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In Vitro Plant Multiplication from Rhizomes of Turmeric (*Curcuma domestica* Val.) and Temoe Lawak (*C. xanthoriza* Roxb.)

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Turmeric (*Curcuma domestica* Val.=*C. longa* L., Zingiberaceae) and "temoe lawak" (*C. xanthoriza* Roxb., Zingiberaceae) are generally used as traditional medicine in Indonesia. In India, Srinivasan[1] studied the content of curcuminoids in turmeric by chromatography. Srimal and Dhawan[2] investigated the pharmacology of curcumin contained in turmeric and proved that curcumin has anti-inflammatory activity, returning the levels of the serum glutamic oxaloacetic transaminase and serum glutamic pyruvic transaminase to normal after its administration to inflamed rats.

In recent years a large scale propagation of plants has been developed by use of the tissue culture technique, and the number of plant species being enabled to multiply by this technique increases annually.

In Zingiberaceae, Hosoki and Sagawa[3] have succeeded for the first time in a clonal propagation of ginger (*Zingiber officinale* Roscue) by culture in vitro on the medium of Murashige and Skoog supplemented with minor elements and vitamins. While a rapid clonal propagation of *Curcuma* species has been desired because of the slow-to-multiply species, there has been no report on tissue culture work on this species.

The present paper describes the micropropagation of plants of two *Curcuma* species, *C. domestica* and *C. xanthoriza*, by the technique of tissue culture.

Materials and Methods

Rhizomes of *C. domestica* Val. and *C. xanthoriza* Roxb. were bought in the common market of Bandung, Indonesia, and then brought to the Laboratory of Radiation Genetics and Chemical Mutagenesis, Faculty of Agriculture, University of Tokyo. They were washed repeatedly with detergent and tap water. Buds which emerged on rhizomes were excised, and their surface was sterilized by immersing in 70% ethanol for 2 min and then in 0.5% sodium hypochlorite (10% Chlorox) for 10 min. After removing a few scale leaves and the basal part, they were immersed in 2% Purelox solution for 5 min and washed three times with sterile distilled water. Buds trimmed in 2 to 3 mm height were cultured on the medium of Ringe and Nitsch[4] with 2% sucrose, 0.8% agar and supplemented with 6-benzyladenine (BA), naphthalene acetic acid (NAA) and/or 2,4-dichlorophenoxyacetic acid (2, 4-D). The pH of the media was adjusted to

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6.0 with KOH or HCl prior to the addition of agar, followed by autoclaving (10.4×10^4 Pa at 121°C for 15 min).

The buds were grown in vitro at 25±1°C in the dark. After formation of root or embryoid they were transferred into the growth chamber (Koitotron) and maintained for 12 hr under light of about 600 to 1,200 lux and for another 12 hr in the dark at 25±1°C in order to stimulate shoot formation.

Results

In this experiment most of the explants were contaminated with fungi, and thus three to four days after innoculation the contaminated explants were sterilized again and recultured. Even after such treatment, a few buds were still infected. Explants grown on the basal medium did not form either shoots, roots or calluses. This result was similar to ginger experiment.[3)]

Curcuma domestica

Explants grown on the medium containing 1.0 mg/*l* BA formed shoots and roots while those cultured on the medium with 10 mg/*l* BA and 1 mg/*l* 2, 4-D or the medium with 10 mg/*l* BA and 15 mg/*l* NAA produced calluses. If calluses were transferred on the medium with 1 mg/*l* BA and 1 mg/*l* NAA a shoot and auxiliary shoots were formed.

Explants cultured on the medium containing 10 mg/*l* BA in combination with 15 mg/*l* NAA produced calluses, embryoids and roots. When they were recultured on the medium with 10 mg/*l* BA and grown in the Koitotron, shoots were formed.

Curcuma xanthoriza

Buds grown on the medium containing 1 mg/*l* BA and 1 mg/*l* NAA formed roots and shoots. Explants cultured on the medium supplemented with 10 mg/*l* BA produced roots and lateral buds. When the buds were grown on the medium enriched with 10 mg/*l* BA and 15 mg/*l* NAA, calluses were induced. If these calluses were transferred to the basal medium, roots were formed.

In order to study the effect of hormones, 2, 4-D and BA, on callus and organ formation, the precultured materials were transferred onto the medium supplemented with 2, 4-D of 0, 0.5, 1 mg/*l* in combination with BA of 0 and 1 mg/*l*. The results are shown in **Table 1**. Materials harvested from the medium supplemented with 10 mg/*l* BA and 15 mg/*l* NAA did not dif-

Table 1. Effect of 2, 4-D and BA on the recultured *C. xanthoriza*.

Material		C (1)	CERS (2)	CERS (3)	CERS (4)
2, 4-D	BA (mg/*l*)				
0	0	R	CR	CR	R
0.5	0	C	CES	ES	ES
1	0	C	S	S	S
0	1	C	CE	S	S
0.5	1	C	CES	CES	none
1	1	C	ES	S	E

C: callus, E: embryoid, R: root, S: shoot.

(1) materials harvested from the medium supplemented with 10 mg/*l* BA and 15 mg/*l* NAA.
(2) materials harvested from the medium supplemented with 1 mg/*l* BA and 1 mg/*l* 2, 4-D.
(3) materials harvested from the medium supplemented with 10 mg/*l* BA.
(4) materials harvested from the medium supplemented with 1 mg/*l* BA and 1 mg/*l* NAA.

ferentiate any organ, except root formation after transferring to the basal medium. The globular embryoids were multiplied on the freshly prepared medium containing 1 mg/*l* BA when the materials first cultured on the medium with 1 mg/*l* 2, 4-D and 1 mg/*l* BA were transferred.

Discussion

Tissue culture has been used to accelerate plantation development, to shorten the breeding cycle, and to rapidly multiply and disseminate limited materials even for the slow-to-propagate species.[5,6]

In this experiment, proliferating embryoids and roots from a bud of rhizome were successfully obtained by the tissue culture technique in vegetatively propagating Curcuma species. This paper is the first report demonstrating the possibility of a rapid and large scale propagation of a clone by the culture of a bud from the rhizome of Curcuma plant. Because the plants obtained by the technique used here are expected to be pathogen free, it adds a useful information to the agricultural industry and medicinal plants.

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《和文要約》

ウコン (*Curcuma domestica* Val.) とクスリウコン (*C. xanthoriza* Roxb.)の根茎培養による大量増殖

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ショウガ科に属するウコンとクスリウコンは根茎によって繁殖する多年生草本であるが，その増殖率は低い．それらの植物の根茎から仮軸分枝芽を切り出し，培養した．6–ベンジルアデニン，ナフタレン酢酸，2, 4–D を添加した Ringe·Nitsch 培地で，カルス，苗条，根を発生させることができた．培地の選択によってウコンとクスリウコンの根茎から大量の種苗を生産することが可能である．