BIOLOGIA PLANTARUM 50 (1): 114-117, 2006

BRIEF COMMUNICATION

Effects of medium composition and culture duration on *in vitro* morphogenesis of sweet potato

L. CHEN*, A. BHAGSARI and J. CARTER

Agricultural Research Station, Fort Valley State University, Fort Valley, GA 31030, USA

Abstract

In vitro morphogenesis of sweet potato (*Ipomoea batatas*) shoot explants after cultures in callus initiation medium (CIM) with two sucrose contents and plant regeneration medium (PRM) with three growth regulator combinations for different durations was studied. After 4 weeks, explants on 5 % sucrose CIM had significantly more shoots but similar or lower root fresh mass and callus fresh mass than those on 3 % sucrose CIM subsequent to transfer for 6 weeks on all three PRM. Cultures transferred to growth regulator-free PRM after 4 and 12 weeks on 5 % sucrose CIM formed plants through organogenesis and embryogenesis, respectively. Embryogenic cultures from 4 weeks on CIM + 10 weeks on callus proliferation medium when transferred to PRM without growth regulator for 4 and 8 weeks produced multiple embryos in the prior and both embryos and shoot buds in the later.

Additional key words: callus growth, *Ipomoea batatas* L. Lam, root growth, shoot regeneration.

Genetic improvement of sweet potato through conventional breeding has been limited by its hexaploid nature and incompatibility (Jones *et al.* 1986). Biotechnological tools such as tissue culture and genetic transformation may be useful for circumventing these problems (Perera and Ozias-Akins 1991, Gama *et al.* 1996). There are several reports of *in vitro* embryogenesis and organogenesis in sweet potato. Plantlets have been regenerated *via* somatic embryogenesis from anther culture (Tsay and Tseng 1979), lateral buds and shoot apices (Liu and Cantliffe 1984, Chee and Cantliffe 1988, Al-Mazrooei *et al.* 1997, Lee *et al.* 2002) and roots (Jarret *et al.* 1984). Sweet potato shoots were obtained through organogenesis from root discs (Carswell and Locy 1984) and petioles (Gosukonda *et al.* 1995).

Effects of the variation in the amounts of sucrose in callus initiation medium (CIM) on regeneration of explants are not known. *In vitro* regeneration methods reported so far produce sweet potato plant by either organogenesis or somatic embryogenesis but not both. Therefore, the present study was conducted to: *1*) assess the influence of sucrose content in the CIM on callus growth, root growth, and shoot regeneration, and *2*) examine if by varying the length of culture during different stages of growth calli could be induced to regenerate by organogenesis, embryogenesis or both.

Explants were obtained from shoot apical meristems (apical dome + 1 - 3 leaf primordia, approximately 0.5 - 1.0 mm in length) of sweet potato [*Ipomoea batatas* (L.) Poir. *ex* Lam] genotype 75-96-1 (from Coastal Plain Experiments Station, Tifton, Georgia). The basal medium was formulated according to Murashige and Skoog (1962; MS). All media were adjusted to pH 5.7 prior to autoclaving at 121 °C for 20 min, solidified with *Phytagel* (0.3 % m/v) (*Sigma Chemical Co.*, St. Louis, USA). Subculture was made every 3 - 4 weeks, unless otherwise indicated. Cultures were incubated in growth chamber at 25 ± 3 °C under 16-h photoperiod (irradiance of 50 μmol m^{-2} s^{-1}).

Received 27 October 2003, *accepted* 3 November 2004.

Abbreviations: CIM - callus induction medium; 2,4-D - 2,4-dichlorophenoxyacetic acid; 2iP - N-isopentenyladenine; KIN - kinetin; NAA - α-naphthaleneacetic acid; PRM - plant regeneration medium; TDZ - thidiazuron.

Acknowledgments: Financial support provided by USDA/CSREES and Agricultural Research Station, Fort Valley State University, GA, USA. The authors are grateful to Drs. Mack Nelson and Bharat Singh for their valuable suggestions.

* Author for correspondence; fax: (001) 478-825-6376, e-mail: chenl@fvsu.edu

To determine the effects of sucrose contents on sweet potato regeneration, shoot tip explants were cultured on CIM containing 8.1 μM α-naphthaleneacetic acid (NAA) + 1.2 μM kinetin (KIN) with 3 % and 5 % sucrose for 4 weeks. The 4-week calluses were transferred onto three different plant regeneration media (PRM) with 2 % sucrose. The three media were: *1*) growth regulator-free, *2*) 0.12 μM N-isopentenyladenine (2iP) + 0.11μM thidiazuron (TDZ), *3*) 0.009 μM 2,4-dichlorophenoxyacetic acid (2,4-D) + 0.009 μM KIN. After 6 weeks on PRM, data were collected on average percent shoot regeneration, average root fresh mass, and average callus fresh mass per explant.

