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ORIGINAL PAPER

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Influence of arbuscular mycorrhizal fungi and kinetin on the response of mungbean plants to irrigation with seawater

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Abstract Increasing use of saline water in irrigation can markedly change the physical and chemical properties of soil. An experiment was carried out to investigate the interaction between the mycorrhizal fungus *Glomus clarum,* isolated from a saline soil, and kinetin on the growth and physiology of mungbean plants irrigated with different dilutions of seawater (0, 10, 20, and 30%). The growth, chlorophyll concentration and sugar content of mycorrhizal plants was greater than that of non-mycorrhizal plants under all conditions (with or without seawater). The dry weight of both mycorrhizal and non-mycorrhizal mungbean plants irrigated with 10% seawater was significantly increased by treatment with kinetin. The mycorrhizal symbiosis increased root:shoot dry weight ratio, concentrations of N, P, K, Ca and Mg, plant height, protein content, nitrogen or phosphorus-use efficiencies, and root nitrogenase, acid or alkaline phosphatase activities of seawater-irrigated mungbean plants, with little or no effect of kinetin. Kinetin treatment generally decreased chlorophyll concentration and sugar content in mycorrhizal plants as well as Na/N, Na/P Na/K, Na/Ca and Na/Mg ratios. Root colonization by *G. clarum* was increased by irrigation with seawater, and kinetin had no consistent effect on fungal development in roots. This study provides evidence that arbuscular mycorrhiza can be much more effective than kinetin applications in protecting mungbean plants against the detrimental effects of salt water.

Keywords Glomus · Growth · Mycorrhiza · Mineral nutrition · Salinity

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Introduction

Saline water is frequently used in situations when there is insufficient good quality water for agriculture. Soil salinity and sodicity are the principal concerns when saline water is used in irrigation as they limit crop productivity and quality (Ayars and Tanji 1999). Soil salinity can also result from the proximity of semi-arid sites to the sea, or due to saline groundwater rising into the root zone and concentrating there when evaporation becomes excessive. In Egypt, about 96% of the land is desert, and saline groundwater creates a serious problem over much cultivated land. Most crops are salt-sensitive, and either cannot survive under conditions of salinity or survive with decreased yields. Plants are stressed in three ways by salinity: (1) low water potential of the root environment leads to water deficits in crop plants, (2) toxic effects of ions, mainly sodium (Na) and chlorine (Cl), and (3) nutrient imbalance caused by decreased nutrient uptake and/or transport to the shoot (Marschner 1995; Adiku et al. 2001). Approaches used to alleviate the deleterious effects of salinity include improvement of saline irrigation water, chemical amendments to soil, and selection of salt-tolerant crop varieties. However, correcting salinity problems is expensive and often represents only a temporary solution. Development of plant cultivars that can produce economic yields under saline conditions (Dasgan et al. 2002; Fooland 1996), as well as phytoremediation procedures that increase, by whatever mechanism, plant tolerance to saline water, are more permanent and complementary solutions (Qadir et al. 2001).

Arbuscular mycorrhizal (AM) associations may increase plant tolerance to salinity (Jalaluddin 1993; Coperman et al. 1996; Smith and Read 1997; Al-Karaki 2000; Ruiz-Lozano and Azcon 2000; Diallo et al. 2001; Yano-Melo et al. 2002). Improved salt tolerance of AM plants has been related to enhanced mineral nutrition, improvement in physiological processes like photosynthesis or water use efficiency, and production of osmoregulators (Auge and Stodola 1990; Ruiz-Lozano and Azcon 2000). Growth regulators such as gibberellic acid (Khan and Rizvi 1994), kinetin (Ungar 1991), and fusicoccin (Pyler and Proseus 1996) are known

to alleviate the inhibitory effects of salinity on plants. Khan et al. (2000) reported that gibberellic acid and kinetin can both alleviate some inhibitory effects of salinity on shoot growth, while root growth is promoted by kinetin. Nemat-Alla et al. (2002) showed that kinetin alleviates stress symptoms and regulates changes in phenolic metabolism of water-logged or saline-treated *Vigna sinensis* and *Zea mays*.

