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RESPONSE OF SWEET PEA TO SALINITY STRESS AND *RHIZOBIUM* INOCULATION

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ABSTRACT: Two pot experiments were conducted at the Experimental Farm of Efficient Productivity Institute, Zagazig University during the two successive seasons of 2002/2003 and 2003/2004 to study the effect of irrigation with saline water at four concentrations (0, 1500, 2500 and 3500 ppm) and three *Rhizobium leguminosarum* inoculation rates (0, 10, and 20 g *Rhizobium* inoculants/ Kg seed) and their interactions on survival percentage, growth, flowering and chemical composition of *Lathyrus odoratus* plant.

As salinity level increased up to the highest level 3500 ppm gradually reduced survival (%), growth and flowering (plant height, branches and leaves production/ plant, plant fresh and dry weights, cluster No/ plant, and flowering stalk length). These effects were associated with reductions in leaf tissue contents of chlorophyll, total N and total carbohydrate. Only 2500 ppm salinity level enhanced flowering date comparing to control (tap water) and the low (1500 ppm) or high (3500 ppm) salinity levels.

Seed inoculation with *Rhizobium leguminosarum* increased leaf chemical constituents of N, chlorophyll and carbohydrate. This was reflected as an enhancing effect on vegetative growth and consequently flowering. Generally, there were no significant differences between the two tested rates of inoculation (10 and 20 g inoculants/ Kg seed).

When *Rhizobium* inoculation was interacted with salinity stress, salinity reduced the enhancing effects of inoculation comparing to inoculation under non saline conditions. Contrary, inoculation enhanced plant salinity tolerance as compare to non inoculated plants. Since, inoculation increased percentage of survived plants, vegetative growth (plant height, branch and leaf No/ plant, and plant fresh and dry weights) and flowering (cluster No/ plant, and flowering stalk length) as well as leaf chemical constituents (chlorophyll, total N and total carbohydrate) under irrigation with salinized water comparing to non inoculation under the same salinity level.

Key words: *Lathyrus odoratus*, saline water irrigation, *Rhizobium leguminosarum* inoculation, plant growth, flowering and leaf chemical composition.

INTRODUCTION

Sweet pea (*Lathyrus odoratus*) plant has long been a favorite of gardeners because of the wonderful fragrance of the clusters of colorful blue, pink, purple, red or white flowers. It is easily to cultivate, grows as annual plant and blooms best in cooler and makes great cut flowers. It belongs to *Fabaceae* family (*Leguminaceae*). As such plants fix the atmospheric nitrogen and should not require a lot of N fertilizer.

Saline conditions disrupt several physiological processes in plant leading to reduction in growth and flowering. For legumes, irrigation pea plants with saline water frequently suppressed growth as: root fresh and dry weights/ plant (Solomon *et al.,* 1989 using medium amended with 120 mM NaCl), as well as number of produced flowers / plant (Abo-Sedera *et al.*, 1992 using 6000 or 9000 ppm NaCl). Also, Yadava and Yadava (1998) stated that pea plant height and pod number/ plant were decreased as salinity level increased from 0 to 60 meq/ litre particularly when induced by chloride. For non legume plants, Abd El-Kafi (1995) studied the effect of irrigation water containing 1000, 2000, or 4000 ppm NaCl on *Salvia officinalis* plant height, branching and fresh weight/ plant; Helal and Khalil (1997) on *Catharanthus roseus* and Ramadan (2001) on thyme plant investigated survival %, plant height, shoot and root fresh and dry weights as affected by saline water irrigation stress, they confirmed the suppressive effects of salinity stress.

Seed inoculation of legumes with proper symbiotic bacteria enhanced plant growth. In this respect, Krishan *et al.* (1995) found that multi-strain inoculants of soybean and pea resulted in promising increases in plant dry weight and percentage of occupied nodules comparing to single strain inoculants. Also, similar enhancements resulted from inoculation were demonstrated on pea by Petel *et al.* (1998) regarding plant height as well as number of branches, leaves and pods/ plant and, El-Mansi *et al.* (2000) testing plant growth as: stem length, branches and leaves No/ plant, as well as shoot and root dry weights/ plant.

Inoculation legume plants with symbiotic bacteria under salinity stress exhibited different responses depending upon the legume species and salinity conditions. However, Siddiqui and Kumar (1987) on peas studied nodule metabolism under salinized and desalinized conditions. They found that salinity decreased N content of nodules (an index of N fixation). Cordovilla *et al.* (1999a) inoculated faba bean and pea with *Rhizobium leguminosarum* under salt stress (100 mM NaCl) and/ or nitrate fertilization (8 mM KNo_3) to test whether plants grown with inorganic nitrogen are more tolerant to salinity than plants entirely reliant upon fixed nitrogen. They demonstrated that pea plants dependent on dinitrogen fixation proved more tolerant to salt stress than N fertilized ones. In other experiment, Cordovilla *et al.* (1999b) proved that salt stress only reduced growth of pea plants inoculated with salt-sensitive *Rhizobium* strain (GRL19), while growth of faba bean was reduced by salt stress regardless of *Rhizobium* strain. Also under salt stress, pea plants showed higher nodule mass and levels of N fixation than faba bean plants.

Consulting the available literature there were no information about the effect of salinity stress or bacterium inoculation on sweet pea plant. So, the aim of this work was to investigate the effect of saline water irrigation on growth, flowering and chemical constituents of sweet pea (*Lathyrus odoratus*) plant in addition to what extent *Rhizobium* inoculation had counteract effect to salinity harmful to utilize the attractive landscaping features of sweet pea plant.

