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Effect of Mycorrhizal Inoculation at Different Salinity Levels on Root Colonization, Growth and Chlorophyll Content of Different Grape Rootstocks (*Vitis* spp)

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ABSTRACT

A pot culture experiment was conducted during 2004-2006 at the College of Agriculture, University of Agricultural Sciences, Dharwad, to investigate effect of arbuscular mycorrhizal (AM) fungus (*Glomus fasciculatum*) inoculation at different salinity levels (0.52, 1.90, 4.33, 6.23 and 7.94 dSm^{-1}) on root colonization, growth and chlorophyll content of four grape rootstocks (Salt Creek, Dogridge, St. George and 1613). The extent of AM response on root colonization, growth and chlorophyll content varied with rootstock species, and with the level of salinity. AM fungus inoculated plants showed significantly higher root colonization percentage, root volume, root length, number of leaves, leaf area, total dry weight, and chlorophyll content. Exposure to salinity stress resulted in decreased root colonization, chlorophyll content and growth of shoots on all rootstocks, but reduction in growth was greatest on St. George.

***Keywords*:** grape, rootstock, salinity, mycorrhiza, root colonization, chlorophyll.

INTRODUCTION

Grape (*Vitis* spp.) is one of the most commercially grown important fruit crops in the world. In India grapes are cultivated at an extent of 40,000 hectares across the country with an estimated production of 1.2 million tonnes (Anonymous, 2003). Maharashtra, Andhra Pradesh, Karnataka and Tamil Nadu in western and southern India, and Punjab, Haryana and Uttar Pradesh in northern India, are the major grape growing states. Over 90 per cent of the area occupied by grape cultivation is found in semi arid regions of Maharashtra, northern Karnataka and Andhra Pradesh. In the last five to six years, grape productivity in these states has been constrained by water scarcity due to regular monsoon failure and soil salinity. Salt-affected soils cover an area of nearly 13.5 M ha in India (Sharma *et al*., 2004) and 173 thousand ha in Karnataka (Sharma, 1998).

Salinity is an environmental stress that limits its growth and development in plants. The response of plants to excess salt is complex and involves changes in their morphology, physiology and metabolism (Shannon *et al*., 1994). In arid and semi-arid regions of the world, limited rainfall, high evapotranspiration, high temperature and inadequate water management contribute to increase in soil salinity. In those areas, plant growth is severely affected by salinity through water deficit, salt-specific damages (Munns and Termaat, 1986) or oxidative stress (Hernandez *et al*., 1995). Plants' capacity to endure the effects of excessive salt in the root zone is the "salt tolerance" of plants. Plants vary in their response to soil salinity and the range of salt concentrations tolerated by crops varies greatly from species to species (Volkmar *et al*., 1998).

Arbuscular mycorrhizal (AM) fungi improve physiological processes, like water absorption capacity of plants by increasing root hydraulic conductivity and favourably adjusting the osmotic balance and composition of carbohydrates (Rosendahl and Rosendahl 1991). Thus, they mitigate the adverse effects of excess salt accumulation in the root (Dixon *et al*., 1993). An experiment was conducted to determine the effect of mycorrhizal inoculation at different salinity on root colonization, plant growth and chlorophyll content of grape rootstocks (*Vitis* spp).

