PATHOGENIC FUNGI FROM SWEET POTATO

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DURING a two-year survey of fruits and vegetables marketed in Mangalore City, South Kanara District, several fungal pathogens were isolated. Samples were collected from the markets during different seasons and those with discernible symptoms were examined directly. Those with incipient symptoms were subjected to incubation in a moist chamber at 28° C and 80% RH. In this paper, some of the hitherto unrecorded pathogens of sweet potato (*Ipomoea batatas* (L.) Lamk.) are listed and the symptoms caused by them described. Each fungus was tested for pathogenicity, using fresh, surface-washed roots and Koch's postulates were proved. The new records are not listed in Biligrami *et al*[1,2], and earlier host indices.

1. *Rhizoctonia solani* Kuhn

   Initial symptoms were pale brown spots on the root surface. Later, the mycelium covered the entire root surface which became soft and rotted.

2. *Penicillium digitatum* Sacc.
   Initially watery, discoloured spots were noticed on the root surface. Later, abundant fine grey mycelium with green-coloured mass of spores was noticed.

3. *Myrothecium roridum* Tode ex Fr.
   Brown patches were formed initially on the root surface with grey coating. Later, the surface of the root was covered by large number of dark sporodochia with slimy spore masses.

4. *Arthrinium phaeospermum* Corda and Ellis
   The disease was characterised by black, circular spots on the root surface. Later, shiny black spore masses were noticed on these spots.

5. *Stemphylium botryosum* Wallr.
   The fungus caused sunken, circular, dark brown spots covered by grey mycelium. In the advanced stages, the roots decayed. A mass of conidia came out with the ooze appearing grey and granular on applying gentle pressure on the spot.

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IMMOBILIZATION OF *BEIJERINCKIA*, A NITROGEN–FIXING BACTERIUM, ON SILICA GEL

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IMMOBILIZATION of enzymes for various applications as biocatalysts has been carried out during the past decade[1]. Recently, binding of whole microbial cells on inert insoluble matrix as biocatalysts has been the object of intensive investigations[2]. Immobilization of denitrifying[3], methanogenic[4] and luminous[5] bacteria was carried out in alginate gels. Species of *Actinoplanes*[2] and *Saccharomyces*[6] were successfully entrapped within cellulose di- and triacetate and on silica beads respectively. Biological nitrogen fixation carried out by certain free-living and symbiotic microorganisms contributes well over 50% of all fixed nitrogen[7]. The potential of such organisms as to their usefulness for the production of fixed nitrogen can be better investigated by using immobilized cells which allow high cell densities and improved stability. *Azotobacter vinelandii* is the only organism immobilized[8] for such purposes so far. An attempt, therefore, to immobilize *Beijerinckia* on silanized and glutaraldehyde activated silica gel has been made in the present investigation.

Silica gel (column chromatography grade, finer than 200 mesh, from Acme's Laboratory Chemicals, India) was prepared for immobilization as suggested by Weetall[9] and Gutcho[10] with some modifications. Five grams of silica gel were added to 30 ml of 1% solution of N-(beta-Aminoethyl) gamma-aminopropyltrimethoxysilane, A-1120 (Union Carbide Corporation, Chemicals and Plastics, New York) in toluene. The mixture was stirred and refluxed continuously for 2 hr. Toluene was decanted and the silica gel residue was repeatedly washed with distilled water followed by acetone. The product was heated for 2 hr

at 120° C. The silanized silica was then mixed with 30 ml of 2.5% glutaraldehyde (grade II from Sigma Chemicals, USA) in 30 mM Tris-HCl buffer (pH 8) and stirred for about 6 hr at room temperature. Glutaraldehyde was decanted and silica was washed 4–5 times with Tris-HCl buffer.

