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Optimised donor plant nutrition improves cryopreservation of shoot-tips of an endangered species - *Gossia fragrantissima* (Myrtaceae)

Jingyin Bao
The University of Queensland
Chris O'Brien
The University of Queensland
Lily Maeve Whelehan
The University of Queensland
Madeleine Gleeson
The University of Queensland
Neena Mitter
Charles Sturt University
Karen Sommerville
Australian PlantBank
Alice Hayward
a.hayward@uq.edu.au

The University of Queensland

Research Article

Keywords: Response surface methodology, Ex situ conservation, Exceptional species, Threatened species, Tissue culture, Myrtle rust

Posted Date: November 2nd, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11545310/v1

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Additional Declarations: No competing interests reported.

Abstract

Gossia fragrantissima (sweet myrtle) is an endangered Australian rainforest tree threatened by habitat loss and the invasive fungal disease myrtle rust. Due to limited wild populations and poor seed production, this study explored cryopreservation of shoot-tips as a long-term conservation strategy for preserving the remaining germplasm. We have previously shown that response surface modelling could deliver a significantly improved basal nutrient medium for *G. fragrantissima* shoot culture. Here for the first time, successful cryopreservation of *G. fragrantissima* apical shoot tips was achieved using an optimised droplet vitrification protocol for two genotypes. While one genotype (0124) showed high regeneration (> 90%) across all treatments, a second genotype (0119) was comparatively sensitive to cryopreservation. We showed that in this cryo-sensitive genotype, an RSM-optimised basal medium supported significantly enhanced cryopreservation outcomes compared to material cultured on the commercially available Woody Plant Medium (WPM; $P < 0.05$). On the RSM-optimised medium, genotype 0119 achieved up to 74% post-cryopreservation regrowth, and 0124 up to 97%. Comparatively, the highest regeneration rates achieved on WPM were 40% and 90%, respectively. This is the first study to show successful cryopreservation of any species from the Gossia genus, which contains other endangered Australian species as well as unique metal hyperaccumulator species. This study revealed that nutritional conditions in culture can be a decisive factor for cryopreservation outcomes and provides a good model for future *in vitro* conservation development for other threatened plants.

Introduction

Australia's endemic plants, accounting for over 80% of Australian flora, have rapidly declined in number and distribution since European colonization, mainly due to habitat clearing, degradation and increasingly disrupted ecosystems (Legge, Rumpff et al. 2023). Myrtaceae is one of the dominant families in Australian ecosystems, comprising 2642 accepted species (Atlas of Living Australia 2026), including many high-profile taxa such as eucalypts (*Eucalyptus* spp.), tea trees and paperbarks (*Melaleuca* spp.), bottlebrushes (*Callistemon* spp.), and myrtles (*Syzygium*, *Rhodomyrtus*, *Gossia*). Myrtaceae species are commonly found in fire-prone ecosystems but are also important components of rainforest and riparian vegetation including in the World Heritage Listed Wet Tropics of Queensland and Great Barrier Reef catchment (Fernandez Winzer, Cuddy et al. 2020, Pegg, Giblin et al. 2025, Department of the Environment 2026, Department of the Environment 2026). They also provide habitat and food resources for native and endangered animals such as the koala (Myerscough 1998, Thornhill, Ho et al. 2015). The introduction of myrtle rust, a fungal disease that impacts over 380 native Myrtaceae species in Australia alone, has greatly challenged the family, driving some already threatened, and even some previously common, Myrtaceae species towards extinction (Makinson, Pegg et al. 2020).

Gossia fragrantissima, or sweet myrtle, is a small subtropical rainforest tree endemic to Eastern Australia. Many *Gossia* species are known to have manganese-hyperaccumulating capacities, with *G. fragrantissima* the only reported multi-metal hyperaccumulator (manganese, nickel, cobalt and zinc), making the genus biologically, ecologically and potentially industrially relevant (Purwadi, Abubakari et al. 2023). *G. fragrantissima* is listed as endangered in State and Federal legislation (Environment Protection and Biodiversity Conservation Act 1999, Snow, Guymer et al. 2003) and other *Gossia* species are in critical decline as a result of myrtle rust (Makinson, Pegg et al. 2020). Despite this, there is currently no dedicated recovery plan for *G. fragrantissima*. *In situ* conservation of this species, while critical (New South Wales Department of Environment 2010), is insufficient to guarantee its survival given increasing risks to already small natural populations, including extreme weather events, fire and myrtle rust. To facilitate conservation of the species, *ex situ* conservation options are being explored. For instance, seed banking research found that the seeds are probably orthodox in storage behaviour, as they can tolerate drying (15% relative humidity) and short-term storage in cold conditions (-20°C) (Sommerville, Errington et al. 2021). However, as myrtle rust impacts flowering and fruiting as well as vegetative growth, seed production and seed quality are likely to decline following infection, limiting the usefulness of seed banking as a conservation strategy (Berthon, Esperon-Rodriguez et al. 2018, Makinson, Pegg et al. 2020). *In vitro* conservation strategies, including tissue culture and cryopreservation, provide complementary approaches under these circumstances. These methods can utilise vegetative plant materials to propagate and store remaining wild germplasm before extinction (Pence, Ballesteros et al. 2020).

In vitro conservation preserves plant germplasm in a usually disease-free and stable environment, and is a resource for genetic banking and future population reinforcement and reintroduction (Kulak, Longboat et al. 2022). Collaborating with the Australian PlantBank, we previously established *in vitro* cultures for *G. fragrantissima* and optimised culture conditions using response-

surface modelling to refine basal nutrient composition of shoot multiplication media (Bao, Gleeson et al. 2025). In this study, cryopreservation of *G. fragrantissima* was explored for the first time, based on this *in vitro* progress, to facilitate long-term conservation. Droplet vitrification was used based on its versatility across a range of plant species, including many woody species (Kartha, Leung et al. 1982, Panis, Piette et al. 2005, O'Brien, Hiti-Bandaralage et al. 2021). Work in other plant species has demonstrated the importance of donor culture health and vigour on cryopreservation outcomes (Benelli, De Carlo et al. 2013, Bettoni, Bonnart et al. 2021). In this study, we quantified the influence of an RSM-optimised basal nutrient media on the survival and regrowth of apical shoot tips following cryopreservation, compared to the commercially available Woody Plant Medium (WPM) (McCown and Lloyd 1981). We simultaneously determined the impact of sucrose pretreatments at 0.2, 0.3, 0.4 M, for 48 h on cryopreservation outcomes. We hypothesised that the RSM-optimised nutritional environment would lead to increased rates of post-cryopreservation recovery, irrespective of sucrose pre-treatment conditions, and that this effect would be transferable between genotypes. This work highlights the fundamental role of donor culture optimisation in plant cryobiology and establishes a feasible long-term cryostorage protocol for an endangered Myrtaceae tree.

