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Mehboobatabassum FH
Department of Agricultural Microbiology, University of Agricultural Sciences, Dharwad, Karnataka, India

P Jones Nirmalnath
Department of Agricultural Microbiology, University of Agricultural Sciences, Dharwad, Karnataka, India

Santhosh GP
Department of Agricultural Microbiology, University of Agricultural Sciences, Dharwad, Karnataka, India

Asif Waratadar
Department of Agricultural Microbiology, University of Agricultural Sciences, Dharwad, Karnataka, India

Corresponding Author:
Mehboobatabassum FH
Department of Agricultural Microbiology, University of Agricultural Sciences, Dharwad, Karnataka, India

Growth and physiological parameters of Vetiver (*Vetiveria zizanioides cv.* Kushnalika) as influenced by the synergistic effect of AM fungi and methylobacterial consortia under salinity

Mehboobatabassum FH, P Jones Nirmalnath, Santhosh GP and Asif Waratadar

Abstract
Soil salinity frequently hinders plant health and productivity in both natural and agricultural system. Among various beneficial microorganisms arbuscular mycorrhizal fungi being used to reduce the negative effects of salinity, the use of arbuscular mycorrhizal fungi (AMF) and Pink Pigmented Facultative Methylotrophs (PPFMs) is considered to be a competent approach for bio-amelioration of salinity stress. In this regard a pot experiment was conducted at Department of Agricultural Microbiology, University of Agricultural Sciences, Dharwad during the year 2020-21 using native soils from Gangavathi of Koppal district having two EC levels *viz.*, 6 and 8 dS m^{-1} to test the synergistic effect of three AM fungal consortia and methylobacterial consortium in influencing the growth and physiological parameters of vetiver plants. The results of the present investigation has revealed that the soil application of saline tolerant UASDAMFSL consortia along with two sprays of methylobacterial consortium at 30 and 45 DAP significantly enhanced the plant growth parameters; plant height (109 cm at 120 DAP), number of leaves (56.35 at 120 DAP), relative chlorophyll content (41.89), dry biomass like shoot dry weight (53.45g/plant) and root biomass (39.67g/plant) compared to uninoculated control (73cm, 34.33, 30.29, 28.26 g/plant and 30.92 g/plant). Thus, our prelimnary findings are of positive indicative of the effectiveness of application of AM Fungi and PPFM on growth and physiological parameters of vetiver even under varied salinity stress condition.

Keywords: Chlorophyll, *Methylobacterium*, arbuscular mycorrhizal fungi, vetiver, salinity

Introduction
Globally soil salinity is one of the major abiotic factors prevailing in arid and semiarid regions transforming seven percent of the earth under salinization. (Ruiz-Lozano *et al.*, 2012) [11]. Indiscriminate application of inorganic chemical fertilizers, use of groundwater for irrigation, flood irrigation practices, and no monocropping are the major reasons for increased soil salinization. Increased soil salinization of arable lands resulted in the loss of 30 percent of agricultural land within the next 25 years and is expected to go up to 50 percent within the next 40 years, with an expected reduction in productivity to the tune of 20 percent. (Abdel-Fattah *et al.*, 2012) [1]. Therefore, Recent studies have revealed that exploitations of microorganisms as a biological strategy for the restoration of salt affected soils. Among the beneficial microorganisms AM fungi and Pink Pigmented Facultative Methylotrophs (PPFMs) employ various mechanisms to mitigate plant salinity stress. For instance, AMF can augment nutrient uptake, increase water uptake, maintain osmotic balance, stimulate antioxidant activities to protect against damage by reactive oxygen species (ROS), increase the photosynthetic rate and regulate hormonal levels to abate the harmful effects of salts on plant growth and development However, these adaptive strategies become inefficient to cope with the rapidly increasing salinity. Furthermore Pink Pigmented Facultative Methylotrophs (PPFMs) labeled as Alfa-proteobacteria are capable of growing on single carbon compounds like methanol and methylamine (Madhaiyan *et al.*, 2012) [8]. They synthesize a wide range of auxins and cytokinins, which are utilized by the host plant for growth and development. In addition Methylotrophic bacteria produce 1-aminocyclopropane-1-carboxylate (ACC) deaminase and induce the production of antioxidant enzymes and osmolytes in plants, which helps in mitigating salinity stress condition.

