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Seed as an Alternative Source of DNA for Molecular Research of Inaccessible Wild *Musa* Species

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Abstract

Most wild species and wild relatives of banana (*Musa* spp.) are found in forests and are thus relatively inaccessible. Their inability to establish in places different from their natural habitats further complicates the use of such wild species for routine molecular research. The present study was therefore undertaken to evaluate seeds as an alternative source of DNA for conservation in comparison with the cigar leaf. Usefulness of seed as a major genetic tool for conservation of seeded wild banana germplasm, standardisation of the stage of seed maturity for DNA extraction and standardisation of the seed DNA extraction protocol were studied. The accessibility of seed DNA as an alternative strategy for leaf DNA was confirmed using molecular markers (RAPD and IRAP). Among different methods of DNA extraction, a modified protocol with cetyltrimethyl ammonium bromide (CTAB) was found to be the best. Partially mature seeds with 70-80% maturity were found to be the best stage for DNA extraction compared to 40-50% and 100% maturity. Removal of the seed coat improved the quality of DNA extracted. Uniformity of seed and leaf DNA was confirmed by using 80 random primers and 10 IRAP primers. The paper tries to analyse the extent of deviation between seed and leaf DNA and possible reasons in view of the breeding behaviour of wild species.

INTRODUCTION

Bananas and plantains (*Musa* spp.) are among the most important crops in the world, also in regions away from the primary centres of origin. Wild *Musa* species are potential sources of resistance to biotic and abiotic stresses and other useful traits. However, the natural habitats of wild *Musa* species and their relatives are being destroyed due to shifting cultivation. In order to conserve the endangered wild species, periodic explorations are being undertaken by NRCB, Trichy in the interior forests of North Eastern States, Andaman and Nicobar islands, Western and Eastern ghats. These areas are difficult to access due to political reasons or insurgency problems (Uma, 2006). Often, germplasm collected under these difficult circumstances later fails to establish under extreme weather conditions, and leaf samples are not available for routine molecular work.

A protocol for the isolation of good quality and quantity DNA from rice seeds has been successfully demonstrated by Kang et al. as early as 1998. Subsequently Crockett et al. (2000) isolated genomic DNA from cabbage seeds and used them in RAPD analysis like leaf DNA. Thangjam et al. (2003) were able to isolate DNA from embryos of *Parkia* spp. using proteinase K in SDS extraction buffer. But still the isolation of high-quality DNA from seeds of banana containing high levels of polysaccharides and polyphenols remains difficult and needs standardisation. In the present study, we have tried to

standardise the protocol for the extraction of DNA from wild banana seeds which are freely available and amenable for storage under proper conditions.

MATERIALS AND METHODS

Seeds at three different stages of maturity, namely 40-50%, 70-80% and 100%, were collected (embryo maturity was calculated based on the days taken for bunch maturity from the day of pollination to full maturity). Three different methods of endosperm extraction were tried for use in DNA isolation. For immature seeds, either the total seed or the liquid endosperm alone was crushed with liquid nitrogen. For mature seeds, the seed coat was removed with slight breaking and the powdered endosperm after sieving was crushed with liquid nitrogen. The endosperm extracted through the above said methods was subjected to four different methods of DNA isolation. DNA purification was done adopting the method of Warude et al. (2003) with minor modifications, wherein proteinase K was used to remove the seed proteins besides RNase. The seed DNA isolated was tested with 80 random primers belonging to OPA, OPB and OPC series and 10 IRAP markers (Teo et al., 2005) using leaf DNA as a check. The gels were scored as either present (1) or absent (0), and the binary data were fed in NTSYS 2.01e to produce the dendrogram.

RESULTS AND DISCUSSION

Extraction of endosperm was very tedious and the DNA quantity was insufficient for routine experiments in case of 40-50% matured seeds. In fully mature seeds, hard seed coat, protein, RNA along with high phenolic content hindered the isolation of pure DNA. Hence, the use of 70-80% matured seeds was found to be the best method as it resulted in pure DNA free from contaminants when extracted using the cetyltrimethyl ammonium bromide (CTAB) method of Gawel and Jarret (1992) with minor modifications. Seed DNA isolated was tested with 80 random primers and 10 IRAP markers in two replicates. A dissimilarity coefficient of 25% was observed between seed and leaf DNA in both marker systems. This is attributed to the inadvertent cross pollination with other varieties while being maintained in the field genebank (Fig. 1 and 2). But in nature, the wild species occur as a population in their natural habitats with self pollination occurring most of the time. This self pollination has led to genetic purity. Under such circumstances, the leaf and seed DNA are expected to be almost 100% similar. Hence, the protocol that was adopted in the present study could be effectively used as an alternative source of DNA for molecular research of inaccessible wild *Musa* species.

