

Some Anatomical Characteristics of Hyperhydic Shoots Occurring in *In Vitro* Culture of Peace Poplar

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Objectives

Populus spp. have been a model system in woody plant biotechnology. Peace poplar is a naturally occurring hybrids between *Populus koreana* and *P. trichocarpa* and is known to be important species for physiological studies. In the present study, we compared some anatomical characteristics for the hyperhydic and normal shoots occurring in shoot proliferation cultures.

Materials and methods

Normal leaves and hyperhydic leaves were removed from the explants *in vitro* and used for microscopic analysis. The explants were fixed at 4°C in 1.5% glutaraldehyde and 1.6% dehydrated through the ethanol series and embedded in Technovit 7100 (Kulzer, Germany). Thin sections (3 µm) obtained using Leica RM 2165 were stained with 0.05% toluidine blue O and observed under Leica DC 300F (Germany). For SEM observation, leaves were collected from normal and hyperhydic plantlets and freeze-dried. Samples were positioned on stub prior to gold sputtering (Ion coater, Eiko IB-3, Japan). Micrographs were taken under a ABT 55 (Japan) scanning electron microscope.

Results and discussion

Hyperhydic shoots (HS) showed better proliferation rate than normal shoot and exhibited water-soaked glassy appearance. Generally, the HS had thick leaves showing darker green in color when compared to the normal shoot. In anatomical characteristics, whereas the width of HS leaves was thicker, the cuticle layer of the leaves was thinner than normal leaves. Other abnormalities in hyperhydic leaves included low cell density, poorly developed spongy tissue, and large stomatal capacity. In addition, the stomata also looked abnormal showing wrinkle around them. On the other hand, normal shoots had thinner leaves, thicker cuticle layer, and well developed spongy parenchyma and palisade cells.