カンキツのアルベドと砂じょう由来のカルスからの器官分化

誌名	名城大学農学部学術報告
ISSN	09103376
巻/号	21
掲載ページ	p. 33-36
発行年月	1985年3月

SHORT COMMUNICATION

Organ Differentiation on Callus Derived from Albedo and Juice Vesicle of Citrus

Naosuke NII* and Takashi OYAMADA*

Abstract

Organ differentiation on callus tissues derived from albedos and juice vesicles of satsuma mandarin (Citrus unshiu Marc.) and Hassaku (C. hassaku hort. ex. Tanaka) was studied on the MS medium without the addition of growth hormones. A liter of basal medium contained the following: nicotinic acid 0.5 mg; pyridoxine HCl 0.5 mg; glycine 2.0 mg; thiamine HCl 0.1 mg; myo-inositol 100 mg; sucrose 30g ; and agar 10 g. Shoot formation was observed in the callus subcultured after one year. The initial signs of differentiation was several globes (2 mm dia.) on the callus tissues from which a shoot meristem developed. Root appeared from these tissues several weeks after transferring to a rooting medium.

key words: organ differentiation ; citrus fruit

Introduction

Citrus callus has been cultured from fruit tissues, leaves, buds, and stem. Grinblat (4) obtained differentiation of shoot-buds and plantlets from in vitro-cultured stem explants of Citrus mandarensis. Kordan (6, 7) showed that potentially unlimited growth in vitro could be attained from juice vesicles of lemon fruit. Schroeder and Spector (12) used explants composed of the fruit mesocarp of citron and noted callus formation. Callus formation and tracheary from citrus juice vesicle have been reported by many workers (2, 5, 10, 11, 13). Explant cultures of juice vesicle or albedo tissues from citrus fruits proliferate in vitro. However, to date there is no report on organ differentiation on callus originating from citrus fruit tissue. Plantlets from callus tissues of citrus leaves and young stems have been successfully obtained (1, 4, 8).

This paper describes means of regenerating plantlets from albedo and juice vesicle of citrus fruits.

Materials and Methods

Citrus fruits, satsuma mandarin (Citrus unshiu Marc.) and Hassaku (C. hassaku hort. ex. Tanaka) were gathered one month after anthesis in 1982. The fruits, 1.5-2.0 cm in diameters, were surface-sterilized by immersion in 70 % ethanol for 5 minutes and rinsed twice with sterile distilled water. Then the fruit was cut, the skin flavedo removed; the albedo and juice vesicles were aseptically isolated with sharp, pointed forceps and razor knife. Tissue pieces of albedo were then cut into 3-5 mm disc without damaging juice vesicles and transferred to culture tubes (25×150 mm) containing 5 ml of MS medium, one disc per test-tube. The basal medium contained MS mineral solution (9), and the following chemicals per liter : nicotinic acid, 0.5 mg ; pyridoxine HCl, 0.5 mg ; glycine, 2.0 mg; thiamine HCl, 0.1 mg; myo-inositol, 100 mg; sucrose, 30 g; and agar, 10 g. The pH of nutrient medium, prior to autoclaving, was adjusted to 5.6 ; sterilization was accomplished

Received Oct. 31, 1984

* Laboratory of Horticultural Science

by autoclaving for 15 minutes at 121°C. The cultures were allowed to develop for three weeks after inoculation in continuous darkness at 25°C, then transferred to a continuous light condition.

Results and Discussion

The callus tissues were easily induced from albedo and juice vesicle collected from young fruit and grown on medium without plant hormones (Fig. 1). Many reseasrchers pointed out that some exogenous plant growth substances played an important role for callus formation from juice vesicles (3, 5, 11, 12). Kato (5) reported that both auxin and cytokinin were necessary for callus induction and growth. However, in this trial, callus was induced from the surface of juice vesicle, without any exogenous supply of plant growth hormones. Consequently it is possible to assume that the callus induction may be caused by endogenously produced natural auxin (s) in a specific tissue like albedo.

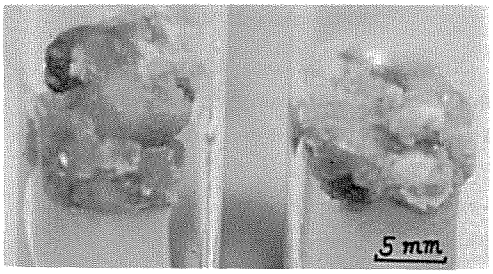

Fig. 1. Typical cultures of callus derived from albedo and juice vesicle. left : satsuma mandarin, right : Hassaku.

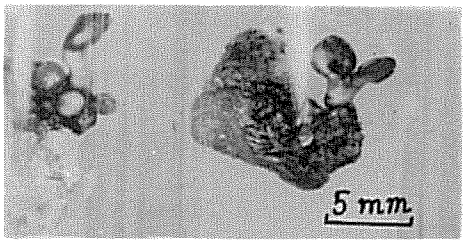

Fig. 2. Shoots developing from explant of callus tissue of albedo and juice vesicle in satsuma mandarin.

To establish a continuous growing callus strain, the mother callus was divided into small fragments for inocula of subcultures three months after beginning tissue culture and cultured under continuous light. When these inocula were explanted to fresh hormone-free MS medium, they enlarged. Root or shoot formation was not observed until ten months after the beginning of the first culture. When the callus culture was continued for a longer period, about one year, several globular structures developed in two test tubes from satsuma mandarin and one from Hassaku (Fig. 2). The typical color of these organs was pale yellow which turned green. These globes continued to increase in volume with time and afterwards developed leaflets. No sign of root differentiation was observed in this medium, although

Table 1. Rooting medium for citrus meristems derived from callus of albedo and juice vesicles.

