A simple and rapid method for isolation of high quality genomic DNA from fruit trees and conifers using PVP

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ABSTRACT

Because DNA degradation is mediated by secondary plant products such as phenolic terpenoids, the isolation of high quality DNA from plants containing a high content of polyphenolics has been a difficult problem. We demonstrate an easy extraction process by modifying several existing ones. Using this process we have found it possible to isolate DNAs from four fruit trees, grape (Vitis spp.), apple (Malus spp.), pear (Pyrus spp.) and persimmon (Diospyros spp.) and four species of conifer, Pinus densiflora, Pinus koraiensis, Taxus cuspidata and Juniperus chinensis within a few hours. Compared with the existing method, we have isolated high quality intact DNAs (260/280 = 1.8–2.0) routinely yielding 250–500 ng/µl (total 7.5–15 µg DNA from four to five tissue discs).

Many studies require isolation of genomic DNA from various kinds of plant species. Because DNA degradation is mediated by secondary plant products such as phenolic terpenoids which may bind to DNA after cell lysis (1), the isolation of high quality DNA from plants containing a high content of polyphenolics has been a difficult problem. We demonstrate an easy extraction process by modifying several existing ones (1–4). The process minimizes manipulations of the samples, and requires no ultracentrifugation to purify DNA separated from the polyphenolics, but optimizes the yield of DNA extracted from a sample. Micrograms to milligrams of DNA can be stored for months at 4°C.

Using this process we have found it possible to isolate DNAs from four different kinds of fruit tree samples, grape (Vitis spp.), apple (Malus spp.), pear (Pyrus spp.) and persimmon (Diospyros spp.) (Fig. 1), and four species of conifers, Japanese red pine (Pinus densiflora), Korean pine (Pinus koraiensis), Japanese yew (Taxus cuspidata) and Chinese juniper (Juniperus chinensis) (Fig. 2) within a few hours.

Initially, we isolated DNA using a modification of the widely used procedure (2,3) or rapid genomic DNA extraction process using PVP (1,4), but found that these methods could not remove polyphenolics from the tree tissues, and that very little DNA was extracted. Compared with the existing method, we have isolated DNAs from grape, pear, apple and persimmon routinely yielding 250–500 ng/µl (total 7.5–15 µg DNA from four to five tissue discs).

All of these DNAs were used for RAPD–PCR analysis using the method previously published by us (5). Arbitrary decamer primers successfully amplified DNA fragments from both those four fruit
Figure 1. Total DNA prepared from apple, grape, pear and persimmon plants. M, DNA size marker (1 kb DNA ladder, GIBCO-BRL). Lanes 1–4: 1, apple (cv. Fuji); 2, grape (cv. Campbell Early); 3, pear (cv. Nijisseiki); 4, persimmon (cv. Fuju).

Figure 2. Total DNA prepared from four different species of conifers. M, DNA size marker (1 kb DNA ladder, GIBCO-BRL). Lanes 1–4: 1, Japanese red pine apple (P. densiflora); 2, Korean pine (P. koraiensis); 3, Japanese yew (T. cuspidata); 4, Chinese juniper (J. chinensis).

Figure 3. RAPD profiles of apple, grape, pear and persimmon DNAs. All reactions had a final volume of 14 µl and contained 1 ng template DNA; 200 µM each of dATP, dCTP, dGTP and dTTP; 0.27 µM primer; 0.028 U Taq DNA polymerase (Promega); 1.5 mM MgCl₂ and 1x reaction buffer (10 mM Tris-HCl pH 8.8, 50 mM KCl and 0.1% Triton X-100). Each reaction mix was overlaid with 14 µl mineral oil. Samples for enzymatic amplification were subjected to 45 cycles of the following thermal profile: 1 min at 94°C, 2 min at 37°C and 3 min at 72°C. Samples were predenatured for 4 min at 94°C and final extension was for 7 min at 72°C. Amplification fragments generated by PCR in a water-bath thermal cycler (FINEPCR, Korea) were separated according to size on 1.2% agarose gels, stained with ethidium bromide. The oligonucleotide primer was 5’-TCCCGAACCG-3’ (UBC #348). M, 1 kb DNA ladder (GIBCO-BRL). Lanes 1–4: 1, apple (cv. Fuji); 2, grape (cv. Campbell Early); 3, pear (cv. Nijisseiki); 4, persimmon (cv. Fuju).

Figure 4. RAPD profiles of four different conifers. PCR reaction is performed using the described protocol in Figure 2. DNAs were amplified using primer 5’-CGCCCGCAGT-3’ (UBC #389). M, 1 kb DNA ladder (GIBCO-BRL). Lanes 1–4: 1, Japanese red pine apple (P. densiflora); 2, Korean pine (P. koraiensis); 3, Japanese yew (T. cuspidata); 4, Chinese juniper (J. chinensis).

Figure 5. Amplification of 13 grape DNAs using the described protocol in Figure 2. DNAs were amplified using primer 5’-CGCCCGCAGT-3’ (UBC #389). M, 1 kb DNA ladder (GIBCO-BRL); lanes 1–13, 13 different grape cultivars. The DNA samples of 32 individual grape cultivars were extracted using this procedure and assayed for RAPD–PCR using 15 primers. We found that 40 reliable RAPD markers selected could be used to calculate both the dissimilarity value and the marker difference that were used to reconstruct the genetic relationships (manuscript in preparation). Figure 5 shows the results of PCR amplification using UBC primer #389 of 13 grape cultivars. This DNA preparation method yielded a predominance of high molecular weight DNA reliably produced PCR amplification products >500 bp in length. Considering all factors involved, this method appears to be the most efficient, reliable and labor-effective DNA isolation procedure from plants containing a high content of polyphenolics such as fruit trees and conifers.

REFERENCES