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Photomixotrophic Growth of *Solanum tuberosum* L. *in vitro* with Addition and Omission of Organic Materials at Thee Initial Sucrose Levels in the Medium

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세 수준의 자당이 첨가된 배지에서 유기물의 첨가 유무에 따른 *Solanum tuberosum* L.의 기내 광혼합영양생장

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Abstract. The most commonly used inorganic nutrient compositions such as Murashige & Skoog medium have been optimized for heterotrophic growth. Therefore, they may not be optimal for photomixotrophic and photoautotrophic growth of plantlets. In photomixotrophic micropropagation, medium sugar level is often lowered, while light and CO_2 levels in vessel are raised, and chlorophyllous explants are used to facilitate photosynthetic carbon acquisition. In a factorial experiment effect of addition (+) and omission (-) of organic materials (OM, 0.5 $g \cdot m^{-3}$ each of thiamine, nicotinic acid and pyridoxine and 100 $g \cdot m^{-3}$ myo-inositiol) combined with three sucrose levels (0, 15, and 30 $kg \cdot m^{-3}$) was tested on the growth of potato plantlets. Each of nodal cuttings with a leaf was cultured on 0.1×10^{-4} m^3 MS agar (8 $kg \cdot m^{-3}$) medium (pH 5.80 before autoclave) in glass test tubes (100 mm×25 mm) capped with a sheet of transparent film with a 6 mm diameter gas permeable filter (5.1 air exchanges $\cdot h^{-1}$). Cultures were maintained in a room for 27 days at 23°C, 50% RH, 350 – 450 $\mu mol \cdot mol^{-1}$ CO_2, 16 $h \cdot d^{-1}$ photoperiod at 130 $\mu mol \cdot m \cdot s^{-1}$ PPFD provided by white cool fluorescent lamps. Growth of potato plantlet in the +OM and –OM treatments were similar, while medium pH was 0.2 scale lower in the latter. Dry weight, % dry matter, and stem diameter enhanced, while shoot to root dry weight ratio, leaf area, chlorophyll concentration per gram dry weight, and medium pH decreased with increasing initial sucrose level. Interaction between OM and sucrose levels was observed in shoot length and medium pH. Results indicate that OM can be omitted from the medium without detrimental effect while addition of sucrose was beneficial for the photomixotrophic growth of potato plantlets under raised light and CO_2 conditions.

Key words : autotrophic, mixotrophic, medium composition, vitamins, inositol

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Introduction

Inorganic nutrient compositions in tissue culture medium such as Murashige & Skoog (MS) medium (Murashige and Skoog, 1962) have been optimized for heterotrophic growth. But they may not be optimal for photomixotrophic and photoautotrophic growths of plantlets. Optimal conditions for photoautotrophic culture have been extensively studied (Kozai et al., 1997). CO_2 and sucrose (Deng and Donnelly, 1993), supporting materials (Jeong et al., 1999; Kirdmanee et al., 1995), initial phosphrous level (Lee and Jeong, 1999), photosynthetic photon flux, and the number of air exchanges per hour of the culture vessel (Kim and Jeong, 2003) have been investigated.

Plant cells, tissues, and organs cultured *in vitro* require sugar, amino acid, and vitamin in media as carbon and energy sources for their growth under heterotrophic and sugar-dependent photomixotrophic conditions. The effects of medium sugar concentrations on their growth have been studied to obtain the maximum dry weight of the plant for a given period of time (Tuskan et al., 1990). Plant cell growth and organ differentiation can be affected by plant regulator in the medium. Amino acid and vitamin in the medium influenced plant cell and callus growth, adventitious embryogenesis, and organ division (Claparols et al., 1993; Ketchum et al., 1995; Li et al., 1995; Pinol et al., 1985; Sheridan, 1977; Wei, 1986).

In photomixotrophic micropropagation, medium sugar level is often lowered, while light and CO_2 levels are raised, and chlorophyllous explants are used to facilitate photosynthetic carbon acquisition. The necessity for the photomixotrophic culture of the chlorophyllous tissues of other organic materials that are usually added to the medium is yet to be examined. In the present study, potato explants were cultured in the treatments with several combinations of addition and omission of thiamine, nicotinic acid, pyridoxine and myo-inositol at 3 initial sucrose levels to investigate their effects on the photomixotrophic growth of potato plantlets.