MATERIALS AND METHODS

This work was conducted at the Experimental Farm of Efficient Productivity Institute, Zagazig University during the two seasons of 2002/2003 and 2003/2004 to study the effect of different levels of saline water irrigation and *Rhizobium leguminosarum* inoculation and their interactions on growth, flowering and chemical composition of *Lathyrus odoratus* plant.

Seeds of the local variety of *Lathyrus odoratus* grown in Zagazig area were used in this study. On October 15th for the two tested seasons, seeds were soaked in tap water overnight then good swelled ones were directly inoculated, according to the inoculation treatments as mentioned later, and planted about 2-3 cm depth at rate of 6 seeds/ one 30 cm diameter plastic pot. Pots were filled with soil mixture analyzed as: 21.8% coarse sand, 49.3% fine sand, 16.6% silt, 10.8% clay, 1.5 organic matter, EC 1.6 m mhos/cm, 0.04% available N, 0.001% available P, 0.06% available K. Pots were watered at sowing and for one month after that by non salinized tap water. The good two seedlings/ pot were selected and the other ones were thinned. About 2 meter sticks were used to support the plants. On November 15th each pot for the all treatments received 2 g of 1:1:1

NPK fertilizer to encourage growth as starter. Also, plants were tied using cotton string when they were about 20 cm tall; this process was repeated every 1 or 2 week, depending on growth rate, throughout the experimental period.

The experimental design was factorial between saline water irrigation (four levels) and *Rhizobium leguminosarum* inoculation (three levels) in a complete randomized block design with three replicates, each replicate contained six pots. Three pots were used for vegetative growth data and the other three for assay flowering aspects. So, the experiment included 12 treatments.

Seeds were directly inoculated by *Rhizobium leguminosarum* inoculants, commercially named "okadein". Inoculation was done just after seed soaking and before planting with three inoculation levels; *i. e.* without inoculation (control), inoculation with 10 g *Rhizobium*m inoculants/ one Kg seed and inoculation with 20 g *Rhizobium* inoculants/ one Kg seed. Arabic gum at 20 % solution was used to facilitate inoculation. *Rhizobium* strain was supplied by the General Organization for Agriculture Equalization Found (GOAEF), Agriculture Research Center, Ministry of Agriculture, Egypt. The viability of inoculants was determined at the Microbiology Laboratory of Fac. Agric. Zagazig Univ. Each one gram inoculants was containing $10^6 \times 4.1$ cells.

On November 15th for the two tested seasons, plants were arranged to receive 4 levels of saline water irrigation, *i.e.* tap water (control), 1500, 2500 or 3500 ppm. Natural salt crust of sea water was used. The chemical analyses of the used sea crust are shown in Table A. The plants were irrigated when needed to maintain soil moisture at about 65% of the field capacity.

Recorded data

Just before flowering, on February 20th for the two seasons, three pots for each replicate (6 plants) were used to record the vegetative growth data which included plant height (cm), branch and leaf numbers/plant as well as fresh and dry weights (g) of shoot (stems and leaves) and root/plant. While, the other three pots (6 plants) were used to record percentage of flowered plants on March 1st, cumulative number of clusters/ plant, florets number/ cluster and cluster stalk length (cm). Clusters were regularly cut every three days and the cumulative number of clusters per plant over season was terminated on April 15th during the two seasons of the experimental period. Also, survival percentage was recorded.

Table A. Chemical analysis of sea water salt crust (water: salt at 5:1)

EC dS/m	Cations, meq/l				Anions, meq/l			
at 25°C	Ca^{++}	Mg^{++}	Na^{+}	K^{+}	HCO^{-}_{3}	CO^{--}_{3}	SO^{--}_{4}	CL^{-}
170.6	9.22	8.44	3000	2.84	4.66	0.00	80.70	2845

Leaf samples for chemical analysis were taken just before flowering, on February 20th for the two seasons and were dried at 70°C for 72 hours, finely ground and wet digested. Total nitrogen percentage was colorimetrically determined according to A.O.A.C (1980) and total carbohydrate percentage was determined according to Dubois *et al.* (1956). Simultaneously, fresh leaves disc samples were prepared from the fourth upper leaf to determine chlorophyll a, b and total chlorophyll (a+b) according to the method described by Wettestein (1957).

Statistical analysis

The collected data were statistically analyzed according to Steel and Torrie (1980). Mean separation was done using Duncan's multiple range test at 5% level (Duncan, 1958).

RESULTS AND DISCUSSION

Survival Percentage

Survival percentage was significantly reduced as salinity increased up to the highest level 3500 ppm (Table, 1). These reductions were significant under 2500 and 3500 ppm comparing to using non saline water (control) or 1500 ppm. Generally, the lowest survival (%) was recorded with the highest water salinity. The reduction of survival percentage under high salinity conditions was previously reported by Rashad (1995) on *Tagetes erecta* and Helal and Khalil (1997) on *Catharanthus roseus* and Ramadan (2001) on thyme plant. This reduction in survival percentage under salinity stress might be due to osmotic inhibition of water absorption, toxicity of one or more specific ions or the combination of the two factors (Lapina, 1967).

