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Molecular and Physiological Characterization of *Aspergillus awamori* Isolated from Different Soil Types of Various Agro Climatic Zones of Karnataka

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A study was undertaken to find the molecular and physiological variability in *Aspergillus awamori* strains isolated from different agroclimatic zones of Karnataka. *A. awamori* strains from different agro climatic zones were isolated, identified and confirmed using standard synaptic keys. Plant growth response studies using tomato as host were conducted. The heights of plants were found to increase steadily with number of days. In plants inoculated with *A. awamori* isolates, the height, number of leaves, fresh and dry weight of roots and shoots, P content and total sugars remained higher than the uninoculated plants. Isolate 1 performed well compared to other isolates. In the protein profile of different *A.awamori* isolates, Rm values of the bands were ranging from 0.006 to 0.54. Almost common bands were observed among the isolates 1, 2, 3 and 4 except some bands, but they differed only in their intensity. Similarity index was more between isolates 1 and 4; 2 and 5; and 4 and 5 (0.94) whereas it was less between isolates 1 and 9, 1 and 10, 5 and 10, 6 and 9, 6 and 10, and 8 and 10 (0.44). The results suggest that protein profiles data can closely separate isolates from different zones.

Key words: *Aspergillus awamori*, Growth response, Tomato and Protein profile.

In soil, lots of microorganisms are at work in mobilizing plant nutrients particularly phosphorus and by producing plant growth promoting substances. An ideal fertile soil is not only characterized by optimum physical and chemical properties condusive for plant growth, but also by microbiological constituents, which are maintained in equilibrium. Occurrence and distribution of phosphate solubilizing microorganisms (PSM) have been found in almost all the soils tested, although their populations vary with different soils, climate and cropping history[1] (Kucey *et al*., 1989).

Aspergillus awamori forms an important component of terrestrial communities. Species of *Aspergillus* belong to the first fungal organisms that were cultivated on artificial media and studied for biochemical properties. These organisms offer several benefits to the host plant such as faster growth, improved nutrition and higher species diversity. Classical methods for systematic studies of this genus have been very successful and have

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provided a relatively good classification. *A. awamori* can be recognized by its distinct conidiophores terminated by swollen vesicle bearing flask shaped philiades. The philiades may be borne directly on the vesicle or on the intervening metulae commonly isolated from the soil, plant debris and house dust. [2]Reddy *et al.* **(**2002) reported that all the isolated species of *Aspergillus* were capable of solubilizing all the natural rock phosphates. [3]Arora and Gaur (1979) tested fungi for their phosphate solubilizing activity. Around the growth of the colony, a zone of clearance of tricalcium phosphate in Pikovaskya's solid medium was found. The solubilization zone was more in case of *A. awamori* followed by *Pseudomonas striata*. Intricate chemical reactions in the soil convert applied phosphatic fertilizers into highly insoluble forms. But, the soil also has fungi that convert these insoluble forms into soluble forms. Such fungi are said to posses, mineral phosphate solubilizing ability that could be alternative to chemical fertilizer.

Molecular techniques, which provide valuable information on the magnitude of genetic variability within and between organisms of different species, have been developed. One such method is based on proteins that can be analyzed using electrophoresis or direct amino acid sequencing. Electrophoretic analysis of proteins has long been a valuable tool in systematic and population genetic studies of bacteria, plants and fungi[4] (Dodd *et al.*, 1996). Electrophoretic analysis of whole cell proteins by one-dimensional protein pattern provides a rough measure of the number and physicochemical properties of gene products. One-dimensional polyacrylamide gel electrophoresis of proteins has been used extensively for identification and classification at the strain and species level[5] (Snider, 1973).

Karnataka state has different soil types viz., red soil, black soil, sandy soil, laterite soil and alluvial soil, and is divided into ten agro climatic zones on the basis of annual rainfall, soil type, cropping pattern and other climatic conditions. The geographical area of Karnataka is classified into ten agro-climatic zones *viz.*, North eastern transition zone, North eastern dry zone, Northern dry zone, Central dry zone, Eastern dry zone, Southern dry zone, Southern transition zone, Northern transition zone, Hilly zone and Coastal zone.

