Plant Cell, Tissue and Organ Culture **44:** 63–70, 1996.

Effect of abscisic acid, osmolarity and partial desiccation on the development of recalcitrant mango somatic embryos

Fernando Pliego-Alfaro[1,2], Mary Joy R. Monsalud[2], Richard E. Litz[2,*], Dennis J. Gray[3] & Pamela A. Moon[2]

[1]*Departamento de Biologia Vegetal, Universidad de Malaga, Malaga, Spain;* [2]*Tropical Research and Education Center, University of Florida, 18905 S. W. 280 Street, Homestead, FL 33031 USA;* [3]*Central Florida Research and Education Center, University of Florida, University Avenue, Leesburg, FL 34748 USA (*requests for offprints)*

Received 16 February 1995; accepted in revised form 19 September 1995

Key words: Mangifera indica L., somatic embryo, dehydration

Abstract

Inhibition of mango somatic embryo growth was induced *in vitro* by treatments for 4 or more weeks with abscisic acid (0–100 μM ABA) with and without high osmolarity provided by mannitol (0–10%). High osmolarity and ABA significantly affected somatic embryo length, precocious germination and the production of good quality secondary somatic embryos. High osmolarity also affected root elongation. Abscisic acid was more effective in suppressing growth and development of $\geq$0.5 cm-length somatic embryos than smaller somatic embryos. Development beyond the heart stage was significantly inhibited by both ABA and mannitol treatments. The recovery of good quality somatic embryos was enhanced by high levels of ABA (100 μM) with and without mannitol (0–5%). Somatic embryos that had been pulsed with ABA were partially desiccated at different relative humidities. Weight loss was affected only by relative humidity; and ABA did not enhance desiccation tolerance.

Abbreviations: ABA - Abscisic acid; 2,4-D - 2,4-Dichlorophenoxyacetic acid; MM1 - Mango maturation medium; RH - Relative humidity

Introduction

Mango (*Mangifera indica* L.) embryos are considered to be recalcitrant, and do not undergo a period of maturation drying during seed development (Roberts, 1973). Mature recalcitrant embryos cannot survive partial dehydration or storage at low temperatures (King & Roberts, 1979). Depending on the species, recalcitrant species cannot be stored for more than a few days or weeks. Recalcitrant embryo maturation, germination and seedling development are viewed as being a single uninterrupted process. Orthodox embryos, in contrast, undergo dehydration to as low as 5% moisture content, and in this state, can survive in storage for many years at ambient or low temperatures (Roberts, 1973).

Abscisic acid and osmolarity both appear to be involved in controlling maturation of orthodox embryos. Using somatic embryo maturation as a model, Bornmann (1993) and Janick *et al.* (1993) suggested that ABA increased endogenous levels of storage proteins and fatty acids; Fujii *et al.* (1993) found that high levels of starch could also be deposited. Although high osmolarity levels have been demonstrated to be beneficial for embryo maturation, their role has not been clear. Increased endogenous ABA levels have occurred in response to osmotic treatments with barley (Morris *et al.*, 1988); however, this was not observed with wheat and rapeseed (Finkelstein & Crouch, 1986; Morris *et al.*, 1988). Sucrose and polyethyleneglycol (PEG) improved the quality of celery somatic embryos, but there was no effect on fatty acid content in either species (Janick *et al.*, 1993). High osmolarity and ABA stimulate embryo maturation of rapeseed (Finkelstein & Crouch (1987), but normal seedling morphology would occur only after high osmolarity treatments. In contrast, Fujii *et al.* (1989) observed better plant recov-

ery with ABA alone. The modes of action of ABA and osmolarity therefore appear to be variable and dependent on the species.

The role of ABA and osmolarity in the development of recalcitrant embryos is not well documented. Pence (1991) demonstrated that endogenous ABA levels increase at the beginning of *Theobroma cacao* L. embryo maturation. Increased production of lipids and fatty acid saturation was observed to be associated with higher ABA levels in cacao embryos (Pence, 1992). Etienne *et al.* (1993) observed that high osmolarity and ABA levels promoted somatic embryo maturation and germination of rubber *Hevea brasiliensis*.

