Influence of AM fungi and *Azospirillum* on fruit nutritional values of *Lycopersicon esculentum*, Mill.

P.V. Sivakumar and P. Thamizhiniyan
Department of Botany, Annamalai University, Annamalai nagar, Chidambaram, Tamil Nadu.

Addresses for communication

P.V. Sivakumar
Email. pvsivadde@rediffmail.com

Received 12 August 2012; accepted 18 August 2012

**Abstract**

Present investigation was carried out to find out the influence of AM fungi and *Azospirillum* on nutritional potential of tomato (*Lycopersicon esculentum*). This study was conducted as field experiment at Sivapuri Village, Annamalainagar, Tamilnadu, India. The influence of VAM and *Azospirillum* on the tomato plant *Lycopersicon esculentum* Mill. In the present study the leaf nutrients and fruit nutrients were higher in VAM + *Azospirillum* group. From this experiment it is clear that the *Azospirillum* along with AM fungi showed better result when compared to control. The main advantage of this association was it does not pollute the soil and also does not show any negative effect to environment and human health.

© 2011 Universal Research Publications. All rights reserved

**INTRODUCTION**

Plants are exposed to very large numbers of microorganisms that are present in the soil and are deposited on leaves and stems. Plants are the prime source of nutrients for microorganisms because they are the main source of organic matter in the environment. They provide nutrients indirectly from plant exudates, the shedding of leaves, pollen, etc. and also from dead plant matter. Mycorrhiza is the mutualistic symbiosis (non-pathogenic association) between soil-borne fungi with the roots of higher plants. In the AM symbiosis, plant defense responses increase transiently in the early phases of the symbiosis and then are suppressed.

Although many studies prove the positive effect of *Azospirillum* sp., their use is still limited by fluctuating performance, often verified in field trials. Hence, there is a need to identify an appropriate inoculation method of *Azospirillum* sp., in which the optimal number of cells remained viable and available to colonize the roots. The inoculation of *Azospirillum* as seed bacterization, root dipping and through soil application is a common practice. Seed inoculation is the most successful technology of bioinoculant inoculation, which provide high population of desirable strain in the close vicinity of the young growing roots. But, this method appears to be difficult to treat the small sized seeds especially for transplanted crops, viz. tomato, chilli, brinjal, tobacco etc. There are no reports of inoculating *Azospirillum* directly to the nursery in case of transplanted crop like tomato which might have the potential of improving the proliferation of the endosymbiont. This method would be simple and easy way of applying the bioinoculant by farmers. Thus, an attempt was made to confirm the beneficial effects of nursery inoculation of *Azospirillum* by keeping the standard root dipping inoculation technique as control. This investigation therefore was aimed at studying the interaction effect of arbuscular mycorrhizal fungi (AMF) and *Azospirillum* sp. that are likely to possess agronomically beneficial traits, viz. N-fixation, P-mobilization and production of plant growth promoting substances for the better growth of tomato crop. So that the present research work has been carried out Influence of AM fungi and *Azospirillum* on fruit nutritional values of *Lycopersicon esculentum*, L.

**2. MATERIALS AND METHODS**

The present investigation was carried on to find out the influence of AM fungi and *Azospirillum* on the nutritional potential of tomato (*Lycopersicon esculentum*).

2.1. **Location of experimental site**

This study was conducted as field experiment at Sivapuri Village, Annamalainagar, Tamilnadu, India. The weather is moderately warm with hot summer months.

