

Investigating cryopreservation options for *Syzygium maire*, a threatened endemic New Zealand tree

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Introduction

Syzygium Gaertn. is the largest genus in the Myrtaceae family. New Zealand is home to one species, *S. maire* (Swamp maire, Maire tawake, Waiwaka). This 16 m tall glabrous tree is endemic and mostly found in waterlogged ground or on margins of streams in lower lying areas, although some populations occur in montane or cloud forests (de Lange *et al.* 2018) (Figure 1). *Syzygium maire* has fleshy red berries which develop over 8–11 months, maturing during early summer in populations at low altitudes (< 200 m.a.s.l.) or in autumn at higher altitudes (> 500 m.a.s.l.; Figure 2). In the higher altitude populations, mature fruits and flowers can be observed on the same plant simultaneously. Globally, *Syzygium* species are valued for medicinal properties (*S. aromaticum* – clove) or as a food source (*S. cumini*, *S. jambos*, *S. malaccense*, *S. samaragense*); similarly, *S. maire* berries have 18 times more antioxidants compared to blueberries (Gould *et al.* 2010). Limited information has been published on the ecology of *S. maire* but honey bees and birds, such as Tui (*Prothemadera novaeseelandiae*), Stitchbird/Hihi (*Notiomystis cincta*) and Bellbird/Korimaki (*Anthornis melanura*) have been observed competing for access to nectar, while the New Zealand Pigeon/Kererū (*Hemiphaga novaeseelandiae*) favours mature berries (Figure 3). *Syzygium maire* is mostly found in the North Island although isolated populations occur in the north-east corner of South Island around Blenheim and Nelson. Clearing and draining of large tracts of forest and wetlands for settlements and pasture resulted in significant loss of habitat. Despite this, *S. maire* was only considered as threatened for the first time in 2018 when it was listed as Nationally Critical due to the predicted impact from Myrtle Rust (*Austropuccinia psidii*) (de Lange *et al.* 2018). This prediction is based on the impact of Myrtle Rust on fleshy fruited Myrtaceae species in Australia. Two species, *Rhodamnia rubescens* and *Rhodomyrtus psidioides*, have been decimated by Myrtle Rust, illustrating that pre-emptive *ex situ* conservation is easier and less expensive if done prior to impacts on reproductive capacity (Sommerville *et al.* 2019).

As *S. maire* seeds are metabolically active and shed at high moisture contents, the only long-term seed

storage option is likely through cryopreservation of zygotic embryos (van der Walt *et al.* 2020a). Key to cryopreservation is the control of the dehydration process and limitation of injury from chemical toxicity during treatments involving concentrated cryoprotectant solutions such as Plant Vitrification Solution (PVS2) (Sakai 2004). In this study we aimed to (a) optimise embryo culture, (b) investigate the impact of desiccation on embryo viability, and (c) examine the effect PVS2 exposure on embryo survival and plantlet development.



Figure 1. Swamps dominated by *Syzygium maire* at Fensham Reserve. Photo: K. van der Walt



Figure 2. Mature *Syzygium maire* berries in habitat. Photo: K. van der Walt



Figure 3. New Zealand Pigeon/Kererū chick swallowing a mature *Syzygium maire* berry. Photo: David Mudge, Nga Manu Trust

Materials and Methods

Syzygium maire fruits were collected over three consecutive summers (2018–2020) from Fensham Reserve and Midhirst (Figure 4). Fruits were mixed with two parts medium grade vermiculite (Ausperl Australia) and stored in airtight containers at 5°C until use. Prior to embryo excision, fruits were surface sterilized by submersion in 50% Janola® (4% sodium hypochlorite) for five minutes. The pulp and seed coat were removed aseptically to expose the radicle tip enabling the excision of the embryonic axes (hereafter referred to as embryos). Once embryos were removed from the cotyledons, these were submerged in 5g/L sodium dichloroisocyanurate (NaDCC). Each sterilisation step was followed by three rinses in sterile distilled water. Sterilised embryos were cultured on solid Murashige and Skoog (MS) medium supplemented with 3% w/v sucrose and incubated at 15/25°C with a 16 h dark/8 h light cycle.

