Effects of Osmotic Agents on Somatic Embryo Maturation and Germination in Sophora davidii (Postprint)

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Abstract

To investigate the effects of different osmotic agents on the maturation and germination of Sophora davidii somatic embryos, this study employed sucrose, maltose, sorbitol, and PEG 6000 as osmotic agents to examine their influence on somatic embryo development, maturation, and germination. The results demonstrated: When embryogenic callus derived from Sophora davidii hypocotyls was inoculated onto MS medium supplemented with 2,4-D 0.2 mg · L ^{1} + NAA 1.0 mg · L ^{1} + 6-BA 2.0 mg · L ^{1} + TDZ 1.0 mg · L ^{1} + sucrose 40 g · L ^{1} + glutamine 100 mg · L ^{1} + phytagel 3 g · L ^{1}, the somatic embryogenesis rate reached 66.21%, with a total embryo count of 79; 7% sucrose enabled somatic embryo maturation rates up to 64.36%, while concurrently increasing the formation of polycotyledonary abnormal embryos; the combination of 2% maltose + 2% sorbitol + 4% sucrose yielded the highest somatic embryo maturation rate of 88.89% with the lowest proportion of abnormal embryos; when cultured with 30 g · L ^{1} PEG, the somatic maturation rate reached its maximum at 82.35%; torpedo-stage somatic embryos were optimal for subculture, achieving somatic embryo germination rates up to 90.58%, and mature somatic embryos cultured on compound sugar medium exhibited the highest rooting rate at 87.47%. These findings establish a theoretical foundation and provide a feasible protocol for Sophora davidii somatic embryo-based seedling propagation.

Full Text

Effects of Penetrant on Maturation and Germination of Somatic Embryos in *Sophora davidii*

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Abstract

To investigate the effects of different penetrants on the maturation and germination of somatic embryos in *Sophora davidii*, this study examined the influence of sucrose, maltose, sorbitol, and PEG (6000) as osmotic agents on somatic embryo development, maturation, and germination. The results showed that when embryogenic callus derived from *Sophora davidii* hypocotyls was cultured on MS medium supplemented with 2,4-D 0.2 mg · L ^{1}, NAA 1.0 mg · L ^{1}, 6-BA 2.0 mg · L ^{1}, TDZ 1.0 mg · L ^{1}, sucrose 40 g · L ^{1}, glutamine 100 mg · L ^{1}, and phytagel 3 g · L ^{1}, the somatic embryogenesis rate reached 66.21% with a total of 79 embryos per gram of callus. A 7% sucrose concentration promoted the maturation rate of somatic embryos to 64.36% but also increased the formation of polycotyledonous abnormal embryos. The combination of 2% maltose + 2% sorbitol + 4% sucrose yielded the highest somatic embryo maturation rate of 88.89% with the lowest proportion of abnormal embryos. Culture with 30 g · L ^{1} PEG resulted in the highest somatic embryo maturation rate of 82.35%. Torpedo-stage somatic embryos were most suitable for subculture, achieving a germination rate of 90.58%. Mature somatic embryos cultured on the compound sugar medium showed the highest rooting rate of 87.47%. These findings provide a theoretical foundation and feasible approach for somatic embryo-based seedling propagation of *Sophora davidii*.

Keywords: *Sophora davidii*, penetrant, somatic embryo, somatic embryo maturation, somatic embryo germination

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Introduction

Somatic embryogenesis via plant tissue culture represents one of the most effective methods for obtaining regenerated plants. This approach offers numerous advantages, including high embryo yields, origin from single embryogenic cells, relative genetic stability, complete structural organization, high regeneration rates, and suitability as a recipient for exogenous gene transformation (Gupta & Pullman, 1991). Consequently, somatic embryogenesis has become a prerequisite for genetic improvement, artificial seed production, rapid propagation, and commercial seedling production (Hu & Liu, 2014). The earliest reports of somatic embryogenesis came from Steward (1958) and Reinert (1959) working with carrot root cultures. Subsequent successes were achieved in various herbaceous plants. However, due to differences in growth patterns and organ structures compared with herbaceous species, somatic embryogenesis in woody plants remains limited. Globally, only about 180 woody plant species have been successfully induced to form regenerated plants through somatic embryogenesis. International research has extensively covered genera such as *Quercus* and *Populus*, as well as species including Norway spruce (*Picea abies*), white spruce (*P. glauca*), and black spruce (*P. mariana*). Commercial applications have been successfully implemented for Douglas fir (*Pseudotsuga menziesii*), loblolly pine (*Pinus taeda*), radiata pine (*Pinus radiata*), Norway spruce (*Picea abies*), Manchurian ash (*Fraxinus mandshurica*), and hybrid tulip tree (*Liriodendron* hybrids). Additionally, somatic embryogenesis systems have enabled gene transfer for disease resistance and stress tolerance in species such as eucalyptus (*Eucalyptus camaldulensis*), tulip tree (*Liriodendron tulipifera*), and European larch (*Larix decidua*) (Chen et al., 2003). To date, however, no research has been reported on *Sophora davidii*.

