was lost and chances of mite infestation and overgrowth of contaminants increased. Similarly, when the leaves pressed and dried under sand were kept between blotters the original green colour faded and the leaves became brittle-dry. In the case of the formaldehyde and alcohol solution method, the green colour was diluted after 6–9 months. The modified fourth method was the best because the specimens retained their original green colour for more than 3 years when kept between papers and free from the overgrowth of other contaminants and mite infestation. The dried green preserved specimens look just like the original leaves and are easy for handling, transportation and maintenance.

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INFLUENCE OF VAM FUNGUS *GLOMUS CALEDONIUS* ON FREE PROLINE ACCUMULATION IN WATER-STRESSED MAIZE

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It is well known that vesicular arbuscular mycorrhizal (VAM) association increases growth and various physiological processes in plants[1–3]. Mycorrhizal association also affects plant–water relations through its influence on the root/shoot hormonal balance[4]. Photosynthesis and photorespiration are significantly affected by mycorrhizal association in water-stressed plants[3]. The VAM-associated physiological changes in the host plant might trigger drought resistance on exposure to water-deficit stress. Free proline accumulation has been correlated with drought resistance in plants[5,6]. The present study was therefore undertaken to ascertain the effect of the VAM fungus *Glomus caledonius* on free proline accumulation in maize under various osmotic stresses.

Seeds of maize (*Zea mays* L. var HS-123), obtained from the Plant Breeding Department of this University, were surface-sterilized by immersing in 95% ethanol for 5 min. They were rinsed in sterile distilled water and planted five seeds per pot filled with 2 kg of a sand : soil mixture (1 : 1); thinning was performed to maintain three seedlings per pot. A phosphorus-deficient soil (3.5 ppm available P) was identified; it was screened through a 2 mm sieve and autoclaved on three alternate days.

The level of phosphorus applied to P-amended soil as single super phosphate but without mycorrhizal inoculum was 60 kg/ha (recommended dose for maize); parallel experiments were carried out with unsterile soil : sand mixture (1 : 1).

An inoculum of *G. caledonius* (Nicol. and Gerd.) Gerdemann and Trappe, obtained from the Rothamsted Experimental Station, UK, was used. It was maintained on maize in sand culture. Each experimental pot was provided with 30 g of VAM inoculum which contained soil, small root fragments, hyphae and approximately 14 spores/g of soil. The indigenous inoculum (IVAM) was prepared from a P-deficient soil by isolation of spores by wet sieving and decanting methods[7]. Spores were suspended in distilled water and diluted to desired numbers. A mixture of IVAM and *G. caledonius* (1 : 1) was given to achieve an inoculum potential similar to that of the introduced VAM; P-amended soil was devoid of mycorrhizal inoculum and served as positive control. Plants were grown in a glass house with day temperatures ranging from 27 to 32°C; water was applied daily to maintain soil moisture to near field capacity. After 5 weeks of growth the rate and percentage of infection were calculated[8,9].

Five-week-old plants were exposed to osmotic potentials of 0, −2, −5 and −10 bar[10,11]. After 8 h exposure to different osmotic potentials, one set of plant sample was employed to measure leaf water potential using a pressure chamber instrument (PMS Instrument Company, Oregon, USA), a second set was employed to estimate free proline content[12].

Under sterile soil, five-week-old maize plants exhibited 48% VAM infection compared to 32% in IVAM + *G. caledonius* treatment; in unsterile soil the percentage root colonization was 85 and 26 for

Table 1 *Effect of leaf water potential on free proline content in maize leaves with/without VAM treatment*

Leaf water potential (bar)	Proline content (mg/g dry wt) Non-VAM	VAM	IVAM + *G. caledonius*	P-amended
Sterile soil				
−3 (control)	0.13 ± 0.01 (100)	0.17 ± 0.04 (128.7)	0.15 ± 0.04 (111.7)	0.18 ± 0.03 (138.3)
−5	1.08 ± 0.07 (100)	2.20 ± 0.09 (203.4)	1.60 ± 0.03 (148.7)	2.62 ± 0.09 (142.6)
−8	2.71 ± 0.16 (100)	4.68 ± 0.19 (172.7)	4.15 ± 0.18 (153.0)	4.80 ± 0.21 (177.0)
−12	4.42 ± 0.23 (100)	5.99 ± 0.17 (135.6)	4.40 ± 0.21 (122.1)	6.41 ± 0.23 (145.0)
Unsterile soil				
−3 (control)	0.13 ± 0.02 (100)	0.14 ± 0.03 (109.4)	0.14 ± 0.01 (108.0)	0.18 ± 0.03 (135.0)
−5	0.85 ± 0.06 (100)	2.12 ± 0.13 (249.6)	0.98 ± 0.04 (115.8)	2.09 ± 0.14 (246.1)
−8	2.32 ± 0.13 (100)	3.87 ± 0.19 (166.5)	3.34 ± 0.19 (143.6)	3.96 ± 0.17 (170.4)
−12	3.89 ± 0.18 (100)	6.24 ± 0.25 (134.9)	4.63 ± 0.21 (119.0)	5.54 ± 0.23 (142.4)

Each value is the mean ± S.E. of three replications; Figures in parentheses are percentage change over non-VAM (control) treatment.

the two treatments respectively. At osmotic potentials of 0, −2, −5 and −10 bar, the leaf water potentials were about −3, −5, −8 and −12 bar respectively in the case of both VAM and IVAM + *G. caledonius* treatments.