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Figures

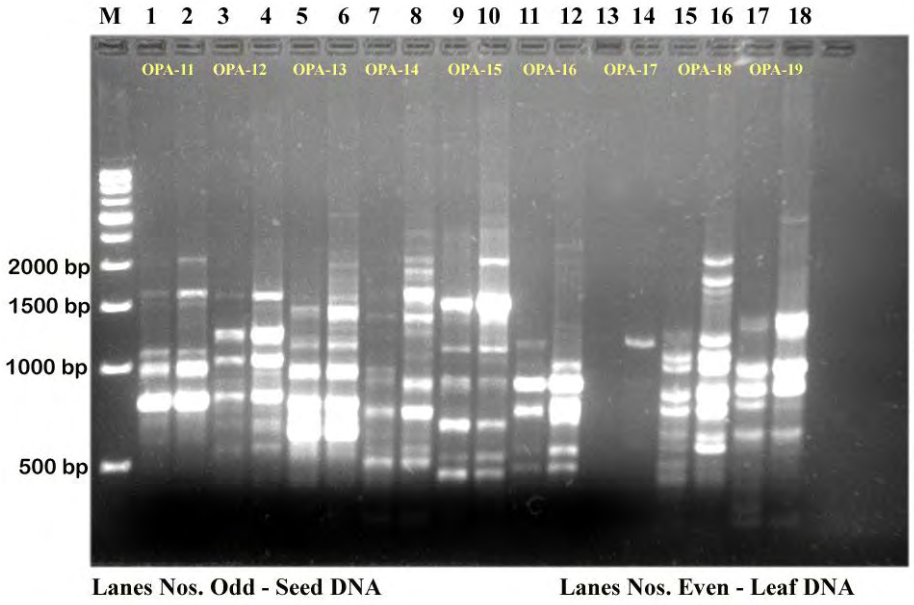

Fig. 1. Banding profile generated using RAPD primers of OPA series.

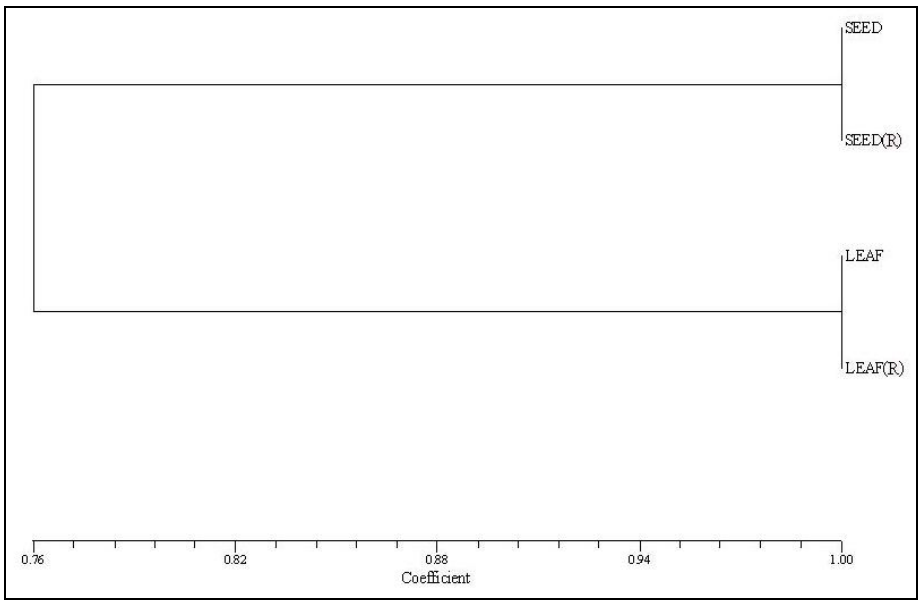

Fig. 2. NTSYS derived dendrogram showing the similarity coefficient between leaf and seed DNA.