Theor Appl Genet (1986) 72:423–428

Screening of *Glycine max* and *Glycine soja* genotypes for multiple shoot formation at the cotyledonary node*

U. B. Barwale [1], M. M. Meyer, Jr. [2] and J. M. Widholm [1]

[1] Department of Agronomy, [2] Department of Horticulture; University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA

Accepted February 3, 1986
Communicated by Hu Han

Summary. To identify genotypes which may give better plant regeneration responses in vitro, multiple shoots were induced from 155 *Glycine max* and 13 *Glycine soja* genotypes from maturity groups '000' to 'VII' on B5 medium supplemented with 1 or 5 μmol benzylaminopurine (BAP). The average number of shoots formed show genotype specific and hormone concentration specific responses, with number of shoots ranging from 1 to 12 for different genotypes. The results were reproducible with different seed lots of the same genotype and genotypes with similar genetic backgrounds responded in a similar fashion. No hybrid vigor was observed, except in one instance of F_1 hybrids between low shoot producers where the number of shoots obtained were higher than either parent. The root forming ability of cuttings of soybean plants grown in vivo showed general agreement with shoot forming ability in vitro. The ability to form multiple shoots appears to be genetically controlled.

Key words: Soybean – Plant regeneration

Introduction

Soybean, [*Glycine max* (L.) Merr.] has been used extensively in tissue culture since the 1960's but regeneration of plants from undifferentiated tissue was not achieved until recently (Christianson et al. 1983). Growth centers, clusters of meristematic cells, embryo-like structures, and embryos have been produced in cultures using different genotypes (Beversdorf and Bingham 1977; Gamborg et al. 1983; Oswald et al. 1977; Phillips and Collins 1981) but these did not develop into whole plants. Major differences among cultivars in their ability to produce embryo-like structures (Christianson et al. 1983) and to produce multiple shoots were seen (Cheng et al. 1980; Saka et al. 1980). Thus, genotype may affect the in vitro regeneration response. Unfortunately, cultivars used in the callus culture work (Oswald et al. 1977) were different from those used for the shoot-culture screening (Saka et al. 1980) so the relationship between the two characteristics is not clear.

The screening of germplasm for multiple shoot formation at the node region may identify genotypes which would give better plant regeneration responses in vitro. In the experiments reported here a system similar to that of Saka et al. (1980) was used to screen genotypes from the soybean germplasm collection. The objective was to determine if genetic differences in shoot formation capacity exist which might be exploited later in soybean tissue-culture plant-regeneration studies.

Materials and methods

Seeds of 155 *Glycine max* (L.) Merr. and 13 *Glycine soja* Sieb. and Zucc., lines differing in seed color and size, flower color, maturity date and genetic background, were sampled for possible regeneration potential (obtained from the soybean germplasm collection at Urbana, Illinois). The seeds were surface sterilized for 20 min in 750 ml of a 0.78% sodium hypochlorite solution containing one drop of Tween 80. They were then rinsed with sterile distilled water three times for 5 min each and placed on B5 medium (Gamborg et al. 1968) in tubes (15 × 2.5 cm) containing 20 ml of solidified medium (6 g/L Bactoagar). The medium was supplemented with 0.125 μmol indole-3-butyric acid (IBA) and either 1 (treatment

* This research was supported by funds from the Illinois Agricultural Experiment Station and Agrigenetics Research Associates

No. 1) or 5 μmol BAP (treatment No. 2). One seed was placed in each tube with the hilum placed directly on the medium. Ten seeds (replication) were used for each treatment (exceptions being where some were not counted due to contamination). The culture tubes were illuminated with cool white fluorescent light of 55 μmol photons m^{-2} s^{-1} for 16 h at 25 °C during the day and 18 °C at night.

The number of shoots formed at the cotyledonary node, including the main shoot, were counted at the end of 4 weeks at two different times using seeds from the same lot for genotypes 'Asgrow A3127', 'Earlyana', and 'McCall'. Similarly, shoots formed at the node region were counted for the other *G. max* and *G. soja* genotypes (listed in Table 3).

Seeds of 'Union', 'Williams', 'Century', 'BSR 201' and 'Pella', each produced at three locations in Illinois in 1984, were also tested.

Reciprocal cross pollinations were made using standard pollination techniques between genotypes ['Illini'×'Voris 285', 'Williams'×'PI 70242-2', 'Earlyana'×'Manchu Mont.', 'Earlyana'×'Wayne']. The seeds from these crosses were tested for multiple shoot formation as described above.

