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Molecular detection and diagnosis of fungal contaminants of recalcitrant seeds: *Quercus robur* L. acorns as a model system

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Summary

Recovery *in vitro* after cryostorage of excised embryonic axes from recalcitrant seeds is frequently compromised by the proliferation of seed-borne mycoflora. Current culture-based methods to ascertain the contamination status of seeds/axes prior to cryostorage are time-consuming and sometimes inaccurate. This study assessed the potential of the PCR as a faster, more sensitive, reliable alternative to detect fungal contaminants. Published universal primer DNA sequences were used to amplify the Internal Transcribed Spacer region (ITS) of the 28S rDNA gene from genomic DNA of the principal fungal contaminants, *viz. Fusarium* sp., *Penicillium* sp. and *Aspergillus* sp., of acorns of South African provenance. The sequences of the amplicons obtained by universal amplification were then used as a basis for the synthesis of genus-specific DNA primers. Subsequent assessment revealed that the primers were specific to the genus for which they had been designed and that fidelity was retained in a complex template consisting of a mixture of seed and fungal genomic DNA. The developed method was shown to be sensitive, the lowest detection limit being 0.1 ng of fungal DNA. The specificity and sensitivity of the PCR was confirmed by the successful detection and discrimination amongst fungi in the embryonic axes of experimentally inoculated seeds.

Introduction

For successful *ex situ* conservation, the duration of seed viability in storage must exceed the natural interval between seed set and germination (Bonner, 1990). For orthodox seeds, preservation is generally easily achieved, as maturation drying on the parent plant allows seeds to retain biological function after extensive natural desiccation (Chin and Roberts, 1980). In contrast to their orthodox counterparts, recalcitrant seeds are characterized by high water content and the maintenance of an active metabolism after shedding (Berjak *et al.*, 1989; Roberts, 1983). Recalcitrant seeds do not have the appropriate mechanisms, or full complement thereof, that confer desiccation tolerance and so are damaged on dehydration, often irreversibly and lethally (Pammenter and Berjak, 1999). Storage of recalcitrant seeds under conditions that maintain water content at, or marginally below, that characterizing the newly shed state is useful only in the short to medium-term, as seeds become increasingly desiccation sensitive and prone to microbial proliferation (Farrant *et al.*, 1986; Calistru *et al.*, 2000; Anguelova-Merhar *et al.*, 2003).

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