Most tropical tree species reproduce from recalcitrant seeds (Chin, 1988; Chin & Roberts, 1980). The absence of developmental arrest of embryos of tree species that have recalcitrant seeds has prevented reforestation in the tropics, and has created the need to establish large living germplasm repositories because seed of these species cannot be stored. In a recent report, Pliego-Alfaro *et al.* (1995) described the responses of adventitious nucellar mango embryos to ABA, osmotic stress and low temperature, as part of an attempt to stimulate developmental arrest in these naturally occurring clonal embryos. In this report, we have described the effects of osmolarity, ABA and partial desiccation on the development of mango somatic embryos.

Materials and methods

Establishment of embryogenic cultures

Immature fruits of polyembryonic mango *Mangifera indica* L. ('Hindi') were collected approximately 40-50 days after pollination from the tropical fruit germplasm collection of the University of Florida, Homestead. Fruit were surface-disinfested for 30 min. in a 30% (v/v) Clorox solution containing 2-3 drops of Tween 20 per 100 ml. The disinfested fruit were washed thoroughly with sterile, deionized water, and each fruit was bisected along its longitudinal axis under sterile conditions. The ovule or immature seed was removed from each fruit, bisected along its longitudinal axis, and the embryo mass was removed and discarded. The ovule halves were placed onto sterile plant growth medium in disposable plastic Petri dishes so that the nucellus was in contact with the medium. After 1 week and at 1-week intervals thereafter, the explanted ovules were subcultured onto fresh medium. The plant growth medium consisted of B5 major salts (Gamborg *et al.*, 1968), MS (Murashige & Skoog, 1962) minor salts and organic compounds, 400 mg l^{-1} glutamine, 60 g l^{-1} sucrose 4.5 μm (1.0 mg l^{-1}) 2,4-D and 2.0 g l^{-1} gellan gum as a solidifying agent (DeWald *et al.*, 1989a). Embryogenic tissue was inoculated into liquid medium of the same formulation in 125 ml Erlenmeyer flasks, and subcultured at 5-day intervals. Suspension cultures were maintained at 100 rpm. All cultures were maintained at 25 °C in darkness.

Maturation of somatic embryos

Somatic embryo maturation was initiated by transferring the embryogenic cultures from liquid embryogenesis medium to liquid medium without 2,4-D (MM1). Normal development was stimulated in 2 ways (Monsalud, 1994):

- Heart-stage somatic embryos (4-6 mm length) were removed from liquid MM1 and subcultured on solid MM1 containing 2.0 g l^{-1} gellan gum and 20% (v/v) filter-sterilized coconut water (DeWald *et al.*, 1989b) in Petri dishes. The cultures were maintained in darkness at 25 °C. These somatic embryos were hyperhydric, and failed to develop normally; however, the somatic embryos generally dedifferentiated and produced apparently normal, opaque, white secondary somatic embryos.
- Heart-stage somatic embryos (4-6 mm length) were removed from liquid MM1, and were partially dehydrated in atmospheres of 95 and 100% relative humidity for 24 and 48 h (Monsalud, 1994; Monsalud *et al.*, 1995) in order to reverse hyperhydricity.

Somatic embryos that were normal in appearance were recultured onto solid MM1 in petri dishes. Torpedo-stage somatic embryos were utilized for all of the following experiments.

Effect of ABA and mannitol on somatic embryo maturation

Individual somatic embryos (5-8 mm) that had been partially dehydrated at high relative humidity in order to promote normal development were used (Monsalud, 1994; Monsalud *et al.*, 1995). Four somatic embryos were cultured on plant growth medium in each 100 × 15 mm Petri dish. The plant growth medium consisted of MM1 that had been modified by the addition of different concentrations of ABA [(+)-cis, trans abscisic acid, Sigma A101z] (0, 1.0, 5.0, 10.0, 50.0 and 100.0

μM) together with mannitol (0, 5.0, 7.5 and 10.0% w/v) in all possible combinations. There were between 7 and 12 replicates of each treatment. The cultures were maintained in darkness at 25 °C, and were subcultured at 4-week intervals. Initial data were recorded 4 days after the beginning of the experiment, and included the length of the somatic embryos and the amount of phenolic compound accululation in the plant growth medium. Measurements recorded after 4 and 8 weeks included somatic embryo length, root length, number of high quality (white, opaque) secondary embryos that formed and germination frequency.

