Isolated from air, Mannanur forest locality, January 1979, OUFA I (IMI 239 544) holotype.

D. sivanesanii Manoharachary and Thulasi Reddy, sp. nov.

Coloniae effuse, obscure fuscae; crescentia in medio agaraceo PSA acidifacto luxurians. Mycelium plerumque aerium et pro parte in medium immerism. Conidiophori macronematici, mononematici, rectivel flexuosi, singulariter emergentes, septati, mediocriter usque obscure fusci, in apice pallidiores, geniculati, cicatricibus paucis instructi, usque 250 μm longi, 3,5–7,5 μ m lati. Conidia aeropleurogenea, solitaria, recta vel incurvata, obclavata, obconico-truncata, pallide fusca usque aureo-fusca, pseudoseptis transversalibus 2–5 praedita, cum centro latiori, in apicem angustum extenuata, 38–76,0 μm longa, ad basin 2–3,8 μ m lata, in centro 11, 4–19,0 μm lata, ad 2,0 μm attenuata rarius conidiophoros secundarios, conidia per proliferationem gerentes, formantia.

Isolata ex area. Locus typi silva Mannanur dicta, Inauario 1979, OUFA 1; holotypus IMI 239 544.

The authors are thankful to Prof. Dr Hanns Kreisel, Department of Biological Sciences, Ernst Moritz Arndt University, Greifswald, GDR for latin translation of the new taxa.

8 October 1982; Revised 30 April 1983.

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TISSUE CULTURE TECHNIQUE TO DEMONSTRATE THE VIABILITY OF DOWNY MILDEW MYCELIUM IN PEARL MILLET SEEDS

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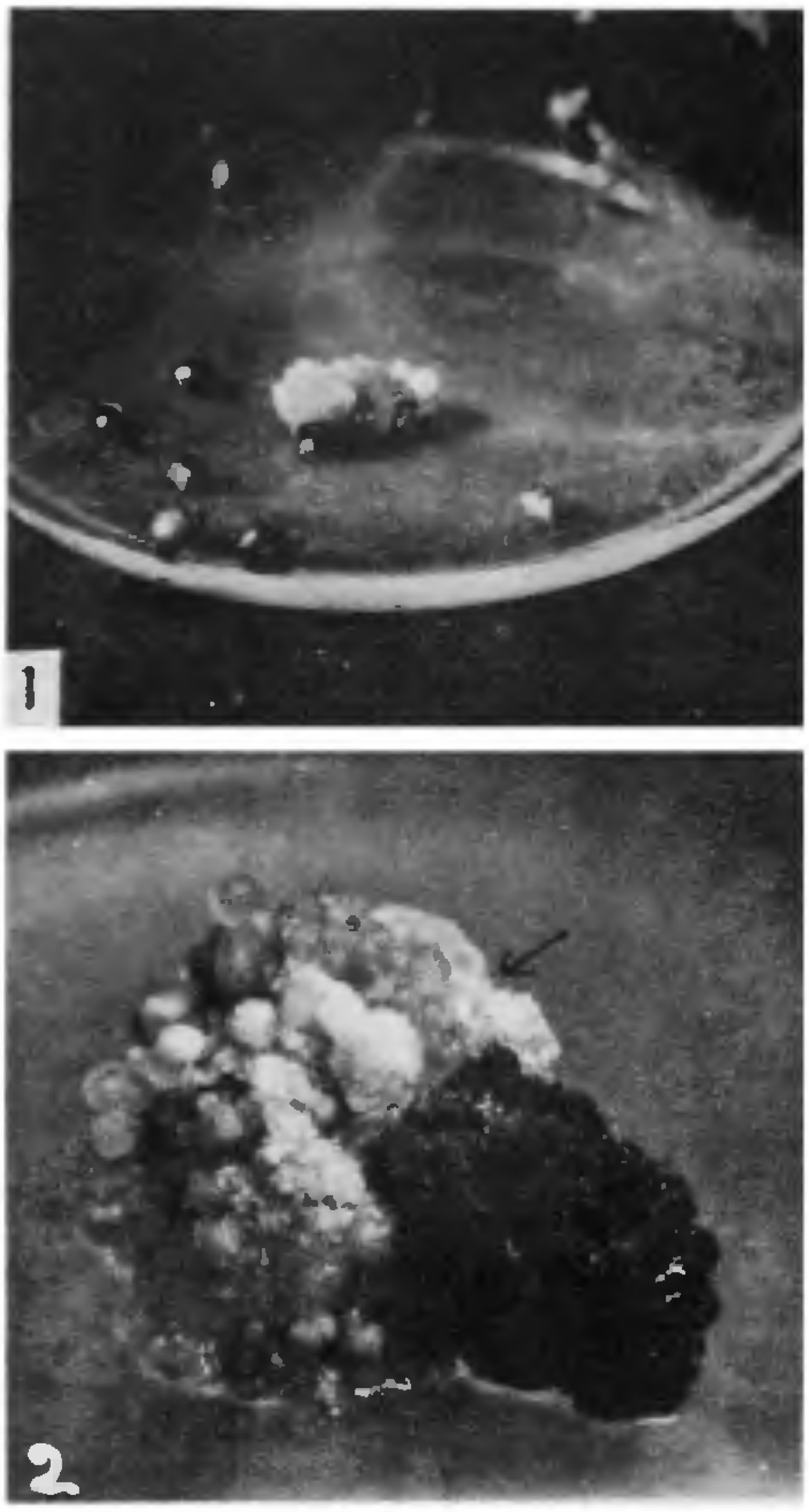
SCLEROSPORA graminicola (Sacc.) Schroet. causes the downy mildew disease of pearl millet and the inoculum occurs in the seeds as oospores sticking to the seed surface and the mycelium in all the seed parts[1-5]. The viability of the oospores has been determined by TTC test[3], but no definite method is available to test the viability of internally seed-borne mycelium. Majority of the workers depended on the seedling symptom test to detect the viability of internally seed-borne downy mildew mycelium[4]. The tissue culture technique to determine the viability of the internally seed-borne mycelium of *S. graminicola* in pearl millet seeds has been worked out in the present investigation. Our investigations show that the mycelium invading the embryo remains viable in dry seeds whereas the viability of the mycelium in the pericarp region decreases as the moisture content of the seed is reduced.

