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Desiccation tolerance and cryopreservation of *Archontophoenix alexandrae* excised embryos at different developmental stages

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Summary

This paper describes research results on relative electrolyte leakage, survival, seedling rate, desiccation tolerance and cryopreservation of *Archontophoenix alexandrae* excised embryos during development. Desiccation tolerance of excised embryos increased gradually from 146 DAF (days after flowering) to 174 DAF and reached the maximum at 174 DAF with a semilethal water content of 0.08 g g^{-1}, but decreased at 181 DAF. Excised embryos of 146-153 DAF with/without water content treatments failed to survive after cryopreservation, but excised embryos of 160-181 DAF drying to suitable water content survived after desiccation and freezing. Drying to 0.25 g g^{-1} (dry weight), survival of excised embryos of 160, 167, 174, 181 DAF was 56%, 63%, 98% and 46% respectively after cryopreservation. Excised embryos formed seedling, but the plantlet formation percentage was lower than the growth rate of seedling. Roots meristems appeared to be more sensitive to desiccation and freezing than shoot meristems. Seeds with/without water content treatments failed to survival after cryopreservation.

Introduction

Archontophoenix alexandrae (F. Muell.) H. Wendl. and Drude is a cultivated plant widely distributed in Fujian, Taiwan, Guangdong, Hainan and Yunnan provinces in China. It is generally used to beautify the environment. Seed is an important germplasm resource. Plant seeds have been divided into two groups according to their desiccation sensitivity and storage characteristics, that is, orthodox seeds and recalcitrant seeds (Roberts, 1973). Recalcitrant seeds can not tolerate desiccation. Berjak *et al.* (1989) gave a definition of recalcitrant seeds as: seeds that are shed wet and cannot be dehydrated or stored. Roberts *et al.* (1984) claimed that the most promising method of germplasm conservation for recalcitrant seeds is storage in liquid nitrogen. It has subsequently been shown that cryopreservation is an appropriate method for long-term storage the germplasm of recalcitrant seeds whilst other methods are not effective (Pence, 1995). For seeds to survive at low temperatures, they must be dried prior to freezing but physical constraints limit the dehydration rate of whole seeds, which leads to the loss of viability at relatively

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high water contents (Farrant *et al.*, 1986). Bajaj (1985) suggested that the germplasm of recalcitrant seeds could possibly be conserved through the cryopreservation of their excised embryos. The embryonic axis is the preferred tissue for use because it is an organized small structure which is able to produce a whole plant from the meristematic tissues contained therein (Chandel *et al.*, 1995). Furthermore, using a suitable drying method, the water content of excised embryonic axes can be reduced to a level which may be much lower than that of whole seeds, for example: 19-30% for lychee axes, 13-20% for longan axes and 16-30% for jackfruit axes whilst the material still retains viability (Fu *et al.*, 1993). Normah *et al.* (1986) found that at water contents of 14-20% and 20-69% of the excised embryos of rubber survived cryopreservation for 24 hours and formed seedlings when cultured *in vitro*. Chin (1988) pointed out that is the first time that true recalcitrant seeds had survived storage in liquid nitrogen. Up to the present time, recalcitrant seeds of many species have been successfully cryopreserved by optimizing water content (Pence, 1995; González-Benito *et al.*, 2002; Touchell and Walter, 2000) and cooling rates (Wesley-Smith *et al.*, 2001; González-Benito *et al.*, 2002) to cryogenic temperatures so that both freezing and desiccation damage are limited.

Desiccation at the proper developmental stage can also be critical to maximizing survival of tissues through cryopreservation (Pence, 1995). Recalcitrant seeds at different developmental stages differ in desiccation sensitivity. Thus linking desiccation tolerance with the various seed developmental stages may be an effective approach of cryopreservation (Fu *et al.*, 1994). In order to determine the optimum level of desiccation for liquid nitrogen exposure, the seeds must be harvested at various time intervals. Drying rate is also an important factor in determining the dehydration tolerance of seeds (Bonner, 1996; Pammenter *et al.*, 1999; Liang *et al.*, 2000; Song *et al.*, 2003). Generally fast-drying has been found to permit recalcitrant seeds or excised axes to survive to lower water contents, and to improve the survival after cryopreservation (Potts and Lumpkin, 1997; Pritchard and Manger, 1998; Wu *et al.*, 2001; Song *et al.*, 2003).