Foliar spray of *methylobacterium* enhanced the antioxidant enzyme activity including polyphenol oxidase (PPO), peroxidase (POD), ascorbate peroxidase (APX), catalase (CAT), and superoxide dismutase (SOD) of snap bean which helps to maintains the ionic balance between K^+ Na^+ (Abd El-Gawad *et al*., 2015) [2] Based on this background mechanisms, The present investigation was aimed to carry out to assess the synergistic impact of AM fungi and PPFM (Pink Pigmented Facultative Methylotroph) on growth and physiological parameters of vetiver under varied salinity stress condition.

Material and Methods

A pot experiment was conducted to know the synergistic effect of AM fungal and methylobacterial consortia on growth and physiological parameters of vetiver (*Vetiveria zizanioides cv.* Kushnalika) under varied levels of salinity. Polythene bags were filled with six kg of saline soils (6 and 8 dS/m) and three AM fungal consortia *viz*., M_1-UASDAMF consortium (Reference), M_2-UASDAMFMS consortium (Moisture stress-tolerant) and M_3-UASDAMFSL consortium (Saline stress stress-tolerant) and M4-uninoculated control, applied @ 50g/pot were inoculated before the planting. Vetiver slips were procured from CIMAP-Bangalore. The PPFM consortium consists of five cultures *viz*., PPFM-18, PPFM-23, PPFM-27, PPFM-33, and PPFM-58 was applied as a spray (P_1- Zero spray, P_2- spray on 30 DAP, and P_3-spray on 30 and 45 DAP). There were 24 treatments and three replications. Plant height was measured from the base of the plant to the tip of the main shoot at 60, 90 and 120 DAP and was expressed in centimeters, Number of leaves was counted at the time of 60, 90 and 120 DAP, physiological parameters like relative chlorophyll content of vetiver were measured at 60 DAP by using SPAD (Soil Plant Analysis Development) meter (SPAD-502 KONICA Japan) and dry biomass were analyzed at 80 DAP.

Statistical analysis

The data collected at different growth stages of the crop were subjected to statistical analysis by using Fischer's method of analysis of variance technique as described by Gomez and Gomez (1984). The level of significance used in the 'F' and 't-test was p= 0.01. Critical difference values were calculated wherever the 'F' test was significant.

Result and Discussion

The plant height and the number of leaves were found to increase steadily with the number of days after planting due to various treatments with three mycorrhizal consortia and methylobacterial consortium at 60, 90, and 120 DAP (table 1 & plate 1, 2 and 3).

The interactive effect between AMF and PPFM at 6 and 8 dS m^{-1} at 60 DAP has revealed that the application of the UASDAMFSL consortium comprising saline tolerant mycorrhizal isolates along with a consortium of methylobacterial spray at 30 and 45 DAP recorded the highest plant height (75 cm and 68 cm at 6 and 8 dS m^{-1} respectively), followed by treatment UASDAMF consortium (Reference) with two sprays of PPFM (68.33 cm and 60.33 cm at 6 and 8 dS m^{-1} respectively). However, the least plant height was observed in uninoculated control (38.27 and 37cm at 6 and 8 dS m^{-1} respectively). Similar results were also recorded at 90 and 120 DAP due to the synergistic effect of UASDAMFSL and methylobacterial consortia in growth promotion of vetiver plants at two salinity levels.

At 60 DAP, the plant received UASDAMFSL consortium with two doses of PPFM spray recorded the highest number of leaves (37.33 and 36 at 6 and 8 dS m^{-1} respectively), followed by UASDAMF consortium with Methylobacterial spray at 30 and 45 DAP at 6 and 8 dS m^{-1} (36.33 and 34 respectively). However, the least number of leaves were recorded in uninoculated control plants (20.33 and 17 at 6 and 8 dS m^{-1} respectively). Similar results were recorded at 90 and 120 DAP due to the interactive effect of UASDAMFSL and methylobacterial consortia in growth promotion vetiver plants at two salinity levels (table 2).

