

## PLANTLET FORMATION FROM CALLUS CULTURES OF COWPEA (*VIGNA SINENSIS* L.)

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VARIOUS cells, tissues and organs from numerous plant species have been cultured successfully to regenerate whole plants<sup>1-5</sup>. The legume family includes many important crop species but regeneration *in vitro* has, until recently, proved to be difficult especially in grain legumes<sup>6,7</sup>. *Vigna sinensis* (Cowpea) is an economically important plant used as vegetable crop in Northern India. Only a few reports are available in the direction of tissue culture studies in this genus<sup>8,9</sup>. The present report deals with the regeneration of *V. sinensis* L. *in vitro* and an assessment of genetic variability characteristic of *in vitro* growth<sup>10-14</sup>.

Seeds of *V. sinensis* obtained from the local market were germinated and different explants such as root, hypocotyl and leaf blade (cotyledonary leaf) of 7-day-old seedlings were washed with distilled water and finally surface-sterilized with 0.1% (w/v) mercuric chloride solution for 5 min followed by a thorough washing with double-distilled water. The leaf explant was prepared in the form of segment excised from lamina of cotyledonary leaf and included central leaf portion along with midrib. The proximal and distal ends of the leaf were not considered for culture. Explants were aseptically transferred onto the solidified Murashige and Skoog's (MS) medium<sup>15</sup>, supplemented with different concentrations and combinations of kinetin and auxins such as IAA, IBA and 2,4-D. Cultures were incubated at 25±2°C in dark for callusing and at 16 h photoperiod/8 h dark for shoot morphogenesis. Calli obtained from different explants were periodically subcultured every 4 weeks. For each treatment 16 replicates were kept and each treatment was repeated at least twice. For cytological investigations regenerated roots (3-4-days-old) were pretreated with 1-4 paradichlorobenzene (3-4 h) followed by overnight fixation in acetic acid:ethanol (1:2), hydrolysed in 1 N HCl at 58-60°C for 15-18 min, and finally stained in 2% aceto orcein:N HCl (9:1). Squashes were prepared in 45% acetic acid. A minimum of 1000 cells were screened for chromosomal studies.

No callusing and rooting responses were observed

with root explant inoculated on MS medium with different concentrations (1, 10 and 100 µM) of auxins (IAA, IBA and 2,4-D) and combinations of auxins and KN (1, 10 and 100 µM) indicating their unsuitability for *in vitro* studies.

Hypocotyl explant too was not found to be very good for tissue culture studies in this species with IAA (1, 10 and 100 µM). Neither callusing nor rooting was observed. With 2,4-D and IBA also, average callusing with slight rooting were observed within 7 days of incubation at lower concentrations (1 µM).

Leaf explant on the other hand was more suitable producing average (45%) to high callusing with different concentrations of 2,4-D (1, 10 and 100 µM), the best (90%) being with the highest concentration (100 µM) within 5-7 days of incubation. However, such high concentration (100 µM) was not found suitable for rooting, the general response being average (3-5 roots per explant within 25 days of incubation) with lower concentrations (1 and 10 µM). With IAA (1, 10 and 100 µM) callusing and root induction remained average 12 days after incubation, the former being 10-35% and the latter being 2-3 per explant respectively. Best rooting along with callusing was observed with all the 3 concentrations (1, 10 and 100 µM) of IBA (figure 1). Rooting either followed callusing or occurred directly from the explant within 6-7 days of incubation.

The combined treatment of IBA (1, 10 and 100 µM) and KN (1, 10 and 100 µM) showed fairly good response of callusing (53-66%) and the average rooting (22-30%) being 4-6 cm within 12-15 days of incubation. These observations indicate that low to moderate concentration of IBA (1 and 10 µM) with high concentration of KN (100 µM) leads to better callus initiation while moderate to high concentration of IBA (10 and 100 µM) with different concentrations of KN (1, 10 and 100 µM) leads to different degrees of rhizogenesis (figure 2), the effect being maximum with 100 µM IBA and 100 µM KN. The callus produced in all the single auxin treatments and combined kinetin and auxin (IBA, IAA and 2,4-D) treatments was soft and friable in texture and white, brownish, or green in colour.