Contrary the previous decreasing effect, the same Table 1 exhibits that *Rhizobium* inoculation enhanced survival (%) comparing to non inoculated plants, with no significant differences between 10 and 20 g *Rhizobium* inoculants/ Kg seed.

When *Rhizobium* acted under salinity stress (Table 2), generally, inoculation enhanced plant salinity tolerance comparing to non inoculated plants under the same salinity level. Since, under 2500 ppm salinity level, survival (%) was increased from 40.3% in non inoculated plants to 61.5% and 65.4% in inoculated ones with 10 and 20 g *Rhizobium* inoculants/ Kg seed, respectively during the first season and from 44.3% with no inoculation to 65.3% and 62.0% with 10 and 20 g inoculants, respectively in the second season. The same trend was true under 3500 ppm salinity level. The inoculation enhancement effects on plant salinity tolerance under the same salinity stress level, generally, did not significantly differ by using 10 or 20 g inoculation rates. This was in confirming during the two tested seasons. Cordovilla *et `al.* (1999a) demonstrated that inoculated pea with *Rhizobium leguminosarum* under salt stress (100 mM NaCl) which dependent on dinitrogen fixation proved more tolerant to salt stress than N fertilized ones. However, the enhancing effect of inoculation on salinity tolerance might be due to the direct or indirect role of fixed N on enhancing metabolic processes leading to more salinity tolerance.

Vegetative Growth

Effect of salinity

Obtained results of Table 1 and Photo 1 indicate that sweet pea plant height, number of branches and leaves/plant, fresh and dry weights of shoots and roots/ plant gradually decreased with increasing saline water concentration up to the highest level of 3500 ppm. Irrigation without saline water (control) recorded maximum values in this respect followed by 1500 ppm saline water, in most cases, during the two tested seasons. Similar results were obtained on pea under salinity stress respecting root fresh and dry weights/ plant (Solomon *et al.*, 1989) and plant height (Yadava and Yadava, 1998). Also, Abd El-Kafi (1995) found that *Salvia officinalis* plant height, branching and fresh weight/ plant were decreased as salinity of irrigation water increased from 1000, 2000, to 4000 ppm NaCl. Helal and Khalil (1997) on *Catharanthus roseus* and Ramadan (2001) on thyme plant recorded salinity inhibitive effects on plant height as well as shoot and root fresh and dry weights. The reduction in plant growth as a result of salinity was attributed mainly to the osmotic inhibition of water absorption (Bernstein and Ayers, 1951).

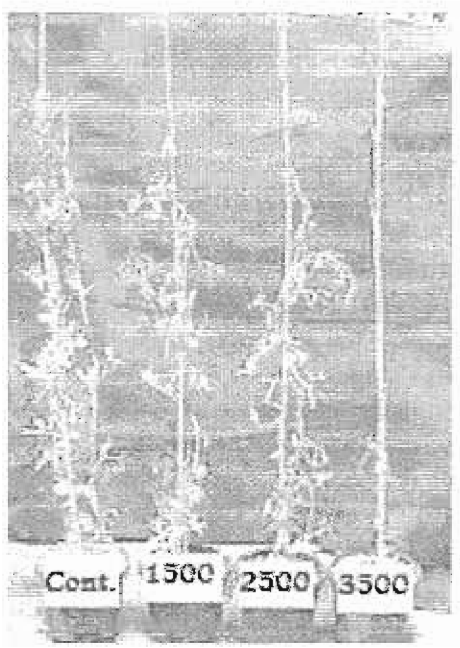

Photo 1: Effect of saline water irrigation (ppm) on vegetative growth of sweet pea plant.
Cont.= Control.

According to Slatyer (1976), the progressive reductions in growth rates as a result of increasing salinity, appeared to be caused primarily by the effect of excess ion accumulation in the treated plants. The direct osmotic effects, acting through the reduction of water availability to the plants, appeared to be of secondary importance. An excess accumulation of electrolytes in plant cells, particularly of ions such as Na^+ or Cl^- resulted in progressive changes in protein hydration. In addition, Schwarz and Gale (1981) stated that prolonged saline stress caused a reduction in photosynthesis, increasing maintenance respiration, and therefore lack of available assimilates, which consequently resulted in growth reduction.

Effect of *Rhizobium leguminosarum* inoculation

Table 1 and Photo 2 clear that inoculated sweet pea plants recorded significant increases, in most cases, in the all studied growth parameters; *i.e.* plant height, number of branches and leaves/ plant, as well as fresh and dry weights of shoot and root/ plant comparing to non inoculated plants during the two growing seasons. Generally, there were no significant differences between the two inoculation levels of 10 and 20 g *Rhizobium inoculants*/ kg seed. Similar enhancements resulted from inoculation were previously recorded on pea by Petel *et al.* (1998) respecting plant height as well as number of branches and leaves / plant, and El-Mansi *et al.* (2000) concerning branch and leaf No/ plant, as well as shoot and root dry weights/ plant. However, the change in root growth rate after inoculation by *Rhizobium* can be suggested to result from the shifts in the content of endogenous IAA and cytokinins in the root. These shifts were caused by the presence of the exogenous phytohormones secreted by root-nodule bacteria in the host-plant growth medium (Akimova *et al.*, 1999). According to Steven (2007) bacteria of the *Rhizobia* stimulate legume plant to form nodules on its roots. Once the bacteria enter these nodules, they convert atmospheric nitrogen to mmonia, a nitrogen compound that plants are able to use. The plant then uses ammonia to assemble amino acids, the building blocks of proteins. As a result, plants that receive excess nitrogen will grow faster, higher, and healthier than those plants that do not.