Materials and Methods

Plant material and growth conditions

In vitro cultures of two genotypes of *G. fragrantissima*, PlantBank accessions P2019-0119 (0119 - previously referred to in Bao, Gleeson et al. (2025) as 0121) and P2019-0124 (0124), were maintained in a culture room with a temperature of 25 ± 1°C and 16 h photoperiod under LED lights (Valoya, L-series model L18, 60 µmol m^{-2} s^{-1}). Cultures were maintained on two basal multiplication media for comparison: (1) Woody Plant Medium (WPM) (McCown and Lloyd 1981) or (2) a Predicted Best medium (PB) for *G. fragrantissima* based on response surface modelling (Box and Wilson 1951, Bao, Gleeson et al. 2025). The PB medium was previously developed and validated as significantly improving the growth parameters and physiological condition of accession (Bao, Gleeson et al. 2025) compared to WPM. It contains altered levels of six basal nutrient factors: vitamins (0.75 × WPM), ammonium nitrate (2.5 × WPM), calcium nitrate (0.5 × WPM), mesonutrients (2.37 × WPM), micronutrients (4 × WPM) and ferric EDTA (3.43 × WPM) (Table 1). Both media were supplemented with 2 µM 6-benzylaminopurine (BAP), 0.2 µM indole-3-butyric acid (IBA), 0.088 M sucrose, and 0.8% agar (Sigma), and pH was adjusted to 6.0 before autoclaving. For maintenance, cultures were transferred to fresh media every four weeks. Unless stated otherwise, all media were sterilized by autoclaving at 121°C for 15 mins.

Shoot tip excision and droplet vitrification cryopreservation procedures

Shoot tip dissection was performed aseptically under a dissection microscope in a laminar flow cabinet. Apical shoot tips (2 mm in length) were excised by peeling off last two visible leaves to expose the meristem (Fig. 1). Dissected apical shoot tips were kept on either a control medium (WPM or PB with 0.088 M sucrose, 0.8% agar, pH 6.0) or a sucrose pretreatment medium (WPM or PB with 0.2 M, 0.3 M, or 0.4 M sucrose, 0.8% agar, pH 6.0) for 48 h in the dark in a culture room with a temperature of 25°C.

All cryopreservation procedures were performed aseptically in a laminar flow cabinet and are summarised in Fig. 2. For osmoprotection, apical shoot tips were placed into 15 mL loading solution (2 M glycerol and 0.4 M sucrose, pH 6.0, filter sterilized) in a 50 mL falcon tube wrapped with aluminium foil and incubated for 20 min at room temperature. The loading solution was then removed and replaced with 15 mL ice-cold plant vitrification solution 2 (PVS2) (30% glycerol (w/v), 15% dimethyl sulfoxide (DMSO) (w/v), 15% ethylene glycol (EG) (w/v), 0.4 M sucrose, pH 6.0, filter sterilized) (Sakai, Kobayashi et al. 1990) and incubated for 20 min on ice. Before the end of the 20 min incubation, shoot tips were placed in drops of fresh ice-cold PVS2 on sterile, pre-cooled aluminium strips (20 · 5 mm) in a sterile petri dish (90 · 15 mm) sitting on an ice block. At the completion of the 20 min PVS2 exposure, the aluminium strips were transferred to pre-cooled cryovials containing liquid nitrogen (LN) which were then submerged in LN. The cryovials were maintained in LN for a minimum of 40 min. To quantify recovery and re-growth, each aluminium strip was removed from LN and placed in one petri dish (60 mm × 15 mm) prefilled with ~ 7 mL unloading solution (1.2 M sucrose, pH 6.0, filter sterilized). Shoot tips were then incubated for a total of 20 min in unloading solution, which was replaced with fresh unloading solution 3 times over the course of the incubation. PVS2-treated control (-LN) shoot tips were loaded for 20 min and treated with ice-cold PVS2 for 20 min before direct unloading as described above.

For regeneration of -LN and + LN samples after unloading, apical shoot tips were placed on top of a sterile filter paper on replate media (WPM or PB medium with 0.3 M sucrose, 0.8% agar, pH 6.0) in petri dishes (60 · 15 mm) sealed with parafilm, wrapped in aluminum foil, and incubated for 24 h in the culture room. Shoot tips were then transferred to regrowth media (WPM or PB with 2 µM BAP, 0.2 µM IBA, 0.088 M sucrose, and 100 mg L^{-1} ascorbic acid, 0.8% agar, pH 6.0, filter sterilized) in petri dishes (60 · 15 mm) and maintained in a dark growth chamber (wrapped with aluminum foil) with a temperature of 25°C for one week. After dark incubation, the foil was removed, and a shade cloth was placed on top of the plates to achieve a light intensity of approximately 25 $mol{\cdot}m^{-2}{\cdot}s^{-1}$. The shade cloth was removed after one week and remaining growth was under 60 $mol{\cdot}m^{-2}{\cdot}s^{-1}$ lights. Each experimental control and treatment was performed on ten apical shoot tips and replicated four times (n = 40).

Shoot tip scoring

Apical shoot tips were scored for 'regrowth', based on the criterion of at least three leaves growing from the shoot apex or axillary buds (Fig. 3), eight weeks after treatment.

