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EFFECT OF SEASONAL VARIATIONS ON MYCORRHIZAL OCCURRENCE AND INFLUENCE OF SALINITY STRESS ON MAIZE AND COWPEA INFECTED BY MYCORRHIZAS AND THEIR ACTIVITIES IN HOST PLANTS

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ABSTRACT

The vesicular arbuscular mycorrhizas (VAM) were isolated from Al-Taif province at three different sites (Al-Hada, Al-Shafa and Wadi-Lea). The seasonal variations of the mycorrhizas indicated that Wadi-Lea have the highest spore density and % of root infection (5850spore/kg oven dry soil and 73.5%. respectively), followed by Al-Hada site (3410spore/kg oven dry soil and 53.5%, respectively) and Al-Shafa showed the lowest values. The results indicated that the soil characteristics and not seasonal variations have a direct influence on mycorrhizal spore density and their infection. The influence of inoculation of VAM spores on the growth of maize and cowpea under different concentrations of salinity, was investigated through some plant growth parameters, photosynthetic pigments and some elements, as well as proline content of the tested plants. The results indicated that every one of the tested plants have different responses to both salinity and mycorrhizal inoculations. However, mycorrhizas have stimulatory effects on the tested parameters. The effect of salinity on mycorrhizas activities on the tested plants (maize and cowpea) revealed that salt concentrations more than 500ppm have a deleterious influence on the mycorrhizal activities on both plants.

INTRODUCTION

Vesicular arbuscular mycorrhizas (VAM) are known to be associated with most agricultural crops and appear over wide ecological zones. The beneficial effects of VAM on plant growth, nutrition, element uptake and different physiological processes have been recognized (Rajapakse et al. 1989). VAM of cultivated lands are affected by various factors such as soil, host plant and its growth phase, environmental conditions, agricultural and horticultural practices. and the seasonal variations (Van Duin et al. 1990; letswart et al. 1992; Shamim et al. 1994). VAM normally inhibit the salt marshes and play important role in reducing loss in plant growth and productivity under salt stress (Pond et al. 1984; Pfeiffer and Bloss, 1988; Sengupta and Chaudhuri, 1990). The increased salinity affects VAM growth and activities on the host plant (Juniper and Abbott, 1993). The present study aimed to isolate and identify VAM from the agricultural soil at Al-Taif province, Saudi Arabia, and to elucidate the effect of some soil characteristics and seasonal variations on VAM. The study included the influence of VAM on the activities of maize and cowpea plants grown under salt stress and also the influence of salinity on the VAM activities on the host plants.

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the plant tissues was determined spectrophometrically (Bates *et al.* 1973). The mycorrhizal activities of spore numbers per plant, inoculum potential (IP) (Liu and Luo, 1994), % of infection per plant, point of entry per cm of root, % of infection along the root and the mycorrhizal structures (vesicular and arbuscular) (Bahabail, 1996), were also estimated.

Each treatment was carried out at least in triplicates and the obtained results are the arithmetic mean. One way ANOVA test at $p \le 0.5$ was used for analysis of data.

RESULTS AND DISCUSSION

Seasonal variations of mycorrhizas in the study area

The results (Table 1) indicated that the studied sites at AI-Taif province (AI-Hada, Al-Shafa and Wadi-Lea) have considerable numbers of spores densities of vesicular arbuscular mycorrhizas (VAM) and considerable mycorrhizal infection of the studied plants. The two-tested mycorrhizal parameters (spore numbers and infection) were varied considerably according to the site, season, humidity and organic matter content as well as potassium and phosphorus of the tested soils. The highest averages of spores density values (5193spore/kg oven dry soil) and infection (65.8%) were registered at Wadi-Lea. Which recorded the highest averages of humidity (12.07%), organic matter (3.2%) and potassium (555ppm), as well as the lowest phosphate content (1754ppm) as compared to the other two sites. On the other hand, Al-Shafa site with the lowest recorded averages of moisture (4.8%), organic matter (1.8%) and potassium (232ppm) and the highest values of phosphate (2333ppm), showed the lowest averages of spore number and infection (2613spore/kg oven dry soil and 29.2% infection, respectively). As the effect of different seasons was considered at the tested sites, it showed different responses according to the study site. At Al-Hada, the highest spore density and root infected were recorded at autumn and the lowest were estimated at winter. While, at Al-Shafa, the winter season was favorable for the highest spore numbers and infection and the summer showed adverse results. However, the summer season was the most adequate for spore numbers and root infection as compared to the winter season. The occurrence of mycorrhizas at different soils of Saudi Arabia was reported (Malibari et al. 1990; Al-Garni and Daft, 1990). In accordance with theses findings, it was indicated that the soil characteristics, stage of host plant growth, and not seasonal variations have a direct influence in mycorrhizal spore density and their infection (Anderson et al. 1984; Van Duin et al. 1990). However, the higher the phosphate content of the soil the lower mycorrhizal spores density and their infection (Mosse, 1973).