The seed germination and storage characteristics of *A. alexandrae* have previously been researched and those studies showed obvious recalcitrant seed storage behavior for the species with the seeds tolerating neither deep desiccation nor low temperatures (Shao *et al.*, 2006). In the present study, an attempt has been made to study the acquisition of desiccation tolerance of *A. alexandrae* excised embryos during development, and the potential for cryopreservation of the excised embryos.

Materials and methods

Plant material

During the full developmental period from November, 2004 to January, 2005, the anthotaxies of *A. alexandrae* were tagged and the ages of seeds expressed as days after flowering (DAF).The fruits from 146 to 181 DAF (at intervals of 7 days) were collected from Xishuangbanna Tropical Botanical Garden, in southern Yunnan Province of China. The seeds of 174 DAF and the excised embryos of different developmental stages were used in the desiccation tolerance and cryopreservation experiments.

Desiccation

The seeds of 174 DAF were buried in activated silica gel in a series of airtight glass desiccators at ambient temperature (15-24°C at night and 24-35°C in the day) for different times (0 h, 6 h, 12 h, 24 h, 36 h, 48 h, 72 h and 96 h). The volume proportion of seeds and silica gel was one to ten. The silica gel was replaced when the gel's colour changed. The embryos were extracted from dried seeds and were used for water content determination.

The embryos of different developmental stages were excised from the fresh seeds which had been disinfected with 75% alcohol for 3-5 minutes. After washing in aseptic water three times, the embryos were excised using shears and nippers. The excised embryos were transferred to an uncovered sterile Petri-dish and dehydrated rapidly by using aseptic air current method (Fu *et al.,* 1993) for different times (0 h, 0.5 h, 1 h, 2 h, 4 h, 6 h, 12 h and 15 h).

Water content determination

Water content of all samples was determined by the oven method at 103±2°C for 17±1h. Presented values represent the mean of three seeds or ten embryos, and were expressed on a g H_2O per g dry matter (g H_2O g^{-1} DW, g g^{-1}) and on a percentage fresh mass basis (%) indicated in parentheses.

Relative electrolyte leakage measurements

Five replicates of excised embryos in batches of 5 each were soaked in 20ml double-distilled water in a tube and conductivity was measured using a DDS-307 conductivity meter. Leakage was measured at the start (a_1) and again after 120 min when conductivity of the bathing solutions of the various treatments reached a plateau (a_2). Thereafter, the tubes were transferred into a pot and boiled for 120min to induce total membrane breakdown and after cooling the conductivity was again measured. (a_3). Leakage data are expressed as a relative electrolyte leakage ($(a_2-a_1)/a_3\times100\%$).

Germination testing and cryopreservation of seeds of 174 DAF

Four replicates of seeds in batches of 25 each with different water content were cultured in Petri-dishes with 1% agar in an incubator at constant 30°C with alternating photoperiod with 14 h light and 10 h dark. The dried seeds with various water contents were put in cryo-tubes and plunged directly into liquid nitrogen. After 24h, the seeds were taken out and thawed at the ambient temperature. They were then cultured in Petri-dishes on 1% agar.

Survival, the growth rate of seedling and cryopreservation of excised embryos

The dried embryos with different water contents of different developmental stages were divided into two groups. Some of the embryos were transferred to sterilized, 2.0-ml capacity cryovials and plunged directly into liquid nitrogen; others were disinfected with 0.1% $HgCl_2$ for 5s and rinsed in aseptic distilled water three times and incubated on MS (Murashige and Skoog, 1962) medium supplemented with 0.2mg/L IAA, 0.1mg/L BA, 30g/L sucrose and 8 g/L agar with the pH adjusted to 5.8 as control. The dried embryos were stored in liquid nitrogen for 24h. The cryo-tubes were then rapidly thawed in a

water bath at 37°C. The dried embryos were disinfected (as above) and incubated on the same MS medium. The embryos were grown under a 10h:14h light:dark photoperiod at 25°C. Survival of ten embryos per treatment was assessed, and embryos were scored as surviving within 1 month. Embryo viability was assessed as: organized growth, which was comparable with normal germination; individual embryo tissue extension; and callus production from the radicle (i.e. unorganized growth) (Pritchard *et al.*, 1995). The growth rate of seedling and shoot length was assessed after 3 months. The seedling grown included radicle elongation ("root"), shoot elongation and plantlet recovery (both radicle and shoot development) (González-Benito *et al.*, 2002).