2.2. **Methods**

The experiment design and details

**Cultivar- Tomato (Lycopersicon esculentum Mill.)**

Sampling days 36, 50 and 75 days

T1—Control (uninoculated)
T_2 – AM Fungi Alone  
T_3 – Azospirillum Alone  
T_4 – AM Fungi + Azospirillum

Tomato seeds (Lycopersicon esculentum) were surface sterilized using 0.02% mercuric chloride, rinsed 2-3 times in sterile distilled water and sown in nursery bed containing a sterilized mixture of soil and sand (3:1, v/v). After 10 days of emergence the plants were transferred to the beds. The following treatments, with five replications each, were included in the study: uninoculated control (T_1), G. fasciculatum (T_2), Azospirillum alone (T_3) and G. fasciculatum + Azospirillum (T_4). All beds were placed under standard conditions (14 h lights (25-27°C), 65% relative humidity) in a completely randomized experimental design. Plants were watered daily. G. fasciculatum inoculations were accomplished by placing 2 g soil inoculum, containing 75 spores g^-1 soil and 50 spores g^-1 soil respectively, where the seeds were to be sown. The Azospirillum strain Azo V6 was cultured in nutrient broth for 72 hours and mixed in pre-sterilized lignite powder @ 30 ml per 100 g. This inoculum was used @ 20 g per m^2 nursery. This quantity was fixed as per field recommendations of 200 g per acre (or 500 g/ha). The Azospirillum suspension was prepared using 200 g of lignite based Azospirillum in five litres of sterilized water. The seedlings were dipped in the Azospirillum slurry for 30 minutes and air dried before transplantation. The population of Azospirillum in the slurry made contained 2.9 x 10^7 cfu per ml of suspension. The seeds were sown immediately. Control plants did not receive any inoculum.

2.3. Biochemical Analysis

Total carbohydrate, total protein, total lipid, total fibre content, vitamin C and carotenoids were estimated by the following methods.

2.3.1. Estimation of Total Carbohydrate

Extraction
Total carbohydrate content in the fruit was determined following the procedure of Dubois et al. (1956). To 5 mg of fruit homogenate add 1ml of distilled water and 1 ml of 5% phenol followed by 5ml of concentrated sulphuric acid. The optical density of the brown colour developed was read after 30 minutes at 490nm in a spectrophotometer and was compared with the standard curve obtained for glucose. Blanks were prepared by mixing 1 ml of distilled water with 1 ml of 5% phenol and 5 ml of concentrated sulphuric acid.

2.3.2. Estimation of Total Protein

Extraction
500 milligram of fruit weighed and macerated with a pestle and mortar with 10 ml of 20 per cent trichloro acetic acid. The homogenate was centrifuged for 15 minutes at 600 rpm. The supernatant was discarded. To the pellet, 5ml of 0.1 N NaOH was added and centrifuged for 5 minutes. The supernatant was saved and made upto 10 ml of 0.1N NaOH. This extract was used for the estimation of protein.

Estimation
From this extract, 1 ml of sample was taken in a 10 ml test tube and 5 ml of reagent B was added. The solution was mixed well and kept in dark for 10 minutes. Later 0.5 ml folin phenol was added and the mixture was kept in dark for 30 minutes. The sample was read at 660 nm in th spectronic -2. Blank prepared without protein sample was used for zero setting. The absorbance value was referred to the standard graph proteins prepared by using 5th fraction of Bouvins’s serum albumen.

Reagents
1. 2% sodium carbonate in 0.1 N NaOH.  
2. 1% copper sulphate mixed with equal volume of 2% sodium potassium tartarate.  
3. Folin phenol reagent.  
5. 20% Trichloro acetic acid.  
6. 0.1 N NaOH solution.

Preparation of Reagents
2% sodium carbonate in 0.1 N NaOH.

Reagent A
400 mg of sodium hydroxide was dissolved in distilled water and made up to 100 ml. To this solution, 2g of sodium carbonate was added.

Reagent B
One per cent of copper sulphate solution mixed with equal volume of 2% sodium potassium tartarate solution.

Reagent C
50 ml of reagent A and 1ml of reagent B mixed fresh at the time of experiment.

2.3.3. Estimation of Total Lipid

Extraction
The weight of lipid was estimated as the difference between the dry weight of the sample (400mg) and the dry weight of the lipidfree substance tested after overnight digestion (extraction) with 5 ml of chloroformmethanol mixture (3:1) following the procedure of Folch et al. (1956).

