Original Papers Plant Tissue Culture Letters, **13**(1), 23-27(1996)

High Frequency Plant Regeneration from Leaf Calli in Sweet Potato cv. Chugoku 25

Motoyasu OTANI*, Masahiro MII** and Takiko SHIMADA*

* *Research Institute of Agricultural Resources, Ishikawa Agricultural College,* Nonoichi-machi, Ishikawa 921, Japan
** *Laboratory of Plant Cell Technology, Department of Horticulture, Chiba University,* 648 Matsudo, Chiba 271, Japan

(Received March 4, 1995)
(Accepted October 21, 1995)

High frequency plant regeneration was established from leaf calli of sweet potato (*Ipomoea batatas* (L.) Lam.) cv. Chugoku 25. Calli were formed from leaf segment on LS medium supplemented with 0. 5 mg/*l* 2, 4-D, 3, 000 mg/*l* yeast extract, 5% (W/V) sucrose and 0. 25% (W/V) gellan gum. Plant regeneration occurred at the frequency of more than 25% by transferring the calli onto the regeneration medium which was LS basal medium without any plant growth regulators. The presence of either abscisic acid (ABA) or silver nitrate ($AgNO_3$) in the callus induction medium promoted shoot regeneration. The optimum concentration was 2 mg/*l* for both ABA and $AgNO_3$, the frequency of plant regeneration being 70 and 73. 3%, respectively.

Introduction

Plant regeneration from calli and tissue cultures is an important step for genetic manipulation in sweet potato. Since sweet potato leaves have a high susceptibility to infection with various wild strains of *Agrobacterium rhizogenes*[1], leaf tissue is also expected to be a suitable material for infection with *Agrobacterium tumefaciens*. Although plant regeneration from leaf tissue-derived callus cultures of sweet potato has already been reported[2,3], low plant regeneration frequencies obtained in these studies may hamper the transgenic plant formation by utilizing disarmed *Agrobacterium tumefaciens* as a vector.

In this paper, we present an efficient plant regeneration method from leaf calli of sweet potato cv. Chugoku 25.

Materials and Methods

1. *Plant materials*

Chugoku 25, a cultivar of *Ipomoea batatas* (L.) Lam. was used. To obtain *in vitro* plants of sweet potato, meristem culture wes performed according to the method of Otani *et al.* (1987). *In vitro* plants were grown on LS medium[4] containing 0. 25% (W/V) gellan gum and lacking plant growth regulators under 4, 800 lux fluorescent light for 16 h at 26°C.

2. *Leaf callus induction and shoot regeneration*

The apical third to fifth fully expanded leaves of *in vitro* plants were cut into rectangular pieces about 10 mm long. These leaf pieces were inoculated for callus induction onto LS medium supplemented with 0. 5 mg/*l* 2, 4-dichlorophenoxyacetic acid (2, 4-D), 3, 000 mg/*l* yeast extract, 5% (W/V) sucrose and 0. 25% gellan gum. In some experiments, various concentrations of abscisic

acid (ABA) (0, 0.5, 2 and 5 mg/l) and $AgNO_3$ (0, 0.2, 5 and 10 mg/l) were added in this callus inducion medium and/or shoot regeneration medium to examine their effect on shoot regeneration. For shoot regeneration, calli formed from leaf explants were transferred onto LS medium supplemented with various concentrations (0, 2, 5 and 10 mg/l) of 6-benzyladenine (BA), 3% (W/V) sucrose and 0.25% (W/V) gellan gum. Plant growth regulators and $AgNO_3$ were added to the media prior to autoclaving and the pH of the media was adjusted to 5.9. The cultures were incubated at 26°C under 4,800 lux fluorescent light for 16 h.

The adventitious shoots regenerated were transferred onto 0.8% (W/V) agar-solidified LS medium without any growth regulators. When the shoots of these plantlets became 100 to 150 mm long, they were transferred to pots containing a 2: 1 (V/V) ratio of, vermiculite and perlite mixture and placed in a container covered with transparent plastic film to maintain a high relative humidity. They were watered twice a week and maintained at 26°C under a 16-h photoperiod with fluorescent lamps (14,000 lux) in a growth chamber for 30 days.

