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Effect of Inorganic salts, Growth Regulators, and Thiamine on the Callus Growth and Plant Regeneration from Long-Term Cultured *Solanum* and *Lycopersicon* Genotypes

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무기염류, 생장조절물질 및 타이아민이 장기간 계대배양한 *Solanum*과 *Lycopersicon*종의 캘러스 생장과 식물체분화에 미치는 영향

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Callus growth and shoot regeneration of *Solanum* and *Lycopersicon* depended on genotype, growth regulators, and thiamine concentrations. *L. penelli* LA 1277, *L. peruvianum* LA 1373 and PI 251301 had the greatest callus growth while *L. hirsutum* LA 1777, *L. esculentum* 'Diego' and 'Red Plum' had the least callus growth. *Lycopersicon peruvianum* genotypes were superior to *L. esculentum* genotypes in regenerating plants. MG medium was more effective in regenerating shoots than MS medium. A low level of IAA (0.2 mg/L) and high level of BA (2 mg/L) resulted in the greatest shoot regeneration. Shoot growth varied depending on thiamine concentration and genotype. Shoot proliferation of *Solanum ptycanthum, Solanum nigrum,* and *L. peruvianum* PI 199380 was best on medium with 20 mg/L thiamine. Regeneration of *L. peruvianum* PI 251301 and PI 128652 was better on medium with 30 and 10 mg/L thiamine, respectively.

Key words: plant regeneration, thiamine

Tissue culture is a valuable tool for propagation, selection and genetic transformation of plants. The practical use of cell culture techniques depends being abe to regenerate plants. Plant regeneration is dependent on medium composition, culture conditions, genotype, explant, and hormones (Jain et al., 1988: Koornneef et al., 1987).

Media constituents including hormones, vitamins, and amino acids were critical for successful plant growth in culture. Thiamine was an essential media component for in vitro growth of some *Solanaceous* plants. For example, addition of thiamine to the medium was beneficial for growth of isolated tomato roots (Gamborg et al., 1968), suspension cultures (Ohira et al., 1976), and long-term callus cultures (Ohira et al., 1973).

Culture conditions such as extended subculturing also can lead to a changes in morphogenetic ability (Armstrong and Phillips, 1988). The progressive decline and eventual loss of the morphogenetic response during long-term culture results from changes in ploidy, an altered hormone balance, or selection of nonmorphogenetic cell types (Koornneef et al., 1987: Negrutiu and Jacobs, 1978).

In some species, morphogenetic capability was not lost after

long-term culture. Shoots regenerated from *L. glandulosum* suspension cultures maintained for over 1 year (Koornneef et al., 1987). Leaf formation of *Arabidopsis thaliana* was induced in long-term callus cultures by transferring to media differing in hormonal composition or glucose concentration (Negrutiu and Jacobs, 1978). The influence of genotype on callus formation and plant regeneration is well-documented in potato (Jarret et al., 1980), *Brassica* species (Jain et al., 1988), and tomato (Koornneef et al., 1987: Locy, 1983).

Both classical breeding methods and protoplast fusion have been used to move the trait governing ability of protoplast and callus cultures to regenerate from *Lycopersicon peruvianum* into *L. esculentum* (Koornneef et al., 1986: Wijbrandi et al., 1988). Thus, undomesticated *Solanaceous* genotypes which easily regenerate from long-term culture can be used to introduce favorable regeneration characteristics into important crop species. Also, these genotypes could be important sources of resistance to herbicide or other stresses (Locy, 1981: Koornneef et al., 1986).

This study determined the effect of genotype, hormone regime and thiamine on growth and plant regeneration from long-term callus cultures.

