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Commentary

Dichloromethane as an Economic Alternative to Chloroform in the Extraction of DNA from Plant Tissues

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Abstract: Processing of large numbers samples of plant tissue samples for molecular mapping and gene tagging requires methods that are quick, simple, and cheap, and that eventually can be automated. Organic solvents used for DNA extraction can represent a significant proportion of the overall cost. In this study we examined dichloromethane as a replacement for chloroform to be used in combination with phenol.

Mapping of plant genomes with RFLP- and PCR-based markers has greatly facilitated tagging of genes for agronomically relevant traits. One major difficulty in applying markers in practical breeding programs is essentially logistic: large numbers of samples must be processed in a short time, using reproducible and simple techniques, preferably at low cost.

The development of RAPD analysis was an important step forward (Williams et al., 1990). This technique obviates blotting of DNA from agarose gels to nylon membranes and subsequent hybridization with radioactive probes. After direct evaluation of band patterns on the gel, the data can be processed using linkage analysis software, such as MapMaker (Landers et al., 1987). These approaches combined with efficient and reproducible DNA extraction provide the basis for automating mapping processes.

Abbreviations: BSA, bovine serum albumin.

Several factors affect the extraction of DNA from plant tissues: the amount of tissue needed and its availability, the number of steps involved, the required purity of the final product, and the chemicals used. Extracted DNA must be digestible by restriction enzymes and amplifiable by PCR. Several methods use phenol and chloroform to extract proteins.

In work on rice, beans, cassava, and leguminous forages, we have adjusted the extraction procedures to each crop, depending on the interfering substances in the tissue (e.g., tannins, latex, polyphenol oxidases, and mucilage). The varied origins of the samples and different uses of the purified DNA have required that we establish different extraction protocols. For example, beans grown in the field contain more tannins than those grown in the greenhouse; tannins may interact with DNA, reducing its yield and quality. Latex and other compounds are the main problem in cassava, which is a member of the family Euphorbiaceae. The fibrous nature of the monocots rice and *Brachiaria* are barriers to high DNA yields.

In all cases we try to use the fastest and least expensive method without sacrificing yield and quality. When possible, we avoid using organic solvents, since they are difficult to dispose of safely. Instead, we apply methods that involve various precipitation and extraction steps, as does the use of xanthogenate (Jhingan, 1992) or the nonionic detergent cetyltrimethylammonium bromide (CTAB) (Dellaporta et al., 1983; Rogers and Bendich, 1985; Murray and Thompson, 1980; Couch and Fritz, 1990). The use of phenol and chloroform may add considerably to costs, which can be reduced somewhat through lower extraction volumes. Another way to economize is to replace chloroform with dichloromethane.

Results and Discussion

In a first attempt to reduce costs and increase efficiency in the extraction of DNA from plant tissues, we cut by half the extraction volume of phenol/chloroform and the reextraction of phenol traces with pure chloroform, giving a volume ratio of 1:2 with respect to the aqueous phase instead of 1:1. No negative effects on extraction quality or efficiency were observed (results not shown). This also reduced the problem of organic solvent disposal.

In the next experiment dichloromethane was subtituted for chloroform in DNA extraction procedures that usually require phenol/chloroform mixtures for protein extraction. Dichloromethane is widely used as an extraction solvent in organic chemistry. It is suitable for DNA extrac-

Table I. Comparison of chloroform with dichloromethane as solvents for DNA extraction. Arrows indicate the most important properties to consider when substituting dichloromethane for chloroform. Data from Bretherick (1986) and the Merck catalog.

Properties	Chloroform	Dichloromethane
Physicochemical		
Formula	$CHCl_3$	CH_2Cl_2
FW (g/mol)	120.4	84.9
➳ Boiling point BP [°C]	61	40
➳ P_{VAP} [mm Hg]	160	340
➳ Polarity $E^{\circ}(Al_2O_3)$	0.40	0.42
UV cutoff [nm]	245	230
Density d [g/mL]	1.483	1.327
Appearance	colorless liquid	colorless liquid
Sol. in water [v/v]	1:200	1:50
Safety Considerations		
Flammability	no	no
➳ Toxicity	anaesthetic, causes headache and nausea, mutagenic, carcinogenic	irritating, causes headache and nausea
➳ Recommended exposure limit (RL) ppm or mL/m^3 (mg/m^3)	10 (50)	100 (360)
Economic Aspects		
➳ Price per liter [US$] FLUKA	$31	$21

tion because it has about the same polarity index as chloroform but, as indicated in Table I, is less toxic and costs only about half as much. The low boiling point and high vapor pressure of dichloromethane require that it be used only at room temperature, the conditions under which the clarification step of most DNA extraction protocols is performed.

