An Efficient System of Embryogenic Suspension Cultures and Plant Regeneration in Sweetpotato

Q.C. Liu[1], H. Zhai[1], D.H. Lu[1], Y. Wang[2], and D.P. Zhang[3]

Genetic transformation offers great potential for improving the disease or pest resistance and nutrition quality of sweetpotato [*Ipomoea batatas* (L.) Lam.]. On-going research in these areas includes developing transgenic sweetpotato with resistance to sweetpotato virus diseases (Beachy et al., 1992; Murata et al., 1998), sweetpotato weevil and other insect pests (Newell et al., 1995; Zhang et al., 1997), and enhanced protein content in the storage root and leaves (Prakash et al., 1998). In spite of the various successful cases in sweetpotato transformation, the lack of an efficient system for regeneration has been a bottleneck for the application of transgenic technology in sweetpotato. The regeneration frequency in sweetpotato is often genotype dependent, ranging from 0 to 85% in tested cultivars (Gosukonda et al., 1995, Zhang et al., 1997). So far only a small number of genotypes have been regenerated, based on the information gathered from the related publications.

In sweetpotato, shoot apices of many genotypes can produce embryogenic callus with somatic embryos on the medium supplemented with 2,4-D. Somatic embryos can also develop into whole plants on the basal medium (Liu and Cantliffe, 1984; Jarret et al., 1984; Liu et al., 1992, 1993, 1997; Tan et al., 1993; Desamero et al., 1994; Otani and Shimada, 1996). However, in most cases the frequencies of somatic embryogenesis were low.

1 Department of Plant Genetics and Breeding, China Agricultural University, Beijing 100094, China.
2 CIP, Beijing, China.
3 CIP, Lima, Peru.

The development of a system of embryogenic suspension cultures has been a focal point for improving the regeneration frequency in sweetpotato. Chee and Cantliffe (1988, 1989), Chee et al. (1990), and Bieniek et al. (1995) succeeded in establishing embryogenic suspension cultures of sweetpotato cultivar White Star. Liu et al. (1997) investigated embryogenic suspension cultures of a Japanese cultivar Kokei No.14 and a Chinese cultivar Xindazi. An improved frequency of plant regeneration from the embryogenic suspension cultures was obtained.

The objective of this research is to develop an efficient system of embryogenic suspension cultures for a wide range of sweetpotato genotypes, which will eventually lead to a universal regeneration protocol for sweetpotato.

Materials and Methods

Plant materials

Eight cultivars of sweetpotato, Gaozi No.1, Koganesengan, Kokei No.14, Lizixiang, Nongdahong, Tamayutaka, Xindazi, and Xushu 18 were used in this study. They are important cultivars in China or Japan. The storage roots were grown in pots in a greenhouse. The young sprouts obtained were used for the isolation of shoot apices.

Induction of embryogenic callus

Young shoots about 30 mm-long were excised, washed with tap water, and sterilized with 70% ethanol for 20 sec and 2% sodium hypochlorite solution for 5 min. The sterilized materials were fully rinsed

with sterile distilled water. Shoot apices of about 0.5 mm in length were isolated with the aid of a dissecting microscope and cultured on MS medium supplemented with 2.0 mg/L 2,4-D, 3.0% sucrose (w/v), and 0.8% (w/v) agar, pH 5.8, at 28°C in the dark. The cultures were examined periodically under a dissecting microscope.

Initiation and maintenance of embryogenic suspension cultures

Embryogenic calli were obtained from shoot apices of 8 cultivars. Six- to eight-week-old embryogenic calli were used for the initiation of embryogenic suspension cultures. Embryogenic calli were crushed into cell-aggregates and free cells through a stainless steel microsieve with mesh size of 0.46 mm. The cells were then placed into a 100 mL Erlenmeyer flask containing 20 mL liquid MS medium with 2.0 mg/L 2,4-D and 3% sucrose (pH=5.8). The cultures were incubated on a reciprocal shaker (100 strokes/min) at 28°C under 13 h of cool-white fluorescent light at 500 lux. Embryogenic suspension cultures were maintained by subculture at a 2-week interval. At each subculture, cell-aggregates over 0.5 mm in size were crushed through a stainless steel microsieve with mesh size of 0.46 mm and then transferred to the fresh medium.

