Effects of Phytagel and Sucrose on Plant Regeneration from Somatic Embryos in Rubber Tree Postprint

Authors: Gu Xiaochuan[1], Xu Zhengwei[1], Cheng Jing[1], He Xinghui[2], Hua Yuwei[1]

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Abstract

Gellan gum and sucrose have significant effects on the regeneration and growth of somatic embryo-derived plantlets. In this study, mature somatic secondary dicotyledonous embryos of Hevea brasiliensis clone Reyuan 7-33-97 were used as material, and MS medium supplemented with 0.23 mol·L^{-1} KT, 0.11 mol·L^{-1} IAA, and 8.7 mol·L^{-1} GA_3 was used as the plant regeneration medium to investigate the effects of different concentrations of gellan gum and sucrose on the regeneration and growth of somatic embryo-derived plantlets in Hevea brasiliensis. The results showed that in the somatic embryo plantlet regeneration medium of rubber tree, different concentrations of gellan gum had significant effects on plantlet regeneration frequency and growth status. At lower concentrations (0-1 g·L^{-1}), the regeneration frequency increased with increasing concentration; however, at higher concentrations (1-4 g·L^{-1}), plantlet growth was inhibited with increasing concentration. When gellan gum was added at 1 g·L^{-1}, plantlet growth was optimal, with a regeneration rate of 86.4$±5.7±9.4±3{-1}),leafemergencewaspromotedbutstemelongationwasinhibited,whereasathighsucroseconcentrations(70–80g
1}),leafemergencewassignificantlyinhibitedbutstemsegmentelongationwaspromoted.Whensucrosewasadde
L{-1},plantletgrowthwasoptimal,with57.6±5.4±12.3{-1},plantstemsandrootswererelativelyrobust.Therefore,inthes
L^{-1}$ and 50 g·L^{-1}, respectively.

Full Text

Preamble

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Title: Effects of Phytagel and Sucrose on the Regeneration Efficiency of Somatic Embryos and Growth of Regenerated Plants in *Hevea brasiliensis*

Authors: GU Xiao-Chuan[1], XU Zheng-Wei[1], CHENG Jing[1], HE Xing-Hui[2], HUA Yu-Wei[1]*

Affiliations: [1] Key Laboratory of Biology and Genetic Resources of Rubber Tree, Ministry of Agriculture, Rubber Research Institute, Chinese Academy of Tropical Agricultural Sciences, Danzhou 571737, Hainan, China
[2] Longjiang Branch, China Hainan Rubber Industry Group Co., Ltd., Baisha 572818, Hainan, China

Abstract

Phytagel and sucrose significantly influence the regeneration efficiency of somatic embryos and the growth of regenerated plants. This study investigated the effects of different concentrations of Phytagel and sucrose on plant regeneration and growth in *Hevea brasiliensis* using mature cotyledonary somatic embryos from clone Reyan 7-33-97 as explants. The regeneration medium consisted of MS basal medium supplemented with 0.23 mol • L^{-1} KT, 0.11 mol • L^{-1} IAA, and 8.7 mol • L^{-1} GA_3. The results demonstrated that Phytagel concentration significantly affected both regeneration frequency and plantlet growth. At lower concentrations (0–1 g • L^{-1}), regeneration frequency increased with increasing Phytagel concentration; however, at higher concentrations (1–4 g • L^{-1}), plant growth was inhibited. The optimal Phytagel concentration was 1 g • L^{-1}, achieving a plant regeneration rate of 86.4 ± 5.7%, with 53 ± 9.4% of plantlets exceeding 5 cm in height and 81.7 ± 3% developing leaves. In contrast, sucrose concentration did not significantly affect regeneration frequency but substantially influenced plantlet growth. Low sucrose concentrations (20–30 g • L^{-1}) promoted leaf emergence while inhibiting stem elongation, whereas high concentrations (70–80 g • L^{-1}) suppressed leaf development but promoted stem elongation. The optimal sucrose concentration was 50 g • L^{-1}, yielding the highest percentages of plantlets over 5 cm tall (57.6 ± 5.4%) and plantlets over 5 cm tall with leaves (46.3 ± 12.3%). Morphologically, plantlets at 50 g • L^{-1} sucrose exhibited robust stems and roots. Therefore, the most suitable concentrations for rubber tree somatic embryo regeneration medium are 1 g • L^{-1} Phytagel and 50 g • L^{-1} sucrose.