Results and discussion

The number of shoots produced at the cotyledonary node were counted at the end of 4 weeks (see Fig. 1 for example). Two experiments with 10 seeds each at both BAP concentrations were conducted at different times using 'Earlyana', 'Asgrow A3127', and 'McCall' seeds from the same lots to check for reproducibility. The number of shoots obtained in the two experiments (Table 1) were similar.

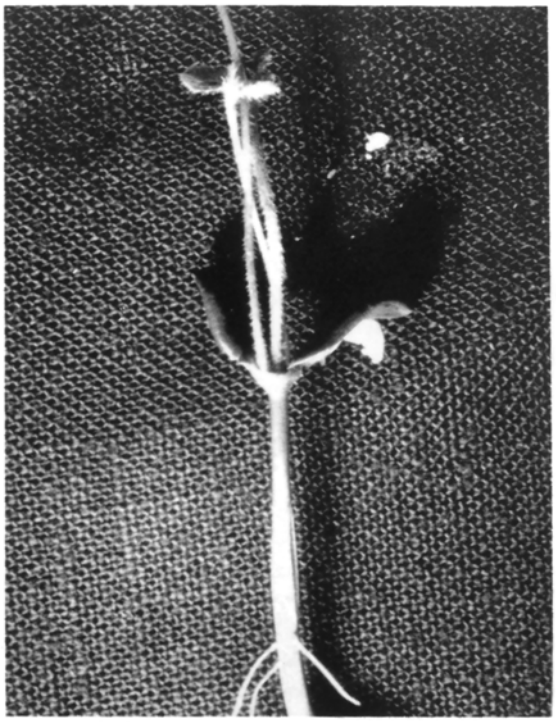

Fig. 1. Shoot formation at the cotyledonary node after 4 weeks in culture. The cultivar used here was 'A3127' and 3 shoots, including the main shoot, were counted for this cotyledonary node

Seeds of five soybean cultivars collected from three locations in Illinois were also tested for multiple shoot formation to establish the genetic basis for the shoot formation response and to test the possibility that environmental effects during seed production could affect the shooting response (Table 2). While the seed lots used showed quite different cold germination percentages, the number of shoots formed were similar for each genotype seed lot indicating that the shoot

Table 1. Effect of BAP concentration on soybean shoot formation done at two different times

Cultivar	Shoot produced[a] BAP treatment			
	1 μmol		5 μmol	
	Expt. 1	Expt. 2	Expt. 1	Expt. 2
'Earlyana'	7.4[b]	8.0	7.7	8.2
'A3127'	4.8	5.0	5.7	6.0
'McCall'	5.9	5.5	5.3	4.3

[a] Values are means from 10 replicates counted four weeks after treatment. The experiment was repeated twice

[b] No significant differences were observed within a genotype and treatment between expt. 1 and expt. 2 at the 95% confidence level

Table 2. Shoot formation counted after 4 weeks using 10 replicates for 5 cultivars grown at 3 locations in Illinois using two concentrations of BAP

Cultivar	Location	BAP treatment		Cold germination[a] (%)
		1 μmol	5 μmol	
'Union'				
	1	4.57±1.60[b]	5.12±0.99	61
	2	3.50±2.00	4.37±1.06	51
	3	4.30±1.30	4.30±0.70	61
'Williams'				
	1	3.80±1.14	4.37±0.74	84
	2	4.60±1.07	4.70±1.30	76
	3	4.85±1.21	4.00±1.60	56
'Century'				
	1	4.60±1.07	4.00±0.50	89
	2	3.62±1.06	4.80±0.90	57
	3	4.00±0.86	5.20±1.38	70
'BSR 201'				
	1	5.40±1.50	5.17±2.00	43
	2	5.12±1.40	5.20±1.70	64
	3	4.40±1.30	7.62±6.00	68
'Pella'				
	1	3.78±1.48	4.50±1.20	66
	2	4.50±1.80	4.62±1.18	85
	3	4.85±1.06	4.40±0.72	56

[a] Cold germination results are courtesy of Joe Lamb, Illinois Crop Improvement Association

[b] SD

forming ability was not influenced by the seed production environment.

There were significant differences between genotypes within maturity groups for shoot formation but there were no differences among maturity groups. For example, maturity group '000' genotypes varied from 4.0 to 7.0 shoots/seed while maturity group 'IV' genotypes varied from 3.1 to 10.4 shoots/seed (Tables 3 and 4). The *G. soja* genotypes did not show significant differences in their ability to form more shoots as the concentration of BAP was changed (Table 4). 'PI 326581' and 'PI 326582A' did produce a higher number of shoots than the other *G. soja* genotypes tested but the *G. soja* genotypes tested did not as a whole perform better than the *G. max* genotypes.