Cotyledonary-stage secondary somatic embryos (3.0 to greater than 10.0 mm length) that were normal in appearance (white, opaque) were removed from dedifferentiating hyperhydric somatic embryos on MM1. Somatic embryos that were morphologically similar in terms of cotyledon length and number, freedom from injury and hyperhydricity were used for these experiments. The somatic embryos were classified in 2 arbitrary sizes, i.e., smaller than 5.0 mm and between 6.0 and 10.0 mm, and were plated on MM1 containing different concentrations of ABA, including 0.0, 100.0, 250.0, 500.0, 750.0 and 1000.0 μM. There were 3 replicates per treatment and 4 somatic embryos per replicate. Initial data were recorded 4 days after the cultures were established and at 4- and 8-week intervals. Initial data included the length of the somatic embryos; thereafter, changes in length and germination frequency of the somatic embryos were measured.

Four weeks after the experiment was begun, somatic embryos that exceeded 10-mm length were partially dehydrated at either 90 or 100% RH for 2 days. There were 3–4 somatic embryos for each treatment. Four to 6 somatic embryos were placed in sterile 100×15 mm petri dishes without plant growth medium. A 4.0-mm-diameter hole was drilled into the cover and was covered with a 0.45 μM acetate filter. The plates were sealed with Parafilm. Fresh weights were recorded. These plates from each treatment were placed in a chamber with 90% RH at 25 °C. Three plates were also placed in germination flats with cells with 1500 ml water and were covered with a clear dome, secured tightly with masking tape. Somatic embryos were dehydrated for 72 h and weighed. The change in fresh weight was determined. The somatic embryos were recultured on MM1, and they were assessed for viability and normal maturation 4 weeks later.

Standard errors were calculated from the collected data, and the significance of different treatments on somatic embryo development was determined uning ANOVA at $\alpha = 0.05$ or 0.01.

The pH of plant growth media was adjusted to 5.8 prior to autoclaving at 121 °C and 1.1 kg cm^{-2} for 20 min. Abscisic acid was dissolved in 0.1 N KOH, diluted with deionized water, filter-sterilized and was added to the media following autoclaving. Media were decanted into sterile disposable plastic Petri dishes (15 ml/60×15 mm dish and 20 ml/100×15 mm dish); Petri dishes were sealed with Parafilm. All plant tissue cultures were maintained in darkness at 25 °C.

Results

Preliminary experiments indicated that maturation, i.e., elongation, germination and root length, of somatic embryos was arrested on plant growth medium containing 100 μM ABA. Four- and 8-week pulses, with either mannitol alone at any concentration or together with ABA, significantly inhibited the enlargement of somatic embryos in relation to the controls (Table 1). The change in somatic embryo length due to a 4-week pulse with ABA was not significant; however, 8-week pulses with ABA significantly inhibited somatic embryo enlargement. The maximum inhibition of growth occurred with the highest concentrations of mannitol (7.5 and 10%), irrespective of the ABA concentration.

Secondary somatic embryogenesis was observed from the hypocotyl bases in many of the treatments (Table 1). Most of these somatic embryos were hyperhydric and their appearance could not be correlated with ABA concentration, mannitol or combinations of the two data (data not shown). The appearance of normal secondary somatic embryos that were white and opaque was associated with 8-week pulses with mannitol and/or ABA, and the relationships were significant (Table 1). The maximum number of normal appearing somatic embryos was recovered from treatments with 5.0 μM ABA and 0–5.0% mannitol.

Precocious germination was strongly inhibited by all concentrations of mannitol and by combinations of mannitol with ABA after 4- and 8-week pulses. A 4-week pulse with ABA alone did not appear to suppress precocious germination except at the highest concentrations tested (50 and 100 μM). The most effective treatments for suppressing precocious germination after a 4-week pulse were 50 and 100 μM ABA together with 5–10% mannitol. Precocious germination, however, was significantly suppressed by 8-

Table 1. Effect of ABA and mannitol on size (cm) of primary singulated somatic embryos and secondary somatic embryo production after 4- and 8- week pulses.