Statistical analysis

SPSS 11.5 was used. Some properties of seeds and fruits during different developmental stages (table 1) were analysed by One-Way ANOVA (analysis of variance) and the effects of desiccation on survival and seedling growth rate of excised embryos (table 2) and on shoot length from control (-LN) and cryopreserved (+LN) excised embryos (table 4) were analyzed by Independent-Samples T Tests to determinate if significant ($P<0.05$) differences occurred.

Table 1. Changes in some physical properties of seeds and fruits during development.

Day after flowering (d)	Colour of fruits	Seed texture	FW/1000 seeds (g)	WC of seeds (%)	DW per seed (mg)
146	light green	soft	446.80±5.13^{a}	48.4±0.94^{a}	240.0± 0.0^{a}
153	green	soft	437.20±1.48^{b}	48.4±0.88^{a}	230.0±10.0^{a}
160	light red	hard	440.10±1.99^{ab}	38.1±0.21^{b}	280.0± 5.0^{b}
167	red	hard	430.80±3.61^{b}	38.0±0.51^{b}	270.0±10.0^{b}
174	deep red	hard	519.03±2.85^{c}	38.6±0.00^{b}	310.0±10.0^{c}
181	deep red	hard	516.40±3.11^{c}	36.9±0.01^{b}	310.0±10.0^{c}

FW, fresh weight; DW, dry weight; WC, water content. Values in each row with the same letters on right corner are not significantly different, LSD test, P=0.05

Table 2. Effects of desiccation on survival and seedling growth rate of excised embryos of material 174 DAF.

Dehydration time (h)	Water content (g g^{-1})	Survival (%)	Seedling growth rate (%)
0	3.52±0.11	100.0±0^{a}	74.4±4.95^{b}
0.5	2.23±0.08	100.0±0^{a}	76.5±6.27^{b}
1	1.35±0.10	100.0±0^{a}	61.4±7.16^{b}
2	0.78±0.05	100.0±0^{a}	54.0±8.21^{b}
4	0.36±0.03	97.8±2.22^{a}	51.5±6.50^{b}
6	0.25±0.03	97.1±2.86^{a}	53.0±5.17^{b}
12	0.14±0.04	82.0±4.67^{a}	54.3±7.19^{b}
15	0.09±0.02	57.7±4.43^{a}	21.5±6.41^{b}

Values in each row with the same letters on right corner are not significantly different, Independent samples test, P=0.05

Table 3. Effects of desiccation on water content and germination percentage of seeds and embryos of material 174 DAF.

Dehydration time (h)	WC of seeds ($g\ g^{-1}$)	WC of embryos ($g\ g^{-1}$)	Germination (%)
0	0.64±0.02	4.41±0.65	80.0±2.31
6	0.50±0.01	2.53±0.40	83.5±2.67
12	0.43±0.01	1.28±0.19	81.3±0.46
24	0.34±0.01	0.70±0.03	57.4±6.63
36	0.27±0.01	0.58±0.06	27.0±1.91
48	0.22±0.00	0.44±0.14	12.0±4.32
72	0.18±0.00	0.16±0.02	0±0.00
96	0.14±0.00	0.07±0.00	0±0.00

The embryos were extracted from desiccated whole seeds

Table 4. Effect of dehydration on the shoot length from control (-LN) and cryopreserved (+LN) excised embryos.

Day after flowering (d)	Dehydration time (h)	Water content ($g\ g^{-1}$)	Shoot length (cm)	
			-LN	+LN
160	6	0.27±0.05	1.90±0.24[a]	1.25±0.14[b]
	12	0.15±0.03	0.98±0.26[a]	0.50±0.17[a]
167	6	0.25±0.04	1.73±0.23[a]	1.88±0.26[a]
	12	0.13±0.03	2.22±0.54[a]	2.30±0.20[a]
174	6	0.25±0.03	0.96±0.13[a]	0.69±0.19[a]
	12	0.14±0.04	1.00±0.23[a]	1.35±0.15[a]
181	6	0.26±0.03	2.91±0.38[a]	1.90±0.20[b]
	12	0.14±0.01	1.43±0.58[a]	2.14±0.39[a]

Values in each row with the same letters on right corner are not significantly different, Independent samples test, P=0.05

Results

Some physical characteristics of seeds and fruits during development

The fruits turned from light green to light red and were deep red at maturity. The texture of seeds changed from soft to hard during development. The fresh weight of 1000 seeds and dry weight per seed increased gradually from 146 DAF to 181 DAF and attained its maximum at 174 DAF with statistically insignificant changes thereafter. The water content decreased during development from 146-160 DAF, but it did not show significant changes thereafter (table 1).