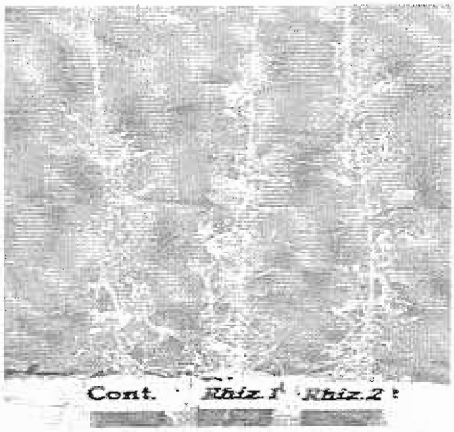

Photo 2: Effect of *Rhizobium* inoculation (g/Kg seed) on vegetative growth of sweet pea plant. Cont. = Control, *Rhiz* 1=10g and *Rhiz* = 20g.

Effect of *Rhizobium* inoculation under salinity stress

Data represented effect of interaction between *Rhizobium* inoculation and saline water irrigation on plant growth (plant height, number of branches and leaves/ plant, and shoot and root fresh and dry weights/ plant) are in Table 2. Generally, increasing the interacted salinity level with any inoculation rate had an inhibition effect on the above studied growth parameters. While, *Rhizobium* inoculation improved plant growth under salinity stress comparing to non inoculated plants under the same salinity level with no significant differences between the two tested inoculation doses of 10 or 20g *Rhizobium* inoculants/ Kg seed in several cases. The highest growth values, generally, were recorded in inoculated plants and irrigated with non saline water comparing to the all other interaction treatments. The inhibitive effects of salinity on growth of inoculated faba bean and pea plants with *Rhizobium leguminosarum* were studied by Cordovilla *et al.* (1999 a and b) in two experiments. They found that salt stress only reduced growth of pea plants inoculated with salt-sensitive *Rhizobium* strain (GRL19), while growth of faba bean was reduced by salt stress regardless of *Rhizobium* strain. For the effect of *Rhizobium* inoculation on growth under salinity stress, also they published that pea plants dependent on dinitrogen fixation proved more tolerant to salt stress than N fertilized ones. Steven (2007) stated that a microorganism ability to fix nitrogen is strongly influenced by soil conditions. Any adverse soil condition or environmental stress that affects plant growth will slow down the nitrogen fixation process.

Table 1. Effect of saline water irrigation and *Rhizobium* inoculation on survival percentage and some vegetative growth characters of sweet pea plant during 2002/2003 and 2003/2004 seasons

Treatments	Survival (%)	Plant height (cm)	Number/ plant		Fresh weight (g/ plant)		Dry weight (g/ plant)	
			Branches	Leaves	Shoot	Root	Shoot	Root
Saline water irrigation (ppm)								
			2002/2003 Season					
Control	100.0 a	118.7a	3.50a	116.7a	22.20a	6.10a	8.20a	1.59a
1500	97.2a	94.3b	2.85b	78.6b	9.49b	1.11b	2.03d	0.72b
2500	55.7b	83.8c	2.89b	60.4c	8.63b	1.09b	3.00c	0.70b
3500	44.3c	54.3d	3.20ab	38.3d	9.78b	1.22b	3.95b	0.75b
			2003/2004 Season					
Control	100.0 a	118.2a	3.21a	108.1a	28.60a	7.08a	9.15a	1.50a
1500	94.4a	91.0b	3.17a	87.3b	14.40b	2.09b	2.99b	0.66b
2500	57.2b	87.3b	2.31b	52.5c	11.50c	1.11c	1.62d	0.44c
3500	45.2c	85.0b	3.30a	61.9c	9.60d	1.01d	2.37c	0.32c
***Rhizobium* inoculants (g/ Kg seed)**								
			2002/2003 Season					
Control	66.2b	74.0c	2.89b	52.7c	11.80a	1.30b	4.11a	0.56b
10	77.8a	99.3a	3.12ab	85.9a	12.80a	2.84a	4.78a	1.10a
20	78.9a	90.1b	3.32a	81.9b	12.90a	3.00a	3.99a	1.16a
			2003/2004 Season					
Control	64.6b	81.3b	2.41c	49.0b	13.10b	1.73b	3.42b	0.52b
10	80.0a	100.1a	3.08b	87.6a	17.00a	3.21a	4.08a	0.84a
20	77.9a	104.7a	3.49a	95.8a	18.00a	3.53a	4.59a	0.83a

Means having same alphabetical letter(s) within each column did not significantly differ according to Duncan multiple range test at 5% level.

