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COMPETITIVE SAPROPHYTIC SURVIVAL OF AZOTOBACTER CHROOCOCCUM IN BLACK COTTON SOIL

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THOUGH the occurrence of dinitrogen fixing bacteria on leaves was reported long back by Ruinen^{1,2}, the survival ability of these organisms in the soil under controlled conditions have not been reported. Hence, an attempt was made to know the saprophytic survival of a phyllosphere (sorghum) strain, *Azotobacter chroococcum* in comparison with a soil isolate *A. chroococcum* IARI M-1.

Ten grams of black soil having following properties, clay content 52%, field capacity 32%, wilting point 15%, pH 8.4, total nitrogen 0.04% and organic carbon 0.582% passed through 70 mm sieve were taken in petri dish in triplicates and sterilized at 160°C for 3 h in hot air oven. The phyllosphere isolate *A. chroococcum* (isolated from sorghum phyllosphere and identified based on morphological characters and its ability to utilize organic compounds as a sole source of carbon and energy³) and a soil isolate *A. chroococcum* IARIM-1 (obtained from the Indian Agricultural Research Institute, New Delhi) were grown in Norris nitrogen-free glucose broth⁴ for 48 h at 30°C. Each petri plate was inoculated with 1 ml each of leaf and soil isolates. In another set 1 ml each of both the cultures were added to the same petri plate to know the competitive saprophytic survival of leaf and soil isolates in mixed culture. Later these plates were moistened with sterile distilled water to field capacity (32%) and loss of moisture during incubation was compensated by adding sterile distilled water.

In mixed culture leaf and soil isolates were differentiated by morphology of the colony because of the variation in the strains. Phyllosphere isolate formed rough, serrated non-pigmented colony (how-

Table 1 Competitive saprophytic survival of *A. chroococcum* of leaf and soil isolates in sterile black cotton soil

Isolate	No $\times 10^5$ g soil				
	0 h initial count	15 days	30 days	45 days	60 days
Control					
Soil isolate	710	283	264.00	27.43	22.33
Leaf isolate	707	270	22.46	2.61	2.16
Mixed					
Soil isolate	730	252	20.77	1.72	1.39
Leaf isolate	707	217	19.23	1.25	1.01
		S.E.m \pm		C.D. at 1%	
For the comparison count		7.1		27.2	

ever pigmentation was observed upon prolonged period of incubation) whereas soil isolate is smooth and pigmented (dark brown). The initial count was taken by following serial dilution technique using Norris nitrogen-free glucose media. The other plates were incubated at 30°C and counts were recorded after 15, 30, 45 and 60 days of incubation.

As can be seen from table 1, soil isolate (IARIM-1) survived alone and in combination with leaf isolate up to 30 days but later there was a drastic reduction in counts in the sterile soil which is not comparable to cultivated soil conditions. It may be concluded that competitive survival of IARIM-1 is not very much altered up to 30 days as compared to the leaf isolate which loses viability about 12 times than the soil isolate in the same duration of incubation. The poor survival of leaf isolate in soil both in controls and mixed cultures may be due to the poor adaptability in soil. The soil isolate seem to be inhibited in mixed culture after 45 days probably due to nutrient competition or metabolic inhibition.

The requirements for the survival of free living nitrogen fixing bacteria in the natural environment depends on: (i) the availability of carbon compounds, (ii) adequate inorganic nutrients (calcium, magnesium, molybdenum), (iii) optimal pH for growth, and (iv) low oxygen tension⁵. Earlier reports indicate that *Azotobacter* remained consistently viable for prolonged periods of dormancy⁶.

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MICROPROPAGATION OF BANANA THROUGH SHOOT TIP CULTURE

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THE technique of plant tissue culture is being employed for the improvement and clonal propagation of economically important plants. *In vitro* techniques applied to *Musa* species are aimed at producing pathogen-free plants by thermotherapy¹ and with multiplication^{2,3}. Cronauer and Krikorian⁴ reported multiplication from excised stem tips and floral apex. In India commercial varieties of banana are seriously affected by bunchy top disease and there is shortage of planting material and pathogen free plants. To overcome these problems, a novel method was tried, viz. *in vitro* culture of shoot tips.

The shoot tips of banana cultivar 'rasabale', with corm tissue, measuring about 1 cm in length, were isolated from field-grown plants. They were surface-sterilized with sodium hypochlorite (13% commercial bleach) for 15 min and then rinsed thrice in sterile distilled water. The shoot tips were reared aseptically on nutrient media after removing 2 or 3 sheathing leaf bases. The nutrient medium used was that of Murashige and Skoog⁵ with 4% sucrose (MS). Supplements like benzyladenine (BA), naphthalene acetic acid (NAA) and coconut water (CW) were added to MS and the pH of the medium was adjusted to 5.8. Initially the shoot tips were reared in liquid MS on a filter paper support and after 3 weeks of culture, they were transplanted to MS agar (0.6%)

media. All the cultures were maintained at 25 ± 2 C and 55–60% relative humidity with 10 h illumination (150–200 lux) daily.

The pale white shoot tips reared on liquid MS containing BA (1, 2 and 5 mg/l) grew further and became green after 3 weeks of culture (figure 1). On transfer to MS agar medium containing BA, multiple shoots were formed. The maximum number of shoots were noted in the case of 5 mg/l of BA. But the resultant shoots did not increase in length. However when the medium was fortified with CW (10% v/v) after 4 weeks 8–14 shoots developed and the growth of these shoots was luxuriant (figure 2). *In vitro* differentiated shoots were isolated individually and transferred to MS + BA (5 mg/l) + CW (10% v/v) where a 10–20-fold increase in the proliferation of shoots occurred (figure 3). Profuse rooting (30–40 roots per shoot) was observed within 2 weeks when they were transferred to MS + NAA (0.5 mg/l) and resulted in plantlets (figures 4 and 5).

Such regenerated plantlets, about 10–14 cm in length, were transferred directly to pots containing soil and were maintained in a glass house for about a week (figure 6). Later they were transferred to field and the survival after transplantation has been 100%. Further performance of these plants in the field is under evaluation. In banana, regeneration of plantlets via multiple shoot production is promising for rapid multiplication of elite plants. Further attempts are being made to regenerate plantlets from shoot tips of different indigenous cultivars.

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(For figures 1–6 and captions, see next page)