The use of PSM has got considerable synergistic effect on growth and development of crop plants[6] (Azcon *et al*., 1978). Many studies on interaction of phosphate solubilizing microorganisms have been conducted in different crop plants. However the information on the compatibility of these organisms in localized soil conditions in promoting growth of plants and biochemical characterization of phosphate solubilizing bacteria and fungi isolated from different soil types of different zones is very much limited. Further, screening of the isolates either individually or in combination is needed to select efficient isolates to improve the plant growth and biomass. Brown (1974) [7]suggested that, the increase in plant growth sometimes observed after inoculation with phosphate solubilizing bacteria were primarily the result of synthesis of plant growth hormones.

The present study invoves Isolation and identification of *A. awamori* from soils of different agro-climatic zones of Karnataka,To study the effect of *A. awamori* on the biological and biochemical characters of tomato plants and to study the variability of *A. awamori* using protein profile.

MATERIAL AND METHODS

The experiments to study the "Molecular and physiological characterization of *A. awamori* isolated from different soil types of various agro climatic zones of Karnataka, were conducted at the Department of Biotechnology, University of Agricultural Sciences, G.K.V.K. Campus, Bangalore, Karnataka, India. The material used and methods followed are described below.

Collection of soil samples from different agro climatic zones of Karnataka

Karnataka state has different soil types viz., red soil, black soil, sandy soil, laterite soil and alluvial soil, and is divided into ten agro climatic zones on the basis of annual rainfall, soil type, cropping pattern and other climatic conditions.

Soil sampling

Four soil samples of 500 grams each were collected randomly from top six-inch layer of soil

from each agro climatic zone and packed in polyethylene bag. They were transferred to Department of Biotechnology, University of Agricultural Sciences, GKVK, for further studies.

Processing of soil sample

The soil samples collected were dried inside the laboratory at 28°C. Four soil samples collected from each soil type were mixed well to get a pooled soil sample for a zone. Totally, ten soil samples were obtained for the study. Each soil sample was sieved through a 1000µ mesh to remove the bigger soil particles and debris. The sieved soil samples were used for the isolation of the organism.

Isolation of *A. awamori* from different zones

A. awamori was isolated by serial dilution method. One gram of soil sample was taken in a test tube. Serial dilutions were made upto 10^{-4}. Dilution plated on Rose Bengal Agar medium, Incubated at 30^0C for 2 days.

Identification of A. awamori isolates

The fungal isolates were subjected to morphological tests as listed in 'The Manual of Soil Fungi' [8](Gilman, 1961). Different tests conducted are outlined below.

Colony morphology

Formation of characteristic colonies by fungal isolates and other characters were taken as a tool for preliminary identification by comparing with the standard.

Microscopic observation

Staining was done for all isolates using cotton blue stain. Cultures grown on Rose bengal agar were used for this purpose**.** The structure of the fruiting bodies and spore arrangement of the isolates were first observed under 100x compound microscope and then photographed using Phase contrast microscope.

Solubilization of insoluble phosphate by *A. awamori* in Sperber's medium

The overnight grown cultures of *A. awamori* isolates were spotted on Sperber's[9] (Sperber, 1957) medium to observe the zone of solubilization by the isolates. The plates were incubated at 30^0C for 36 hours.

Inoculum preparation

A. awamori isolates were grown separately, in a 250 ml flask containing 100ml potato dextrose broth for 2 days. The grown cultures were homogenized and 15 ml of each of the solution was given to each pot.