μM ABA	% mannitol	Initial SE length	4-week SE length	8-week SE length	8-week 2ndary SE
0	0	0.7±0.0	1.2±0.1	1.7±0.1	1.0±0.2
0	5.0	0.6±0.0	0.8±0.1	1.2±0.1	3.8±1.1
0	7.5	0.6±0.0	0.7±0.1	1.0±0.1	1.8±0.5
0	10.0	0.6±0.0	0.7±0.3	0.8±0.0	0.2±0.1
1.0	0	0.7±0.1	1.2±0.1	1.8±0.1	2.5±1.2
1.0	5.0	6.0±0.0	0.9±0.1	1.4±0.1	2.1±0.9
1.0	7.5	0.6±0.0	0.7±0.0	0.9±0.1	0.3±0.1
1.0	10.0	0.6±0.0	0.8±0.1	0.9±0.1	0.0±0.0
5.0	0	0.7±0.1	1.2±0.1	1.7±0.1	3.4±1.0
5.0	5.0	0.8±0.1	0.7±0.1	1.1±0.1	3.6±1.5
5.0	7.5	0.6±0.0	0.7±0.0	0.9±0.1	0.0±0.0
5.0	10.0	0.6±0.0	0.7±0.0	0.9±0.1	0.0±0.0
10.0	0	0.7±0.0	1.1±0.1	1.6±0.1	1.7±0.6
10.0	5.0	0.6±0.0	0.8±0.1	1.2±0.1	0.4±0.2
10.0	7.5	0.6±0.0	0.7±0.1	1.1±0.1	0.6±0.3
10.0	10.0	0.6±0.0	0.7±0.0	0.9±0.0	0.1±0.0
50.0	0	0.7±0.0	0.9±0.1	1.3±0.1	2.0±0.7
50.0	5.0	0.7±0.0	0.8±0.0	1.0±0.1	0.4±0.2
50.0	7.5	0.6±0.0	0.7±0.0	0.9±0.1	0.0±0.0
50.0	10.0	0.6±0.0	0.7±0.0	0.9±0.1	0.0±0.0
100.0	0	0.7±0.0	0.9±0.1	1.2±0.1	0.2±0.1
100.0	5.0	0.6±0.0	0.6±0.0	0.9±0.1	0.0±0.0
100.0	7.5	0.6±0.0	0.7±0.0	0.9±0.1	0.0±0.0
100.0	10.0	0.6±0.0	0.7±0.0	0.9±0.1	0.0±0.0

Source	parameter	DF	MS	S(PR>F)	R^2
SE initial length	rep	11	0.2970	0.0001	0.0478
	ABA	5	0.1227	0.1611	0.0090
	mannitol	3	0.4714	0.0004	0.0207
	mannitol* ABA	23	0.1204	0.0176	0.0445
SE 4-wk length	rep	11	0.2851	0.0148	0.0304
	ABA	5	0.3894	0.0123	0.0189
	mannitol	3	7.2751	0.0001	0.2115
	mannitol* ABA	23	1.11424	0.0001	0.2547
SE 8-wk length	rep	11	0.4179	0.0993	0.0218
	ABA	5	0.8985	0.0034	0.0260
	mannitol	3	16.625	0.0001	0.2894
	mannitol* ABA	23	2.7136	0.0001	0.3622
secondary SE 8-wk	rep	9	24.225	0.0977	0.0218
	ABA	5	32.579	0.0508	0.0163
	mannitol	3	62.006	0.0055	0.0176
	mannitol* ABA	23	26.023	0.0122	0.0598

Data represent mean length (cm) ± standard error. Somatic embryo = SE.

Table 2. Effect of ABA and mannitol on germination frequency (%) and root elongation (cm) of primary singulated somatic embryos after 4- and 8- weeks.

