

Tissue Culture Studies in Turmeric

M. S. Kuruvina Shetty, Padmaja Haridasan and
R. D. Iyer

CENTRAL PLANTATION CROPS RESEARCH INSTITUTE,
KASARAGOD 670 124, KERALA.

ABSTRACT

Single sprouting buds of turmeric clone 15B were cultured on a modified Murashige and Skoog's medium containing sucrose (40g/l), kinetin (0.2 to 0.5 mg/l), adjusted to pH 5.6 and gelled with 7 g/l of agar agar. On subsequent transfer to the medium of same composition, these individual plants developed a marked swelling at the base. During the second month of culture, 5 to 25 shoots were produced from a single explant.

At the point of swelling, a soft, translucent callus was produced. The callus could easily be separated from the developing plant. When subcultured and exposed to light, the callus grew further, simultaneously producing several buds that turned green and later developed into plantlets. The rate of multiplication was high and could be used for initial multiplication of the promising clones.

In vitro culture studies of *Curcuma longa*, selection No. 15B ($2n=63$) were undertaken with the following objectives.

1. Rapid clonal multiplication of the individual elite plants of breeders' stock to reduce the time lag between identification of the clone, its evaluation and final release to farmers, thus providing a large amount of initial planting material.

2. Induced mutagenesis using single cell cultures to isolate chimera-free mutant types for various characters including yield and curcumin content.

3. Screening the clones and mutants for resistance to rhizome rot and leaf spot diseases.

Materials and methods

One of the improved clones of turmeric, selection No. 15B obtained from the Project Co-ordinator, All India Co-ordinated Spices and Cashewnut Improvement Project, was employed for the present study. Rhizomes were moistened and kept in humid condition in dark, for sprouting. When the sprouts were about two cm. they were removed from the rhizomes, treated with a non-ionic detergent, Triton x-100 (SIGMA), and thoroughly washed in glass distilled water. They were surface sterilised in 25% chlorine water and subsequently washed in sterile distilled water. The enclosing sheaths (1-2) of the sprouts were removed and individual sprouts were inoculated in culture tubes containing 20 ml of Murashige and Skoog's

medium (1962) supplemented with kinetin (0.2 mg/l), benzyl aminopurine (0.4 mg/l) GA_3 (0.01 mg/l) and sucrose (40 g/l). The pH of the medium was adjusted to 5.6 and gelled with agar (7 g/l). The cultures were incubated at $26 \pm 1^\circ C$ in light from 40 W fluorescent tubes (Philips 4300° K) for 12 hr cycle of light and darkness.

For regeneration of plantlets, the callii were separated carefully from the explant and inoculated on the medium described above.

Results and discussion

Within a week of inoculation, the sprouts showed visible signs of growth, and 2-3 fleshy roots penetrated into the medium. The first leaf emerged completely in three weeks time. Meanwhile the base of the explant swelled up. Fresh shoots started appearing after six weeks in culture. The swollen condition of the stem was either due to the production of callus as a prelude to the production of buds or the sprouts themselves. The shoots arose in two ways:

a) *Through an intermediary callus* :

A translucent, soft, friable callus was produced from the base of the plant, not essentially from the cut surface. As the callus grew in size, the shoot buds emerged from the periphery of the callus. Further addition of callus tissue was observed from the base of the fresh shoots (Fig. 1).

b) *Direct regeneration of multiple shoots* :

Some of the shoot bud explants did not produce callus at all. Instead produced a bunch of adventitious shoots differentiated from the basal region of the explant, looking like a tuft of grass (Fig. 2 & 3). The process of regeneration appears to be similar to that of suckering in monocots.