Protein profiles of *A. awamori*

Isolates were grown overnight at 37^0C in 100ml potato dextrose broth under shaking condition. Soluble proteins were extracted by grinding 100mg of freeze-dried mycelium with pestle and mortar with or without liquid nitrogen and 5ml of buffer solution (0.1M Tris-HCl, pH 6.8). The mixture was centrifuged for 20 min at 17000 rpm and the supernatant was collected. The protein content was estimated as described by [10]Lowry *et al.* (1951) with bovine serum albumin as standard protein. Protein content was adjusted to 100µg/ml of sample. SDS-PAGE was used for analysis.

Response of tomato for *A. awamori* isolated from different soil types of agro climatic zones of Karnataka

Two weeks old seedlings were raised in plastic trays (30x20x10cms) and transplanted in to pots containing sterile sand soil mixture (1:1 w/w). The plants were grown for 60 days by watering as and when required. There were three replications each treatment. The treatments of experiment includes: T_0 =Control, T_1=*A. awamori*$_1$, T_2=Aa_2, T_3=Aa_3, T_4=Aa_4, T_5=Aa_5, T_6=Aa_6, T_7=Aa_7,T_8=Aa_8 ,T_9=Aa_9 ,T_{10}=Aa_{10} ,T- Treatments for isolates of zone 1 to 10. The observations with respect to the growth parameters such as plant height, number of leaves, shoot fresh weight and dry weight, root fresh weight and dry weight were recorded at different intervals. The plant height was measured from the soil surface to the tip of the growing point at 15, 30, 45 and 60 days interval. The numbers of fully opened leaves were recorded at 15, 30, 45 and 60 days interval. The harvested plants were weighed and then the shoot fresh weight root fresh weight was recorded and expressed as grams per plant. The harvested plants were dried in an oven at 60°C for 4 days to attain constant weight and then the dry weight of shoot and root were recorded and expressed as grams per plant. Plant phosphorus concentration was estimated colorimetrically following the vanadomolybdate yellow colour method[11] (Jackson, 1973). Total plant sugar was estimated colorimetrically following the phenol sulphuric acid method[11] (Dubios *et al*., 1996).

The data obtained from the experiments were subjected to one-way analysis of variance for completely randomized design (CRD) using MSTAT-C software. The treatment means were

separated by Duncan's Multiple Range test (DMRT) a 5% level of significance[12] (Little and Hills, 1978).

RESULTS

Investigations were carried out on the molecular and physiological characterization of *A. awamori* isolated from different soil types of various agro climatic zones of Karnataka. Results of experiments conducted in glass house and laboratory are presented below.

Collection of soil samples from different agro climatic zones of Karnataka

Karnataka state has different soil types viz., red soil, black soil, sandy soil, laterite soil and alluvial soil, and is divided into ten agro climatic zones on the basis of annual rainfall, soil type, cropping pattern and other climatic conditions as shown in Figure 1. In order to study the molecular variability of *A. awamori* in the soils of different agro climatic zones of Karnataka the details of the soils taken for the study are given in Table 1.

Isolation and identification of *A. awamori*

A. awamori were isolated from different agro climatic zones of Karnataka by growing in the Rose bengal agar media, by serial dilution plate method. For isolation, preliminary identification was carried out by morphological observations of the fungal colonies such as colour, mycelial growth pattern, colour of the spores etc. All the check isolates and the standard strains formed yellowish-white fungal colonies initially and turned to complete black colour after sporulation due to colour of the spores.

Microscopic examination

All the isolates exhibited the typical spore arrangement on the conidial heads as that of the standard reference strain, in which the spores were arranged linearly on the conidial head and also spores.

Phosphate solubilizing efficiency of different isolates of *A. awamori*

The Phosphate solubilizing efficiency of different isolates of *A. awamori* was tested on modified Sperber's medium. All the isolates were found to have good solubilizing ability. The zone of solubilization produced by these isolates was measured and was presented in Table 2.