Results for free proline content under various treatments are presented in table 1. In all the cases, proline content increased markedly with decrease in leaf water potential. At a leaf water potential of −12 bar, there was an approximately 33-fold increase in proline level over the control value. Similar results have been reported for other plants[6]. This increase of proline is probably associated with the inhibition of the oxidation of proline into glutamic acid in water-stressed leaves[13]. At all water-stress treatments the accumulation of proline was generally greater in P-amended plants than in plants given VAM, IVAM + *G. caledonius*, and non-VAM treatments. Proline accumulation is known to be correlated with drought resistance in a number of plants[5,6]. Thus, the increased accumulation of proline in P-amended, and VAM and IVAM + *G. caledonius* treated maize plants than in non-VAM treated plants may reflect greater drought resistance.

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MICROSPOROGENESIS IN *RHIZOPHORA LAMARCKII* MONTR.

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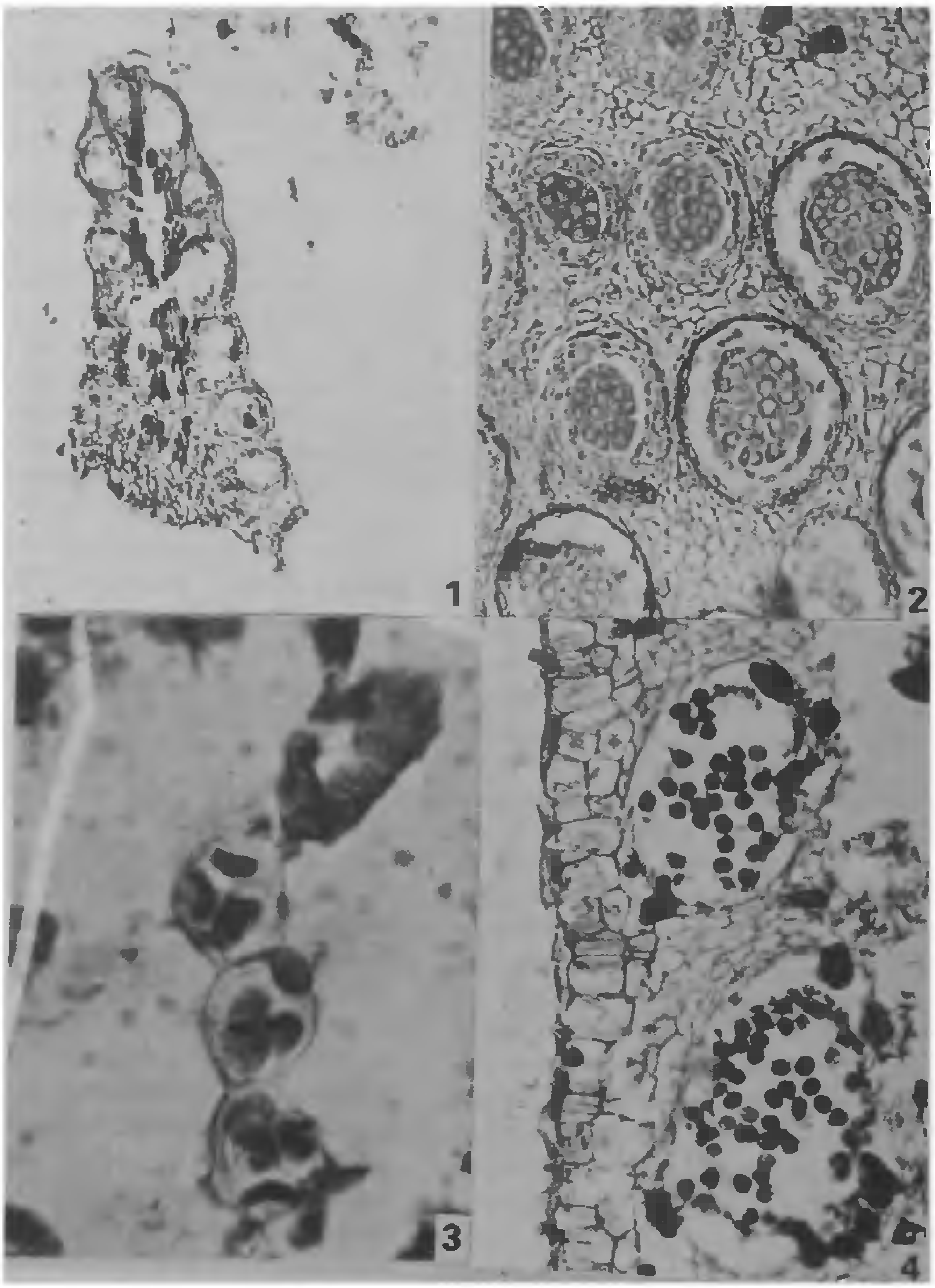
MANGROVES form a unique type of coastal vegetation, reaching their climax under tropical condi-



Figures 1–4. *Rhizophora lamarckii* Montr. **1.** TS of anther showing distribution of microsporangia; **2.** Tangential view of the anther with locules at different stages of development; **3.** Tetrahedral tetrads, one a degenerating triad and another with a microspore being liberated; and **4.** Mature anther with fibrous endothecium.