Plant Recovery from Embryonic Axes of Deteriorated Peanut Seed for Germplasm Renewal[1]

J.B. Morris*[2], S. Dunn[3], and R.N. Pittman[2]

ABSTRACT

Embryo axes explants from deteriorated seed of peanut (*Arachis hypogaea* L.) were incubated at a 16 hr photoperiod at 26 C on an MSB5 medium containing MS salts, B5 vitamins, 20 g/L sucrose, and 8 g/L agar. Five to 8-wk-old plants regenerated from embryonic axes were transplanted to Jiffy pots in the greenhouse. Thirty-two samples of deteriorated seed between 2 and 32 yr old were evaluated. Significant differences in organogenesis were observed between different seed accessions. Shoots and roots were recovered from 74 and 36%, respectively, of embryonic axes explants. In the same experiment, seed producing plants were recovered from 14- to 31-yr-old deteriorated seed of E-2, PI 275704, Macrocarpa, G33, G34, G64, Strain No. 5, TMV 3, PI 290608, PI 295981, Sekelembwe, PI 298879, PI 337300, and PI 371850, by *in vitro* rescue of embryonic axes, while no plants were recovered from seed of 31 seed accessions germinated in the field. The *in vitro* rescue of embryonic axes can significantly increase the recovery of germplasm from deteriorated seed of peanut.

Key Words: *Arachis hypogaea,* peanuts, tissue culture, germination, seed longevity, seed viability.

The USDA, ARS Plant Genetic Resources Conservation Unit is dedicated to acquiring, conserving, characterizing, evaluating, documenting, and distributing the genetic resources of crops, including cultivated peanut. The long-term preservation of seed at germplasm storage facilities requires a large amount of land and labor (19). More than 7400 accessions of the U.S. cultivated peanut germplasm collection are stored as seed at 4 C and 40% relative humidity at the USDA, ARS, PGRCU in Griffin, GA.

Seed longevity is influenced by genotype, seed moisture content, storage conditions including specific light requirements and low temperatures, seed cultur-ing conditions including endogenous inhibitors and embryo immaturity, and harvesting and processing methods (3, 7, 11, 12). Even under optimum conditions seeds lose their viability and germplasm losses occur during seed storage (19). Most peanut seeds do not germinate after 10 to 12 yr in storage. Embryos of inviable seeds may possess the potential to initiate development but may be inhibited from reaching maturity due to a failure of nutritional support tissue (18). This can be circumvented by embryo axis excision and *in vitro* culture.

A tissue culture protocol for recovery of plants from

[1]Research supported by the USDA, ARS, SAA, and the Univ. of Georgia, Griffin, GA 30223-1797.

[2]Agronomist/Legume Curator and Peanut Curator, USDA, ARS, Plant Genetic Resources and Conservation Unit, Griffin, GA 30223-1797.

[3]Lab Tech. I, Dept. of Plant Pathology, Georgia Exp. Stn., Univ. of Georgia, Griffin, GA 30223-1797.

*Corresponding author.

embryonic axes of deteriorated seed from wild *Arachis* species has been developed (4). Embryo culture for testing seed germinability has been conducted in other crops (13, 14, 23). Some peanut genetic resources could be lost forever if such a technique is not applied to cultivated peanut. This report describes the use of embryo axis rescue from deteriorated seed accessions of cultivated peanut.

Materials and Methods

Effect of Genotype on Embryonic Axes Organogenesis. Seed accessions from the 49 *Arachis hypogaea* L. genotypes used in this study had been stored at 4 C and 40% relative humidity in paper envelopes inside metal trays for 2 to 32 yr (Table 1). Embryonic axes were excised from peanut seeds, surface-sterilized in 2.5% sodium hypochlorite (20% clorox bleach) plus 10 drops/L of Tween 20, and rinsed for 10 min in sterile distilled water. Embryonic axes were cultured radical down in 25 x 150-mm culture tubes containing 15 mL of MSB5 medium containing MS salts (14) B5 vitamins (5), 20 g/L sucrose, and solidified with 8 g/L agar (Sigma Chemical Co., St. Louis, MO). The media pH was adjusted to 5.8 prior to the addition of agar and autoclaving at 120 C and 105 kg/cm^2 for 15 min. Explants were incubated at a 16-hr photoperiod (100 mmolm^2/sec from cool-white fluorescent lamps) at 26 C.

