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GENOTYPE EFFECTS ON PROLIFERATIVE EMBRYOGENESIS AND PLANT REGENERATION OF SOYBEAN

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SUMMARY

Proliferative somatic embryogenesis is a regeneration system suitable for mass propagation and genetic transformation of soybean [*Glycine max* (L.) Merr.]. The objective of this study was to examine genotypic effects on induction and maintenance of proliferative embryogenic cultures, and on yield, germination, and conversion of mature somatic embryos. Somatic embryos were induced from eight genotypes by explanting 100 immature cotyledons per genotype on induction medium. Differences in frequency of induction were observed among genotypes. However, this step was not limiting for plant regeneration because induction frequency in the least responding genotype was sufficient to initiate and maintain proliferative embryogenic cultures. Six genotypes selected for further study were used to initiate embryogenic cultures in liquid medium. Cultures were evaluated for propagation of globular-stage tissue in liquid medium, yield of cotyledon-stage somatic embryos on differentiation medium, and plant recovery of cotyledon-stage embryos. Genotypes also differed for weight and volume increase of embryogenic tissue in liquid cultures, for yield of cotyledon-stage embryos on differentiation medium, and for plant recovery from cotyledon-stage embryos. Rigorous selection for a proliferative culture phenotype consisting of nodular, compact, green spheres increased embryo yield over that of unselected cultures, but did not affect the relative ranking of genotypes. In summary, the genotypes used in this study differed at each stage of plant regeneration from proliferative embryogenic cultures, but genotypic effects were partially overcome by protocol modifications.

Key words: somatic embryogenesis; conversion; desiccation; *Glycine max*.

INTRODUCTION

Proliferative embryogenic cultures of soybean were developed by Finer and Nagasawa (1988) and consisted of dense, globular-stage clumps of embryogenic tissue which were rapidly propagated and maintained by routine subculture in liquid medium at low inoculum densities. Recently, these cultures have proven amenable to transformation (Finer and McMullen, 1991), although plant regeneration and transformation from this system have been reported for only one genotype (cv. Fayette). Genotype has considerable influence on the efficiency of plant regeneration via somatic embryogenesis of soybean (Komatsuda and Ohyama, 1988; Komatsuda et al., 1992) and thus may influence the application of proliferative embryogenic cultures.

As is the case with zygotic embryogenesis (Kermode, 1990; Hughes and Galau, 1989), somatic embryogenesis has several distinct developmental stages. Inasmuch as plant recovery is potentially limited at any stage of regeneration, it is essential to quantify all stages when examining the effect of experimental variables such as genotype. Large differences in embryogenic potential among genotypes, for example, are of little relevance if the embryos derived from prolific genotypes are incapable of conversion to plants. The objective of this study was to evaluate the extent of genotypic effects on induction, growth, and differentiation of proliferative embryogenic cultures, and on recovery of fertile plants from mature somatic embryos. The importance of selecting high-quality, embryogenic tissue for overcoming genotype limitations was also investigated.

MATERIALS AND METHODS

Plant material. Eight genotypes (Century, Centennial, Davis, Hutcheson, Lee, Peking, Williams 82, and Masshokutou kou 502 [= PI 417138]) were selected to represent a diversity of germplasms and maturity groups. Ten soybean plants from each genotype were planted in a greenhouse, with staggered planting dates to synchronize anthesis between the different maturity groups. In addition, flowers were removed from early flowering genotypes until all genotypes were in bloom.

Induction of somatic embryos. Immature pods containing embryos 3 to 5 mm in length were harvested over a 1-mo. period. Pods were surface-sterilized for 30 s in 70% 2-propanol, followed by 10 min in 1.05% sodium hypochlorite and three rinses with sterile water. Cotyledon explants were excised by removing the embryonic axis and pushing the cotyledons out of the seed coat. Cotyledons 2 to 3 mm in length were cultured with the abaxial surface in contact with induction medium which consisted of MS salts (Murashige and Skoog, 1962), B5 vitamins (Gamborg et al., 1968), 6% sucrose, 40 mg/liter 2,4-dichlorophenoxyacetic acid (2,4-D), and pH 7.0 (MSD40 medium). Twenty explants were cultured on 30 ml of medium dispensed in a 100 × 20-mm plastic disposable petri dish. Five dishes (replicates) per genotype were prepared, sealed with Nescofilm (Karlan Research Products, Corp., Santa Rosa, CA), and incubated at 28° C with a 23-h photoperiod. Illumination was provided by cool white fluorescent fixtures providing 5 to 10 $\mu E \cdot m^{-2} \cdot s^{-1}$. The proportion of explants responding was evaluated after 6 wk in culture. Responses scored were the propor-

tion of explants with any evidence of globular-stage embryos, and the proportion with proliferative embryogenic tissue.