Seed samples were collected from partially malformed earheads of pearl millet (IP 7042 variety) with 10% moisture content grown in the downy mildew nursery at Mysore. From each sample, 1000 seeds were washed thoroughly with distilled water and surface-sterilized using 0.1% mercuric chloride solution for 3 min followed by 6 washings in sterile-distilled water (20–30 min). After drying on sterile blotters, the seeds were transferred to 100 ml Earlenmeyers flask, 10 into each, containing 30 ml of Murashige and Skoog medium[10] with 5 ppm of 2,4-D and 3 ppm of NAA along with 20 mg/l of ascorbic acid under aseptic conditions. The flasks were incubated at $20 \pm 1^\circ$ C under 12 hr light and 12 hr dark conditions. Also seeds at milky stage (40% moisture) from partially malformed earhead were surface-sterilized in a similar way and 1000 seeds were transferred to the same medium, 10 seeds to each flask.

To determine the percentage of seed infection, 1000 seeds from the same seed sample have been macerated using the modified embryo extraction technique[3]. The percentage of seeds having mycelium in the pericarp and embryo has been accurately noted.

The callus initiation took place 3 days after placing on to the medium in dry mature seeds and the callus originated from the hypocotyl region of the seedling (figure 1). In a few cases, the callus initiated directly from the seed tissue without the seedling emergence. After 30–45 days of incubation, thin, white mycelial growth of *S. graminicola* was observed on the callus tissue of the 0.1% seeds (figure 2). The seeds at the milky stage took more time for callus initiation (8–10 days). However, in this case, about 1% of the seeds showed mycelial growth of *S. graminicola* on the callus tissue. In some instances, the primary callus turned brown at the initial stages and after subculturing, on the same medium, some of them developed healthy callus and a clear network of mycelial growth was observed on this secondary callus (figure 2). Growth of *S. graminicola* mycelium was confirmed by squashing the infected callus tissue and observing asexual and sexual structures.

The results of maceration technique showed that about 20% of dry seeds contained mycelium in the pericarp whereas 0.1% of seeds contained mycelium in the embryonic tissue.



Figures 1 & 2 1. Callus initiation from pearl millet seeds collected from half malformed earheads. **2.** *S. graminicola* mycelium on secondary callus originating from primary callus of pearl millet. (Arrow indicates mycelial presence.)

At present, the only standardised technique available to detect the seed-borne mycelium of downy mildew of pearl millet is the embryo extraction method[3]. No doubt, this technique is reliable for detection, but is not practicable when the seed sample size is very small. When 10–100 breeder's seeds or foundation seeds are brought to a research station, a breeder may like to test the health of such seeds and may not afford to lose it. In such cases the tissue culture technique can suitably be employed and from the callus tissue plantlets can be raised again to save the valuable germplasm.