Effects of desiccation on the survival and rate of seedling establishment from excised embryos

Without an imposed drying treatment, excised embryos of 146-174 DAF showed 100% survival, but the survival of 181 DAF embryos decreased to 93%. The survival of excised embryos decreased with extended dehydration. However, the desiccation tolerance is different during the different development. When the water content fell to 0.25 g g^{-1}, the embryos survival from 146-181 DAF was 0%, 0%, 64.5%, 66.7 %, 97.1 %, 43 %.The semi-lethal water content of embryos from 146-181 DAF was 0.4, 0.54, 0.2, 0.19, 0.08, 0.26 g g^{-1} respectively (figure 1). It was obvious that the desiccation tolerance of embryos increased gradually from 146 DAF to 174 DAF and reached a maximum at 174 DAF, but decreased again at 181 DAF. The growth rate of seedling showed a similar trend as the embryo survival (data not shown), but the rate of growth of seedlings was lower than the embryo survival (take 174 DAF for example, table 2). This indicated that some embryos can survive dehydration but can not form seedlings.

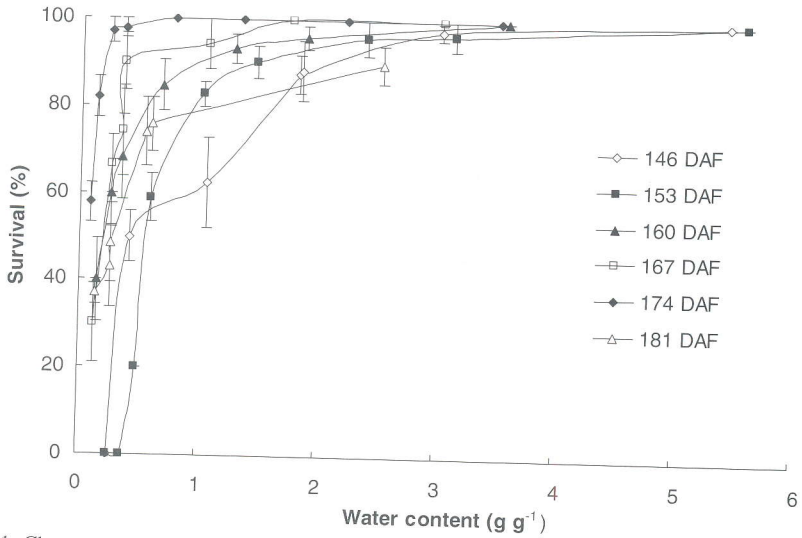

Figure 1. Changes in survival of excised embryos of different developmental stages in response to dehydration to different water contents.

Changes in electrolyte leakage of excised embryos of different developmental stages

Without dehydration, the electrolyte leakage from embryos of 146-174 DAF seeds retained a similar level, but the leakage of 181 DAF embryos was higher. With increasing desiccation time the electrolyte leakage increased at every developmental stage, but the electrolyte leakage of immature embryos increased quicker than mature embryos. When dehydrated to the same water content, the immature embryos had a higher relative electrolyte leakage than the partially mature or fully mature embryos. The leakage of 181 DAF embryos was higher before dehydration, but increased slowly during dehydration (figure 2). The increase of relative electrolyte leakage was in accordance with the decrease of survival percentage (figure 1, 2 and 3).

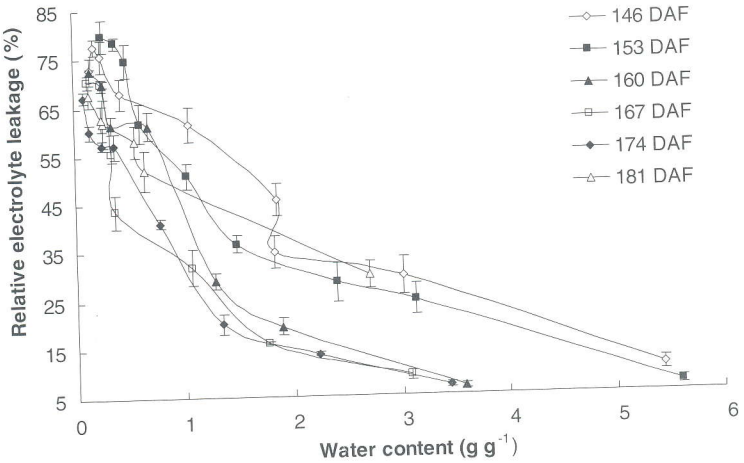

Figure 2. Changes in relative electrolyte leakage of excised embryos of different developmental stages in response to desiccation to different water contents.