To demonstrate the utility of dichloromethane for DNA purification, we compared its ability to extract protein and phenol with that of chloroform, and we evaluated the quality of the extracted DNA in restriction enzyme digestion and PCR amplification. No interference with enzymatic reactions was detected when using DNA extracted from different crops. Fig. 1 shows digestion of genomic DNA from tepary bean (*Phaseolus acutifolius*) and common bean (*P. vulgaris*) with the restriction enzymes *Eco* RI and *Bam* HI; no difference in the quality of digestion was detected when dichloromethane was substituted for chlo-

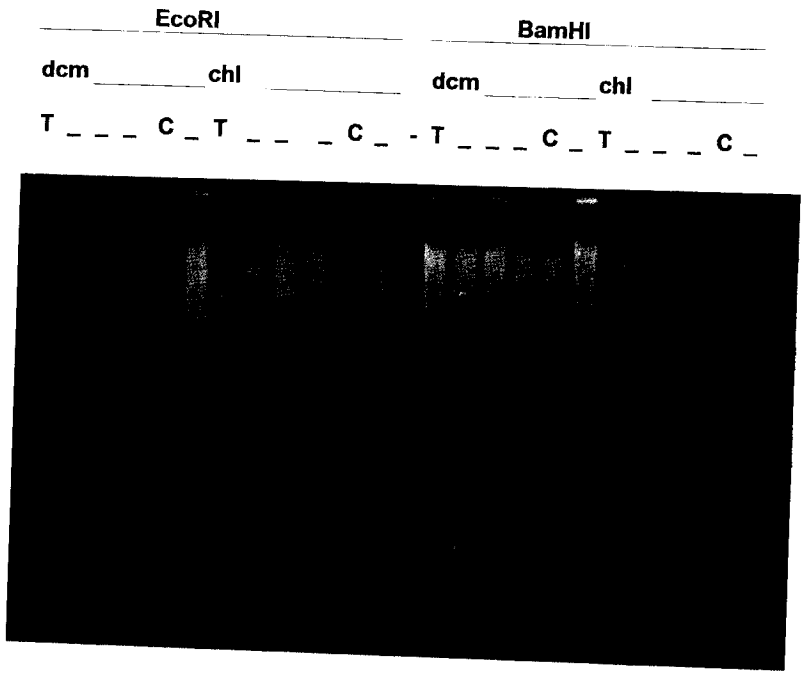
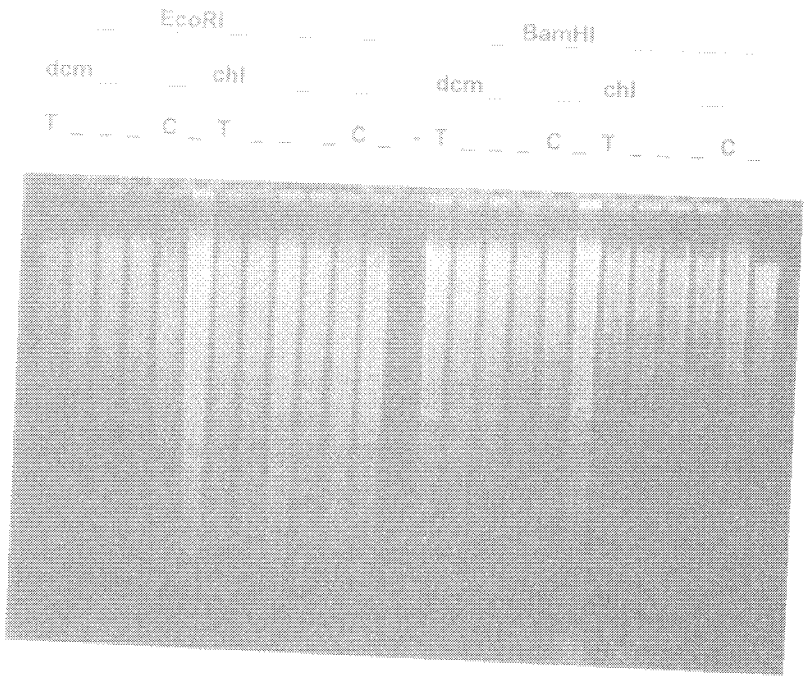