Initiation, maintenance, and growth of proliferative embryogenic cultures. After 6 wk on induction medium, three to five clumps of globular-stage tissue, either attached or unattached to the cotyledon explant, were transferred to a triple-baffle 125-ml Erlenmeyer flask containing 35 ml of 10A40N medium (Finer and Nagasawa, 1988). At least seven liquid cultures from each genotype were initiated. Cultures were shaken on an orbital shaker at 125 rpm under continuous fluorescent light with an intensity of 22 $\mu E \cdot m^{-2} \cdot s^{-1}$. At monthly intervals large, high quality, embryogenic clumps were manually divided and subcultured at a low inoculum density (two 3-mm clumps per flask) as described by Finer and Nagasawa (1988). Six genotypes (Century, Davis, Hutcheson, Lee, Peking, and PI 417138) with similar culture history were selected for further study after three subcultures in 10A40N medium at monthly intervals. Two equally sized embryogenic clumps per genotype were subcultured into each of five replicate flasks of 10A40N medium. After 1 mo., growth was measured on a volume basis and on a fresh weight basis. Clumps of tissue were approximately spherical, and volume was calculated using the formula $^4/_3\pi r^3$. Thus, two clumps of 3-mm diameter were equal to a packed cell volume (PCV) of 28 μl (Finer and Nagasawa, 1988). Embryogenic tissue was blotted dry on sterile filter paper before weighing.

Embryo yield. After volume estimation and weight measurement, all tissue from each of the five replicate flasks per genotype was manually subdivided into approximately 1-mm diameter clumps and transferred to solid MSM6AC medium (MS salts, B5 vitamins, 6% maltose, 0.5% activated charcoal, 0.2% Gelrite, and pH 5.8) to permit histodifferentiation. After 28 days, cotyledon-stage embryos with distinct root and shoot poles were counted, separated, and transferred individually to MSM6 medium (MSM6AC without charcoal) for maturation.

Germination, conversion, and fertility. After 28 days on MSM6 medium, embryos were partially desiccated in dry petri dishes for 5 days (Hammatt and Davey, 1987; Parrott et al., 1988). Partially desiccated embryos were then transferred to MSO medium (MS salts, B5 vitamins, 3% sucrose, pH 5.8) for germination. After 25 days on MSO medium, embryos were evaluated for germination, which was defined as the emergence of pubescent structures at the shoot apex. For evaluation of conversion, all germinated embryos were first transferred to 50 ml of fresh MSO medium dispensed in GA-7 vessels (Magenta Corp., Chicago, IL). After 2 to 3 wk, all seedlings were individually transferred to 6.35-cm pots containing a 1:1 mixture of sand and Hyponex (Hyponex Corp., Maryville, OH) potting soil. Pots with seedlings were placed in GA-7 vessels enclosed with a GA-7 coupler and transferred to a growth chamber (relative humidity of 75%, 12-h photoperiod, 25° ± 2° C, and light intensity of 200 $\mu E \cdot m^{-2} \cdot s^{-1}$). After 1 to 2 wk of growth in soil, plants were acclimatized by gradual removal of the coupler over a 2- to 7-day period. Plants were scored as converted if they a) survived uncovered at ambient humidity for at least 1 week, b) were at least 10 cm tall, and c) had at least two trifoliolate leaves (Fujii et al., 1989). Ten plants from each genotype meeting these criteria were transplanted in a greenhouse to assess survival and fertility.