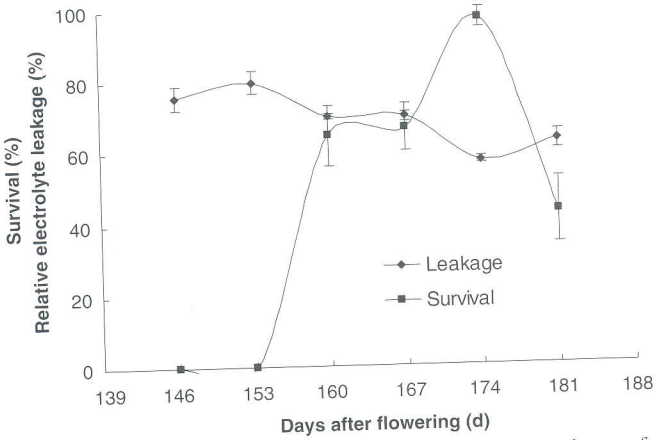

Figure 3. Changes in relative electrolyte leakage and survival of excised embryos of different developmental stages in response to desiccation to 0.25 g g^{-1}.

Effects of desiccation on water content and germination percentage of seeds and embryos of material 174 DAF

The water content of non-desiccated seeds of material 174 DAF was 0.64 g g^{-1} (39%) and that of embryos extracted from desiccated seeds was 4.42 g g^{-1} (80.7%) respectively. The water content of the isolated embryos was consistently higher than that of the intact seeds for all desiccation times. The germination percentage of non-desiccated seeds was 80% and this increased after dehydration. With increasing dehydration time, the germination percentage decreased rapidly. The semi-lethal water content of seeds and embryos was 0.32 g g^{-1} and 0.66 g g^{-1} respectively whilst the lethal water content of seeds and embryos was 0.18 g g^{-1} and 0.16 g g^{-1} respectively (table 3).

Effects of cryopreservation on survival of mature seeds
No germination was obtained with desiccated and cryopreserved seeds of 174 DAF, whatever the desiccation period tested (data not shown).

Effects of cryopreservation on survival, the growth rate of seedlings and shoot length of excised embryos of different developmental stages
No germination was obtained with desiccated and cryopreserved excised embryos of 146-153 DAF, whatever the desiccation period tested. Excised embryos of 160-181 DAF did not survive cryopreservation when at high water content, but at relatively low water content they survived the freezing and thawing process. Excised embryos of 160, 167, 174, 181 DAF desiccated to 0.25 g g^{-1} moisture levels could be successfully cryopreserved with a survival of 56%, 63%, 98% and 46% respectively. The corresponding growth rate of seedlings was 33.9%, 33.3%, 70.6% and 26.6% respectively. After further desiccation to 0.13 g g^{-1}, the survival of 160-181 DAF decreased to 21.3%, 22%, 83.3% and 24.4%, and corresponding seedling rate was 6%, 6%, 54.4% and 27.5%. Survival of excised embryos of 174 DAF with a water content of 0.25 g g^{-1} showed the maximum survival percentage (98%) after LN exposure. It therefore appears that material taken from 174 DAF was the best developmental stage and 0.25 g g^{-1} was the suitable water content for cryopreservation (figure 4).

Excised embryos subsequently formed seedlings, but the plantlet formation percentage was lower than the actual growth rate of the seedlings. Some of embryos displayed abnormal development with shoot growth but no root development whereas other embryos had root growth but no shoot development. The rate of plantlet recovery (both root and shoot development) decreased especially after considerable desiccation or cryopreservation. 60-80% embryos did not develop roots but developed normal shoots. The seedling recovery after freezing was similar to the control when excised embryos of 167-181 DAF were desiccated to 0.25 g g^{-1} (figure 5). The shoot length after cryopreservation were not significantly different from the control (6h of 160, 181 DAF is exceptive) (table 4).