MATERIALS AND METHODS

In Vitro Culture

Callus of *Solanum ptycantum* Dun, *Solanum nigrum* L., *Lycopersicon peruvianum* Mill accessions PI 126945, PI 251301, LA 1373, LA 1374, *L. hirsutum* Humb LA 1777, *L. penelli* LA 1277, and *L. esculentum* Mill cultivars Carmen, Diego, and Red Plum were used in the experiments. The callus had been established from cotyledons and grown on Thomas and Pratt (1982) medium with 2,4-D (2,4-dichlorophenoxyacetic acid) (2 mg/L) and BA (6-benzyladenine) (1 mg/L) for approximately 1 year. There were a minimum three replicates in all 3 experiments, and each experiment was repeated.

Inorganic Salts Effects on Callus Growth and Shoot Differentiation.

The objective of the first experiment was to determine the optimum inorganic salt composition for growth of *Solanaceous* genotypes. The experiment was a factorial design. Four media were evaluated: 1) Murashige and Skoog salts (Murashige and Skoog, 1962) plus Gamborg vitamins (Gamborg et al., 1968) medium (MG): 2) Murashige and Skoog medium (MS): 3) Gamborg medium (GM): and 4) White medium (WM) (White, 1963). Two hormone regimes also were evaluated. The hormone regimes were: 2 mg/L of 2,4-D and 1 mg/L of BA: or 2 mg/L of IAA and 2 mg/L of BA. The pH of all media was adjusted to 5.7 before addition of 7.5 g/L of agar. The growth conditions were 16 hr of light and 25°C.

The experiment was initiated by transferring 100 mg of callus from Thomas and Pratt medium onto the experimental media. Callus weight was determined after 30 days, when the cultures were subcultured to fresh medium. Sixty days after initiation the number of shoots and their length were determined.

The Effect of Plant Growth Regulators on Shoot Regeneration

A second experiment was conducted to determine the optimum IAA concentration, and whether BA or 2iP (6-(dimethylallylamino) purine) provided the greatest shoot production. Calli were transferred onto MG medium containing different growth regulator concentrations. The cytokinin treatments were either: none, 2 mg/L BA, or 2 mg/L 2iP. Three IAA concentrations between 0 and 2.0 mg/L were also evaluated. The experiment was a factorial design. Calli were subcultured after 30 days and shoot number and length were determined after 60 days.

Thiamine Effects on Shoot Regeneration and Growth

A final experiment was conducted to determine the optimum concentration of thiamine for regeneration of *Solanaceous* shoots. Calli were transferred onto culture vessels containing 30 ml of MG medium with 0.2 mg/L IAA and 2mg/L BA. The experimental treatments were thiamine concentration and genotype. The MG medium contained thiamine ranging from 0 to 200 mg/L. Representative genotypes from the previous experiments were used. These include: *Solanum ptycanthum, Solanum nigrum, Lycopersicon esculentum* Diego, and *L. peruvianum* accessions PI 199380, PI 251301, and PI 128652. Shoot number and length were determined after 30 days.