Table 2. Effect of interaction treatments between saline water irrigation and *Rhizobium* inoculation on survival percentage and some vegetative growth characters of sweet pea plant during 2002/2003 and 2003/2004 seasons

Interaction treatments		Survival (%)	Plant height (cm)	Number/ plant		Fresh weight (g/ plant)		Dry weight (g/ plant)	
Rhizobium (g/Kg seed)	Saline water (ppm)			Branches	Leaves	Shoot	Root	Shoot	Root
				2002/2003 Season					
0	0.00	100.0a	96.1b	2.23b	85.4cd	21.20b	2.50b	7.76a	0.78cde
0	1500	91.6a	88.7b	2.47c	58.6ef	8.21cd	0.76c	1.78a	0.29f
0	2500	40.3d	63.4c	2.46c	33.3fg	8.98cd	0.86c	2.86a	0.51ef
0	3500	32.8d	47.7d	3.40b	33.5fg	8.78cd	1.08c	4.03a	0.68def
10	0.00	100.0a	135.8a	2.94bc	120.1b	25.7a	7.84a	9.98a	2.22a
10	1500	100.0a	92.8b	3.20b	86.5cd	10.4cd	1.45c	2.44a	0.89cde
10	2500	61.5b	98.1b	3.19b	86.7cd	6.48d	0.92c	2.63a	0.46ef
10	3500	50.0c	70.6c	3.16b	50.4efg	8.76cd	1.13c	4.06a	0.83cde
20	0.00	100.0a	124.1a	4.33a	144.7a	19.7b	7.96a	6.86a	1.76b
20	1500	100.0a	101.5b	2.88bc	90.8c	9.84cd	1.13c	1.87a	0.98cd
20	2500	65.4b	90.0b	3.03bc	61.20de	10.4cd	1.48c	3.50a	1.15c
20	3500	50.3c	44.8d	3.03bc	31.1g	11.7c	1.45c	3.76a	0.75cde
				2003/2004 Season					
0	0.00	100.0a	90.8c	2.67a	65.7a	22.0b	2.99c	7.87a	0.91b
0	1500	83.3b	89.2c	2.72a	65.0a	14.0cd	1.99de	2.50a	0.56c
0	2500	44.3e	68.0d	1.70a	26.0a	10.4e	0.89f	1.26a	0.36cd
0	3500	31.0f	77.3cd	2.58a	39.2a	5.85f	1.07ef	2.06a	0.24d
10	0.00	100.0a	142.3a	3.33a	135.7a	32.9a	8.60b	9.50a	1.70a
10	1500	100.0a	90.8c	3.10a	89.5a	13.2cde	2.26cd	2.82a	0.84b
10	2500	65.3c	77.1cd	2.43a	53.8a	10.5e	1.16ef	1.80a	0.55c
10	3500	55.0d	90.1c	3.48a	71.2a	11.4de	0.82f	2.23a	0.27d
20	0.00	100.0a	121.6b	3.63a	122.9a	31.0a	9.65a	10.0a	1.87a
20	1500	100.0a	93.0c	3.71a	107.5a	15.9c	2.03cde	3.66a	0.59c
20	2500	62.0c	116.8b	2.79a	77.6a	13.5cd	1.29def	1.81a	0.43cd
20	3500	49.6de	87.6c	3.85a	75.2a	11.4de	1.14ef	2.82a	0.45cd

Means having same alphabetical letter(s) within each column did not significantly differ according to Duncan multiple range test at 5% level.

Flowering Aspects

Effect of salinity

Irrigated sweet pea plants with the moderate salinity level of 2500 ppm were the earlier in flowering (as percentage of flowered plants on 1st March), while the highest saline concentration of 3500 ppm delayed flowering comparing to using non saline water or the low salinity level (1500 ppm) during the two seasons (Table, 3). The other recorded flowering aspects in the same Table 3 and Photo 3; *i.e.,* produced number of clusters/ plant, florets No/ cluster and cluster stalk length (cm) were gradually reduced as salinity level increased up to the highest level 3500 ppm. This means that 2500 ppm salinity level enhanced flowering date, but lowered quality of the produced flowers. Abo-Sedera *et al.* (1992) found that irrigation pea plant with 6000 or 9000 ppm NaCl or Na_2So_4, reduced number of produced flowers / plant. However, the acceleration of flowering under 2500 ppm salinity level might be resulted from the caused retardation in vegetative growth; consequently the metabolites might be directed to the reproductive process. While the inhibitive effects of salinity on the other flowering aspects might be due to the suppressive effects of salinity on plant vegetative growth (Table, 1) which reflected as little metabolites for flowers production and growth.

Effect of *Rhizobium leguminosarum* inoculation

Rhizobium inoculation at 10 or 20 g inoculants/ Kg seed tended to enhance sweet pea flowering as: flowering (%) on 1st March, produced cluster No/ plant and florets No/ cluster as well as cluster stalk length comparing to non inoculated plants during the two seasons. This effect was only significant in respect to cluster stalk length during the first season (Table, 3). This enhancing effect on flowering was expected as a result of stimulative effects of *Rhizobium* inoculation on plant height, branching, leaves production as well as shoot and root dry weight as mentioned before (Table, 1).

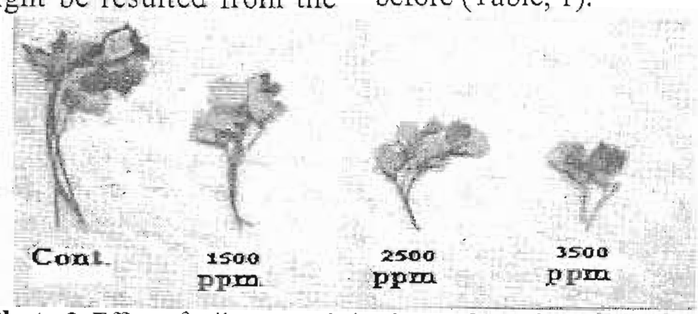

Photo 3: Effect of saline water irrigation on flowering of pea plant.