A *Beijerinckia* strain originally isolated in the laboratory from cotton plants (*Gossypium hirsutum* var. Varalaxmi) leaf surfaces, capable of reducing 30 to 35 nmol of C_2H_2 per mg protein/hr was used for immobilization. When grown in Burk's modified nitrogen-free (BMN) medium[11] with sucrose as the sole carbon source, the organism produced copious amounts of slime which interfered with the pelleting of cells and subsequent processing for immobilization. The cells therefore were transferred to nutrient broth where no slime was produced and immobilization was feasible. Cultures from BMN medium slants were directly inoculated to 200 ml of nutrient broth and incubated at 30° C on a rotary shaker for three days *i.e.* until the cells reached late logarithmic phase. Three-day old cultures were found suitable for immobilization in the present study. The cells were harvested, washed and resuspended in Tris-HCl buffer to give approximately 10^7 cells/ml. A 50-ml bacterial suspension was added to the modified silica gel and the mixture was stirred intermittently for 12 hr at 4° C. The silica gel with immobilized cells was washed with the same buffer until all free cells were removed. The bacteria adsorbed were determined by the difference between the initial concentration and that remained in solution after adsorption. Viability of the cells was qualitatively checked by spreading the silica gel on nutrient agar plates. Quantification of viable cells here becomes difficult and erroneous as the cells are tightly bound to the particles.

For scanning electron microscopy, the immobilized cells were dehydrated using ethanol-water mixtures of gradually increasing ethanol concentration (30, 50, 70 and 100%) followed by distilled acetone. The dehydrated samples were coated with carbon (about 100 Å) followed by gold-palladium (about 200 Å) and examined in a Cambridge Stereoscan 150 scanning electron microscope operating at an accelerating voltage of 15 kV.

Whole cell immobilization methods previously investigated include entrapment in various resins[12] and alginate gels[5]. However, these organic carriers may be susceptible to microbial attack while inorganic carriers such as silica gel are not. Attempts to immobilize *Beijerinckia* on other carriers such as nylon flakes, glass beads and quartz sand were unsuccessful. The method employed in the present investigation resulted in the immobilization of about 90% of the

