafy (1989), Kandeel and Elwan (1991) and Abdel-Hafiz Ahmed (2001) on marjoram plants.Also, the results presented in Table (3) show that treatment with 10 µM putrescine considerably increased all the percentage of oil constituents over control under normal or saline conditions up to 25% sea water followed by a sharp decrease under 40% sea water and all other putrescine treatments. This results might be due to the positive effects of di-amine putrescine and polyamines in the plant metabolism and phyisiological processes on the marjoram plants grown under saline conditions. In accord with these results, Hanfy *et al.* (2002) reported that essential oil percentage of myrtus plants increased by increasing salinity, while its components fluctuated due to undergoing to both salinity stress and polyamines [putrescine and spermidine] foliar application at 0.1 and 0.2 ppm.

**Table(3): Effect of putrescine foliar application treatments on essential oil components (%) in marjoram plants grown under different levels of salinity (% sea water) [combained analysis for two seasons].**

| Putrescine (µM) | % Sea Water | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | α-Pinene | β- Pinine | Limonene | Cineole | Linalool | α-Terpineol | Terpinene-4-ol | Citronellol | Linalyl-acetate | α-Terpineol acitate | Geraniol | α-Terpenolene | Unidentified (U) |
| | Control | | | | | | | | | | | | |
| Control | 5.85 | 13.45 | 13.65 | 1.11 | 1.46 | 57.22 | 1.73 | 0.59 | 0.47 | 3.89 | 1.52 | 0.75 | 0.88 |
| 10 | 7.25 | 16.97 | 17.01 | 1.41 | 1.87 | 73.82 | 2.19 | 0.79 | 0.59 | 4.95 | 1.88 | 0.93 | 1.18 |
| 15 | 4.71 | 12.64 | 11.94 | 1.02 | 1.23 | 51.67 | 1.65 | 0.47 | 0.35 | 3.41 | 1.41 | 0.66 | 0.74 |
| 20 | 4.23 | 11.71 | 11.79 | 0.94 | 1.11 | 46.99 | 1.51 | 0.32 | 0.22 | 3.15 | 1.29 | 0.54 | 0.61 |
| | 10% | | | | | | | | | | | | |
| Control | 5.16 | 12.22 | 12.34 | 1.32 | 1.27 | 59.96 | 1.87 | 0.45 | 0.53 | 3.54 | 1.41 | 0.84 | 0.74 |
| 10 | 6.78 | 15.41 | 15.56 | 1.48 | 1.59 | 77.48 | 2.45 | 0.59 | 0.69 | 4.61 | 1.76 | 1.19 | 0.91 |
| 15 | 4.35 | 11.45 | 11.67 | 1.11 | 1.17 | 53.85 | 1.74 | 0.34 | 0.41 | 3.29 | 1.28 | 0.71 | 0.61 |
| 20 | 4.09 | 10.55 | 10.89 | 1.04 | 1.08 | 48.96 | 1.66 | 0.21 | 0.34 | 2.95 | 1.19 | 0.65 | 0.53 |
| | 25% | | | | | | | | | | | | |
| Control | 4.86 | 10.99 | 11.75 | 1.54 | 1.19 | 97.11 | 1.99 | 0.33 | 0.61 | 3.11 | 1.23 | 0.92 | 0.65 |
| 10 | 6.29 | 13.98 | 15.56 | 1.99 | 1.49 | 84.59 | 2.48 | 0.51 | 0.79 | 4.19 | 1.64 | 1.19 | 0.89 |
| 15 | 4.11 | 10.23 | 9.95 | 1.32 | 1.07 | 58.65 | 1.87 | 0.22 | 0.52 | 2.91 | 1.11 | 0.81 | 0.51 |
| 20 | 3.85 | 9.69 | 9.71 | 1.19 | 0.94 | 52.74 | 1.75 | 0.11 | 0.45 | 2.75 | 0.99 | 0.69 | 0.44 |
| | 40% | | | | | | | | | | | | |
| Control | 4.15 | 9.38 | 9.54 | 1.06 | 1.03 | 51.32 | 1.68 | 0.26 | 0.39 | 2.92 | 1.08 | 0.66 | 0.51 |
| 10 | 3.87 | 9.11 | 9.32 | 0.91 | 0.89 | 44.82 | 1.44 | 0.20 | 0.27 | 2.78 | 0.94 | 0.48 | 0.42 |
| 15 | 3.41 | 8.74 | 8.94 | 0.75 | 0.66 | 41.65 | 1.29 | 0.15 | 0.21 | 2.66 | 0.75 | 0.31 | 0.29 |
| 20 | 2.95 | 8.16 | 8.53 | 0.62 | 0.49 | 37.29 | 1.11 | 0.10 | 0.12 | 2.34 | 0.61 | 0.19 | 0.18 |