Keywords: Phytagel, sucrose, embryoids, rubber tree, plant regeneration

Introduction

Natural rubber is a crucial strategic material and industrial raw material currently derived primarily from latex of *Hevea brasiliensis*. Significant yield variations exist among different rubber tree planting materials. Somatic embryo seedlings represent a novel planting material propagated directly from somatic embryos using anthers or integuments of high-yielding varieties as explants. In 1978, Chinese scientist Wang Zeyun first successfully cultivated somatic embryo seedlings worldwide, and field data both domestically and internationally have demonstrated that these seedlings represent a juvenile planting material superior to first-generation seedling stocks and second-generation budded stocks in terms of yield, stress resistance, and uniformity. This makes them promising third-generation planting materials for large-scale application. In 2007, Hua Yuwei et al. first achieved large-scale propagation of somatic embryos through cyclic proliferation.

Various factors influence somatic embryo regeneration and plant growth, including medium composition, osmotic potential, embryo status, and physical pretreatments before germination. Gelling agents and carbohydrates directly affect medium osmotic potential and significantly impact embryo regeneration and plant growth. Higher agar concentrations enhance water retention capacity, limit water absorption by embryoids, reduce embryoid water content, and thereby improve regeneration frequency while reducing vitrification. Additionally, gelling agent type and concentration affect the availability of magnesium, calcium, zinc, manganese, and potassium ions in the medium, further influencing regeneration and growth. Sucrose, serving as both an osmotic regulator and energy source, also significantly affects embryo maturation and regeneration. Becwar et al. (1995) identified the key to somatic embryo germination as transferring embryos to low-sucrose, hormone-free germination medium. Similarly, in longan somatic embryo regeneration, high sucrose concentrations favored embryo maturation while low concentrations promoted plant regeneration.

To date, no studies have investigated the effects of Phytagel and sucrose on rubber tree somatic embryo regeneration and plant growth. Therefore, this study examined different concentrations of Phytagel and sucrose in rubber tree somatic embryo regeneration medium to determine their impacts and identify the most suitable concentrations for *Hevea brasiliensis*.

Materials and Methods

Experimental Materials

Mature secondary cotyledonary somatic embryos of *Hevea brasiliensis* clone Reyan 7-33-97 were used as experimental material, cultured according to the method described by Hua et al. (2010).

Chemicals and Containers

Chemicals for preparing modified MS macro-elements, micro-elements, and iron salts, as well as sucrose, were purchased from Guangzhou Chemical Reagent Factory. Chemicals for modified MS organic components and plant hormones were obtained from Shanghai Bioengineering Technology Co., Ltd. Phytagel was purchased from Sigma-Aldrich Company. Conical ventilated test tubes were customized according to the patent "A Conical Ventilated Test Tube" (Application No.: 201320062994.0).

Experimental Methods

Mature secondary cotyledonary embryos larger than 1 cm in diameter were inoculated into conical ventilated test tubes containing plant regeneration medium and cultured at 28 °C under a 16-hour photoperiod. Plant regeneration and growth were assessed 45 days after inoculation. The regeneration medium consisted of MS medium supplemented with 0.23 mol • L^{-1} KT, 0.11 mol • L^{-1} IAA, and 8.7 mol • L^{-1} GA_3. Phytagel concentrations ranged from 0 to 4 g • L^{-1}, while sucrose concentrations ranged from 0 to 80 g • L^{-1}. Each experiment comprised three replicates, with 21 tubes per replicate and one embryoid per tube.