Regeneration of plants from embryogenic suspension cultures

Twenty weeks after the initiation protocol and at 4-week intevals cell-aggregates about 1.0 mm in size were transferred to solid MS medium supplemented with 2,4-D (2.0 mg/L). This condition is suitable for the proliferation of cell-aggregates and the formation of somatic embryos at 28°C in the dark. Four to six weeks after transfer, the embryogenic calli obtained with somatic embryos were further cultured on MS medium supplemented with 1.0 mg/L ABA for 4 to 5 weeks to induce the germination of somatic embryos at 28°C under 13 h of cool-white fluorescent light at 3000 lux. Plantlets from somatic embryos developed into whole plants on the basal medium.

Results

Formation of embryogenic callus

The incubated shoot apices responded actively to the culture conditions. After 3 to 4 days, most of them started to produce white, friable callus. This callus was non-embryogenic and grew rapidly. A few of shoot apices directly formed embryogenic callus. This type of embryogenic callus grew much slower than the non-embryogenic callus. Four to five weeks after incubation, embryogenic calli were formed on the surface of the non-embryogenic calli (Figure 1). These two kinds of embryogenic calli (the one directly formed from the shoot apices and the one formed on the surface of the non-embryogenic calli) were easily distinguishable from the non-embryogenic calli because of their bright-yellow and compact appearance. Both embryogenic calli were used to initiate embryogenic suspension cultures. All of the eight tested cultivars successfully formed an embryogenic callus, although there is variation in the frequency of embryogenic callus formation (Table 1).

Establishment of embryogenic suspension cultures

Using embryogenic calli derived from shoot apices of eight cultivars, we have developed a system of embryogenic suspension cultures, which has high potential for regeneration. When maintained in liquid MS medium containing 2.0-mg/L 2,4-D, the cells proliferated rapidly and dispersed well (Figure 2). The embryogenic suspension cultures consisted of bright-yellow and compact embryogenic cell-aggregates and free cells. After 20 weeks of initiation, approximately 20,000 embryogenic cell-aggregates about 1.0 mm in size were obtained from a single embryogenic callus derived from a shoot apex in all eight cultivars.

Regeneration of plants from embryogenic suspension cultures

After 20 weeks of the initiation protocol, the regeneration ability of embryogenic

Table 1. Formation of embryogenic callus from shoot apices in eight sweetpotato cultivars.

Cultivar	Origin	Shoot apices Incubated	Shoot apices forming embryogenic callus	
		No.	No.	%
Gaozi No.1	China	57	16	28.1
Koganesengan	Japan	27	5	18.5
Kokei No.14	Japan	115	98	85.2
Lizixiang	China	89	61	68.5
Nongdahong	China	56	29	51.8
Tamayutaka	Japan	53	14	26.4
Xindazi	China	30	5	16.7
Xushu 18	China	32	7	21.9

suspension cultures was tested every 4 weeks. By the transfer of cell-aggregates (about 1.0 mm in size) to solid MS medium supplemented with 2.0 mg/L 2,4-D, the cell-aggregates proliferated rapidly into typical embryogenic calli with large numbers of somatic embryos (Figure 3). When the obtained embryogenic calli with somatic embryos were further cultured on MS medium supplemented with 1.0 mg/L ABA, somatic embryos germinated and formed plantlets (Figure 4).

The regeneration frequency peaked at about 24 weeks after initiation when 100% of the cell-aggregates regenerated into plants in all eight cultivars. After that, the regeneration frequency gradually decreased, as some of the cell-aggregates became non-embryogenic. However, there is cultivar difference in this regard. For example, Xindazi still maintained a frequency of up to 100% as long as 36 weeks after initiation.

Discussion

The structure, color, and pattern of the embryogenic calli formation from shoot apices of the eight cultivars were very similar to those reported by Liu et al. (1992 and 1993). The formed embryogenic calli were used to initiate embryogenic suspension cultures. The embryogenic suspension cultures proliferated rapidly and dispersed well in MS medium containing 2.0 mg/L 2,4-D. By using the suspension culture for 20 weeks, approximately 20,000 embryogenic cell-aggregates about 1.0 mm in size can be produced per embryogenic callus derived from shoot apices. The suspension cultures can maintain their embryogenic ability for at least 24 weeks.

The long-term maintenance of embryogenic suspension cultures can be achieved by the re-cultivation of embryogenic cell-aggregates isolated from the suspension cultures. Additionally, several factors are important for successfully maintaining an embryogenic culture.