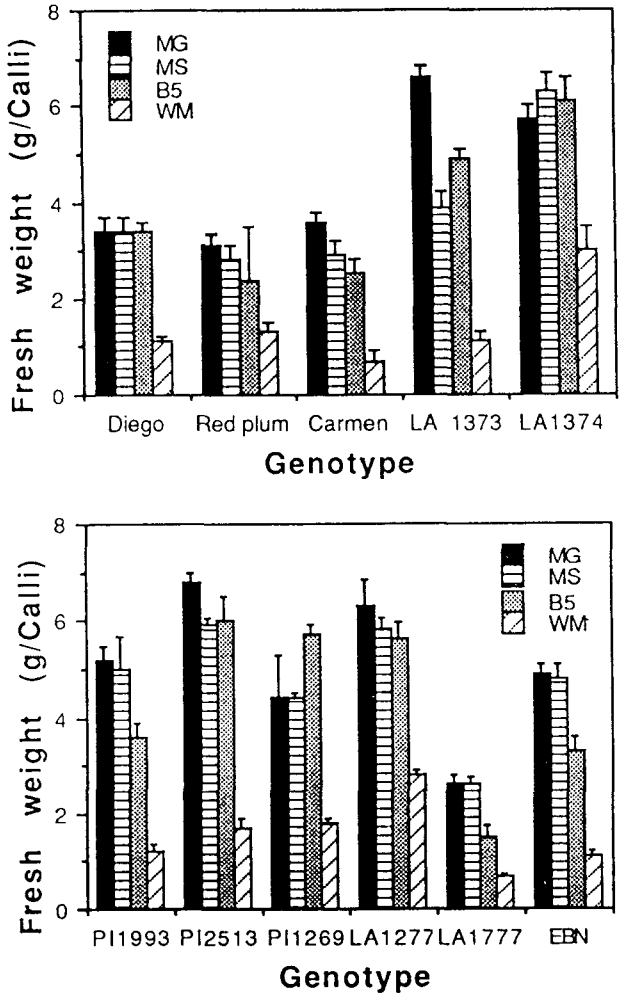

Figure 1. The effect of media on callus growth of *Solanum* and *Lycopersicon* genotypes. The weights were measured 30days after transferring. BA at 1 mg/l and 2, 4-D at 2 mg/l were the Plant growth regulators. The genotypes were *L. esculentum* cultivars Diego, Red plum, and Carmen: *L. peruvianum* accessions LA 1373, LA 1374, PI 199380, PI 251301, and PI 126945l *L. penelli* LA 1277: *L. hirsutum* LA 1777: and *Solanum ptycanthum* EBN. Bars represent the standard error of mean.

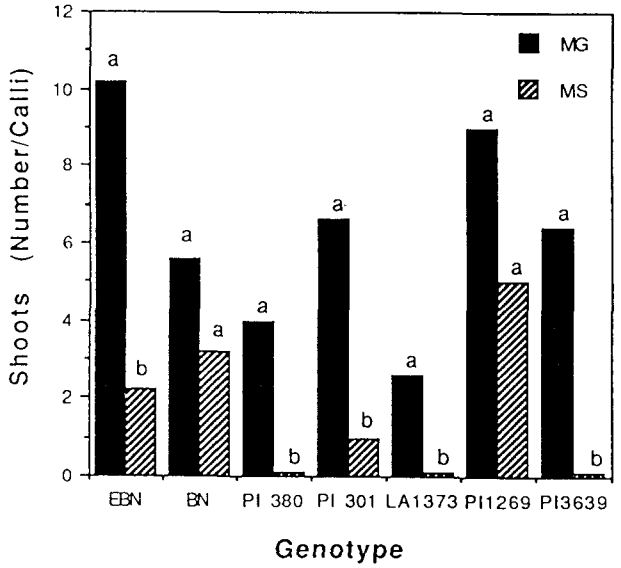

Figure 2. Effect of MG and MS medium on shoot regeneration of *Solanum ptycanthum* and *Lycopersicon* genotypes after 60 days of subculturing. Media contained 0.2 mg/l IAA and 2 mg/l BA. The genotypes were *Solanum ptycanthum* EBN: *Solanum nigrum* BN: *L. peruvianum* accessions PI 199380, PI 251301, LA 1373, PI 126945, and OI 363945. The same letters within genotypes are not significantly different at 5% level.

RESULTS AND DISCUSSION

Inorganic Salts Effects on Callus Formation and Shoot Differentiation.