Statistical analysis

All statistical analysis and data manipulation were performed in R version 4.5.3 (2026-03-11) (R Core Team 2026). Percentage regeneration data were transformed using an arc sine transformation. Data for the two genotypes and PVS2 control and LN exposed shoot tips were analysed separately, in 4 groups. Data were tested for normality within these groups using Q-Q plots and a Shapiro Wilk test. Where normality was established, one-way analysis of variance (ANOVA) was performed to determine significant differences among treatments at confidence level $p \leq 0.05$. Where data were not normal, a non-parametric Kruskal-Wallis test (Kruskal and Wallis 1952) was performed to determine significant differences among treatments at confidence level $p \leq 0.05$. Percentage regeneration and significance levels are reported to 2 significant figures.

Results

This study evaluated the effects of basal medium nutrient composition (WPM vs. PB) of both the donor culture media and the regrowth media, as well as sucrose pretreatment, on the regrowth of shoot-tips from genetically independent accessions (0119 and 0124) of *G. fragrantissima* after cryopreservation. The PB media was previously developed and validated as significantly improving the multiplication and health of accession 0119 compared to WPM (Bao et al., 2025). Representative images showing the health of the cultures on WPM and PB media prior to shoot tip excision can be seen in Fig. 4.

After excision, sucrose pretreatment, and loading, shoot tips were exposed to PVS2 solution alone (-LN) or subjected to full cryopreservation with LN to assess treatment responses (Fig. 2).

Basal media optimisation significantly enhanced cryopreservation outcomes in one genotype

Basal medium had a significant effect on post-cryopreservation regeneration in genotype 0119 (Fig. 5). There was no significant effect of sucrose pre-treatment at any concentration on LN or -LN regeneration rates. The highest LN regeneration rate for 0119 was achieved following culture on PB medium supplemented with 0.3 M sucrose for 48 hours, with an average regeneration rate of 74%.

In genotype 0124, post-cryopreservation (+ LN) regeneration rates on both media, and for all pre-treatments, exceeded 80% (Fig. 6). There was no significant effect of either basal medium or sucrose preculture concentration. The highest regeneration rate was again achieved with PB medium paired with a 0.3 M sucrose preculture for 48 hours, which resulted in a mean regeneration of 97%. The highest regeneration rate for WPM was 90%, recorded for shoot tips treated with 0.2 M sucrose preculture.

Discussion

In this study, cryopreservation of the endangered Australian native tree species *G. fragrantissima* was achieved for the first time using the droplet vitrification protocol. This represents the first successful cryopreservation of a species within the genus *Gossia*. High post-cryopreservation regrowth of up to 97% was achieved using a Predicted Best (PB) medium developed through Response Surface Methodology (RSM) for both donor culture maintenance and regrowth stages (Bao, Gleeson et al. 2025). This high regrowth percentage is notable given the generally low to moderate post-cryopreservation recovery rates reported for slow-growing woody species and, in particular, woody species within the Myrtaceae (Hardstaff, Sommerville et al. 2022). As described by Bao, Gleeson et al. (2025), PB was developed by optimising six factors found in standard WPM (WPM vitamins, NH_4NO_3, $Ca(NO_3)_2$, mesonutrients, micronutrients, FeEDTA) to levels predicted to support the best growth responses of *G. fragrantissima in vitro*. Validation of the PB formulation demonstrated that cultures maintained on PB showed improved shoot morphology and vigour in comparison to those maintained on WPM, which frequently exhibited signs of physiological stress, including basal browning, shoot tip necrosis, and chlorosis (Bao, Gleeson et al. 2025).

In the more cryo-sensitive genotype, 0119, for which the PB was originally validated (Bao, Gleeson et al. 2025), shoot tips from cultures maintained on PB had significantly higher regrowth after cryopreservation compared to those from WPM. This is consistent with the broader cryopreservation literature, where culture physiological health is widely recognised as a key determinant of successful cryopreservation and regeneration (Benelli, De Carlo et al. 2013, Bettoni, Bonnart et al. 2021). However, most studies have focused on the negative impacts of *in vitro* ageing, with comparatively little attention given to actively optimising basal medium compositions (Harding, Johnston et al. 2009, Ochatt, Lambardi et al. 2021).

While the improved culture medium resulted in improved cryopreservation results in 0119, the same was not true for 0124 in this study. In comparison with 0119, this genotype showed very high regeneration rates across all the treatments tested. While shoot tips from PB medium performed slightly better, there was no significant improvement from WPM. The effect of the PB medium on this genotype may have been dampened by its more robust and healthy growth on both media, or by an overall strong cryo-tolerance of this genotype.

Where nutrition has previously been considered in the context of cryopreservation, it has typically focused on modifications to the regrowth medium rather than optimisation of the shoot multiplication medium before the cryopreservation protocol is applied (Popova, Kulichenko et al. 2023). Basal nutrient composition can be critical in regrowth media after cryopreservation to support explant recovery from oxidative and osmotic stress as well as repair of membrane damage caused during cryopreservation (Malhotra, Bansal et al. 2024). Across species, explant regeneration on iron-reduced and ammonium-reduced media often improves cryopreservation results, such as in rice (*Oryza sativa*), silver birch (*Betula pendula*), and lemon (*Citrus limon*) (Benson, Lynch et al. 1995, Popova, Kulichenko et al. 2023). Iron in the regrowth medium has been suggested to act as a potent catalyst for production of reactive oxygen species (ROS) and therefore is not thought to be beneficial in facilitating oxidative stress recovery after cryopreservation, as shown in blackberry (*Rubus* spp.) (Uchendu, Leonard et al. 2010, Thi Tuyet Le, Brumbarova et al. 2019, Ren, Wang et al. 2021). Likewise, ammonium toxicity has been widely acknowledged in higher plants, potentially related to cellular pH change, ion imbalance, auxin disruption, and oxidative stress, whereas nitrates can partially counteract ammonium toxicity, despite not being discussed in the context of cryopreservation (Britto and Kronzucker 2002, Esteban, Ariz et al. 2016). In this study, the donor culture medium as well as the regrowth medium contained elevated Fe EDTA (3.43 × WPM), NH_4NO_3 (2.5 × WPM), and micronutrients (4× WPM), yet post-cryopreservation regrowth was improved on this medium compared to WPM.