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MATERIALS AND METHODS

Cuttings of four grape rootstocks *viz.*, Dogridge, Salt Creek, St. George and 1613 were rooted in nursery beds at College of Agriculture, University of Agricultural Sciences, Dharwad (situated 15°–26' North latitude and 70°–07' East longitude). Experimental design used was factorial CRD, with four replications, wherein a total of four plants per treatment were grown. The inoculation of AM fungus to grape cuttings was done in the nursery bed using five grams of inoculum (*G. fasciculatum*) per cutting consisting of 19 infective propagules (chlamydospores) per gram of inoculum at five centimeters depth. After putting a thin layer of soil on the inoculum, grape cuttings of about 25-30 cm long, having four nodes, were placed and two buds of the cuttings covered with soil. Two months old rooted cuttings were transferred to a 6" x 9" size polythene bags and allowed to grow for four months (until they attained pencil size shoot girth). At this stage the rooted cuttings were removed from polythene bags and imposed to different levels of salinity (0.52, 1.90, 4.33, 6.23 and 7.94 dSm^{-1}), which were obtained from naturally salt affected soil in Gangawati Agricultural Research Station of the University of Agricultural Sciences, Dharwad (Raichur district, Northern Karnataka). To maintain level of salinity, plants were given a measured quantity of water and kept under salinity stress condition for four months. Per cent root colonization was determined following the procedure outlined by Phillips and Hayman (1970). Leaf area was determined using Portable Area Meter, Model LI-3000A, LI-COR. Chlorophyll content of the leaves was determined following dimethyl sulfoxide (DMSO) method. At the end of the experimental period, plants were carefully removed from earthen pots and shoot and root parts were separated and the roots washed in water. In order to obtain dry weight of shoot and root, fresh tissues were dried at $70^{o}C$ in an oven for 24 hours till constant weight was reached.

RESULTS AND DISCUSSION

Mycorrhizal inoculation and salinity stress had strong effects on growth, root colonization and leaf chlorophyll content. Growth parameters (number of leaves per vine, leaf area, root volume, root length and total dry weight) and per

Table 1. Effect of mycorrhizal inoculation and different salinity levels on root colonization, root volume and root length of grape rootstocks

AMF inoculation	Root stocks	Root colonization (%)						Root volume (cc)						Root length (cm)					
		Salinity levels						Salinity levels						Salinity levels					
		S0	S1	S2	S3	S4	Mean	S0	S1	S2	S3	S4	Mean	S0	S1	S2	S3	S4	Mean
M0	R1	45.00	40.00	40.00	36.00	31.00	38.40	24.00	21.22	20.36	18.00	15.25	19.76	90.30	79.30	47.27	46.28	40.28	60.69
	R2	48.00	43.00	42.00	40.00	35.00	34.60	21.17	15.18	15.12	14.50	12.28	15.65	45.42	38.30	34.32	23.27	20.25	32.31
	R3	44.00	43.00	43.00	41.00	36.00	34.20	30.33	24.17	23.19	21.17	18.25	23.42	60.30	40.30	34.30	22.30	19.67	35.37
	R4	44.00	42.00	42.00	41.00	36.00	33.80	26.95	18.25	18.22	15.08	14.47	18.59	50.28	41.28	32.33	30.27	26.34	36.10
	Mean	45.25	42.00	41.75	39.50	34.50	35.25	25.61	19.70	19.22	17.19	15.06	19.36	61.58	49.80	37.05	30.53	26.64	41.12
M1	R1	52.00	51.00	49.00	45.00	40.00	39.40	30.15	24.17	23.19	18.15	17.00	22.53	81.30	74.28	67.28	59.28	51.60	66.75
	R2	66.00	63.00	62.00	57.00	52.00	49.60	24.22	18.05	17.32	15.17	12.22	17.39	46.27	32.28	28.30	20.30	17.67	28.96
	R3	65.00	62.00	61.00	59.00	54.00	49.40	35.12	32.82	30.18	21.02	20.16	27.86	65.13	45.23	43.28	27.30	23.15	40.82
	R4	78.00	78.00	76.00	72.00	67.00	60.80	33.00	30.12	24.17	18.22	17.48	24.60	62.33	48.27	36.25	25.28	22.01	38.83
	Mean	65.25	63.50	62.00	58.25	53.25	49.80	30.62	26.29	23.71	18.14	16.71	23.09	63.76	50.02	43.78	33.04	28.61	43.84
	For comparison of rootstocks and salinity																		
	R1	48.50	45.50	44.50	40.50	35.50	38.90	27.08	22.69	21.77	18.08	16.13	21.15	85.80	76.79	57.28	52.78	45.94	63.72
	R2	57.00	53.00	52.00	48.50	43.50	42.10	22.69	16.62	16.22	14.84	12.25	16.52	45.84	35.29	31.31	21.78	18.96	30.64
	R3	54.50	52.50	52.00	50.00	45.00	41.80	32.73	28.49	26.69	21.09	19.21	25.64	62.72	42.77	38.79	24.80	21.41	38.10
	R4	61.00	60.00	59.00	56.50	51.50	47.30	29.98	24.18	21.19	16.65	15.98	21.60	56.31	44.78	34.29	27.78	24.17	37.46
	Mean	55.25	52.75	51.88	48.88	43.88	42.53	28.12	23.00	21.47	17.66	15.89	21.23	62.67	49.91	40.42	31.79	27.62	42.48
		S.Em±	CD 5%							S.Em±	CD 5%					S.Em±	CD 5%		
	M	0.61	1.72					M		0.30	0.83			M		0.42	1.20		
	R	0.86	2.43					R		0.42	1.18			R		0.60	1.69		
	S	0.96	2.72					S		0.47	1.32			S		0.67	1.89		
	M x R	1.22	3.44					M x R		0.59	1.67			M x R		0.85	2.39		
	M x S	1.36	3.84					M x S		0.66	1.86			M x S		0.95	2.67		
	R x S	1.93	5.43					R x S		0.94	NS			R x S		1.34	3.78		
	M x R x S	2.73	7.68					M x R x S		1.32	NS			M x R x S		1.90	5.35		