Effect of continuous selection on proliferative embryogenic cultures. In the experiments described above, embryogenic cultures were evaluated after two subcultures on 10A40N medium despite variation for culture phenotype among and within genotypes. To assess the importance of culture phenotype on growth and embryo yield, preliminary experiments were conducted in which various types of tissue from suspension cultures of Peking were tested for stability in 10A40N medium over subculture periods and for differentiation on MSM6AC medium. A stable, fast-growing culture phenotype, which consisted of green, dense, spheres of friable, nodular tissue and which differentiated into relatively large numbers of normal cotyledon-stage embryos on MSM6AC medium, was identified after 6 monthly cycles of selection and subculture (Fig. 1 *A*). These cultures are referred to as "homogeneous cultures." Using homogeneous cultures of Peking as a model, cultures with a similar phenotype were developed for all genotypes on which rigorous selection for desired morphology was imposed.

Homogeneous cultures of Peking, Century, and PI 417138 which had been subcultured in 10A40N medium for 8 to 10 mo. were chosen for further evaluation. Compared to their heterogeneous counterparts, these cultures grew rapidly, requiring subculture at 3-wk intervals instead of 4. Differentiation and maturation of somatic embryos, and the assessment of embryo yield were accomplished as previously described, whereas protocols for conversion, and the data recorded for germination and conversion were modified as follows. Replicated tests for germination were conducted, with the mature somatic embryos derived from a single flask representing a replicate. Germination was evaluated at 5-day intervals for 25 days. Average time to germination was calculated from the following formula:

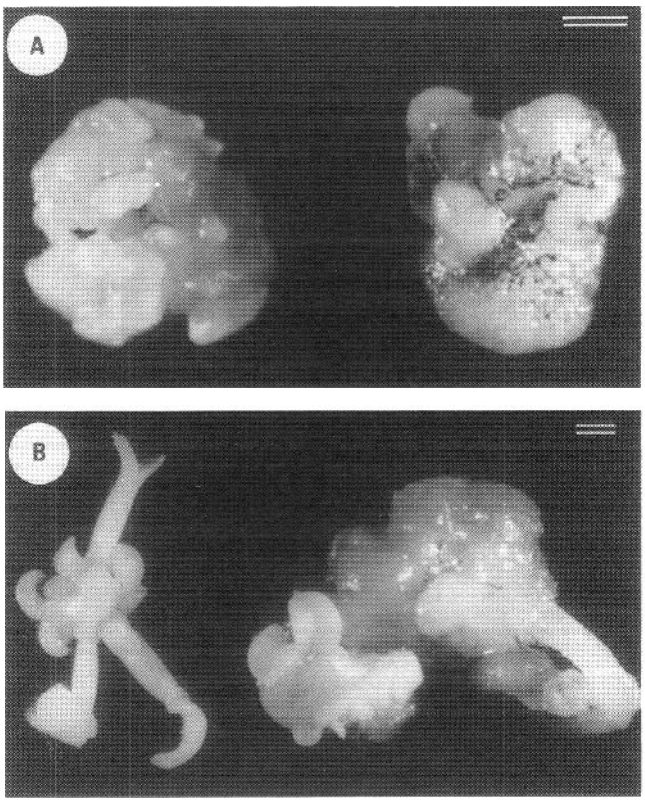

FIG. 1. *A*, comparison of representative tissue clumps of homogeneous (*left clump*) and heterogeneous (*right clump*) suspension cultures. *B*, response of homogeneous (*left clump*) and heterogeneous (*right clump*) suspension cultures after 1 mo. on differentiation medium (MSM6AC). Tissue is of cultivar Peking. *Bar* = 1 mm.

$$\text{Mean days to germination} = \frac{N_1T_1 + N_2T_2 + \cdots N_xT_x}{\text{total embryos germinating}}$$

where N is the number of embryos with shoots per time interval, and T is the total time after imbibition (Hartmann and Kester, 1975; Buchheim et al., 1989). Final percent germination was recorded at 25 days, at which time 50 seedlings from each genotype were selected at random and transferred directly to potting mixture. Acclimatization, as previously described, was started 10 days after transfer to soil. Conversion was scored after 17 days in soil, which was 6 wk after the end of the desiccation treatment. At least four plants from each genotype were transplanted in a greenhouse to test fertility.