μM ABA	% mannitol	4-weeks % germ	4-weeks root length	8-weeks % germ	8-weeks root length
0	0	42.5±7.8	1.3±0.1	82.1±7.1	1.2±0.1
0	5.0	18.8±9.2	1.1±0.4	75.0±9.5	1.7±0.2
0	7.5	0.0±0.0	0.6±0.3	45±5.0	1.2±0.3
0	10.0	2.8±2.8	0.7±0.1	39.8±8.3	1.3±0.2
1.0	0	31.2±9.2	1.2±0.1	84.4±8.1	1.6±0.2
1.0	5.0	10.0±10.0	1.0±0.5	65.8±10.9	1.6±0.4
1.0	7.5	9.2±4.7	0.6±0.1	45.8±9.6	1.4±0.3
1.0	10.0	2.8±2.8		27.8±10.1	1.0±0.3
5.0	0	36.1±7.8	1.2±0.1	92.9±4.6	1.9±0.1
5.0	5.0	0.0±0.0	0.4±0.1	36.5±10.3	1.2±0.3
5.0	7.5	10.7±10.7	0.9±0.3	20.2±13.1	2.7±0.4
5.0	10.0	3.1±3.1	0.3±0.0	13.5±7.0	0.8±0.2
10.0	0	33.3±7.2	1.4±0.3	65.6±8.1	1.6±0.2
10.0	5.0	10.7±7.4	1.3±0.6	32.1±7.1	2.6±0.4
10.0	7.5	10.7±7.4	0.7±0.3	29.5±9.4	1.4±0.3
10.0	10.0	2.5±2.5	0.6±0.3	13.7±4.5	1.2±0.2
50.0	0	18.8±10.3	1.2±0.5	59.5±10.5	1.6±0.2
50.0	5.0	0.0±0.0	2.0±0.3	13.9±4.4	1.3±0.3
50.0	7.5	3.1±3.1	1.0±0.1	5.0±5.0	0.5±0.5
50.0	10.0	0.0±0.0		0.0±0.0	
100.0	0	12.5±6.7	1.1±0.4	37.5±12.9	2.1±0.3
100.0	5.0	0.0±0.0		0.0±0.0	
100.0	7.5	0.0±0.0	0.6±0.1	0.0±0.0	0.6±0.3
100.0	0.0±0.0		0.0±0.0		

Source	parameter	DF	MS	S(PR>F)	R^2
Root length 4-wk	rep	11	0.3565	0.6453	0.0942
	ABA	5	1.5955	0.1185	0.0336
	mannitol	3	1.4051	0.0197	0.1012
	ABA*mannitol	23	0.4337	0.4862	0.1978
Germ. 4-wk	rep	11	0.0354	0.6096	0.0471
	ABA	5	0.0980	0.0385	0.0592
	mannitol	3	0.8430	0.0001	0.3052
	ABA*mannitol	23	0.1483	0.0001	0.4117
Root length 8-wk	rep	11	1.0111	0.3543	0.0372
	ABA	5	1.5955	0.1185	0.0326
	mannitol	3	2.7812	0.0265	0.0341
	ABA*mannitol	23	2.0125	0.0008	0.1646
Germ. 8-wk	rep	9	0.0784	0.7719	0.0329
	ABA	5	1.0669	0.0001	0.2491
	mannitol	3	2.4726	0.0001	0.3464
	ABA*mannitol	23	0.6125	0.0001	0.6578

Data represent means ± standard error.

week pulses with mannitol, ABA and combinations of ABA with mannitol (Table 2). The most effective treatments for suppressing precocious germination after an 8-week pulse were 50 and 100 μM ABA with 7.5 or 10% mannitol, respectively. Among the somatic embryos that germinated, root length was apparently suppressed by mannitol after a 4-week pulse (Table 2); however, the differences among treatments were not significant. Combinations of ABA and mannitol sig-

Table 3. Effect of ABA on development of singulated secondary somatic embryos of mango.

μM ABA	Initial SE length cm	4-week SE length cm	4-week germ. %	8-week length cm	8-week germ. %	12-week germ. %
<0.5 cm somatic embryos						
0	0.5±0.0	1.2±0.3	16.7	1.2±0.2	27.3	71.4
100	0.6±0.0	0.7±0.1	0.0	0.9±0.1	0.0	50.0
250	0.5±0.0	0.7±0.1	0.0	0.8±0.1	0.0	100
500	0.5±0.0	0.8±0.1	0.0	0.8±0.1	0.0	50.0
750	0.5±0.0	0.6±0.1	0.0	0.6±0.1	0.0	25.0
1000	0.6±0.0	0.7±0.1	0.0	0.8±0.1	0.0	77.8
Source	parameter		DF	MS	S(PR>F)	R^2
replicate	initial length		2	0.0056	0.6901	0.0119
	#1 subculture		2	0.0192	0.6066	0.0162
	#2 subculture		2	0.0239	0.8825	0.0073
ABA	initial subculture		5	0.0109	0.6071	0.0579
	#1 subculture		5	0.4931	0.0121	0.2181
	#2 subculture		5	0.3472	0.0751	0.2654
>0.5 cm somatic embryos						
0	0.9±0.0	1.7±0.2	10.0	2.2±0.3	66.7	66.7
100	0.9±0.1	1.0±0.1	0.0	1.1±0.1	11.1	25.0
250	0.9±0.1	1.1±0.7	0.0	1.2±0.2	0.0	25.0
500	0.9±0.1	1.1±0.1	0.0	1.1±0.1	0.0	
750	0.9±0.0	1.0±0.0	0.0	1.1±0.0	50.0	
1000	0.8±0.0	1.0±0.0	0.0	1.0±0.1	0.0	100
Source	parameter		DF	MS	S(PR>F)	R^2
replicate	initial length		2	0.0692	0.0754	0.0946
	#1 subculture		2	0.0281	0.8330	0.0069
	#2 subculture		2	0.0589	0.8429	0.0077
ABA	initial length		5	0.0143	0.7720	0.0489
	#1 subculture		5	0.6495	0.0001	0.4031
	#2 subculture		5	1.6262	0.0001	0.5347