Table 3. Effect of saline water irrigation and *Rhizobium* inoculation (g/ Kg seed) on some flowering aspects of sweet pea plant during 2002/2003 and 2003/2004 seasons

Treatments	Flowering@ (%)	Cluster No/ plant	Florets No/ cluster	Cluster stalk length (cm)
Saline water irrigation (ppm)				
		2002/2003 Season		
Control	44.4c	35.7a	3.14a	18.30a
1500	56.3b	26.2b	3.11a	12.40b
2500	77.1a	10.6c	2.50b	4.69c
3500	26.5d	6.16c	2.03b	4.37c
		2003/2004 Season		
Control	51.2b	39.7a	3.13a	17.30a
1500	51.0b	33.3b	3.11a	9.99b
2500	81.9a	16.0c	2.22b	6.40c
3500	34.9c	7.81d	2.09b	4.47d
Rhizobium inoculants (g/ Kg seed)				
		2002/2003 Season		
Control	49.6a	18.4a	2.72a	9.46b
10	52.1a	20.3a	2.64a	9.86ab
20	51.5a	20.2a	2.73a	10.50a
		2003/2004 Season		
Control	54.7a	23.2a	2.42a	8.87a
10	54.6a	24.5a	2.63a	9.80a
20	55.0a	24.8a	2.86a	9.95a

Means having same alphabetical letter(s) within each column did not significantly differ according to Duncan multiple range test at 5% level.
@ Flowering percentage was recorded on March 1st.

Effect of *Rhizobium* inoculation under salinity stress

Table 4 indicates that the highest flowering (%) was found in irrigated plants with 2500 ppm saline water concentration combined with any inoculation level (0, 10 or 20 g *Rhizobium* inoculants/ Kg seed), this effect was significant in the first season. This enhancing effect on flowering time was associated with significant reductions in cluster production/ plant and cluster stalk length comparing with inoculated or non inoculated plants and irrigated with non saline water. Generally, inoculation reduced the harmful effects of saline water irrigation. Since, under the same salinity

Table 4. Effect of interaction treatments between saline water irrigation and *Rhizobium* inoculation on some flowering aspects of sweet pea plant during 2002/2003 and 2003/2004 seasons

Interaction treatment		Flowering@	Cluster No/ plant	Florets No/ cluster	Cluster stalk length
Rhizobium (g/ Kg seed)	**Saline water** (ppm)	(%)			(cm)
			2002/2003 Season		
0	**0.00**	45.9cd	31.80ab	3.44a	17.30a
0	**1500**	53.9bc	27.80b	3.00a	11.90b
0	**2500**	70.9ab	7.38cd	2.58a	4.63c
0	**3500**	27.8de	7.02cd	1.85a	3.89c
10	**0.00**	45.0cde	37.60a	2.89a	18.40a
10	**1500**	56.5bc	25.70b	2.99a	12.60b
10	**2500**	81.2a	11.80cd	2.70a	4.00c
10	**3500**	25.8e	6.10cd	1.98a	4.33c
20	**0.00**	42.4cde	37.60a	3.10a	19.10a
20	**1500**	58.4bc	25.40b	3.33a	12.60b
20	**2500**	79.3a	12.70c	2.23a	5.43c
20	**3500**	26.1e	5.36d	2.25a	4.88c
			2003/2004 Season		
0	**0.00**	51.6a	33.40ab	3.15a	16.30a
0	**1500**	51.2a	31.70b	2.94a	9.75bc
0	**2500**	78.5a	17.80c	1.88a	4.93de
0	**3500**	37.4a	10.10cd	1.73a	4.51de
10	**0.00**	51.7a	43.40a	3.14a	17.20a
10	**1500**	51.6a	35.00ab	3.32a	10.70b
10	**2500**	82.6a	13.40cd	1.96a	7.09cd
10	**3500**	32.5a	6.41d	2.09a	4.08e
20	**0.00**	50.2a	42.40a	3.10a	18.30a
20	**1500**	50.3a	33.30ab	3.07a	9.44bc
20	**2500**	84.6a	16.70cd	2.81a	7.18cd
20	**3500**	34.9a	6.92d	2.45a	4.82de

Means having same alphabetical letter(s) within each column did not significantly differ according to Duncan multiple range test at 5% level.

@ Flowering percentage was recorded on March 1st.

concentration inoculation enhanced cluster No/ plant and cluster stalk length. Inoculation at 10 or 20 g *Rhizobium* inoculants/ Kg seed combined with non saline water treatments recorded the highest values of cluster No/ plant and cluster stalk length with no significant differences between the two used inoculation levels in most cases. Interaction treatments between *Rhizobium* inoculation and the used salinity levels did not exhibit significant effects on produced florets No/ cluster during the two seasons. The enhancing effect on flowering time under 2500 ppm salinity concentration combined with any inoculation level might be due to the caused restriction of vegetative growth under these interaction treatments (as mentioned above) which in turn shift plants to the reproductive habit, while the inhibitive effects of the same treatments on quality of produced flowers (clusters No/ plant and cluster stalk length) might be due to the inadequate metabolites for proper flowers growth. Regarding the effect of inoculation on the salinity stress harmful, Cordovilla *et al.* (1999a) stated that inoculated pea plants with *Rhizobium* were more tolerant to salt stress than N fertilized ones.