Table 1. Characteristics features of agro climatic zones of Karnataka*

Zone	Name	Soil type	Rainfall (mm/yr)	Temp. (°C)	Humidity %	Sand %	Silt %	Clay %	pH	CEC Centi-mols
1	North Eastern Transition Zone	Laterite soil	860	31.1-20.7	65.0	55.7	10.1	34.2	8.07	30.00
2	North Eastern Dry Zone	Sandy clay loam	526-754	22.3-33.5	63.0	14.3	24.7	61.0	8.5	63.00
3	Northern Dry Zone	Clay loam	585	32.4 -21.9	67.0	17.86	17.98	64.16	9.17	44.68
4	Central Dry Zone	Red sandy loam to Black soil	456-717	30.8-20.7	73.0	34.66	28.96	36.38	9.54	47.82
5	Eastern Dry Zone	Red sandy soil	674-889	29.2-18.6	71.0	72.97	4.93	22.1	5.31-6.21	16.5
6	Southern Dry Zone	Red sandy soil	670.6-888.6	29.1(max)	61.6	71.45	4.62	23.93	6.72	15.5
7	Southern Transition Zone	Red sandy loam	619-1300	30-19.2	81.0	76.17	5.86	17.94	5.8	-
8	Northern Transition Zone	Black soil	780	30.1-18.0	76.0	16.35	36.38	57.27	6.72-7.85	64.0
9	Hilly Zone	Red loam to Red Sandy loam	904-3695	25.2-16.6	89.0	69.7	20.5	9.79	5.3	20.5
10	Coastal Zone	Lateritic	4000	30.5-23.5	96.5	48.46	12.76	38.78	5.2	25.56

*Source: Department of Soil Science, UAS, GKVK, Bangalore

Protein profile of *A. awamori* isolates

The data on the protein banding patterns of *A. awamori* isolates are presented based on the Relative mobility value (Rm), similarity index and intensity of the bands is presented in Table 3, Table 4 and Fig. 2. Rm value of the bands ranged from 0.006 to 0.54. Among these isolates, isolate 10 was different (one extra band in 0.35, 3.10 and 3.80) from other isolates but rather more similar to isolate 9 as both have a common band in 1.20, while it is absent in all other isolates. Almost common bands were observed between the isolates 1, 2, 3 and 4 except some bands, but they differed only in their intensity. Similarity index was more between isolates 1, 4; 2, 5 and 4, 5 (0.94) whereas it was less between isolates 1, 9; 1, 10; 5, 10; 6, 9; 6, 10 and 8, 10 (0.44).

Elucidean distances and dendrogram was constructed (Figure 3) using Statistica software. Two clusters were illustrated. The first cluster contains a single group. The first group had the isolates 10 and 9, while the second cluster is subdivided into 2 groups wherein isolates 8 and 7 form one group. On the other hand the second cluster also had the two groups. Within that first group forms the sub group with isolate 3 and 6, and in second group also sub group was formed by isolate 5 and 2 with isolates 4 and 1.

Response of tomato plants to *A. awamori* isolates

The data pertaining to the influence of *A. awamori* isolates from ten agro climatic zones on plant height and number of leaves of tomato are presented in Table 5.

Table 2. Solubilization zones produced by different *A. awamori* isolates

Isolates	Diameter of Solubilization zone (mm)
1	21.7
2	24.5
3	21.7
4	21.5
5	23.7
6	19.0
7	19.5
8	20.0
9	23.5
10	24.7*
Standard**	24.7

* Isolate with maximum diameter of solubilization zone
** *A. awamori*
Source: Department of Agriculture Microbiology, GKVK, Bangalore

Table 3. Band intensity and Rm value in protein profile of *A. awamori* isolates

Isolates/bands	Rm value	1	2	3	4	5	6	7	8	9	10
1	0.006	+	+	+	+	+	+	+	+	++	+++
2	0.013	+	+	+	+	+		+	++	+++	+++
3	0.026			+				++	+	++	+++
4	0.033	++	++	++	+		+				++
5	0.040		++	+			+			+++	+++
6	0.046										++
7	0.053		+	+	+					+	++
8	0.080							+		+	
9	0.100				++					++	
10	0.110	++	++	++	++	+	+	+	++	+++	++
11	0.150									++	
12	0.160	-								++	+
13	0.300								+	+	+
14‘	0.320								+		
15	0.340	++	++		++	+	+		++	+++	+++
16	0.410										++
17	0.500										+
18	0.540	+	+		+	+					