Shoot regeneration was initiated in the form of green shoot buds directly from the explant with 1 µM IBA + 10 µM KN. However, proper shoot development occurred with higher concentration of IBA (100 µM) and 10 µM KN after third subculture (figures 3-5), indicating thereby that shoot elongation



Figures 1–7. 1. Direct rooting from leaf explant with  $1\ \mu\text{M}$  and  $10\ \mu\text{M}$  IBA respectively (14–17 days after inoculation); 2. Multiple rooting and callusing from leaf explant with  $100\ \mu\text{M}$  IBA +  $1\ \mu\text{M}$  KN; 3–5. Shoot organogenesis with  $1\ \mu\text{M}$  IBA +  $10\ \mu\text{M}$  KN (after third subculture); 6. Diploid ( $2n = 22$ ), dividing cells of the 3–4-days-old roots from regenerated plant ( $\times 1250$ ), and 7. A cell in root showing aneuploid chromosome number ( $\times 1250$ ).

was favoured at higher IBA/KN ratio. Rooting of the *in vitro* regenerated shoots was obtained simultaneously with shoot elongation in the medium containing ( $100\ \mu\text{M}$  IBA +  $10\ \mu\text{M}$  KN) 7 days after subculture. Shoot regeneration was not observed in

the present study in combined treatments of IAA/KN and 2,4-D/KN.

The regenerated plants showed among the normal diploid chromosome numbers (MI-18.13) (figure 6), with an occurrence of about 1% of aneuploid ones (figure 7). Interestingly, the chromosome number of the roots of the plant regenerated from calli showing aneuploidy revealed a lower chromosome number than most frequently seen in culture. This situation is somewhat similar to the one observed in *Nicotiana*<sup>16</sup>. Polyploid chromosome numbers, however, have not been observed in the regenerated plants in the present study. The present study indicates that such genetically aberrant plantlets of *V. sinensis* showing variability in ploidy levels and/or aneuploid chromosome numbers may be assessed for the possible selective advantage of the chromosome complement. This aspect is under investigation.

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### LEUCOCOPRINUS FRAGILISSIMUS (BERK. & BR.) PAT., A NEW FIND IN ORISSA, INDIA

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MEMBERS of the genus *Leucocoprinus* are terrestrial in habitat. They are found mostly on forest debris and are sub-tropical in distribution. The genus contains thirteen well-defined species<sup>1,2</sup>. *L. cepaestipes* is a common species reported from Maharashtra, West Bengal, Gujarat and Madras<sup>3</sup>. In a survey of agaric flora of Orissa from 1980 to 1983 only *L. fragilissimus* was encountered. The species was earlier reported from Ceylon<sup>2</sup>.

Methodology: For taxonomic details and identification of the fungus, Pegler<sup>2</sup> was followed, while colour terminology was according to Ridgway<sup>4</sup>. The new record from India was ascertained using Manjula's<sup>3</sup> list of Basidiomycetes.

*Leucocoprinus fragilissimus* (Berk. & Br.) Pat., *Essai Taxon*: 171 (1900).

Pileus 3.5–5.5 cm broad, convex to planoconvex, fragile; surface yellow, glabrous, often covered with minute yellowish granules, with a circular brownish patch on the umbo; margin sulcate-striate, appendiculate. Lamellae free, sub-ventricose, moderately distant, collariate, thin, 2–2.5 mm broad, whitish