nificantly suppressed root elongation after an 8-week pulse.

The accumulation of phenolic compounds in the plant growth media was slightly less after 4 weeks in comparison with the initial readings (data not shown); however, there was no difference between the control and the treatments with mannitol alone. A 4-week pulse with ABA alone or together with mannitol resulted in significantly more accumulation of phenolic compounds in the medium (data not shown).

Abscisic acid inhibited enlargement of small cotyledonary secondary somatic embryos (≤5.0 mm) after pulses of 1 and 2 months; however, the differences among treatments were not significant. The growth of later-stage somatic embryos (5.0–10.0 mm) was significantly suppressed by all ABA concentrations relative to the control after 4- and 8-week pulses (Table 3). Precocious germination of both sizes of somatic embryos was significantly inhibited by ABA relative to the control for up to 8 weeks. There was less precocious germination involving early-stage somatic embryos after ABA pulses of 1 and 2 months than with the corresponding treatments involving later-stage somatic embryos and in comparison with untreated somatic embryos. After a 12-week pulse, there were no sig-

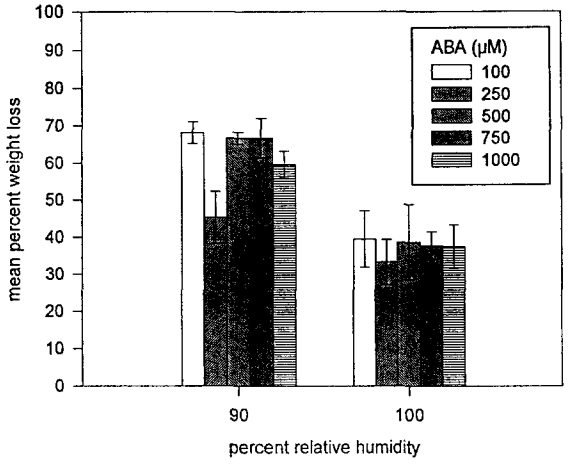

Fig. 1. The effect of different ABA concentrations on dehydration of mango somatic embryos at different relative humidities. Bars = SE.

nificant differences for germination frequency among treatments or between somatic embryo sizes.

Somatic embryos (10–15 mm) that had been pulsed with ABA for 1 month were dehydrated for 72 h at different RH. The percent weight loss was significantly affected by % RH, and there was more water loss from treated somatic embryos at 90% RH than at 100% RH (Fig. 1). Abscisic acid had no effect on water loss, and failed to enhance desiccation tolerance. Four weeks after partial dehydration, there was no significant difference for precocious germination between dehydrated and nondehydrated somatic embryos, regardless of the ABA pulse. After 8 weeks, all of the somatic embryos, both control and partially dehydrated, germinated precociously.