+Less band intensity ; ++ Moderate band intensity ; +++ High band intensity

The plants inoculated with *A. awamori* isolates, increased plant height compared to uninoculated plants throughout the observation period. However, the heights differed significantly among the plants inoculated with various isolates. The least plant height (11.00cms) was recorded in uninoculated control plants while maximum height (22.50cms) was recorded in plants inoculated with isolate 1, at 60 days after transplanting, which was followed by plants inoculated with isolate 3. The number of leaves was found to increase progressively with time. It was observed that number of leaves in inoculated plants was always higher than the control. Maximum number of leaves (18.00) was observed in plants inoculated with the isolate 1 and least number of leaves (7.33) was found in uninoculated plants. However, there was no significant difference in the number of leaves among the inoculated treatments except isolate 1, which had maximum number of leaves.

The fresh weight and dry weight of the plants harvested after 60 days after transplanting are presented in Table 6. The total fresh weight and dry weight in the plants inoculated with *A. awamori* isolates were higher than uninoculated plants. Maximum shoot and root fresh weight was recorded in plants inoculated with the isolate of zone 1 (14.37 g and 1.56 g). It was superior over

Table 4. Similarity index of *A. awamori* isolates based on protein profile analysis

	1	2	3	4	5	6	7	8	9	10
1	-									
2	0.83	0								
3	0.88	0.83	0							
4	0.94	0.88	0.83	0						
5	0.88	0.94	0.77	0.94	0					
6	0.77	0.72	0.77	0.72	0.66	0				
7	0.66	0.72	0.66	0.72	0.77	0.55	0			
8	0.77	0.72	0.77	0.72	0.77	0.66	0.77	0		
9	0.44	0.61	0.55	0.50	0.55	0.44	0.77	0.55	0	
10	0.44	0.50	0.55	0.50	0.44	0.44	0.55	0.44	0.66	-

Table 5. Plant height and number of leaves of Tomato as influenced by *A. awamori* isolates

Treatment	Plant height (cm) at different intervals				Number of leaves at different intervals			
	15 DAT	30 DAT	45 DAT	60 DAT	15 DAT	30 DAT	45 DAT	60 DAT
Control	3.91	4.75	7.33	11.00	4.00	5.06	06.66	07.33
T1	6.26	9.93	14.16	22.50*	5.66	9.28	11.33	18.00*
T2	6.33	9.00	12.83	17.50	5.26	8.53	9.66	14.25
T3	7.03	9.91	13.66	20.50	5.33	8.36	11.00	16.50
T4	6.56	9.60	13.33	20.30	6.00	9.23	13.66	16.83
T5	7.18	8.80	13.83	20.10	5.13	8.06	10.33	15.76
T6	7.00	9.08	11.16	19.50	5.35	8.16	09.66	14.32
T7	7.33	9.06	13.33	19.76	5.23	8.50	09.54	16.78
T8	6.66	8.26	12.33	19.00	6.00	8.26	12.33	14.37
T9	6.33	8.10	11.50	17.50	5.20	7.73	09.33	13.41
T10	6.10	8.05	12.16	18.00	5.16	7.96	09.28	13.66
S.Em ±	0.240	0.394	0.512	0.766	0.328	0.374	0.638	0.676
CD (p=0.05)	0.710	1.163	1.511	2.262	0.969	1.105	1.882	1.994

DAT: Days after transplanting
* Isolate with maximum response
T1 to T10: Treatments for isolates from zone 1 to 10

all other treatments. However, the fresh weight differed significantly among the plants inoculated with various *A. awamori* isolates. Minimum shoot and root fresh weight (5.99 g and 0.37 g) was recorded in uninoculated plants. Maximum shoot and root dry weight was recorded in plants inoculated with isolate from zone 3 (2.46 g and 0.70 g) respectively, which was significantly higher over all other treatments and minimum shoot and root dry weight (0.97 g and 0.14 g) was recorded in uninoculated plants. No significant difference in the total dry weight was observed in plants inoculated with all other isolates of *A. awamori* except isolates 7, 9 and 10 that showed lower dry weight compared to other treatments.