Mycorrhizal treatment (M): M0=Uninoculated, M1=Inoculated,
Rootstocks (R): R1 = Dogridge, R2=St. George, R3=Salt Creek, R4=1613; Salinity levels (S): S0= Control (0.52), S1= 1.90, S2=4.33, S3=6.23, S4=7.94 dSm^{-1}

Table 2. Effect of mycorrhizal inoculation and different salinity levels on number of leaves, leaf area and total dry weight of grape rootstocks

AMF inoculation.	Root stocks	Number of leaves						Leaf area (cm^2)						Total dry weight (g)					
		Salinity levels						Salinity levels						Salinity levels					
		S0	S1	S2	S3	S4	Mean	S0	S1	S2	S3	S4	Mean	S0	S1	S2	S3	S4	Mean
M0	R1	15.33	12.33	12.00	8.67	5.67	10.80	19.20	16.39	14.13	13.61	12.55	15.18	14.16	9.02	8.01	6.22	5.68	8.62
	R2	13.67	12.67	11.67	11.33	7.33	11.33	18.59	13.50	11.01	10.56	9.34	12.60	7.41	3.94	3.61	2.80	2.36	4.02
	R3	16.00	14.33	13.00	11.00	7.33	12.33	27.91	26.55	22.00	15.51	14.55	21.30	18.31	14.17	11.99	9.39	8.73	12.52
	R4	19.00	17.00	16.67	9.00	5.67	13.47	35.25	25.78	23.90	20.43	19.72	25.02	11.92	8.84	7.04	5.23	4.74	7.55
	Mean	16.00	14.08	13.33	10.00	6.50	11.98	25.24	20.56	17.76	15.03	14.04	18.52	12.95	8.99	7.66	5.91	5.38	8.18
M1	R1	20.67	20.33	18.00	14.67	9.67	16.67	29.42	22.77	20.88	20.42	19.71	22.64	14.66	12.32	9.73	7.06	6.50	10.05
	R2	16.00	14.33	12.33	10.33	6.67	11.93	28.60	20.29	19.60	11.08	9.88	17.89	10.88	6.80	4.51	4.10	3.62	5.98
	R3	16.67	15.67	15.67	9.33	6.33	12.73	53.41	43.35	26.79	22.70	22.11	33.67	20.04	16.75	12.70	11.62	10.91	14.40
	R4	23.00	18.00	17.33	16.33	10.67	17.07	37.91	34.84	30.64	27.67	27.35	31.68	14.78	11.98	8.53	5.60	5.09	9.20
	Mean	19.08	17.08	15.83	12.67	8.33	14.60	37.34	30.31	24.48	20.47	19.76	26.47	15.09	11.96	8.87	7.10	6.53	9.91
	For comparison of rootstocks and salinity																		
	R1	18.00	16.33	15.00	11.67	7.67	13.73	24.31	19.58	17.51	17.01	16.13	18.91	14.41	10.67	8.87	6.64	6.09	9.34
	R2	14.83	13.50	12.00	10.83	7.00	11.63	23.60	16.90	15.31	10.82	9.61	15.25	9.15	5.37	4.06	3.45	2.99	5.00
	R3	16.33	15.00	14.33	10.17	6.83	12.53	40.66	34.95	24.40	19.10	18.33	27.49	19.18	15.46	12.35	10.51	9.82	13.46
	R4	21.00	17.50	17.00	12.67	8.17	15.27	36.58	30.31	27.27	24.05	23.54	28.35	13.35	10.41	7.79	5.42	4.92	8.38
	Mean	17.54	15.58	14.58	11.33	7.42	13.29	31.29	25.43	21.12	17.75	16.90	22.50	14.02	10.48	8.27	6.50	5.95	9.04
		S.Em±	CD 5%							S.Em±	CD 5%					S.Em±	CD 5%		
	M	0.43	1.20						M	0.25	0.72			M	0.02	0.05			
	R	0.60	1.70						R	0.36	1.01			R	0.02	0.07			
	S	0.67	1.90						S	0.40	1.13			S	0.03	0.08			
	M x R	0.85	2.40						M x R	0.51	1.43			M x R	0.03	0.10			
	M x S	0.95	2.68						M x S	0.57	1.60			M x S	0.04	0.11			
	R x S	1.35	3.79						R x S	0.80	2.26			R x S	0.05	0.15			
	M x R x S	1.91	NS						M x R x S	1.14	3.20			M x R x S	0.08	0.21			