2.3.4. Estimation of Total fibre content

The total fiber content was determined by the neutral detergent fibre method (Goering and Van soest,1970; Jhonson and Marlet, 1986).

2.3.5. Estimation of Vitamin C

Vitamin C was estimated by the 2,4-dinitrophenylhydrazine method in conjunction with spectrophotometric measurement (Osborne, 1985).

2.3.6. Estimation of Carotenoids

The method of extraction and HPLC analysis was described by Wills et al. (1988). The extraction method involved the blending of produce in acetone, shaking with diethyl ether and water, and collecting the organic layer which was washed with water, dried over anhydrous sodium sulphate, and reduced in volume under vacuum. The extract was saponified to remove chlorophylls and hydrolyse carotenoid esters (Curl, 1953; Nudin, 1989) to quantitatively hydrolysing a range of carotenoid types. Saponification was performed at room temperature with methanolic potassium hydroxide and, after washing with water, the organic fraction was dried over sodium sulphate and evaporated under vacuum at room temperature. The carotenoid residue was dissolved in a mixture of ethanol:methyl-terbutyl-ether and after making up to volume was filtered and immediately analyzed by HPLC–
mass spectrometry. Analyses were made with a Hewlett-Packard HP11100. The stationary phase was YMC Carotenoid C-30 5 mm, 4.6 mm _250 mm. The drying gas flow was 6 L/min, the nebulizer pressure was 40 psig, the drying gas temperature was 325 8C, the vaporizer temperature was 450 8C, the capillary voltage was 2500 V and the corona current was 3 mA. The mobile phase was a mixture of methanol (A): methyl-terbutyl-ether (B) (50%, v/v). The eluent flow was: 1 mL/min. The linear gradient was: t = 0 min 10% B; t = 2 min 10% B; t = 10 min 20% B; t = 20 min 70% B; t = 22 min 10% B; t = 25 min 10% B. The interface between the LC and MS was APCI (atmospheric pressure chemical ionization) positive (fragmentor 100 V). Peak identification was based on the comparison of HPLC retention times and mass spectra with chemical standards (lutein, b-carotene and lycopene), which were purchased from IMATRA, S.A. Other carotenoids (9-cis-b-carotene, lycopene isomers and neurosporene) were identified through their mass spectra by comparing their retention times with those in the literature. The amount for each individual carotenoid was calculated from chromatographic responses to standard solutions (in the cases in which the chemical standard was available). 9-cis-b-carotene was quantified as b-carotene, and lycopene isomers and neurosporene were quantified as lycopene.

Table 1. Showing the influence of AM fungi and Azospirillum on fruit nutritional values of Lycopersicon esculentum, L.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>T1 Control</th>
<th>T2 AM Fungi Alone</th>
<th>T3 Azospirillum Alone</th>
<th>T4 AM Fungi + Azospirillum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total carbohydrate (g)</td>
<td>2.18±0.3</td>
<td>2.14±0.2</td>
<td>2.19±0.1</td>
<td>2.18±0.0</td>
</tr>
<tr>
<td>Total Protein (g)</td>
<td>1.05±0.0</td>
<td>1.05±0.02</td>
<td>1.05±0.00</td>
<td>1.05±0.00</td>
</tr>
<tr>
<td>Total Lipid (g)</td>
<td>0.42±0.0</td>
<td>0.42±0.00</td>
<td>0.43±0.00</td>
<td>0.42±0.02</td>
</tr>
<tr>
<td>Total fibre content (g)</td>
<td>1.60±0.1</td>
<td>1.61±0.02</td>
<td>1.62±0.00</td>
<td>1.65±0.00</td>
</tr>
<tr>
<td>Vitamin C (mg/100g)</td>
<td>21.04 ± 1.10</td>
<td>21.06 ± 1.15</td>
<td>21.08 ± 1.18</td>
<td>21.05 ± 1.18</td>
</tr>
</tbody>
</table>