To demonstrate the viability of the seed-borne downy mildew mycelium, the generally used method is seedling symptom test[4]. But, it requires an elaborate system of environment-controlled chambers to carry out seed-transmission studies. But, in the present technique, the same thing can be achieved in any ordinary laboratory and is also economically feasible. This tissue culture technique can be well adopted in the quarantine laboratories to detect the obligate parasites. When strict biotrophic fungus like *S. graminicola* has expressed itself on the tissue culture, this technique can be utilised for other pathogens of the same calibre.

Pure axenic growth of the downy mildew of pearl millet has not been established yet and the use of tissue culture is in progress to grow these pathogens *in vitro*[6,9]. In view of the lack of a suitable technique for raising the dual cultures, the infected seeds form important tools to study the host-parasite systems *in vitro*.

The percentage of seeds with viable mycelium has been found to be 0.1% in dry seeds which is directly correlated with the percentage of embryonal infection. In seedling symptom test, the transmission rate of the same pathogen in the seed was 0.1% and this was also correlated to the percentage of embryo infection and the mycelium colonising in the pericarp was declared to be inactive[8]. In dry, mature seeds generally, the callus originated from the hypocotyl region of the seedling and the mycelium expressed on the callus should have originated from the embryonic tissue. However, it seems seed moisture plays an important role in the viability of the seed-borne inoculum of downy mildews[11]. Infected seeds put on the medium at milky stage, expressed high percentage of mycelial viability than the dry infected seeds and this confirms the role of moisture in the viability of mycelium in the pericarp.

In certain cases, the failure of pathogen to express on the primary callus was due to the secretion of some phenolic substances which inhibit the growth of the fungus[7]. The expression of mycelium on the secondary callus obtained by subculturing the primary callus clearly demonstrates the ability of the mycelium to remain dormant during unfavourable conditions and to regain its activity during favourable conditions.

The first author acknowledges financial assistance from the Council of Scientific and Industrial Research, New Delhi.

18 October 1982

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SEEDLING HANDEDNESS IN *CAJANUS CAJAN* (L.) MILL SP IN RELATION TO SEED POSITION

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THIS note describes the results of the possible effect of the position of seed in the pod on seedling handedness in *Cajanus cajan* (L.) Mill sp., whose seedling handedness was first described by Rao and Bahadur[1]. Recently, Bahadur *et al*[2] studied the various aspects of seedling handedness in relation to yield.

Twenty five healthy pods selected at random representing 5 cultivars of *C. cajan* obtained through the courtesy of ICRISAT were used without considering the seedling handedness of the parent. The seeds from each pod were numbered starting from the fruit stalk. They were sown in small plots (2×2 m) and suitably numbered for the pod and the seed within it. Handedness of the seedling was scored when the seedlings emerged following the technique used earlier[1], and the data analysed and interpreted.

Table 1 summarizes data on seedling handedness in relation to seed position in 25 pods of 5 varieties of *C. cajan*. A perusal of the data shows that the seed in the first position of all the cultivars but one, produced excess of left-handed seedlings and the same trend is shown even by the seed at the second position. It is only at the third position that the seed showed a uniform excess of right-handed seedlings. In the fourth position, however, the seed arrangement with regard to seedling handedness appears to be haphazard and bizzare with excess of left-handed seedlings in some pods and right-handed seedlings in other irrespective of the cultivars studied.

A perusal of the compounded data given in table 2 shows that in almost all the varieties, the position of the first and second seed in the pod gave high percentage of left-handed seedlings, although on the total, the first seed showed a slight excess of left-handers. The position of the third seed uniformly showed an excess of right-handed seedlings not only within the 5 cvs examined but even on the total. As pointed out earlier, the seed at the fourth position showed a tendency to wobble, and the excess of left-handed is seen in some cvs and RH in others, but on the whole an excess of right-handed seedlings was observed. A study of the combined data strangely showed equality of left and right-handed seedlings. Thus seedling isomerism is correlated with mathematical isomerism and it is because of this rule that the seedling character perhaps cannot be fixed[3].

What controls the seed position that which in turn controls seedling handedness is a mystery, but the reasons appear to be morphogenetical and are laid down at the time of ovule initiation. Whether this has anything to do with enantiomer selectivity of certain metabolites during ovule morphogenesis is a matter of conjecture but such a possibility is not ruled out.

More experiments in this direction are needed to establish the genetic purity of seedling handedness, since earlier attmempts despite repeated selection of the desired seedling type have failed[1].

It may be of interest to point out that left and right ovule position in flowers of *Medicago sativa* influences the seed set and out-crossing rates. Whether the same holds true of *Cajanus* is presently not known[5].

18 January 1983

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