In studies using reduced iron and ammonium in recovery media, it is implicit that the basal medium used for shoot growth is, if not fully optimised, at least suitable for the species. In the case of *G. fragrantissima* accession 0119, it may be that the original commercial medium was so far removed from the optimum nutrition for this genotype that any small detriment from excess iron or ammonium in the PB regrowth medium was obscured by the vast improvement in health resulting from pre-culture on an optimised medium. Therefore, to investigate whether *G. fragrantissima* is truly insensitive to reduced ammonium or iron in regrowth, one would have to compare plants grown exclusively on PB medium, and test PB-based recovery media with reduced ammonium and iron.

Indeed, ammonium- or iron-reduced media are not universally applied in cryopreservation of plant species (Panis, Piette et al. 2005, Cui, Yu et al. 2017). Although excessive iron may contribute to increasing oxidative stress, iron is also essential for fundamental physiological functions such as chlorophyll synthesis and electron transport (Therby-Vale, Lacombe et al. 2022). As a multi-metal

hyperaccumulator, *G. fragrantissima* may require different baseline levels of iron and other metals (such as manganese, cobalt, nickel and zinc) to maintain normal physiology, as reported in other metal-hyperaccumulating species (Gao, Yu et al. 2025). Its constitutively enhanced metal uptake, combined with antioxidant defences mediated by metals like iron, could help buffer ROS stress after LN exposure, potentially explaining why higher iron and micronutrient concentrations in PB may be beneficial (Millaleo, Reyes-Diaz et al. 2010). Moreover, nitrate is simultaneously increased in PB with increased ammonium, which may potentially reduce ammonium toxicity by balancing pH and improving ammonium assimilation (Li, Yan et al. 2024). Overall, the PB medium formulation determined by RSM led to considerable improvements in culture health, and regrowth following cryopreservation, for *G. fragrantissima*, and especially for the genotype used to optimise the PB medium. To our knowledge, this is one of the first demonstrations that basal medium nutrient optimisation of donor cultures can significantly improve cryopreservation outcomes, presumably by directly enhancing donor physiological condition. While individualised optimisation of *in vitro* media is unlikely to be essential for all species, nor feasible when cryobanking thousands of accessions, this nevertheless highlights the potential of this method for key precious and/or exceptional species.

Other methods of improving plant donor vigour have been investigated previously, such as reducing the duration of subculture intervals. For instance, in a raspberry species (*Rubus humulifolius*), regrowth after shoot-tip cryopreservation was only achieved from one-month-old cultures and not from two-month-old cultures (Edesi, Tolonen et al. 2020). Extended subculture intervals usually correlate with reduced culture health (e.g. increased shoot tip necrosis) due to the depletion of nutrients in the medium (Liu, Deng et al. 2024). Since shoots are typically maintained with subculture intervals of no longer than eight weeks, extended intervals may reduce vigour and thereby negatively affect cryopreservation outcomes (Phillips and Garda 2019, Edesi, Tolonen et al. 2020).

In addition to the benefits of reducing subculture intervals, a few studies have demonstrated that a liquid overlay (e.g. liquid hormone-free basal medium with or without sucrose) on normal tissue culture media can increase donor culture vigour and subsequent cryopreservation outcomes, particularly when combined with improved regeneration conditions (e.g. nitrogen-deprived regrowth medium), such as in Chrysanthemum (*Chrysanthemum* spp.), mint (*Pogostemon yatabeanus*) and figwort (*Scrophularia kakudensis*) (Lee and Kim 2022, Lee, Park et al. 2023, Lee, Park et al. 2024). It was proposed by Kim (2023) that donor culture vigour has a critical role in buffering cryoprotectant toxicity and dehydration stress, and that culture vigour can be improved through tissue culture protocol optimisation, selection of suitable explants, and incorporation of pretreatments.

Though RSM has been used most widely in optimisation of nutrition, this method could be applied to other variables, potentially speeding up the optimisation of the other factors such as liquid overlay, pretreatments, and subculture interval discussed above. However it is relevant to note here that changing the nutrition alone was sufficient to induce enhanced cryopreservation recovery in this genotype, irrespective of pre-treatments. Hence RSM-optimised media may enable simpler protocols to be developed for otherwise difficult species.

Sucrose pretreatment, which has been incorporated in shoot-tip cryopreservation of many woody species, such as sour cherry (*Prunus cerasus*) and avocado (*Persea americana*) (Barraco, Chatelet et al. 2012, O'Brien, Hiti-Bandaralage et al. 2021), was also trialled in this study. Pretreatment of donor explants with sugar prior to cryopreservation promotes the accumulation of soluble sugars, most notably sucrose, within plant tissue, which is associated with greater tolerance to dehydration and freezing stress (Kaviani 2011, Folgado, Panis et al. 2015). During pretreatment, sucrose functions as a stabilising solute and osmotic agent, promoting osmotic dehydration and helping maintain cell membrane and protein structure during dehydration and cold stress (Folgado, Panis et al. 2015, Tarkowski and Van den Ende 2015). The results of the current study suggest that sucrose pretreatment is not necessary to achieve satisfactory regeneration after cryopreservation in *G. fragrantissima.* This result is somewhat surprising considering the species is native to subtropical rainforest where leaf tissues could be expected to be desiccation sensitive. However, pretreatment on 0.3 M sucrose for two days did result in a modest improvement in regeneration rates compared to other concentrations, though the difference was not significant. Shatnawi, Johnson et al. (2004) reported that a 0.75 M sucrose pretreatment for one day was critical to achieving approximately 60% regrowth following cryopreservation of *Syzygium francissi* (Myrtaceae) shoot tips.

Five other *Gossia* species are listed as critically endangered in Queensland (ALA), none of which have cryopreservation protocols established and all of which are potentially susceptible to myrtle rust (Pegg, Perry et al. 2012). The Australian Government's Threatened Species Action Plan outlines a goal to have all myrtle rust susceptible species conserved in *ex situ* collections by 2032

(Commonwealth of Australia 2022). Considering the previously limited number of examples of successful cryopreservation for Australian Myrtaceae genera (Hardstaff, Sommerville et al. 2022), this study achieved a critical step towards reaching the 2032 target. The topic of optimised basal growth media in relation to cryopreservation is greatly under-studied and may be key to accelerating the conservation of other Endangered species not only in Australia, but globally.