Fig. 1 Restriction digestion analysis of genomic DNA extracted using dichloromethane or chloroform. 5 mg total genomic DNA from tepary bean (*Phaseolus acutifolius*) (t) and common bean (*P. vulgaris*) (c) were extracted with dichloromethane (dcm) or chloroform (chl), digested with *Eco* RI and *Bam* HI, separated on a 0.8% agarose gel, and stained with ethidium bromide.

roform in the extraction procedure. Bean leaves, especially tepary bean leaves, are good tissues for testing extraction quality because their composition usually poses problems. We have also used dichloromethane successfully with other crops at CIAT, including cassava (*Manihot esculenta* Crantz), rice (*Oryza sativa*), and various tropical pasture legumes and grasses (results not shown).

RAPD analysis was performed with DNA extracted using dichoromethane. Fig. 2 shows PCR amplification of genomic DNA from tepary bean and common bean. No difference was detected between DNA extracted with dichloromethane and that with chloroform.

About the same amount of protein was extracted in consecutive steps with the two solvents. Protein was extracted in three steps from a DNA/BSA mixture (5 mg/mL each). The amount of protein extracted in the



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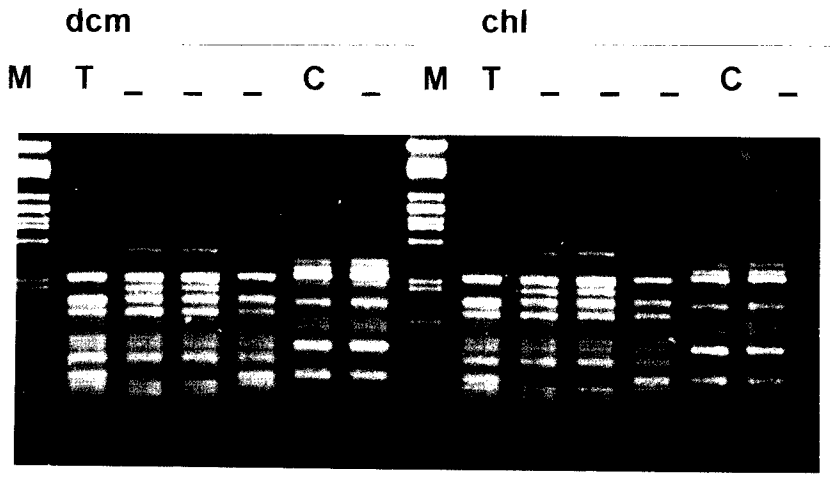

Fig. 2 RAPD analysis of DNA extracted using dichloromethane or chloroform. PCR amplification was performed using a standard RAPD protocol. Operon primer AI 08 was used on tepary bean (*Phaseolus acutifolius*) (t) and common bean (*P. vulgaris*) (c) total genomic DNA, using dichloromethane (dcm) or chloroform (chl) during extraction. M, molecular weight marker, λ *Pst* I.

organic phase is visible in the right half of Fig. 3, which shows the first and the third extraction steps. The same amount was extracted with dichloromethane and chloroforn. The spectral differences between the two solvents, below 230 nm, are due to their respective UV cutoffs.

The purity of the preparations and their extracting power were judged according to three criteria:

- Absorbance ratios (260/280 nm) were between 1.8 and 1.9.
- Absorbance ratios (230/280 nm) were around 1, with higher ratios indicating contamination with protein.
- Second-derivative UV spectra of DNA could detect changes in the slope of the zero-order spectra that were otherwise not apparent (Mach et al., 1992), as demonstrated by the detection of phenol traces after extraction with chloroform and dichloromethane using this type of analysis (Fig. 4).

