PROCEDURE FOR STUDY OF IMMUNOCHEMICAL PROPERTIES OF ENDOSPERM TISSUE OF PINUS STROBUS L.[1/]

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PROCEDURE

Seed Germination

Forty seeds were germinated from each of six single tree collections (Table 1). Seeds were stored at -20C from collection until immunochemical analysis. All seeds were soaked in 10 percent H202 48h prior to placing under germination conditions. Germination occurred over a two-week period under 20h photoperiod at 28C. When the radicle protruded 1-2 mm from the seed coat, seeds were stored at 4C until preparation for analysis (4 to 6 days). One set of germinated seeds was used for injection into rabbits and a second set was used in testing for antigenic differences.

Table 1. Sources of eastern white pine seeds used in immunochemical analysis.

New Hampshire Clonal Seed Bank	Years Storage	I.U.F.R.O. Section 221/	Years Storage
S-3 Claremont Co., N.H.	8	2512 Ashland Co., WI.	12
E-1 Rindge Co., N.H.	8	2580 Newaygo Co., MI.	12
0-1 Coos Co., N.H.	8	2614 Greenbriar Co., WV.	10

[1/] Seed supplied by H.B. Kriebel, O.A.R.D.C., Wooster, OH. 44691

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Preparation of Endosperm

Excised endosperms were washed and homogenized in DH2O containing 1mM PMSF (phenyl methyl sulfonyl-fluoride) in a glass teflon homogenizer. The homogenate was centrifuged 10 min at 25,000 x gravity and the supernatant recovered. Protein content was determined by absorbance at 280 nm. (Table 2). Six endosperms per tree were used in all preparations. Seed preparations were maintained on ice at all times.

Table 2. Protein quantity at A280 in endosperms of six sources of eastern white pine. [1/]

Source	mg/ml protein
0-1	2.5-3.0
E-1	2.5-3.0
S-3	2.5-3.0
2580	1.0
2512	0.4
2614	0.4

[1/] Based on six endosperms per source.

Antibody Production

Homogenate containing approximately 0.5 mg/ml of protein was diluted to 1.0 ml with DH2O. A few crystals of A1C13 were added to precipitate the protein and the suspension was emulsified with an equal volume of Freund's adjuvant. Female New Zealand white rabbits were injected intramuscularly at several sites. After six weeks, rabbits were boosted with another 0.5 mg of protein in incomplete adjuvant. Rabbits were bled seven days after the boost. Serum was collected and the immunoglobulin fraction precipitated with ammonium chloride at 50 percent of saturation.

Electrophoresis

SDS- polyacrylamide slab gel electrophoresis was performed according to Laemmli (1970) and stained with Coomassie Brilliant Blue R.

Antibody Labeling

Protein run in gels were transferred to nitrocellulose paper electrophoretically according to Towbin et al. (1979). The nitrocellulose blots were washed with 0.9 percent NaC1 in 10 mM tris Cl pH 7.4 with 10 mg/ml bovine serum albumin added. The immunoglobulin fraction was diluted 1:100 and added to the blots overnight with agitation.

Blots were washed for 6h with the same buffer and treated with 1 ^{125} labeled Stapholococcus Protein A overnight using 1 uCi per blot. After extensive washing, blots were dried, placed on Kodak 0M-1 x-ray film with a Kodak Lanex intensifying screen for 3 days. These autoradiograms were developed in Kodak x-ray film developer.

LITERATURE CITED

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