SHORT COMMUNICATION

Manipulation of desiccation-sensitive axes of wampee (*Clausena lansium*) to facilitate increased dehydration tolerance

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Abstract

The plumules of newly-excised wampee embryos, which are more sensitive to dehydration than the roots, became more resistant to water loss when axes were allowed to sprout on woody plant medium [WPM; McCown and Lloyd (1981) *Hortscience* **16**, 453] before being dried. Pre-treatment of sprouting axes (seedlings) with sucrose incorporated in the WPM enhanced survival. Although the roots withered following further dehydration of seedlings cultured on WPM containing 60% sucrose, excised plumules were capable of generating adventitious roots when a combination of 10 mM α-napthaleneacetic acid and 10 mM indole-3-butyric acid was used during subsequent *in vitro* incubation.

Keywords: Axis, desiccation sensitivity, recalcitrant seed, sucrose pre-treatments, wampee (*Clausena lansium*), woody plant medium

Introduction

Wampee (*Clausena lansium*) seeds cannot tolerate dehydration (Fu *et al*., 1989; Hoffmaun and Steiner, 1989) and are termed recalcitrant (Roberts, 1973). As seed development progresses, the desiccation tolerance of wampee seeds increases gradually, reaching a maximum 67 days after anthesis (DAA), approx. the beginning of physiological maturity (Fu *et al*., 1994). In an earlier study (Fu *et al*., 1989), wampee seeds died when the average water content decreased from 82% to 33–34% (fresh mass basis [fmb]). However, Berjak *et al*. (1992) have shown that recalcitrant seeds at different developmental stages differ in their degree of desiccation sensitivity.

To conserve the genetic resources of species producing recalcitrant seeds, the most promising method is storage in liquid nitrogen (Roberts *et al*., 1984) which requires drying of the material (usually excised embryonic axes) before freezing. This is difficult to achieve with desiccation-sensitive material, but Lu and Fu (1997) found that the vigour index of wampee at the optimal developmental stage was increased after pre-culturing with sucrose, and the moisture content for axis lethality declined.

The objectives of the present study were to facilitate dehydration of excised wampee axes and to identify an explant with the potential of normal plant development. These are necessary preliminary steps before cryopreservation trials can be undertaken.

Materials and methods

Fruits of *Clausena lansium* (Lour.) Skeels cv. Jixin were hand-harvested when seeds were at physiological maturity. Seeds were removed from the fruits by hand, washed with tap water and mixed with the fungicide, chlorothalonil (tetrachloroisophthalonitrile). After slight drying on the bench at room temperature, seeds were stored in polythene bags at 15°C until use.

Prior to excision of the embryonic axes, the chlorothalonil was removed. The excised axes were sterilized by a 3 min immersion in 0.1% $HgCl_2$ and

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https://doi.org/10.1017/S096025850000043X Published online by Cambridge University Press

then washed five times with sterile water. Axes were incubated on woody plant medium (WPM; McCown and Lloyd, 1981) with 3% sucrose at 25°C with a 12 h photoperiod (800 lux).

After 3 weeks, the sprouting axes formed 1–2 mm long plumules with 10 mm long hypocotyl roots and were either dehydrated immediately using silica gel or transferred to media containing 27% sucrose and incubated for 5–8 d. Sprouting axes were also further dehydrated after incubation on this medium by placing them on WPM containing 55% sucrose for 5 additional days followed by transfer to media with up to 80% sucrose. The final dehydration regime incorporated the use of silica gel after the axes had been cultured on a medium containing 60% sucrose. For all silica gel treatments, axes were placed on filter paper overlying this desiccant within closed desiccators.

After dehydration using the final regime, axes with withered roots and those that were rootless were incubated in half-strength WPM containing combinations of the plant growth regulators (PGRs) NAA (α-naphthaleneacetic acid), IBA (indole-3-butyric acid) and BA (6-benzylaminopurine) for the induction of adventitious roots.

Water content, determined gravimetrically, is expressed as a percentage on a fresh mass basis.

Results

Water content and survival of wampee axes

Sprouting axes (seedlings) cultivated on WPM for 2–3 weeks had an average water content of 82%. After dehydration using silica gel, the water content declined to 47%, 30% of the seedlings lost viability, 60% exhibited withered roots and only 10% retained a normal appearance (Table 1). With further dehydration, no normal seedlings survived, the proportion that lost viability increased and the declining percentage of survivors all showed withered roots. However, the hypocotyl–plumule portions of the surviving seedlings were far less adversely affected by dehydration, and this was the criterion for categorizing seedlings as viable. When water content was monitored for roots separately from the surviving hypocotyl–shoot portion of dehydrated sprouting axes, high levels of hydration were observed in the latter (Table 2).

Table 1. Survival of sprouting wampee axes after desiccation.

Water content (%)	% normal seedlings	% seedlings with withered roots
82	100	0
47	10	60
36	0	30
30	0	28
24	0	25
20	0	11

Table 2. Changes in the water content of roots and hypocotyl–shoots of sprouting wampee axes during desiccation.

Dehydration (h)	Root water content (%)	Hypocotyl–shoot water content (%)
0	85	81
1	74	76
2	67	75
4	46	72
6	24	70
10	7	63

When the sprouting axes were pre-cultured for 7 d on media containing 27% sucrose prior to dehydration with silica gel, all survived and appeared normal despite dehydration to an average water content of 47% (Table 3). Further, at each successively lower water content assessed, a considerably greater proportion of seedlings with withered roots survived compared with the situation where sprouting axes were not pre-treated with sucrose (Table 3 cf. Table 1).