This experiment was a completely randomized design with a maximum of four single plant replicates per genotype. Data recorded included the number of embryo axes with shoots, roots, callus, and buds (data taken 4 to 8 wk after culture initiation).

Effect of Transferred Genotypic Explants on Organogenesis. Embryonic axes that were green after 1 to 12 mo in culture that did not produce shoots were transferred to MSB5 medium supplemented with 1 mg/L BAP. Shoots (4-6 cm long) produced initially from embryonic axes or after transfer to fresh BAP-containing media that did not produce roots were transferred onto MSB5 media after slicing the shoot at its base.

This experiment was a completely randomized design with a maximum of 92 single plant replicates per genotype. Data recorded included the number of cultures producing shoots, roots, callus, and shoot buds. Data was recorded 4 to 8 wk after the subculture period.

Effect of Genotype on Plant Survival and Seed Production. Five to 8 wk after embryonic axes or shoots were cultured, plants that developed were transferred to the greenhouse. Culture medium was washed from the roots prior to the plants being transferred into 10-cm-diameter Jiffy pots (A. H. Hummert Seed Co., St. Louis, MO) containing potting soil. Plants were placed on a mist bench for 1 wk to acclimate them to greenhouse conditions. Surviving plants were transplanted to 163-cm-diameter plastic pots (A. H. Hummert Seed Co., St. Louis, MO) and transferred to the greenhouse.

This experiment was a completely randomized design with a maximum of 92 single plant replicates per genotype. Data included the number of regenerated plants (5 mo after transfer to soil medium), and the number of seeds produced from these plants. In a preliminary investigation, seed from 31 accessions were planted in the field with 0% germination.

In all experiments, analysis of variance was conducted and standard errors of the mean were estimated on the data to evaluate the effects of genotypes and explants (22).

Results and Discussion

Effect of Genotype on Embryonic Axes Organogenesis. Highly significant differences ($P < 0.01$, F test) were observed in the ability of embryonic axis explants from different deteriorated peanut seed accessions to undergo organogenesis (Table 1). The number of explants that produced shoots, roots, callus, and buds was greatest for runner genotypes, intermediate for bunch genotypes, and lowest for valencia genotypes. The number of embryonic axes that produced shoots, roots, and buds without callus was greatest for plant introductions (PIs) 270981, 288124, 298834, 337300, 372335, 494782, 496440, and 496450 (Table 1). These results indicate considerable variation in the ability of different genotypes to undergo organogenesis. Previous investigators also observed genotype to be a factor influencing the *in vitro* performance of peanut (8, 15, 17, 18, 21). Genotypic differential factors influencing *in vitro* performance have also been reported for *Stylosanthes guyanensis* (Aubl.) Sw. (12) and several *Trifolium* species (9).

This experiment utilized a small number of seeds for each genotype. This was necessary due to the small number of seed available from the deteriorated seed accessions.

Effect of Transferred Genotypic Explants on Organogenesis. Embryonic axes that produced callus or had shoot buds which did not elongate were transferred to MSB5 medium supplemented with 1 mg/L of BAP. Highly significant differences ($P < 0.01$, F test) were observed in the ability of transferred explants from different peanut genotypes to produce shoots (Table 2). All explants produced shoot buds except PI 290605 and PI 290608. The greatest number of cultures producing shoots was observed for the bunch and runner genotypes PIs 275498, 288123, 288133, 288178, 290605, 290607, 290608, 290614, 372335, and 468138. Shoots that developed without roots were transferred to MSB5 medium. The greatest percentage of shoots with roots after transfer to MSB5 were from the runner types PI 290605 (100%) and PI 298879 (50%) and the bunch type PI 290607 (100%). Roots were recovered from 36% of the embryonic axes explants. Callus production was observed in all genotypes except PIs 290605 (runner), 290607 (bunch), 290608 (bunch), 298879 (runner), 337300 (runner), and 496443 (unknown type).