Materials and Methods

Culture Conditions

A general description of the culture condition is given in Table 1. Single node cutting with a small leaf and virtually no roots, and 65 mg in mean fresh weight of potato

Table 1. Materials and environmental conditions used in the experiment.

Plant material	
Species	Potato, *Solanum tuberosum* L. Benimaru
Explant	Single node cutting with a small leaf
Fresh weight	65 mg/explant
Plant density	1 explant per vessel
Type	Glass test tube (100 mm × 25 mm)
Volume	0.47×10^{-4} m^3
Cap	A sheet of transparent film with a 6 mm gas permeable filter
Number of air exchanges	4.2 · h^{-1}
Culture medium	
Basal composition	Murashige and Skoog (1962)
Volume	0.1×10^{-4}m^3 per vessel
Gelling agent	Agar, 8 kg · m^{-3}
pH	5.8 before autoclaving
Growth regulators	None
Growth chamber	
Air temperature	23°C
Relative humidity	70%
Radiation source	Cool-white fluorescent lamps
Photoperiod	16 h · d^{-1}
PPFD level*	130 μmol · m · s^{-1}
CO_2 concentration	350 – 450 μmol · mol^{-1}

*PPFD: Photosynthetic photon flux density

(*Solanum tuberosum* L. cv. Benimaru) plantlets were subcultured *in vitro* at the late-multiplication stages, which had been cultured under conventional heterotrophic culture conditions and were used as explants. Each explant was transplanted on standard MS medium with 8 kg · m^{-3} agar and no plant growth regulator in the vessel. The pH of the medium was adjusted to 5.80 before autoclaving. The volume of the medium dispensed was 10 mL per vessel.

The culture was conducted using flat bottom glass test tube (inside volume: 47 mL) with a gas permeable, transparent film under a photosynthetic photon flux (PPF) of 130 μmol · m · s^{-1} (ca. 9 klx) on the empty culture shelf in the culture room. Micro-porous polypropylene film (brand name : TPX film, Mitsui Petrochemical Co.) was used to cap the test tubes. A hole (6 mm in diameter) was made at the center of square TPX film (the length of the side: 7 cm).

The number of air exchanges per hour of the vessel containing 10 mL medium with the cap was 5.1 h^{-1} on the average. CO_2 concentration outside the vessel (in side the culture room) ranged from 350 to 450 μmol · mol^{-1}. The chamber air temperature and relative humidity were maintained at about 23°C and 50% through day and night, respectively. The photoperiod was 16 h^{-1}.

Fresh weight, dry weight, the number of leaves, leaf area, shoot length, stem diameter and medium pH were measured on 28th day after the start of the experiment. Percent dry matter and shoot to root dry weight ratio were calculated based on dry and fresh weights, and shoot and root dry weights, respectively. The chlorophyll contents per dry weight (mg · gDW^{-1}) of potato plantlet were determined according to the methods described by Arnon (1949).

Table 2. General description of the treatment.

Treatment code*	Organic material	Sucrose (kg · m^{-3})
A	+	0
B	–	0
C	+	15
D	–	15
E	+	30
F	–	30

* +: addition, –: omission; addition treatment solution contained (in g · m^{-3}) 100 gmyo-inositol, each of 0.5 thiamine-HCl, nicotinic acid, and pyridoxine-HCl as organic materials.

Treatments

The description of six treatments prepared is shown in Table 2. In a factorial experiment effect of addition (+) and omission (–) of organic materials (OM, 0.5 g · m^{-3} each of thiamine, nicotinic acid, and pyridoxine, and 100 g · m^{-3} myo-inositiol) combined with three sucrose levels (0, 15, and 30 kg · m^{-3}) was tested on the growth of potato plantlets. The numeral letters following denoted sucrose concentrations in the medium. The number of explants per treatment was 50. Data was analyzed by t-test at the 5% level.