Mycorrhizal treatment (M): M0=Uninoculated, M1=Inoculated,
Rootstocks (R): R1 = Dogridge, R2=St. George, R3=Salt Creek, R4=1613; Salinity levels (S): S0=Control (0.52), S1= 1.90, S2=4.33, S3=6.23, S4=7.94 dSm^{-1}

Table 3. Effect of mycorrhizal inoculation and different salinity levels on chlorophyll 'a', 'b', and total chlorophyll content of grape rootstocks

AMF inoculation	Root-stocks	Chlorophyll 'a' content (mg g fr. wt.$^{-1}$)						Chlorophyll 'b' content (mg g fr. wt.$^{-1}$)						Total chlorophyll content (mg g fr. wt.$^{-1}$)					
		Salinity levels						Salinity levels						Salinity levels					
		S0	S1	S2	S3	S4	Mean	S0	S1	S2	S3	S4	Mean	S0	S1	S2	S3	S4	Mean
M0	R1	1.52	1.47	1.35	0.98	0.58	**1.18**	0.78	0.75	0.61	0.40	0.35	**0.63**	2.30	2.22	1.95	1.38	0.93	**1.76**
	R2	1.31	1.23	1.20	0.77	0.74	**1.05**	0.56	0.52	0.47	0.42	0.34	**0.47**	1.87	1.75	1.21	1.62	1.11	**1.51**
	R3	1.63	1.53	1.38	1.21	0.72	**1.29**	1.08	0.86	0.65	0.55	0.49	**0.79**	2.71	2.39	2.04	1.75	1.22	**2.02**
	R4	1.64	1.56	1.53	1.22	0.73	**1.34**	1.14	1.01	0.86	0.56	0.51	**0.89**	2.78	2.57	2.39	1.79	1.24	**2.15**
	Mean	**1.52**	**1.45**	**1.36**	**1.05**	**0.69**	**1.22**	**0.89**	**0.79**	**0.65**	**0.48**	**0.42**	**0.70**	**2.42**	**2.23**	**1.90**	**1.63**	**1.12**	**1.86**
M1	R1	1.55	1.50	1.38	1.32	0.79	**1.31**	0.83	0.76	0.64	0.60	0.55	**0.71**	2.37	2.26	2.01	1.91	1.34	**1.98**
	R2	1.53	1.50	1.33	1.32	0.80	**1.29**	0.80	0.77	0.60	0.59	0.54	**0.69**	2.33	2.27	1.92	1.91	1.34	**1.95**
	R3	1.65	1.56	1.51	1.44	0.87	**1.41**	1.33	0.89	0.83	0.69	0.64	**0.94**	2.98	2.45	2.34	2.14	1.51	**2.28**
	R4	1.64	1.58	1.54	1.55	0.88	**1.44**	1.17	0.99	0.91	0.89	0.86	**0.99**	2.80	2.57	2.45	2.44	1.74	**2.40**
	Mean	**1.59**	**1.53**	**1.44**	**1.41**	**(0.83)**	**1.36**	**1.03**	**0.85**	**0.74**	**0.69**	**0.65**	**0.83**	**2.62**	**2.39**	**2.18**	**2.10**	**1.48**	**2.15**
	For comparison of rootstocks and salinity																		
	DG	1.53	1.48	1.36	1.15	0.69	**1.24**	0.80	0.76	0.62	0.50	0.45	**0.67**	2.33	2.24	1.98	1.65	1.13	**1.87**
	SG	1.42	1.37	1.26	1.05	0.77	**1.17**	0.68	0.65	0.53	0.50	0.44	**0.58**	2.10	2.01	1.57	1.77	1.23	**1.73**
	SC	1.64	1.54	1.45	1.33	0.79	**1.35**	1.21	0.88	0.74	0.62	0.57	**0.86**	2.85	2.42	2.19	1.95	1.36	**2.15**
	1613	1.64	1.57	1.53	1.39	0.81	**1.39**	1.16	1.00	0.88	0.73	0.68	**0.94**	2.79	2.57	2.42	2.11	1.49	**2.28**
	Mean	**1.56**	**1.49**	**1.40**	**1.23**	**0.76**	**1.29**	**0.96**	**0.82**	**0.70**	**0.59**	**0.54**	**0.76**	**2.52**	**2.31**	**2.04**	**1.87**	**1.30**	**2.01**
		S.Em±	CD 5%							S.Em±	CD 5%					S.Em±	CD 5%		
	M	0.03	0.07						M	0.004	0.011			M	0.03	0.08			
	R	0.04	0.10						R	0.005	0.015			R	0.04	0.11			
	S	0.04	0.12						S	0.006	0.017			S	0.05	0.13			
	M x R	0.05	0.15						M x R	0.008	0.022			M x R	0.06	NS			
	M x S	0.06	NS						M x S	0.009	0.024			M x S	0.06	NS			
	R x S	0.08	0.23						R x S	0.012	0.034			R x S	0.09	0.25			
	M x R x S	0.12	0.33						M x R x S	0.017	0.049			M x R x S	0.13	NS			

