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CALLUS INDUCTION AND PLANT REGENERATION FROM LEAF EXPLANT OF *CORNUKAEMPFERIA AURANTIFLORA* MOOD & LARSEN

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Abstract

Young leaves of *Cornukaempferia aurantiflora* Mood & Larsen were used for the initiation of callus growth on Murashige Skoog medium supplemented with 3% sucrose and various concentrations of 2,4-dichlorophenoxyacetic acid (2,4-D) in the light. The highest number of callus forming, percentage of callus formation and average weight of callus were obtained from young leaves cultured on the medium supplemented with 2.0 mg/l 2,4-D. Shoots were successfully regenerated on the medium with concentrations of 2,4-D and BA added.

Introduction

The genus *Cornukaempferia* is the new genus in Zingiberaceae family from Thailand, described by Mood & Larsen (1997, 1999). Three species, *C. aurantiflora, C. longipetiolata* and *C. larsenii* (Saensouk *et al.*, 2007a) have been recognized. This genus is listed as rare and it is endemic to Thailand. Its distribution is restricted to only few provinces in the northeastern and northern parts of the country. *C. aurantiflora* has been used by local people in northeastern Thailand to treat infected hemorrhoids and laryngitis which is common in Thai children.

In nature, the plants in this family are vegetatively propagated through rhizomes. In a vegetatively propagated plant like *Cornukaempferia*, the risk of systemic infections with rootknot nematodes, bacterial wilt and *Fusarium* from the propagules is very high. Thus, the application of tissue culture can be used to produce large amounts of disease-free plantlets. The objective of this study was to establish a system for vegetative propagation of this rare plant species through tissue culture. This is the first report of callus induction and plant regeneration from leaf tissue of *C. aurantiflora*.

Materials and Method

Young leaves of *C. aurantiflora* (Fig. 1) were collected from natural habitats, then washed with running tap water, rinsed with 70% (v/v) ethyl alcohol for 30 seconds, sterilized with 0.9% sodium hypochlorite containing 2 drops of Tween 20 for 15 seconds, followed by three washes with sterilized distilled water. The young leaves were cut into 1x1 cm^2 pieces and cultured on MS medium (Murashige & Skoog, 1962) supplemented with 3% sucrose, 0.7% agar and 0, 0.1, 0.5, 1, 2 and 4 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D) in the light for 16 weeks. Callus was transferred to regeneration medium i.e., MS medium, with 0, 0.1 and 0.2 mg/l 2,4-D and 0, 2 and 5 mg/l benzyladenine (BA) added for 8 weeks. The cultures were incubated at 25±2 ^{o}C under white, fluorescent light (2,000 lux) with a 16 h photoperiod. All the experiments were conducted using complete randomized design (CRD) with 20 replicates each containing one explant per culture tube. Data were analyzed using ANOVA and the mean separation was achieved by the Duncan's multiple range test (DMRT). The test of statistical significance was performed at 5% level using the SPSS program (version 11.5).

Fig 1. *Cornukaempferia aurantiflora*. A. Habit & B. Flower. (scale bars A = 10 cm and B = 1 cm).

Results and Discussions

Young leaves were cultured on MS medium with various levels of 2,4-D for induction of callus in the light. The callus formed on the basal part of the explants which contained the meristematic tissue. The callus was soft in texture, friable in structure and yellowish white. The greatest number of callus forming was obtained from young leaves cultured on the medium supplemented with 2.0 mg/l 2,4-D where the percentage of callus formation

and average weight of callus were 95% and 2.50 g respectively (Fig. 2 and Table 1). These results differed from those of Ilahi & Jabeen (1987) who obtained callus from shoot buds, rhizome explants with shoot primordia and juvenile shoots of *Zingiber officinale* on MS medium supplemented with 0.5 mg/l BAP and 0.5 mg/l 2,4-D. They stated that the callus forming from various tissues were morphologically different. Soft and friable calli developed from shoot primordia, whereas hard and compact calli developed from 2 week old buds on treatment with the same concentrations of hormones. Babu *et al.* (1992) reported callus formation from the young leaves of *Z. officinale* cultured on MS medium containing 2-5 mg/l 2,4-D. Vincent *et al*., (1992) developed highly embryogenic callus from rhizomes with vegetative buds from *Kaempferia galanga* on MS medium supplemented with 0.5 mg/l BAP and 1.0 mg/l 2,4-D. Kackar *et al.* (1993) induced callus formation from young leaf segments of ginger on MS medium added with dicamba. Samsudeen *et al*., (2000) induced *Z. officinale* anthers to develop callus on MS medium supplemented with 2-3 mg/l 2,4-D. Salvi *et al*., (2001) induced callus from the leaf base of *Curcuma longa* on MS medium supplemented with 2 mg/l dicamba, 2 mg/l picloram or 5 mg/l NAA in combination with 0.5 mg/l BA. Ventura *et al.* (2003) induced callus of *C. longa* to form from culturing the basal part of the stem of the plantlets developed *in vitro* on the MS medium supplemented with 1.5 mg/l 2,4-D and 0.2 mg/l BA. Prakash *et al*., (2004) obtained semi-friable callus from leaf sheath explants of *Curcuma amada* on MS medium with 2 mg/l 2,4-D added. Rahman *et al*., (2004) observed the highest percentage of callus induction of *K. galanga* on MS medium supplemented with 1.5 mg/l 2,4-D and 1 mg/l BA. Guo & Zhang (2005) induced embryogenic callus from *Z. officinale* shoot tips on MS agar medium supplemented with 1 mg/l 2,4-D and 0.2 mg/l kinetin, which contained only half the concentration of NH_4NO_3. In addition, Tan *et al*., (2005) established that a single medium formulation of MS supplemented with 3 mg/l 2,4-D was the only medium out of eight formulations to result in the complete somatic embryogenesis process for the culture of *Boesenbergia rotunda*. Saensouk *et al*., (2007b) induced callus from young leaves of *Cornukaempferia larsenii* on MS medium supplemented with 3% sucrose and various concentrations of 2,4-D in light and dark conditions. The greatest number of callus forming, percentage of callus formation and average weight of calluses were obtained from young leaves cultured on a medium supplemented with 0.5 mg/l 2,4-D in the light. Moreover, Brown (1990) reported that the induction of the callus, is easily obtained from young tissue with meristematic activity or parenchymatous cells. In addition the response depends on the chemical nature, the combination and the concentration of plant growth regulators and varies according to the plant species and to the origin of the plants (Brown 1990; Raja *et al.*, 2009; Yasmin *et al.*, 2009; Hussain *et al.*, 2010; Munazir *et al.*, 2010).