Discussion

Effects of desiccation on the survival, seedling rate of excised embryos
The desiccation tolerance of recalcitrant seeds of other species is different at different developmental stages (Chandel *et al.*, 1995). The decrease in water content towards the end of development is accompanied by an increase in desiccation tolerance in most recalcitrant seeds (Pammenter and Berjak, 1999). The semi-lethal water content of *A. alexandrae* excised embryos of 146-174 DAF decreased gradually, but increased at 181 DAF (figure 1). It was obvious that the desiccation tolerance of excised embryos increased gradually from 146 DAF to 174 DAF and reached the maximum at 174 DAF, but decreased at 181 DAF. Finding the developmental stage with high desiccation tolerance could increase survival of cryopreservation. It was evident from excised embryos of the 174 DAF materials that they could tolerate desiccation to the greatest degree. This developmental stage was in fact the physiologically mature stage. A similar phenomenon has been demonstrated in *Aesculus hippocastanum* and *Clausena lansium* seeds (Tompsett and Pritchard, 1993;

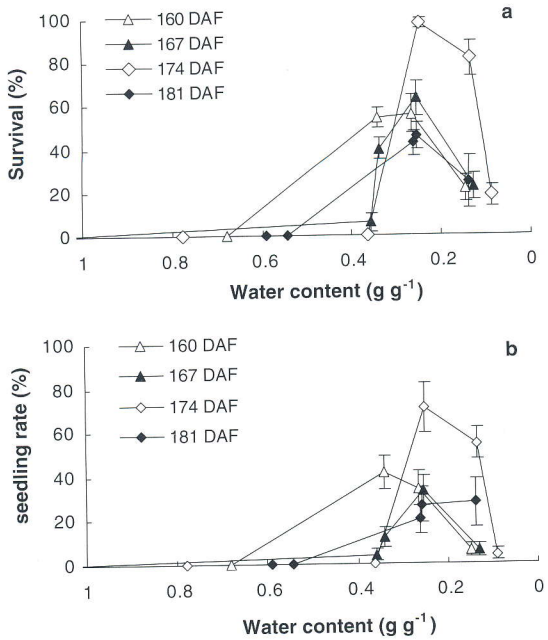

Figure 4. Changes in survival and the growth rate of seedlings developed from excised embryos of different developmental stages after liquid nitrogen storage.

Fu *et al.*, 1994). Walters (2000) suggested that dry matter accumulation is an important feature in conferring tolerance and most of the tolerance in developing embryos appears to be acquired during dry matter accumulation. Similarly the acquisition of desiccation tolerance by oil palm immature embryos was associated with an accumulation of dry matter (Aberlenc-Bertossi *et al.*, 2003). However, it appears that not all recalcitrant seeds react in the same manner. Once they have become germinable, the desiccation tolerance of seeds of *Avicennia marina* did not change during further development (Farrant *et al.*, 1993). In our study, a decrease in survival also appeared among the seeds aged by delaying harvest after physiological maturation (181 DAF, figure 1). A similar response has been demonstrated in *Clausena lansium* and *Litchi chinensis* seeds. The desiccation tolerance of *Clausena lansium* and *Litchi chinensis* seeds was greatest at 67 and 84 days after anthesis respectively, but decreased in further development (Fu *et al.*, 1994).

The growth rate of seedlings was lower than the survival percentage (table 2). A great deal of investigation has suggested that dehydration treatment influenced the development process of embryos into seedlings (Pritchard and Prendergast, 1986; Pritchard, 1991; Poulsen, 1992). Similarly in our study, the damage from the dehydration treatment influenced the development process from embryo to seedling.

Changes in electrolyte leakage of excised embryos of different developmental stages

Relative electrolyte leakage of excised embryos increased with desiccation (figure 2). Pammenter *et al.* (1991, 1993) suggested that in recalcitrant seeds, increased electrolyte