Table 2. Showing the influence of AM fungi and Azospirillum on carotenoids (µg/g dry weight) of Lycopersicon esculentum, L.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>T1 Control</th>
<th>T2 AM Fungi Alone</th>
<th>T3 Azospirillum Alone</th>
<th>T4 AM Fungi + Azospirillum</th>
</tr>
</thead>
<tbody>
<tr>
<td>All trans lutein</td>
<td>8 ± 2</td>
<td>10 ± 4</td>
<td>14 ± 2</td>
<td>10 ± 1</td>
</tr>
<tr>
<td>b-carotene</td>
<td>73 ± 2</td>
<td>75 ± 2</td>
<td>76 ± 2</td>
<td>76 ± 2</td>
</tr>
<tr>
<td>9-cis b-carotene</td>
<td>5 ± 2</td>
<td>6 ± 1</td>
<td>8 ± 1</td>
<td>8 ± 1</td>
</tr>
<tr>
<td>Lycopene</td>
<td>350 ± 34</td>
<td>353 ± 30</td>
<td>358 ± 21</td>
<td>352 ± 16</td>
</tr>
<tr>
<td>Lycopene Isomers</td>
<td>122 ± 14</td>
<td>125 ± 10</td>
<td>129 ± 8</td>
<td>125 ± 14</td>
</tr>
<tr>
<td>Neurosporene</td>
<td>25 ± 8</td>
<td>28 ± 2</td>
<td>31 ± 2</td>
<td>31 ± 2</td>
</tr>
<tr>
<td>TOTAL</td>
<td>583 ± 60</td>
<td>597 ± 49</td>
<td>616 ± 35</td>
<td>591 ± 39</td>
</tr>
</tbody>
</table>

3. RESULTS
3.1. Fruit Nutritional values
The nutritional values of the fruit analysed include total protein, total carbohydrates, total lipid, total fibre content and vitamin C. The values were expressed as in grams (g). The quantity of vitamin C was expressed as mg/100mg. The variations in the nutritional value of the fruit were summarized in table 1 and 2.

3.2. Total carbohydrate
The value of total carbohydrate content in T1 group was 2.18 ± 0.3g after 35 days. The value slightly increased to 2.19 ± 0.1g after 75 days. The highest value of 2.23 ± 0.1 was recorded in T4 group after 75 days (Table 6; Fig.13).

3.3. Total Protein
The total protein content showed 1.05g in T1 group. Protein content showed a non significant variation in all the groups at various periods of experiment. The details were given in table 1.

3.4. Total Lipid
The total lipid content ranged from 0.42 – 0.45g from T1 to T4 group. Like proteins the total lipid value also showed a non significant variation at all periods of the experiment.

More details were given in table 1.

3.4. Total Fibre Content
The total fibre content in T1 group was 1.60, 1.61 and 1.62 for 35, 50 and 75 days respectively. The value showed an increasing trend in T2, T3 and T4 groups. The details were given in table 1.

3.5. Vitamin C
The quantity of vitamin C in control group (T1) ranged from 21.04 – 21.08 mg/100g. The values showed an increasing trend from control to various groups. The vitamin C content showed a significant variation from T1 to T4 groups. The variations in quantity were given in table 1.

3.6. Carotenoids
The various carotenoids analysed include all trans lutein, b-carotene, 9 cis – b-carotene, lycopene, lycopene isomers and neurosporene. The HPLC chromatogram of tomato carotenoids were given in table 2. The values were expressed as in µg/g dry weight. The total carotenoids in T1 group ranged from 583 – 616 µg/g dry weight. In T2 group the value increased to 584 – 617 µg/g dry weight. T3 group also showed the value as in T1 group. The T4 group showed the total carotenoid value of 591 – 621 µg/g dry weight.
REFERENCES


Source of support: Nil; Conflict of interest: None declared