A significant interaction between cultivars and BAP treatments was observed. Cultivars such as 'Goku' and 'PI 31122' produced < 5.0 shoots at 1 μmol and > 10 at 5 μmol BAP while 'Chico' and 'PI 32033' produced the same number of shoots at both concentrations of BAP. Thus, cultivar specific and hormone concentration specific responses were observed.

Because differences among genotypes were observed, the genetic backgrounds of some genotypes were examined to establish if there were any genetic relationships among cultivars which produced a high number or low number of shoots (Table 5). Genotypes were ranked as good when 10 or more shoots were produced, intermediate when six to nine shoots were produced, and poor when five or less shoots were produced at either one of the concentrations of BAP used. The poor responder, 'Williams', was a parent of both 'A3127' and 'Crawford' both of which responded poorly. 'Ada' and 'Blackhawk', both high shoot producers, have both 'Mukden' and 'Richland' as parents. 'Richland' (high shoot producer) and 'Lincoln' (low shoot producer) are present in the parentage of 'Wayne' (intermediate shoot producer) which is a parent of 'Williams' (poor shoot producer). The other genotypes in 'Williams' background also include 'Lincoln' (poor), 'Richland' (high) and 'Adams' (poor) which might explain the poor response of 'Williams'. The combination of four parents 'Wayne' × [('Lincoln'² × 'Richland') × 'Adams'] can produce either an intermediate ('Woodworth') or a poor ('Williams') shoot producing genotype. The genotypes mentioned here are listed in Table 3. One can conclude that, in general, there is a parental influence on the multiple shoot production response indicating that the response is under genetic control.

To determine if hybrid vigor for shoot forming ability occurred, F_1 hybrid seeds from several crosses were examined (Table 6). The hybrid between 'Illini' × 'Voris 295' (both low shoot producers) showed significantly higher numbers of shoots than either parent. The

Table 3. Mean number of shoots produced per seed for several soybean genotypes grown in B5 medium containing 1 or 5 μmol BAP and counted after 4 weeks with 10 replicates in each treatment

Maturity group	Genotype	BAP treatment	
		1 μmol	5 μmol
000	'PI 180519'	6.29	7.00
	'PI 180521'	5.75	6.89
	'PI 181519'	6.00	7.00
	'PI 189963'	4.33	5.78
00	'Ada'	6.00	10.00
	'Altona'	6.44	9.25
	'Crest'	6.00	9.71
	'Flambeau'	5.67	5.67
	'Maple Presto'	6.40	5.89
	'McCall'	5.90	5.33
	'Pagoda'	9.17	8.83
	'PI 30685'	4.56	8.38
	'PI 30694'	6.25	6.25
	'PI 54855'	5.38	9.25
	'PI 132214'	6.13	8.89
	'PI 227327'	7.00	6.56
	'PI 232997'	4.78	3.89
	'PI 404155A'	8.70	10.50
0	'Capital'	5.43	7.00
	'Chico'	7.57	7.12
	'Clay'	5.89	6.22
	'Comet'	6.38	7.57
	'Dawson'	6.10	9.10
	'J-82'	5.60	4.50
	'Ozzie'	4.90	4.30
	'PI 30692'	4.13	12.67
	'PI 32033'	6.90	6.75
	'PI 79739'	8.38	10.25
	'PI 89001'	8.33	8.14
	'PI 152361'	3.92	6.33
	'PI 188866'	4.80	7.00
	'PI 227327'	7.00	6.56
	'PI 232994'	7.00	5.50
I	'A-100'	4.40	4.80
	'Blackhawk'	6.40	10.00
	'Bombay'	6.13	7.00
	'Chippewa 64'	4.29	5.38
	'Earlyana'	7.43	7.78
	'Giant Green'	6.57	8.14
	'Habaro'	6.25	7.33
	'J-88'	6.00	5.43
	'Manchu' (Montreal)	–	3.50
	'Manchuria'	6.25	5.83
	'Mandarin'	4.22	5.38
	'Medium Green'	5.29	6.00
	'Mendota'	5.89	9.71
	'OAC 211'	6.80	6.71
	'PI 03609'	6.29	6.89
	'PI 31122'	4.67	12.67
	'PI 36653'	7.50	10.00
	'PI 70017'	6.20	8.43
	'PI 81765'	3.50	6.71
	'PI 229354'	6.25	6.86
	'PI 291276'	6.50	7.00
	'Soysota'	6.22	9.50
	'Wisconsin black'	6.13	9.00

continued overleaf

Table 3 (continued)