First, the embryogenic culture needs to be subcultured in a timely fashion. Our previous study shows that delaying the subculture diminished the embryogenic ability of the cell-aggregates. Liu et al. (1997) reported that at 21 weeks after initiation, only 61.1% of cell-aggregates of Kokei No.14 were still embryogenic if the suspension cultures were not subcultured in a timely way.

Second, the proliferation of cell-aggregates on solid MS medium with 2.0 mg/L 2,4-D is of critical importance for achieving a high frequency of somatic embryo formation and plant regeneration.

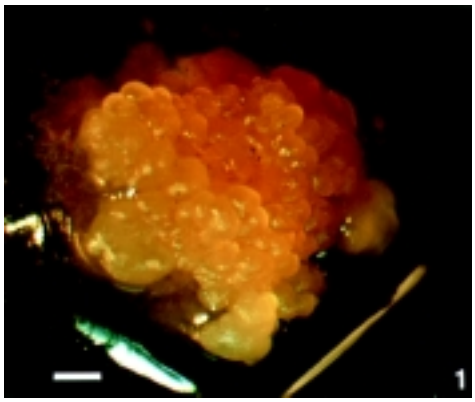

Figure 1. Embryogenic callus derived from a shoot apex of sweetpotato cultivar Lizixiang on MS medium supplemented with 2.0 mg/L 2,4-D (scale bar = 1.0 mm).

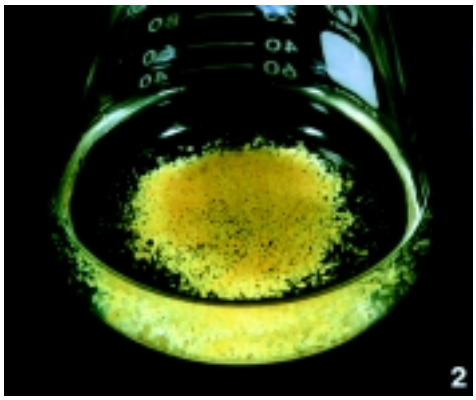

Figure 2. Embryogenic suspension cultures of sweetpotato cultivar Lizixiang proliferating in MS medium containing 2.0 mg/L 2,4-D.

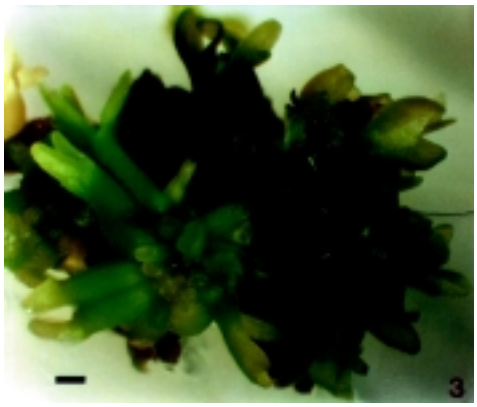

Figure 3. Somatic embryos formed on embryogenic callus derived from a cell aggregate of sweetpotato cultivar Lizixiang on MS medium supplemented with 2.0 mg/L 2,4-D (scale bar = 1.0 mm).

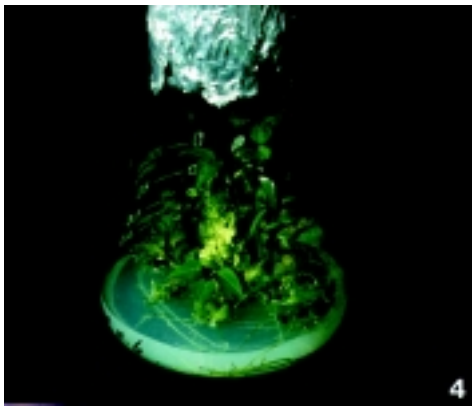

Figure 4. Germination of somatic embryos and formation of plantlets of sweetpotato cultivar Lizixiang on MS medium supplemented with 1.0 mg/L ABA.

Third, the size of cell-aggregates, which were transferred to 2,4-D solid medium, is also important to obtain a high regeneration frequency. Chee and Cantliffe (1989) reported that the percentage of somatic embryo formation decreased as the size of the cell-aggregates decreased. They found that sweetpotato somatic embryos only formed on cell-aggregates at least 0.18 mm in size and cell-aggregates 0.71-1.0 mm in size gave the maximum percentage of somatic embryo formation (up to 70%). Our results indicated that 100% of cell-aggregates about 1.0 mm in size produced somatic embryos and plants in all 8 tested cultivars.