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Plant	Salinity (ppm)	VAM (-/+)		Pigments (mg/g D.Wt.)						
			Height (cm)	Leaf rea (cm ²)	Shoot D.Wt. (g/plant)	Root D.Wt. (g/plant)	Chl. a	Chl.b	Carote- noids	Total
Zea mays (Maize)	None (control)	-+	45 75	55 90	0.40 0.82	0.30	5.89 6.83	4.63 5.06	2.30 2.82	12.82 14.71
	500	-+	54 61	76 80	0.51 0.68	0.26 0.47	6.34 7.55	5.10 5.53	2.82 3.02	14.26
	1500	- +	52 60	60 68	0.45 0.63	0.23 0.44	5.80 7.68	5.20 6.75	2.87 2.97	13.87 17.40
	2500	-+	45 57	51 64	0.33 0.47	0.21 0.41	5.44 5.91	4.30 6.55	1.98 2.72	11.72 15.18
	3500	-+	40 55	40 60	0.31 0.39	0.20 0.32	5.43 5.46	3.21 5.05	1.94 2.61	10.58
	L.S.D.		4.32	5.99	0.138	0.168	1.75	1.36	0.54	3.09
Vigna sinesis (Cowpea)	None (control)	-+	68 93	12 14	0.39 0.62	0.074 0.170	4.20 4.09	3.26 3.45	1.27 0.98	8.73 8.52
	500	- +	62 76	10 13	0.33 0.53	0.068 0.154	5.91 4.75	5.05 3.59	2.22 1.53	13.18 9.87
	1500	- +	59 75	9 13	0.30 0.49	0.032 0.096	6.75 7.09	5.88 6.83	2.64 2.42	15.27 16.34
	2500	- +	56 70	8 11	0.23 0.32	0.045 0.076	5.11 7.02	4.35 6.19	1.42 2.24	10.88 15.45
	3500	- +	49 51	6 7	0.17 0.19	0.038 0.055	3.43 6.69	2.38 5.74	0.63 2.07	6.44 14.50
	L.S.D.		5.66	1.32	0.0819	0.031	1.95	1.76	0.51	4.5

 Table 2. Effect of mycorrhizal spore (VAM) inoculation (+) or none (-) on some growth parameters and pigments content of maize and cowpea under different concentrations of salinity.

Table 3. Effect of mycorrhizal spore (VAM) inoculation (+) or none (-) on mineral and proline contents of maize and cowpea under different concentrations of salinity.

Plant	Salinity	VAM (-/+)	Elements (ppm)						
	(ppm)		Na	N	Р	к	Ca	Mg	(mg/g D.Wt.)
	None (control)	- +	4.28 3.29	13.10 15.09	0.52 1.21	14.04 15.73	3.14 3.08	6.56 7.73	0.1401
	500	- +	6.07 5.18	15.84 16.28	0.40 1.18	15.50 16.81	5.17 5.15	6.89 6.99	0.2334
Zea mays (Maize)	1500	- +	9.15 8.39	17.03 18.31	0.40 1.16	17.80 19.17	8.85 8.09	5.49 6.76	0.4191
Zea (M	2500	- +	13.09 12.83	18.81 18.98	0.41 1.17	18.90 20.22	11.14 10.99	6.11 5.67	0.6315
	3500	-+	14.13 13.19	20.31 20.45	0.40 1.08	21.39 23.03	14.94 14.98	5.88 4.85	0.9425
	L.S.D.		1.31	1.74	0.098	1.50	1.18	1.95	0.04
	None (control)	-+	5.62 6.19	14.19 16.02	0.63 1.21	12.08 14.17	4.11 4.08	7.16 8.17	0.2028
sis	500	- +	7.22 8.51	16.16 18.75	0.71 1.31	12.79 15.08	5.90 5.11	8.62 9.12	0.4329
sines vpea)	1500	- +	11.57 13.13	17.05 18.09	0.73 1.40	15.20 17.30	7.16 6.82	7.78 8.09	0.8621
Vigna sinesis (Cowpea)	2500	- +	13.89 14.80	20.21 20.18	0.62	16.18 19.46	9.63 9.00	7.91 7.38	1.1382
	3500	- +	14.46 15.33	22.58 23.78	0.51 1.13	18.33 21.03	11.22 10.49	6.20 5.95	1.3601 1.2291
	L.S.D.		1.58	2.09	0.17	1.73	1.21	0.81	0.12