Callus growth when averaged over all genotypes differed depending on media. MG and MS media were consistently the most effective in promoting callus growth while White medium was the least effective (Figure 1 and 3-A). The callus weight of the 11 genotypes averaged 3 times more on MG medium than on WM. Callus growth was also varied depending on the genotype. *L. penelli* LA 1277, L. peruvianum LA 1373 and PI 251301 had the best callus growth, averaging 6 g per callus, while *L. hirsutum* LA 1777, *L. esculentum* Diego, and Red Plum had the least growth, averaging 3 g per callus. Locy (1983) also reported that callus growth of *L. peruvianum* and *L. glandulosum* was better than that of *L. esculentum, L. hirsutum, L. pimpinellifolium,* and *L. cheesmannii.*

Shoot proliferation occurred on media containing IAA and BA. Differences in shoot production depended on the medium and genotype (Figure 2). *Solanum ptycanthum* and *Solanum nigrum* were regenerated both on MS and MG media (Figure 3-C). No genotypes regenerated on GM or WM. Overall, MG medium was more effective than MS in regenerating shoots. MG and MS media mainly differ in thiamine concentrations, with MG medium containing 100 times more thiamine than MS medium (MS contains 0.1 mg/L and MG contains 10 mg/L).

Effects of Plant Growth Regulators on Shoot Regeneration

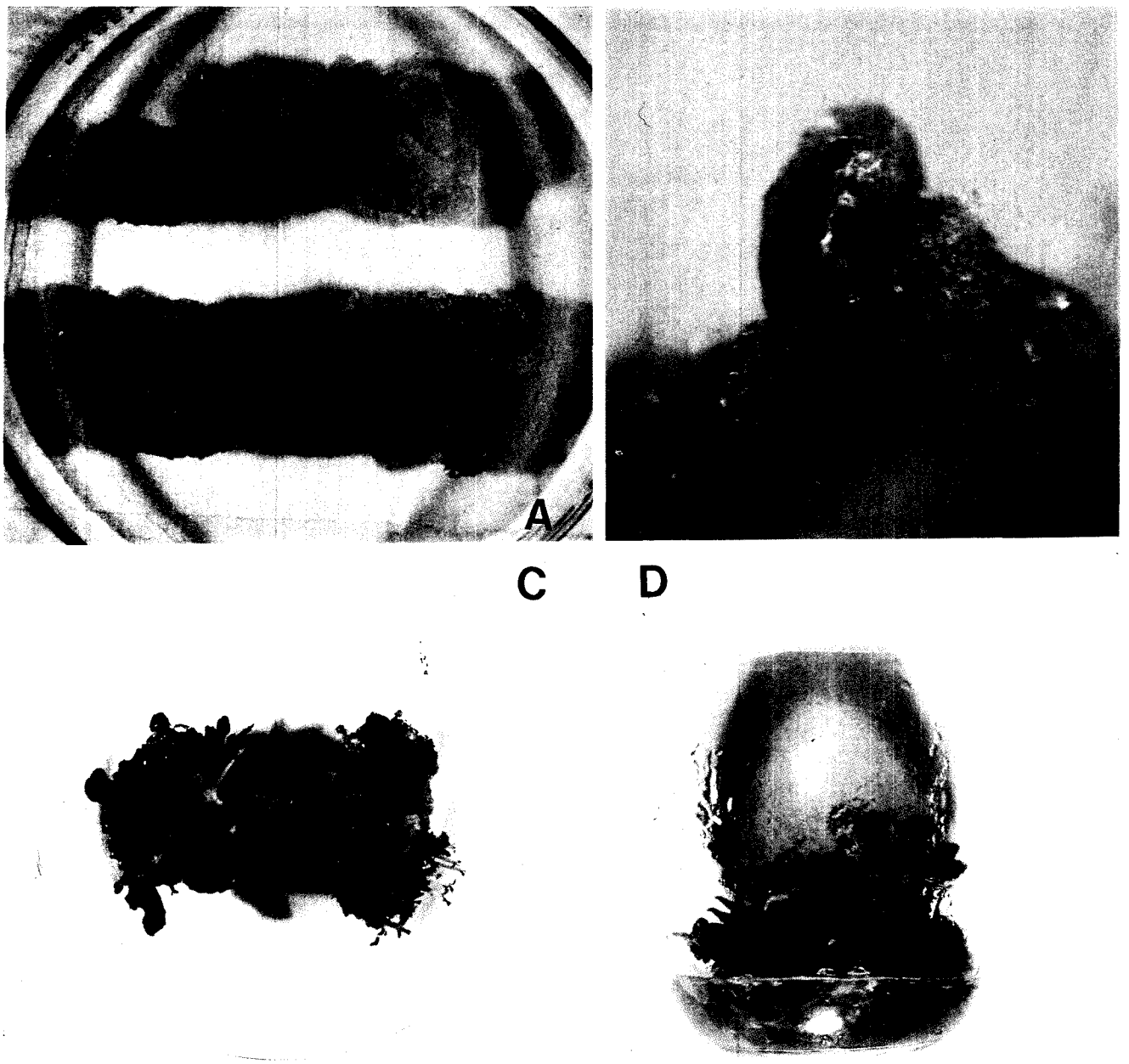

Figure 3. A: Callus proliferated on White (leaf above), Gamborg (right above), MS (left below), and MG (right below) medium. B: Shoot primordia induced from callus of *L. peruvianum* PI 199380. C: Plantlets regenerated from callus of *S. ptycanthum*. D: Multiple shoots regenerated from callus of *L. peruvianum*.

When calli were transferred from media with 2,4-D to regeneration media containing IAA and BA, the calli changed color from grey to green. Shoot primordia were initiated soon after the change in calli color (Figure 3-B). No shoots were formed on IAA alone without a cytokinin; but a cytokinin such as BA or 2iP without IAA did result in shoots (Table 1). In general, a low level of IAA with BA or 2iP resulted in the greatest number of shoots (Figure 3-D). Shoot regeneration was better with combinations of IAA and BA than combinations of IAA and 2iP. The optimum combination of hormones in this study was IAA at 0.2 mg/L and BA at 2.0 mg/L.

Many other studies have reported the optimal shoot regeneration media for *Solanaceous* species to contain low levels of IAA (0.2 to 1mg/L) and high levels of BA (2 to 5 mg/L) (Kurtz and Lineberger, 1983; DeLanghe and