Results and Discussion

1. *Effect of BA on shoot regeneration from leaf calli*

Friable calli pale yellow in color were initiated within 7 days from almost all of the leaf explants

Table 1. Effect of BA on shoot regeneration from leaf callus of sweet potato cv. Chugoku 25.

BA concentration of regeneration medium*[1] (mg/l)	No. of calli transferred*[2]	No. of calli forming shoots (%)
0	30	14 (46.7)
2	50	17 (34.0)
5	23	8 (34.8)
10	24	3 (12.5)

*[1] Regeneration medium: LS medium supplemented with 30 g/l sucrose and 2.5 g/l gellan gum.

*[2] Leaf calli were produced on LS medium supplemted with 0.5 mg/l 2, 4-D, 3,000 mg/l yeast extract, 50 g/l sucrose and 2.5 g/l gellan gum.

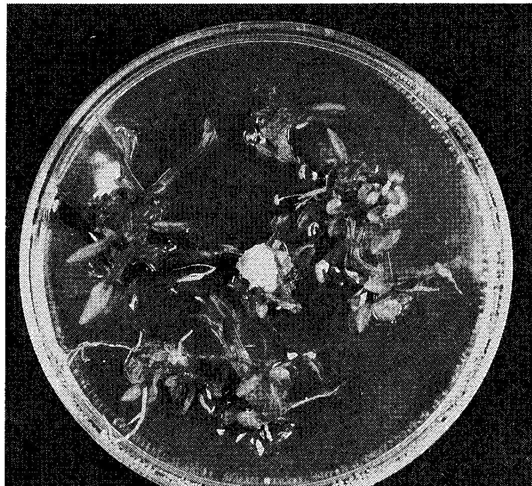

Fig. 1 Adventitious shoot formation from leaf calli of sweet potato cv. Chugoku 25 on LS medium without any plant growth regulators.

on LS medium containing 0. 5 mg/*l* 2, 4-D and 3, 000 mg/*l* yeast extract.

Shoot regeneration from callus cultures is generally induced by the addition of cytokinin to the culture medium[5]. In *Ipomoea trichocarpa*, a wild relative of sweet potato, our previous study also showed that shoot regeneration from leaf-calli was only induced on the media containing more than 2 mg/*l* BA, and that 10 mg/*l* was the most effective[6]. In the present experiment on the effect of BA, however, the highest frequency (46. 7%) of shoot regeneration from leaf-calli was obtained in the medium without BA and the addition of BA to the regeneration medium acted inhibitorily with the increase of its concentration (**Table 1, Fig. 1**). These findings suggest that leaf calli of sweet potato contained enough endogenous cytokinin and that exogenous supply of a supra-optimum concentration of cytokinin inhibited the shoot regeneration.

2. *Effect of ABA on shoot regeneration from leaf calli*

Calli formed from leaf explants on callus induction media containing various concentrations of ABA (0, 0. 5 2 and 5 mg/*l*) were transferred onto the shoot regeneration medium which was LS medium containing no plant growth regulators. Neither callus induction nor growth were affected by the addition of ABA to the callus induction medium. However, the addition of ABA was effective for shoot regeneration especially at 2-5 mg/*l* (**Table 2**). In both *I. trichocarpa* and *I. trifida*, we also obtained similar results in which shoot formation from the calli was achieved only when the callus induction medium containing 5 mg/*l* ABA was used (unpublished data). Therefore, it seemed that the presence of ABA in the callus induction medium might be an important factor for promoting shoot regeneration from leaf calli in the *Ipomoea* genus.

Table 2. Effect of ABA on shoot regeneration from leaf callus of sweet potato cv. Chugoku 25.