The 260/280 ratio is the most widely used criterion, but the 230/280 ratio is generally more sensitive to contamination with protein.

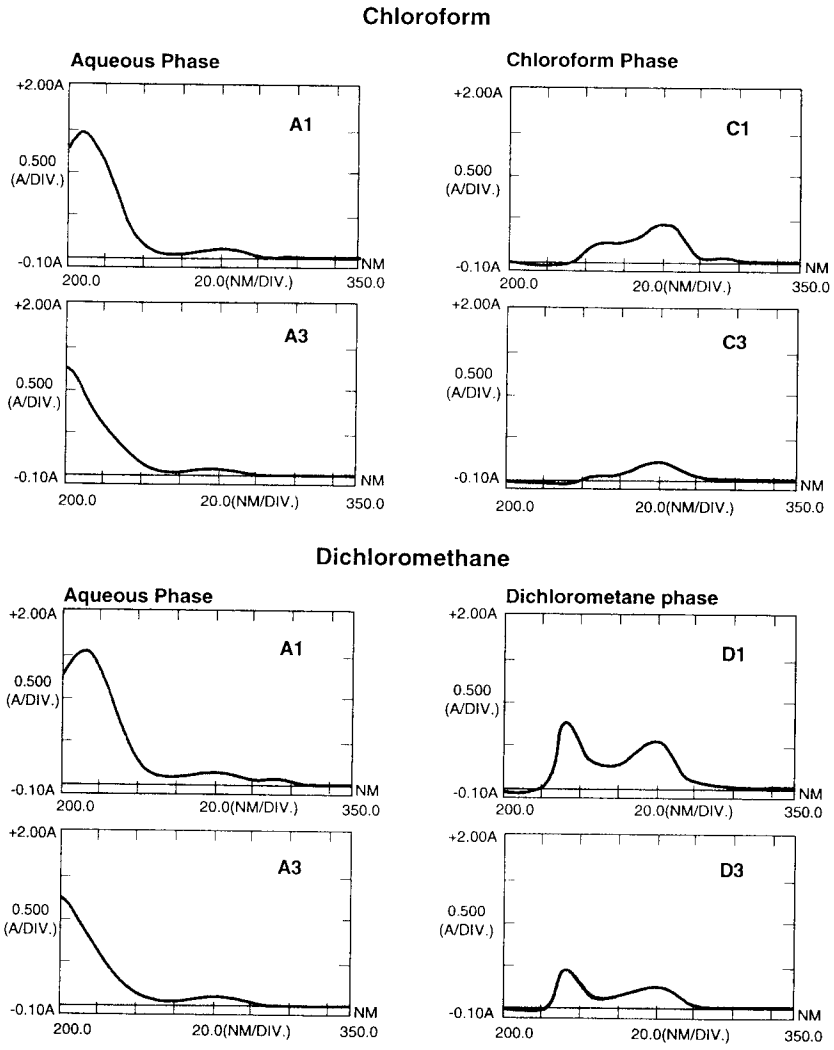

Fig. 3. Protein extraction power of dichloromethane vs. chloroform. A DNA / BSA mixture containing 5 mg / mL DNA and 5 mg / mL BSA was extracted three times with dichloromethane or chloroform. The UV spectra of the first and third aqueous (A1 and A3) and organic phases (D1 and D3 for dichloromethane, and C1 and C3 for chloroform) was recorded.