Mungbean (*Vigna radiata* L. *Wilczik*) is a fast-maturing N-fixing leguminous crop with relatively low fertilizer requirements and good drought tolerance. Mungbean is widely grown, particularly in developing countries, where it is consumed directly or used as a source of starch. Mungbean seeds surpass lentil and broad bean in calcium (Ca), iron (Fe) and vitamin A and contain nearly the same amount of protein. The introduction of new, high-yielding, fast-maturing food crops such as mungbean may help reduce the food gap in developing countries like Egypt (Ashour et al. 1996). The objective of the present work was to study the effects of inoculation with a salt-tolerant isolate of *Glomus clarum* and foliar application of kinetin on the growth and physiology of mungbean plants irrigated with different concentrations of seawater.

Materials and methods

A reclaimed calcareous soil obtained from a surface layer (0–20 cm) in the Burg El-Arab region, Egypt, was used. The soil (pH 7.9), which was dried then ground and sieved (2.0 mm), contained 63.9% sand, 2.9% silt and 34.2% clay, 34.9% $CaCO_3$, 120 mg/kg total nitrogen, 14 mg/kg available nitrogen, 21 mg/kg available phosphorus, 4.5 mg/kg available potassium and 1.8 mho/m EC.

Mycorrhizal inocula consisted of soil, spores, mycelium and infected root fragments, obtained from an open pot culture (*Allium cepa* L.) of *G. clarum* Nicolson and Schenck, previously isolated from a saline soil and provided by the Botany Department, Faculty of Science, Mansoura University, Egypt.

The experiment was conducted in a greenhouse between June and August 1998. Seeds of mungbean (*V. radiata* var. 2010 from the Agronomy Department, Agricultural Research Center, Giza, Egypt) were surface-sterilized with 7% calcium hypochlorite for 20 min and then washed with distilled water. Seeds were sown at a rate of four per pot (30-cm diameter containing 2 kg soil). Seedlings were thinned to one seedling/pot 7 days after sowing. The soil was inoculated with *G. clarum* before sowing by placing 10 g mycorrhizal inoculum (10 spores g^{-1}) 3 cm below the surface of the soil. Non-inoculated pots were supplied with a filtered washing of the inoculum to supply the same microflora other than the mycorrhizal fungus. One-half of the plants were sprayed (25 ml/plant) with a 0.5-mM kinetin solution 1 and 2 weeks after sowing (Nemat-Alla et al. 2002). All pots received NH_4NO_3 and K_2SO_4 at a rate of 60 kg/fed and 48 kg/fed, respectively. Plants for each treatment were watered with an equal amount of different concentrations of seawater to maintain soil moisture near field capacity. The experimental outlay was two mycorrhizal treatments (non-inoculated control and inoculated with *G. clarum*), two kinetin treatments (non-treated and treated), and four seawater concentrations (0, 10, 20 and 30%). Four replications per treatment were used and the plants were harvested 8 weeks after planting.

Growth and physiological parameters

At the end of the experiment, plant height, root and shoot dry matter as well as root-to-shoot ratio (R/S) were measured. Nitrogen (N) was extracted from plants with sulfuric acid using the semi-micro Kjeldahl method (Jackson et al. 1973). Phosphorus (P) was extracted by nitric-perchloric acid digestion and measured using the vanadono-molybdophosphoric colorimetric method (Jackson 1967). Potassium (K) and sodium (Na) were assayed using a flame spectrophotometer, while calcium (Ca) and magnesium (Mg) were determined by atomic absorption (Allen et al. 1984). The sugar and protein content of plant tissues were estimated according to Naguib (1963) and Bradford (1976), respectively.

The photosynthetic pigments chlorophyll *a* and *b* were extracted from leaves and determined by the method of Harborne 1984. Shoot – and P-use efficiency was determined as the ratio of the shoot dry weight (milligrams) produced per milligram of total shoot N or P (Ruiz-Lozano and Azcon 2000).