Sophora davidii, a leguminous deciduous shrub, serves as a pioneer species for soil and water conservation in the karst regions of southwestern China and arid northern regions (Li et al., 2009; He, 2007; He et al., 2018). The roots, stems, leaves, flowers, and seeds of this species have medicinal value, offering heat-clearing, throat-soothing, heatstroke-relieving, and anti-inflammatory effects (Mao et al., 2009). With numerous small, dense flowers, *S. davidii* also provides an excellent spring nectar source and serves as a natural "green food" (Fan et al., 2005). Field investigations have revealed that despite abundant flowering and fruiting, natural seedling establishment is poor due to hard seed coats (Wu et al., 2014) with poor water and air permeability and seed dormancy characteristics (Wu et al., 2018), limiting population regeneration. Somatic embryogenesis could provide an effective solution to the challenges of slow sexual propagation. However, previous studies on *S. davidii* have identified low induction and germination rates as major obstacles. Building on prior research, this study aimed to optimize culture conditions for *S. davidii* somatic embryos to improve maturation and germination. We investigated optimal concentrations and ratios of auxins and cytokinins for embryo differentiation, screened suitable culture conditions, and addressed low germination rates by adjusting medium osmotic pressure and composition to identify essential nutrients and key factors during germination, thereby establishing a theoretical and practical foundation for somatic embryo-based seedling propagation of *S. davidii*.

Materials and Methods

1.1 Experimental Materials

Based on previous research, embryogenic callus induced from *Sophora davidii* hypocotyls on MS medium containing 2,4-D 2.0 mg · L ^{1}, TDZ 0.5 mg · L ^{1}, 6-BA 0.5 mg · L ^{1}, sucrose 40 g · L ^{1}, glutamine 100 mg · L ^{1}, and agar 7 g · L ^{1} was used as experimental material.

1.2 Somatic Embryo Induction

Embryogenic callus was transferred to somatic embryo differentiation medium consisting of MS + 2,4-D 0.2 mg · L^{1} + NAA 1.0 mg · L^{1} + 6-BA 2.0 mg · L^{1} + TDZ 1.0 mg · L^{1} + sucrose 40 g · L^{1} + glutamine 100 mg · L^{1} + phytagel 3 g · L^{1} (pH 5.8). Cultures were maintained under 20 mol · m^{2} · s^{1} light intensity with a 14 h · d^{1} photoperiod at 25°C. After 40 days, somatic embryogenesis rate, total embryo number per gram fresh weight of callus, and embryo numbers at different developmental stages were recorded.

Somatic embryogenesis rate (%) = (Number of callus pieces producing somatic embryos / Number of inoculated embryogenic callus pieces) × 100%

Somatic embryo number (embryos per gram callus, embryos · g^{1}) = Total somatic embryo count / Fresh weight of callus

1.3 Somatic Embryo Maturation Culture

Globular embryos obtained from differentiation culture were inoculated onto maturation media. The following formulations were compared: sucrose (2%, 3%, 4%, 5%, 6%, 7%); maltose (2%, 5%, 7%) + sucrose 4%; sorbitol (2%, 5%, 7%) + sucrose 4%; maltose 2% + sorbitol 2% + sucrose 4%; and PEG6000 (20, 30, 40, 50, 60, 70 g · L^{1}). All maturation media contained ABA 15 g · L^{1}, NAA 1.0 mg · L^{1}, 6-BA 2.0 mg · L^{1}, TDZ 1.0 mg · L^{1}, glutamine 100 mg · L^{1}, phytagel 3 g · L^{1}, and activated charcoal 2 g · L^{1}. Each treatment consisted of 10 culture vessels with 4–6 embryos per vessel. Cultures were maintained under 20 mol · m^{2} · s^{1} light intensity with a 14 h · d^{1} photoperiod at 25 ± 1°C. After 45 days, embryo development was observed and mature embryo numbers were recorded.

1.4 Somatic Embryo Germination

Somatic embryos at different developmental stages obtained from section 1.2 were inoculated onto germination medium consisting of 1/3 MS, maltose 2% + sorbitol 2% + sucrose 4%, activated charcoal 2 g · L^{1}, and phytagel 3 g · L^{1} (pH 5.8). After 50 days of culture under 20 mol · m^{2} · s^{1} light intensity, germination status was observed. Normal seedlings were counted to calculate germination rate, and seedling quality was assessed.

