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Plant Science 170 (2006) 634–647
PLANT SCIENCE
www.elsevier.com/locate/plantsci

Identification and quantitative analysis of stage-specific organic acids in loblolly pine (*Pinus taeda* L.) zygotic embryo and female gametophyte

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Received 11 July 2005; received in revised form 28 October 2005; accepted 31 October 2005
Available online 22 November 2005

Abstract

Stage-specific analyses of 32 organic acids were completed across the range of seed development for zygotic embryo and female gametophyte (FG) tissues of loblolly pine (*Pinus taeda* L.). Tissue was analyzed in triplicate from two open-pollinated families, grown in different locations and years, by gas chromatography/mass spectrometry (GC/MS). Significant changes in organic acids occurred over time, with both seed collections showing similar trends in change of organic acid content. Although concentrations were not always similar, embryo and female gametophyte tissues generally showed similar patterns of change in organic acid content over time. The major organic acids contributing to osmotic potential were malic early in seed development and oxalic late in seed development. This data provides suggestions for stage-specific media composition changes for each step in the somatic embryogenesis protocol.

Keywords: Loblolly pine; *Pinus taeda*; Embryo development; Organic acids; Megagametophyte; Female gametophyte

1. Introduction

Loblolly pine (LP, *Pinus taeda* L.) is the major commercial species in the southern U.S., ranging from New Jersey to Texas, with 1–1.5 billion seedlings planted annually [1]. LP is dominant on 11.7 million ha and comprises approximately 23% of the total U.S. harvest of all woody species [2].

Forest productivity can be increased by planting tree farms with large numbers of elite, high-value trees. Methods to propagate large numbers of genetically superior conifer trees are needed. Clonal propagation by somatic embryogenesis (SE) can capture the benefits of breeding or genetic engineering programs to improve wood quantity, quality, and uniformity. Factors currently limiting commercialization of SE for LP include low initiation, low culture survival, culture decline causing low or no embryo production, and the inability of somatic embryos to fully mature, resulting in low germination and slow growth of somatic seedlings.

In pine seed, the zygotic embryo grows and develops within the female gametophyte (FG). Somatic embryos, however, are cultivated in the absence of this tissue, and the culture medium must provide nutrients and developmental signals. The more closely the culture medium resembles the environment of the FG, the more likely vigorous somatic embryos will develop. Nutritional, osmotic, hormonal, and gaseous environments surrounding an embryo control growth. Optimization of these environments is critical for the growth and development of high-quality, vigorous somatic embryos. Most often media optimization is accomplished through a process of empirical trial and error. An alternative approach is to use analyses of zygotic tissues to provide targets for the growth medium. In following this approach to improve LP somatic embryo growth, the Forest Biology Group at the Institute of Paper Science of Technology has performed stage-specific analyses of LP tissues for abscisic acid [3] and metals [4].

Organic acids are important constituents of plants and can accumulate in large amounts as dissolved free anions. While

DOI of original article: 10.1016/j.plantsci.2005.10.018.
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0168-9452/$ – see front matter
doi:10.1016/j.plantsci.2005.10.019

several organic acids are present in all plants as part of the citric acid cycle (also known as the tricarboxylic acid cycle or Krebs cycle), organic acids also play important roles in many other plant pathways and processes including: glycolysis, the glyoxylate cycle, the shikimic acid pathway, the C_2 oxidative photosynthetic carbon cycle, the C_4 carbon cycle, crassulacean acid metabolism, and stomatal opening and closing. Fruit ripening and flavor development also involves changes in organic acids [5]. In some plant tissues, specific organic acids are known to play a role in disease and pest repulsion, root exudation for formation of soluble metal complexes to enhance nutrient acquisition, and metal detoxification [6–8]. However, the role of organic acids in seed and embryo development is poorly understood. We report here the results of an investigation on quantitative analysis of organic acids present in stage-specific female gametophyte and embryo tissues of LP. Preliminary reports on part of this work were presented in Pullman et al. [9].

2. Materials and methods

2.1. Plant material

LP cones were collected weekly throughout the sequence of embryo development from two open-pollinated mother trees. Seeds from Union Camp tree UC5-1036, located in a seed orchard near Bellville, GA, were collected in 1998. Seeds from tree 7-56 were collected from a Weyerhaeuser seed orchard near Lyons, GA in 2002. Cones were shipped in a cooler with blue ice to IPST and received within 24–48 h of collection. Cones were opened and seeds isolated. Seeds were cracked using a hemostat, pried open with fine-tipped forceps, and the integument and nucellus tissue removed exposing the female gametophyte.

