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INTERIM PRESERVATION OF PLANT SPECIMENS FOR DNA UTILIZATION

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Abstract - The use of fungicides and a bactericide were investigated for the preservation of leaves (spinach) for subsequent extraction of DNA. After 7 days at 35• C, only the spinach leaves sprayed with a 5% chlorox solution yielded good DNA. Fair to good DNA was obtained from treatments with copper oleate and streptomycin sulfate powder. Fair DNA was obtained from treatments using sulfur powder, Maneb Mn & Zn powder and mothballs (naphthalene). Little or no DNA was obtained from Na azide, Na cacodylate, phenol, and 0.5 M EDTA treatments.

Key Word Index- DNA preservation, degradation

Introduction

In order to secure DNA from plants for systematic and evolutionary studies, an investigator is often presented with the problem of collecting plant materials (often leaves) from distant sites and then trying to preserve them until they can be returned to the lab for DNA extraction. Doyle and Dickson (1987) reported on several strategies to preserve plant leaves for subsequent DNA extraction. None of the chemical preservatives were useful. They did get good DNA from dried leaves of Solanum, even after 3 months and from Glycine after 10 months but not after 26 months. We recently found that the DNA in spinach leaves (air dried at 42^{o} C, then stored in a herbarium cabinet at 22^{o} C) was good after 2 months but highly degraded after 5 months.

Although a vial of 'DNA preservative' would be an ideal solution for the preservation of leaf materials on long field trips or for shipment from country to country, the search for a 'magic solution' has been quite elusive. Pyle and Adams (1989) examined 25 treatments and found no chemical solutions that would preserve the DNA for even a few days. We, therefore, began to explore other alternatives for preservation. In this paper we report on treatments using fungicides and a bactericide for the short-term storage of foliage for subsequent DNA extraction.

Experimental

Preparation of Plant Material: Leaves from fresh, unwashed spinach (Spinacia oleracea) were cut into pieces weighing approximately 0.45 g. The leaf pieces were placed inside pint-size heavy duty Ziploc freezer bags (Dow Chemical) into which a preservative was then added. These preservatives are listed in Table 1. The bags were placed inside a plant dryer for 7 days and the temperature kept at 35^{o} C.

Liquid treatments, including the control with tap water, were added to the bags using an atomizer. The atomizer was sprayed 25 times into the bag (about 2 ml of liquid). For the dry powder treatments, 1/4 teaspoon of powder was placed inside the bag and shaken to distribute the powder over the leaf surface. Naphthalene was added to the bag in the form of a single mothball. The following products were used: Na azide (Sigma S-2002); Na cacodylate (cacodylic acid sodium salt-trihydrate, Kodak 15404); copper oleate solution (American Brand copper fungicide); sulfur powder (Hi-Yield brand, 90% sulfur); Maneb powder (Hi-Yield Brand, zinc ion and manganese ethylene bisdithiocarbamate); phenol

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(Mallinckrodt 0025); Streptomycin powder (Ferti-lome Brand, fire blight treatment).

DNA Extraction and Analyses

The hot CTAB procedure (Doyle and Dickson, 1987; Doyle and Doyle, 1987) was used with minor changes (Pyle and Adams, 1988). After extraction, the DNA was resuspended in 0.5 ml of 1X TE (1mM Tris HCl, 0.1 mM EDTA, pH 7.2) buffer.

For gel electrophoresis, DNA was mixed in various concentrations with 1X TBE and 1/5 volume of loading buffer [15% Ficoll (Sigma F-2637), 0.05% bromphenol blue, and 0.10 M EDTA in 1X TBE]. Ten μl of this mixture was loaded onto a 0.6% agarose gel (Sigma A-6013) containing 0.5 μl/ml of ethidium bromide and electrophoresed for 30 minutes at 100 V (10 V/cm), submerged in running buffer (1X TBE, 0.5 μg/ml ethidium bromide). Concentrations of the extracted DNA's were judged by comparison with unrestricted lambda DNA (Sigma D-0144) at various known concentrations. Gels were photographed under short wave UV light using a Polaroid direct screen camera (DS34).

Results and Discussion

Only the sample preserved in chlorox gave DNA comparable to DNA from fresh leaves (Table 1). The DNA from chlorox preserved sample was still much less concentrated than DNA from fresh spinach. Tap water only, Na cacodylate solution, phenol, and 0.5 M ETDA solution treatments did not yield any detectable DNA (Table 1). The Na azide (3 % solution) treated leaves had a poor DNA yield with very poor quality (Table 1). The copper oleate solution seems to have promise as these leaves resulted in good yields of DNA with good quality.

The sulfur powder, and Maneb Mn and Zn powder treatments resulted in only fair yields and fair quality (Table 1). The mothball (naphthalene) was used to generate a non-oxidizing atmosphere by the release of naphthalene vapors inside the bag. This resulted in a fair DNA yield with very good quality. The streptomycin sulfate powder treated leaves had a fair to good yield of DNA with very good quality (Table 1). Streptomycin sulfate might be very useful if used in combination with a fungicide to prevent the growth of bacteria on the specimen.

It is interesting to note that all the storage methods involving powders yielded fair or good DNA. The leaves felt dry upon removal from the baggies. It is possible that preservation by desiccation may have been as important as any biocidal action of the preservatives. The DNA in these samples, however, is not as concentrated as DNA from press-dried (42^o C), air-dried (22^o C), or desiccated spinach (Pyle and Adams, 1988). The DNA also does not contain proportionately as much large DNA as does the extract from dried samples (Pyle and Adams, 1988). The use of additional powders and combinations will be considered in a future study.

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Table 1. Comparisons of fungicides and a bactericide for short storage of fresh spinach leaves for DNA extraction after 7 days at 35° C. NA = Not analyzable due to only trace amounts of DNA.

Treatment	DNA yield	DNA quality
1. Fresh spinach leaves	excellent	excellent
2. Control (sprayed with tap water)	none	NA
Fungicides:		
3. Chlorox (5% soln in tap water)	good	excellent
4. Na azide (3% soln in tap water)	poor	very poor
5. Na Cacodylate (1% soln in tap water)	none	NA
6. Copper oleate solution	fair-good	good
7. Sulfur powder	fair	fair
8. Maneb Mn & Zn powder	fair	fair
9. Phenol	none	NA
10. Naphthalene	fair	very good
11. 0.5M EDTA	none	NA
Bactericide:		
12. Streptomycin sulfate powder	fair-good	very good