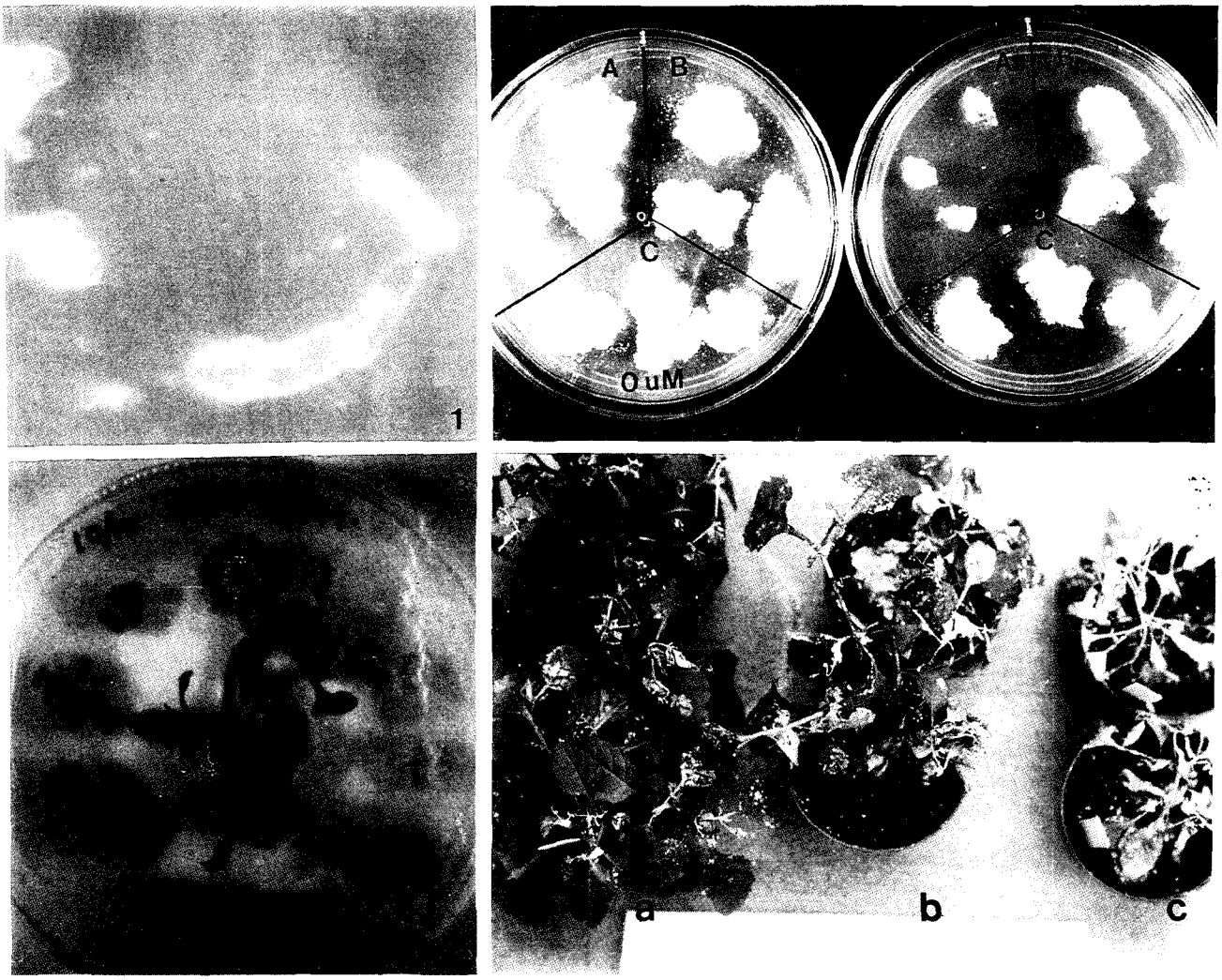

Figure 4. The suspension cultured cells of *Solanum ptycanthum* (1). The growth of callus on MS medium containing 0 μM and 2 μM(2). The cell lines are: unselected cell line (A): EBN-5 (B): EBN-26 (C). Regeneration of acifluorfen tolerant cell line on media with 4 μM acifluorfen (3). The segragation of acifluorfen tolerance in selfed progenies of an acifluorfen tolerant somaclone (EBN-12E) (4). a, b, and c represent tolerant, intermediate tolerant, and sensitive, respectively.

DeBruijne, 1976). Combinations of benzyladenine (BA) and indoleacetic acid (IAA) were more effective in inducing shoot differentiation than those of BA and naphthaleneacetic acid (NAA) (Kartha et al., 1976) or combinations of IAA and Kinetin (DeLanghe and DeBruijne, 1976).

Although, Kartha et al., (1976) reported IAA was better than synthetic auxins for differentiating shoots from leaf segments of tomato, in our study high concentrations of IAA decreased the number of shoots. Kurtz and Lineberger (1983) have also reported that low concentrations of IAA (0.2 or 1 mg/L) medium increased shoot regeneration, while high levels of IAA inhibited it. The reason for the difference in auxin response may be due to differences in culture age or the use of leaf segments by Kartha et al. (1976), while we used long-term callus cultures growing on 2,4-D containing medium.

The optimum hormone regime also depended on the genotype. Some genotypes such as those of *Lycopersicon esculentum* did not regenerate. For the genotypes regenerating on 0.2 mg/L IAA and 2.0 mg/L BA the number of shoots per callus ranged from 15 in *Lycopersicon peruvianum* PI 363945 to less than one shoot per callus in *L. peruvianum* LA 1373. Modifying the hormone regime did not overcome the inability to regenerate *L. esculentum* genotypes from

Table 1. Effect of different cytokinins and concentrations of IAA on shoot regeneration from callus of *Solanum* and *Lycopersicon* genotypes after 60 days.