Phosphorus content

The phosphorus content of shoot and root in the plants inoculated with the *A. awamori*

Table 6. Total biomass of tomato plants as influenced by *A. awamori* isolates

Isolates	Fresh weight (g/plant)			Dry weight (g/plant)		
	Shoot	Root	Total	Shoot	Root	Total
Control	5.99	0.37	6.36	0.97	0.14	1.11
1	14.37	1.56	15.93	2.39	0.73	3.12*
2	9.43	0.83	10.26	1.60	0.53	2.13
3	13.22	1.06	14.28	2.46	0.70	3.16
4	11.98	1.15	13.13	2.21	0.71	2.92
5	9.23	1.10	10.33	1.84	0.56	2.40
6	9.36	1.19	10.55	1.61	0.68	2.29
7	8.03	0.88	8.91	1.49	0.56	2.05
8	10.05	0.84	10.89	1.99	0.61	2.60
9	8.68	0.63	9.31	1.01	0.41	1.42
10	7.90	0.60	8.50	1.52	0.50	2.02
S.E.M ±	1.593	0.217	1.810	0.272	0.095	0.367
CD (p=0.05)	4.700	0.641	5.341	0.802	0.279	1.081

Table 7. Phosphorus concentration and total sugar of shoot and root of tomato as influenced by *A. awamori* isolates

Zones	Shoot P (mg/plant)	Root P (mg/plant)	Total (mg/plant)	Shoot Total sugar (mg/plant)	Root Total sugar (mg/plant)	Total (mg/plant)
C	0.017	0.004	0.021	0.94	0.030	0.970
T 1	0.225	0.065	0.290*	3.22	0.320	3.540*
T 2	0.128	0.031	0.159	1.82	0.076	1.896
T 3	0.123	0.021	0.144	2.85	0.083	2.933
T 4	0.159	0.039	0.198	2.62	0.091	2.711
T 5	0.081	0.028	0.109	1.76	0.088	1.848
T 6	0.104	0.027	0.131	1.79	0.096	1.886
T 7	0.094	0.022	0.116	1.55	0.078	1.628
T 8	0.081	0.021	0.102	2.19	0.079	2.269
T 9	0.123	0.023	0.146	1.62	0.067	1.687
T 10	0.127	0.025	0.164	1.37	0.061	1.431
SEM+	0.083	0.018	0.101	0.374	0.018	0.392
CD at 5%	0.121	0.019	0.092	1.102	0.053	1.155

C –Control

T_{1}-T_{10}- Treatments from 10 different zones

isolates are presented in Table 7.The shoot P content in the inoculated plants was found to differ significantly from the control plants. No significant difference was observed within the treated plants. The maximum shoot P content (0.255 grams/plant) was observed in the plant inoculated with isolate of zone 1 while minimum shoot P (0.017 grams/ plant) was observed in the uninoculated plants.The root P content of inoculated plants was found to be higher than the uninoculated plants. There was no significant difference in the root P content of the inoculated plants with isolates of zones 9, 10 and 6, 7. The maximum root P (0.065 grams/plant) was found in the plants inoculated with isolate 1, and minimum root P (0.004 grams/plant) was observed in the uninoculated plants.