To determine the effect of desiccation on embryo survival, excised embryos were desiccated in a laminar air flow cabinet for 0, 1, 2, 3, 4, 5 and 6 hours. For each desiccation treatment embryo viability was determined through germination as described above, while moisture content was calculated after drying at 103°C for 17 h and expressed on wet weight basis.

Solutions used for vitrification protocol included loading solution (LS), PVS2 and washing solution (WS). LS consisted of MS with 2.0 M glycerol and 0.4 M sucrose. PVS2 consisted of MS with 0.4 M sucrose, 30% w/v glycerol, 15% w/v ethylene glycol and 15% w/v dimethyl sulfoxide (DMSO). WS consisted of MS with 0.75 M sucrose. Sterilised embryos were placed in LS for 20 min then transferred to PVS2 for various incubation times (30, 60 or 90 min). The LS and PVS2 steps were conducted at room temperature and 0°C. After PVS2 incubation embryos were soaked in WS for 20 min and transferred to culture medium for viability assessment. All experiments were conducted using 10 embryos and replicated four times.

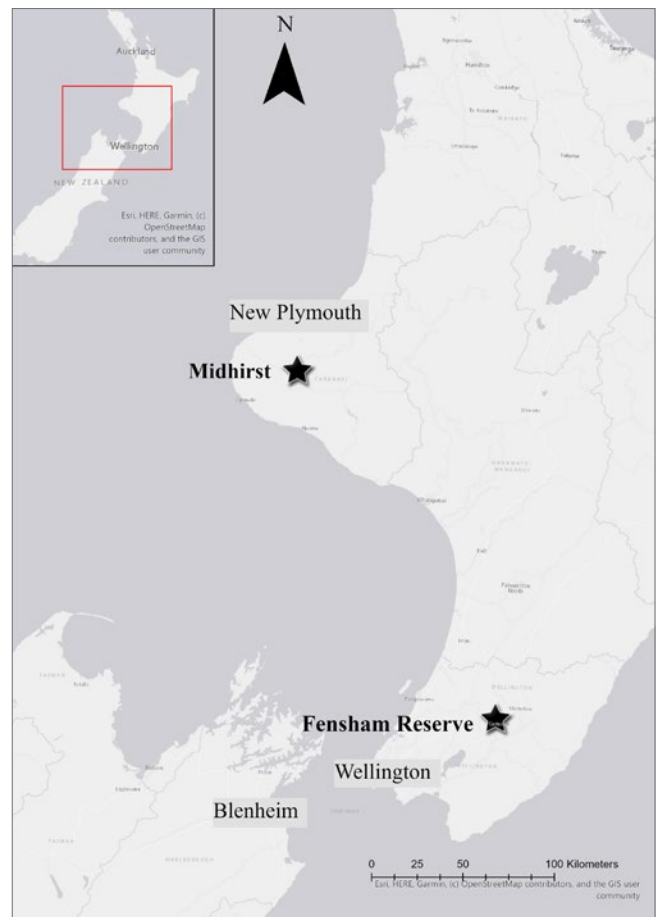


Figure 4. Locality of Fensham Reserve and Midhirst where *Syzygium maire* fruits were collected. Image: K. van der Walt

Results

Untreated embryos (controls) had high moisture content ($64.38 \pm 0.5\%$) and 100% viability. Radicle growth was observed after seven days and the first set of leaves were fully developed within 20 days (van der Walt *et al.* 2020b). Desiccating embryos for up to 2 h had no significant impact on viability or moisture content. However, embryos desiccated for 3 h had a significant reduction in moisture content ($25.4 \pm 1.3\%$) and although viability loss was recorded ($86.6 \pm 15.3\%$), it was not significant. Low survival was associated with further desiccation (Figure 5).

Exposure to PVS2 had a significant effect on embryo survival with lowest survival (63.3%) associated with 90 min exposure to PVS2. Survival rate was not affected by temperature (0°C or 20°C). Plantlet development was also negatively affected by PVS2 exposure, with some embryos showing radicle, but not shoot, development following exposure to PVS2 for 60 min or longer (Figure 6).