Genotypea	No cytok[a]	mg/L BA IAA(mg/L)			2mg/L IPA			LSD
	0.2	2.0	0	0.2	2.0	0	0.2	2.0	5%
	(Number / Calli)								
S. ptycanthum	0	0	0.8	10.4	9.8	1.2	2.4	5.6	2.7
S. nigrum	0	0	1.6	7.0	5.8	1.4	2.2	2.2	1.2
L. peruvianum									
LA 1373	0	0	1.7	0.6	0.4	0.0	1.6	2.2	1.1
PI 126945	0	0	0.0	5.0	2.4	0.0	4.5	0.0	1.2
PI 199380	0	0	3.3	14.2	5.4	1.3	8.4	4.0	1.6
PI 251301	0	0	1.0	6.8	5.0	2.0	2.0	1.7	1.1
PI 363945	0	0	1.2	15.0	5.2	0.8	9.8	2.6	1.9
LSD 5%	0	0	1.0	2.4	2.5	1.0	1.6	2.5	

[a] The medium contains no cytokinins.

Table 2. Effect of different levels of thiamine on the number of shoot from callus of *Solanum* and *Lycopersicon* after 30 days. The media consisted of MG medium with 0.2 mg/L and 2 mg/L BA.

Genotypea	Thiamine (mg/L)								LSD
	0	0.01	1	10	20	30	60	200	5%
	No. of Shoot								
S. ptycanthum	8.0	6.4	8.2	10	17.3	11.3	8.0	6.1	3.0
S. nigrum	1.6	3.6	3.7	6.6	7.9	2.3	3.9	2.1	4.1
L. peruvianum									
PI 199380	1.4	0.7	2.9	2.3	6.8	7.1	2.2	1.7	2.3
PI 251301	0.9	1.3	1.7	2.0	1.8	2.9	1.7	1.3	2.9
PI 126945	2.8	4.7	5.8	4.2	2.9	3.4	0.3	1.7	1.2
PI 128652	0.7	0.3	0.6	3.0	0.3	0.0	2.3	0.0	1.0
LSD 5%	3.5	2.6	2.9	2.7	3.3	3.3	2.2	1.7	

[a] The medium contains no cytokinins.

Table 3. Effect of thiamine concentration on shoot growth of plants initiated from callus of *Solanum* and *Lycopersicon* genotypes. Shoot length was determined 30 days after subculturing.

Genotype	Thiamine (mg/L)								LSD
	0	0.01	1	10	20	30	60	200	5%
	Shoot Length (mm)								
S. ptycanthum	7.3	7.7	14.1	10.9	11	13	4.8	6.6	4.6
S. nigrum	3.3	5.2	5.1	8.6	13.4	5.4	5.7	4.3	7.3
L. peruvianum									
PI 199380	2.2	3.0	4.2	4.2	9.8	10.3	3.0	2.3	4.4
PI 251301	1.1	1.8	4.1	5.0	1.0	3.9	2.2	2.3	2.9
PI 126945	2.2	4.4	6.6	6.0	7.0	4.0	0.5	4.0	3.3
PI 128652	4.0	1.0	1.0	6.0	0.4	0.0	3.0	0.0	3.2
LSD 5%	4.9	3.9	4.6	5.2	6.7	5.0	3.3	3.6	

long-term callus culture. Compared to L. esculentum, Locy (1983) also reported *L. peruvianum* was much easier to regenerate from long-term callus culture. The regenerative capacity of *L. esculentum* was lost much sooner after initiation of long-term callus culture than *L. peruvianum*. This loss may be due to characteristics of the *L. esculentum* genome which causes different cellular development in culture. *L. esculentum* cells might require very specific cultural conditions for regeneration whereas culture conditions for regeneration of *L. peruvianum* may be far less (Koornneef et al., 1987). Reduced sensitivity of *L. esculentum* cells to exogeneously applied auxins or cytokinins may also contributed to a decline in the morphogenetic response (Wochock and Wetherell, 1972). Muhlback (1980) reported an imbalance of regulating factors which are produced or accumulated during long-term cultures as the cause of the fail of regeneration from protoplast-derived calli in tomato.

