MATERIALS AND METHODS
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3.1. Study localities:

Six different coastal localities of south-east coast of Tamil Nadu state, India (Figs. 1&2) were selected for the study of mycorrhizal status of salt marsh and dune plants including mangroves and their colonizing VA-mycorrhizal fungi. They were:

1. Nagapattinam
2. Point Calimere
3. Vedharanyam
4. Velanganni
5. Muthupet estuary
6. Pitchavaram

Nagapattinam (Lat. 10° 47.50 'N; long. 79° 50.30 'E), Point Calimere (Lat. 10° 17.45 'N; long. 79° 51.30 'E), Vedharanyam (Lat. 10° 22.30 'N; long. 79°-51.30 'E), Velanganni (Lat. 10° 47.50 'N; long. 79° 50.30 'E) and Muthupet estuary (Lat.10° 17.45 'N; long. 79° 51 'E) of Nagai Quaid-e-milleth district and Pitchavarm (Lat. 11° 26 'N; Long. 79° 48 'E) of South Arcot Vallalar district (Figs. 1&2) are the coastal areas of south-east coast of Tamil Nadu, India. The average annual atmospheric temperature during the years of study (1993-94) varied between 32.3°C and 24.2°C (Max.42°C; min. 16°C). Annual rainfall was 330mm and the major precipitation was between the months of October-December.

3.1.1. Selection sites:

Salt marsh of Nagapattinam, Point Calimere, Vedharanyam and Velanganni, plants were surveyed for colonization by VAM fungi at three sites from
each locality (Plates. 1 - VI). Site selection was based on the floristic composition and general soil characteristics.

The three study sites of each locality were:

**Nagapattinam:**
- Site. 1 - Maraimalai nagar area
- Site. 2 - Light house area
- Site. 3 - Kallar area

**Point Calimere:**
- Site. 1 - Muniappaneri area
- Site. 2 - Light house area
- Site. 3 - Turtle pan area

**Vedharanyam:**
- Site. 1 - Puthu santhai area
- Site. 2 - Arkattuthurai area
- Site. 3 - Pushpavanam area

**Velanganni:**
- Site. 1 - Serudhur area
- Site. 2 - Prathaparamapuram area
- Site. 3 - Kallimedu area

The sites are located within 6 to 10 km by road from each other. At each study site, an area of 500 m$^2$ was chosen for sampling. Care was taken to include the different growth which Rao (1971) reported as successional stages in a salt marsh flora. The plant species for study were selected based on their relative abundance (Hussain et al., 1984). All the sites were surveyed for VAM occurrence during 1994-1995.
The other two study localities, Pitchavaram and Muthupet estuary, mangrove plants were surveyed for colonization of VAM fungi at three sites from each locality (Plates. VII & VIII). Site selection was based on the floristic composition and general soil characteristics.

**Pitchavaram:**
- Site. 1 - Periakadavu area
- Site. 2 - Karithurai area
- Site. 3 - Chinnavaikkal area

**Muthupet estuary:**
- Site. 1 - Sethukuda area
- Site. 2 - Koraiyar bank area
- Site. 3 - Pamaniyar bank area

At each site, sampling area was not less than 1 km. All the study sites were surveyed for VAM occurrence during 1993-1994 in relation to four different seasons (pre-monsoon; monsoon; post-monsoon and summer).

3.2. **Field sample collection:**

At Nagapattinam from three study sites, totally twenty six plant species belonging to fifteen families of angiosperms, at Point Calimere from three sites, totally thirty plant species belonging to fifteen families of angiosperms, at Vedharanyam, from three study sites, totally fifteen plant species belonging to nine families and at Velanganni from three study sites, totally twenty two plant species belonging to twelve families of angiosperms (Table-3&4) were selected for study. At Pitchavaram, from three study sites, totally twenty mangrove plant species belonging to eleven families of angiosperms (Table-5) and at Muthupet estuary, from three study sites, totally eight mangrove plant species belonging to seven families of angiosperms (Table-6) were
selected for study. Totally fortyone plant species belonging to eighteen families of angiosperms of salt marsh and dune plants were selected at the four study localities of Nagai Quaid-e-milleth district and twenty mangrove plant species belonging to eleven families of angiosperms were selected at Pitchavarm and Muthupet estuary. The plants were identified to species level (Gamble, 1935; Mathew, 1983; Bennet, 1987). At each study site of six localities, 3-5 vigorous appearing mature plants per species were selected for study by their collection of roots and root-zone soil samples (Koske, 1987).

