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Rapid Biochemical Test for Seed Germinability

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Sugar and aminoacids were investigated in sterile distilled water after *Pinus pinea* seeds had been soaked in it during 24 hours at 37° C. Germination viability decreased with the ageing of the seeds and was accompanied by sugar and aminoacids increase in the exudate. The sugar content from seeds with loss of germinability was about 50 to 80 times greater than that from seeds with good germinability.

Paper chromatography showed that there was an increase in mono, di, oligosaccharides and amino-acids in seeds without germinability, but these substances were only found as trace quantities in viable seeds.

The methods already described by TKAYANAGI and MURAKAMI to determine germinability could be applied with some modifications to *Pinus pinea* seeds. The presence of sugar in the exudate could be detected by urine sugar test after 24 hours at 37° C. It was necessary to concentrate the exudate till a final volume of 2 ml.

Deterioration of seed during storage is an important problem for plant breeders, seedsmen, and farmers. Since a decline in vigor usually precedes viability loss, a sensitive measure of vigor would be very useful. Biochemical measures of seed vigor have included: Analysis of free fatty acids, enzymatic activity, CROCKER and HARRINGTON (4) tetrazolium method LAKON (5), and respiratory tests WOODSTOCK (10-12). Some physical measurements in dry seeds such as their density and electrical conductivity/capacitance ratio have also been studied. They are comparatively expensive, time consuming and need elaborate techniques. Recent proposals for practical tests of seed viability and vigor, have been based either on chemical determination of reducing sugars TAKAYANAGI and MURAKAMI (9), or on increase in electrical conductivity leachates, BRADNOCK and MATTHEWS (3).

The aim of this work was to find whether the method described by TAKAYANAGI and MURAKAMI for rape seed, could be applicable to an oil seed such as the *Pinus pinea* one, which contains a

smaller amount of reducing sugars SANZ *et al.* (8) and a harder coat.

Materials and Methods

Pinus pinea seeds collected in various years were kept in the laboratory at 20° C. The experiences were performed in 1974.

Samples of 10 g of seeds stored during several years were used; seeds surfaces were sterilised in sodium hypochlorite solution (1 % avaible chlorine) for ten minutes, immersed in sterile distilled water and then soaked in 20 ml of distilled water for 24 hours at 37° C and afterwards washed with water three times. The washings were collected and made up to 50 ml. In this solution sugar was measured by the methods of ASHWELL (1) and MORRIS (2, 7) and described by TAKAYANAGI and MURAKAMI (9).

The exudates solutions in which the seeds were soaked were concentrated by vacuum to a final volume of 2 ml. These solutions were analysed for aminoacids and sugars by descending paper chromatography on Whatman n.° 1 with triple development. Solvent was n-butanol:acetic acid:water (4:1:5). Detecting reagenst were Bencidine for sugar and nynhidrine for aminoacids.

Table I. *Sugar contents exuded from seeds stored in various conditions.*

Storage conditions	Germination (%)	Exuded sugars from seeds*
Seeds collected in 1974 (room temperature)	56	330
Seeds collected in 1973 (room temperature)	31	680
Seeds collected in 1972 (room temperature)	26	710
Seeds collected in 1974 (heated at 100° C for 3 days)	00	25,020
Seeds collected in 1974 (heated at 90° C for 3 days)	00	17,560

* Exuded sugars are expressed as µg glucose/g dry weight.

Results and Discussion

It is known that high temperatures adversely affect germination of several types of seeds. In this way, *P. pinea* ones heated af 90° of 100° C for 3 days, lost their germinability capacity. M. HONDUVILLA (6). The amount of sugar exuded in seeds treated at 90° C and 100° C for 3 days, were about fifty to eighty times greater than in those with good germinability (table I).

In addition, ageing of seeds is connected to a germination viability decrease. Decline of germination is matched by an increase in the amount of sugar content in the exudate (table I).

The paper chromatography results show that mono, di and oligosaccharides are abundant in the exudate of seeds having lost their viability but monosaccharides are only observed as trace amounts in viable seeds (fig. 1). Significant increasing in sugar contents in the exudate of seeds with no germinability is matched by a great aminoacid levels (fig. 2). These results are in agreement with the findings of ADEBONA and ODU (1) in cowpea seeds *(Vigna ungiculata L.)*. This sugar increase in deteriorating seeds might be a consequence of senescence.

Modifications to TAKAYANAGI test have been introduced after detecting the presence of sugars in pine seeds. The exudate sugar could be detected, after 24 hours of soaking at 37° C and concentrated at a final volume of 2 ml, by urine sugar test (paper or tablet). These methods were established on a basis of colour change: colour did not change in seeds with sufficient germinability, however in seeds having lost their germinability the yellow or blue colour turned green or green and

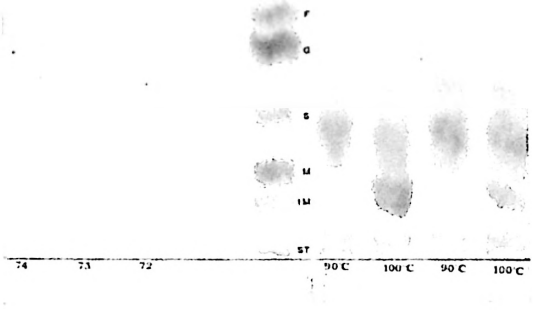

Fig. 1. *Diagram of paper chromatography of saccharides from pine seeds.*
Chromatography was carried out as indicated in methods. F, fructose; G, glucose; S, sucrose; M, maltose; IM, isomaltose; ST, stachyose.

