Improved Production Procedures for Somatic Embryos of Sweetpotato for a Synthetic Seed System

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Additional index words. somatic embryogenesis, suspension culture, cell aggregates, *Ipomoea batatas*

Abstract. Embryogenic callus of sweetpotato [*Ipomoea batatas* (L.) Lam.] disassociates in liquid medium to form a heterogeneous population of embryogenic and nonembryogenic cell aggregates of varying sizes. To improve embryo production, such cell aggregate populations were obtained by manually fragmenting calli 5 to 10 mm in size into liquid medium. The resulting suspensions were analyzed and the embryogenic fraction identified. The percentage of embryogenic aggregates and the percentage of aggregates forming embryos decreased with decreasing aggregate size. Thus, 76% of the 710- to 1000- μ m-diameter aggregates but only 14% of the 180- to 250- μ m aggregates had embryogenic potential. However, only 20% of the 710- to 1000- μ m aggregates and only 2% of the 180- to 250- μ m aggregates actually formed embryos. Conversely, embryogenic callus and embryo production per milligram of cultured embryogenic callus increased quadratically with decreasing aggregate size. Individual torpedo-stage embryos were produced from cell aggregates 180 to 250 μ m in size.

Sweetpotato, a major food crop and potential biomass crop, is conventionally propagated using stem cuttings. This propagation method is costly since intensive labor and large nurseries and storage facilities are required. The lack of resistance to virus diseases further complicates the maintenance and multiplication of virus-free stocks. Mass production of disease-free plant material can be expected using tissue culture. The production of synthetic seed, through somatic embryogenesis, and direct seeding would reduce the production cost of this vegetatively propagated crop.

The application of somatic embryogenesis to synthetic seed production will require many

embryos per amount of callus cultured in suspension and the formation of individualized embryos. Callus proliferation in sweetpotato suspension cultures occurs through cycles of growth and fragmentation (Chée and Cantliffe, 1989a). This situation is similar to that described for carrot (*Daucus carota* L.) suspension cultures (Halperin and Jensen, 1967; Jones, 1974; McWilliam et al., 1974). Embryogenic and nonembryogenic cell aggregates of various sizes are released when calli undergo fragmentation in suspension culture (Chée and Cantliffe, 1989a) or when calli grown on agar-solidified media are fragmented manually. In sweetpotato, embryos are produced from the released individual embryogenic cell aggregates (Chée and Cantliffe, 1989b). Embryo yields might be better optimized if the callus and embryo production potentials of cell aggregates of diverse sizes were known. Then, the appropriate suspension culture fractions could be either used to produce embryos or recycled for embryogenic callus production.

Sieving of *D. carota* callus dispersed in liquid medium and suspension cultures has been used to study the ontogeny of somatic

Received for publication 9 Aug. 1991. Accepted for publication 15 July 1992. Univ. of Florida, Agricultural Experiment Station Journal Series no. 9343. This work was supported in part by an IFAS/Gas Research Institute cooperative grant. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked advertisement solely to indicate this fact.

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Table 1. Distribution according to size of cell aggregates composing embryogenic calli of sweetpotato and nature of growth from subcultured cell aggregates after 21 days.

Cell aggregate diam (µm)	Distribution of cell aggregates from fragmented calli ²			Growth after subculture of cell aggregates of each fraction ³					
	Total no. aggregates (%)	Aggregates producing embryogenic callus (%)	Aggregates producing nonembryogenic callus (%)	Embryonic callus			Nonembryonic callus		
				Embryogenic type aggregates [*] (%)	Aggregates producing embryogenic callus ^w (%)	Amount produced/embryogenic aggregates (mg)	Amount produced/mg embryogenic aggregate (mg)	Aggregates producing nonembryogenic callus ^w (%)	Amount produced/aggregate (mg)
1000-710	1	6	1	55	76	9.0	24	52	38
710-500	16	39	11	40	33	7.2	50	38	33
500-355	13	20	6	26	21	6.7	153	26	17
355-250	16	20	7	21	17	5.9	358	24	13
250-180	15	15	5	11	14	5.0	829	17	11
180-125	11	0.1	20	NA	NA	NA	NA	NA	NA
125-90	28	0	50	NA	NA	NA	NA	NA	NA
Avg. total no. ^w	8992	1223	4978	NA	NA	NA	NA	NA	NA
F	NA	NA	NA	L**	L**Q**	L**	L**Q**	L**	NS

²Average of five samples of five callus lines.