Statistical Analysis

Plant regeneration frequency and growth were evaluated using regeneration indices (germination rate, rooting rate, and germination plus rooting rate) and growth indices (percentage of plants exceeding 5 cm in height, percentage of plants with roots longer than 5 cm, and percentage of plants with leaves). Germination rate represents the percentage of germinated embryoids among total inoculated embryoids; rooting rate represents the percentage of rooted embryoids; germination plus rooting rate represents the percentage of embryoids that both germinated and rooted; the percentage of plants exceeding 5 cm in height represents the proportion of plantlets taller than 5 cm among total inoculated embryoids; the percentage of plants with roots longer than 5 cm represents the proportion with root length exceeding 5 cm; the percentage of plants with leaves represents the proportion developing leaves; and the percentage of plants exceeding 5 cm in height with leaves represents the proportion meeting both criteria.

Results

Effects of Phytagel on Somatic Embryo Regeneration and Plant Growth

Phytagel concentration significantly influenced somatic embryo regeneration. Germination rates, rooting rates, and combined germination-rooting rates of rubber tree embryoids cultured on medium containing 1–4 g • L^{-1} Phytagel were significantly higher than those on medium with 0–0.5 g • L^{-1}. However, no significant differences were observed among the 1–4 g • L^{-1} treatments. Following the principle of using the lowest effective concentration, 1 g • L^{-1} Phytagel was deemed optimal for rubber tree somatic embryo regeneration, achieving germination, rooting, and combined rates of 86.4%, 97.4%, and 86.4%, respectively, all at high levels (Table 1 and Figure 1 [Figure 1: see original paper]).

Phytagel concentration also significantly affected regenerated plant growth. The percentages of plants exceeding 5 cm in height and plants with leaves were significantly higher at 1–4 g • L^{-1} compared to 0–0.5 g • L^{-1}, indicating that 1–4 g • L^{-1}

Phytagel promotes growth. However, excessive concentrations inhibited growth. At 1 $g \bullet L^{-1}$, the percentage of plants over 5 cm tall reached its maximum (53 ± 9.4%), while at 4 $g \bullet L^{-1}$, cultures exhibited malnutrition, stunted growth, premature leaf yellowing and abscission, and complete growth inhibition, with 0% of plants exceeding 5 cm (Figure 1.F). Considering all growth parameters, 1 $g \bullet L^{-1}$ Phytagel was optimal for regenerated plant growth. Overall, 1 $g \bullet L^{-1}$ Phytagel in rubber tree somatic embryo regeneration medium simultaneously promoted both high regeneration frequency and vigorous plant growth.

Effects of Sucrose on Somatic Embryo Regeneration and Plant Growth

Medium containing sucrose was superior to sucrose-free medium for both embryo regeneration and plant growth. However, statistical analysis revealed no significant differences in germination rates, rooting rates, or combined germination-rooting rates among media containing 5-80 $g \bullet L^{-1}$ sucrose (Table 2), indicating that sucrose concentration within this range did not significantly affect regeneration frequency.

In contrast, sucrose concentration significantly influenced regenerated plant growth (Table 2). The percentage of plants exceeding 5 cm in height increased significantly with sucrose concentration, peaking at 59% at 80 $g \bullet L^{-1}$. The percentage of plants with roots longer than 5 cm peaked at 10 $g \bullet L^{-1}$ and showed no significant differences across the 10–80 $g \bullet L^{-1}$ range. The percentage of plants with leaves initially increased then decreased with sucrose concentration, reaching its peak at 20–40 $g \bullet L^{-1}$. Morphologically, low sucrose concentrations resulted in stunted plants with poor root and leaf development, exhibiting malnutrition and premature leaf yellowing and abscission (Figure 2 [Figure 2: see original paper] a, b, c), while excessively high concentrations inhibited leaf development (Figure 2.f). These results demonstrate that sucrose, as a primary nutrient, promotes plantlet growth within an optimal concentration range by enhancing stem elongation, root development, and leaf formation.