Somatic embryo germination rate (%) = (Number of embryos germinated into seedlings / Number of inoculated embryos) × 100%

Somatic embryo seedling quality (mg) = Fresh weight of germinated seedling - Fresh weight of inoculated embryo

1.5 Rooting Culture and Plant Regeneration

Mature somatic embryos obtained from different osmotic treatments were transferred to rooting medium consisting of 1/2 MS + NAA 0.2 mg · L ^{1} + activated charcoal 2 g · L ^{1} + sucrose 30 g · L ^{1} + agar 7 g · L ^{1}. Complete plants were obtained and acclimatized before transplantation to pots containing substrate (humus soil:perlite = 2:1). Plant survival rate was recorded after 30 days.

Plant survival rate (%) = (Number of surviving transplanted plants / Total number of transplanted plants) × 100%

Results

2.1 Somatic Embryo Induction

After inoculating embryogenic callus onto MS + 2,4-D 0.2 mg · L ^{1} + NAA 1.0 mg · L ^{1} + 6-BA 2.0 mg · L ^{1} + TDZ 1.0 mg · L ^{1} + sucrose 40 g · L ^{1} + glutamine 100 mg · L ^{1} + phytagel 3 g · L ^{1} medium, callus growth was visible within the first 10 days, appearing soft and slightly granular [Figure 1: see original paper]A. With extended culture, the callus surface became light yellow with smooth protrusions. After approximately 30 days, cylindrical protrusions elongated longitudinally [Figure 1: see original paper]B, and young somatic embryos began to develop [Figure 1: see original paper]C. After 40 days, high-frequency somatic embryogenesis was achieved, producing globular, heart-shaped, torpedo, and a few cotyledonary embryos. Statistical analysis revealed a somatic embryogenesis rate of 66.21% with 79 embryos per gram of callus, including 52 globular, 18 heart-shaped, 8 torpedo, and 4 cotyledonary embryos. Among the cotyledonary embryos, one had a single cotyledon, one had multiple cotyledons, and two exhibited normal morphology.

2.2.1 Effects of Sucrose on Somatic Embryo Maturation

Sucrose serves as both an energy source and osmotic regulator in plant tissue culture, with 4% sucrose used as the control. As shown in Table 1 , somatic embryo maturation improved with increasing sucrose concentration, reaching a maximum maturation rate of 64.36% at 7% sucrose. The lowest maturation rate (35.58%) occurred at 2% sucrose. Both low and high sucrose concentrations adversely affected normal embryo development. At low concentrations, embryos exhibited poor growth, appearing transparent and water-soaked with weak viability, eventually browning and dying. High sucrose concentrations promoted polycotyledonous abnormal embryo formation and clustered monocotyledonous-like embryos sharing a common embryonic axis, which could not develop further. Optimal sucrose concentrations of 5-6% produced robust, enlarged embryos with elongated hypocotyls.

2.2.2 Effects of Compound Sugars on Somatic Embryo Maturation

The effects of different maltose, sucrose, and sorbitol combinations on somatic embryo maturation are presented in Table 2 . In maltose-sucrose combinations, maturation rate increased with maltose concentration, peaking at 86.27% with 7% maltose, though this also yielded the highest abnormal embryo rate (22.73%). At 2% maltose, the maturation rate was 57.41% with 22.58% abnormal embryos, while 5% maltose produced the lowest abnormal embryo proportion (10.81%). In sorbitol-sucrose combinations, mature embryo numbers also increased with sorbitol concentration, reaching 78.18% maturation at 7% sorbitol with 18.60% abnormal embryos. Medium sorbitol concentration (5%) yielded 71.93% maturation with the lowest abnormal embryo rate (9.76%). The combination of 2% maltose + 2% sorbitol + 4% sucrose achieved the highest maturation rate (88.89%) with the lowest abnormal embryo proportion (6.25%). These results demonstrate that both sugar type and concentration significantly affect somatic embryo maturation rate and abnormal embryo frequency.

2.3 Effects of PEG on Somatic Embryo Maturation

As an osmotic agent, PEG typically induces water stress, inhibits protein synthesis, blocks cell division, and accelerates somatic embryo development. Table 3 shows the effects of different PEG concentrations on somatic embryo maturation. The highest maturation rate (82.35%) with only 9.52% abnormal embryos occurred at 30 g · L ^{1} PEG. PEG concentrations exceeding 30 g · L ^{1} decreased maturation rates, with only 41.51% maturation and 31.82% abnormal embryos at 70 g · L ^{1} PEG.