Maturity group	Genotype	BAP treatment	
		1 μmol	5 μmol
II	'Amsoy'	4.42	5.09
	'Amsoy 71'	5.25	5.44
	'Beeson 80'	5.20	7.60
	'Black Eyebrow'	5.80	9.00
	'Century'	5.90	5.33
	'Corsoy'	4.69	5.63
	'Goku'	4.57	10.14
	'Harosoy 63'	4.00	4.50
	'Henry'	7.73	8.86
	'J-103'	5.30	5.33
	'J-105'	5.29	6.11
	'Kanum'	3.00	1.20
	'Korean'	4.33	–
	'Linman 533'	9.00	8.00
	'Madison'	6.40	8.90
	'PI 01547'	4.50	4.00
	'PI 31409'	5.00	6.47
	'PI 70242-2'	11.75	12.00
	'PI 80488-1'	5.43	5.20
	'PI 82532'	6.00	5.75
	'PI 84992'	6.33	9.00
	'PI 86046'	4.25	7.00
	'PI 135590'	6.16	5.86
	'PI 181533'	6.25	4.71
	'PI 253650A'	4.11	7.70
	'PI 358313'	5.60	9.71
	'PI 416865'	5.43	6.63
	'Richland'	7.19	10.33
	'Seneca'	–	4.67
	'Sloan'	5.50	3.00
	'Voris 285'	2.00	4.00
	'Wells'	4.89	6.13
III	'A.K. (Harrow)'	5.67	4.00
	'A3127'	4.82	5.69
	'Adams'	5.67	1.50
	'Columbia'	6.67	8.43
	'Cumberland'	5.90	5.80
	'Dunfield'	6.63	9.00
	'Elf'	4.20	5.00
	'Illini'	3.25	3.75
	'Lincoln'	4.63	3.00
	'Manchu'	6.29	3.67
	'Oakland'	6.60	5.60
	'PI 02108'	4.14	6.67
	'PI 31678'	6.25	9.00
	'PI 54583'	6.75	8.33
	'PI 153292'	7.00	–
	'PI 181535'	6.00	4.50
	'PI 229336'[b]	3.78	5.67
	'PI 243532'	5.44	7.29
	'PI 361063'	10.22	9.78
	'PI 390936'	4.57	5.89
	'PI 416868A'	2.75	6.40
	'Wayne'	6.55	5.69
	'Williams'	5.07	3.73
	'Woodworth'	6.33	7.25
IV	'Carlin'	7.29	10.29
	'Chief'	5.25	4.00
	'Columbus'	3.38	4.88
	'Crawford'	5.13	4.00
	'Desoto'	5.37	4.80

Table 3 (continued)

Maturity group	Genotype	BAP treatment	
		1 μmol	5 μmol
IV	'Gibson'	5.33	4.40
	'Lawerence'	4.00	4.60
	'Mitchell'	4.75	6.14
	'Norredo'	3.50	–
	'Patoka'	8.20	4.50
	'PI 31557'	4.50	4.00
	'PI 70467'	4.33	4.78
	'PI 157431'	5.71	8.60
	'PI 181539'	5.20	4.43
	'PI 303037'[d]	5.00	3.17
	'PI 361103'	3.40	7.25
	'PI 398349'[c]	5.25	9.00
	'PI 399028'[d]	4.50	5.80
	'PI 404165'	6.50	5.56
	'PI 404185'	6.56	9.57
	'PI 408294A'	3.50	6.29
	'PI 408332A'[c]	3.67	3.29
	'PI 417207'[c]	3.00	5.00
	'PI 423763'[d]	3.00	4.50
	'PI 424188B'[c]	3.89	4.83
	'PI 424226'[d]	3.00	4.67
	'PI 424367'[c]	6.33	7.57
	'PI 424499C'	6.75	6.00
	'PI 424592'[c]	5.33	6.33
	'Sooty'	–	9.80
	'Union'	6.30	6.10
	'Wabash'	5.50	8.33
	'Wilson'	6.25	5.50
V	'Dare'	5.85	5.67
VII	'Braxton'	5.70	8.13

[a] No data taken

[b, c, d] Good, intermediate, poor rooters, respectively, as determined in a experiment by Hilderbrand and Collins (1983)