Effects of further sucrose pre-treatments, followed by dehydration with silica gel, on subsequent growth of sprouting wampee axes

Following a 7 d incubation on WPM containing 27% sucrose, seedlings were transferred to each of several media incorporating progressively elevated sucrose concentrations (Table 4). As the sucrose concentration increased, the water content of the sprouting axes decreased. Use of WPM containing 60% sucrose brought about dehydration to an average water content of 37%, while still facilitating 100% normal seedling production (Table 4). However, lower water contents (£20%) are desirable for subsequent cryopreservation, which was the ultimate goal of the present dehydration studies. This degree of dehydration could not be achieved by incubation of the sprouting axes on medium incorporating 70% or more sucrose, which, in any case, proved to be lethal.

Table 3. Effect of pre-culturing on 27% sucrose-enriched WPM on the survival of sprouting axes.

Water content (%)	% normal seedlings	% seedlings with withered roots
69	100	0
47	100	0
36	0	95
30	0	76
26	0	53
21	0	28

https://doi.org/10.1017/S096025850000043X Published online by Cambridge University Press

Table 4. The growth of sprouting wampee axes cultured on WPM incorporating various concetrations of sucrose.

% sucrose	Dehydration period (d)	Water content (%)	% normal seedlings	% seedlings with withered roots
27	7	69	100	0
55	5	46	100	0
60	2	37	100	0
65	2	36	78	0
70	2	31	0	0
75	2	28	0	0
80	2	23	0	0

When sprouting axes that had been pre-treated with 60% sucrose for 2 d were then dried with silica gel for 12 h, 57% survived as seedlings with withered roots, although the water content had been lowered to an average of 18% (Table 5). Despite the absence of normal seedlings, such desiccated sprouted axes were considered suitable for ongoing studies to generate explants suitable for wampee germplasm cryopreservation.

Induction of root growth from the rootless sprouting axes

Adventitious root induction was attempted using either that portion of the sprouted axis remaining after removal of the withered roots or using the excised plumule only. Explants were cultured on WPM containing combinations of NAA, IBA and BA at various concentrations (Table 6). Inclusion of BA in the medium promoted bud formation, but no induction of adventitious roots. In contrast, when media contained both NAA and IBA, adventitious roots formed from the cut surfaces of the explants.

Table 5. The growth of sprouting wampee seedlings monitored after 1 month in culture following desiccation with high concentrations of sucrose and 12 h drying with silica gel.

% sucrose conc.	Water content (%)	% normal seedlings	% seedlings with withered roots
55	25	0	64
60	18	0	57
65	17	0	0

Table 6. Effect of plant growth regulators (PGRs) on the generation of adventitious roots from explants from which the withered roots had been removed.

PGRs added to WPM			% explants with adventitious roots	% explants with bud induction
NAA (mg l^{-1})	IBA (mg l^{-1})	BA (mg l^{-1})		
0.1	0	0.5	0	71
0.5	0	0.5	0	67
2	0	0.5	0	62
0	0	0.5	0	58
0.5	0	0	17	0
2	2	0	54	0
10	10	0	69	0

Discussion

When axes of *C. lansium* were isolated, they tolerated dehydration to lower water contents than did whole seeds (Fu *et al.*, 1989), as has been recorded for recalcitrant seeds of other species (Berjak *et al.*, 1990). Therefore, the use of excised axes is preferable for manipulations aimed at increasing (however transiently) tolerance to dehydration. The presence of appropriate levels of soluble sugars has been emphasized in the phenomenon of inherent desiccation tolerance in a variety of organisms and structures, including seed embryos (Crowe *et al.*, 1984; Hoekstra *et al.*, 1989; Leopold *et al.*, 1992; Obendorf, 1997). Sucrose pre-treatments increase the desiccation tolerance of isolated embryonic axes in other species as well (Dumet and Berjak, 1997; Yap *et al.*, 1997).

As wampee seeds become desiccated, solute leakage increased greatly due to possible membrane damage (Song and Fu, 1997). Sucrose pre-culture has been reported to cause accumulation of sugars in microspore embryos of *Brassica napa* (Uragami et *al.*, 1993); this could increase the stability of membranes under dehydrating conditions. The present results indicate that sucrose pre-treatment followed by dehydration has considerable potential to improve the ability of axes from wampee seeds to withstand increased water loss. Other methods, such as the application of ABA, $CaCl_2$ and KCN, have also been used to lower desiccation sensitivity in wampee seeds (Xiang and Fu, 1997).

In fresh (non-sprouted) embryonic axes of wampee seeds, shoot meristems were most sensitive to drying and therefore died first. In contrast, the plumule of sprouting axes endured dehydration while the root withered rapidly. The present investigation showed that sprouting axes with a living plumule but withered root retained the capacity for new root formation. Pre-culture of the sprouting axes using high concentrations of sucrose in the medium not only appeared to confer greater desiccation tolerance, but also desiccated the specimens. Further dehydration with silica gel caused the roots to wither, but plumules excised from such seedlings retained the ability for adventitious root production when cultured on WPM with the appropriate combination and concentration of auxins.

If these rooted explants are able to form normal plants, then plumules excised from axes that have been sucrose pre-treated and silica-gel-dried afford

https://doi.org/10.1017/S096025850000043X Published online by Cambridge University Press

the potential for cryopreservation of the germplasm of wampee, *Clausena lansium*.

Acknowledgements

This project was supported by the Natural Science Foundation of Guangdong Province.

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Received 10 January 2000
Accepted after revision 17 May 2000