To evaluate the influence of callus initiation duration on morphogenesis pattern, shoot tip explants were cultured on CIM with 5 % sucrose for 4 and 12 weeks. Cultures were then transferred onto growth regulator-free PRM with 2 % sucrose for plant regeneration. Somatic embryogenesis and organogenesis was determined after 6 weeks.

To assess the impact of culture duration during plant regeneration phase on morphogenesis pattern, 14-week somatic embryogenic cultures from shoot tip explants were cultured on the CIM with 5 % sucrose for 4 weeks and on callus proliferation medium (CPM, 5.4 μM NAA + 4.6 μM KIN + 5 % sucrose) for 10 weeks. Afterward, they were transferred onto growth regulator-free PRM with 2 % sucrose and cultured for 4 or 8 weeks without subculture.

Data were analyzed by analysis of variance (*ANOVA*) using Statistical Analysis System (SAS Institute 2000). Mean values of the data were separated with the least significant difference method at $P < 0.05$.

After 4 weeks, explants on 5 % sucrose CIM had fewer roots and more friable calli than on 3 % sucrose CIM. When transferred onto PRM, calli initiated on 5 % sucrose CIM regenerated significantly more shoots than those initiated on 3 % sucrose in all three PRM (Table 1). The shoot regeneration of explants firstly grown on CIM with 5 % sucrose and transplanted on PRM with no growth regulator, PRM + 2,4-D + KIN, and PRM + 2iP + TDZ PMR was 72.2, 59.6, and 51.5 %, respectively. Huang and Liu (1998) have also observed increased rice shoot regeneration with a high sucrose concentration in medium. However, root fresh mass of regenerants on all PMR was significantly greater when explants were grown on CIM with 3 % sucrose than 5 % sucrose. Also, callus fresh masses of explants from 3 % sucrose medium were significantly higher than those from the 5 % sucrose medium. Similarly, a high sucrose concentration in culture medium decreased rice callus mass (Al-Khayri and Al-Bahrany 2002).

Results showed that the duration of culture of explants on CIM determined whether they would undergo organogenesis or embryogenesis. Plant regenerated through organogenesis in explants cultured for only 4 weeks on 5 % sucrose CIM, but through embryogenesis when the duration was increased to 12 weeks. The cells capable of division and proliferation were generated from shoot tips on 5 % sucrose CIM. After a culture period of 4 weeks, slow growing, flat, and compactly tight calli were formed. When these calli were transferred onto growth regulator-free PRM, plants were obtained *via* organogenesis (Fig. 1). But when the calli were subcultured onto fresh 5 % sucrose CIM and cultivated for further 8 weeks, they appeared nodular, embryogenic-like, friable, and bright yellow. Upon transfer to the growth regulator-free PRM, these calli regenerated *via* somatic embryogenesis (Fig. 2). The frequency of explants developing embryos was only 42.1 % compared to 100 % in case of organogenesis. Though the frequency of plant regeneration is higher through organogenesis, somatic embryogenesis is still preferred as it is more suitable for genetic transformation and results in higher number of plants. Results of this study agree with the findings of Ranch and Pace (1988) and (Carman 1990) that the plant regeneration is an archetypal event and shoot organogenesis is only a modification of the process of embryo induction and development. The length of the initial culture treatment with KIN + NAA modified the proportions among the different types of morphogenic structures was also reported in quince (D'Onofrio and Morini 2003/4).

Explants cultured on 4-week CIM + 10-week CPM

Table 1. Effect of sucrose content in callus initial medium (CIM) and growth regulators in plant regeneration medium (PRM) on the average percent shoot regeneration (APSR), average root fresh mass (ARFM), average callus fresh mass (ACFM) of sweet potato in plant regeneration medium (PRM). Means marked with the same letter in the same plant regeneration medium are not significantly different according to the least significant difference test ($P < 0.05$).