Mycorrhizal treatment (M): M0=Uninoculated, M1=Inoculated,
Rootstocks (R): R1 = Dogridge, R2=St. George, R3=Salt Creek, R4=1613; Salinity levels (S): S0= Control (0.52), S1= 1.90, S2= 4.33, S3= 6.23, S4= 7.94 dSm^{-1}

cent root colonization decreased in all the rootstocks with increase in salinity stress from 0.52 to 7.94 dSm^{-1}. Mycorrhiza inoculated plants recorded significantly higher root colonization percentage, root volume and root length (Table 1), number of leaves per vine, leaf area and total dry weight (Table 2), and chlorophyll content (Table 3) compared to non-mycorrhizal plants.

Several greenhouse studies showed that grapevines inoculated with indigenous AM fungi had higher pruning weights and root weights (Linderman and Davis, 2001), and more compact, highly branched roots than non-mycorrhizal grapevines (Schellenbaum *et al*., 1991). Munns and Termaat (1986) suggested that growth inhibition in the long term exposure to increased salinity condition was related to lower photosynthetic area which will eventually become too low to support continuing growth. Munns (1993) proposed that accumulation of salt in the old leaves accelerated their death, and loss of these leaves decreased the supply of carbohydrates or growth hormones to meristematic regions, thereby inhibiting growth. Zekri (1991) concluded that salinity reduced shoot growth by suppressing leaf initiation and expansion as well as internode growth and by accelerating leaf abscission. The present study showed that salinity treatment caused significant decreases in leaf number of shoots. Decreases in the number of leaves were not only related to the growth inhibiting effects of salt, but also to the injurious effects of salt due to defoliation of the damaged leaves. Ramanujalu *et al*. (1993) observed gradual decrease in the contents of chlorophyll 'a' and chlorophyll 'b' with increase in the salt intensity in mulberry, wherein relatively higher rate of depletion was found with chlorophyll 'a' than chlorophyll 'b'.

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