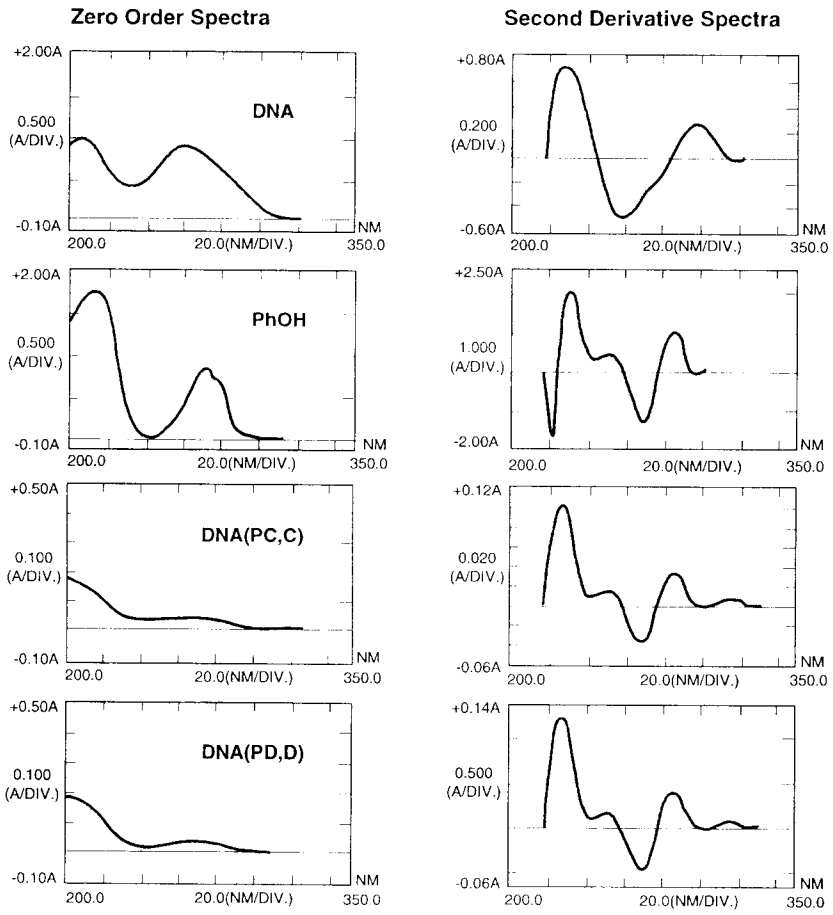

Fig. 4. Detection of traces of phenol after extraction with dichloromethane and chloroform using second-order derivative spectra. From top to bottom, aqueous DNA solution (5 mg/ml), aqueous phenol solution; DNA extracted with phenol/ chloroform and chloroform; DNA extracted with phenol/ dichloromethane and dichloromethane. The spectra were recorded with a Shimadzu UV 160A model spectrophotometer.

Adding the criterion of second-derivative spectra may help avoid problems during restriction digestion of DNA samples extracted under extraction protocols that involve phenol. Contamination with phenol is not detectible using the other criteria. Our analysis demonstrates that the partition coefficient of phenol in a biphasic system of water / chloroform (or dichloromethane) makes it necessary to reextract or precipitate and wash the DNA carefully to produce a phenol-free sample.

We are convinced that dichloromethane offers a viable alternative to chloroform in protocols for DNA extraction in which organic solvents are used.

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References

Bretherick, l. 1986. *Hazards in the Chemical Laboratory*, 4th edition. The Royal Society of Chemistry, London.

Couch, J.A. and P.J. Fritz. 1990. Isolation of DNA from plants high in polyphenolics. Plant Mol. Biol. Reptr. 8:8-12.

Dellaporta, S.L., J. Wood and J.B. Hicks. 1983. A plant DNA minipreparation: Version II. Plant Molec. Biol. Reptr. 1:19-21.

Jhingan, A.K. 1992. A novel technology for DNA isolation. Methods Molec. Cell. Biol. 3:15-22.

Landers, E.S., P. Green, J. Abrahamson, A. Barlow, M.J. Daly, S.E. Lincoln and I. Newburg. 1987. Mapmaker: An interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174-181

Mach, H., R. Middaugh, R.V. Lewis. 1992. Detection of proteins in DNA samples with second-derivative absorption spectroscopy. Anal. Biochem. 200:20-26.

Murray, M.G. and W.F. Thompson. 1980. Rapid isolation of high-molecular-weight plant DNA. Nucl. Acids Res. 8:4321.

Rogers, S.O. and A. J. Bendich. 1985. Extraction of DNA from milligram amounts of fresh, herbarium and mummified plant tissues. Plant Mol. Biol. 5:69- 76.

Williams, J.G.K., A.R. Kubelik, K.J. Livak, J.A. Rafalski and S.V. Tingey. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucl. Acids Res. 18:6531-6535.