Total sugar content of shoot

The total sugar content of shoot and root in the plants inoculated as well as the uninoculated plants are presented in Table 7. The total sugar content of shoot content was maximum in plants inoculated with isolate 1 (3.22 mg). Control significantly differed over other treatments. No significant difference was observed among the treatments. Least sugar content (0.94 g) was observed in uninoculated plants. The total sugar content of root was maximum in plants inoculated with isolate 1 (0.32 mg). Control significantly differed over other treatments. No significant differences were observed between other treatments. Least sugar content was observed in control (0.03 mg).

Fig. 1. Different Agro-climtic zones of Karnataka

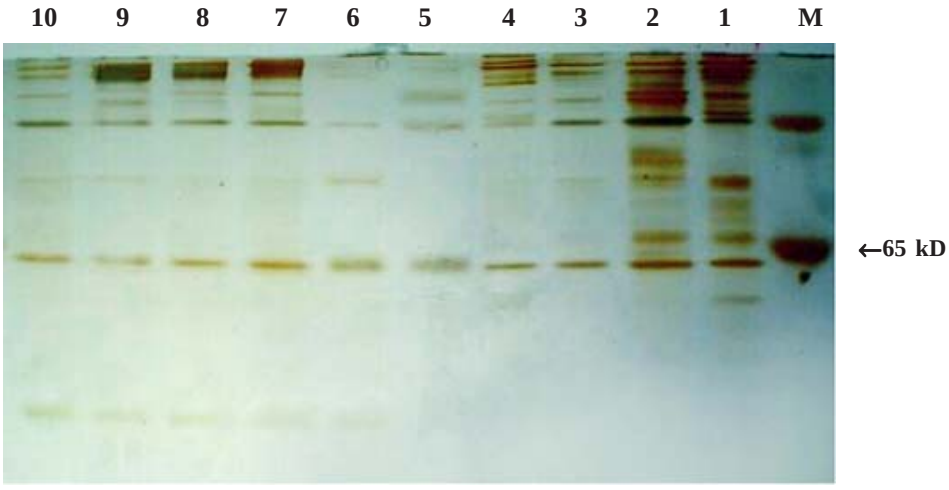

Fig. 2. Protein profile to *Aspergillus awamori* isolates

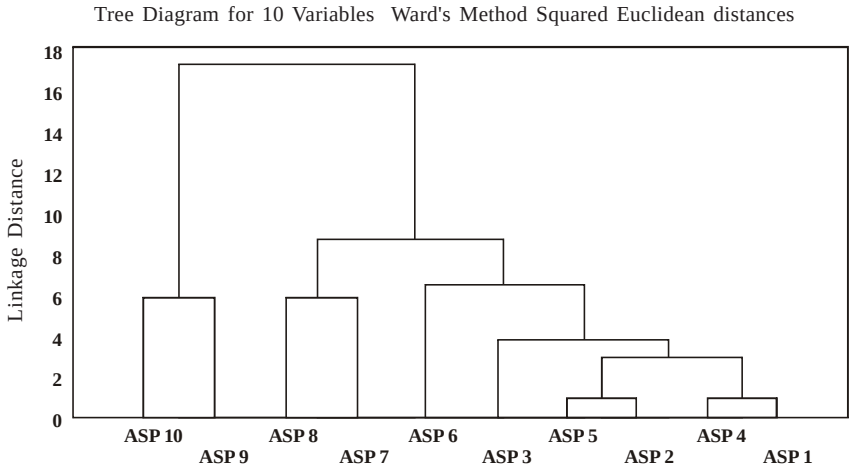

Fig. 3. Dendrogram of *A. awamori* (ASP) isolates obtained by protein profile analysis

DISCUSSION

In the present investigation, *A. awamori*, a phosphate solubilizer, has been isolated from various agro climatic zones of Karnataka. Efficiency of P solubilization by *A. awamori* was studied in *in vitro* conditions for confirmation of the isolates and then in a green house experiment using all the isolates to identify efficient isolates using tomato as the indicator plant and the strains were designated as efficient, based on the zone of solubilization.