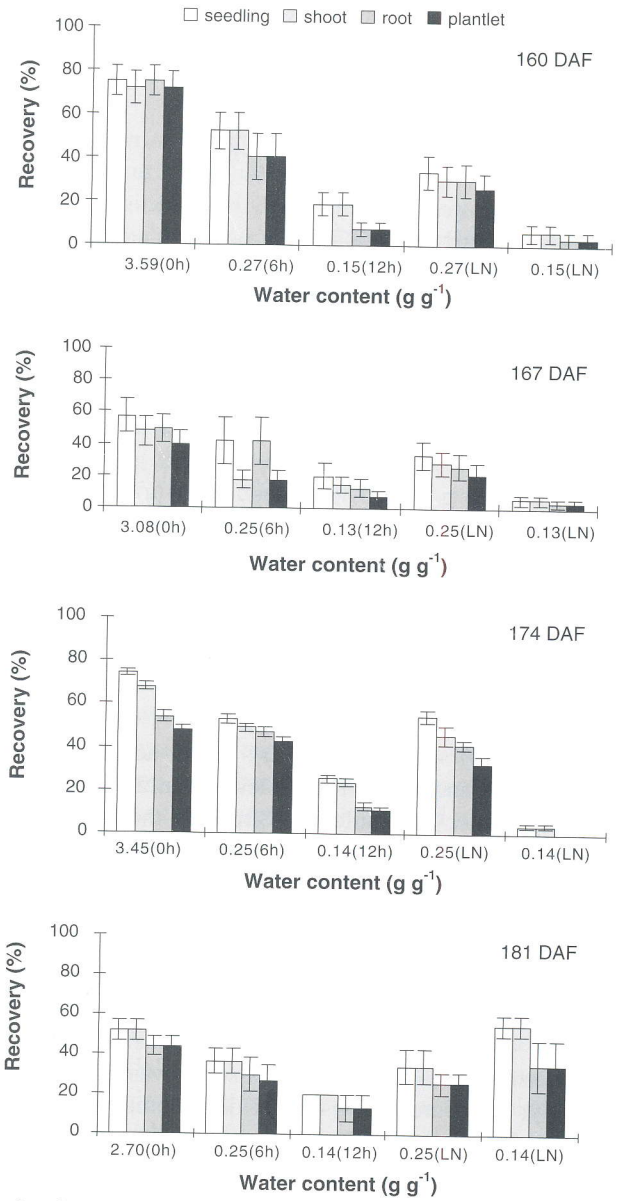

Figure 5. Response of excised embryos to desiccation for different times and cooling in liquid nitrogen.

leakage from cells is directly linked to plasma membrane abnormalities, and is a good indicator of viability. In our studies relative electrolyte leakage was used to evaluate the extent of drying damage to plasma membranes. The immature embryos had a higher relative electrolyte leakage than the partially mature or fully mature embryos, this was perhaps due to the fact they had not developed completely and had low cellular membrane

integrity so that the embryos leaked electrolytes more easily than the more mature ones (Yang *et al.*, 2004). The present results support the conclusions of Pukacka and Wójkiewicz (2002) concerning changes of percentage germination and electrolyte leakage of Norway maple seeds during development. The increase of relative electrolyte leakage was associated with a concomitant decrease in survival percentage (figure 1, 2, 3). An increase of relative electrolyte leakage and decrease of survival percentage also appeared among the seeds aged by delayed harvest after physiological maturation (181 DAF, figure 1, 2). In southern Yunnan Province of China, *A. alexandrae* fruits and seeds ripen in an environment of high humidity and temperature. Some researchers have pointed out that high humidity and temperature environments may readily cause seeds to age and to lose vigor more or less (Walters, 2000).

Cryopreservation of seeds and excised embryos of different developmental stages

No germination was obtained with desiccated and cryopreserved seeds, whatever the desiccation period tested. But excised embryos of 160-181 DAF could survive after proper dehydration treatment and freezing. This indicated that excised embryos are an appropriate propagule for long-term storage the germplasm of *A. alexandrae* seeds. This response could have been due to higher water content in the embryo of *A. alexandrae* than in the rest of the seed. Even if water content of seed has reduced to low level, that of the embryo remains high (table 3). Therefore in seeds with a differential in water content across tissues, lethal ice crystals will form in the embryo (high water content) when the whole seed is frozen in liquid nitrogen. Similarly desiccated conical explants (containing the axes) of *Quercus faginea* failed to survive cryopreservation despite low water content due to similar reason (Gonzalez-Benito *et al.*, 1992). A similar difference in water content between whole seed and axes, when dried within the fruit, has been reported in *Quercus rubra* (Prichard, 1991).