We have succeeded in the development of an efficient system of embryogenic suspension cultures and plant regeneration in eight sweetpotato cultivars. This system of embryogenic suspension cultures has an excellent application potential in genetic transformation for cultivar improvement, as

well as in cryopreservation of sweetpotato germplasm.

Literature Cited

Beachy R.N., C. Malpica and G. Clark. 1992. Coat protein mediated resistance: Strategy for genetic engineering virus resistance in sweetpotato. In: Hill, W., C.K. Bonsi and P.S. Loretan (eds.). Sweetpotato for the 21st century. Proc. Int. Symposium, June 2-6, 1991, Tuskegee University, Tuskegee, AL, USA. p. 507-513.

Bieniek, M.E., R.C. Harrell, and D.J. Cantliffe. 1995. Enhancement of somatic embryogenesis of *Ipomoea batatas* in solid cultures and production of mature somatic embryos in liquid cultures for application to a bioreactor production system. Plant Cell Tiss. Org. Cult. 41:1-8.

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

____. 1989. Composition of embryogenic suspension cultures of *Ipomoea batatas* Poir. and production of individualized embryos. Plant Cell Tiss. Org. Cult. 17:39-52.

Chee, R.P., J.R. Schultheis, and D.J. Cantliffe. 1990. Plant recovery from sweetpotato somatic embryos. Hortsci. 25(7):795-797.

Desamero, N.V., B.B. Rhodes, D.R. Decoteau, and W.C. Bridges. 1994. Picolinic acid induced direct somatic embryogenesis in sweetpotato. Plant Cell Tiss. Org. Cult. 37:103-110.

Gosukonda, R.M., C.S. Prakash, and A.P. Dessai. 1995. Shoot regeneration in vitro from diverse genotypes of sweetpotato and multiple shoot production per explant. HortSci. 30:1074-1077.

Jarret, R.L., S. Salazar, and R.Z. Fernandez. 1984. Somatic embryogenesis in sweetpotato. Hortsci. 19:397-398.

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

Liu, Q.C., T. Kokubu, and M. Sato. 1992. Varietal differences of somatic embryogenesis in shoot tip culture of sweetpotato, *Ipomoea batatas* (L.) Lam. Japan. J. Breed. (Suppl. 2) 42:8-9.

Liu, Q.C., J.Q. Luo, H.Y. Zhou, and S.Y. Lu. 1993. High frequency somatic embryogenesis and plant regeneration in sweetpotato, *Ipomoea batatas* (L.) Lam. J. Agr. Biotech. 1(1):84-89.

Liu, Q.C., K.X. Mi, D.H. Lu, H.Y. Zhou, and Z. Fu. 1997. Establishment of embryogenic cell suspension cultures in sweetpotato, *Ipomoea batatas* (L.) Lam. Acta Agr. Sinica 23(1):22-26

Murata, T., Y. Okada, A. Saito, T. Kimura, M. Mori, M. Nishiguchi, K. Hanada, J. Saika, and H. Fukuoka. 1998. Transformation by direct gene transfer in sweetpotato [*Ipomoea batatas* L. (Lam)]. In: Proceedings of International Workshop on Sweetpotato Production System toward the 21st Century held 9-10 Dec. 1997 at Miyakonojo, Miyazaki, Japan. p. 159-180.

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

Newell, C.A., J.M. Lowe, A. Merryweather, L.M. Rooke, and W.D.O. Hamilton. 1995. Transformation of sweetpotato (*Ipomoea batatas* (L.) Lam.) with *Agrobacterium tumefaciens* and regeneration of plants expressing cowpea trypsin inhibitor and snowdrop lectin. Plant Sci. 107:215-227.

Otani, M. and T. Shimada. 1996. Efficient embryogenic callus formation in sweetpotato (*Ipomoea batatas* (L.) Lam.). Breeding Sci. 46:257-260.

Prakash, C.S, M. Egnin, and J. Jaynes. 1998. Biotechnology touches a Third World crop: Improving protein content in sweetpotato. Agricultural Biotech International Conference held 9-12 June 1998 at Saskatoon, Saskatchewan, Canada. p. 42. (Abstract)

Tan, F., K.P. Li, L.Q. Lan, and Q.T. Zhang. 1993. Somatic embryogenesis and plant regeneration in sweetpotato. Acta Agr. Sinica 19:372-375.
Zhang, D.P., A. Golmirzaie, and G. Cipriani. Developing weevil resistance in sweetpotato with genetic transformation. In: International Potato Center Program Report 1995-96, CIP, Lima, Peru. p. 205-210.