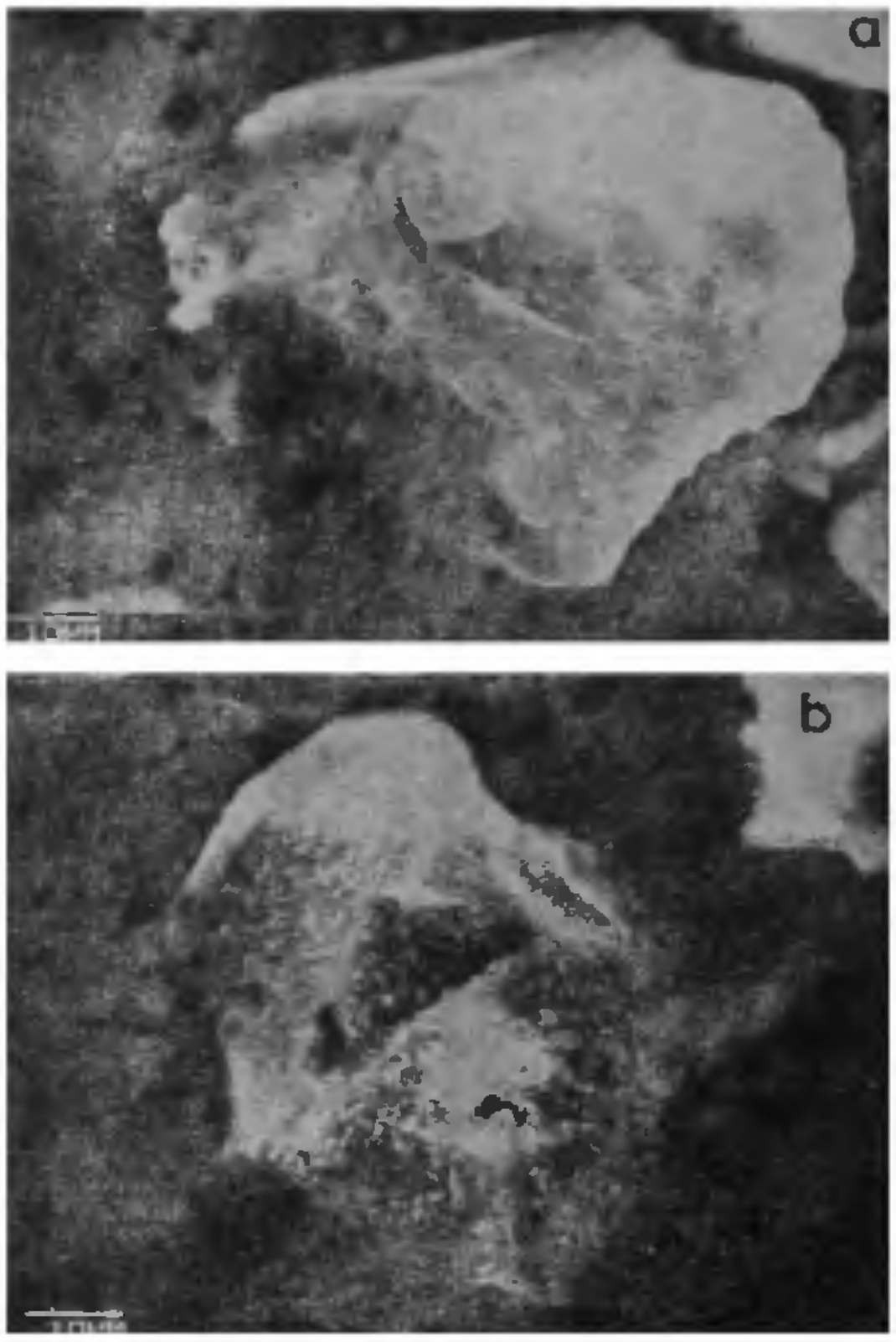

Figure 1. Scanning electron micrographs of **(a)** silanized, glutaraldehyde activated silica gel particle, and **(b)** the same with immobilized *Beijerinckia* cells.

cells that were initially used and these cells were viable after fixation. A scanning electron micrograph with immobilized *Beijerinckia* cells is shown in figure 1. Immobilization of *A. vinelandii* on resins[8] involved ionic interaction while *Beijerinckia* in the present study was covalently attached to silica gel. This is expected to permit operation over a wider range of pH and salinity conditions. The kinetic and other studies relating to nitrogenase (the enzyme involved in nitrogen fixation) may be carried out by transferring these immobilized cells to nitrogen-free medium. Further studies are underway with immobilized cells to elucidate those conditions required to maintain nitrogen fixation activity.