At the lethal point of seeds of 174 DAF, the water content of embryos extracted from desiccated seeds was 0.16 g g^{-1} (table 3). But the excised embryos (excised from seeds before desiccation) of same development stage could survive at lower water content. The survival remained 83% at 0.13 g g^{-1} and 58% even at 0.09 g g^{-1} (figure 1). This means that the water content of excised embryos can be reduced to much lower level than that of the whole seed thereby meeting the conditions required for cryopreservation. Some typical recalcitrant species express similar characteristics to those demonstrated in the present study. The excised embryonic axes of wampee seeds on rapid drying survived 100% at 0.13 g g^{-1}, and the semi-lethal water content was 0.09 g g^{-1} (Wu *et al.*, 2001). The excised embryonic axes of mango seeds can tolerant rapid desiccation to 11.8%, and the survival still remained around 80% (Fu *et al.*, 1990). Still *et al.* (1994) reported the survival of wildrice at water contents as low as 0.07 g g^{-1}.

As mentioned by Dumet *et al.* (1997), it seems reasonable to suppose that there might be an optimal developmental stage that would facilitate successful cryopreservation, and that this is likely to be at full maturity. Mature embryonic of *Landolphia kirkii* were more tolerant of cryopreservation than immature axes (Vertucci *et al.*, 1991). Similar observations were reported for the cryopreservation of cocoa and jackfruit embryonic axes (Chandel *et al.*, 1995). From the present study, excised embryos of 146-153 DAF could not

survive whatever the water content was, but excised embryos of 160-181 DAF desiccated to appropriate water contents could survive storage in liquid nitrogen for 24 h. Otherwise, survival after cryopreservation increased with excised embryos change to maturity and reached the maximum (98%) at 174 DAF with 0.25 g g^{-1} and decreased thereafter (figure 4). This was consistent with the rule of desiccation tolerance. The developmental stage with high desiccation tolerance achieved high survival after cryopreservation too. It was obvious that finding the developmental stage with high desiccation tolerance could increase survival of cryopreservation.

Stanwood (1983) has published a wide range of the high moisture freezing limit (HMFL) from a low of 9.6% for sesame to a high of 28.5% for bean. HMFL is the seed water content range in which the seeds do not freeze and survive liquid nitrogen. But the safe water content of recalcitrant seeds was usually higher than HMFL. Chin (1988) stated that if the excised embryonic axes of some recalcitrant seeds can be decreased lower than their HMFL, they could possibly be cooled and stored in liquid nitrogen. In this regard the embryonic axes of longan seeds at 18% water content can survive after being stored in liquid nitrogen for 24 h, and the survival was 25% or 50% by direct plunge or by steps into liquid nitrogen respectively (Fu *et al.*, 1993). Tea embryonic axes desiccated to 13 and 10% moisture levels could be successfully cryopreserved with a survival percentage of 95 and 83%, respectively. Similarly, in jackfruit the axes with 14% water content could be cryopreserved with 30% survival (Chandel *et al.*, 1995). In our study, 0.25 g g^{-1} was the suitable water content for cryopreservation, and survival decreased when water content was higher or lower than 0.25 g g^{-1}. It was therefore obvious that optimum water content was necessary to ensure the successful cryopreservation.

The growth rate of seedlings developed from excised embryos with proper dehydration treatment was high after cryopreservation, but exhibited higher proportion abnormal plantlets (figure 5). Some of embryos only had shoot elongation and no radicle elongation, whilst others only had radicle elongation and no shoot elongation. Abnormal plantlets were produced mostly by the dehydration treatment. Many embryos did not form roots even though shoots elongated normally, thus revealing that roots meristems appeared to be more sensitive to desiccation and freezing than shoot meristems. Differential sensitivity to desiccation of root and shoot axes was also observed with *Aesculus* and *Castanea* axes, with roots being more sensitive in *A. hippocastanum* and shoots in *A. glabra* and *C. sativa* (Pence, 1992). This response has also been observed, however, with embryo axes of *Araucaria hunsteinii*, *Quercus rubra, Quercus robur*, in which shoot meristems were more sensitive to desiccation than root meristems (Pritchard and Prendergast, 1986; Pritchard, 1991; Poulsen, 1992). It is not known if there is an inherent physiological difference in the tolerance of these tissues to drying or freezing, if the root and shoot germinate at different rates, or if the tissues dehydrate at different rates, expressing different responses after desiccation for the same time.

At the optimum water content, for example, excised embryos of 160-181 DAF desiccated for 6 h, the seedling recovery of control and cryopreservation did not express an evident change (figure 5). And comparing the shoot length of cryopreserved with the control, they were not significantly different (6h of 160 DAF, 181 DAF is exceptive, table 4). This means that cryopreservation did not effect the growth of seedling.

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