Conclusion

This is the first report of successfully cryopreserving *G. fragrantissima* apical shoot tips using a droplet vitrification protocol. Apical shoot tips sourced from *G. fragrantissima* cultures maintained on a basal-nutrient-optimised medium supported up to 97% regrowth after cryopreservation in a cryo-tolerant genotype and an improvement from 40% to 74% regeneration in a cryo-sensitive genotype. This study revealed the critical role of optimising donor plant nutrition and regrowth medium for successful cryopreservation outcomes in *G. fragrantissima*. This protocol can be used as a starting point for cryopreservation of other threatened Myrtaceae and the approach of improving basal media for cryopreservation outcomes is a novel method of cryopreservation optimisation for other exceptional species.

Declarations

This project was supported by Logan City Council and the Australian Research Council Linkage Program (LP210200907). Jingyin Bao was funded by an Australian Government Research Training Program (RTP) Scholarship administered by The University of Queensland. The authors have no relevant financial or non-financial interests to disclose.

Author Contribution

All authors contributed to the study conception and design. Experiments were carried out by J.B., C.O.B., and L.M.W. Data collection and analysis were performed by L.M.W. The first draft of the manuscript was written by J.B. and L.M.W. MG, KS, and AH commented and made edits on previous versions of the manuscript. All authors read and approved the final manuscript.

Acknowledgement

The authors thank Professor Ricardo Mancera, Dr Bryn Funnekotter, Dr Lyndle Hardstaff, Dr Cathy Offord, Lee-Anne Veage, and Dr Geoff S Pegg for their valuable discussions and insights during the development of this study. We also thank Amanda Rollason for initiation of the original in vitro cultures of the two genotypes on which the study was based.

Data Availability

All data supporting the findings of this study are available within the paper and its Supplementary Information.

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Tables

Table 1 Nutrient quantities in WPM and PB media, showing the differences between WPM and PB as a multiplier, so that (mg/L in WPM) x (WPM > PB multiplier) = mg/L in PB. Darker shading in the multiplier column indicates greater increase in concentration

	mg/L in WPM	WPM > PB multiplier	mg/L in PB (WPM x multiplier)	Referred to in text as:
Ammonium nitrate	400	2.5	1000	Ammonium nitrate
Na_2 EDTA•$2H_2O$	37.3	3.43	127.94	Iron
Ferrous sulfate•$7H_2O$	27.85	3.43	95.53	Iron
Calcium chloride, Anhydrous	72.5	2.37	171.83	Mesonutrient
Magnesium sulfate, Anhydrous	180.7	2.37	428.26	Mesonutrient
Potassium phosphate, monobasic	170	2.37	402.9	Mesonutrient
Calcium nitrate	386	0.5	193	Calcium nitrate
Boric acid	6.2	4	24.8	Micronutrient
Cupric sulfate•$5H_2O$	0.25	4	1	Micronutrient
Manganese sulfate•H_2O	22.3	4	89.2	Micronutrient
Molybdic acid (Sodium Salt) •$2H_2O$	0.25	4	1	Micronutrient
Zinc sulfate•$7H_2O$	8.6	4	34.4	Micronutrient
Glycine (Free Base)	2	0.75	1.5	Vitamin
myo-Inositol	100	0.75	75	Vitamin
Nicotinic acid (Free Acid)	0.5	0.75	0.375	Vitamin
Pyridoxine•HCl	0.5	0.75	0.375	Vitamin
Thiamine•HCl	1	0.75	0.75	Vitamin
Potassium sulfate	990	1	990	Potassium sulfate

Figures

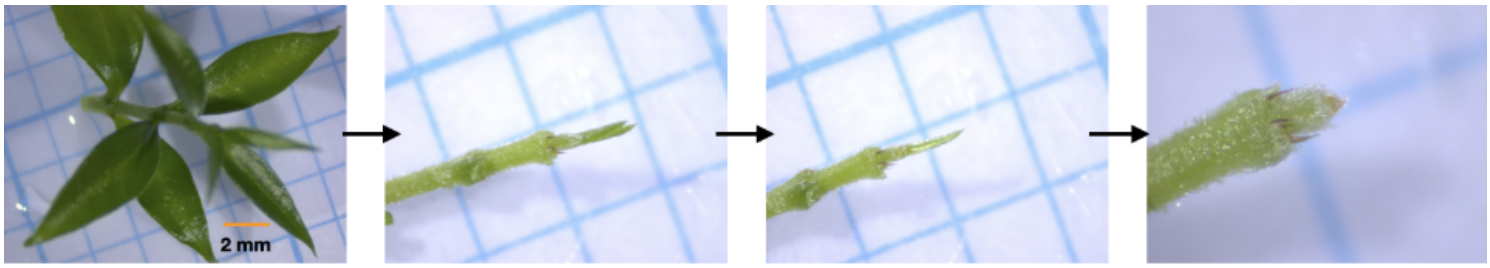


Figure 1

Stepwise excision and isolation of in vitro G. fragrantissima apical shoot tips for cryopreservation experiments