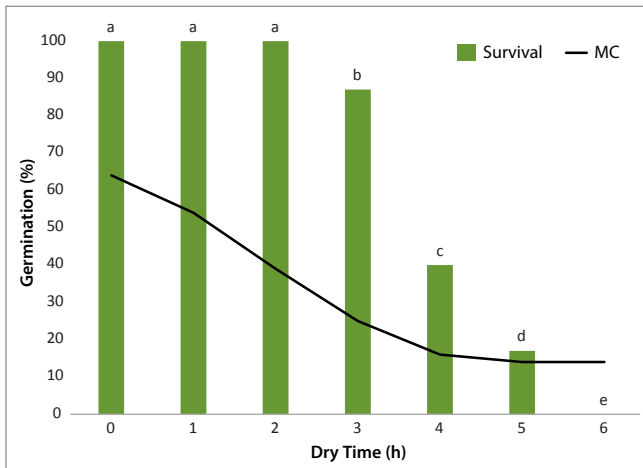


Figure 5. Germination (%) of *Syzygium maire* embryos desiccated for 0–6 h. Values followed by the same letter do not differ significantly (Fisher's, $P < 0.05$; $N = 10$; MC = Moisture Content).

Conclusion

These results demonstrate that *S. maire* embryos are metabolically active, shed at high moisture contents and although initial viability is high, desiccation is detrimental to survival. Exposure to PVS2 for longer than 30 min negatively impacted embryo viability and plantlet development therefore additional steps to optimise embryo survival could include the use of the novel droplet vitrification method which will limit exposure to PVS2 (van der Walt *et al.* 2020b).

Acknowledgements

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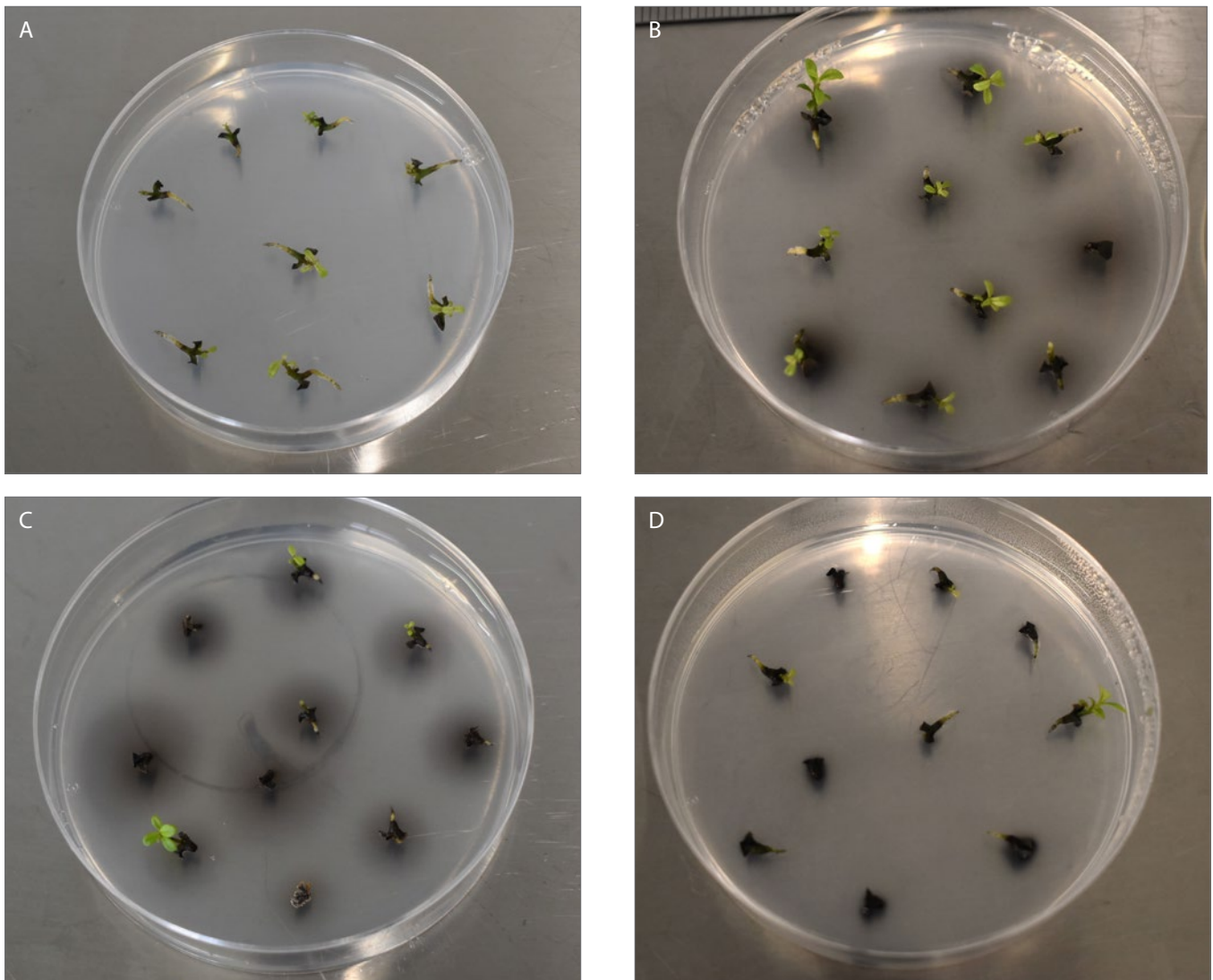