RESULTS

Induction. A portion of immature cotyledon explants from all genotypes tested produced globular-stage somatic embryos after 6 wk on MSD40 medium. Induction frequencies among genotypes were different and ranged from 46% for PI 417138 to 94% for Centennial (Fig. 2). A fraction of the somatic embryos on embryogenic explants underwent proliferative embryogenesis with secondary somatic embryos arising from the apical region of primary so-

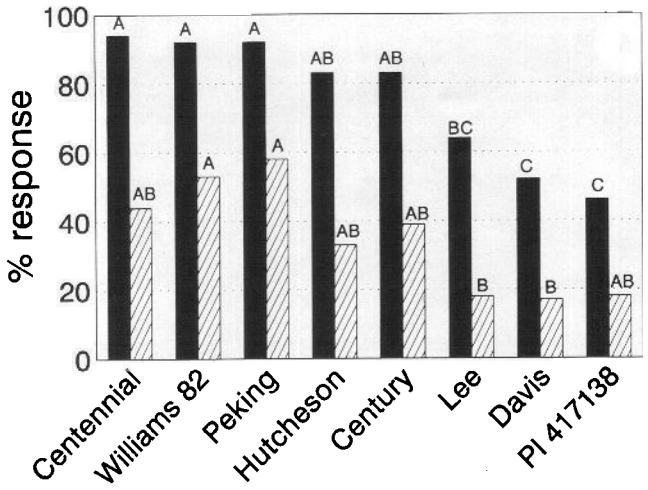

FIG. 2. Mean frequency of embryogenesis (*solid bars*) and mean frequency of repetitive embryogenesis (*hatched bars*) from immature cotyledon explants of soybean. Means are from 5 to 6 reps. Comparisons were made using Fisher's Protected LSD test on arcsin $y^{0.5}$ transformed data. *Bars* of the same pattern with different letters are different at $P = 0.05$.

matic embryos as described by Finer (1988). The frequency of explants with proliferative embryogenic tissue was different among genotypes and ranged from 17% for Davis to 58% for Peking (Fig. 2). Tissue scored as embryogenic varied in color from pale yellow to green. Differences in friability of embryogenic clumps and the size of constituent globular embryos were also evident.

Growth of proliferative embryogenic tissue in 10A40N medium. Clumps of embryogenic tissue, arrested at the globular stage, rapidly proliferated in shaking cultures of 10A40N medium. Cultured tissue and spent medium varied qualitatively within and among flasks. High quality embryogenic tissue was green and had a nodular, homogeneous, friable, and compact morphology (Fig. 1 *A*). Dense spheres of embryogenic tissue up to 1 cm in diameter were apparent after 1 mo. of culture. Excessive turbidity, tissue necrosis, lack of friability, and postglobular differentiation were characteristic of some cultures. From previous experience these characteristics were considered detrimental for maintenance of long-term cultures and for the subsequent recovery of mature, differentiated embryos (Fig. 1 *B*). Therefore, tissue for subculture was rigorously selected for the high quality traits just described. Nevertheless, over the time course of this study, some cultures retained one or more of these negative characteristics. For purposes of discussion these cultures are henceforth referred to as "heterogeneous cultures" (*see* below). When genotypes were ranked relative to each other according to the phenotypic characteristics described above, rankings from highest to lowest quality were as follows: PI 417138 > Lee = Peking = Davis > Hutcheson > Century. In addition to qualitative differences, genotypes differed for final fresh weight and for final packed cell volume (Table 1). Final mean fresh weights ranged from 68 to 176 mg, representing a 5- to 10-fold increase over the initial inoculum of 15 ± 3 mg. Final mean PCV ranged from 257 to 613 μl, representing a 9- to 22-fold increase over the initial estimate of 28 μl PCV.

Embryo yield of heterogeneous cultures. Clumps of globular-stage somatic embryos differentiated into clumps of cotyledon stage somatic embryos after transfer from 10A40N medium to MSM6AC medium. Differentiated somatic embryos were of diverse morphologies and included all types described by Buchheim et al. (1989). Any structure with at least one distinct cotyledon and a hypocotyl-root axis after 28 days on MSM6AC medium was scored as a somatic embryo. Embryo yield was different among genotypes, and ranged from an average of 2 somatic embryos/flask for Century to an average of 63 somatic embryos/flask for PI 417138 (Table 2).

TABLE 1

GROWTH OF HETEROGENEOUS SUSPENSION CULTURES AFTER 4 WK IN 10A40N MEDIUM[a]

Genotype	Mean PCV, μl	Mean Fresh Weight, mg
Century	613a[b]	176a
PI 417138	431abc	128ab
Lee	447ab	100bc
Davis	329bc	92bc
Hutcheson	257c	68c

[a] Five replicate flasks were inoculated with 15 ± 3 mg (28 μl PCV) of tissue.