Discussion

Maturation of nucellar mango embryos removed from polyembryonic ovules and grown *in vitro* was reported to be arrested by pulses of 750–1750 μM ABA, 7.5–12.5% mannitol and combinations of ABA with mannitol (Pliego-Alfaro *et al.*, 1995). The effects of mannitol and ABA differed, however, in that there was a 4-week residual effect of ABA, but no such effect with mannitol. In the current study, mannitol was significantly more effective than ABA in suppressing mango somatic embryo development. Mango somatic embryos were sensitive to much lower exogenous levels of both ABA and mannitol than nucellar embryos. Maturation of somatic embryos was suppressed by 100 μM ABA in contrast with 750 μM ABA needed for suppression of growth of nucellar embryos. Mannitol at 10% was toxic for somatic embryos, although nucellar embryos survived exposure to 12.5% mannitol (Pliego-Alfaro *et al.*, 1995). Our results therefore concur with other studies that indicate that osmolarity and ABA affect maturation in different ways (Fujii *et al.*, 1989), and suggest that recalcitrant somatic embryos may not be suitable models for studying *in ovulo* embryo maturation (Pliego-Alfaro *et al.*, 1995).

In order to maximize the effectiveness of ABA during orthodox somatic embryo maturation, it is necessary to apply it at a critical stage of embryo development. According to McKersie *et al.* (1989), ABA should be applied to alfalfa somatic embryos during the early cotyledonary stage. Increase in size of advanced-stage mango somatic embryos (5.0–10.0 mm) was significantly inhibited by ABA in comparison with earlier-stage somatic embryos (≤5.0 mm). In addition, precocious germination was suppressed by ABA in ≤5.0 mm somatic embryos in comparison with 5.0–10.0 mm somatic embryos. Thus, two ABA-sensitive growth responses of recalcitrant mango somatic embryos are dependent on and can be characterized on the basis of their stage of development.

Abscisic acid has been shown to confer desiccation tolerance in orthodox somatic embryos (Carman, 1988; Welbaum *et al.*, 1990). However, viviparous seeds that are mutants of plants that normally produce orthodox seeds produce very little ABA, and have been demonstrated to be unable to tolerate desiccation (Karssen *et al.*, 1983; Koornheef *et al.*, 1989). Etienne *et al.* (1993) reported that *Hevea brasiliensis* recalcitrant somatic embryos acquired desiccation tolerance as a result of treatment with high levels of ABA and sucrose; however, no such relationship was evident in the current study. Pence (1992) also demonstrated that decreased moisture levels in mature recalcitrant cacao embryos in comparison with immature embryos is not associated with dessication tolerance. Abscisic acid-treated mango somatic embryos germinated precociously following dehydration in comparison with undehydrated somatic embryos. Thus, there appear to be clear differences in mode of action of ABA during development of recalcitrant versus orthodox embryos.

Altough desiccation tolerance was not observed in preconditioned recalcitrant mango somatic embryos, the extended maintenance of somatic embryos *in vitro* was possible on plant growth medium containing high levels of mannitol with and without ABA. Develop-

mental arrest without dehydration of mango somatic embryos was most clearly demonstrated as a result of an 8-week pulse with mannitol and ABA. Mannitol was more effective than ABA in suppressing somatic embryo development; however mannitol, unlike ABA, was earlier shown to have no residual effect on development after nucellar embryos were subcultured onto MM1 (Pliego-Alfaro *et al.*, 1995). Mango somatic embryos germinated precociously after osmotic stress unless 100 μM ABA was also present. The control of precocious root formation was also described by Monsalus *et al.* (1995), who showed that precocious germination of partially dehydrated hyperhydric mango somatic embryos could be prevented by the presence of ABA in the plant growth medium. These results could provide the basis for a strategy to induce developmental arrest for short and medium term storage of recalcitrant somatic embryos.

Acknowledgements

The authors would like to express their gratitude for support provided by the International Board for Plant Genetic Resources (IBPGR), now the International Plant Genetic Resources Institute (IPGRI), Rome, Italy. Florida Agricultural Experiment Station Journal Series No. R - 04398.

References

Bornmann CH (1993) Maturation of somatic embryos. In: Redenbaugh K (ed) Synseeds: Applications of Synthetic Seeds to Crop Improvement (pp 105–113). CRC Press, Boca Raton

Carman JG (1988) Improved somatic embryogenesis in wheat by partial simulation of the *in ovulo* oxygen, growth regulators and dessication environments. Planta 175: 417–424

Carron MP & Enjalric F (1985) Somatic embryogenesis from inner integument of the seed of *Hevea brasiliensis* (Kunth., Mull., Arg.). Comp. Rend. Ser. III 300: 653–658