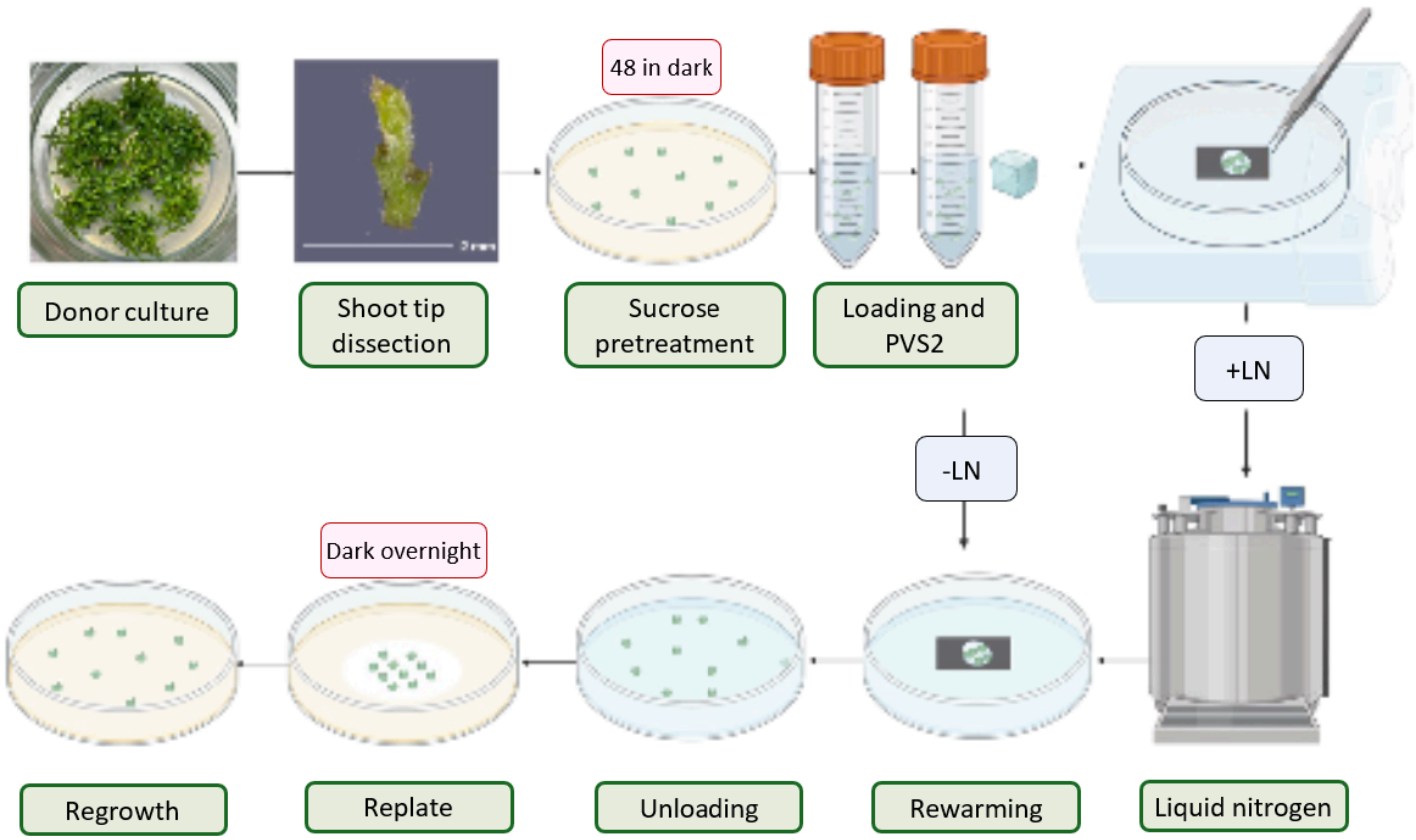


Figure 2

Schematic diagram of the droplet vitrification cryopreservation procedure for PVS2-treated G. fragrantissima apical shoot tips under control (−LN) and liquid nitrogen (+LN) treatments