[b] Comparisons were made using Fisher's Protected LSD test. Means within a column followed by different letters are different at the 0.05 level.

Germination, conversion, and fertility of heterogeneous cultures. Partially desiccated embryos exhibited a variety of responses after 25 days on MSO medium. The vast majority of somatic embryos enlarged considerably and turned green, although a few were necrotic or bleached, and presumably dead. Responses of surviving embryos were categorized as follows: a) no roots or shoots, b) roots-only, c) shoots-only, or d) roots and shoots. Shoots were defined as any pubescent apical structure because their morphology and size varied widely. In preliminary experiments (data not shown), structures with no roots or shoots or with roots-only after 25 days on MSO rarely progressed to seedling growth if subcultured on fresh medium. In contrast, structures with shoots-only usually developed vigorous root systems upon subculture to fresh medium or to soil. Thus, "germination" was scored as those structures with shoots-only and those with both roots and shoots. Germination frequency was different among genotypes and ranged from 11% for Century to 74% for PI 417138 (Table 3 and Fig. 3).

TABLE 2

YIELD OF COTYLEDON-STAGE SOMATIC EMBRYOS DERIVED FROM HETEROGENEOUS SUSPENSION CULTURES[a]

Genotype	Total	Range	Mean
PI 417138	314	37–97	63a[b]
Lee	202	21–55	40ab
Peking	140	9–68	28bc
Davis	99	1–42	20bcd
Hutcheson	45	0–27	9cd
Century	9	0–4	2d

[a] Values are from five replicates per genotype. A replicate equals all tissue from a single suspension culture transferred to differentiation medium (MSM6AC).

[b] Comparisons were made using Fisher's Protected LSD test. Means followed by different letters are different at the 0.05 level.

TABLE 3

PERFORMANCE OF PARTIALLY DESICCATED SOMATIC EMBRYOS DERIVED FROM HETEROGENEOUS SUSPENSION CULTURES

	Germination[a] of Somatic Embryos				Conversion[b] of Somatic Embryos				Conversion of Germinated Somatic Embryos			
Genotype	*n*	Obs[c]	Exp[d]	%	*n*	Obs	Exp	%	*n*	Obs	Exp	%
PI 417138	210	156	99.49	74	210	127	76.54	60	156	127	120.00	81
Peking	140	76	66.33	54	140	51	51.02	36	76	51	58.46	67
Hutcheson	45	18	21.32	40	45	16	16.40	36	18	16	13.85	89
Lee	195	59	92.38	30	195	46	71.07	24	59	46	45.38	78
Davis	87	15	41.22	17	87	10	31.71	11	15	10	11.54	67
Century	9	1	4.26	11	9	0	3.28	0	1	0	0.77	0
		$\chi^2 = 124.02^{**}$				$\chi^2 = 94.83^{**}$				$\chi^2 = 11.61^{ns}$		

[a] Structures with shoots-only or with roots and shoots.
[b] Conversion = those seedlings which survived ambient humidity, were at least 10 cm tall, and had at least two trifoliolate leaves after 1 mo. in soil.
[c] Observed.
[d] Expected value assuming equal response for each genotype.
** Significant at the 0.01 level of probability. [ns] Not significant 0.05 level of probability.

All germinated embryos were tested for conversion. Conversion frequencies were calculated from both conversion per somatic embryo and conversion per germinated somatic embryo. Conversion frequency of somatic embryos ranged from 0% for Century to 60% for PI 417138, whereas the conversion frequency of germinated embryos ranged from 0% for Century to 89% for Hutcheson. Chi-square analysis indicated that genotypes were different for conversion of somatic embryos but not for conversion of germinated somatic embryos (Table 3). Ten converted plants each of Davis, Lee, Hutcheson, Peking, and PI 417138 were selected at random and transplanted in a greenhouse. All plants survived and were fertile, as determined by normal seed set.

Embryo yield of homogeneous cultures. Homogeneous cultures of Peking, Century, and PI 417138 differentiated asynchronously into masses of cotyledon-stage embryos during incubation on MSM6AC medium. Embryo yield was different among genotypes and ranged from a mean of 72 somatic embryos per flask of Century to a mean of 310 somatic embryos per flask of PI 417138 (Table 4).

