inefficient strain M8. The endogenous values for C_2H_2 reduction of calli were ten times less than the lowest activity observed with any bacterial association, indicating that the values are not influenced by the endogenous C_2H_2 reduction or C_2H_4 production activity of the calli. These studies suggest that it is not only the infectivity for which a strain of *Rhizobium* differs but the nitrogen fixing ability is also associated with the host it recognises for nodulation. A strain manipulated for cross infectivity therefore, will not be efficient in all the hosts infected.

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REGENERATION OF RHIZOME SEGMENTS OF *REGNELLIDIUM DIPHYLLUM* LINDM. IN ASEPTIC CULTURES

DURING the recent comparative *in vitro* studies on a few Phyletically unrelated ferns (both homo- and heterosporous), *Regnellidium diphyllum* has proved to be an excellent experimental material for morphogenetic studies[1].

Regnellidium diphyllum, the monotypic Brazilian genus belonging to the family Marsiliaceae is an aquatic heterosporous fern with globular sporocarps containing numerous gradate sori. Rhizome segments used as inocula were obtained from sexually reproduced sporophytes raised in sterile cultures on Knop's medium supplemented with 3% sucrose. Equal-sized rhizome segments, with and without nodal parts were cut and inoculated separately in Knop's medium supplemented with 3% sucrose. All the cultures were maintained under 12 h photoperiod at 25° 2° C.

Some variations regarding the activation and growth pattern of rhizome segments with and without nodal part were observed. Segments with nodal part were the first to sprout, while the rhizome segments without nodal part remained quiescent for about two months. Further growth of these segments was interesting. Segments with nodal part, after a few days of inoculation, turned brown. A week later, swelling up of rhizome segments was observed and the first sign of growth was evidenced by the production of multicellular, uniseriate haris. Subsequently, a normal sporophyte was formed from each of these segments.

Segments of the internodal parts behaved quite differently. They turned brown and remained in the same state for two months. Their prolonged cultures resulted in the production of small-sized cylindrical structures herein designated as 'protophylls' and chlorophylous, positively geotropic roots. These 'protophylls' were comprised of compactly arranged sporophytic cells with discoid chloroplasts without any vascular supply and stomata. A large number of multi-cellular, uniseriate hair was seen on the surface of these 'protophylls'.

In the present investigations, it is seen that the regeneration response of the rhizome segments, with and without nodal part intact is different on the same nutrient medium. Rhizome segments with nodal part regenerated whole plants; on the other hand, those without nodal part produced 'protophylls' only. This differential response may be explainable on the basis of the presence-absence of the quiescent bud. Segments with nodal part intact, on coming in contact with the nutrient medium, resumed mitotic activity. As a result, the endogenous auxin level is raised, which in turn, helps in differentiating the whole plant. The rhizome segments without nodal parts, regenerating into whole plant is limited by the low endogenous auxin level and consequently the 'protophylls' are formed.

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BAITED RAGI CULM CULTURE, AN EASY TECHNIQUE FOR MAINTENANCE OF VIRULENCE IN *HELMINTHOSPORIUM NODULOSUM* BERK. & CURT.

REPEATED subculturing and/or their survival in soil saprophytically, resulted in the loss of virulence and subsequent sporulation in *Helminthosporium nodulosum* Berk. and Curt. which causes pre- and post-emergence seedling blight[1] in ragi (*Eleusine coracana* Gaertn.).

Since the virulence of the pathogen was correlated with its sporulating capacity, a technique to maintain the culture in a sporulating condition has been developed. It was also found that this was an easy method for harvesting spores in the preparation of spore suspensions, without mycelial bits.