3.2.1. Soil samples:

Root-zone soil samples were collected at 0-30cm soil depth (Dickman et al., 1984) a region of maximum mycorrhizal incidence. In the case of dune sands, samples were obtained by removing soil from a depth of 0-40cm. All samples were collected by using a 13cm shovel. The samples were collected in triplicates and each soil sample was placed in a plastic bag that was sealed and brought to the laboratory and kept at 5-10°C (Koske and Halvorson, 1981). A portion of soil from each sample bag was utilized for the estimation of gravimetric soil moisture. Previously weighed soil samples were oven dried at 105°C for 48 h and dry weights were obtained. The water loss was expressed as percent of the dry weight (Dickman et al., 1984). Another portion was earmarked for the estimation of other physico-chemical characteristics (pH, ECse, percent organic matter, total N, available P, K, Ca, Mg, Na, and Cl⁻). From the remaining portion 100g was used to estimate VAMF spore numbers per sample bag.

3.2.2. Soil characteristics:

For the study of general soil characteristics of the three study sites of each locality at Nagai Quaid-e-milleth district and of the three study sites each of Pitchavaram of South Arcot Vallalar district. Soil samples were collected at each site,
mixed thoroughly and analysed for soil texture, pH, EC<sub>se</sub>, N, P, K at the Soil Testing Laboratory. Tamil Nadu Rice Research Institute, Aduthurai, Thanjavur district, Tamil Nadu following standard methods (Jackson, 1973; Sarma et al., 1984).

3.3. Root samples:

To check the mycorrhizal status of the sampled plant species, feeder root samples were collected, washed free of attached soil particles, cut into several small fragments and fixed in FAA (Phillips and Hayman, 1970) in the field itself. The FAA fixed root segments of 1 cm length, cleared, stained and then percent root colonization for each plant species was estimated. The clearing and staining of non-pigmented and pigmentend roots were done following the method of Phillips and Hayman (1970) and Kormanik et al. (1980) respectively as described below.

3.3.1. Trypan blue method (Phillips and Hayman, 1970):

The 1 cm long root segments were first washed thoroughly in distilled water and then placed in 10% KOH and heated to 90°C for 15-30 min. They were then washed in distilled water and then immersed in alkaline H<sub>2</sub>O<sub>2</sub> for 10-15 min. Then they were washed in distilled water and acidified with 5N HCl for 2-5 min. Then the root bits were stained in 0.05% trypan blue in lactophenol for 15-30 min. and the excess of stain was removed with clear lactophenol and observed.

3.3.2. Acid fuchsin method (Kormanik et al., 1980):

The 1 cm long root segments prefixed in FAA were washed thoroughly in distilled water and then transferred to 10% KOH solution. They were autoclaved for 10-15 min., washed thoroughly in distilled water and then immersed in alkaline H<sub>2</sub>O<sub>2</sub> for 10-15 min. Then they were washed in distilled water, acidified with 1% HCl and
stained with 0.01% acid fuchsin in acetic acid for 15-30 min. The excess stain was removed with clear lactophenol and observed.

3.4. Isolation and identification of VAM fungal spores and sporocarps:

The root-zone soils, sand from different sites of six study localities were analysed by the following method.

3.4.1. Wet-sieving and decanting technique (Gerdemann and Nicolson, 1963):

100 g of soil was suspended in 500 ml of water. The suspension was passed through a series of sieves of the following pore dimensions collecting the residues at each sieve 710, 400, 250, 106, 75 and 45 μm. The residue from 710 μm sieve were examined under a dissecting microscope for sporocarps and attached hyphae and spores on root pieces. The residue collected from 400 and 250 μm sieves were examined for sporocarps and large spores under dissecting microscope. The residues from 106, 75 and 45 μm sieves were used for observing small and detached spores under a compound microscope. After preliminary observation, the residues from each sieve of the above mentioned size were collected in beakers of water and passed through circular Whatman No. 1 filter paper.

