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A TISSUE CULTURE TECHNIQUE FOR SEED GERMINATION AND ASEXUAL PROPAGATION OF THE JELLY-FIG (*FICUS PUMILA* L. VAR. *AWKEOTSANG* (MAK.) CORNER)[1]

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Abstract

Near one hundred percent of cultured fresh immature seeds of jelly-fig (*Ficus pumila* L. var. *awkeotsang* (Mak.) Corner) germinated as soon as 4 days later. Stem segments excised from germinated seedlings were used as explants for *in vitro* micropropagation. Four plants per explant within two months was achieved on a medium containing hyponex (N:P:K=7:6:16) 5 g/l, MS microelements and 3% sucrose. Micropropagated plants were successfully transferred to the soil.

Key words: Tissue culture; Awkeo-jelly; *Ficus pumila* L. var. *awkeotsang* (Mak.) Corner; germination; asexual propagation.

Introduction

Jelly-fig (*Ficus pumila* L. var. *awkeotsang* (Mak.) Corner) is an indigenous dioecious plant of Taiwan and the source of the unique refreshing jelly called "Awkeo". The high methoxyl pectin and pectinesterase which cause the formation of jelly are contained in the seeds of the pistillate fruit (Huang and Chen, 1979). The "Awkeo-jelly" is very popular in Taiwan but plantings are limited and do not meet the needs of growing markets. Sun-dried mature seeds which were traditionally used for propagation do not easily germinate (Hu *et al.*, 1986). At present, vegetative cuttings is used mainly in propagation (Hu and Liu, 1985), this imposes limitations for the breeding of high quality varieties. In this study, tissue culture methods were applied for obtaining top seed germination rates and asexual propagation.

Materials and Methods

Both sun-dried mature seeds (DMS) (Fig. 1) and fresh immature seeds (FIS)

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(Fig. 2) were cultured. Immature fruits were corrected from the central mountain area of Taiwan in November, 1985 one month prior to maturation. Mature seeds and fruits were seperately sterilized in 75% ETOH for 2 min, then in 1% sodium hypochlorite solution [NaOCl] for 20 min, and rinsed three times in sterile water. Fresh immature seeds were aseptically removed from sterilized fruits. Both of sterilized DMS and FIS were cultured on solid or in liquid MS medium (Murashige and Skoog, 1962) with 15 ml quantity in a 50 ml flask. The effect of gibberellic acid (GA_3) at either 0, 10^{-3}, 10^{-2}, 10^{-1}, and 1 mg/l was tested. Liquid cultures were shaken at 80 rpm. Germinated seeds were subsequently transferred to solid MS medium for further development. All cultures were incubated at 25±2°C with 12 h light (2000 lux) daily. Each experiment was carried out at least 2 times.

For asexual propagation, 7-10 cm long seedlings were cut into four to six segments (Fig. 3) with either an apex or a node. The basal ends of the segments were inserted into the medium for rooting. Media tested with MS microelements included diluted MS major salts (1/4 MS, 1/2 MS and hyponex 5 g/l (N:P:K=7:6:16, Hydroponic Chemical Co., Inc.). Growth regulators n-benzyladenine (BA) and indole-3-butyric acid (IBA) were tested at 0, 0.1, 0.5, and 1.0 mg/l in hyponex medium. Plants rooted in culture were transplanted to plastic pots in a 2:1 (v/v) soil/sand mixture, kept under high humidity for 2 weeks, watered with 0.5 mg/l IAA, and then transferred to a green house.

Results and Discussion

Germination of DMS did not occur until after two months of culture in liquid medium (Fig. 4). On the other hand, FIS started to germinate after only 4 days in liquid culture (Fig. 5) or 10 days on solid culture, with germination rate as high as 100% after 2 weeks on hormone free media (Table 1). Gibberellic acid (GA_3) was

Table 1. *Germination rate of dried mature seeds (DMS) and fresh immature seeds (FIS) of (**Ficus pumila** L. var. **awkeotsang** (Mak.) Corner) when cultured on GA_3 containing liquid medium**

GA_3 (mg/l)	Total No. of seeds germinated	Total No. of seeds cultured	Germination rate	
	DMS	FIS	DMS	FIS
0	9/720	878/880	1.25%	99.77%
0.001	5/360	1,010/1,030	1.38%	98.05%
0.01	4/400	456/460	1.00%	99.13%
0.1	2/400	228/429	0.50%	53.15%
1.0	0/400	209/521	0.00%	40.12%

* MS medium (1962) without agar.