Table 4. Mean number of shoots produced by *Glycine soja* genotypes when grown on B5 medium supplemented with 1 or 5 μmol BAP for 4 weeks. Genotypes belonging to different maturity groups were chosen

Maturity group	Entry	BAP treatments	
		1 μmol	5 μmol
II	'PI 326581'	7.50±2.07[a]	10.13±3.09
II	'PI 326582A'	11.00±1.41	5.80±2.59
II	'PI 342618A'	5.75±1.98	4.88±1.36
II	'PI 339732'	3.50±1.85	5.83±1.17
IV	'PI 339735A'	5.60±2.07	4.67±1.15
IV	'PI 366122'	–[b]	6.00±0.00
IV	'PI 366123'	3.50±1.64	3.67±1.12
V	'PI 339731'	6.00±1.41	5.00±0.63
V	'PI 339871A'	3.75±1.50	2.14±1.46
V	'PI 349647'	4.60±2.41	5.00±2.55
VI	'PI 378683'	8.00±0.00	–
VII	'PI 407065'	9.00±0.00	8.00±1.41
X	'PI 393551'	3.00±0.00	1.00±0.00

[a] SD

[b] Data not recorded

Table 5. Genetic background of some of the genotypes screened for multiple shoot formation

Genotypes producing ≥ 10.00 shoots	
'Carlin'	From 'Dunfield' (PI 36846)
'Ada'	'Merit' × 'Norman' = [('Blackhawk' × 'Capital') × 'Norman']
'Blackhawk'	'Mukden' × 'Richland'
'Richland'	From 'Manchuria'
Genotpyes producing 6–9 shoots	
'Comet'	'Pagoda' × 'Mandarin'
'Clay'	'Capital' × 'Renville'
'Earlyana'	'Rogue' in 'Dunfield'
'Habaro'	'PI 20405'
'Wayne'	[('Lincoln'[2] × 'Richland') × ('Lincoln' × 'CNS')] × 'Clark'
'Wells'	['Harosoy' × ('Lincoln' × Ogden')] × ['Blackhawk' × 'Harosoy']
'Woodworth'	'Wayne' × [('Lincoln'[2] × 'Richland') × 'Adams']
'Union'	'Williams'[5] × (['Wayne'[6] × 'Clark 63') × 'Wayne'] × [('Clark'[6] × 'T201') × ('Clark'[6] × 'T245')] × ('Wayne'[10] × 'Kanrich'))
Genotypes producing ≤ 5 shoots	
'A3127'	'Williams' × 'Essex'
'Columbus'	('Lincoln' × 'Ogden') × 'Clark'
'Crawford'	'Williams' × 'Columbus'
'Elf'	'Williams' × 'Ransom'
'Kanum'	'PI 84668-1'
'Sloan'	[('Lincoln'[2] × 'Richland' × 'Korean') × ('Renville' × 'Capital')] × ('Amsoy' × 'Wayne')
'Williams'	'Wayne' × [('Lincoln'[2] × 'Richland') × 'Adams']
'Lincoln'	'Mandarin' × 'Manchu'

Table 6. Number of shoots formed by the hybrid soybean seeds grown on the two concentrations of BAP as compared to either parent. The number of shoots formed at the cotyledonary node regions were counted 4 weeks after the cultures were initiated

Genotype	BAP treatments	
	1 μmol	5 μmol
1. 'Illini'	3.2	3.6
'Illini' × 'Voris 295'	6.3 (4)[a]	9.3
'Voris 295'	2.0	4.0
2. 'Williams'	5.0	3.7
'Williams' × 'PI 70242-2'	4.0 (3)	5.5 (2)
'PI 70242-2' × 'Williams'	6.0 (1)	–[b]
'PI 70242-2'	11.7	12.0
3. 'Earlyana'	7.4	7.7
'Earlyana' × 'Manchu Montreal'	8.4 (11)	9.2 (12)
'Manchu Montreal' × 'Earlyana'	5.5 (8)	9.7 (8)
'Manchu Montreal'	–	3.5
4. 'Earlyana'	7.4	7.7
'Earlyana' × 'A3127'	6.3 (8)	6.4 (8)
'A3127'	4.8	5.6
5. 'Earlyana'	7.4	7.7
'Earlyana' × 'Wayne'	6.0 (3)	–
'Wayne' × 'Earlyana'	9.0 (2)	8.0 (1)
'Wayne'	6.5	5.6

[a] Number of seeds used for each treatment given in ( ). Other genotypes had 10 seeds each
[b] Data not recorded