3.4.2. Wet-sieving and filtration method for dune sands (Koske and Walker, 1984):

100 g of sand was placed in a 1500 ml beaker to which was added 600 ml of water. The sand-water mixture was shaken vigorously to 30 seconds, the sand allowed to settle and the water solution quickly decanted into a Buchner funnel holding Whatman No. 4 filter paper 9 cm in diameter. Suction was applied and the paper removed as the spores got collected on it.
The spores collected on the filter papers were then spread on petri dishes and examined under appropriate magnifications of a compound microscope for spore count. The spore number from the root-zone soil sample of each plant species was counted in replicates of 3-6, averaged and tabulated per 100g soil. For identification of spores and sporocarps, individual healthy ones were picked up from the filter paper, mounted on slides in lactophenol, observed under different magnification of a compound microscope and photomicrographs were taken. The spores and sporocarps were identified using the synoptic key of Gerdemann and Trappe (1974), Hall and Fish (1979), Trappe (1982), Berch and Trappe (1987), Schenck and Perez (1987) and Raman and Mohankumar (1988).

3.5. Study of correlation between VAMF variables and physico-chemical characteristics of the soil:

This study was carried out in the twenty and twenty-six VAM plant species identified at the three study sites of each locality of Nagapattinam and Point Calimere respectively.

Pearson product moment correlation (Sokal and Rohlf, 1973) was estimated to examine the relationship (a) between VAMF spore abundance and physico-chemical characteristics pH, ECse, percent organic matter, total N, available P, K, Na, Ca, Mg and Cl⁻ and (b) among the physico-chemical characteristics of the root-zone soils of VAM plant species at Nagapattinam and Point Calimere separately. All estimations made in triplicates and the data were averaged.
3.6. Comparative study of VAM status of eight common plant species at four study localities of Nagai Quaid-e-milleth:

Eight plant species common in four study localities viz., Nagapattinam, Point Calimere, Vedharanyam and Velanganni were selected for this study. At each locality, the roots and root-zone soils of eight plant species were collected. The roots of each plant species collected fixed in FAA, cleared and stained in 0.05% Trypan blue (Phillips and Hayman, 1970) and percent root colonization estimated (Giovannetti and Mosse, 1980). The VAM spores from soils were wet sieved, decanted (Gerdemann and Nicolson, 1963) and counted per 100g soil, percent root colonization of various fungal structures were observed in roots and the spore density values were recorded. The above values were estimated in four replicates and the results were averaged.

3.7. Study of the effect of soil depth and seasonal change on VAM spore population and colonization of Aeluropus lagapoides and Spinifex littoreus:

To understand the phenology of VAM formation and spore production at different seasons in relation to soil depth, the experiments were conducted from two study localities viz., Nagapattinam and Point Calimere located at Nagai Quaid-e-milleth district, Tamil Nadu, during May 1994 to April 1995. The sampling was done at monthly intervals from May 1994 to April 1995. The average atmospheric temperature during the years of study (1994-95) varied between 34.5°C and 20.2°C. The roots of Aeluropus lagapoides and Spinifex littoreus along with the 500g of rhizosphere soil were taken separately from the top of 10cm in each case. The samples were processed immediately for assessment of VAM colonization and spore extraction. To observe the effect of soil depth on VAM, the roots and soils sampled at different vertical column (soil depth) i.e., 0-10, 10-20, 20-30 and 30-40cm were analyzed for spore population and
VAM colonization in May 1995. The roots were fixed in FAA, cleared, stained and mounted following the procedure of Phillips and Hayman (1970). The percentage of root length colonized by VAM was found with a grid line intersect method (Giovannetti and Mosse, 1980). The relative abundance of mycelium, arbuscules and vesicles of mycorrhizal fungi in host tissues were recorded. The extraction of VAM fungal spores from rhizosphere soil was done by wet-sieving and decanting technique (Gerdemann and Nicolson, 1963) and the spore population was calculated per 100g of dry soil.

3.8. Pot experiments:

Pot experiments in sterilized soil have been reported to be valuable by providing much useful information on the role of VAM in growth and mineral nutrition (Krishna and Bagyaraj, 1982; Abbott et al., 1984) by demonstrating the apparent differences in the effectiveness of VAM fungi.

In this study, pot experiments using sterilized soil were performed for

a. Bioassay and mass inoculum production experiments

b. Selection of an efficient VAMF strain and

c. Salt tolerance experiments.