Chemical Composition

Effect of salinity

Data in Table 5 illustrate that chlorophyll a, b and total (a+b) chlorophyll (mg/ g fresh weight) as well as total nitrogen and total carbohydrates percentages in sweet pea leaf tissues significantly, in most cases, and gradually decreased with increasing salinity concentration up to the highest level of 3500 ppm. The highest values in this respect were recorded by using non saline water followed by 1500 ppm level. Moursi *et al.* (1977) stated that, the depression in the content of total carbohydrates was due to the production of relatively high energy by increasing respiration to overcome the relatively low availability of water and nutritional elements in saline medium. Also, the depression effect of salinity on the water content of leaves usually resulted in a decrease in the rate of photosynthesis and consequently a reduction in carbohydrate accumulation. In addition, Maharaj *et al.* (1990) recorded significant decreases in sugar (total, reducing and non-reducing) and N contents in pea cotyledons with increasing salt and water stress compared to the control at all stages of seedling growth. Fedina *et al.* (1994) explain reduction of carbohydrates in NaCl salinity stressed pea plants as a result of increasing photorespiration and decreasing photosynthesis. However, lacking N, as a constituent in chlorophyll molecule, in salt affected plants may be in turn had an adverse effect on chlorophyll synthesis leading to more reduction in chlorophyll content in leaf tissues.

Effect of *Rhizobium leguminosarum* inoculation

Presented data in Table 5 show that *Rhizobium* inoculation at 10 or 20 g/kg seed significantly, in most cases, increased chlorophyll a, b, and total (a+b) chlorophyll (mg/ g fresh weight) as well as total nitrogen and total carbohydrates percentages in sweet pea leaf tissues in both seasons comparing to non inoculated plants. The enhancing effect of *Rhizobium* inoculation on leaf chemical constituents was expected since, *Rhizobium* in nodules will fix the atmospheric N and supply plants with it. N will contribute in synthesis of enzymes, proteins and chlorophyll leading to more plant viability and more accumulation of N, chlorophylls and carbohydrates. However, El-Mansi *et al*. (2000) recorded similar increases in leaf contents of chlorophyll a, b and total (a+b) as well as N% by pea seed *Rhizobium* inoculation. Also, Vasilieva *et al*. (1999) stated that inoculation pea plants with *Rhizobium leguminosarum* generally increased nitrate content in leaves throughout the growth cycle.

Effect of *Rhizobium* inoculation under salinity stress

Concerning the effect of interaction between *Rhizobium* inoculation and saline water on chlorophylls (mg/ g fresh weight) as well as total N and total carbohydrates percentages, obtained results in Table 6 show that, in general, increasing the interacted salinity concentration under any inoculation level reduced chlorophyll a, b and total chlorophyll as well as total N and total carbohydrates in sweet pea leaf tissues. On the other side, inoculation at 10 or 20 g *Rhizobium* inoculants/ Kg seed under any salinity concentration increased all studied chemical constituents with no significant differences between the two inoculation levels comparing to non inoculated plants under the same salinity level. The reductions in N and subsequently chlorophyll and carbohydrates by increasing salinity stress under any inoculation level may explain as a result of the adverse effects of salinity on nodulation and N fixation. Siddiqui and Kumar (1987) on peas studied nodule metabolism under salinized and desalinized conditions. They found that salinity decreased N content of nodules, an index of N fixation. Steven (2007) on pea mentioned that any adverse soil condition or environmental stress that affects plant growth will slow down the nitrogen fixation process. While, the enhancing effect of inoculation on leaf chemical constituents may be due to the additional supplying of fixed N as compare to non inoculated plants.

Table 5. Effect of saline water irrigation and *Rhizobium* inoculation on some leaf chemical constituents of sweet pea plant during 2002/2003 and 2003/2004 seasons

Treatments	Total N (%)	Chlorophyll (mg/ g fresh weight)			Total carbohydrate (%)
		a	b	Total (a+b)	
Saline water irrigation (ppm)					
			2002/2003 Season		
Control	3.31a	1.81a	0.41b	2.23a	18.8a
1500	2.19b	1.24b	0.55a	1.80b	17.6a
2500	2.16b	1.06c	0.30c	1.36c	16.0b
3500	1.73c	0.85d	0.27c	1.12d	12.9c
			2003/2004 Season		
Control	3.54a	1.81a	0.60a	2.41a	17.3a
1500	2.70b	1.47b	0.64a	2.12b	15.6b
2500	2.29c	1.04c	0.29b	1.33c	14.4bc
3500	1.61d	0.49d	0.24b	0.74d	13.9c
***Rhizobium* inoculants (g/ Kg seed)**					
			2002/2003 Season		
Control	1.77b	1.17b	0.34b	1.51b	15.1b
10	2.58a	1.28a	0.40a	1.68a	17.0a
20	2.69a	1.28a	0.41a	1.70a	16.9a
			2003/2004 Season		
Control	2.04b	1.17a	0.41b	1.58b	14.9a
10	2.72a	1.22a	0.45a	1.67a	15.8a
20	2.84a	1.23a	0.46a	1.69a	15.2a

Means having same alphabetical letter(s) within each column did not significantly differ according to Duncan multiple range test at 5% level.