Sucrose [%]	Growth regulators in PRM	[μM]	Number of explants	Shoot regeneration [%]	Root fresh mass [mg explant^{-1}]	Callus fresh mass [mg explant^{-1}]
5	0	0	39	72.2^{a}	147.7^{a}	593.0^{a}
	2iP + TDZ	0.120 + 0.110	37	51.5^{a}	100.3^{a}	820.7^{b}
	2,4-D + KIN	0.009 + 0.009	37	59.6^{a}	143.7^{a}	610.7^{a}
3	0	0	39	4.9^{b}	160.7^{a}	750.7^{b}
	2iP + TDZ	0.120 + 0.110	38	8.3^{b}	203.0^{b}	783.7^{b}
	2,4-D + KIN	0.009 + 0.009	39	13.5^{b}	221.3^{b}	886.3^{b}

when transferred on growth regulator-free PRM for 4 weeks, regeneration occurred via embryogenesis but if the duration was extended to 8-weeks, plants were produced through both embryogenesis (Fig. 3) and organogenesis (Fig. 4). In tissues maintained on PRM for 8 weeks, both multiple embryos and multiple shoot buds (Fig. 4) developed around 7th week. However multiple shoot buds later developed into abnormal plantlets (Fig. 5). According to Brown *et al.* (1989) and Brown *et al.* (1995) embryogenesis and organogenesis are competing organogenic events.

In summary, results indicated that higher sucrose content in CIM enhanced callus friability, suppressed root growth, but significantly improved shoot regeneration in sweet potato. It was also observed that the morphological development pattern can be altered by changing the periods of culture on different growth stage medium.

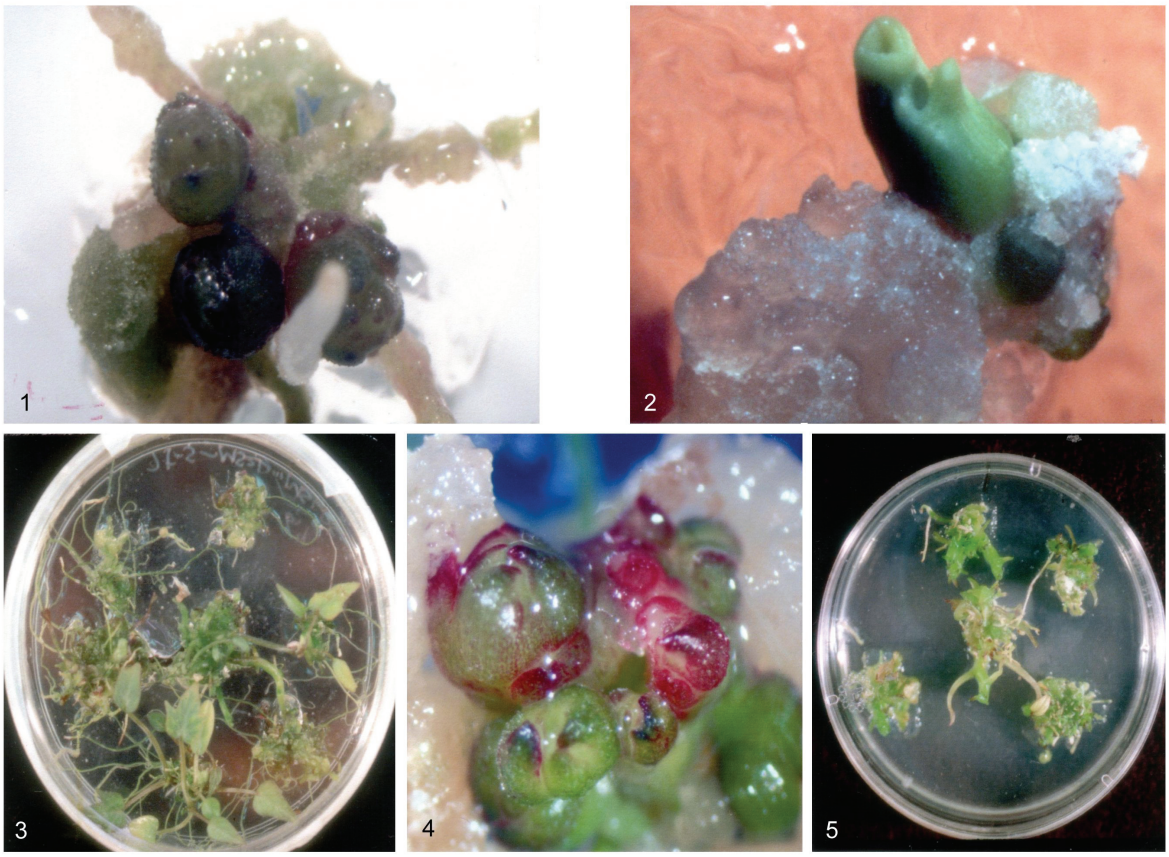

Fig. 1. Shoot buds emerged after the 4-week callus cultures from shoot tip of sweet potato explants transferred onto plant regeneration medium (PRM).
Fig. 2. Somatic embryos were produced after the 12-week callus cultures from shoot tip of sweet potato explants transferred onto plant regeneration medium (PRM).
Fig. 3. Somatic embryos in sweet potato 14-week embryogenic cultures converted into normal plantlets after 4 week on plant regeneration medium (PRM).
Fig. 4. Multiple shoot buds were produced from sweet potato 14-week embryogenic cultures after 8 week on plant regeneration medium (PRM).
Fig. 5. Multiple shoot buds developed into abnormal plantlets.