## CONCLUSION

The obtained results during the two growing seasons clearly emphasized that, the absolute superiority of the low concentration of putrescine [10 μM] compared either with untreated control treatment or with the other putrescine treatments at [15 and 20 μM]. The results emphasized its superiority in inducing higher degree of salt tolerance and high promoting effects on its growth, essential oil percentage, oil yield, essential oil components and the high accumulation of the ABA and protective substances, i.e. sugars, proline and amino acids in the shoot of marjoram plants. This accumulation was positively correlated with the increase in the salt level up to 25% sea water in the medium and improve the adaptation to salinity. Also, the obtained results confirmed positive response of IAA and $GA_3$ synthesis to 10 μM putrescine treatment, indicating that the lower level of putrescine [10 μM] seems to be the most suitable concentration for enhancing plant growth and development through stimulation of auxin biosynthesis. The obtained data suggested that the lower rate of putrescine [10 μM] may be successefully applied to improve salinity tolerance of marjoram plants, but it must be applied widely and after precise study to approach its optimal effectiveness in improving of salinity tolerance.

Further physiological studies are needed to elucidate that the role of di-amine putrescine in regulating the uptake and accumulation of different solutes, is attributed to some alternations in the properties of the cell membranes.

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# زيادة تحمل نباتات البردقوش للملوحة باستخدام الرش بالبتروسين

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أجريت تجارب الأصص خلال موسمين متتاليين ٢٠٠١ و ٢٠٠٢ لدراسة تأثير الرش بالبتروسين [ صفر، ١٠، ١٥،٢٠ ميكرومول] في تحسين مقاومة الملوحة لنباتات البردقوش و ذلك تحت تأثير مستويات مختلفة من ماء البحر [ صفر، ١٠%،٢٥%، ٤٠٪] .

وقد ثبت من نتائج البحث أن الرش بـ ١٠ ميكرومول بتروسين أدت إلى الحصول على زيادة معنوية عالية في طول النبات و عدد الأفرع و الوزن الطازج و الجاف للعشب و النسبة المئوية للزيت الطيار و محصول الزيت للنبات تحت الظروف العادية و الملحية إلى مستوى ٢٥% ماء بحر وقد ثبت أيضاً من نتائج التحليل الكيماوي للمجموع الخضري أن تحمل الملوحة إلى مستوى ٢٥% ماء بحر و الذي ظهر بدرجة عالية في معاملة الرش بـ ١٠ ميكرومول بتروسين قد خلقت جهدا اسموزيا أكثر سالبية داخل أنسجتها (عملية التنظيم الأسموزي) و ذلك بتراكم كميات كبيرة و متزايدة من عناصر النتروجين و الفسفور و البوتاسيوم و الكالسيوم و المغناسيوم مع الإحتفاظ بنسبة البوتاسيوم إلى الصوديوم عند أعلى مستوى لها، بالإضافة إلى تراكم كميات كبيرة من السكريات و البرولين و الأحماض الأمينية الحرة في الأنسجة النامية و التي فاقت كثيرا مثيلتها في نباتات جميع المعاملات الأخرى بما فيها (معاملة المقارنة حتى مستوى ٢٥% ماء بحر على الأقل ) ، و قد صاحب هذا حالة خاصة للتوزيع الداخلي للهرمونات النباتية في صالح المزيد من تحمل النباتات لهذه الملوحة و تمثل هذا في تراكم الجبرلينات ة الأوكسينات و حمض الأبسيسك في المجموع الخضري لهذه النباتات. يتضح من النتائج المتحصل عليها من هذا البحث مدى إمكانية إستخدام الرش بالبتروسين بنجاح في تحسين صفة تحمل الملوحة لنباتات البردقوش.