The significant increase in plant height of vetiver could be due to positive interactions between native AMF and PPFM consortia with host plants at two salinity stress levels *viz*., 6 dS m^{-1} and 8 dS m^{-1}. The results of the present study are in close conformity with Kadian *et al*. (2013), who reported that plants inoculated *with Glomus mossae* along with *Acaulospora laevis* at varied levels of salinity (4-12 dS m^{-1}) recorded the highest plant height followed by, single inoculation of *G. mossae* and uninoculated control plants. It is also evident that an increased level of salinity stress from 4 to 12 dS m^{-1} in non-mycorrhizal plants resulted in decreased plant growth parameters which may be due to the spending of energy to restrain the noxious effects of salts (Sharifi *et al*., 2007) [12].

Inoculation of UASDAMF and PPFM consortia with two doses has significantly influenced the relative chlorophyll content (41.89 and 38.31 at 6 and 8 dS m^{-1} respectively), followed by treatment receiving two doses of PPFM spray and UASDAMFSL consortium (40.67 and 38.00 at 6 and 8dS m^{-1}) and the lowest chlorophyll content was observed in uninoculated control (31.32 and 30.29 at 6 and 8 dS m^{-1} respectively) (table 3).

Giri *et al*. (2007) [7] revealed that the chlorophyll content in the leaves of mycorrhized plants increased under saline conditions, while reduced chlorophyll content in non-mycorrhized plants may be due to the enhanced activity of chlorophyllase enzyme, responsible for chlorophyll degrading enzyme under stressed conditions (Ozturk *et al*., 2002).

Furthermore, the vetiver plants received dual inoculation of UASDAMFSL and methylobacterial spray significantly influenced the shoot dry weight (53.45 and 47.91 at 6 and 8 dS m^{-1} respectively), which is followed by UASDAMF consortium with two doses of PPFM spray (50.21 and 48.70 at 6 and 8 dS m^{-1} respectively) and the lowest shoot dry weight was observed in uninoculated control (30.34 and 28.94 at 6 and 8 dS m^{-1} respectively). Similar observations were recorded with root biomass (39.67 and 36.27 at 6 and 8 dS m^{-1} respectively). The lowest root biomass was observed in uninoculated control (28.00 and 27.67 at 6 and 8 dS m^{-1} respectively) (table 4).

An increase in biomass owing to mycorrhization has been reported in numerous plant species of economic importance (Gianinazzi *et al*. 1989). Both the host plants showed higher growth (shoot biomass) when inoculated by *Glomus macrocarpum* than by *Glomus fasciculatum* though colonization by *G. macrocarpum* was less than *Glomus. fasciculatum.*

Table 1: Influence of AM fungal and methylobacterial consortia on plant height of vetiver at different levels of salinity

Treatments	Plant height (cm)																					
	60 DAP							90 DAP								120 DAP						
	S_1			S_2				S_1			S_2					S_1			S_2			
	P_1	P_2	P_3	P_1	P_2	P_3	Mean of M	P_1	P_2	P_3	P_1	P_2	P_3	Mean of M	P_1	P_2	P_3	P_1	P_2	P_3	Mean of M	
M_1	46.00	56.67	68.33	44.67	56.00	60.33	54.83	90.00	91.67	107.33	84.33	90.67	99.67	93.94	94.33	101.33	110.00	91.67	98.67	101.67	99.44	
M_2	42.67	45.67	53.33	40.67	53.67	52.33	48.06	84.00	89.00	96.67	81.33	80.67	96.67	88.06	80.67	89.67	99.00	78.67	96.33	97.33	90.28	
M_3	51.67	60.33	75.00	50.67	61.00	68.00	61.11	93.67	99.00	110.00	90.00	95.33	106.00	99.00	106.00	104.00	115.33	94.33	104.33	109.00	105.50	
M_4	38.27	41.67	40.33	37.00	41.00	39.33	39.60	68.66	73.00	72.67	66.66	71.33	76.67	71.38	73.00	79.00	82.00	71.33	77.00	80.33	77.28	
Mean of S	51.41			50.39				89.58			86.61					94.39			91.86			
Mean of P	43.95		52.00			56.75		82.25		86.33			95.71			86.37		93.79		99.21	
	S.Em. +			C.D. (p = 0.01)				S.Em. +			C.D. (p = 0.01)					S.Em. +			C.D. (p = 0.01)			
C.D. of S (Salinity)	0.24			0.91				0.28			1.03					0.41			1.55			
C.D. of P (PPFM)	0.29			1.11				0.34			1.26					0.50			1.90			
C.D. of M (AMF)	0.34			1.29				0.39			1.46					0.58			2.19			
C.D. of S x P	0.42			1.58				0.48			N/A					0.71			2.68			
C.D. of S x M	0.48			1.82				0.55			2.06					0.82			3.10			
C.D. of P x M	0.59			2.23				0.68			2.53					1.00			3.79			
C.D. of S x P x M	0.83			3.15				0.96			3.57					1.41			5.36			