Immediately after harvest, part of the root system of non-AM and AM plants was washed carefully with water at 4°C to remove adhering soil particles, and quantitatively assayed for soluble acid and alkaline phosphatase activities (Gianinazzi-Pearson and Gianinazzi 1976). Values of phosphatase activities were recorded as units ml^{-1} min^{-1}. Nitrogenase activity was measured in root samples using the acetylene reduction assay (Hardy et al. 1973), and recorded as nmol C_2H_4 g dry $root^{-1}$ h^{-1}.

The remainder of the root system was cut into 0.5- to 1.5-cm segments, cleared in 10% KOH and stained with Trypan Blue in lactophenol (Phillips and Hayman 1970). The frequency (F%) and intensity (M%) of root colonization, and arbuscule development in the colonized region of roots (A%), were estimated by the method of Trouvelot et al. (1986).

Statistical analysis

The data were statistically analyzed as a complete randomized block design. Means were compared using the least significant difference (LSD; multiple *t*-test) procedure (Steel and Torri 1960) at $P<0.05$. The effects of mycorrhizal inoculation, seawater concentration and kinetin application on plant parameters were compared by analysis of variance (ANOVA) (Hicks 1983).

Results

The dry weight of mungbean plants colonized by *G. clarum* was higher than non-mycorrhizal plants irrigated either without seawater or with different levels of seawater (Table 1). Application of kinetin improved growth of non-AM plants only at 10% seawater, and at 10 and 20% seawater for AM plants. Kinetin did not affect growth of plants in the absence of seawater. The R/S ratio was increased in mycorrhizal plants treated with seawater, and highest R/S values were obtained in the presence of kinetin. The average height of the mungbean plants decreased with increasing levels of salinity from seawater (Table 1). The height of AM plants was greater than that of non-AM plants, irrespective of the presence or absence of kinetin.

No significant reduction in the total chlorophyll content of plants treated with increasing seawater concentrations was observed as compared to controls (Table 2). However, the total chlorophyll content of AM plants was always higher than that of non-AM plants. A similar trend was observed for chlorophyll *a* and *b*. Sugar content was not significantly affected by increased seawater concentrations in either AM or non-AM plants, regardless of the presence of kinetin.

Table 3 shows that significant decreases in N, P and K content, a slight increase in Ca and Mg, and a significant accumulation of Na occurred in non-AM plants upon raising the concentration of seawater during irrigation. On the other hand, the N, P, K, Ca and Mg content of AM plants were increased by raising the seawater content of the irrigation water, irrespective of the presence or absence of kinetin. Moreover, the results also indicate that mycorrhizal mungbean had lower Na/N, Na/P Na/K, Na/Ca and Na/Mg ratios than non-AM plants, and that these ratios were much lower in the presence of kinetin. The results shown in Table 4 indicate that increasing seawater concentrations had a negative effect on the protein content of all plants. Although the protein content in AM plants decreased with increasing seawater salinity level, it remained much higher than that of non-AM plants. Increasing seawater concentrations also had a negative effect on the values for – and P-use efficiencies, as well as alkaline and acid phosphatase activities, in non-AM plants while these values were relatively unaffected in AM plants, irrespective of the absence or presence of kinetin. Nitrogenase activity was reduced in non-AM plants at higher seawater levels whilst it was stimulated in AM plants, irrespective of the presence or absence of kinetin.

Seawater stimulated mycorrhiza development in mungbean (Table 5). Frequency of mycorrhizal root segments (F%), intensity of mycorrhizal colonization in root tissues (M%) and arbuscule frequency in root systems (A%) were higher at all three seawater levels than in control plants. Kinetin did not appear to have any consistent effect on mycorrhiza development, independent of the seawater concentration in the irrigation water.