References

Al-Khayri, J.M., Al-Bahrany, A.M.: Callus growth and proline accumulation in response to sorbitol and sucrose-induced osmotic stress in rice. - Biol. Plant. **45**: 609-611, 2002.

Al-Mazrooei, S., Bhatti, M.H., Henshaw, G.G., Tayor, N.J., Blakesley, D.: Optimization of somatic embryogenesis in fourteen cultivars of sweet potato [*Ipomoea batatas* (L.) Lam.]. - Plant Cell Rep. **16**: 710-714, 1997.

Brown, D.C.W., Finstad, K.I., Watson, E.M.: Somatic embryogenesis in herbaceous dicots. - In: Thorpe, T.A. (ed.): *In Vitro* Embryogenesis in Plants. Pp. 245-415. Kluwer Academic Publishers, Dordrecht 1995.

Brown, F.C., Brooks, F.J., Pearson, D., Mathias, R.J.: Control

of embryogenesis and organogenesis in immature wheat embryo callus using increased medium osmolarity and abscisic acid. - J. Plant Physiol. **133**: 727-733, 1989.

Carman, J.G.: Embryogenic calls in plant tissue cultures: occurrence and behavior. - *In Vitro* cell. dev. Biol. Plant **26**: 746-753, 1990.

Carswell, G., Locy, R.D.: Roots and shoot initiation by leaf, stem, and storage root explants of sweet potato. - Plant Cell Tissue Organ Cult. **3**: 229-236, 1984.

Chee, R.P., Cantiliffe, D.J.: Selective enhancement of *Ipomoea batatas* Poir. embryogenic and non-embryogenic callus growth and production of embryos in liquid culture. - Plant Cell Tissue Organ Cult. **15**: 149-159, 1988.

D'Onofrio, C., Morini, S.: Simultaneous regeneration of different morhogenic structures from quince leaves as affected by growth regulator combination and treatment length. - Biol. Plant. **47**: 321-325, 2003/4.

Gama, M.I.C.S., Leite, R.P., Jr., Cordeiro, A.R., Cantliffe, D.J.: Transgenic sweet potato plants obtained by *Agrobacterium tumefaciens*-mediated transformation. - Plant Cell Tissue Organ Cult. **46**: 237-244, 1996.

Gosukonda, R.M., Porobodessai, A., Prakash, C.S., Peterson, C.M.: Thidiazuron-induction adventitious shoot regeneration of sweet potato (*Ipomoea batatas*). - *In Vitro* cell. dev. Biol. Plant **31**: 65-71, 1995.

Huang, W.L., Liu, L.F.: Promotion of shoot regeneration from rice (*Oryza sativa* L.) callus induced on the medium containing high concentration of sucrose. - Chinese Agron. J. **8**: 91-100, 1998.

Jarret, R.L., Salazar, S., Fernandez, Z.R.: Somatic embryogenesis in sweet potato. - HortScience **19**: 397-398, 1984.

Jones, A., Dukes, P.D., Scahlk, J.M. (ed.): Breeding Vegetable Crops. - AVI Publishing Co., Westport 1986.

Lee, I.Y., Lee, I.S., Lim, Y.P.: Variations in sweetpotato regenerates from gamma-ray irradiated embryogenic callus. - J. Plant Biotechnol. **4**: 163-170, 2002.

Liu, J.R., Cantliffe, D.J.: Somatic embryogenesis and plant regeneration in tissue cultures of sweet potato (*Ipomoea batatas* Poir.). - Plant Cell Rep. **3**: 112-115, 1984.

Murashige, T., Skoog, F.: A revised medium for rapid growth and bioassays with tobacco tissue cultures. - Physiol. Plant. **15**: 473-497, 1962.

Perera, S.C., Ozias-Akins, P.: Regeneration from sweetpotato protoplasts and assessment of growth conditions from flow-sorting of fusion mixtures. - J. amer. Soc. hort. Sci. **116**: 917-922, 1991.

Ranch, J., Pace, G.M.: Science in the art of plant regeneration from cultured cells: An essay and a proposal for a conceptual framework. - Iowa State J. Res. **62**: 537-541, 1988.

Tsay, H.S., Tseng, M.T.: Embryoid formation and plantlet regeneration from anther callus of sweet potato. - Bot. Bull. Acad. sin. **20**: 117-122, 1979.