ABA concentration of callus induction medium*1 (mg/*l*)	No. of calli transferred*2	No. of calli forming shoots (%)
0	50	13 (26. 0)
0. 5	50	24 (48. 0)
2	50	35 (70. 0)
5	50	31 (62. 0)

*1 Regeneration medium: LS medium supplemented with 30 g/*l* sucrose and 2. 5 g/*l* gellan gum.

*2 Leaf calli were produced on LS medium supplemented with 0. 5 mg/*l* 2, 4-D, 3, 000 mg/*l* yeast extract, 50 g/*l* sucrose and 2. 5 g/*l* gellan gum.

3. *Effect of $AgNO_3$ on shoot regeneration from leaf calli*

Callus induction and callus growth were not affected by the addition of $AgNO_3$ to the callus induction medium. By contrast, the presence of 2 mg/*l* $AgNO_3$ in the callus induction medium was effective for promoting shoot regenertion from the calli (**Table 3**). However, higher concentrations of $AgNO_3$ (more than 5 mg/*l*) in the callus induction medium had no or rather an inhibitory effect on the regeneration. The beneficial effect of $AgNO_3$ has been reported in pollen embryo formation from anther cultures of Brussels sprouts[7,8] and tetraploid wheat[9], shoot regeneration from callus cultures of wheat, *Nicotiana plumbaginifolia*[10] and maize[11], and direct shoot regeneration from cotyledonary explants of Chinese cabbage[12] and sunflower[13]. In those plant species, the stimulation of morphogenesis occurred by the addition of 1. 3 to 17 mg/*l* $AgNO_3$. $AgNO_3$ is known to be a potent inhibitor of ethylene action in plants[14]. Therefore, endogenous ethylene of the leaf explants of sweet potato may have acted inhibitorily to the induction of calli with good regeneration

Table 3. Effect of $AgNO_3$ on shoot regeneration from leaf callus of sweet potato cv. Chugoku 25.

Concentration of $AgNO_3$ in the medium		No. of calli transferred	No. of calli forming shoots
Callus induction medium*[1]	regeneration medium*[2]		
(mg/l)	(mg/l)		(%)
0	0	53	14(26.4)
0	2	31	5(16.1)
0	5	29	7(24.1)
0	10	30	4(13.3)
2	0	30	22(73.3)
5	0	25	6(24.0)
10	0	25	7(28.0)
2	2	32	21(65.6)
5	5	54	8(14.8)
10	10	33	6(18.2)

*[1] Callus induction medium: LS medium supplemented with 0.5 mg/l 2, 4-D, 3, 000 mg/l yeast extract, 50 g/l sucrose and 2.5 g/l gellan gum.

*[2] Plant regeneration medium: LS medium supplemented with 30 g/l sucrose and 2.5 g/l gellan gum.

ability, which might be overcome by $AgNO_3$ in the callus induction medium.

4. *Characterization of regenerated plants*

All of regenerated plants survived with this acclimatization procedure. Among 100 regenerated plants obtained, only one plant was morphologically abnormal. This abnormal plant showed dwarfness(**Fig. 2-a**) and failed to produce tuberous roots(**Fig. 2-b**). Despite this abnormal plant, the frequency of the somaclonal variation seems to be relatively low. Therefore, the plant

