

ANATOMICAL CHANGES DURING ACCLIMATIZATION IN SOMATIC EMBRYOGENESIS-DERIVED PLANTS OF *HEVEA BRASILIENSIS*

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In *Hevea brasiliensis*, though high frequency plant regeneration protocols *via* somatic embryogenesis have been developed, establishment of these plants in the field has been difficult. A comparison of the anatomical characters of healthy and weak plants derived through somatic embryogenesis, with bud-grafted field-grown plants was carried out in detail. Scanning electron microscopy studies on leaves of healthy plants confirmed the presence of epicuticular wax and its continued increase during hardening. In acclimatized plants, the pattern was identical to that of field-grown control plants. Leaves of *in vitro* weak plants showed less epicuticular wax. Before hardening, stomatal frequency of the *in vitro* healthy plants was higher than that of the weak ones, but the size of the stomatal aperture did not vary significantly. After acclimatization, stomatal frequency of healthy plants was comparable to, or slightly higher than, that of the control plants. Vascular continuity and distribution of latex vessels in the *in vitro* plants are also discussed.

Keywords: Acclimatization, Anatomical changes, *Hevea brasiliensis*, *In vitro* culture, Somatic embryogenesis.

Acclimatization is a process by which tissue culture plants adapt to the uncontrolled external environment, during which normal photosynthetic activity and water relations have to be developed (Desjardins *et al.*, 1995). This process can generate stress (Van *et al.*, 1998). Although the cultural conditions of tissue culture promote rapid growth and development of tissues, the formation of abnormal characteristics like altered leaf morphology, altered mesophyll structure, poor photosynthesis, non-functional stomata and marked decrease in cuticular wax are very common during *in vitro* culture (Ziv, 1986).

Upon transplantation, tissue culture plantlets with abnormal leaf development show low survival, mainly due to water loss and desiccation (Ziv *et al.*, 1987). Several reports have indicated that epicuticular wax and stomata in the leaves of tissue culture plants are inadequate or inoperative and are considered to be a major factor responsible for excessive water loss resulting in low survival rate of such plants during hardening (Brainerd and Fuchigami, 1982; Sutter and Langhans, 1982; Sutter, 1988).

Besides micropropagation, the potential use of somatic embryogenesis in molecular genetic manipulation through transgenic