18cm dia earthen-ware pots were used to carry out bioassay-cum-inoculum production and selection of an efficient VAM cum salt tolerance experiments. Pots were filled with 5kg of sandy loam: sand mixture (1:1 v/v) deficient in P (5mg P kg⁻¹ soil extracted with NH₄F and HCl, Olsen and Decan, 1965) with a pH of 7.4. Nitrogen as urea (35mg/kg soil) was applied as basal application to soil contained in the earthen-ware pots irrespective of the treatments. Sandy loam soils filled in the pots
irrigated with Ruakura nutrient solution added at the rate of 50ml per 2.5L substrate once in 8 days (Smith et al., 1983) was most suitable for VAM mass inoculum production (Sreenivasa and Bagyaraj, 1988 b).

3.8.1. Host plants used:

For bioassay and mass inoculum production of VAM experiments, *Coleus aromaticus* Benth. and *Ocimum sanctum* L. were used as the host plants. For growth experiments involving selection of an efficient strain of VAM and salinity tolerance studies, sesame (*Sesamum indicum* L. cv. Co.1) was used.

Seeds of *Ocimum sanctum* L. and *Sesamum indicum* L. cv. Co.1 were obtained from the office of the Assistant Director of Agriculture, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu. The cuttings were used for *Coleus aromaticus*. Seeds and cuttings were surface sterilized in 5% chloramine T for 5 min. and repeatedly washed in sterile water before sowing.

3.8.2. VAM inocula used:

For mass inoculum production, selection of a efficient saline strain and salinity tolerance experiments, seven VAM fungal inocula were used. They were *Glomus aggregatum*, *Glomus ambisporum*, *Glomus geosporum*, *Gigaspora margarita*, *Acaulospora scrobiculata*, *Sclerocystis clavispora* and *Scutellospora calospora*. The Point Calimere isolates of *Glomus aggregatum*, *Glomus geosporum* and *Sclerocystis clavispora* were isolated from the mycorrhizal roots of *Spinifex littoreus*, *Aeluropus lagopoides* and *Phyla nodiflora*; *Glomus ambisporum*, *Gigaspora margarita*, *Scutellospora calospora* and *Acaulospora scrobiculata* were isolated from the mycorrhizal roots of *Spinifex littoreus* through bioassay experiments outlined below:
3.9. Bioassay for infection efficiency and mass inoculum production of VAM:

In the search for salinity tolerant VAM fungal endophytes, it was found that *Phyla nodiflora* was found to be growing in the highest saline conditions [EC$_{se}$ 3.1 and Cl'1640ppm] in light house area of Point Calimere (Table-11) and also was found that *Spinifex littoreus* was found to be growing in the saline sandy soils of Nagapattinam (Table-10). The associated VAM fungal endophytes of mycorrhizal roots of these two plant species were bioassayed in *Ocimum sanctum* L. and *Coleus aromaticus* Benth. (Plate. XXVII) for their infection efficiency and for selection for better host for mass inoculum production. Among the VAM fungal endophytes associated with the mycorrhizal roots of *Phyla nodiflora* and *Spinifex littoreus*, VAM spores of *Glomus aggregatum*, *Glomus ambisporum*, *Glomus geosporum*, *Gigaspora margarita*, *Sclerocystis clavispora*, *Scutellospora calospora* and *Acaulospora scrobiculata* were selected for bioassay as these seven species alone were found to be associated with greater number of host plants at Point Calimere and Nagapattinam localities in the above order (Table-4). The spores of these seven VAM species from both plant species were isolated from the root-zone soils by wet sieving and decanting technique (Gerdemann and Nicolson, 1963).

18 cm dia. earthen pots were filled with autoclaved sandy loam : sand mixture (1:1 v/v) were chosen. N as urea at 35mg/kg soil was added per pot. Surface sterilized seeds of *Ocimum sanctum* and cuttings (approximate 5 cm length with two nodes) of *Coleus aromaticus* were sown in pots. Before sowing mycorrhizal inoculum as spores of seven VAM fungal species (Point Calimere and Nagapattinam isolates) were inoculated in *Ocimum sanctum* and *Coleus aromaticus* plants. Uninoculated plants were kept as control. Plants were harvested after ninety days and the percentage of root colonization, plant height, shoot and root dry weights were recorded. Spores in
the root-zone soils were collected after the 90th day by wet-sieveing and decanting (Gerdemann and Nicolson, 1963). These were used for confirmation and to test the efficiency of infection.