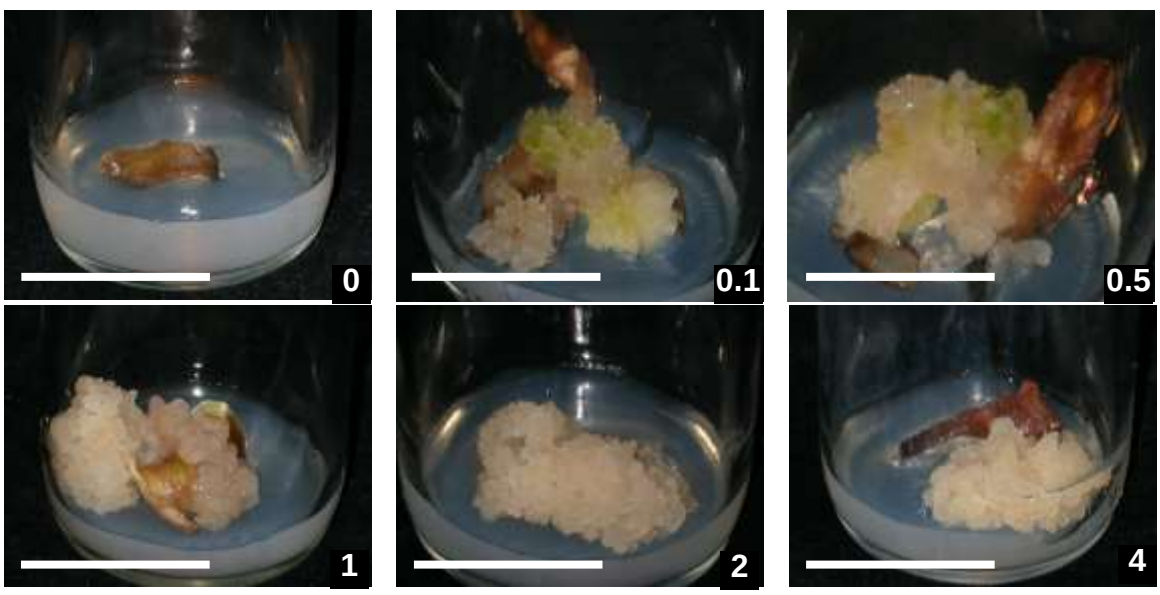

Fig. 2. Callus induction from leaf explants of *Cornukaempferia aurantiflora* on MS medium with 0, 0.1, 0.5, 1, 2 and 4 mg/l 2,4-D added (scale bars = 2.5 cm).

Table 1. Effect of 2,4-D on callus induction from leaf explants of *Cornukaempferia aurantiflora* in the light for 16 weeks.

2,4-D (mg/l)	No. of explants	No. of callus forming	% of callus formation	Average weight of callus (g) mean ± SE
0	20	0 a	0	0 a
0.1	20	4 b	20	2.10 ± 0.34 bc
0.5	20	15 d	75	2.03 ± 0.22 b
1	20	18 e	90	2.46 ± 0.35 d
2	20	19 e	95	2.50 ± 0.29 d
4	20	12 c	60	2.12 ± 0.33 c

*In each column the values with the different letters differ significantly (p=0.05) as determined by DMRT