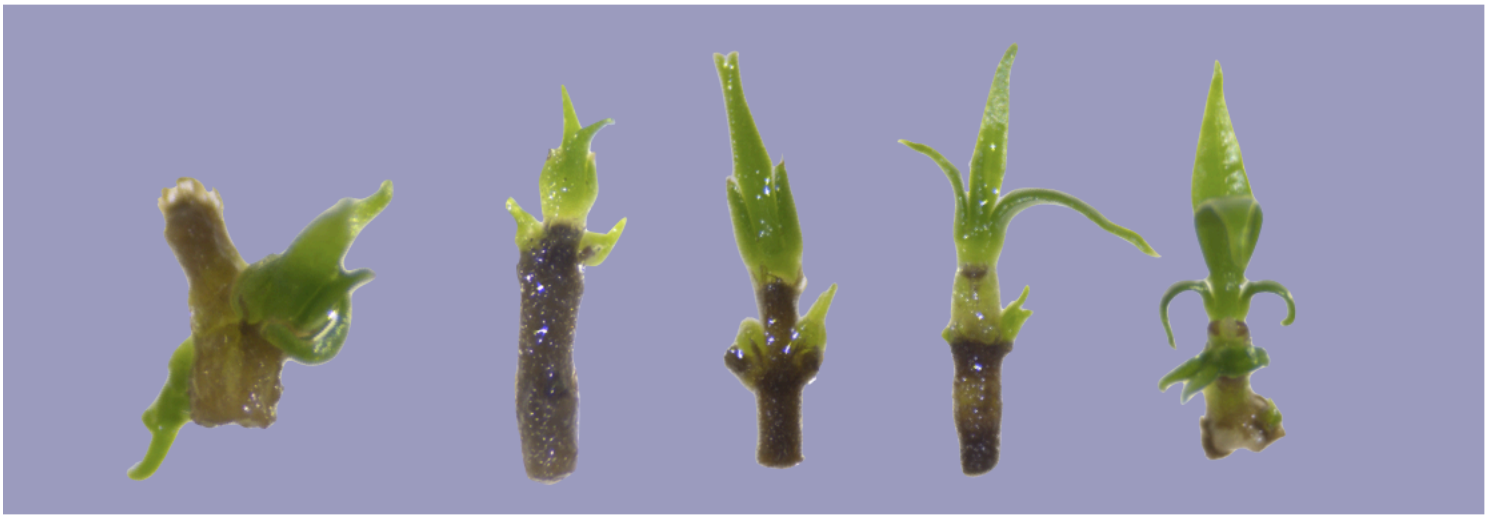


Figure 3

Photo criteria for ‘regrowth’ of Gossia fragrantissima apical shoot tips

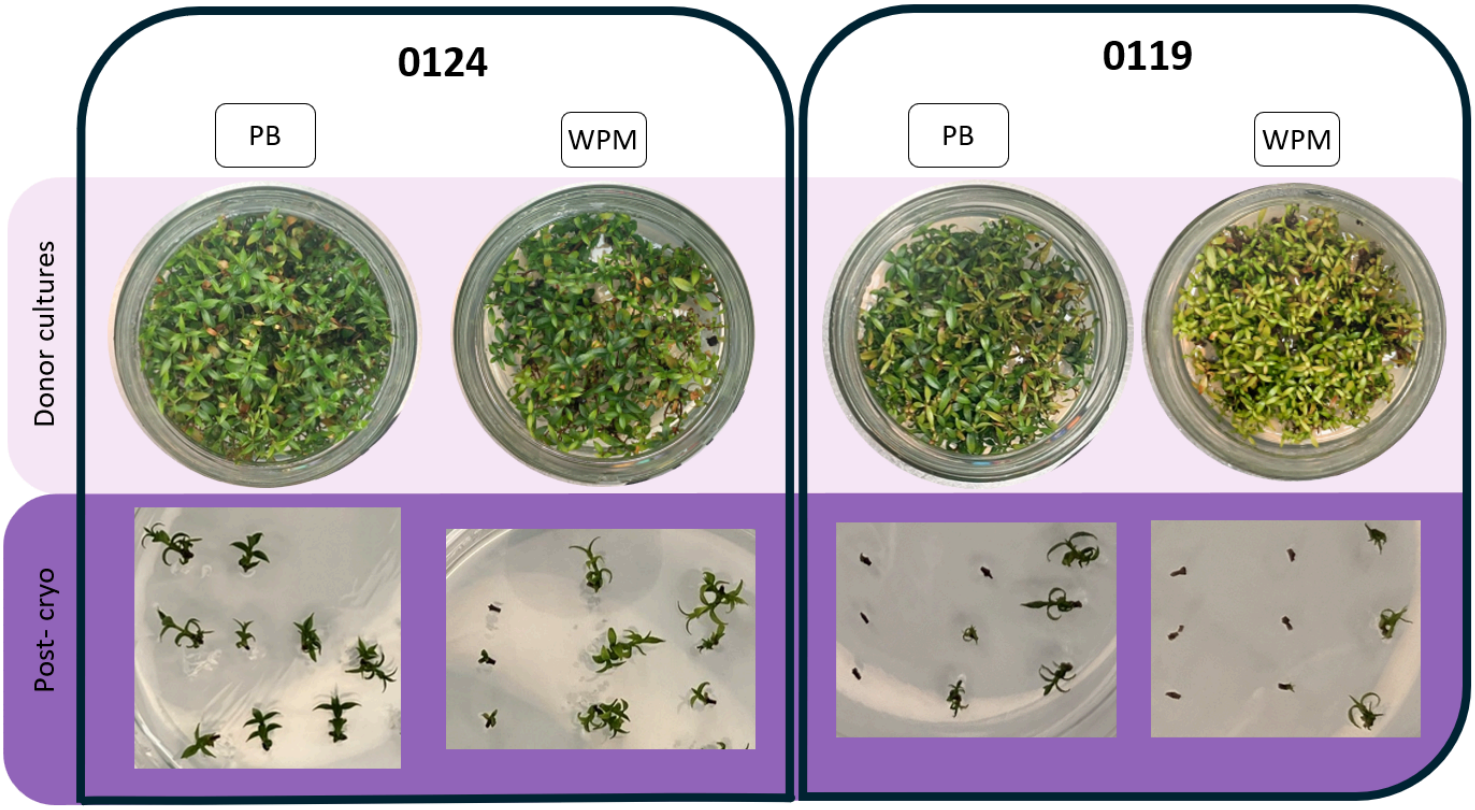


Figure 4

Representative photographs of 0124 (left) and 0119 (right) cultures grown on WPM and PB medium before and 2 months after cryopreservation. Donor cultures are shaded in light pink (top), and cultures after cryopreservation are shaded darker purple (bottom)