Fungi are usually identified on the basis of the morphological characters shown by fruiting bodies, spores, vegetative mycelia etc. Similarly in this study, *A. awamori* was isolated from the soils collected from different zones, by growing in the Rose bengal agar medium. For isolation, preliminary identification tests were carried out in which the fungal isolates were subjected to morphological tests as listed in 'The manual of Soil Fungi' [13](Gilman, 1961). These were identified based on the formation of characteristic colonies by fungal isolates. The strains formed whitish-yellow fungal colonies initially and completely black after sporulation due to black colour of the spores. For identification of the isolates, confirmatory test was conducted on *A. awamori* isolated from all the soil types. All the isolates exhibited the typical spore arrangement on the conidial heads and fruiting body structure. This has shown that all the isolates belonged to the same strain. These *A. awamori* isolates were capable of utilising inorganic insoluble P provided in Sperber's medium. Similar results were reported by [14]Arora and Gaur (1979). They tested the PSM and found a zone of clearance of tricalcium phosphate around the growth of the colony in Pikovaskya's solid medium. The solubilization zone was more in *A. awamori* followed by *Pseudomonas striata*, and they also observed that fungal isolate was found to be more effective than bacteria.

In the present study, both qualitative and quantitative differences were observed in the protein profile of different *A. awamori* isolates which is presented in Figure 1. The data on the protein banding patterns of *A. awamori* isolates are presented based on the Rm value, similarity index and intensity of the bands (Table 3 and 4). Rm value of the bands ranged from 0.006 to 0.54. Among these isolates, isolate 10 is different (one extra band in 0.35, 3.10 and 3.80) from other isolates but rather more similar to isolate 9 (have common band in 1.20). Almost common bands were observed between the isolates 1, 2, 3 and 4 except some bands, but they differed only in their intensity. Similarity index was more between isolates 1, 4; 2, 5 and 4, 5 (0.94) whereas it was less between isolates 1, 9; 1, 10; 5, 10; 6, 9; 6, 10 and 8, 10 (0.44).

Two clusters were illustrated from the dendrogram obtained as presented in Figure 2. The first cluster contains a single group. The first group

had the isolates 10 and 9, while the second cluster is subdivided into 2 groups wherein isolates 8 and 7 form one group. On the other hand the second cluster also had the two groups. Within that first group forms the sub group with isolate 3 and 6, and in second group also sub group was formed by isolate 5 and 2 with isolates 4 and 1. This result upholds the study conducted by [15]Avio and Giovannetti (1998). The above results suggest that protein profiles data can closely separate isolates from different zones. These results agree with the work done by several scientists such as [16]Aly *et al.* (2003) wherein the protein profile data of *Fusarium* isolates of cotton obtained from different areas clearly separated the isolates.

Biological variability studies were also conducted using tomato growing in the sterilised soil. In this study, different geographical isolates of *A. awamori* were used as inoculants. The biological and biochemical changes were recorded in tomato plants. The present investigation has showed that tomato plants inoculated with *A. awamori* isolates grew taller as compared to uninoculated plants. Similar result was reported by [17]Sundaram *et al.* (2002) who concluded that *A. awamori* inoculation to rice variety Savithri increased P solubilization in the rhizosphere and yield components under lowland conditions. Also, [18]Shiva Kumar *et al.* (2003) reported that *A. awamori*, in the presence of compost in the soil significantly increased the biomass, pod weight and oil content of groundnut when compared to the plants inoculated with compost alone. The increase in height in these plants may be due to an increase in phosphorus uptake as well as other minor nutrients and PGPR activity[19] (Brown, 1974).

However, the plants differed significantly in height in response to some isolates within the treatments but the highest was seen in case of isolate 1. Since, all the isolates belong to the same species of *A. awamori*, their effect on growth in terms of height may not be as significant as those usually observed in the plants inoculated with different species or genera or in combination with other beneficial microorganisms. The earliest report of increasing P uptake and dry weight of plants through inoculation of phosphate solubilizing organisms was made by [20]Gerestson (1948).

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