influences on the tested mycorrhizal activities on both plants. The influence of salinity on mycorrhizas activities was studied by some workers (Pfeiffer and Bloss, 1988; Juniper and Abbott, 1993). It was reported (McMillen et al. 1998) that the hyphal growth of some vesicular arbuscular mycorrhizal fungi is inhibited by increasing concentrations of NaCl in soil and this may influence the formation of mycorrhizas. As the average of the different tested fungal activities at the tested salinity was considered, it was clearly indicated that the mycorrhizal activities on the host plants depend mainly on the infected plant and to a lesser extend on the salinity. Thus instead of % of infection/plant of cowpea in absence of salinity (control) was higher (81%) than in maize (76%), the first plant showed about 30% average of decrease and the latter had about 33% decrease, in presence of salinity. However, the other investigated activities showed higher decreases in cowpea than in maize plants. It is also worthy to note that all the averages of the tested activities (under salt stress) showed lower values as compared to control treatment except % of infection along the root of maize which showed average of about 17% increase.

Plant	Salinity (ppm)	% of infection/ plant	Vesicular structures/ cm of root	Arbusculr structures (%)	Points of entry/cm Of root	% of infection along the root	Length of root (cm)	No. of spores/ plant	IP**
	None	70	10		0	20	185	650	1700
Zea mays (Maize)	(control) 500	76 68	13	29	9 11	39 63	219	652 736	4722 5773
	1500	54	10	27	8	52	171	620	4382
ea may (Maize)	2500	45		11	5	39	149	581	2518
See	3500	37	8	6	5 3	28	123	439	1546
	L.S.D.	4.73	1.75	3.80	2.19	5.38	14.63	51.26	351.76
	Average*	51	9	19.5	6.75	45.5	165.5	594	3554.8
	None								
5	(control)	81	10	33	12	57	91	624	2698
es (e	500	84	11	35	13	59	114	696	3360
Vigna sinesis (Cowpea)	1500	63	7	15	6	43	83	552	1631
	2500	52	4	8	5	29	52	360	828
	3500	28	3	5	4	22	46	288	610
	L.S.D.	5.38	2.88	3.50	2.81	3.91	7.46	16.41	289.94
	Average*	56.75	6.25	15.75	7	38.25	73.8	474	1607.2

Table 4. Effect of salinity on mycorrhizas activities on Zea mays (maize) and Vigna sinesis cowpea).

Average* = Average of the tested salinity

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IP** = Infection potential

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تأثير التغيرات الموسمية علي تواجد الميكوريزا واستجابة الذرة واللوبيا ونشاط الميكوريزا للملوحة

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قسم علوم الأحياء ـ كلية العلوم ـ جامعة الملك عبدالعزيز جدة ـ المملكة العربية السعودية .

تم عزل الميكوريزا الحويصلية من ثلاث مناطق مختلفة بمحافظة الطائف (الهدا ـ الشفا ـ وادي ليـا). أوضحت التغيرات الموسـمية أن وادي ليـا يحتـوى علـي أكثر كثافة لجراثيـم الميكوريـزا والنسـبة المئوية لإصابة الجذور تبعها منطقة الهدا ثم الشفا والتي أظـهرت أقـل الأرقـام المسـجلة. أظـهرت النتائج أن خواص التربة وليست التغـيرات الموسـمية فـي المنـاخ هـي ذات التـأثير المباشـر علـي كثافة الجراثيم ومدى قابليتها للعدوى.

تم دراسة تـأثير إصابـة الـذرة واللوبيـا بجراثيـم الميكوريـزا الحويصليـة تحـت تركـيزات مختلفـة مـن الملوحة علي دلالات النمو وصبغات البناء الضوئي وبعض العناصر المعدنية وكذلك البرولين. أوضحت النتائج أن كل من نباتي الدراسة أظهر استجابة مختلفـة لكـل مـن الملوحـة والإصابـة بـالميكوريزا. وكان للميكوريزا تأثيرا محفزا علي الدلالات المختبرة. أثرت الملوحة علي نشاط الميكوريزا بالعائلين وكان تركيز الملوحة فوق ٥٠٠جزء في المليون أكثر خطورة علي نشاط الفطر علـي كـل مـن الـذرة واللوبيا.