During maturation on MSM6 medium, approximately 25% of somatic embryos from PI 417138 were associated with an additional flush of cotyledon-stage embryos, which formed from a clump of globular-stage tissue attached to the root pole of the original embryo. As many as 12 additional somatic embryos differentiated in association with a primary embryo after 28 days on MSM6. An average of 159 additional somatic embryos per replicate flask were scored, which, when added to previous values, resulted in an average of 470 differentiated somatic embryos/flask of PI 417138. Differentiation of additional somatic embryos was occasionally observed in association with maturing embryos of Peking and Century; however, the contribution of additional embryos to total embryo yield was negligible for these genotypes.

Germination, conversion, and fertility of homogeneous cultures. The germination frequency of partially desiccated embryos was different among genotypes, ranging from 10% for Century to 57% for PI 417138. The average time to germination was not different among genotypes (Table 5). Most germinated embryos were alive after 17 days in soil, with survival ranging from 66% for Century and Peking to 78% for PI 417138. Conversion frequency

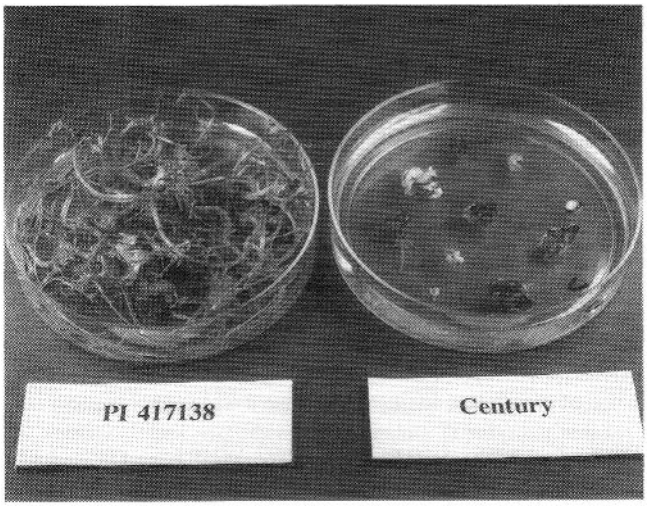

FIG. 3. Response of partially desiccated somatic embryos after 25 days on germination medium (MSO).

TABLE 4

YIELD OF COTYLEDON-STAGE EMBRYOS FROM HOMOGENEOUS SUSPENSION CULTURES

Genotype	Rep[a]	Total	Range	Mean
PI 417138	6	1861	195–434	310a[b]
Peking	6	689	49–194	115b
Century	5	370	13–129	72b

[a] All tissue from a single suspension culture transferred to differentiation medium.
[b] Comparisons were made using Fisher's Protected LSD test. Means within a column followed by different letters are different at the 0.05 level.

TABLE 5

PERFORMANCE OF PARTIALLY DESICCATED EMBRYOS DERIVED FROM HOMOGENEOUS SUSPENSION CULTURES

	Germination[a]			Survival[b]			Conversion[c]		
Genotype	Mean Frequency (%)	Mean Rate (days)	n^d	Observed	Expected	%	Observed	Expected	%
PI 417138	57a	11.2a	50	39	36	78	17	11	34
Peking	34b	10.4a	50	33	36	66	10	11	20
Century	10c	10.0a	47	33	34	66	4	10	8
				$\chi^2 = 1.81^{ns}$			$\chi^2 = 9.51^*$		

[a] As described in Table 3. Values are from 5 to 6 replicates. A replicate was all partially desiccated embryos derived from a single flask of homogeneous cultures. Comparisons were made using Fisher's Protected LSD test. Means within a column followed by different letters are different at the 0.05 level.

[b] Seedlings that survived ambient humidity but did not meet other conversion criteria after 17 days in soil.

[c] As described in Table 3.

[d] Number of germinated embryos transferred to soil.

* Significant at 0.05 level. [ns] Not significant at 0.05 level.

was different among genotypes and ranged from 8% for Century to 34% for PI 417138 (Table 5). At least four regenerated plants from each genotype were transplanted in a greenhouse and all plants were fertile.