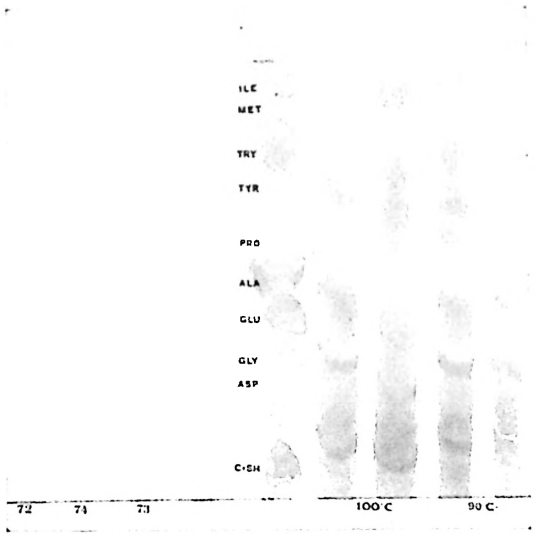

Fig. 2. *Diagram of paper chromatography of aminoacids from pine seeds.*
Chromatography was carried out as indicated in methods. ILE, isoleucine; MET, methionine; TRY, tryptophan; TYR, tyrosine; PRO, proline; ALA, alanine; GLU, glutamic acid; GLY, glycine; ASP, aspartic acid; CYSH, cysteine.

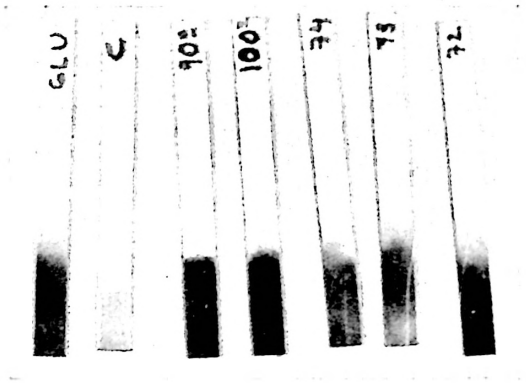

Fig. 3. *Reaction of sugar using «urine paper test».*
Glu, glucose 5 mg/ml (dark green); C, water (yellow); 90°, 100° exudates from the seeds heated at 90° C and 100° C for 3 days, respectively (dark green -no germinability); 72, 73, 74 exudates from seeds collected in 1972, 1973 and 1974 (light green, light green and yellow respectively).

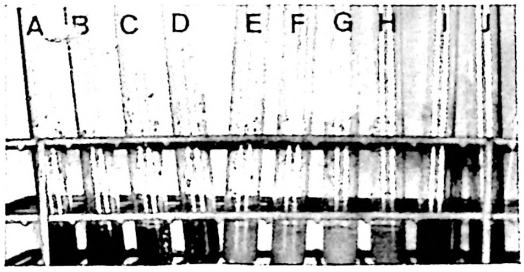

Fig. 4. *Reaction of sugar using «urine tablet test».*
A, B, C, D glucose, 2, 1, 0.5 and 0.25 % respectively (brown); E, water (blue); F, G, H exudates from seeds collected in 1972, 7973 and 1974 (blue); I, J exudates from seeds heated at 90° C and 100° C days (Brown-No germinability).

successively brown when reacting with a greater glucose amount (paper or tablet) (figs. 3-4).

Resumen

Semillas de *Pinus pinea* se agitaban en agua destilada estéril, durante 24 horas a 37° C y en estos líquidos se investigaban aminoácidos

y azúcares. Cuando la edad de la semilla aumenta, la germinación de las mismas disminuye. Esta disminución en poder germinativo viene acompañada de un incremento en el contenido de azúcares y aminoácidos en el exudado. Siendo esta cantidad de azúcar 50 a 80 veces superior en semillas que han perdido su germinabilidad respecto a las mismas con buena germinación.

El análisis cromatográfico muestra un incremento en semillas que han perdido su capacidad germinativa de mono, di y oligosacáridos, así como en aminoácidos. Estas sustancias sólo se encuentran como trazas en el caso de semillas viables. El método ya sugerido por Takayanagi para detcctar germinabilidad, puede ser aplicado con algunas modificaciones para la semilla de pino. La presencia de azúcares en el exudado puede detectarse con un test para analizar azúcar en orina después de 24 horas a 37° C, siendo necesario concentrar el exudado a un volumen de final de 2 ml.

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