'Wayne' × 'Earlyana' (both intermediate shoot producers) cross also produced higher numbers of shoots than either parent but the reciprocal cross of 'Earlyana' × 'Wayne' did not show higher shoot formation. The other three hybrids showed similar or lower shoot forming ability when compared to either parent. Thus, the only hybrid which performed better than the parents was the cross between two poor shoot producers. This may indicate that there is a maximum number of shoots which can be formed. However, the greatest number seen with the hybrids was not close to the maximum number of 12 shoots seen in the genotype screening experiment as described below. It is also possible that certain genotype combinations may show better combining ability for this trait. One must also be cautious in interpreting these results since only a limited number of F_1 plants were tested (Table 6).

In an experiment performed by D. Hildebrand and G. Collins (personal communication) many *G. max* genotypes were grown in the field and the plants subsequently cut off at the soil level at the R_6 stage. The cut stems were dusted for a short period with 'Hormex' (rooting powder containing 0.3% IBA) and grown in sand in a mist chamber. The roots were examined after nine days and were ranked as good, intermediate or poor rooters by visual observation (Table 3). 'PI 229336' (maturity group III, Table 3) was the only good rooter tested here and when tested for multiple shoot production performed as an intermediate shoot producer. The intermediate and poor rooters performed poorly in the cotyledonary node assay. Two exceptions were seen, 'PI 398349', an intermediate rooter was also an intermediate shoot producer, and 'PI 399028', a poor rooter responded intermediately in the shoot screen. A similar pattern for the number of shoots and roots formed was seen when the results of rooting and multiple shoot formation experiments were compared. As the number of shoots produced decreased, the number of roots produced decreased also.

Three of the 56 genotypes used by Beversdorf and Bingham (1977) were reported to have a greater capacity to develop embryo-like structures. When these three genotypes, 'Wayne', 'Corsoy', and 'Chippewa 64', were used in our multiple shoot assay they performed intermediately or poorly.

In the experiments reported here the highest number of shoots (approximately 12) were produced by PI's '404155A', '30692', '31122', and '70242-2', whereas

'Kanum' and 'PI 393551' were the poorest performers producing one to three shoots in both BAP treatments.

In these experiments we screened 155 soybean genotypes to identify those which produced high numbers of shoots. This screening was carried out with the hope that the high shoot producing genotypes may form tissue cultures from which plants can be regenerated more easily. Preliminary results with a suspension culture system similar to that of Phillips and Collins (1981), in which calli from good, intermediate, and poor shoot producers were used, has shown that, in general, there is a direct relationship between the number of embryoids produced in suspension and the number of shoots produced in the cotyledonary node assay (Kerns et al. 1986). Thus this shoot screening system may identify genotypes which will be more likely to undergo plant regeneration in culture.

Acknowledgements. Special thanks is given to Dr. C. D. Nickell for his advice and help. We also thank Joe Lamb from the Illinois Crop Improvement Association for providing seeds grown at three locations and to Dr. R. Bernard for providing the other seeds.

References

Beversdorf WD, Bingham ET (1977) Degrees of differentiation obtained in tissue cultures of *Glycine* species. Crop Sci 17:307–311

Cheng TY, Saka H, Voqui-Dinh TH (1980) Plant regeneration from soybean cotyledonary node segments in culture. Plant Sci Lett 19:91–99

Christianson ML, Warnick DA, Carlson PS (1983) A morphogenetically competent soybean suspension culture. Science 222:632–634

Gamborg OL, Davis BP, Stahlhut RW (1983) Somatic embryogenesis in cell cultures of *Glycine* species. Plant Cell Rep 2:209–212

Gamborg OL, Miller RA, Ojima K (1968) Nutrient requirements of suspension cultures of soybean root cells. Exp Cell Res 50:151–158

Kerns HR, Barwale UB, Meyer MM Jr, Widholm JM (1986) Correlation of cotyledonary node shoot proliferation and somatic embryoid development in suspension cultures of soybean (*Glycine max* L. Merr.). Plant Cell Rep (in press)

Oswald TH, Smith AE, Phillips DV (1977) Callus and plantlet regeneration from cell cultures of ladino clover and soybean. Physiol Plant 39:129–134

Phillips GC, Collins GB (1981) Induction and development of somatic embryos from suspension cultures of soybean. Plant Cell Tissue Organ Culture 1:123–129

Saka HT, Voqui-Dinh TH, Cheng TY (1980) Stimulation of multiple shoot formation on soybean stem nodes in culture. Plant Sci Lett 19:193–201