³Statistical analysis was by the component of variance method; arcsin-square root transformation was used for percent analysis; F test of significance was used; **, significant at $P = 0.01$; NS, nonsignificant; NA, not applicable; L, linear trend; Q, quadratic trend; 10 replicates per treatment; 50 aggregates per replicate.

^{*}Yellow aggregates of cytoplasmic cells; visually estimated at culture inception.

^wAggregates without growth were not counted; some aggregates grew both embryogenic and nonembryogenic callus.

Table 2. Effect of cell aggregate size on embryo production at 21 days in sweetpotato.²

Cell aggregate diam (µm)	Avg aggregates/replicate (no.)	Aggregates with embryos (%)	Embryos produced/aggregate with embryos (no.)	Embryos produced/mg aggregate with embryos (no.)
1000-710	10	20	8.8	23
710-500	44	10	4.9	37
500-355	52	4	3.8	83
355-250	54	3	2.0	121
250-180	60	2	1.3	223
F		L**	L**	L**Q**

²Statistical analysis was by the component of variance method; arcsin-square root transformation was used for percent analysis. F test of significance was used; **, significant at $P = 0.01$; L, linear trend; Q, quadratic trend; 10 replicates per treatment.

embryogenesis (Halperin, 1966; Halperin and Jensen, 1967; Halperin and Wetherell, 1964), the influence of population density (Halperin, 1964, 1967), and the effects of media constituents (Halperin and Wetherell, 1965) and to synchronize embryo development from homogeneous cell aggregate populations (Fujimura and Komamine, 1979; Halperin, 1966). In this paper we report the distribution, according to size, of cell aggregates in embryogenic callus of *I. batatas* and the effect of cell aggregate size on the quantitative and qualitative aspects of callus and embryo production.

Embryogenic callus of *I. batatas* 'White Star' was obtained from shoot apices and proliferated on agar-solidified medium (Chée and Cantliffe, 1988a). Eight-week-old calli 5 to 10 mm in diameter were collected into a layer at the bottom of a 50-ml beaker containing 10 ml of liquid basal medium. The calli were fragmented into their natural component cell aggregates by applying slight pressure with the edge of a glass slide. The resulting suspension was fractionated using phosphor-bronze sieves with square mesh openings of 1000, 710, 500, 355, 250, 180, 125, and 90 µm. Each fraction of cell aggregates was resuspended separately in petri dishes containing 5 ml of liquid basal medium. The number of embryogenic and non-

embryogenic cell aggregates in each fraction were first estimated based on appearance (Chée and Cantliffe, 1989a) from five random sample visual counts at $\times 7$ to $\times 40$ magnification. Then, two 1-ml aliquots of cell aggregates were plated on agar-solidified medium designed either for embryogenic callus growth or embryo formation (Chée et al., 1992). Plastic petri dishes, 60 $\times$ 15 mm, with 12 ml of medium per dish were used. Incubation was at 27°C in darkness with unmonitored light interruptions during daily observations. The cultures were evaluated for callus or embryo production at 21 days. The whole procedure was repeated five times, starting with the collection of 8-week-old calli from a new source each time. Thus, 10 samples of cell aggregates in each size range were cultured for callus growth and 10 samples for embryo production. In calculating the yields per amount of embryogenic callus cultured, we assumed that cell aggregates were spherical and that their distribution was Gaussian within each fraction. Thus, the median diameter was taken as the average diameter of aggregates within a fraction, i.e., for fraction 710 to 1000 µm, the diameter used was 855 µm. The density (mass per unit volume) of embryogenic cell aggregates was estimated at 1.146 by using sucrose gradients.