Table 6. Effect of interaction treatments between saline water irrigation and *Rhizobium* inoculation on some leaf chemical constituents of sweet pea plant during 2002/2003 and 2003/ 2004 seasons

Interaction treatment		Total N	Chlorophylls (mg/ g fresh weight)			Total carbohydrate
Rhizobium (g/ Kg seed)	Saline water (ppm)	(%)	a	b	Total (a+b)	(%)
			2002/2003 Season			
0	**0.00**	2.14bcd	1.69a	0.37bc	2.06a	17.4a
0	**1500**	1.83cd	1.19a	0.45b	1.64a	16.1a
0	**2500**	1.56d	1.04a	0.29d	1.33a	14.8a
0	**3500**	1.55d	0.75a	0.26d	1.01a	12.0a
10	**0.00**	3.77a	1.89a	0.43b	2.32a	19.9a
10	**1500**	2.28bc	1.27a	0.61a	1.88a	18.3a
10	**2500**	2.77b	1.04a	0.31cd	1.36a	15.5a
10	**3500**	1.51d	0.91a	0.24d	1.15a	14.1a
20	**0.00**	4.01a	1.86a	0.44b	2.31a	19.1a
20	**1500**	2.48bc	1.27a	0.60a	1.88a	18.4a
20	**2500**	2.16bcd	1.09a	0.31cd	1.40a	17.7a
20	**3500**	2.12bcd	0.90a	0.31cd	1.21a	12.4a
			2003/2004 Season			
0	**0.00**	2.65a	1.72b	0.52d	2.24bc	15.9a
0	**1500**	2.13a	1.38c	0.60cd	1.99d	15.7a
0	**2500**	1.95a	0.49d	0.29e	1.24e	14.6a
0	**3500**	1.44a	0.62e	0.24e	0.86f	13.4a
10	**0.00**	3.86a	1.83ab	0.55cd	2.38b	18.1a
10	**1500**	2.79a	1.53c	0.70ab	2.24bc	15.8a
10	**2500**	2.46a	1.09d	0.30e	1.39e	14.6a
10	**3500**	1.77a	0.43f	0.26e	0.69g	14.9a
20	**0.00**	4.10a	1.88a	0.73a	2.61a	17.9a
20	**1500**	3.19a	1.50c	0.63bc	2.13cd	15.4a
20	**2500**	2.46a	1.10d	0.27e	1.37e	14.1a
20	**3500**	1.63a	0.44f	0.23e	0.67g	13.5a

Means having same alphabetical letter (s) within each column did not significantly differ according to Duncan multiple range test at 5% level.

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استجابة بسلة الزهور لإجهاد الملوحة والتلقيح بالريزوبيم

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أُجريت تجربتي أصص في المزرعة التجريبية لمعهد الكفاية الإنتاجية ، جامعة الزقازيق خلال الموسمين المتتاليين ٢٠٠٢/٢٠٠٣ ، ٢٠٠٣/٢٠٠٤ لدراسة تأثير أربع تركيزات لملوحة ماء الري (صفر، ١٥٠٠، ٢٥٠٠، ٣٥٠٠ جزء في المليون) وثلاث معدلات من التلقيح بالريزوبيم (صفر ، ١٠ ، ٢٠ جم لقاح ريزوبيم/ كجم بذرة) وتفاعلاتهم على نسبة البقاء والنمو والإزهار والتحليل الكيمياوى لنباتات بسلة الزهور.

أدت الزيادة في تركيز ملوحة ماء الري حتى المستوى المرتفع ٣٥٠٠ جزء في المليون إلى نقص تدريجي في نسبة البقاء والنمو والإزهار (ارتفاع النبات وإنتاج الأفرع والأوراق /

نبات والوزن الغض والجاف للنبات وعدد العناقيد الزهرية / نبات وطول الحامل الزهري)، وكانت هذه التأثيرات لها علاقة بانخفاض محتوى أنسجة أوراق النبات من الكلوروفيل والنيتروجين الكلى والكربوهيدرات الكلية، وقد أدى الري بمستوى ملوحة ٢٥٠٠ جزء في المليون إلى تبكير الإزهار مقارنةً بالكنترول (الري بماء الصنبور) وبمستوى الملوحة المنخفض (١٥٠٠ جزء في المليون) وأيضاً المرتفع (٣٥٠٠ جزء في المليون).

أدى تلقيح البذور بالريزوبيم إلى زيادة النيتروجين والكلوروفيل والكربوهيدرات بأنسجة أوراق النبات، وانعكس ذلك على تحسين النمو الخضري وبالتالي الزهري. لم تظهر اختلافات معنوية، عموماً، بين معدلي التلقيح المختبران ١٠ ، ٢٠ جم لقاح/ كجم بذرة.

أدت الملوحة إلى تقليل التأثيرات المنشطة للتلقيح (عندما أجرى التلقيح بالريزوبيم تحت تأثير إجهاد الملوحة) مقارنة بالتلقيح تحت الظروف الغير ملحية. وعلى العكس من ذلك ، أدى التلقيح بالريزوبيم إلى زيادة مقاومة النبات للتأثيرات الضارة للملوحة مقارنةً بالنباتات الغير ملقحة، ذلك حيث أدى التلقيح تحت تأثير الري بالماء المالح إلى زيادة نسبة النباتات المتبقية حية والى زيادة النمو الخضري (ارتفاع النبات وعدد الأفرع والأوراق/ نبات والوزن الغض والجاف للنبات)، والإزهار (عدد العناقيد الزهرية / نبات وطول الحامل الزهري)، بالإضافة إلى زيادة محتوى الأوراق من الكلوروفيل والنيتروجين الكلى والكربوهيدرات الكلية مقارنةً بالنباتات الغير ملقحة والنامية تحت نفس مستوى الملوحة.