High contamination of cultures was reported when rhizomes or vegetative buds were used as explants for initiation of the culture. By using leaf tissue as explants this problem was eliminated almost completely. In *Cornukaempferia aurantiflora*, 2,4-D was used for the induction of callus from leaf explants, when callus was transferred to MS medium with varying concentrations of 2,4-D and BA added they were cultured for 8 weeks, callus could be regenerated. Plant regeneration occurred on medium with 0.1 and 0.2 mg/l 2,4-D and 2 and 5 mg/l BA added, after 8 weeks of culture. The highest percentage of shoot formation (50%), the highest number of shoot per explant (3.42) and the highest height of shoots (4.53 cm) were obtained from young leaves cultured on the medium supplemented with 0.1 mg/l 2,4-D and 5 mg/l BA in the light (Fig. 3 and Table 2). Varying types and concentrations of auxin and cytokinin have been successfully used to regenerate plantlets from callus of several other species of Zingiberaceae. In *Z. officinale*, Malamug *et al.* (1991) reported plant regeneration from shoot tip callus on MS medium with 1 and 3 mg/l 2,4-D added. Callus could also be regenerated from the young leaf explants of *Z. officinale* on MS medium supplemented with 0.2 mg/l 2,4-D and 5 mg/l kinetin or 5 mg/l BA (Babu *et al.*, 1992). Vincent *et al.* (1992) induced callus formation from *K. galanga* rhizome explants on MS medium supplemented with 0.5 mg/l BAP and 1.0 mg/l 2,4-D. Callus subcultured on MS medium supplemented with 0.1 mg/l BAP and 1.0 mg/l NAA produced small globular embryoids, which further developed into plants when transferred to hormone free medium. Kackar *et al*., (1993) induced embryogenic callus from young leaf segments of *Z. officinale* from *in vitro* shoot cultures. Among the four auxins tested (IAA, NAA, 2,4-D and dicamba), on MS medium, dicamba at 2.7 µM was the most effective in inducing and maintaining embryogenic cultures. Efficient plant regeneration was achieved when embryogenic cultures were transferred to MS medium containing 2 mg/l BA. Samsudeen *et al*., (2000) was able to regenerate plantlets from callus of *Z. officinale* anther on MS medium supplemented with 5-10 mg/l BA and 0.2 mg/l 2,4-D. In addition, Prakash *et al*., (2004) cultured semi-friable callus from leaf sheath explants of *Curcuma amada* on MS medium containing 2 mg/l BA and 0.5 mg/l NAA which produced optimum shoot initiation and development.

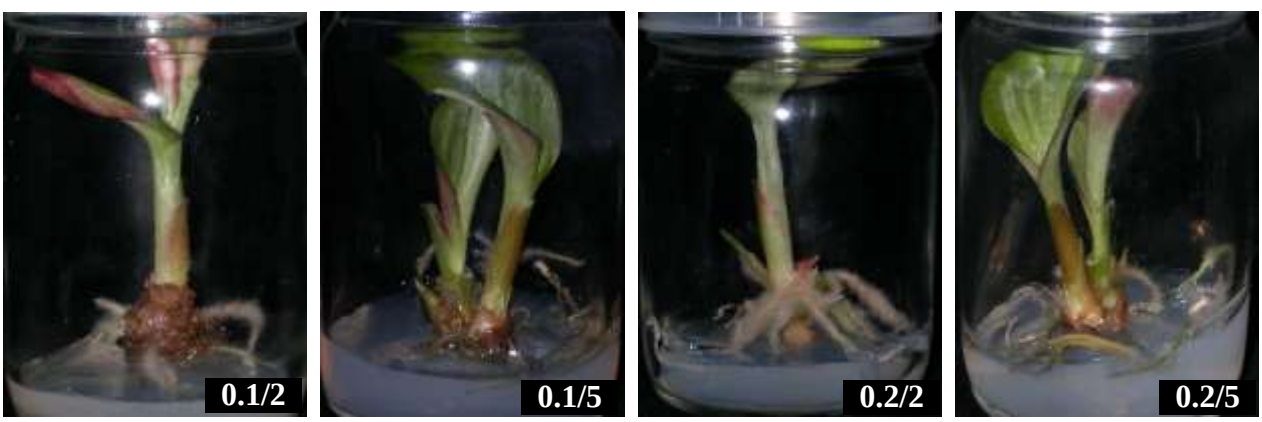

Fig. 3. Plant regeneration from leaf explants of *Cornukaempferia aurantiflora* cultured on MS medium with 2,4-D and BA added.

Table 2. Effect of 2,4-D and BA on shoot formation from explants of *Cornukaempferia aurantiflora* callus for 8 weeks.

2,4-D (mg/l)	BA (mg/l)	No. of explants	% of shoot formation	Number of shoots per explants	Height of shoots (cm)
0	0	20	0	0a	0a
0.1	2	20	15	1.56 ± 0.08 b	3.41 ± 0.19 b
0.1	5	20	50	3.42 ± 0.13 d	4.53 ± 0.12 d
0.2	2	20	35	2.37 ± 0.15 c	3.85 ± 0.07 c
0.2	5	20	20	1.87 ± 0.22 b	3.67 ± 0.32 b

*In each column the values with the different letters differ significantly (p=0.05) as determined by DMRT.

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