Table 1 Effect of different dilutions of seawater on dry weight, root shoot ratios and plant height of mycorrhizal and non-mycorrhizal mungbean plants with and without foliar application of kinetin. *R/S* Root-to-shoot ratio, *LSD* least significant difference

	Dry weight (g plant^{-1})	R/S	Plant height (cm plant^{-1})
Control			
Non-mycorrhizal plant	3.6	0.02	40.1
Mycorrhizal plant	10.9	0.01	51.3
Non-mycorrhizal plant+kinetin	3.8	0.02	35.5
Mycorrhizal plant+kinetin	11.6	0.02	56.6
10% Sea water			
Non-mycorrhizal plant	2.7	0.04	25.3
Mycorrhizal plant	13.3	0.2	50.5
Non-mycorrhizal plant+kinetin	6.2	0.01	28.8
Mycorrhizal plant+kinetin	16.2	0.23	46.6
20% Sea water			
Non-mycorrhizal plant	1.9	0.04	24.5
Mycorrhizal plant	10.1	0.22	38.6
Non-mycorrhizal plant+kinetin	3.0	0.01	18.1
Mycorrhizal plant+kinetin	14.6	0.25	39.2
30% Sea water			
Non-mycorrhizal plant	1.1	0.01	21.8
Mycorrhizal plant	6.7	0.22	27.3
Non-mycorrhizal plant+kinetin	2.2	0.02	16.9
Mycorrhizal plant+kinetin	8.7	0.26	39.2
LSD $P \leq 0.05$	2.7	0.013	6.31

Table 2 Effect of different dilutions of seawater on chlorophyll concentration and sugar content of mycorrhizal and non-mycorrhizal mungbean plants with and without foliar application of kinetin

	Chlorophyll concentration (μg g^{-1})			Sugar content (g plant^{-1})
	Total	*a*	*b*	
Control				
Non-mycorrhizal plant	2.44	1.29	0.79	1.56
Mycorrhizal plant	5.1	2.98	2.03	4.72
Non-mycorrhizal plant+kinetin	3.72	1.71	1.8	1.64
Mycorrhizal plant+kinetin	5.185	2.83	2.23	4.51
10% Sea water				
Non-mycorrhizal plant	2.16	1.1	0.67	0.72
Mycorrhizal plant	4.85	2.74	2.06	5.0
Non-mycorrhizal plant+kinetin	2.39	1.13	0.97	0.94
Mycorrhizal plant+kinetin	3.82	1.42	2.17	3.05
20% Sea water				
Non-mycorrhizal plant	1.75	0.96	0.45	0.65
Mycorrhizal plant	4.3	2.32	1.83	5.25
Non-mycorrhizal plant+kinetin	1.84	1.12	0.53	0.78
Mycorrhizal plant+kinetin	2.75	1.16	1.36	3.85
30% Sea water				
Non-mycorrhizal plant	1.66	0.89	0.43	0.63
Mycorrhizal plant	3.62	2.05	1.55	6.59
Non-mycorrhizal plant+kinetin	1.74	1.1	0.45	0.66
Mycorrhizal plant+kinetin	2.75	1.16	1.36	3.32
LSD $P \leq 0.05$	0.43			3.26

Table 3 Effect of different dilutions of seawater on percent mineral contents of mycorrhizal and non-mycorrhizal mungbean plants with and without foliar application of kinetin

	%N	%P	%K	% Ca	% Mg	% Na
Control 0.0%						
Non-mycorrhizal plant	1.75	0.15	1.21	0.81	0.63	0.47
Mycorrhizal plant	1.94	0.19	1.39	0.98	0.79	0.42
Non-mycorrhizal plant +kinetin	1.75	0.16	1.24	0.83	0.75	0.44
Mycorrhizal plant+kinetin	2.13	0.19	2.43	1.56	1.1	0.41
10% sea water						
Non-mycorrhizal plant	1.78	0.16	1.86	0.83	0.65	0.68
Mycorrhizal plant	2.42	0.28	2.29	1.39	1.12	0.44
Non-mycorrhizal plant+kinetin	1.82	0.13	1.52	0.83	0.81	0.62
Mycorrhizal plant+kinetin	2.63	0.30	2.95	2.1	1.87	0.45
20% sea water						
Non-mycorrhizal plant	1.26	0.09	1.15	1.3	0.75	0.98
Mycorrhizal plant	2.98	0.41	2.29	1.96	1.41	0.47
Non-mycorrhizal plant+kinetin	1.8	0.09	1.2	1.28	0.82	0.83
Mycorrhizal plant+kinetin	3.6	0.41	3.1	2.87	2.12	0.47
30% sea water						
Non-mycorrhizal plant	0.98	0.09	1.62	1.35	0.90	1.36
Mycorrhizal plant	2.81	0.33	2.29	2.88	1.61	0.47
Non-mycorrhizal plant+kinetin	1.87	0.08	1.1	1.35	0.93	1.1
Mycorrhizal plant+kinetin	3.12	0.33	2.99	3.24	2.53	0.45
LSD $P \leq 0.05$	1.04	0.001	0.71	1.06	0.73	0.01