The FG was slit, pried open, and the dominant embryo or mass of embryos removed. Individual embryos were quickly observed through a dissecting microscope, evaluated for stage of development [10,4] (Fig. 1), sorted by stage and tissue type, and placed in vials partially immersed in liquid nitrogen. Stage 9 embryos were categorized by the week they were collected: 9.1 (stage 9, week 1), 9.2 (stage 9, week 2), etc. Twenty similar-staged embryos or FG were collected per vial, frozen, and stored at −70 °C until analyzed. Collections for each tree and tissue type spanned from stage 1, collected 2–3 weeks after estimated fertilization, to stage 9.11, shortly before cone harvest.

2.2. Organic acid analysis as trimethylsilyl derivatives by gas chromatography/mass spectrometry

With the often small amount of tissue available (μg to mg quantities), especially in early stages of seed development, a

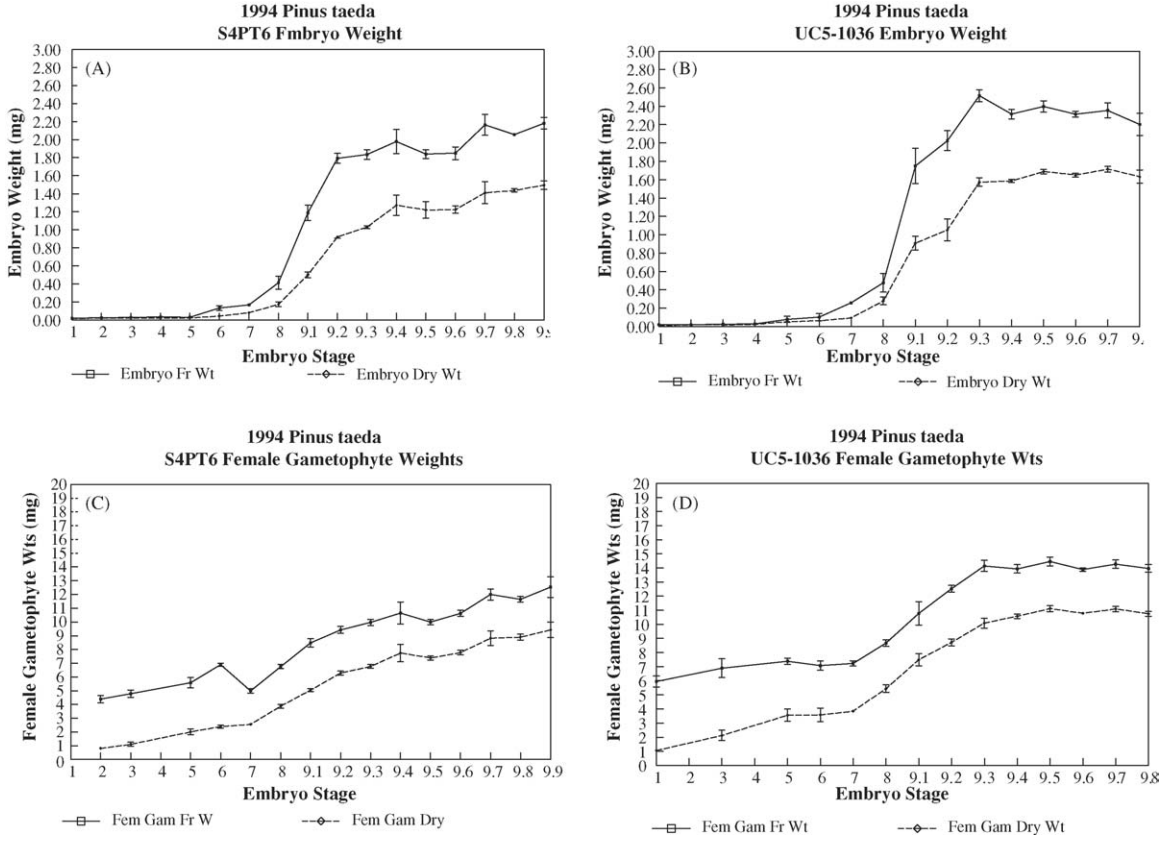

Fig. 1. *Pinus taeda* embryo and female gametophyte tissue fresh and dry weights during seed development in 1994. (A) Embryo fresh and dry weights for S4PT6 seed. (B) Embryo fresh and dry weights for UC 5-1036 seed. (C) Female gametophyte fresh and dry weights for S4PT6 seed. (D) Female gametophyte fresh and dry weights for UC5-1036 seed.