Figure 6. *Syzygium maire* embryo survival and plantlet development 10 weeks after exposure to PVS2 at room temperature for (a) 0 min, (b) 30 min, (c) 60 min and (d) 90 min.

seed was collected. Funding for this project by the Ministry for Primary Industries (MPI), New Zealand (Project 18608) is greatly appreciated. Bill Clarkson (private) and James Jones (Otari) are thanked for fieldwork assistance while Cliff John provided access to land. The work was undertaken at the Otari Garden with the findings of this work being communicated with representatives of Port Nicholson Trust of Wellington.

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News from the Australian Seed Bank Partnership

Ten years of an Australian *ex situ* seed conservation partnership

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The Global Strategy for Plant Conservation (GSPC) provides the plant conservation community with a clear set of targets, goals and objectives under which conservation actions can be identified and delivered. The commencement of the second decade of the GSPC coincided with another formal collaboration for which Australia continues to enjoy local, national and international support—the establishment of the Australian Seed Bank Partnership.

For many years prior to 2010, the major *ex situ* conservation seed banks across Australia collaborated through the Australian Conservation and Research Network (AusCAR), providing opportunities to share information and collaborate on critical research into the Australian native flora. Following almost a decade of funding and capacity building through the Millennium Seed Bank Project of the Royal Botanic Gardens, Kew, UK, the seed banks and conservation organisations of AusCAR transitioned to a new partnership arrangement. In 2010, The Council of Heads of Australian Botanic Gardens with substantial assistance from Dr Lucy A. Sutherland, established the Australian Seed Bank Partnership (the Partnership), a formal collaborative network of conservation seed banks and flora-focussed organisations operating across Australia.

The Partnership's national program has largely been guided by the GSPC, with specific Australia-based priorities and actions identified through the Partnership's Business Plan. This ambitious, ten-year Business Plan outlined strategies, actions, priorities and key outcomes intended to guide seed collections, storage, research and restoration work. The Business Plan provided a focus for ensuring the Partnership's work remains relevant to our vision of a future where Australia's native plant diversity is valued, understood and conserved for the benefit of all.

While the \$12 million Business Plan remained largely unfunded throughout its implementation, substantial investments were made by Partner institutions, governments and philanthropic granting organisations and individuals to support our work. Millions of dollars of investment from Partner institutions have been directed towards staff and facilities with major upgrades to equipment and critical infrastructure.

Beyond cash investments, the backbone of the Partnership continues to be the people. Many seed bank staff, students and volunteers have contributed their expertise, enthusiasm and dedication to ensuring Australia's native flora are provided the best possible opportunities for survival, both *ex situ* and *in situ*.