Table 3. Fresh weight (FW), percent dry matter (DM), stem diameter (SD), number of leaf (NL), shoot to root (S/R) dry weight (DW) ratio per potato plantlet, and medium pH on day 28 as affected by addition and omission of myo-inositol, vitamin and amino acid and with three initial sucrose levels.

Treatment code*	FW (mg/plantlet)	DM (%)	SL (mm)	SD (mm)	NL	S/R DW ratio	Medium pH
+OM0	359	7.6	45	1.3	10	6.2	4.7
–OM0	379	6.4	40	1.4	10	6.0	4.8
+OM15	394	10.2	42	1.6	10	5.0	4.7
–OM15	430	8.9	44	1.6	10	4.8	4.4
+OM30	437	11.0	46	1.6	9	3.9	4.6
–OM30	377	11.7	49	1.5	9	5.0	4.3
Organic mater. (OM)	NS**	NS	NS	NS	NS	NS	*
Sucrose (S)	**	**	**	**	*	**	*
Interaction (OM S)	NS	NS	**	NS	NS	NS	*

* +: addition, –: omission; addition treatment solution contained (in g · m^{-3}) 100 myo-inositol, each of 0.5 thiamine-HCl, nicotinic acid pyridoxine-HCl as organic material. The symbol following OM denoted organic material of the media.
** NS, *, **: Nonsignificant and significant at $P = 0.05$, 0.01, respectively.

Results

The growth of potato plantlet *in vitro* on day 28 is shown in Table 3. Fresh weight per potato plantlet was the greatest in treatment +OM30 and was the smallest in treatment +OM0. Fresh weight was not affected by addition or omission of thiamine, nicotinic acid, pyridoxine, or myo-inositol as organic materials. However, fresh weight increased gradually and significantly with the increasing sucrose concentrations.

Dry matter and stem length per potato plantlet was the greatest in treatment -OM30 and was the smallest in treatment -OM0. Dry matter increased with the increasing sucrose concentration under the addition of organic materials. Stem diameter, the number of leaf, and shoot to root dry weight ratio were affected by different sucrose concentrations in the medium. In potato (Nakayama et al. 1991), total assimilation per plant dry weight was 8~10 times greater with presence than absence of the sugar in the medium. On the other hand, omission of organic materials as sucrose and vitamins was better than addition of organic materials for fresh weight and dry weight of *Limomium* spp. cv. Misty Blue (Lee, 1998).

Figs. 1, 2, and 3 show the changes in dry weight, leaf area, and chlorophyll concentration of potato plantlet *in vitro* on day 28. Dry weight increased with elevated sugar concentrations regardless of the addition or omission of thiamine, nicotinic acid and pyridoxine and myo-inositol as organic materials (Fig. 1). Leaf area did not changed significantly at sucrose concentrations of 0 and 15 kg · m^{-3} in both addition and omission of organic materials. However, gradual decrease in leaf area was detected with increasing sucrose concentrations (Fig. 2). Chlorophyll concentration also decreased gradually with the increasing sucrose concentration, but it seems to be not affected by addition or omission of organic materials.

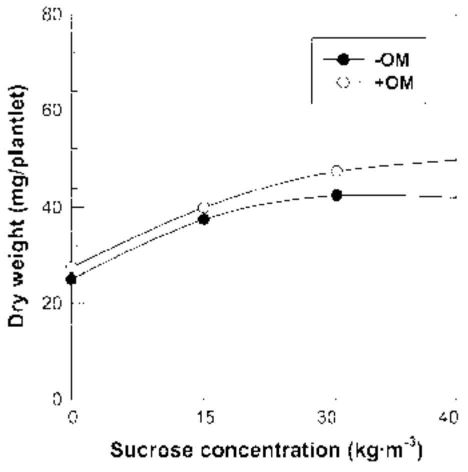

Fig. 1. Dry weight of potato plantlets on day 28 as affected by addition (+OM) and omission (–OM) of organic materials at 3 initial sucrose levels.

