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BROWNING MECHANISMS AND FACTORS OF INFLUENCE IN *IN VITRO HEVEA* CALLI CULTURES

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The biochemical changes associated with tissue browning in *in vitro* plant culture and the influence of medium conditions and explant origin on callus browning were studied. Some anti-browning agents were also tested. The activities of peroxidase (POD), soluble and bound polyphenol oxidase (PPO_s and PPO_b), NADH-quinone reductase (NQR), superoxide dismutase (SOD), catalase (CAT) and glutathion reductase (GR) were studied. As the culture aged, browning was accompanied by a marked increase in POD, PPO_s, PPO_b and SOD activity. NQR was higher in the seeds and showed little variation after 10 days of culture, whereas high CAT activity in the explants decreased considerably to the point that it was difficult to measure after 40-50 days' culture. The concentration of hormones in the callogenic induction and the embryo initiation media were found to be a crucial factor in browning and also controlled the embryogenic potential. At lower hormone concentrations, browning was always accompanied by a marked increase in POD, PPO_s, PPO_b and SOD activity and although pale-coloured calli obtained at these concentrations were 100% embryogenic, there were more brown calli than pale calli. Calli obtained in media with low hormonal content and the corresponding pale highly embryogenic calli were characterised by very high SOD activity. Calli from different seed parts (funicle, median or distal) reacted differently at different hormone concentrations in the medium. A study of 7 standard clones with different browning potential revealed that the brownest clones generally showed high PPO_s, PPO_b, POD and GR activity. CAT activity was found to be higher in the least brown calli than in pale calli or slightly necrotic, non-embryogenic calli. A certain number of culture medium additives effectively reduced browning. Phenol traps, such as polyvinylpyrolidone (Polyclar AT,PVP), an absorbant resin (Amberlite XAD) and antioxidant agents, such as ascorbid acid, were able to reduce tissue browning and favour growth of embryogenic calli to various degrees.

Key words: *Hevea*, Antioxidants, Callus browning, Embryogenesis, Enzymes, Toxic oxygen

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INTRODUCTION

Many studies aimed at obtaining somatic embryos of *Hevea* have been carried out using calli from the inner tegoments of immature fruits (Carron and Enjalric, 1985; Carron *et al.*, 1992). However, callus frequently undergoes browning in *in vitro* culture which leads to tissue degeneration and the stopping of embryogenic development.

The analysis of some biochemical factors considered to be linked with bro-

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