3.10. **Selection of an efficient saline strain of VAM using sesame (Sesamum indicum L. cv. Co-1):**

The screening experiments were conducted after the survey to assess the soil collected from study sites for mycorrhizal colonization and plant growth response. *Sesamum indicum* L. cv. Co-1 seedlings which is responsible to VA-mycorrhizae (Selvaraj and Subramaniyan, 1988; Sulochana et al., 1988) has chosen as the test plant.

The experimental design was a completely randomized factorial combination of nine different treatment combinations

1. Control (NM + NS)
2. Control (NM + S)
3. *Glomus aggregatum* + S
4. *Glomus geosporum* + S
5. *Glomus ambisporum* + S
6. *Gigaspora margarita* + S
7. *Sclerocystis clavispora* + S
8. *Scutellospora calospora* + S
9. *Acaulospora scrobiculata* + S

A growth and physiological study of sesame in pot inoculated with seven strains of VAM fungi (isolated from saline study sites) viz., *Glomus aggregatum* (PC-3 isolate), *Glomus geosporum* (PC-1 isolate), *Glomus ambisporum* (PC-2 isolate) *Gigaspora margarita* (NM-1 isolate), *Sclerocystis clavispora* (PC-1 isolate), *Scutellospora calospora* (NM-2 isolate) and *Acaulospora scrobiculata* (NM-3 isolate)
in salinized and non-saline soils were conducted. (Plate. XXVIII). Seedlings were germinated in a sandy loam soil containing 3.3 ppm extractable P which had been sterilized by autoclaving twice for 1h on consecutive days. Two weeks after germinated seedlings were transplanted to 18cm dia. earthen-ware pots containing 5kg of sterilized sandy loam; sand soil mixture 1:1 (v/v) and inoculated by placing 10g of *Ocimum sanctum* L. or *Coleus aromaticus* Benth. sample roots. Mycorrhizal inoculum containing 700 spores per 100ml of soil and root segments of *Ocimum sanctum* or *Coleus aromaticus* colonized by saline strain of VAM fungi was placed 2cm below the soil surface before sowing the seeds. The leachate flowing out of the 45μm sieve containing associated microorganisms was added to non-mycorrhizal treatments.

Plants were watered daily with tap water and after 3 weeks were irrigated with saline solution containing 20mM each of NaCl (Pond *et al.*, 1984; Kannan, 1989). A non-inoculated, salinized control was watered with the saline solution and a non-inoculated non-salinized control was watered with tap water only. The plants were kept growing in the case house watered as and when necessary and harvested on 85th day of growth.

The following morphological and nutritional parameters were studied at harvest on 85th day.

1. Plant height, dry weight and root: shoot biomass,

2. shoot P content and

3. percentage of mycorrhizal colonization in roots, number of VAM spores per 100g of root-zone soil and mycorrhizal effect.
All the above parameters were estimated as per the following procedures outlined below:

3.10.1. **Estimation of growth, nutritional and physiological parameters**:

In the growth experiment involving selection of an efficient saline strain and salinity tolerance of VAM to sesame, the following parameters were studied.

3.10.2. **Plant dry weight**:

The plant samples were first air-dried and then cut into their respective root and shoot portions. They were then dried in a hot air oven at 60-70°C to constant weight. The root and shoot weights recorded for each plant and summed up to calculate the plant dry weight.

In bioassay, selection of an efficient VAM and salinity tolerance experiments, the data for total plant dry weights (g/plant) were tabulated.