Note: Salinity level 1 (S_1) = 6 dS/m P_1 = zero spray
Alinity level 2 (S_2) = 8 dS/m P_2 = PPFM spray @ 30 DAP
P_3 = PPFM spray @ 30 and 45 DAP
M_1 = UASDASMF consortium (Reference)
M_2 = UASDAMFMS consortium (Moisture stress)
M_3 = UASDAMFSL consortium (saline stress)
M_4 = UIC Un inoculated control

Table 2: Influence of AM fungal and methylobacterial consortia on number of leaves of vetiver at different levels of salinity

Treatments	Number of leaves																					
	60 DAP							90 DAP								120 DAP						
	S_1			S_2				S_1			S_2					S_1			S_2			
	P_1	P_2	P_3	P_1	P_2	P_3	Mean of M	P_1	P_2	P_3	P_1	P_2	P_3	Mean of M	P_1	P_2	P_3	P_1	P_2	P_3	Mean of M	
M_1	28.33	30.33	36.33	24.33	30.00	34.00	30.22	43.33	46.33	58.00	39.00	44.67	54.33	47.78	45.67	52.67	62.67	43.00	48.67	55.33	51.17	
M_2	22.33	26.00	27.00	21.00	22.00	25.00	23.89	38.33	41.00	44.67	35.67	40.00	47.35	41.17	44.33	44.00	53.00	41.67	44.00	50.67	46.28	
M_3	30.00	33.00	37.33	26.00	31.00	36.00	32.22	45.33	50.33	58.67	43.33	49.00	56.00	50.50	47.33	55.00	67.00	44.67	50.00	56.35	53.33	
M_4	20.33	21.00	26.00	17.00	21.67	29.67	22.61	29.67	33.00	41.33	19.00	27.00	32.00	31.78	34.33	38.33	43.33	22.67	34.00	35.00	36.28	
Mean of S	28.00			24.97				44.17			36.60				48.97			44.56				
Mean of P	23.67		26.88			31.17		37.79		41.41			49.20		41.71		45.83			52.75		
	S.Em. +			C.D. (p = 0.01)				S.Em. +			C.D. (p = 0.01)					S.Em. +			C.D. (p = 0.01)			
C.D. of S (Salinity)	0.19			0.72				0.17			0.65					0.20			0.74			
C.D. of P (PPFM)	0.23			0.88				0.21			0.80					0.24			0.90			
C.D. of M (AMF)	0.27			1.02				0.24			0.92					0.28			1.04			
C.D. of S x P	0.33			1.24				0.30			1.13					0.34			1.28			
C.D. of S x M	0.38			1.44				0.34			1.30					0.39			1.47			
C.D. of P x M	0.46			1.76				0.42			1.59					0.48			1.80			
C.D. of S x P x M	0.66			2.49				0.59			2.25					0.68			2.55			

Note: Salinity level 1 (S_1) = 6 dS/m P_1 = zero spray
Salinity level 2 (S_2) = 8 dS/m P_2 = PPFM spray @ 30 DAP
P_3 = PPFM spray @ 30 and 45 DAP
M_1 = UASDASMF consortium (Reference)
M_2 = UASDAMFMS consortium (Moisture stress)
M_3 = UASDAMFSL consortium (saline stress)
M_4 = UIC Un inoculated control

Table 3: Relative chlorophyll content of Vetiver as influenced by AM fungal and methylobacterial consortia at different levels of salinity