Table 5 Effect of different dilutions of seawater on mycorrhiza development (as indicated by Trypan Blue staining) in mungbean plants inoculated with *Glomus clarum* isolated from a saline soil, with and without foliar application of kinetin. *F%* Frequency of mycorrhizal root segments, *M%* intensity of mycorrhizal colonization, *A%* arbuscule frequency in root systems

Treatment	F (%)	M (%)	A (%)
Mycorrhizal plants			
Control	68	21	11
10% Sea water	78	36	19.2
20 % Sea water	74	24	16
30 % Sea water	71	23	17.1
Mycorrhizal plants+kinetin			
Control	68	19	11.3
10% Sea water	81	40	22
20 % Sea water	75	26	17.4
30 % Sea water	73	21	17.2
LSD $P \leq 0.05$	3.36	0.72	1.2

The ANOVA analysis of data (Table 6) confirmed the significant effects of seawater salinity on plant dry weight, plant height, K, Ca and Na accumulation, and protein content in mungbean. Table 6 also reveals that the combined effects of AM with kinetin and salinity, or with kinetin and salinity are similar to the effect of AM alone for the different growth and physiological parameters measured in the mungbean plants.

Table 4 Effect of different dilutions of seawater on protein content, nitrogen (N) and phosphorus (P)-use efficiencies, root nitrogenase and phosphatase enzyme activities of mycorrhizal and non-mycorrhizal mungbean plants with and without foliar application of kinetin

	Protein content (mg plant^{-1})	N-use efficiency	P-use efficiency	N_2-use activity (nmol C_2H_4 g^{-1} dry root^{-1} h^{-1})	Phosphatase activities (U^{-1} ml^{-1} min^{-1})	
					Alkaline	Acid
Control						
Non mycorrhizal plant	11.25	41.4	42.6	75.1	325	1,220
Mycorrhizal plant	13.4	72.9	84.6	82.7	520	1,635
Non-mycorrhizal plant+kinetin	11.38	43.7	42.9	75.0	320	1,110
Mycorrhizal plant+kinetin	13.68	73.1	84.3	82.4	515	1,610
10% sea water						
Non mycorrhizal plant	12.4	32.9	35.0	77.9	300	1,192
Mycorrhizal plant	21.13	73.6	85.0	116.5	610	1,640
Non-mycorrhizal plant+kinetin	13.21	33.0	31.9	80.4	310	1,205
Mycorrhizal plant+kinetin	22.63	72.0	82.3	117.9	627	1,605
20% sea water						
Non mycorrhizal plant	9.6	26.4	24.5	64.7	300	1,068
Mycorrhizal plant	19.37	69.7	86.1	108.3	570	1,585
Non-mycorrhizal plant+kinetin	10.2	27.8	24.7	74.6	302	1,042
Mycorrhizal plant+kinetin	18.3	74.7	83.5	107.8	570	1,498
30% sea water						
Non-mycorrhizal plant	7.42	25.1	23.0	59.3	290	923
Mycorrhizal plant	16.5	71.8	80.3	108.0	556	1,421
Non-mycorrhizal plant+kinetin	9.6	26.4	22.3	63.9	267	936
Mycorrhizal plant+kinetin	16.6	72.4	81.2	105.2	549	1,490
LSD $P \leq 0.05$	4.31	10.65	13.51	8.3	15.6	10.92