3.10.3. **Estimation of mineral content in root and shoot**:

The dried root and shoot samples were ground in a fine powdered with pestle and mortar and passed through a 60μm mesh sieve. The powdered plant material was taken to Environmental Resources Research Centre, Tiruvananthapuram, Kerala and estimation of P content in shoot (Jackson, 1973) was done in the selection of an efficient strain of VAM experiment, while estimation of N,P,K, (Jackson, 1973) and Zn & Ca (Lindsay and Norvell, 1978) in the saline tolerance experiment.
3.10.4. **Estimation of percent root colonization and VAMF spores from root-zone soil:**

This was done in bioassay, selection of efficient strain of VAM fungi and salinity tolerance experiments of sesame following the method of Krishna and Dart (1984).

\[
\text{Percent root colonization} = \frac{\text{Number of VAM positive segments}}{\text{Total number of segments scored}} \times 100
\]

The collected spores by wet-sieving and decanting technique were then spread on Whatman No.1 filter paper, finder appropriate magnification counted in replicates of 3-5, averaged and tabulated per 100g.

3.10.5. **Mycorrhizal effect was calculated as follows:**

\[
\text{Mycorrhizal Effect} = \frac{\text{Dry weight of VAM inoculated plant}}{\text{Dry weight of non-inoculated plant}} \times 100
\]

3.11. **Study of the effect of VAM, *Glomus aggregatum* (PC-3 isolate) on salinity tolerance of sesame:**

An experiment was conducted to determine the tolerance levels of salinity for sesame by growing them at different levels of NaCl salinity (Plate. XXIX). Plants were grown under salinization was done following the method of Pond et al. (1984) with suitable necessary modifications as given below.

The experimental design was a completely randomized factorial combination. Ten sets of treatment each with three replicates were maintained. There were:
The sesame (*Sesamum indicum* L. cv. Co.1) seeds were surface sterilized in 5% chloramine T for 5 min. and after several changes in sterile distilled water were sown in earthen-ware pots (18cm dia.) containing sterilized (by autoclaving twice for 2h on two consecutive days) soil : sand mixture (1:1 v/v). Mycorrhizal inoculum contained 700 spores per 50ml and root segments of *Ocimum sanctum* colonized by *Glomus aggregatum* was placed 2cm below the soil and sand surface before sowing the seeds. The leachate flowing out of the 45μm size containing associated microorganisms was added to non-mycorrhizal treatments. Surface sterilized seeds of sesame were then placed above the pad of mycorrhizal inoculum 3 per pot. The irrigation with 1/4 strength Rakura’s solution was done as and when necessary which contained up to 20th day of seedling growth. Seedlings were then thinned to one per pot. Salinization was done on 20th day of growth at different levels of NaCl viz., 20, 40, 60, 80mM. For each treatment triplicates were maintained and the experiment was terminated on 85th day. The pots were irrigated with sterilised deionised water on other days as and when required.

The following morphological, physiological, nutritional and yield parameters were estimated at harvest on 85th day.
1. Plant height, root and shoot dry weights.

2. Proline content in leaf.


5. Total proteins, total soluble sugars, aminoacids, lipids, phenols and orthodi-hydroxy phenols in root and shoot.

6. Percent mycorrhizal colonization in roots and number of VAMF spores per 100g of root-zone soil.

7. Seed yield per plant, 1000 seed weight, plant height, plant dry weight per plant, number of capsules per plant, capsule length and percentage of oil content per 10g seed.

3.12. **Estimation of leaf proline content**:

The estimation of leaf proline content was done in the salinity tolerance experiment of sesame. The estimation was carried out in the third leaves from the apex in the sesame plants grown for 85 days in different mycorrhizal treatments with salinity.
3.12.1. Procedure for determination of leaf proline content:

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Reagents</th>
<th>Experimental details</th>
<th>Wavelength</th>
<th>Reference</th>
</tr>
</thead>
</table>
| 1.     | Acid-ninhydrin reagent*:  
6 M Orthophosphoric acid - 20 ml  
Glacial acetic acid - 30 ml  
Ninhydrin - 1.25g | Third leaves from the apex of the plants were used. 10mg leaf samples were ground in a pestle and mortar in 3ml of 3% sulphosalicyclic acid. The extract was made upto 5ml with the same acid. To this, 2ml of ninhydrin reagent was added and kept in a water bath at 100°C for 1h. Reaction was terminated in an ice bath. The proline-ninhydrin complex was extracted using 10ml of toluene in a separating funnel. The toluene layer containing the proline-ninhydrin complex was read in a spectrophotometer. Proline content in the samples was computed from a standard curve. The experiment was conducted in three replicates for each treatment. | 515 nm | Bates et al. (1973) |
| 2.     | 3% sulphosalicyclic acid | | | |
| 3.     | Toluene | | | |

* Used within 24h of preparation. Note: Spectrophotometric readings were taken using Spectronic-20 (Bausch and Lomb).