Treatments	Relative chlorophyll content (SPAD values)						
	S_1			S_2			
	P_1	P_2	P_3	P_1	P_2	P_3	Mean of M
M_1	38.28	39.08	41.89	36.45	36.45	38.31	38.41
M_2	33.29	35.25	37.33	33.33	34.22	37.55	35.16
M_3	38.03	38.47	40.67	36.23	35.59	38.00	37.83
M_4	31.32	33.07	34.52	30.29	30.30	34.51	32.33
Mean of S	36.68			35.19			
Mean of P	34.65		35.30			37.85	
	S.Em. +			C.D. (p = 0.01)			
C.D. of S (Salinity)	0.10			0.36			
C.D. of P (PPFM)	0.12			0.44			
C.D. of M (AMF)	0.13			0.51			
C.D. of S x P	0.16			0.62			
C.D. of S x M	0.19			0.72			
C.D. of P x M	0.23			0.88			
C.D. of S x P x M	0.33			1.25			

Note: Salinity level 1 (S_1) = 6 dS/m P_1 = zero spray
Salinity level 2 (S_2) = 8 dS/m P_2 = PPFM spray @ 30 DAP
P_3 = PPFM spray @ 30 and 45 DAP
M_1 = UASDASMF consortium (Reference)
M_2 = UASDAMFMS consortium (Moisture stress)
M_3 = UASDAMFSL consortium (saline stress)
M_4 = UIC Un inoculated control

Table 4: Total dry biomass as influenced by AM fungal and methylobacterial consortia at different salinity levels (180 DAP)

Treatments	Shoot dry weight (g plant^{-1})								Root biomass (g plant^{-1})						
	S_1			S_2					S_1			S_2			
	P_1	P_2	P_3	P_1	P_2	P_3	Mean of M	P_1	P_2	P_3	P_1	P_2	P_3	Mean of M
M_1	45.32	48.88	50.21	43.12	47.09	48.70	47.22	32.00	35.96	37.53	29.67	32.35	35.33	33.81
M_2	41.30	45.15	48.12	38.82	43.05	44.27	43.45	30.33	32.67	35.63	28.67	31.19	33.52	32.00
M_3	46.50	50.87	53.45	44.11	46.94	47.91	48.30	33.67	36.27	39.67	31.67	34.74	36.27	35.38
M_4	30.34	31.11	37.19	28.94	29.48	28.26	30.89	28.00	34.67	35.92	27.67	30.92	32.00	31.53
Mean of S	44.04			40.00					34.27			31.74			
Mean of P	39.81		42.82		44.76				29.95		33.46		35.61		
	S.Em. +			C.D. (p = 0.01)					S.Em. +			C.D. (p = 0.01)			
C.D. of S (Salinity)	0.18			0.70					0.10			0.36			
C.D. of P (PPFM)	0.22			0.85					0.12			0.44			
C.D. of M (AMF)	0.26			0.99					0.14			0.51			
C.D. of S x P	0.32			1.21					0.17			0.62			
C.D. of S x M	0.37			1.39					0.19			0.72			
C.D. of P x M	0.45			1.71					0.24			0.88			
C.D. of S x P x M	0.64			2.41					0.33			1.24			

Note: Salinity level 1 (S_1) = 6 dS/m P_1 = zero spray
Salinity level 2 (S_2) = 8 dS/m P_2 = PPFM spray @ 30 DAP
P_3 = PPFM spray @ 30 and 45 DAP

M_1 = UASDASMF consortium (Reference)
M_2 = UASDAMFMS consortium (Moisture stress)
M_3 = UASDAMFSL consortium (Saline stress)
M_4 = UIC Un inoculated control

Plate 1: General view of Experiment at 180 DAP

Plate 2: Plant height as influenced by UASDAMFSL consortium (Saline stress) and PPFM consortium spray at 30 and 45 DAP moderate saline soil (6 dS m^{-1}) at 180 DAP

Plate 3: Plant height as influenced by UASDAMFSL consortium (Saline stress) and PPFM consortium spray at 30 and 45 DAP moderate saline soil (6 dS m^{-1}) at 180 DAP

Conclusion

The present investigation revealed that the *Vetiveria zizanioides* inoculated with AM fungal and methylobacterial consortia were found to be superior to uninoculated plants. Growth parameters like, plant height, number of leaves, chlorophyll content and dry biomass differed significantly in all saline levels. The interaction between AMF and PPFM inoculation UASDAMFSL, UASDAMF (Reference) and UASDAMFMS consortia with two doses of PPFM spray stimulated the growth parameters over uninoculated plants at varying salinity. Thus, our prelimnary findings are of positive indicative of the effectiveness of AM Fungi and PPFM under varied salinity stress condition.

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