In suspension cultures obtained by fragmenting embryogenic callus in liquid media, 14% (1223/8992) of the total aggregates were embryogenic (Table 1). The 90- to 180-µm fraction contained 39% of all cell aggregates, of which only 0.1% were embryogenic. The 500- to 1000-µm fraction contained 17% of all cell aggregates and accounted for 45% of the total amount of embryogenic callus.

The percentage of cell aggregates producing embryogenic callus and the amount of embryogenic callus produced per cell aggregate decreased with decreasing cell aggregate size (Table 1). However, when callus production was standardized per milligram of cell aggregate, the amount of embryogenic callus produced increased with decreasing cell aggregate size. Thus, embryogenic callus production per unit mass was 35 times greater for 180- to 250-µm than for 710- to 1000-µm cell aggregates. The percentage of cell aggregates in each fraction producing nonembryogenic callus also decreased with decreasing cell aggregate size (Table 1). However, there was no statistically significant trend between the amount of nonembryogenic callus produced and cell aggregate size.

The visual classification of cell aggregates as either embryogenic (yellow, firm, small

cytoplasmic cells) or nonembryogenic (white, loose, vacuolated cells), made at the time the cultures were established, closely paralleled the percentage of cell aggregates that later produced embryogenic callus as expected (Table 1). The calli classifications were based on previously published work (Chée and Cantliffe, 1988a, 1989a; Liu and Cantliffe, 1984). Compact white cell aggregates with smooth surfaces were also observed. They possibly represented an embryogenic to non-embryogenic transition stage and were classified as nonembryogenic.

As cell aggregate size decreased, so did the capacity of the aggregate to produce embryos (Table 2). However, on a standardized weight value of 1 mg of aggregate, the embryo production per milligram was 10 times greater for 180- to 250- μ m cell aggregates than for 710- to 1000- μ m cell aggregates. Individual embryos were produced from cell aggregates 180 to 250 μ m in diameter.

Sweetpotato embryogenic suspension cultures are initiated from embryogenic callus that proliferates through cycles of fragmentation into cell aggregates and growth of aggregates into new calli (Chée and Cantliffe, 1989a). The production potential of suspension cultures can be calculated from the number of embryogenic cell aggregates in each size fraction of fragmented calli (Table 1) and from the callus and embryo yields of the subcultured fractions (Tables 1 and 2). The shares of potential embryogenic callus and embryo production were, respectively, 50% and 64% for the 500- to 1000- μ m fraction and, respectively, 50% and 36% for the 180- to 500- μ m fraction.

Our results demonstrate that, in sweetpotato, embryogenic callus and embryo production per unit mass of cultured callus is greater from 180- to 250- μ m aggregates than from larger aggregates. The 180- to 250- μ m fraction also produced individualized embryos. However, sweetpotato somatic embryos initiated on large cell aggregates develop to the torpedo- and cotyledonary-stage aggregates while they remain arrested at the globular stage on small cell aggregates (Chée and Cantliffe, 1988a, 1989a, 1989b). In sweetpotato, only torpedo- and cotyledonary-stage embryos produce plants (Chée and Cantliffe, 1988b). Consequently, we are using in our current synthetic seed system the larger (500 to 1000 μ m) aggregates for embryo production and the smaller cell aggregates for callus production until media and incubation conditions are defined for embryo development to a more advanced stage on small aggregates.

Our present findings are significant to programs for synthetic seed systems through somatic embryogenesis because they demonstrate the importance of cell aggregate size in producing high yields of embryogenic callus and embryos. Further, our data underline the importance of continued research for defining culture conditions and protocols for homogeneous liquid culture of cell aggregates of desirable sizes.

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