Chin HF & Roberts EH (1980) Recalcitrant Crop Seeds, Tropical Press, Kuala Lumpur

Chin HF (1988) Recalcitrant Seeds A Status Report, International Board for Plant Genetic Resources, Rome

DeWald SG, Litz RE & Moore GA (!989a) Optimizing somatic embryo production in mango. J. Amer. Soc. Hort. Sci. 114: 712–716

DeWald SG, Litz RE & Moore GA (1989b) Maturation and germination of mango somatic embryos. J. Amer. Soc. Hort. Sci. 114: 837–841

Etienne H, Montoro P, Michaux-Ferriere N & Carron MP (1993) Effects of desiccation, medium osmolarity and abscicic acid on the maturation of *Hevea brasiliensis* somatic embryos. J. Exp. Bot. 44: 1613–1619

Finkelstein RR & Crouch MC (1986) Rapeseed embryo development in culture on high osmoticum is similar to that in seeds. Plant Physiol. 81: 907–912

Finkelstein RR & Crouch MC (1987) Hormonal and osmotic effects on developmental potential of maturing rapeseed. HortScience 22: 797–800

Fujii JAA, Slade D & Redenbaugh K (1989) Maturation and greenhouse planting of alfalfa artificial seeds. In Vitro Cell. Dev. Biol. 25: 1179–1182

Fujii JAA, Slade, D & Redenbaugh K (1993) Planting artificial seeds and somatic embryos. In Redenbaugh K (ed) Synseeds: Applications of Synthetic Seeds to Crop Improvement (pp 183–202). CRC Press, Boca Raton

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

Janick J, Kim YH, Kitto S & Saranga Y (1993) Desiccated synthetic seeds. In Redenbaugh K (ed) Synseeds: Applications of Synthetic Seeds to Crop Improvement (pp 409–425). CRC Press, Boca Raton

Karssen Cm, Brinkhorst-van der Swan DLC, Breekland AE, & Koornheef M (1983) Induction of dormancy during seed development by endogenous abscisic acid: studies on abscisic acid deficient genotypes of *Arabidopsis thaliana* (L.) Heynh. Planta 157: 158–165

King MW & Roberts EH (1979) The storage of recalcitrant seeds. Achievements and possible approaches, International Board for Plant Genetic Resources, Rome

Koornheef M, Hanhart CJ, Hilhorst HWM & Karssen CM (1989) *In vivo* inhibition of seed development and reserve protein accumulation in recombinants of abscisic acid biosynthesis and responsiveness mutanta in *Arabidopsis thaliana*. Plant Physiol. 90: 463–469

McKersie BD, Senaratna T, Bowley SR, Brown DCW, Krochko JE & Bewley JD (1989) Application of artificial seed technology in the production of hybrid alfalfa (*Medicago sativa* L.) In Vitro Cell. Dev. Biol. 25: 1183–1188

Monsalud MJR (1994) Control of hyperhydricity in 'Hindi' mango somatic embryos. M.S. Thesis, University of Florida, Gainesville

Monsalud MJ, Mathews H, Litz RE & Gray DJ (1995) Control of hyperhydricity of mango (*Mangifera indica* L.) somatic embryos. Plant Cell Tiss. Org. Cult. 42: 195–206

Morris P, Weiler E & Maddock S (1988) Determination of endogenous abscisic acid levels in immature cereal embryos during *in vitro* culture. Planta 173: 110–116

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

Pence VC (1991) Abscisic acid in developing zygotic embryos of *Theobroma cacao*. Plant Physiol. 95: 1291–1293

Pence VC (1992) Abscisic acid and the maturation of cacao embryos *in vitro*. Plant Physiol. 98: 1391–1395

Pliego-Alfaro F, Litz RE, Moon PA & Gray DJ (1995) Effect of abscisic acid, osmolarity and temperature on *in vitro* development of recalcitrant mango (*Mangifera indica* L.) nucellar embryos. Plant Cell Tiss. Org. Cult. (in press)

Roberts EH (1973) Predicting the stoage life of seeds. Seed Sci. Technol. 1: 499–514

Welbaum Ge, Tissaoui T & Bradford KJ (1990) Water relations of seed development and germination in muskmelon (*Cucumis melo* L.). III. Sensitivity of germination to water potential and abscisic acid during development. Plant Physiol. 92: 1029–1037