## Qualitative and quantitative detection of soluble nucleotides and their derivatives in cereal seedlings by means of paper chromatography

During recent investigations on the relationship between the content of soluble nucleotides and cold resistance in seedlings of *Triticum aestivum* L. and also in some research on low temperature effects upon seedlings of *Zea mays* L., free purine and pyrimidine compounds could be detected by paper chromatography in much greater numbers than had previously been possible. Investigations by other separative methods, in most cases, considered merely the soluble nucleotides but not their ribosides and bases[1–3].

*Experimental*

Homogenates of coleoptiles and roots of dark-grown seedlings of wheat (2 days, 22°) and maize (3 days, 27°) were extracted in ethanol (83 %) at 0° for 3 h or 1 h respectively[4]. After filtration through bacterial filters (G 5 Schott-glass) or Celite-columns, optimal quantities of extracts were applied to paper strips (Schleicher & Schüll 2043 b mgl, or VEB Niederschlag FN 3) of 4 × 60 cm (wheat) or wedge-strips (maize) and developed by the descending technique at 20°, using isobutyric acid–acetic acid–water (19:0.4:11)[5] as solvent, the time of development being 18 to 20 h depending upon the shape of the strips. During each separation procedure additional strips were developed with authentic substances. Spots were localized by the photoprint technique[6] and identified by comparing their $R_F$ values with those of the authentic substances and by their co-chromatography with 0.05 ml extract from the same starting point.

The bases guanine, hypoxanthine and uracil, and their nucleosides and nucleotides, however, could not be separated in this way. Therefore, the following additional methods were used for identification: re-chromatography of eluates of the spots or their hydrochloric acid hydrolysates in suitable solvents (*e.g.* LINSKENS[7] No. 3, 5, 22, 27, 28, 29), after first being evaporated in vacuum; high-voltage electrophoresis[8] on Schleicher & Schüll paper 2043 b gl, 10 × 60 cm; chemical–analytical reactions and measurement of ultraviolet absorption spectra of some extract components[9]. Quantitative estimation was carried out by elution of the substances in water and subsequent determination by spectrophotometry of the eluates.

*Results and discussion*

*Qualitative evaluation.* The following compounds were identified: nucleotides: GTP, UTP, CTP, ATP, CTP, UDP, ADP, GMP, IMP, UMP, CMP, AMP; ribosides: guanosine, inosine, uridine, cytidine, adenosine; bases: guanine, hypoxanthine, uracil, cytosine, adenine[9]. $R_F$ values are given in Table I.

Differences in tri- and diphosphates are due to different shapes of the paper strips, above $R_F$ 0.3 the differences vanish. The sequence of the compounds on the chromatograms, however, remains the same, independent of the shape or make of the paper or of the plant material used in experiments, and agrees well with results cited in the literature[7,10,11]. Thus, IMP and CMP could be detected originally in wheat (*cf.* BERGKVIST[1] and KEYS[2]) and several nucleoside tri- and diphosphates were identified in maize (*cf.* GRÄSER[5]). Several spots on the chromatograms contain some flavonoid

TABLE I

QUALITATIVE EVALUATION OF PURINE AND PYRIMIDINE COMPOUNDS FROM EXTRACTS OF MAIZE AND WHEAT SEEDLINGS

Mean $R_F$ values of authentic substances were calculated from 20 to 40 measurements. + = substance present; — = substance not found in extract.

| *Authentic substances* | *$R_F$ values* | | *Substances identified from extracts of* | |
|---|---|---|---|---|
| | *Wedge strips 3 × 60 cm* | *Normal strips 4 × 60 cm* | *Maize* | *Wheat* |
| ITP | 0.13 | 0.08 | — | — |
| CTP | 0.14 | 0.08 | + | — |
| UTP | 0.15 | 0.08 | + | + |
| GTP | 0.15 | 0.08 | + | — |
| IDP | 0.17 | 0.09 | — | — |
| GDP | 0.18 | 0.09 | + | — |
| UDP | 0.19 | 0.11 | + | + |
| ATP | 0.19 | 0.11 | + | + |
| ADP | 0.24 | 0.17 | + | + |
| I-5′-MP | 0.30 | 0.27 | + | + |
| G-5′-MP | 0.30 | 0.27 | + | + |
| U-5′-MP | 0.30 | 0.28 | + | + |
| C-5′-MP | 0.32 | 0.31 | + | + |
| A-5′-MP | 0.41 | 0.40 | + | + |
| Guanosine | 0.46 | 0.46 | — | + |
| Inosine | 0.49 | 0.47 | — | + |
| Uridine | 0.48 | 0.48 | + | + |
| Cytidine | 0.53 | 0.52 | + | + |
| Guanine | 0.54 | 0.57 | — | + |
| Hypoxanthine | 0.58 | 0.57 | + | + |
| Uracil | 0.60 | 0.57 | + | + |
| Cytosine | 0.60 | 0.59 | + | + |
| Adenosine | 0.76 | 0.74 | + | + |
| Adenine | 0.82 | 0.83 | + | + |

glycosides which were not identified *e.g.* with $R_F$ values 0.69 and 0.95 in wheat, and 0.45 and 0.73 in maize.

*Quantitative evaluation.* Separation by means of the isobutyric acid solvent permits quantitative recording of four groups of substances: nucleoside tri- and diphosphates, nucleoside monophosphates, ribosides and bases; with the exception of the first group, in most cases adenine and cytosine derivatives may also be separated from the residual substances. After eluting substance-containing zones with distilled water (24 h) the optical density of the solutions was measured at a wavelength of 260 nm. Since paper blanks not only depend on the size of the spots but also increase with decreasing wavelength in the region of 260 to 220 nm, precise correction is necessary, in particular when determining ultraviolet absorption spectra. In repeated extract separations the deviation ($s$) of a measured extinction from a mean value may rise up to 20% for each spot; whilst standard errors ($s_m$) in most cases do not exceed 10 %. The lower the extinction values, the greater is, usually, the deviation and standard error[9]. If substances are only available in low concentrations, statistical evaluation of the quantitative differences of the same compounds in different series of treatments becomes difficult. Reliable information on the quantitative determination

of purine and pyrimidine derivatives by means of paper chromatography requires, therefore, precise knowledge of the limits of the method.

Only about 75 % of ultraviolet absorption in extracts is caused by the compounds investigated; the residue is due largely to flavonoids which are also soluble in ethanol. Using the method described above flavonoids did not overlap the purine and pyrimidine compounds in wheat and did not disturb the estimation, but in maize quantitative evaluation of uridine ($R_F$ 0.45) was hindered. Even aromatic or heterocyclic amino acids, which also show absorption maxima within the ultraviolet range, only interfere at high concentrations ($>$ 50 $\mu$g per spot). In seedlings of wheat and maize interfering amounts of such compounds do not occur.

During extraction a certain amount of nucleoside tri- and diphosphates is lost by decomposition; even during chromatographic separation about 15 % of these compounds are hydrolysed to monophosphates and ribosides by the acid solvent. Under strictly constant experimental conditions these quantitative changes may be neglected, if merely relative values are to be compared. In determining absolute values, however, they must be taken into account. Derivatives of adenine and uracil usually prevail. Thus the practicality of quantitative, and partly, even of qualitative detection of compounds occurring only in small quantities (*e.g.* ITP, GTP, CTP, GDP or CDP) is limited or much impeded, because the quality of separation also depends on the concentration of extracts.

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