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lower values than non-infected plants and their values increased with salinity. Vigna sinesis (cowpea) have different patterns of responses to both salinity and mycorrhizal inoculations. Where as the salinity increases the growth parameters showed gradual decrease and mycorrhizal inoculation were responsible for higher growth parameters of height, leaf area, shoot and root systems dry weights (36.8, 16.7, 59 and 129.7%, respectively). The mycorrhizal inoculations were stimulatory for the growth parameters of maize (monocotyledon) than those of cowpea (dicotyledon). 1500ppm of salinity were favorable for higher accumulation of the tested photosynthetic pigments and mycorrhizal inoculations induce the pigment formation at the higher salinity (more than 1500ppm). Generally, the fungal infection induces the accumulation of the tested elements than the fungal free plants. The elements content increased as salinity increases. A salinity of 1500ppm was responsible for maximal accumulation of P, whereas 500ppm salinity was so for Mg. On the other hand, proline contents showed different patterns as compared to that of maize, where it increases with the salinity increase. So, it is safely (according to the present findings) to conclude that, for the tested plants, every plant have different responses to both salinity increase and mycorrhizal inoculations in its growth patterns, photosynthetic pigments, elements content and proline accumulation. The stimulatory effect of mycorrhizas on the growth patterns of some plants was recorded by some workers (Pfiffer and Bloss, 1988; Jalaluddin, 1993). It was reported that mycorrhizal inoculations resulted in increased levels of the photosynthetic pigments (Johnson, 1984) and decrease in the pigments content of the plants under salt stress due to inhibitory effect of accumulation of the ions on the pigments biosynthesis (Strogonov, 1962). It has been indicated that under normal cultivation conditions, the plants inoculated by mycorrhizal spores were able to accumulate nitrogen (Vaast and Zasosk, 1992). Other workers has been indicated the increased levels of P, K, Na and Mg as a result to mycorrhizal inoculations (Pond et al. 1984). Accumulation of K in plants, under salt stress, play a role in ionic equilibrium in the cytoplasm (Jeschke, 1984), and the toxicity of Na ions reduced through pumping K (Allen and Cunningham, 1983). The role of proline accumulation as a result to salt stress was reported (Stewart, 1983; Shaddad and Zidan, 1989). These reports are in harmony with previous results, which indicated that mycorrhizas play an important role in plant tolerance for salt stress (Pfeiffer and Bloss, 1988; Rosendahl and Rosendahl, 1991).

Effect of salinity on mycorrhizas activities on maize and cowpea

The results (Table 4) revealed that a salinity of 500ppm was optimal for the different tested mycorrhizal activities on both maize and cowpea as compared to the control soil. Salt concentrations more than 500ppm have deleterious

rable	1. Average of seasonal variations of some soil characteristics and number of mycorrhizal
	spores/10g oven dry soil, as well as percentage of root infection of the cultivated plants by
	mycorrhizas in the tested sites at AI-Taif province.

12(2))			S	No. of	1			
Site	Season	pH Moisture content (%)		Organic matter (%)	K (ppm)	P (ppm)	spores/10 g dry soil	% of root infection
Al-Hada	Autmn	8.51	8.78	3.30	297	1875	36.1	55.3
	Winter	8.36	8.94	2.70	217	3584	21.6	30.9
	Spring	8.42	10.51	3.21	316	3269	29.8	43.6
	Summer Average:	8.28 8.39	8.20 9.11	3.23 3.11	283 278.25	1717 2611.3	34.1 30.4	53.3 45.78
Al-Shafa	Autmn	8.39	7.05	1.92	226	2894	26.3	31.1
	Winter	8.42	5.23	1.83	174	2316	29.6	38.1
	Spring	8.20	3.82	1.62	192	2264	26.7	28.5
	Summer Average:	8.16 8.29	3.09 4.80	1.82 1.80	334 231.5	1857 2332.8	21.9 26.13	19.0 29.18
Wadi-Lea	Autmn	8.80	10.96	3.56	556	1938	56,4	73.8
	Winter	8.16	13.31	2.86	502	2295	40.1	50.3
	Spring	8.21	14.31	3.17	548	1400	52.7	65.5
	Summer Average:	8.31 8.37	9.69 12.07	3.20 3.20	614 555	1381 1753.5	58.5 51.93	73.5

