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SHORT COMMUNICATION

ISOZYME EXTRACTION FROM MATURE OIL PALM (*Elaeis guineensis, Jacq.*) LEAVES FOR ELECTROPHORETIC STUDIES

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ABSTRACT

The oxidation of phenolics, due to the action of the phenoloxidase complex, to form quinones and tannins is commonly observed during extraction of plant isozymes and organelles. These products are powerful enzymatic inhibitors. To overcome such a process when working with mature Oil Palm leaves, a method that combines efficient techniques for grinding, homogenization and storage is presented. Good results were obtained for isozyme activity through starch gel electrophoresis for Malate dehydrogenase, Isocitrate dehydrogenase, Phosphogluco mutase, Phosphogluco isomerase and Acid phosphatase.

The medium for isozyme extraction from adult plant tissues requires careful formulation that depends on the species being studied (Anderson, 1968).

Phenolics, that are spatially separated from phenoloxidases (E.C. 1.10.3.1.) in intact tissues, are rapidly oxidized to quinones and condensed tannins during extraction. These products powerfully inhibit plant enzymes, an important consequence when one works with mature leaf tissues (Anderson, 1968; Loomis, 1969, 1974). Such a problem is not observed to a great extent during isozyme extraction from oil palm pollen but can be limiting when other tissues must be employed, as in the case of pollen production difficulties or for additional studies such as gene regulation during development.

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The methodology presented here is an adaptation for the oil palm of a combination of methods developed by Kelley and Adams (1977), Pitel and Cheliak (1985a e b) and Ghesquiére *et al.* (1987).

For our purposes, the leaf sample is composed of six leaflets located at the central part of the number one leaf of adult palms. After removing the central and marginal nervures the material is cut into three pieces, cleaned and immediately lyophilized for 48 hours. After drying, the leaflets are ground in an electric mill, finely sieved and stored at -20°C.

Tissue (300 mg of lyophilized leaf) is ground to a fine powder, with the aid of washed sand in a previously chilled mortar and pestle, in liquid nitrogen as described by Mitton *et al.* (1979). The green powder thus produced is transferred to a 15 ml tube to which is added 0,6 ml of soluble PVP 10% (polyvinyl pyrrolidone. MW 360.000), 3 ml of a buffer solution, 0.1 M kH_2PO_4, pH 7.2, containing 0.1 M cysteine an 0,01 M di-tio-erytreitol (DTE). After homogenization in a mixer, the material is centrifuged at 35,000 g for 30 minutes at 4°C. The supernatant is lyophilized for 24 hours for future use.

When necessary, 10 mg aliquots are ressuspended in 100 μl of the above buffer solution at 4°C and immediately applied to wicks and subjected to horizontal starch gel electrophoresis.

Results with this methodology are comparable with those obtained by Ghesquiére (1983) working with pollen for six isozyme systems: Malate dehydrogenase, Isocitrate dehydrogenase, Phosphogluco dehydrogenase, Phosphogluco isomerase, Phosphogluco mutase and Acid phosphatase.

Trials with other species such as the American Oil Palm (*Elaeis oleifera* H.B.K. Cortés) and Macaúba (*Acrocomia spp*) were very promising, denoting the potential of this method.

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RESUMO

A oxidação de fenóis, pela ação das fenol-oxidases, produzindo taninos e quinonas é comumente observada durante a homogenização de tecidos vegetais, especialmente no manuseio de folhas de plantas adultas. Tais produtos são potentes inibidores enzimáticos. Visando a recuperação da atividade enzimática presente em folhas adultas de dendezeiros submetidas a homogenização é apresentada metodologia que combina formas eficazes de maceração, homogeneização, uso

de agentes redutores, polímeros e estocagem. Bons resultados foram obtidos no estudo de seis sistemas enzimáticos, a saber: Malato desidrogenase, Isocitrato desidrogenase, Fosfoglicomutase, Fosfoglico desidrogenase, Fosfoglico isomerase e Fosfatase Ácida.

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