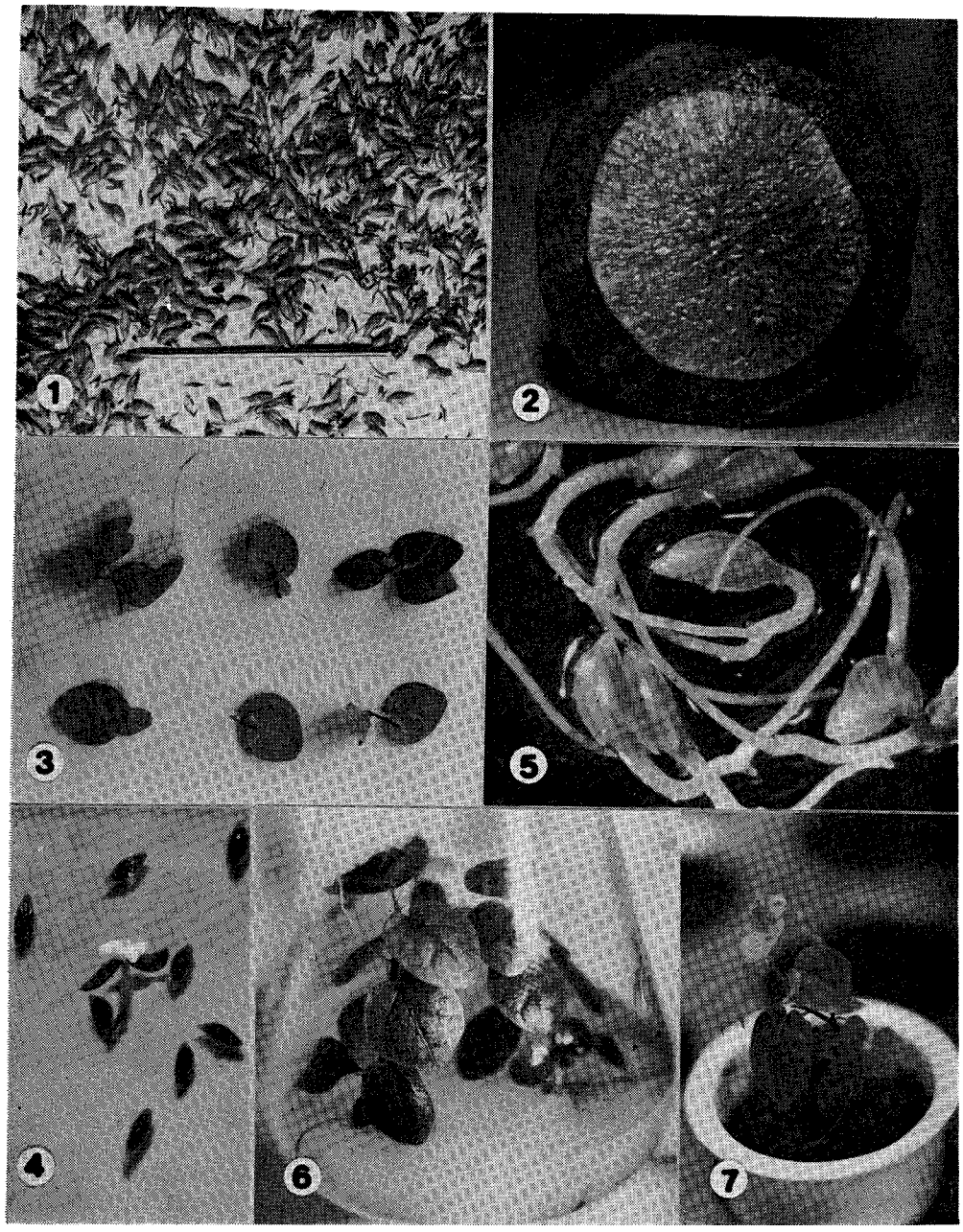

Fig. 1. The dried mature jelly-fig seeds (DMS). (×1.8)
Fig. 2. The fresh immature jelly-fig seeds (FIS) within a gree fruit. (×1.5)
Fig. 3. The explants obtained from jelly-fig seedlings for asexual propagation. (×1.3)
Fig. 4. The DMS rarely germinated after 2-month culture. (×3.7)
Fig. 5. Rapid germination of FIS after 7-days culture. (×7.4)
Fig. 6. Proliferation of stem segments into young plants after 2-month culture period. (×1.5)
Fig. 7. Growth of new leaves from young plants after transferring to the soil. (×1.4)

tested in as much as it has been used for breaking the dormancy of several types of seeds (Wareing and Phillips, 1970). However, GA_3 had little effect on stimulation of germination and adversely inhibited germination at concentrations higher than 0.1 mg/l. When cultured in 1.0 mg/l GA_3 medium, no DMS germinated, but the FIS with the rate of 40%. In conclusion, it is more convenient and economical to use fresh immature seeds due to their high and rapid germination rate.

The stem segments of the jelly-fig grew into new plantlets after 2 months of culture on agar media (Fig. 6). Leaves and roots developed as a result of the extension of axillary buds at the base of the leaves. Best results were obtained on hyponex medium. The percentages of successful rooting and shooting were: hyponex medium (91.9%), 1/4 MS (60.5%), 1/2 MS (50.4%) and MS (50.6%). Addition of hormones BA or IBA resulted in the wilting of leaves and insufficient production of roots even at low concentrations. Davies and Joiner (1978) reported that IBA stimulated rooting of leaf-bud cuttings of *Ficus pumila*, but IBA at the concentrations tested harmed the growth of jelly-fig axillary bud and rooting. In this study, the optimum cloning efficiency was obtained in the hormone-free hyponex medium.

All young plants transferred to soil grew new roots and leaves after 2 weeks in pots (Fig. 7). Since four to six stem segments can be cut from one proliferated plant at 2 month intervals, a single jelly-fig plant could theoretically yield more than 16000 plants after one year through micropropagation.

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愛玉子種子發芽及無性繁殖之組織培養

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愛玉子（*Ficus pumila* L. var. awkeotsang (Mak.) Corner）是臺灣特有的植物，其種子爲愛玉凍（Awkeo-jelly）的原料，但發芽率低。利用未成熟果實內的種子從事培養，四天後其發芽率可至 99 %。此法將有助於人工育種時小苗之培育。取愛玉子實生苗含腋芽之莖段，扦插於含 MS 微量無機鹽，5 g/l Hyponex (N:P:K=7:6:16)，3 %蔗糖及0.8%洋菜粉之簡單培養基上，兩個月後卽見腋芽生長並發根成小苗，可達到試管大量繁殖的目的。