Chemical	mg/l	Chemical	mg/l
KH_2PO_4	979.2	$ZnSO_4 \cdot 7H_2O$	1.0
Na_2NO_3	122.4	Na_2MoO_4	0.1
KCl	90.0	Inositol	100.0
$MgSO_4 \cdot 7H_2O$	442.8	Nicotinic acid	0.5
$Ca(NO_3)_2 \cdot 4H_2O$	623.0	Pyridoxine HCl	1.0
KNO_3	533.3	Thiamine HCl	1.0
NH_4NO_3	480.0		
Fe-EDTA	10.0	Sucrose	30,000
H_3BO_3	3.0	Agar	8,000
$MnSO_4 \cdot H_2O$	2.0		

pH adjusted to 5.4-5.5, prior to autoclaving.

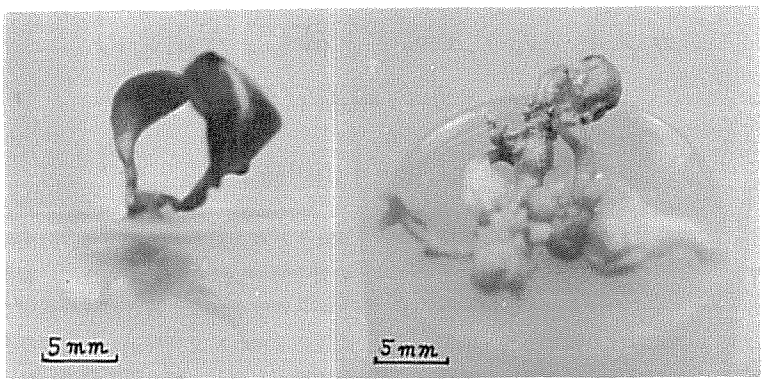

Fig. 3. Rooting of callus with regenerated shoots in satsuma mandarin (left) and Hassaku (right).

new leaf and stem appeared in both species. These new meristems with some callus attached were subcultured on a rooting medium (Table 1). Root initiation occurred about three weeks after inoculation giving rise to plantlets (Fig. 3). Thus, regeneration of plantlets from albedo and juice vesicles required about one year.

Acknowledgement

The authors wish to thank Messrs. S. Isoe and K. Yamada for their invaluable assistance.

Literature Cited

1) Chaturredi, H. C. and G. C. Mitra. (1974) Clonal propagation of citrus from somatic callus cultures. Hortsci. 9 : 118-120.
2) Einset, J. (1978)Citrus tissue culture. Stimulation of fruit explant cultures with orange juice. Plant Physiol. 62 : 885-888.
3) Erner, Y., O. Reureni and E. E Goldschmit. (1975) Partial purification of a growth factor from orange juice which affects citrus tissue culture and its replacement by citric acid. Plant Physiol. 56 : 279-282.
4) Grinblat, U. (1972) Differentiation of citrus stem in vitro. J. Amer. Soc. Hort. Sci. 97 : 599-603.
5) Kato, Y. (1980) Studies on juice vesicle isolation from mature and immature citrus fruit. J. Japan. Soc. Hort. Sci. 49 : 36-40.
6) Kordan, H. A. (1959) Proliferation of excised juice vesicles of lemon in vitro. Science. 129 : 779-780.
7) Kordan, H. A. (1963) Growth characteristics of citrus fruit tissue in vitro. Nature (London). 198 : 867-869.
8) Machida, H., A. Ooishi and T. Hosoi. (1974) Clonal multiplication of citrus through tissue culture. Bull. Fac. Agri. Shizuoka Univ. 24 : 15-21.
9) Murashige, T. and F. Skoog. (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15 : 473-497.
10) Murashige, T., R. Nakano and D. P. H. Tuker. (1967) Histogenesis and rate of nuclear change in citrus limon tissue in vitro. Phytomorphology. 17 : 469-476.
11) Murashige, T. and D. P. H. Tuker. (1969) Growth factor requirements of citrus tissue culture. Proc. First Int. Citrus Symp. 3 : 1151-1161.
12) Schroeder, C. A. and C. Spector. (1957) Effect of GA and IAA on growth of excised fruit tissue. Science 126 : 701-702.
13) Unger, J. W. and K. A. Feng. (1978) Growth and differentiation of juice vesicles of orange grown in vitro. Amer. J. Bot. 65 : 511-515.

カンキツのアルベドと砂じょう由来のカルスからの器官分化

新居直祐・小山田高士

摘　　要

温州ミカンとハッサク果実のアルベド組織と砂じょうから誘起したカルスにおける器官分化について，植物ホルモン無添加のＭＳ培地を用いて検討した。基本培地にビタミン類（ニコチン酸0.5mg/l，ピリドキシン0.5mg/l，グリシン2.0 mg/l，チアミン0.1 mg/l，イノシトール100 mg/l），スクロース30g/l と寒天 10g/l を加えた。枝葉の形成は，組織培養を開始してから約１年後に，継代培養したカルスから観察された。器官形成の過程で，カルス上に直径約２mmにまで発達した数個の不定胚様の球状の組織が出現し，枝一芽に発達した。その器官を新しい培地に継代培養したところ，数週間後に，これらの組織から根が出現した。