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SHORT COMMUNICATION

ISOLATION AND PROPERTIES OF NUCLEIC ACIDS FROM SOYBEAN LEAF TISSUE

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Abstract—RNA is quantitatively hydrolyzed from extracted soybean leaf tissue by treatment with 0·33 N KOH for 7 hr at 37°. Subsequent neutralization and precipitation with ethanol is necessary to remove a DNA fraction and other unidentified interfering substances. Sixty to seventy per cent of the total DNA is hydrolyzed by this mild alkaline treatment and is partly eluted with the RNA fraction. Total DNA may be quantitatively hydrolyzed from extracted leaf tissue with 5% trichloracetic acid (TCA), without prior treatment with alkali and estimated colorimetrically.

INTRODUCTION

POTENTIAL residue problems and accumulation of toxic intermediates emphasize the need for a clearer understanding of the effects of pesticide chemicals on the physiology of exposed plants. This is especially true for sensitive species such as soybeans, an economically important crop. Since some herbicidal chemicals affect nucleic acid synthesis[1] or enzyme systems, including catalase[2,3] associated with protein synthesis,[4] these effects need to be examined.

Present investigations were undertaken to determine if DNA and RNA could be hydrolyzed simultaneously, and estimated from the same hydrolysate, rather than from different ones.[5,6] This objective was not achieved, but the modifications necessitated by the character of soybean nucleic acid resulted in the development of a procedure whereby both DNA and RNA can be determined more rapidly than by using other methods.

RESULTS

Hydrolysis of RNA

Earlier investigations[7] indicated that a 7 hr treatment of extracted apple leaf tissue with 0·33 N KOH effectively hydrolyzed essentially all of the RNA into acid-soluble nucleotides. Extension of this work to soybean leaf tissue indicated that this treatment also hydrolyzes soybean leaf RNA into spectrophotometrically identifiable nucleotides. Lesser periods of time are inadequate.

Subsequently we showed[8] that adjusting the pH of the alkaline hydrolysate to 5·0 and

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addition of one vol. of ethanol along with two drops of $MgCl_2$ removed substances that interfered with RNA determinations. It was unnecessary with the soybean hydrolysate to use $MgCl_2$, as alcohol by itself precipitated the maximum amount of interfering substances. The main interfering substance was judged, on the basis of a positive color test[5] to be DNA. Some absorption is also due to non-nucleic acid components that are precipitated by the alcohol.

Hydrolysis of DNA

KOH digestion of animal material[9] solubilizes both RNA and DNA, and thus both nucleic acids may be determined on the same aliquot. In our investigations it has been found necessary to determine DNA separately since complete hydrolysis of DNA in plant leaf tissue usually requires heating with NaOH prior to treatment with trichloroacetic acid (TCA). Fresh bean leaf tissue does not require this treatment[10] and therefore studies comparing TCA hydrolysis, either directly or on alkali-treated tissue, were extended to soybean. Essentially no differences were found, indicating that alkaline treatment prior to TCA hydrolysis is not necessary. The precipitate remaining after the KOH digestion was hydrolyzed with TCA and the DNA in this fraction (KOH-insol.) added to that found in the KOH supernatant (KOH-sol.) gave good agreement with that found by direct analysis (Table 1).

TABLE 1. DNA CONTENT OF SOYBEAN LEAF TISSUE*

KOH-sol.	KOH-insol.	Sum of 1 and 2	Direct TCA hydrolysis
920	570	1490	1440
900	595	1495	1445
935	585	1520	1485

* DNA values expressed as μg/g dry wt. Each value represents an average of three determinations.

As indicated in Table 1, the sum of the two DNA fractions equalled that found for the total with an error amounting to less than 3 per cent. Differences of this magnitude or greater have been reported by other workers[11,12] for DNA replicates of the same sample. Thus either method may be used for estimating the DNA content of leaf tissue.

EXPERIMENTAL

Preparation of Leaf Material

Leaf tissue was prepared by lyophilization in an apparatus described by Millikan and Thomas.[13] The dried leaf tissue was then passed through a 60 mesh screen and extracted as described previously.[5]

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Hydrolysis of RNA

Fifty-mg portions of air-dried solvent-extracted leaf powder are weighed into 50 ml tubes. These are wetted with about 0·5 ml of ethanol and 5 ml of 0·33 N KOH added. After incubation at 37° for 7 hr the tubes are centrifuged and the supernatant drawn off. The precipitates are washed with pH 8·5 water, centrifuged and the supernatants combined.

After neutralization with perchloric acid and the addition of one vol. of ethanol, the samples are stored overnight in the cold. The precipitates are then centrifuged, washed and the supernatants combined and made to 25 ml volume. RNA is determined in these solutions after passage over Dowex 1-X8 columns and elution with 0·12 N HCl.

Hydrolysis of DNA

Extraction of total DNA. 100 mg of air-dried, solvent-extracted leaf powder is weighed into 50 ml centrifuge tubes, wetted with 0·5 ml of ethanol and 10 ml of 5 % trichloracetic acid (TCA), heated for 20 min in a boiling water bath, cooled, centrifuged and the supernatant saved. After washing with 5 % TCA the supernatants are combined and brought to 25 ml volume. DNA was determined by a modification of the method of Webb and Levy.[11]

Estimation of DNA in DNA hydrolysate. DNA in the supernatant used for RNA determination may be determined after neutralization and prior to the addition of ethanol. Sufficient TCA must be added to make it 5 per cent and then DNA determined as for the total. DNA in the precipitate after 0·3 N KOH treatment also must be determined. This is done in the same manner as that for the total. The sum of these two fractions will equal that found for the total.