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Identificação de Clones de Castanheiro através de Marcadores RAPD

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Resumo

O Castanheiro Europeu (*Castanea sativa*) é uma espécie com grande importância ecológica e económica. Em Portugal, tem-se verificado um grave declínio na área desta espécie. Os principais causadores da mortalidade do Castanheiro são os fungos *Phytophtora cinnamomi* e *Cryphonectria parasitica* causadores das doenças da tinta e do cancro do Castanheiro, respectivamente. O Instituto Nacional de Investigação Agrária possui uma colecção de clones de Castanheiro que não está caracterizada geneticamente. O objectivo deste trabalho foi estabelecer um método de detecção de polimorfismos no DNA que permita uma rápida identificação e discriminação de um conjunto de 17 clones de Castanheiro. Iniciou-se pela optimização da técnica de extracção de DNA desta planta lenhosa, tendo-se obtido um DNA genómico de elevado peso molecular, com um bom grau de pureza e com um alto rendimento. A técnica de obtenção de polimorfismos utilizada foi a técnica RAPD (Random Amplified Polymorphic DNA). Na optimização desta técnica, as condições de reacção de amplificação do DNA foram estandardizadas com a utilização das "Ready- To-Go PCR beads" (Pharmacia) e foram optimizadas variáveis experimentais, tais como as concentrações de DNA genómico e de magnésio na reacção de amplificação. Desta forma, obtiveram-se padrões RAPD reprodutíveis e com boa resolução em geles de agarose.

Para a obtenção de marcadores RAPD foram testados 60 primers arbitrários dos kits comerciais OPA, OPE e OPH (Operon Technologies). Do conjunto destes 3 kits de primers arbitrários, foram seleccionados 24 primers que produzem 90 marcadores

RAPD com óptima resolução em geles de agarose, são altamente reprodutíveis e que permitem uma fácil e rápida identificação dos 17 clones de Castanheiro. O número de bandas polimórficas geradas por primer varia entre 1 e 6 e o seu peso molecular varia entre 450bp e 3500bp. Dos 24 primers escolhidos, 16 eram do kit OPA, 7 eram do kit OPE, e somente 1 era do kit OPH.

Com os 90 marcadores RAPD, também foi realizada uma primeira abordagem ao estudo das relações genéticas entre os 17 clones de Castanheiro e destes com 3 espécies de Castanheiro (*Castanea sativa, C. crenata e C. mollissima*) recorrendo ao coeficiente de similaridade de Nei & Li (1979) e ao método de análise de 'cluster' UPMGA. Esta análise sugere que os clones de Castanheiro em estudo são híbridos entre *C. sativa* e *C. crenata*.

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