Discussion

Proliferative embryogenic suspension cultures were established from all tested genotypes, and fertile plants were recovered from all genotypes subjected to the complete regeneration protocol. Moreover, stable, high-quality embryogenic cultures were developed and maintained for three genotypes (Peking, Century, and PI 417138) on which selection for culture phenotype was practiced. Given the diversity of genotypes used in this study, this regeneration system should be widely applicable. However, the magnitude of several responses (induction, growth, embryo yield, germination, and conversion) essential for efficient plant recovery was different among the genotypes studied.

Previous studies have documented genotype effects on induction of somatic embryogenesis from immature cotyledon explants of soybean using a variety of protocols (Komatsuda and Ohyama, 1988; Komatsuda, 1990; Komatsuda and Ko, 1990; Komatsuda et al., 1991; Parrott et al., 1989; Ranch et al., 1985; Shoemaker et al., 1991), but this is the first report in which proliferative embryogenesis has been quantified in soybean. The role of genetic effects has been questioned, with differences of induction frequencies among genotypes attributed to environmental conditions more favorable for early maturing genotypes (Shoemaker et al., 1991). No relationship between maturity group and induction of embryogenesis was apparent in this study. The synchronization of flowering, explant collection, and culture that was accomplished here may have reduced environmental variability attributable to maturity group effects.

Still unknown is whether genotype differences for induction capacity are stable over a wide range of conditions, or whether the conditions used here are favorable for specific genotypes. The smallest-seeded genotype, PI 417138, was among the least inducible of all genotypes studied (Fig. 2). Developmental stage of cotyledon explants is known to be critical for induction (Lippmann and Lippmann, 1984; Lazzeri et al., 1985; Ranch et al., 1985), and for this reason uniform explants of 2 to 3 mm were prepared from immature seeds of 3 to 5 mm in length. However, selection of explants from uniform seeds may not have ensured uniform developmental status among genotypes differing in mature seed size. Thus, genotypic differences for induction capacity might be altered by selection of explants based on criteria other than equal seed length. Komatsuda et al. (1992) for example, selected immature embryos for culture which were one-half the length of mature seeds. In fact, genotype-specific induction frequencies may be of little relevance to transformation protocols that utilize proliferative embryogenic cultures. Numerous cotyledon explants may be cultured with reasonable ease, and only a low percentage need respond to provide sufficient material for expansion and long-term propagation of cultures.

The basis for genotype differences in growth of heterogeneous cultures may be explained in part by differences in the quality of embryogenic tissue among genotypes. Embryogenic tissue differed considerably in size of constituent embryos, extent of proliferation, friability, and color. PI 417138 had both the highest qualitative evaluation and the highest embryo yield, whereas Century had the lowest qualitative rating and embryo yield (Table 2). The low qualitative ranking, faster growth, and poor embryo yield from suspension cultures of Century were probably due to a predominance of poorly or non-embryogenic tissue. In support of this idea, Finer and Nagasawa (1988) previously observed that non-embryogenic tissue was faster growing than embryogenic tissue. Rankings of tissue quality and embryo yield were the same for all five genotypes, suggesting that enrichment for superior embryogenic tissue might be accomplished by simple inspection and selective subculture. Finer and Nagasawa (1988) previously reported on the variability of soybean suspension culture tissue and the need to carefully select high-quality tissue for experimentation.

Accordingly, after several cycles of selection, stable, homogeneous cultures were developed for Peking, Century, and PI 417138. For each genotype, growth of homogeneous cultures was obviously more rapid than that of heterogeneous cultures. No quantitation of growth was attempted because it was neither limiting nor a reliable indicator of embryo yield in the previous evaluation of heterogeneous cultures. Although not directly comparable, embryo yield of homogeneous cultures was also considerably higher than that of heterogeneous cultures, but genotype rankings remained unchanged (Tables 2 and 4). Inasmuch as the morphology of homo-

geneous cultures was similar for PI 417138, Peking, and Century, genetic factors other than those that result in distinct culture phenotypes must contribute to differences in growth rate and embryo yield among genotypes. In this regard, other studies have shown that PI 417138 has superior embryogenic capacity when subjected to a variety of culture conditions very different from those described here (Komatsuda and Ohyama, 1988; Komatsuda et al., 1991). Thus, PI 417138 may be an appropriate source of "embryogenesis" genes for introgression into agronomic genotypes with lower embryo yield.