Fresh, healthy 10 cm long culms of highly susceptible variety, Hamsa, to *Helminthosporium nodulosum* were collected from 60 day-old plants, washed first with tap water and then treated with 0·01% aqueous mercuric chloride for one minute. These culms were then dipped in 250 ppm streptomycin solution for 5 min and buried in sterilized soils infested with *Helminthosporium nodulosum*. The moisture level of the soil was maintained at 50% by adding sterile distilled water[2] at 28° C ± 2° C. The culms were removed after 6 days, washed thoroughly first with tap water and then with sterile distilled water. The culms were then observed under stereo-microscope for the lesions on the culms; such culms were then incubated on moist blotter for 6 days. The fungus sporulated heavily on the culms and at this stage spores can be harvested with the help of a fine brush or by gentle tapping of the culms on a butter paper for inoculation or the culms can be dried and wrapped with butter paper and preserved in a refrigerator as stock culture. This can be maintained for 2 to 3 years without much loss in viability, and on subculturing on potato dextrose agar (PDA) at any time during this period gave sporulation equivalent to the fresh isolates from infected material.

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DOES SOIL INHIBIT BIOLOGICAL NITROGEN FIXATION?

THE sensitivity and convenience of the acetylene reduction technique for assaying nitrogen fixation has stimulated interest in the study of nitrogen fixing microorganisms and in recent years many a new diazotrophs have been described[1,2]. Excepting perhaps the root-nodule bacteria within the nodules, other diazotrophs live in close contact with soil particles and at the interphase of soil particle and microorganisms many biochemical reactions take place[3]. It has been well realized that the activity of soil microorganisms are tremendously modified by the sorptive interactions between the microbes and soil particles[4]. Recently, Takahashi[5] from Japan observed that nitrogen fixation in *Azotobacter vinelandii* was inhibited considerably by the presence of soil. There appears to be little work so far done on this area and hence the present investigation was undertaken.

Pure cultures of *Azotobacter chroococcum*, *Beijerinckia indica* and *Derxia gummosa* were grown in 100 ml of N-free Waksman broth No. 77 (devoid of calcium carbonate) Becking's broth and Campelo and Doberreiner's broth respectively[6] over a gyrotary shaker at 28° C. The cells were harvested by centrifugation at log phase and dispensed in sterile, 0·1 M phosphate buffer (pH 7·0). Air dried, pound and sieved clay loam soil obtained from rice paddies and coarse sand collected from river bed were used. Two ml of the cell suspension (Ca 87 × 10^7, 2·5 × 10^7 and 25 × 10^7 cells/ml respectively of *A. chroococcum*, *B. indica* and *D. gummosa*) was taken in 20 ml vials fitted with needle puncture rubber stoppers. Weighed quantities of soils were added to the vials and mixed thoroughly. Air in the vessels was replaced by nitrogen. Maintaining an internal O_2 pressure at 0·02 atm, 10% (V/V) of pure acetylene was injected into the vial. After 6 h of incubation at 28° C, 1 ml of gas sample was withdrawn from the vial and fed to a Perkin Elmer (Model F. 15) Gas Chromatograph fitted with Porapak (80–100 mesh) column and flame ionization detector. Nitrogenase activity was computed by measuring the ethylene peaks[7]. For the other experiment designed to study the effect of different adsorbants like resins and activated charcoal on nitrogenase activity, one g of the adsorbant after suitable regeneration was mixed with 2·0 ml of the bacterial cell suspension. Assay conditions and methods were the same as detailed above. For finding out the minimum number of viable cells required for detecting N-ase activity, the cell suspension was diluted manifolds and the assay performed. Cell counts were made following plate count method[8]. Wherever needed the biomass was also determined.

The results presented in Table I revealed that the N-ase activity of the three organisms has been considerably inhibited by the addition of soil. The inhibition was strickingly more with clay loam than coarse sand. When 2 g of the clay loam was added there was practically no N-ase activity. The clay loam contained 35·6% of clay and 9·3% silt with a CEC of 38·5 me/100 g which caused the adsorption of of the cells whereas in coarse sand only a small fraction was fine sand and hence poor adsorption. That microbial cells in an adsorbed state exhibit