RAPD Reactions from Crude Plant DNA

Adding RNase A as a "Helper Enzyme"

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Abstract

As opposed to standard polymerase chain reaction (PCR) using specific primers, genome analysis involving short random primers, for example RAPD, may yield inconsistent results if crude plant DNA preparations are used as the template. When RNase A, a thermostable enzyme, was added to such reactions, highly repeatable banding patterns were obtained from crude plant DNA, thus speeding up analyses substantially.

Index Entries: RAPD; RNase A; plant DNA; PCR.

1. Introduction

Polymerase chain reaction (PCR) techniques, in general, do not require highly purified DNA templates. This feature is especially useful for work on plant DNA *(1)*, which often needs to be purified extensively for other purposes, e.g., restriction analysis. PCR-based genome analysis applying random primers, known as random amplified polymorphic DNA (RAPD) *(2)*, arbitrarily primed PCR (AP-PCR) *(3)*, single-primer amplification reaction (SPAR) *(4)*, or DNA amplification fingerprinting (DAF) *(5)*, on the other hand, has been reported to be sensitive to relatively small variations in DNA quality and quantity *(6,7)*, especially in plants *(8)*. Adherence to strictly controlled reaction conditions has been recommended before regarding banding patterns as reliable. RNA contamination of DNA preparations has been reported to be one cause of inconsistencies *(9,10)*.

It is shown here that by adding RNase A to RAPD reaction mixes, the quality of banding patterns is improved substantially, allowing for the use of crude plant DNA preparations in RAPD analyses.

2. Material and Methods

Crude DNA was prepared from somatic embryos and needles of Norway spruce (*Picea abies* L. Karst) *(11)*, from needles of other coniferous species

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(various larch, fir, and pine species), and from leaves of *Quercus* species. Plant tissue (a few mg fresh weight) was ground in microcentrifuge tubes using tightly fitting pestles (Kontes, Vineland, NJ) in the presence of extraction buffer (450 µL of 50 m*M* Tris-HCl, pH 8.0, 1 m*M* EDTA, 0.25*M* NaCl, 1% w/v SDS, 1% w/v polyethylene glycol (PEG) mol wt 8000, 0.2% v/v β-mercaptoethanol). After a short incubation at 55–60°C, DNA was precipitated from the supernatant in 0.7*M* NaCl, 10% w/v PEG (end concentrations), washed in 70% ethanol, and dissolved in 300 µL (embryos) or 1000 µL of either 10 m*M* Tris-HCl, pH 8.0, 10 m*M* Tris-HCl, pH 8.0, containing 10 n*M* EDTA, or distilled water.

RAPD analysis was carried out in a reaction mix (15 µL per sample) containing (final concentrations): enzyme (*Taq* from Promega, Madison, WI, 0.067 U/µL, or DynaZyme from Finnzymes Oy, Espoo, Finland, 0.05 U/µL), 1X supplier's buffer, tetramethylammonium chloride (20 µ*M*), dNTPs (200 µ*M* each), primer (single 10-mers from Operon, Alameda, CA; 400 n*M*), RNase A as indicated (from Boehringer Mannheim, Vienna, Austria; 0.3 µg/3 µL DNA solution in 15 µL reaction buffer), 3 µL of DNA preparations and distilled water. The standard program in a MJ Research (Watertown, MA) thermocycler was: 3 cycles of 94°C, 1 min; 35°C, 3 min; slow heating (1°C/4 s) to 65°C, 72°C, 2 min; 51 cycles of 94°C, 40 s, 38°C, 1 min; 72°C, 2 min; further 10 min at 72°C, and 4°C until recovery and electrophoresis.

Banding patterns were analyzed in agarose gels (1.25% w/v of a 4:1 mix of NuSieve GTG [FMC, Rockland, ME] and UltraPure [Life Technology, Gaithersburg, MD] respectively) including ethidium bromide (0.75 µg/mL gel) in 0.5X TBE, and photographed over UV (Polaroid 655 films).

3. Results and Discussion

There was considerable variation in both quality and quantity of DNA preparations. Without RNase treatment, certain DNAs did not allow amplification of any bands at all, or they did only sporadically. Others yielded short length bands (<400 bp) only. This effect was not a result of differing DNA concentrations in the preparations. The overall percentage of failed reactions (approx 20%) was unsatisfactory. Rather than further purifying the DNA preparations, RNase A (a heat-stable enzyme) *(12)* was added directly to the amplification reactions. In early experiments, an incubation at 65°C for 15 min preceded RAPD reactions, but this proved unnecessary. Also, DNA concentration in the preparations did not have to be adjusted. Stable RAPD banding patterns were obtained over a 1000-fold dilution range of DNA (not shown).

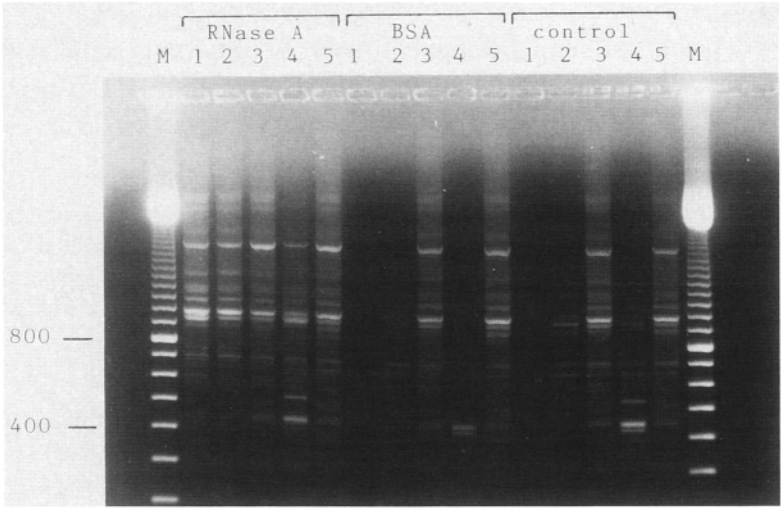

Fig. 1. Effect of RNase A addition to DNA amplification reactions with random primers. DNA from somatic embryos (lanes 1 and 2) and plant needles (lanes 3–5) of Norway spruce; amplified in the presence of RNase A or BSA. Primer OPA-19; M, mol-wt marker (100-bp ladder, Pharmacia, Uppsala, Sweden).

Figure 1 shows that the effect of RNase A addition was specific and not owing to the presence of higher levels of protein. Bovine serum albumin (BSA) was added at the same concentration (20 ng/μL) to a set of controls.

The advantage of adding RNase A directly to the RAPD reactions is the fact that no further steps like incubation or removal of the enzyme by phenol/chloroform extractions are necessary. Crude DNA "quick-preps" can be used directly, thereby saving considerably on time and labor in large-scale screening projects, for example breeding programs involving high numbers of progeny, or population surveys.

There is reason to suggest that the assisting role of RNase A during RAPD reactions is primarily a result of the rapid degradation of RNA. Gymnosperm embryos can contain high levels of RNA, which is rapidly degraded upon germination *(13)*. Apparently, RAPD-inhibiting amounts of RNA were also present in the needle and leaf DNA preparations. RNA may inhibit RAPD reactions by blocking primer annealing sites on the template DNA, or by competing with primers for thermostable polymerase *(14)*, which seems to be limiting in RAPD reactions. Another explanation may be the (albeit low) reverse transcriptase activity of *Taq* polymerase *(15)*.

RNase A addition has also improved RAPD reactions from fungal DNA *(Cryphonectria parasitica)* in this laboratory.

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