Previous studies have documented the relatively high frequency of "germination" and mature plant recovery from primary somatic embryos of PI 417138 (Komatsuda and Ohyama, 1988; Komatsuda et al., 1992). These attributes are also characteristic of somatic embryos derived from proliferative embryogenic cultures, since PI 417138 had the highest germination and conversion capacity (Table 3). Conversion from germinated somatic embryos derived from heterogeneous cultures was not different among genotypes (Table 3), which suggests that structures scored as germinated were functionally equivalent across genotypes when subjected to this conversion protocol. With the exception of Century, from which there was only a single germinated embryo, the frequency of germinated embryos that converted was high, ranging from 67 to 89%.

Germination and conversion frequencies of somatic embryos derived from homogeneous cultures of Peking and PI 417138 were lower than those from heterogeneous cultures, yet genotype rankings were identical (compare Tables 3 and 5). The basis for differences in germination frequency based on the quality of cultures is unclear, because protocols were identical for both sources of embryos. For germinated embryos derived from heterogeneous cultures, the time on MSO medium and soil and the acclimatization conditions were varied according to the vigor of individual plantlets. The conversion protocol for somatic embryos derived from homogeneous cultures was modified by eliminating a passage on MSO medium, and by standardizing both the time in soil and the acclimatization conditions (*see* Materials and Methods). These changes probably contributed to the reduction in conversion frequency, but they also showed that conversion can be accomplished more rapidly. Inasmuch as homogeneous cultures were 2 to 4 times older than heterogeneous cultures, it is also possible that absolute culture age or the number of subcultures reduced germination and conversion frequencies. Survival frequencies are given to show that many germinated embryos retain the potential to meet the conversion criteria if allowed to continue growth in soil (Table 5). It should also be noted that structures meeting the germination criteria were chosen at random to test conversion. As described, the morphology and size of shoots varied widely, and obviously superior germinated embryos were conspicuous. Selection of such germinated embryos would greatly improve conversion frequency.

In conclusion, whole plant regeneration of soybean from proliferative embryogenic cultures was evaluated at several distinct stages, and there were genotype effects for each stage. PI 417138 was clearly the most regenerable genotype, as judged by superior embryo yield, germination, and conversion. This genotype should be useful for transformation studies, where many other variables limit the recovery of transgenic plants. Unfortunately, PI 417138 is a semiwild variety once classified as *Glycine gracilis* and is of little agronomic value, although it is sexually compatible with improved cultivars of *G. max*. To be useful for crop improvement, transformation of this genotype will require subsequent backcrossing to elite lines. Alternatively, the regeneration capacity of PI 417138 could be introgressed into elite lines before transformation, although this is a long-term strategy. A third approach would be to optimize the limiting steps of regeneration for recalcitrant genotypes such as Century. This seems feasible because the limited embryo yield of the most unresponsive genotype, Century, was improved by selection for homogeneous cultures (Tables 2 and 4).

Other recent studies have also demonstrated that embryo yield is not limiting for plant regeneration from proliferative embryogenic cultures of soybean. Wright et al. (1991) showed that many plants could be recovered from single cotyledonary and torpedo-stage somatic embryos through shoot-bud propagation on medium containing 6-benzyladenine. This procedure eliminates the requirement of producing large numbers of cotyledon-stage somatic embryos to ensure plant recovery, but the time and resources required for embryo-to-plant conversion is increased by the additional steps of shoot-bud culture. Liu et al. (1992) showed that explants derived from cotyledon-stage somatic embryos could be re-induced to undergo a cycle of somatic embryogenesis on media containing 2,4-D or α-naphthaleneacetic acid. Both of these studies differ from the present study by including an additional step for propagation of regenerable tissue. However, all three studies are similar in that they initially utilize proliferative embryogenic cultures arrested at the globular-stage. The most efficient regeneration system in terms of time and resources would involve direct conversion of cotyledon-stage somatic embryos derived from the initial globular-stage cultures. The homogeneous cultures described here result in large numbers of cotyledon-stage somatic embryos for even the least responsive genotype (i.e. Century). Therefore, it will now be possible to optimize germination and conversion directly from cotyledon-stage somatic embryos of recalcitrant genotypes such as Century.

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