2.4 Somatic Embryo Germination

Somatic embryos at different developmental stages exhibited varying germination capacities when cultured on hormone-free germination medium. Globular embryos required progression through heart-shaped, torpedo, and cotyledonary stages before seedling development, taking approximately 40 days and producing relatively weak seedlings. Heart-shaped embryos similarly developed through torpedo and cotyledonary stages. Some cotyledonary embryos turned off-white, senesced, and browned before dying. In contrast, torpedo-stage embryos converted to cotyledonary embryos within 10 days, with cotyledons gradually expanding, turning green, hypocotyls elongating, and primary roots initiating growth. Seedlings developed within approximately 20 days, showing rapid development and robust growth. As shown in Table 4 , torpedo-stage embryos demonstrated the highest germination capacity (90.58%) with seedling quality of 103 mg per plant, while globular-stage embryos showed the lowest germination rate (69.77%) with seedling quality of 81 mg per plant.

2.5 Rooting Culture and Plant Regeneration

Mature somatic embryos from different osmotic treatments were transferred to rooting medium [Figure 2: see original paper]A. Embryos cultured on the maltose + sorbitol + sucrose combination medium exhibited the highest rooting rate (87.47%), with some seedlings developing 5-6 lateral roots in addition to the primary root, showing robust hypocotyls, thick roots, and expanded cotyledons [Figure 2: see original paper]B. Sucrose-only medium produced lower rooting rates (77.51%), while PEG-cultured embryos showed intermediate rooting rates (82.13%). Seedlings [Figure 2: see original paper]C were acclimatized by opening culture vessel lids for 7 days before transplantation to greenhouse pots. Maintaining humidity above 80% and avoiding direct sunlight for the first 20 days resulted in seedling survival rates exceeding 92% after 30 days.

Discussion

Various sugars, including sucrose, glucose, fructose, maltose, mannitol, and sorbitol, are added to plant tissue culture media to provide essential C, H, O, and N elements while regulating osmotic pressure. Sucrose is the most commonly used sugar at concentrations of 2-5%, with higher concentrations required for specialized cultures such as anther or young embryo culture, as it plays a crucial role in somatic embryo development. Cell and protoplast cultures often require additional sugars beyond sucrose for optimal growth. Xu et al. (2017) demonstrated that 60 g • L ^{1} maltose was more suitable than sucrose for somatic embryo development and maturation in nematode-resistant red pine. Qi et al. (2004) reported that 3% maltose combined with 4% PEG (4000) was superior to sucrose-PEG combinations for *Larix principis-rupprechtii*, achieving 57.89% rooting frequency and significantly improved embryo quality. Gu et al. (2018) found that sucrose concentration did not affect embryoid regeneration rate but significantly influenced plant height and leaf emergence in rubber tree embryoids. In this study, different sugars and combinations affected somatic embryo maturation, with 5-6% sucrose producing robust, enlarged embryos with elongated hypocotyls, while excessive sucrose concentrations increased maturation rates but also elevated abnormal embryo proportions.

PEG has been widely reported as an osmotic regulator promoting somatic embryo maturation by inducing cellular dehydration, increasing intracellular solute concentration, and accelerating embryo development. Sun (2009) reported that 70 g • L ^{1} PEG significantly promoted somatic embryo maturation and germination in *Fraxinus mandshurica*. Our findings indicate that moderate PEG concentrations enhance somatic embryo maturation, while excessive concentrations reduce maturation rates and increase abnormal embryo proportions. Appropriate PEG-induced osmotic stress may inhibit normal protein synthesis while inducing stress protein production, thereby regulating metabolism, suppressing callus cell division, accelerating embryonic development, and promoting precocious maturation while inhibiting germination of mature embryos (Gao, 2001).

In conclusion, *Sophora davidii* hypocotyl-derived embryogenic callus cultured on MS + 2,4-D 0.2 mg · L^{1} + NAA 1.0 mg · L^{1} + 6-BA 2.0 mg · L^{1} + TDZ 1.0 mg · L^{1} + 4% sucrose + glutamine 100 mg · L^{1} + phytagel 0.3% achieved a somatic embryogenesis rate of 66.21%. Sucrose concentration affected both embryo maturation and abnormal embryo frequency. The combination of 2% maltose + 2% sorbitol + 4% sucrose yielded the highest maturation rate (88.89%), while 30 g · L^{1} PEG produced an 82.35% maturation rate. Torpedo-stage embryos were optimal for subculture, and post-acclimatization seedling survival exceeded 92%. These results provide a theoretical foundation and practical protocol for somatic embryo-based seedling propagation of *Sophora davidii.*

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