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NEOCENTRIC ACTIVITY IN *PENNISETUM ORIENTALE* RICH

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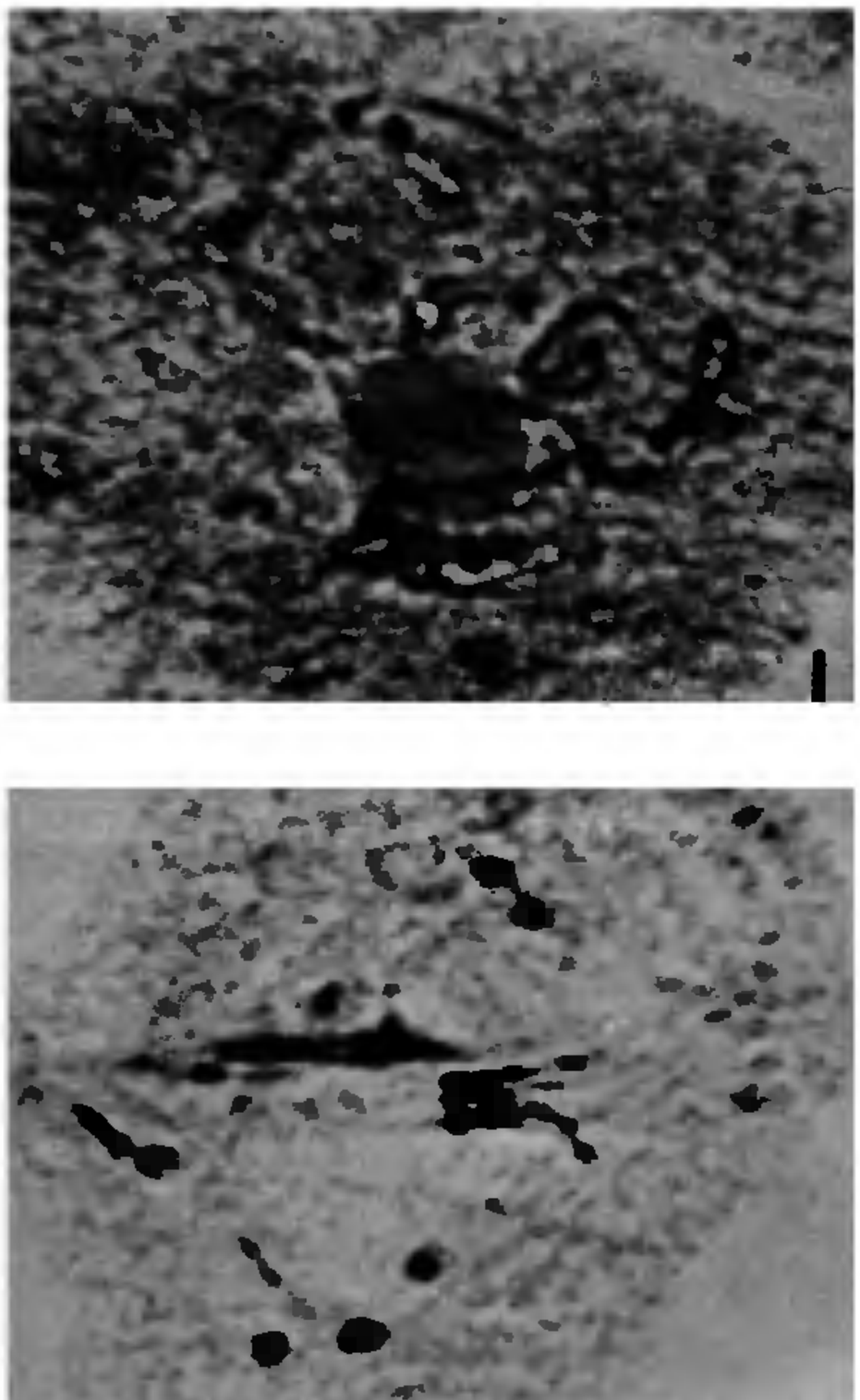
THE present paper concerns with the report of neocentric activity, for the first time in a new triploid race of *Pennisetum orientale* Rich $2n = 27$ which is a valuable, evergreen, highly nutritious and drought-resistant forage grass.

During mitosis and meiosis the spindle fibers attach themselves to the kinetochore component of the centromere, thereby indicating that no other site on the chromosome has this property. An exception to this general rule has been noticed earlier in meiotic cells of maize[1,2] and rye[3]. In these, abnormal chromosomes with heterochromatic knobs exhibit at or near these segments localized kinetic activity similar to that of normal centromere. *P. orientale* also can now be added to this list.

In *P. orientale*, some pachytene chromosomes had heterochromatic knobs at both the ends and in others at only one end (figure 1). At metaphase I the chromosomes manifested enormous stretching and spindle fibers attached themselves at regions other than the centromere (figures 2 and 3). The strength of the neo-



Figures 1–3. 1. Meiotic stages in triploid *P. orientale*. Pachytene stage showing heterochromatic knobs × 1650. **2.** and **3.** Metaphase I showing stretching of chromatin and neocentric activity × 1500.