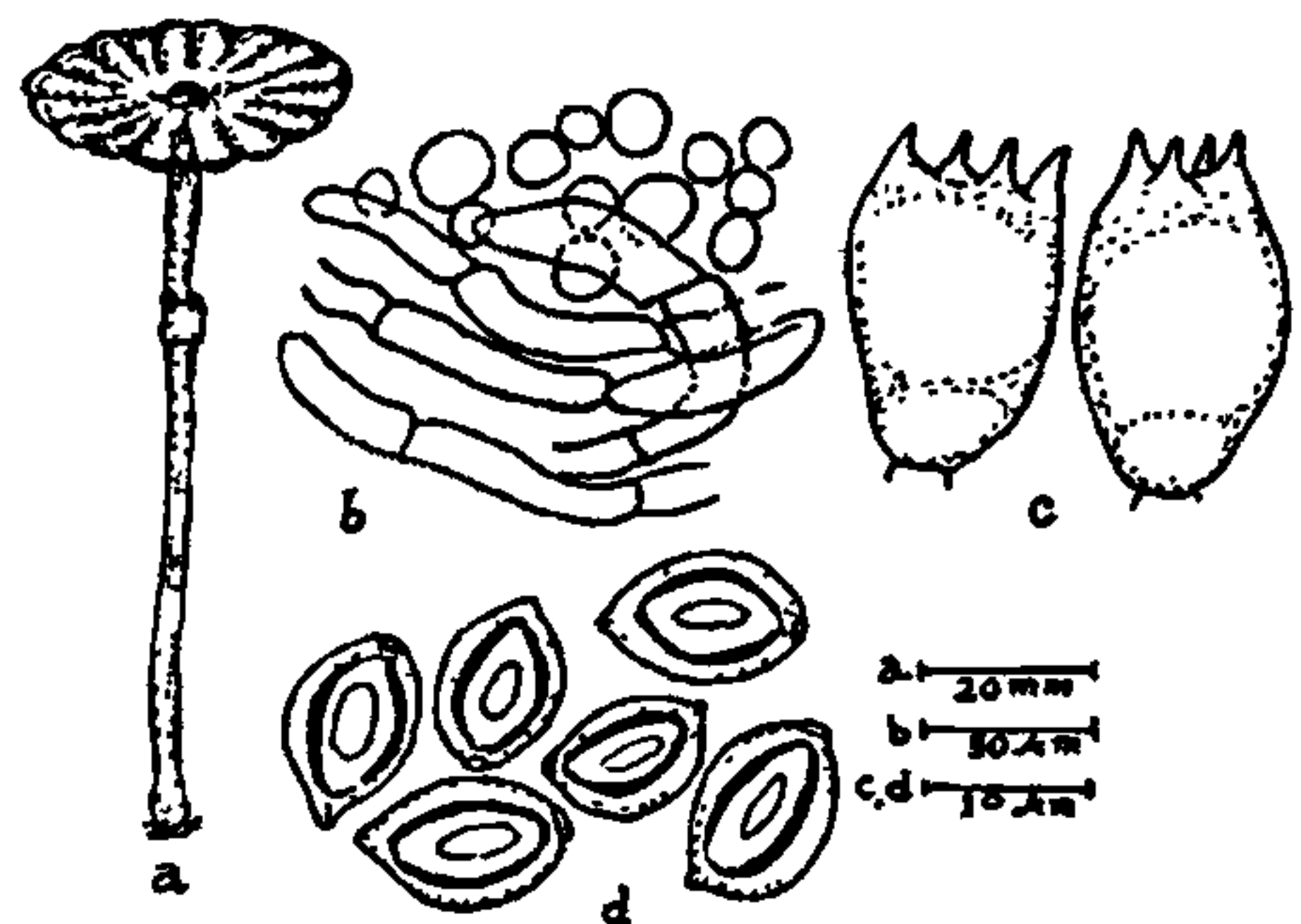


Figure 1a–d. *Leucocoprinus fragilissimus*. a. Habit; b. Pileal epicutis; c. Basidia, and d. Spore.

yellow; lamellulae absent. Stipe 80–110 × 2–2.5 mm, cylindrical, equal, sub-bulbous, pubescent, covered with powdery granules. Annulus superior, movable, falls with age, single, thin, yellowish, powdery. Context papery, white, subdeliquescent, unchanging, interwoven, thin-walled, hyaline hyphae of 8–21 μm diam. Spore print white. Spores 8–12.5 × 6.6–8.8 μm, ovoid to broadly ellipsoid (Q = 1.25 to 1.5), broad truncate germ pore, hyaline, smooth, complex wall, dextrinoid, with a large refractive oil guttule. Basidia 18–23 × 12–15.5 μm, subglobose to wedge-shaped, hyaline, four-sterigmate. Cystidia not traced out. Sub-hymenium layer poorly developed. Hymenophoral trama regular, hyaline hyphae 2.2–4.4 μm broad. Pileal epicutis thin-walled, hyaline, sphaerocyst, 13–21 μm diam., intermixed with hyaline hyphae 6.5–12.5 μm broad. Stipe tissue elongated, hyaline hyphae 10–20 μm broad. Clamp connections absent.

Basidiomata: Solitary, on leaf litter of *Shorea robusta* Gaertn.; Kiriburu Hill Top, District Keonjhar, Orissa; alt. 810 m; leg. M. K. Das; 9.7.1983; H.C.I.O. 36835.

The specimen is deposited at the Herbarium Cryptogamae Indiae Orientalis, Division of Mycology and Plant Pathology, IARI, New Delhi. This is the first report of the species from Orissa, India, and is a new Indian record.

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