Thiamine Effect on Shoot Regeneration and Growth

When media were compared, we found that Murashige and Skoog salts with Gamborg vitamins were more effective for shoot regeneration of *Solanaceous* species than Murashige and Skoog vitamins. The main difference between these two vitamin formulas were the amount of thiamine. In this study, we also found that shoot regeneration depended on thiamine levels (Table 2). Most *Solanaceous* genotypes produced more shoots on high levels of thiamine (10 to 30 mg/L) than low levels of thiamine (0.01 to 1 mg/L). Modifying the thiamine concentration of the media did not overcome the inability of *L. esculentum* 'Diego' to regenerate. *Solanum ptycanthum*, *Solanum nigrum*, and *L. peruvianum* PI 199380 produced the most shoots with 20 mg/L thiamine. Regeneration of *L. peruvianum* PI 251301 and PI 128652 was better on medium with 30 mg/L and 10 mg/L, respectively. *L. peruvianum* PI 126945 did not have an optimum thiamine concentration for

regeneration. Increasing thiamine concentrations above 60 mg/L resulted in fewer and shorter shoots. Shoot growth varied depending on thiamine concentration and genotype (Table 3). In general, the optimum thiamine concentration for shoot growth was similar to the optimum concentration for shoot production.

Many researchers have found that an external supply of thiamine was beneficial for growth of suspension (Ohira et al., 1976) and long-term callus cultures (Ohira et al., 1973). Thiamine was also reported to be essential for tobacco callus growth. Linsmaier and Skoog (1965) reported that tobacco callus yields were consistently higher and the appearance of the tissue was improved after treatment with thiamine. The requirement for thiamine in our culture could be because of reduced biosynthesis. Nishi (1974) reported that ephera callus cultures which lost their biosynthesis ability, could not grow without thiamine in the media.

In our study, callus formation, plant regeneration, and shoot growth differed depending on the genotype, media, growth regulator concentration, and thiamine concentration. If combinations of growth regulators and thiamine were optimized, it would be possible to get more efficient plant regeneration and growth from long-term callus cultures. This improved regeneration could be important when callus cultures were used in selection.

적 요

일반적으로 캘러스상태로 오랫동안 계대배양시 식물체분화능력이 상실되거나 감소한다. 캘러스배양이나 현탁배양을 이용하여 제초제 및 각종 저항성 식물체를 선발하고저 할 때 장시간이 소요되므로 장기간 계대배양된 세포나 캘러스로 부터 식물체를 분화시키는 체계를 확립하는 것이 중요하다. 따라서 본 실험은 2년 이상 캘러스상태로 기내에서 장기간 계대배양한 수종의 *Solanum*과 *Lycopersicon*종으로부터 캘러스 생장 및 식물체분화에 미치는 무기염류, 생장조절물질 및 타이아민의 효과를 조사하였다. 캘러스 생장 및 식물체분화에는 MG배지가 다른 MS, Gamborg, White배지보다 더 효과적이었으며 낮은 농도의 IAA와 높은 농도의 BA가 조합처리 되었을때 높은 식물체분화율을 보였다. 타이아민이 첨가되었을때가 무처리보다 식물체분화와 생장을 증가시켰으며 타이아민의 농도가 증가함에 따라 식물체분화와 생장을 증가시켰으며 그 농도는 10 mg/L에서 30 mg/L까지가 적합한 것으로 보였다.

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