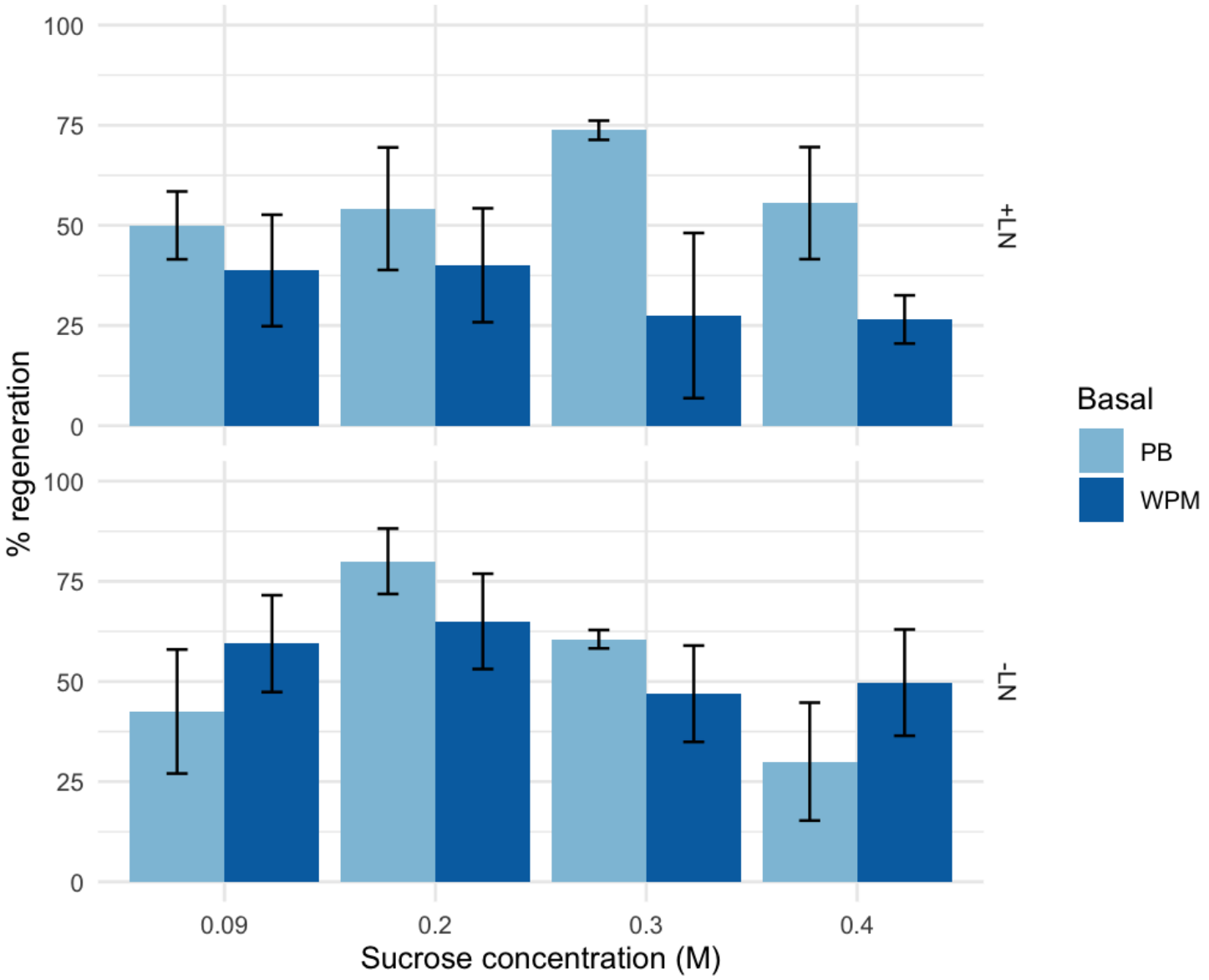


Figure 5

Post-cryopreservation regeneration rates for *G. fragrantissima* genotype 0119, recorded 8 weeks after treatment. Shoot tips were harvested from donor plants cultured on response surface methodology-optimised Predicted Best (PB) medium or commercially available Woody Plant Medium (WPM) and pre-cultured on different sucrose levels (0.09-0.4 M) prior to immersion in liquid nitrogen (+LN) or immediate replating on recovery media (-LN). For each bar, n= 4 replicates of ten shoot tips each. Basal medium had a significant effect on regeneration rate (P = 0.01) in LN treatment but not in -LN treatment. No significant differences were found between specific sucrose treatments in either +LN or -LN treatments

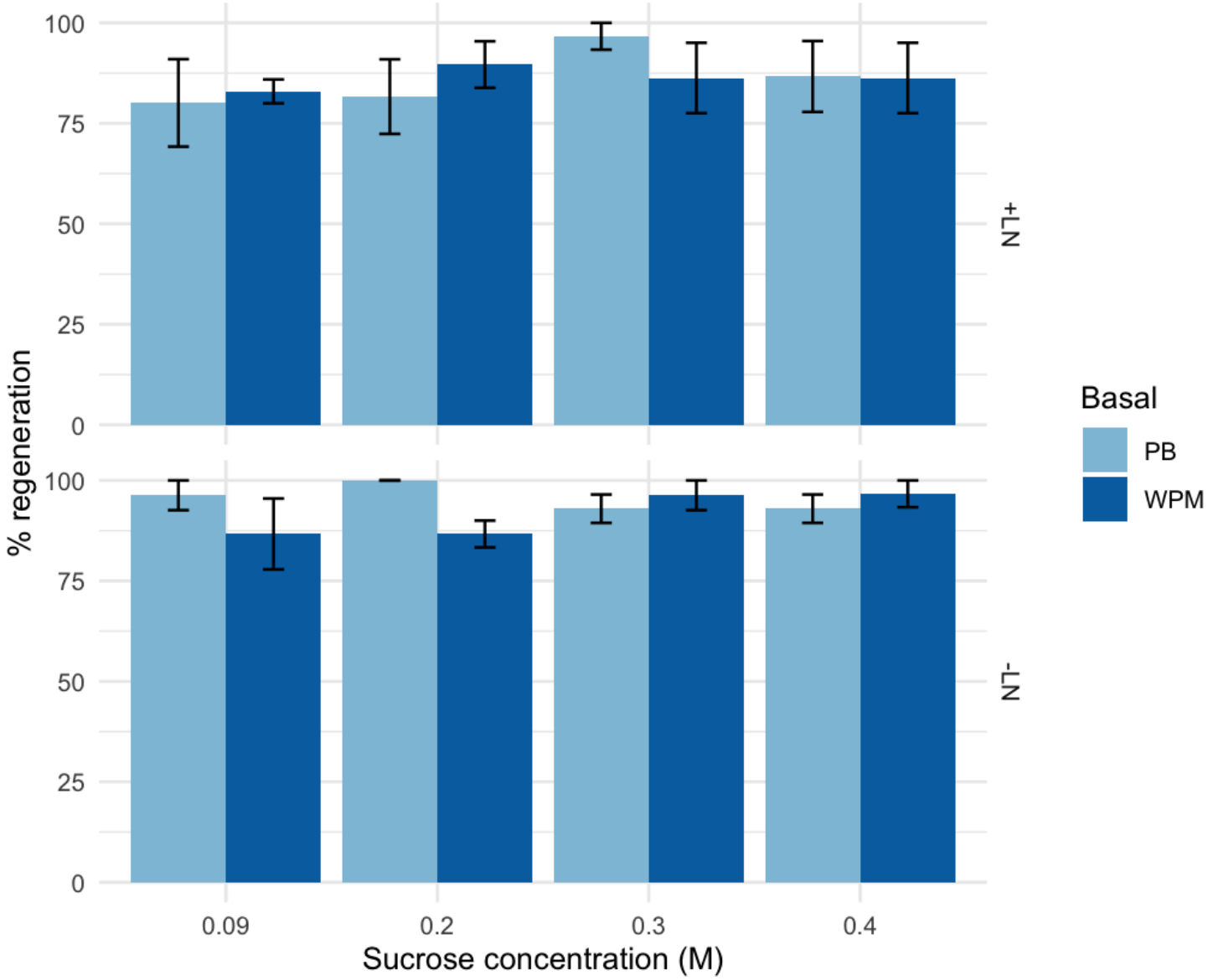


Figure 6

Post-cryopreservation regeneration rates for *G. fragrantissima* genotype 0124, recorded 8 weeks after treatment. Shoot tips were harvested from donor plants cultured on response surface methodology-optimised Predicted Best (PB) medium or commercially available Woody Plant Medium and pre-cultured on different sucrose levels (0.09-0.4 M) prior to immersion in liquid nitrogen (+LN) or immediate plating on recovery media (-LN). For each bar, n= 4 replicates of ten shoot tips each

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- jbcryodataclean.csv