To identify the optimal sucrose concentration, a refined study was conducted using 20–80 $g \bullet L^{-1}$ sucrose (Table 3). Consistent with previous results, low sucrose (20–30 $g \bullet L^{-1}$) promoted leaf emergence but inhibited stem elongation, whereas high sucrose (70–80 $g \bullet L^{-1}$) suppressed leaf development but promoted stem elongation. Detailed analysis revealed that the percentage of plants exceeding 5 cm in height peaked at 57.6% with 50 $g \bullet L^{-1}$ sucrose, and the percentage of plants over 5 cm tall with leaves also peaked at 46.3% at this concentration. Morphologically, plantlets at 50 $g \bullet L^{-1}$ exhibited robust stems and roots (Figure 3 [Figure 3: see original paper]), establishing 50 $g \bullet L^{-1}$ as the optimal sucrose concentration.

Discussion

Phytagel Significantly Affects Somatic Embryo Regeneration and Plant Growth

Plant tissue culture requires semi-solid medium to support explants, and gelled media provide this essential physical support. Various gelling agents are available, with Phytagel being a superior option due to its good aeration, low gelling temperature, minimal impurities, and reduced vitrification, though its cost is relatively high. Previous rubber tree somatic embryo regeneration studies have reported using Gelrite, Phytagel, and agar at varying concentrations, with substantial effects on regeneration frequency (Table 3). Hua et al. (2010) achieved the highest reported regeneration frequency of 85.4% using 2.2 $g \bullet L^{-1}$ Phytagel. Our detailed investigation found that 1 $g \bullet L^{-1}$ Phytagel yielded a maximum regeneration frequency of 86%, slightly higher than Hua et al. (2010) and representing the highest reported rate to date. At this concentration, the percentages of plants exceeding 5 cm in height and plants with leaves were also significantly higher than at 2.0 $g \bullet L^{-1}$ and other treatments, demonstrating that concentrations above the optimum inhibit regeneration and growth. Furthermore, reducing Phytagel concentration helps lower production costs for seedling propagation.

Role of Sucrose in Somatic Embryo Regeneration and Plant Growth

Carbohydrates play crucial roles in plant tissue culture as energy sources, carbon sources, and osmotic agents. Various sugars have been compared, with sucrose being optimal, followed by glucose, maltose, and raffinose, while fructose is less effective and mannose and lactose are the least suitable. Previous studies have reported sucrose effects on rubber tree callus and embryoid induction, but not on embryoid regeneration and plant growth. Our study found that sucrose concentration did not affect regeneration efficiency, as no significant differences were observed in germination rates, rooting rates, combined rates, or percentages of plants with roots exceeding 5 cm across 5–80 $g \bullet L^{-1}$ sucrose. Therefore, previously reported variations in regeneration rates were not attributable to sucrose concentration (Table 3). However, significant differences in the percentages of plants exceeding 5 cm in height and plants with leaves indicated that sucrose substantially affects stem elongation and leaf development. Low sucrose concentrations (below 10 $g \bullet L^{-1}$) were inadequate for both height increase and leaf emergence, suggesting insufficient carbohydrate supply. As sucrose concentration increased, plant height increased significantly, but leaf number gradually decreased. At 80 $g \bullet L^{-1}$ sucrose, plant height reached its maximum while the percentage of plants with leaves reached its minimum, suggesting that abundant sucrose in the medium may substitute for photosynthetic products, reducing the need for leaf development to supply energy. Thus, sucrose concentration is a critical factor balancing stem height and leaf development. Our refined experiments identified 50 $g \bullet L^{-1}$ as the optimal sucrose concentration for rubber tree somatic embryo regeneration medium, maximizing both the percentage of plants exceeding 5 cm in height (57.6%) and the percentage of plants over 5 cm tall with leaves (46.3%), while producing robust stems and roots.

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Note: Figure translations are in progress. See original paper for figures.