3.12.2. Estimation of acid and alkaline phosphatases activity in roots:

The procedure for the above estimation has been outlined below and this estimation was done only in salinity tolerance VAM experiment of sesame.
<table>
<thead>
<tr>
<th>St. No.</th>
<th>Compounds</th>
<th>Experimental details</th>
<th>Wavelength (nm)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Acid phosphatase</td>
<td>Sample (cell free extract) - 0.2 ml 10 mM P-nitrophenyl phosphate (PNPP) in 0.1 M acetate buffer pH 4.0 - 0.5 ml 0.5 mM MgCl₂ - 0.1 ml</td>
<td>405</td>
<td>Gianinazzi-Pearson and Gianinazzi (1976) modified by Krishna (1981).</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Incubated for 30 min. at 30°C. Reaction terminated by addition of 5 ml of 0.05 M NaOH. For control, no substrate was added. Standard: p-nitrophenol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Alkaline phosphatase</td>
<td>Sample (cell free extract) - 0.2 ml 10 mM p-nitrophenyl phosphate (PNPP) in 0.05 M Tris-citric acid buffer pH 8.5 - 0.9 ml 0.5 mM MgCl₂ - 0.1 ml</td>
<td>405</td>
<td>Gianinazzi - Pearson and Gianinazzi (1976) modified by Krishna (1981).</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Incubated for 30 min. at 30°C. Reaction terminated by addition of 5 ml of 0.05 M NaOH. For control, no substrate was added. Standard: p-nitrophenol</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: All colorimetric readings were taken using Spectronic - 20 (Bausch and Lomb).

3.13.1 Procedure for quantification of soluble phosphatases:

Three replicates of plants of each treatment were harvested after 85 days growth for analyses. Immediately after harvesting, roots were carefully washed in ice cold distilled water to remove all soil debris and used immediately for phosphatase analyses.

The soluble root enzymes by macerating the detached roots (1:1 w/v) in a precooled mortar at 4°C using 0.1 M borate buffer (pH 8.8) plus glutathione. The macerate was centrifuged at 30,000 x g for 30 min. and the soluble acid and alkaline phosphatase activities in the cell free extract were determined.
Soluble phosphatase activity in 0.2ml of free extract was determined quantitatively by measuring the amount of p-nitrophenol (PNP) released by the phosphatases from the substrate p-nitrophenyl phosphate (PNPP). Amount of PNP released was estimated using a standard curve.

Activities of acid and alkaline phosphatases were expressed at mU/ml root extract where U, one enzyme unit, is that enzyme activity which transforms 1 mole of substrate in min. under specific experimental conditions.


The leaf nitrate reductase activity was assayed in vivo by vacuum infiltration technique of Klepper et al. (1971). Both young and matured fresh leaves were washed in distilled water blotted dry and cut into 1mm wide strips. A known quantity of the leaf bit was placed in side arm test tube containing 5ml of infiltration medium. The medium composed of 0.92m KNO₃, 0.1M Phosphate buffer pH 7.5 (Jaworski, 1971) and 1% n-propanol (v/v). The test tube containing the media and tissues were rubber stoppered and connected to a vacuum pump. Alternate evacuation and readdition of air was performed for 2 min. and care was taken to keep the material well wettered and completely submerged in the medium. The infiltration carried out at 0 - 10°C.

After infiltration, the test tubes were wrappered with aluminium foil and incubated at 30°C in a water bath with gentle shaking. After 30min. of incubation, 0.5ml of aliquotes were taken to determined the amount of nitrite released into the medium. Nitrite was measured as the colour complex developed by the sample in the presence of 0.3ml 1% sulfanilamide in 3N HCl and 0.3ml of 0.02% N (1-Napthyl) ethylene diamino dihydrochloride (NEDD). After 20min., volume was made upto 5ml
with distilled water. Extinction was read at 540nm and quantified with nitrite standard. The blank contained sulfanilamide (NEDD) and the infiltration medium in the place of the sample. The nitrate reductase activity was expressed as n mol of nitrite produced per gram fresh weight.