Table 6 ANOVA analysis of data for growth and physiological parameters and mycorrhiza levels of mungbean plants treated with kinetin and sea water. *AM* Arbuscular mycorrhizal

Variables	AM (M)	Kinetin (K)	Salinity (S)	M × K	M × S	S × K	S × M × K
Dry weight	**[a]	ns	**	*	**	ns	*
Plant height	*	ns	*	ns	**	ns	ns
Chlorophyll content	*	ns	ns	*	ns	ns	*
Sugar content	ns	ns	ns	ns	*	ns	*
Nitrogen	*	ns	ns	*	*	ns	**
Phosphorus	**	ns	ns	*	**	ns	**
Potassium	*	*	**	*	**	ns	*
Calcium	*	ns	*	*	**	ns	*
Magnesium	*	ns	ns	*	*	ns	*
Sodium	*	ns	**	*	**	ns	**
Protein	**	ns	*	*	*	ns	*
Nitrogenase	**	ns	ns	*	*	ns	*
Alkaline phosphatase	**	ns	ns	**	*	ns	*
Acid phosphatase	**	ns	ns	**	*	ns	*

[a]Non-significant
**Highly significant, $P \leq 0.01$;
* significant, $P \leq 0.01$

Discussion

Mycorrhizal symbiosis is a key component in helping plants to cope with adverse environmental conditions. The beneficial effects of different mycorrhiza on plant growth under saline conditions have been demonstrated in various plant species (Diallo et al. 2001; Yano-Melo et al. 2002; Muhsin and Zwiazek 2002). In this study, the overall growth and measured physiological parameters of mungbean plants irrigated with different levels of seawater increased upon mycorrhizal association with *G. clarum* as compared to non-AM plants. The results also indicate that application of kinetin can improve the growth of AM and non-AM plants at levels of seawater of 10–20%. These findings are in agreement with previous results (Mathur and Vyas 1990; Jalaluddin 1993; Nemat-Alla et al. 2002) and suggest that management of AM symbiosis together with kinetin application may help in overcoming the detrimental growth effects of salt stress induced by sequential irrigation with seawater.

Nutrient imbalances in plants can result from salt stress in various ways. Imbalances may result from the effect of salinity on nutrient availability, competitive uptake, transport or partitioning within the plant, or may be caused by physiological inactivation of a given nutrient. In the present investigation, it is interesting to note that seawater salinity caused a nutrient imbalance in both AM and non-AM mungbean plants, which showed lower and higher concentrations of the macronutrient elements N, P and K, respectively. Similar observations have been made in previous studies (Jarstfer et al. 1998; Al-Karaki 2000; Yano-Melo et al. 2002; Rao and Tak 2002). In non-AM plants, Na content increased to a greater extent relative to N, P, K, Ca and Mg levels with seawater irrigation. These results are in agreement with the previous work of Khan et al. (2000) who found that nutrient deficiencies can occur in plants when high concentrations of Na^+ reduce amounts of available K^+, Mg^{2+} and Ca^{2+}, displace membrane-bound Ca^{2+}, or have a direct toxic effect by interfering with the function of K^+ as a cofactor in various metabolic reactions. It is of interest that the Na^+ content of AM mungbean plants remained constant at the three levels of seawater tested, and relatively similar to that of non-AM plants growing in the absence of seawater. These results emphasize the protective effect for plants against salinity of AM, and indicate that this effect may be mediated through AM lowering Na/N, Na/P Na/K, Na/Ca and Na/Mg ratios in mungbean plants.

In conclusion, the present study indicates that AM formed by a salt-tolerant fungus can significantly increase the dry weight, height, chlorophyll, sugar and protein content, – and P-use efficiencies, nitrogenase, and acid and alkaline phosphatase activities, of mungbean plants under saline stress conditions. Based on these results, mycorrhizal symbiosis appears to be a prime factor for growth and survival (certain aspects of which may be increased with foliar application of kinetin) of mungbean plants under salinity stress. Accordingly, it is conceivable that AM may make a critical contribution to crop production under conditions where saline waters are used for irrigation.

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