Similar variation in the ability of different peanut genotypes to undergo organogenesis has been reported for other cultivated peanuts (8). The use of cotyledon, hypocotyl, or leaf explants for germplasm rescue of deteriorated seed may be more difficult because these explants require adventitious shoot formation (4). Cucumber cotyledonary explants from deteriorated seed did not regenerate plants (1). In this study, embryonic axes from deteriorated seed did not always develop plants. However, plants were still recovered from some of these embryonic axes by transferring callus tissue, buds, or shoots to adventitious shoot, and shoot elongation media

Table 1. Organogenesis from embryonic axes of peanut.[a]

PI	Cultivar	Country of origin	Type[b]	Seed age	Shoots/ embryo	Axes with		
						Roots	Callus	Buds
				yr		------ No. ------		
162596	No. 6456	Argentina	B	16	1/5	0	1	0
264163	E-2	Australia	B	31	7/7	5	3	4
270829	MJ 371	Rhodesia	B	21	1/4	1	0	1
274191	RCM 384	Bolivia	B	2	3/4	3	0	0
274248	Tripoli-2	Libya	B	32	0/5	0	1	1
288133	G64	India	B	14	3/4	2	3	1
290565	Strain No. 5	India	B	14	3/4	3	1	3
290607	Unknown	France	B	10	1/4	1	0	0
290608	Unknown	Nigeria	B	15	1/2	1	0	1
290609	Unknown	Nigeria	B	11	2/4	2	1	0
290614	Manfredi-102	Argentina	B	23	3/4	0	1	1
292956	Jumbo Giant	Rhodesia	B	16	2/10	0	2	1
295185	Egyptian Giant	Israel	B	15	0/4	2	0	1
207530	Kichusa	Zaire	V	18	1/4	1	1	0
288120	G21	India	V	10	4/4	4	2	2
288124	G34	India	V	24	3/3	2	0	1
275498	Jinju	Rep. of Korea	R	16	3/4	1	2	2
275499	Sasa	Rep. of Korea	R	26	1/5	1	0	2
279953	Macrocarpa	Brazil	R	14	4/4	3	1	2
279954	Nhambiquara Verme	Brazil	R	14	4/4	4	1	2
288123	G33	India	R	29	4/4	1	3	3
288178	GC7	India	R	14	3/4	0	4	2
288212	Nhambiquara	India	R	14	3/4	2	1	0
288213	Nhambiquara	India	R	14	4/4	4	4	1
290605	TMV 3	India	R	23	0/4	1	0	1
290634	Unknown	India	R	15	4/4	4	1	1
290682	Kairiyo Handachi	Japan	R	15	3/4	3	0	1
295981	Unknown	Nigeria	R	23	3/4	3	0	0
298834	Sekelembwe	S. Africa	R	24	2/2	2	0	0
298879	Unknown	S. Africa	R	23	2/4	2	0	2
300241	Virginia	Nigeria	R	10	1/2	1	0	0
300242	LBU 598	Nigeria	R	6	2/4	3	0	2
337300	Unknown	Brazil	R	14	4/4	3	0	2
371850	Unknown	Israel	R	13	2/4	2	1	1
372335	Chimbwila	Nigeria	R	20	2/2	0	0	1
468138	Unknown	Zimbabwe	R	10	4/4	4	3	0
468252	Unknown	Bolivia	R	9	1/3	2	0	1
261914	RCM 447-1 Guanaco	Argentina	R/B	16	1/4	1	0	0
275704	Unknown	Brazil	R/B	14	3/3	2	3	2
298865	Chitanga Spreading	S. Africa	R/B	12	2/3	2	1	1
494782	Unknown	Zambia	UN	10	4/4	4	0	1
494792	Unknown	Zambia	UN	19	4/4	4	1	4
496415	Moaga	Burkina Faso	UN	7	1/4	1	0	1
496439	Nassara Nagouri Poilinga	Burkina Faso	UN	7	4/4	2	3	4
496440	Nassara Nagouri Tanguin	Burkina Faso	UN	7	2/2	2	0	0
496443	Nassara Nagouri Tembo	Burkina Faso	UN	7	3/4	3	0	2
496450	X4	Burkina Faso	UN	7	2/2	2	0	2
270981	F1-5	Rhodesia	UN	14	4/4	4	0	3
407489	Unknown	Senegal	UN	16	0/5	0	1	1
494789	Chipata	Zambia	UN	9	2/4	3	3	4

[a]Nonsignificant age effect.

[b]B = bunch type, V = valencia type, R = runner type, R/B = runner/bunch type, and UN = unknown.