3.15. Estimation of total soluble sugars, proteins, free aminoacid, lipids, ortho-dihydroxy and total phenols in root and shoot samples:

Immediately after harvesting, the roots and shoots were washed carefully in water to remove the adhering soil particles and were then ground well (known weight) in pestle and mortar and extracted with ethanol following the procedure outlined by Radhakrishnan et al. (1955). Estimation of total soluble sugars, proteins, free amino acids, lipids, ortho-dihydroxy and total phenols in the roots and shoots were carried out as follows:

3.15.1. Procedure for quantification of different compounds in root and shoot samples:

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Compounds</th>
<th>Extraction medium</th>
<th>Experimental details</th>
<th>Wave length (nm)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Total soluble sugars</td>
<td>80% ethanol</td>
<td>Sample - 0.1 ml 5% phenol - 1.0 ml Conc. H₂SO₄ - 5.0 ml Total volume made upto 10ml with double distilled water. Standard-glucose.</td>
<td>490</td>
<td>Dubois et al., (1956)</td>
</tr>
<tr>
<td>2.</td>
<td>Lipid</td>
<td>Chloroform : Methanol 2:1 (V/V)</td>
<td>250mg of sample homoginized with extraction solvent. Filtered through filter paper. Filterate vortexed with sodium sulphate then it was taken in a preweighed beaker and dried by boiling. Dried extract weighed by substract in the initial weight form the final weight amount was expressed in μg g⁻¹ fresh wt.</td>
<td>--------</td>
<td>Sato and Murata, (1988)</td>
</tr>
<tr>
<td>Sl. No.</td>
<td>Compounds</td>
<td>Extraction medium</td>
<td>Experimental details</td>
<td>Wave length (nm)</td>
<td>Reference</td>
</tr>
<tr>
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</tr>
<tr>
<td>3.</td>
<td>Total proteins</td>
<td>distilled water</td>
<td>10% TCA precipitated, centrifuged, pellet, dissolved in 0.1N NaOH. Sample - 0.5 ml 0.1% Copper sulphate - 0.5 ml 12.5% Sodium carbonate- 2.5 ml 25% Folinphenol reagent - 0.5 ml Total volume made upto 5.0 ml and the reaction mixture kept in dark for 30min. before measurement. Standard- Bovine serum albumin.</td>
<td>660</td>
<td>Lowry et al., (1951).</td>
</tr>
<tr>
<td>4.</td>
<td>Total free aminoacids</td>
<td>80% ethanol</td>
<td>Sample - 1.0 ml 80% phenol - 1.0 ml Kept in boiling water bath for 10min. 5% ninhydrin - 0.2 ml Kept again in boiling water bath for 15min. Total volume made upto 10ml with 60% ethanol. Standard - L - leucine</td>
<td>575</td>
<td>Troll and Canan (1953).</td>
</tr>
<tr>
<td>5.</td>
<td>Ortho-dihydroxy phenol</td>
<td>80% ethanol</td>
<td>Sample - 1.0 ml 0.05 N HCl - 1.0 ml Arnow’s reagent - 1.0 ml Distilled water - 10.0 ml 1 N NaOH - 2.0 ml Standard - Catechol.</td>
<td>515</td>
<td>Mahadevan, (1966).</td>
</tr>
<tr>
<td>6.</td>
<td>Total phenols</td>
<td>80% ethanol</td>
<td>Sample - 1.0 ml 10% Folin Phenol reagent - 3.0 ml 20% Sodium carbonate - 2.0 ml Total volume made upto 25ml with distilled water. Standard - Catechol.</td>
<td>650</td>
<td>Swain and Hillis, (1959)</td>
</tr>
</tbody>
</table>

Note: All Colorimetric readings were taken using spectronic - 21 and the experimental details were carried out at room temperature (30±1°C).
3.16. **Microscopy and photomicrography:**

All measurements and characterization of the root squashes, spores and sporocarps were done using Carl-Zeiss (Germany) research microscope with stage and ocular micrometers. All photomicrographs including those of root squashes, spores and sporocarps were taken using Nikon Optiphot microscope (Japan). Images were recorded in 100 ASA Konica colour film.

3.17. **Statistical analyses:**

Pearson product moment correlation, one way and two-way ANOVA Student’s ‘t’ test (Sokal and Rohlf, 1973) were performed.