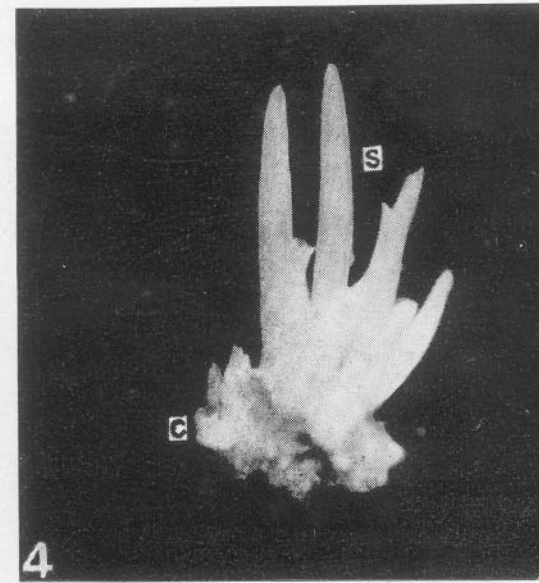
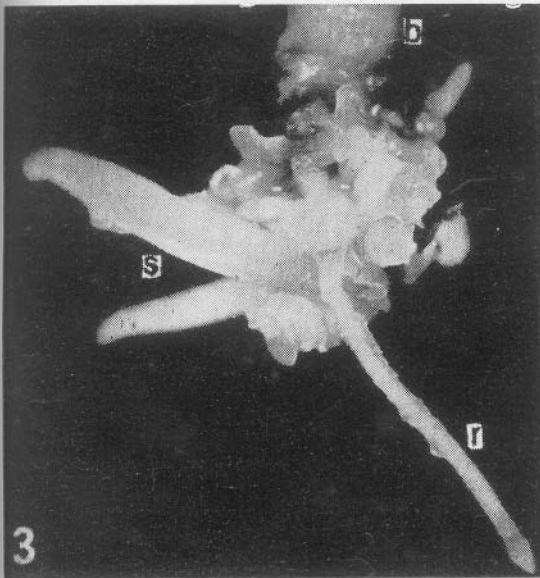
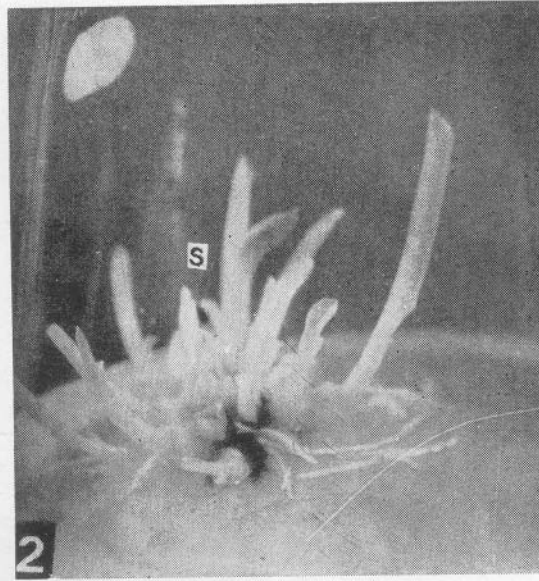
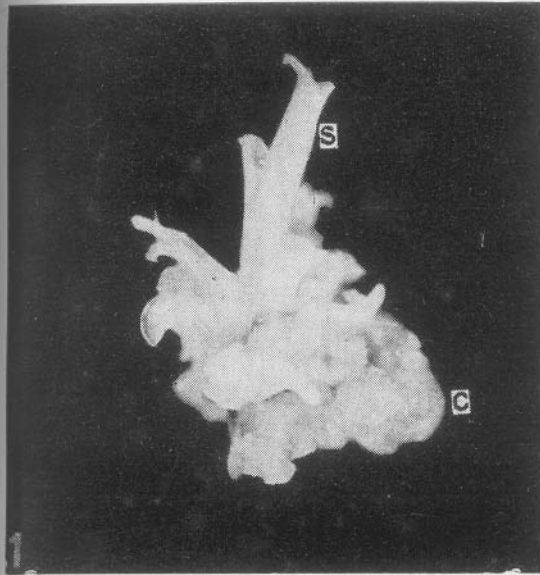
The rate of production of shoot was very high through the callus, when compared to direct suckering. Explants from the same lot and under identical conditions of cultures behaved in both the ways described above. In some cases the new shoots regenerated first by suckering and later through an intermediary callus (Fig. 4).

Regeneration of plantlets from isolated callus

As described above, the majority of the shoot bud explants produced callus which could be easily separated from the base of the explant. Callus pieces of 6-10 mg were inoculated on the same medium described above. Initially the callus grew rapidly for 2-3 weeks before several tiny plantlets could be seen emerging from the periphery of the callus mass. These plantlets grew further, turned green, produced a healthy root system and could be separated and cultured individually.

Nadgouda et al. (1978) reported the successful *in vitro* propagation of Tekurpeta and Duggirala cultivars of turmeric achieving high rate of multiplication (1: 2,00,000) and field establishment (65%). According to their observation, the plantlets arose without production of the intermediary callus, through the swellings at the base of the primary sprout. In the present study, the production of the callus was a regular feature in most of the cultures. Wherever the callus was not produced, the number of suckers was less (5-7/culture tube). Where the regeneration was through callus, upto 25 plantlets/culture tube and an average 10-12 plantlets could be obtained during a

Figs. 1-4 Stages in the *in vitro* development of plantlets in *C. longa*



1. Regeneration of plantlets from the callus. 2. Suckering *in vitro*. 3. Close-up of an explant showing regeneration of multiple shoots. 4. Suckering and regeneration of plantlets in the same explant.

b, base of the explant; c, callus; r, root; s, shoot.

culture period of 6-8 weeks. These cultures could help the breeders in accelerating the clonal multiplication of planting material for initial evaluation of superior clones, identified on single plant basis.

Acknowledgements

We are grateful to Dr. N. M. Nayar, Director and Dr. M. C. Nambiar, project Co-ordinator (spices and cashewnut), CPCRI for providing facilities for the work.

REFERENCES

- MURASHIGE, T. AND SKOOG, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.* 15: 473-497.
- NADGOUDA, R. S., MASCARENHAS, A. F., HENDRE, R. R. AND JAGANNATHAN, V. 1978. Rapid multiplication of turmeric (*Curcuma longa* Linn.) plants by tissue culture. *Indian J. Exp. Biol.* 16: 120-122.

DISCUSSION

P. N. Ravindran (CPCRI): It is well known that many chromosomal aberrations take place during the process of callus regeneration, and the progenies may have an altered genetic constitution than the parental clones. Then do you think you can confidently recommend your *in vitro* seedlings to farmers? Can you guarantee the seedlings are true to types?

M. S. Kuruvina Shetty (CPCRI): Chromosomal aberrations are more common where callus is produced using 2, 4 - D. We have not used it. More over, turmeric being entirely vegetatively propagated, minor changes may not really matter so long as the clonal characteristics are not changed. Chromosome counts from shoot tips of a few plantlets produced *in vitro* revealed only the normal chromosome number ($2n = 63$)