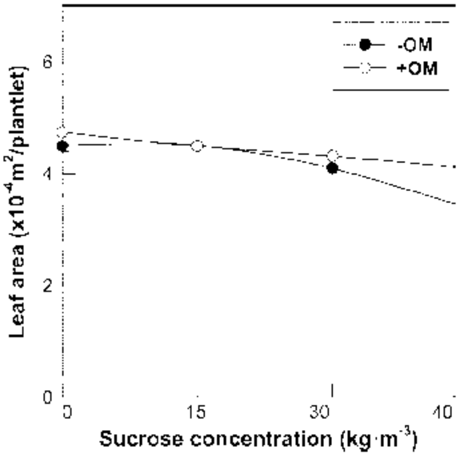

Fig. 2. Leaf area of potato plantlets on day 28 as affected by addition (+OM) and omission (–OM) of organic materials at 3 initial sucrose levels.

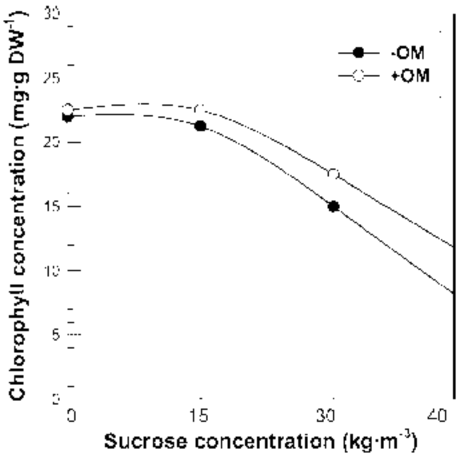

Fig. 3. Chlorophyll concentration of potato plantlets on day 28 as affected by addition (+OM) and omission (–OM) of organic materials at 3 initial sucrose levels.

적 요

식물의 조직배양에서 가장 널리 이용되는 MS 배지

등의 무기성분은 종속영양에 적합하도록 조성되었다. 그러므로 이들 배지가 혼합영양이나 독립영양 배양에는 부적합 할 수도 있다. 광혼합영양 미세증식에서 배지에 첨가되는 당의 수준은 낮추고 배양기의 광과 CO_2 수준은 올리며, 광합성의 의한 탄소의 획득을 증진시키기 위해 엽록소가 있는 절편체를 이용한다. Factorial 실험에서 유기물(OM, m^3 배지당 각각 0.5 g의 thiamine, nicotinic acid 및 pyridoxine과 100 g · m^{-3} myo-inositiol)의 첨가(+)와 생략(–) 및 세 가지 수준의 당(0, 15 및 30 kg · m^{-3})이 감자 소식물체의 생장에 미치는 영향을 조사하였다. 단엽단절 절편체를 0.1×10^{-4} m^3의 MS 아가(8 kg · m^{-3}) 배지(증기소독 전 pH 5.80)배지가 담긴 유리 시험관(100 mm × 25 mm)에 치상하여 직경 6 mm의 가스투과 필터(5.1 air exchanges · h^{-1})가 부착된 투명한 필름으로 봉하여 배양하였다. 배양체는 27일간 23℃, 50% RH에서 16 h · d^{-1}의 명기 동안에 cool white 형광등으로 130 μmol · m · s^{-1} PPFD의 광과 350 – 450 μmol · mol^{-1}의 CO_2가 공급되는 배양실에서 배양되었다. 유기물이 공급된 처리(+OM)와 공급되지 않은 처리(–OM)에서 감자 소식물체의 생육은 유사하였으나 배지의 pH는 후자에서 0.2 더 낮았다. 배지에 첨가된 당의 수준이 증가할수록 건물중, % 건물율 및 경경은 증진된데 반해 신초와 뿌리의 건물중 비율, 엽면적, 건물중당 엽록소 함량 및 배지의 pH는 감소하였다. 유기물과 당 수준간의 상호작용도 신초길이와 배지 pH에서 관찰되었다. 이상의 결과로부터 광도와 CO_2 농도를 높인 광혼합영양배양에서 감자 소식물체의 생장에는 배지에 첨가되는 당은 유익하지만 그 이외의 유기물은 첨가하지 않아도 악영향이 없다는 것은 나타낸다.

주제어 : 자가영양, 혼합영양, 배지조성, 비타민, 이노시톨

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