Light Quality Effects on Germination and Conversion of Southern Pine Somatic Embryos

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While induction of somatic embryogenesis for the major southern pines and production of somatic seedlings from these cultures has been reported, major bottlenecks remain that prevent the adaptation of somatic embryogenesis for mass cloning of these trees. These bottlenecks include culture initiation, embryogenic culture capture, continued culture proliferation, somatic embryo development, maturation, germination and conversion to somatic seedlings. Each of these bottlenecks must be overcome for somatic seedlings to be delivered for planting in large numbers, at an acceptable cost and, perhaps most importantly, on a predetermined timetable. One of the most serious bottlenecks standing in the way of industry-wide adoption of this technology is the production of vigorous somatic seedlings from somatic embryos produced by the cultures (i.e. the maturation, germination and conversion steps).

The objective of the experiments described here was to determine the effects of light quality on maturation, germination and conversion of loblolly pine *(Pinus taeda)*, slash pine *(P. elliottii)* and longleaf pine *(Pinus palustris)* somatic embryos and on early growth of the resulting somatic seedlings. Evidence of a synergistic effect between light quality and high CO_2 on in vitro growth was reported in a patent by Tisserat et al. (2000), although this work did not involve pine somatic seedlings.

In a first, preliminary experiment, six slash pine lines were generated following a protocol based on that of Smith (1996). Briefly, cultures, which were maintained by serial transfer on semisolid EDM6 medium (Smith 1996), were proliferated in liquid EDM6 medium for three weeks, then cultured on EMM2 medium (Smith 1996) in the dark until they reached the early cotyledonary stage. Then, embryos were cultured for 6 weeks on Pre-germination Medium (Smith 1996), followed by 6 weeks on Germination Medium (Smith 1996), under different light treatments provided by either standard cool white fluorescent bulbs at full-strength or with 1 or 2 layers of shade cloth, or by LEDs, using a Percival E30-LED Plant Growth Chamber. LEDs supplied pure red (670 nm) wavelengths. In this experiment, somatic embryos cultured under cool white fluorescent light with no shade cloth began germination (i.e. radicle elongation) earlier than other treatments, but those cultured under red light ultimately had the highest germination frequency after 100 days (57% versus 48% for cool white). In addition, embryos germinated under red light developed green hypocotyls and cotyledons, while those germinated under cool white fluorescent light had red hypocotyls and cotyledons.

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For the second experiment, somatic embryos from 5 loblolly pine lines and 1 slash pine line, produced using the same protocol described above, were cultured under cool white fluorescent light (90 $\mu mol \cdot m^{-2} \cdot sec^{-1}$) and red light at two intensities (24 $\mu mol \cdot m^{-2} \cdot sec^{-1}$ and 59 $\mu mol \cdot m^{-2} \cdot sec^{-1}$). Red light at both intensities gave higher overall germination frequencies (63-65%) compared to the cool white fluorescent light (25%). Furthermore, both red light treatments allowed germination of somatic embryos from two clones that failed to germinate under cool white fluorescent light. Germinants under both red light treatments had longer tap roots than those germinated under cool white fluorescent light and those germinated under the higher intensity red light had significantly more first order lateral roots.

In the final experiment, mature loblolly, slash pine and longleaf pine somatic embryos, produced as described above, underwent 3 weeks of treatment on Pre-germination Medium under either red or blue (470 nm) light. Embryos were then transferred to germination medium, with half of the embryos coming from the red light environment maintained under red light, while the rest were transferred to blue light. Embryos initially incubated on Pre-germination Medium under blue light were divided between blue and red light treatments when transferred to Germination Medium. A continuous cool white fluorescent light treatment was also included as a control. After 6 weeks, the embryos cultured under red light throughout had the highest germination and conversion percentages (76% and 48%, respectively), and those cultured under blue light throughout the lowest (25% and 15%, respectively). Reciprocal transfers (red to blue, blue to red) gave intermediate results, although embryos cultured on Pre-germination Medium under blue light, followed by culture on Germination medium under red light had similar germination and conversion rates to those cultured continuously under red light. Cool white light was inferior to red light for germination of somatic embryos, but allowed a higher frequency of epicotyl elongation. However, very large standard errors for the light quality treatment effects, probably due to the inclusion of 3 species in the experiment, made conclusions regarding statistical significance of the treatments difficult to interpret.

Preliminary experiments combining elevated light intensity (up to 330 $\mu mol \cdot m^{-2} \cdot sec^{-1}$) with elevated CO_2 (up to 1300 ppm) indicated that raising these two variables may produce more vigorous somatic seedlings once they are transferred to potting mix, compared to the standard treatments (100 $\mu mol \cdot m^{-2} \cdot sec^{-1}$ and ambient CO_2, 300 ppm). Our future plans call for combining light quality and elevated CO_2 during in vitro culture to test for their effects on somatic embryo germination and conversion.

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