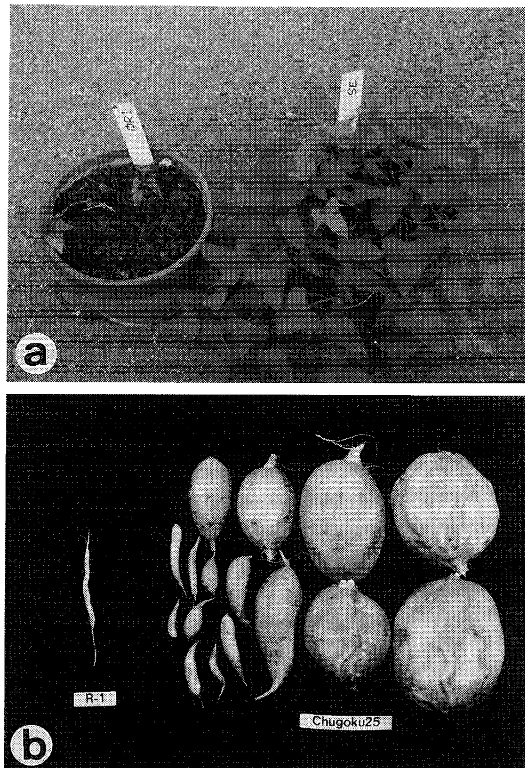

Fig. 2 (a) Aerial parts and (b) tuberous roots of a dwarf mutant regenerated from leaf callus (left) and control plant (right) of Chugoku 25.

regneration system established in this study could be efficiently used for genetic transformation studies.

References

1) Otani, M., M. Mii, T. Handa, H. Kamada, T. Shimada, 1994. Plant Sci., **94**: 151-159.
2) Sehgal, C. B., 1975. Beitr. Biol. Pflanzen, **51**: 47-52.
3) Liu, J. R., D. J. Cantliffe, 1984. Plant Cell Rep., **3**: 112-115.
4) Linsmaier, E. M., F. Skoog, 1965. Physiol. Plant., **18**: 100-127.
5) Flick, C. E., D. A. Evans, W. R. Sharp, 1983. In "Handbook of Plant Cell Culture" Volume 1 (eds. by Evans, D. A., W. R. Sharp, P. V. Ammirato, Y. Yamada), p. 13-81, Macmillan, New York.
6) Otani, M., T. Shimada, 1988. Japan. J. Breed., **38**: 205-211.
7) Biddington, N. L., R. A. Sutherland, H. T. Robinson, 1988. Ann. Bot., **62**: 181-185.
8) Ockendon, D. J., R. McClenaghan, 1993. Plant Cell Tissue Organ Cult., **32**: 41-46.
9) Ghaemi, M., A. Sarrafi, G. Alibert, 1994. Plant Cell Tissue Organ Cult., **36**: 355-359.
10) Purnhauser, L., P. Medgyesy, M. Czako, P. J. Dix, L. Marton, 1987. Plant Cell Rep., **6**: 1-4.
11) Songstad, D. D., D. R. Duncan, J. M. Widholm, 1988. Plant Cell Rep., **7**: 262-265.
12) Chi, G. L., E. C. Pua, 1989. Plant Sci., **64**: 243-250.
13) Chraibi, B. K. M., A. Latche, J. P. Roustan, J. Fallot, 1991. Plant Cell Rep., **10**: 204-207.
14) Beyer, E. M., 1976. Plant Physiol., **58**: 268-271.

《和文要約》

サツマイモ品種中国25号の葉切片由来カルスからの高頻度な植物体再生

大谷基泰*・三位正洋**・島田多喜子*

* 石川県農業短期大学 農業資源研究所
** 千葉大学 園芸学部

サツマイモ品種中国25号の葉切片由来カルスからの植物体再分化条件を検討した．0.5 mg/l 2, 4-D, 3, 000 mg/l yeast extract, 5%(W/V)sucrose および 0.25%(W/V)gellan gum を添加した LS 培地上で葉切片よりカルスを誘導した．これらのカルスを植物生長調節物質を添加していない LS 培地に移植したところ，25%以上のカルスから不定芽が形成された．再分化培地への BA の添加はカルスからの不定芽形成を促進しなかった．カルス培地に ABA もしくは $AgNO_3$ を添加することによって，カルスからの不定芽の再分化が著しく向上した．ABA(2 mg/l) または $AgNO_3$(2 mg/l) を添加した培地で不定芽形成率は最も高く，それぞれ 70% と 73.3% であった．葉片由来カルスから再分化した植物体100個体の形質を調査したところ，形態的に異常なものが1個体だけ得られたが，それ以外は全て正常であった．