Effect of inoculation of VAM spores on the growth of maize and cowpea under different concentrations of salinity

The influence of mycorrhizal spore inoculation on some growth parameters, photosynthetic pigments and elements content as well as proline concentrations of the tested crop plants, Zea mays (maize, as a monocotyledonous plant) and Vigna sinesis (cowpea, as a dicotyledonous plant), grown under different concentrations of salinity was tested. The results (Tables 2, 3) revealed that in case of maize plants free from mycorrhizas, a salinity of 500ppm was responsible for the highest growth parameters of height, leaf area, shoot and root systems dry weights. The same figures were recorded for the photosynthetic pigments of chlorophyll a, b and carotenoids. On the other hand, the elements content of Na, N, K and Ca showed parallel increase as the salinity increase, while the opposite was clear in case of P and Mg contents. As proline content was considered, its values were in concomitant decrease with salinity increase. Ineffectiveness of maize with mycorrhizal spores resulted in higher growth parameters of plant height, leaf area, shoot and root systems dry weights, as compared with non-infected plants (66.7, 38.9, 105 and 273.3%, respectively). However, as the salinity increased the previous parameters showed a parallel decrease, but their values still higher than the non-infected plants. The pigment content of chlorophyll a, b and carotenoids showed higher values than the non-infected plants and increased with salinity up to1500ppm, then followed by parallel decrease as the salinity increased more than 1500ppm. The presence of mycorrhizas induced the accumulation of N, P, K, Ca and Mg as the salinity increased as compared to plants free from them. However, the fungi reduce the Na content of the plants, instead of its I ncreased content with salinity. While, proline content showed

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MATERIALS AND METHODS

Soil and plant samples

Soil samples (approximately 3kg) and root systems of the standing crops were collected from three different agricultural sites (AI-Hada, altitude 2000m, AI-Shafa, altitude more than 2500m and Wadi-Lea, altitude less than 1500m) at AI-Taif province, Saudi Arabia (AI-Garni and Daft, 1990). Soil pH was determined using a Beckman pH meter with a glass electrode. The moisture content was estimated after drying to constant weight at 105°C. Organic matter was determined by the method described by Peach and Tracey (1956). Mycorrhizal spores were extracted from the soil samples (2kg) using the wet sieving and decanting technique (Gerdemann and Nicolson, 1963). The number of spores was estimated by filtering one mI of spore suspension into a gridded paper and then counted under a binocular microscope. The root system of each standing plant was gently washed to remove all the soil particles, then stained in Trypan blue (Phillips and Hayman, 1970).

Identification of the mycorrhizal spores

Representative spore samples (replicated three times) were taken from each sample for identification purposes. The endophytes were identified to the genus level on the basis of spore characteristics and the relationship of the spore to the associated hyphal attachments. Using the keys of Hall and Fish (1979), Trappe (1982) and the manual for the identifications of VA mycorrhizal fungi by Schenck and Perez (1988). The spores under identification appeared to belong to *Glomus* spp.

Plant culture technique and infection

The tested plants *Zea mays* (maize, as a monocotyledonous) and *Vigna sinesis* (cow- pea, as a dicotyledonous) were grown in horticultural sterilized, washed sand mixed with rock phosphate (0.2g/kg sand) in 13cm pots. Approximately 250spores/pot were added. Two seeds were planted in each pot, reduced to one after two weeks germination. Plants were fed twice weekly with 60ml (water holding capacity) of the Hogland's nutrients solution (Downs and Heilmers, 1975), which contains different concentrations of salinity (0.0 (control), 500, 1500, 2500, 3500ppm) of a mixture of NaCI and CaCl₂ (1:1). After 15 weeks of growth in the green house $(28\pm2^{\circ}C)$ (Al-Garni and Daft, 1990), the plants were harvested and the following parameters were recorded: plant height, root system length, leaf area (using the planometer), shoot and root systems dry weights, chlorophyll a,b and carotenoids (Metzner *et al.* 1965), the elements of the ash of the shoot system of Na, K, Ca and Mg (by atomic absorption flame emission spectrometer Shimadzu AA-670), N (Nessler reagent, Delory, 1949), and P (Woods and Melon, 1941). The free proline in