Table 2. Organogenesis of transferred peanut genotypes from callus or shoot buds.[a]

PI	Cultivar	Country of origin	Type[b]	Seed age	Shoots/ embryo	Axes with Roots[c]	Callus	Buds[d]
				yr	no.	------ no. ------		
162596	No. 6456	Argentina	B	16	3/10	0	2	3
288133	G64	India	B	14	77/92	24	49	27
290607	Unknown	France	B	10	2/2	2	0	2
290608	Unknown	Nigeria	B	15	4/5	1	0	0
290614	Manfredi-102	Argentina	B	23	10/13	1	3	4
275498	Jinju	Rep. of Korea	R	16	12/13	1	3	2
275499	Sasa	Rep. of Korea	R	26	0/3	0	1	1
288123	G33	India	R	29	19/20	3	2	2
288178	GC7	India	R	14	9/12	3	6	4
290605	TMV 3	India	R	23	2/2	2	0	0
298879	Unknown	S. Africa	R	23	2/4	2	0	2
337300	Unknown	Brazil	R	14	2/3	0	0	1
372335	Chimbwila	Nigeria	R	20	10/10	3	6	3
468138	Unknown	Zimbabwe	R	10	10/10	3	1	4
275704	Unknown	Brazil	R/B	14	8/14	2	4	8
496439	Nassara Nagouri Poilinga	Burkina Faso	UN	7	3/4	1	2	1
496443	Nassara Nagouri Tembo	Burkina Faso	UN	7	0/2	0	0	1

[a]Nonsignificant age effect.
[b]B = bunch type; V = valencia type; R = runner type; R/B = runner/bunch type; and UN = unknown.
[c]Data represent totals at the end of the subculture period on MSB5 media.
[d]Data represent totals at the end of the subculture period on MSB5 + 1 mg/L BAP.

(MSB5 + 1 mg/L BAP), or root inducing media (MSB5).

Effect of Genotype on Plant Survival and Seed Production. Highly significant differences ($P < 0.01$, F test) were observed in the ability of small plantlets transferred into the greenhouse from different peanut accessions to regenerate into mature plants (Table 3). The greatest percentage of regenerated plants per embryonic axis was observed for the runner types PI 295981 (75%) and PI 337300 (75%) and the bunch type PI 290565 (75%). Sixty percent of the plants transferred to the greenhouse survived of which 41% produced seeds. The greatest number of seeds produced per embryonic axis was observed for the bunch types PI 264163 (47 seeds) and PI 290565 (48 seeds) and the runner type PI 371850 (62 seeds). The greatest percentages of plants regenerated and seed number produced per transferred explant was observed for PIs 290605 (runner), 290608 (bunch), and 337300 (runner). These observations indicate that considerable variation exists in the ability of different peanut genotype explants to undergo plant regeneration and seed production.

Arachis hypogaea seeds held in long-term storage should be regenerated on average every 10 yr. However, if seeds have been stored for extended periods (11 to 30 yr) or in suboptimal conditions, rescue of embryonic axes from seeds can significantly increase germplasm recovery from deteriorated accessions. Embryo axis rescue of cultivated peanut was successful because seeds can fail to germinate for reasons other than total tissue death (4, 20).

Table 3. Plant survival and seed production from plants regenerated from embryonic axes of peanut.[a]

PI	Primary explants Embryos	Plants	Seed	Subcultured tissues Explants	Plants	Seed
	---------- no. ----------					
264163	7	2	47			
274191	4	2	9			
288133	4	2	21	92	5	30
290565	4	3	48			
290608				5	3	48
288124	3	1	29			
279953	4	1	10			
288123	4	1	31			
290605				2	2	72
295981	4	3	39			
298834	2	1	9			
298879	4	2	34			
300241	2	1	7			
300242	4	1	32			
337300	4	3	18	3	3	44
371850	4	2	62			
372335				10	3	34
275704				4	2	17
494782	4	1	12			
496439	4	2	11			
496443	4	1	12			
496450	2	1	9			

[a]Nonsignificant age effect.

Embryos or embryonic axes have shoot meristems and may be the best explant for germplasm recuperation from depreciated seeds. *In vitro* culture of seeds, embryos, or embryonic axes has been used to rescue interspecific hybrids (6, 16), identify virus-free plants (2), provide meristems for microprojectile bombardment (20), provide shoot tips and meristems forcryopreservation (10), and break seed dormancy (3). Similar methods may be useful for rescuing germplasm from degenerated seed accessions of other crop species (4). In conclusion, embryonic axes from deteriorated peanut seed provide an excellent explant source for peanut germplasm renewal and recovery. Organs including shoots, buds, and roots developed rapidly from these peanut seed and plants can be transferred to the greenhouse or field as early as 5 wk after culture initiation. Plants obtained from embryonic axes appeared normal and true-to-type. These attributes make embryo axis rescue a promising method for obtaining plants and seed from deteriorated seed accessions.

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