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Short Communications

The Acid Soluble Nucleotides of *Vicia Faba* II

M. F. ABDEL-WAHAB and S. A. EL-KINAWI

Radioisotope Centre, A.E.E., Dokki, Cairo, U.A.R.

In a previous work from this department the acid soluble nucleotides, obtained by perchloric acid (PCA) extraction, of *Vicia Faba* plant just before fruiting, were studied [1]. The yield was found to be low at that stage of growth; this urged us to estimate the acid soluble nucleotides in the plant at different stages of germination.

In the last few years some important papers have been published about the metabolism of the acid soluble nucleotides in some plants and yeast [2-4]. Some difficulties arising in the separation from interfering substances of the nucleotides in plants in the pure form were overcome [5]. Previous methods developed primarily for the isolation of nucleotides from animal tissue proved to be unsatisfactory for the isolation of these compounds from plants. An improved method for the isolation of the acid soluble nucleotides from plant tissue has been described by Bergkvist [2]. The application of this method to *Vicia Faba*, seeds, pods, and seedling, has established the occurrence of ten nucleotides, *viz.* cytidine monophosphate, diphosphopyridine nucleotide, adenosine monophosphate, guanosine monophosphate, uridine monophosphate, adenosine diphosphate, a sugar derivative of uridine diphosphate, adenosine triphosphate, uridine triphosphate, and guanosine triphosphate.

Extracts prepared by directly homogenising plant material from any source in ice-cold perchloric acid demonstrated complex ultraviolet absorption spectra and hardly any separation of the acid soluble nucleotides from impurities [2]. Chromatography or ionophoresis at this stage proved also to be impracticable. Bergkvist overcame this difficulty by adsorbing the nucleotides quantitatively on Norite, and recovered them by repeated elution with 25 % aqueous ethanol containing 0.5 % ammonia. Spectrophotometric analysis of the combined eluates showed that the nucleotides were mixed with large quantities of non-nucleotides material [5].

In our laboratories a similar method was used where the whole organ was homogenised in 10 % PCA and reextracted with 5 % PCA in a Waring Blender. The nucleotides were isolated from the acid extract by the method used before [1]. After extraction and purification from the non-nucleotide fractions, the nucleotides were adsorbed on a strong base anion exchanger in the formate form. The individual nucleotides were eluted with formic acid solutions containing increasing concentrations of sodium formate. Elution of the nucleotides was followed by measuring the optical densities of the effluent fractions at 260 mμ and 270 mμ. The optical densities were plotted against the effluent volume. The extinction obtained from the eluate was plotted for the seeds, pods and seedlings, one, two, three and four weeks old. An example is shown in Fig. 1. The investigation was carried out on plants grown out of doors. The radioactivity of the effluent fractions was also determined. At all stages of germination the peaks of the extinction curves and the radioactivity curves coincided. This confirms the previous results that no reduced nucleotides are found in *Vicia Faba* [1]. The fact that in the course of germination ten distinct peaks

Table 1.

Compound	μmoles of nucleotides at different stages					
	seeds	1st week	2nd week	3rd week	4th week	Pods
CMP	2.00	1.00	1.50	1.00	0.32	0.08
DPN	0.30	0.20	0.60	0.20	0.28	0.07
AMP	0.28	0.30	0.50	0.21	0.50	0.10
GMP	0.20	0.22	0.20	0.24	0.26	0.08
UMP	0.32	0.32	0.30	0.22	0.30	0.07
ADP	0.21	0.20	0.20	0.23	0.28	0.08
UDPx	0.08	0.22	0.50	0.24	0.24	0.12
ATP	0.22	0.44	0.72	0.26	0.24	0.08
UTP	0.12	0.45	0.98	0.23	0.08	0.20
GTP	0.13	0.46	0.25	0.24	0.09	0.18

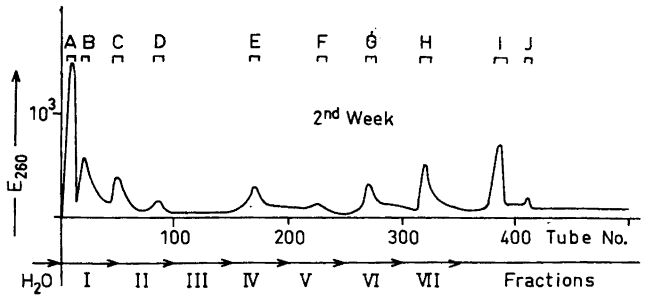

Fig. 1.

were observed, confirmed that no other nucleotides could exist.

Characterisation of the nucleotides was obtained by the following procedures:

1. Paper chromatography identification of the nucleotides and their bases after hydrolysis.
2. The position of the phosphate group on the different nucleotides was confirmed by oxidation with periodate [6].
3. Estimation of phosphorus for the different nucleotides [7].

Table 1 summarises the results obtained by plotting the extinction of the nucleotides at 260 mμ against the effluent fractions at different stages of germination.

Fig. 1 shows the absorption chromatogram at 260 mμ of the effluent fractions, collected by an automatic fraction collector, against the tube number. The material was obtained from 150 g of whole fresh *Vicia Faba* plants (2nd week seedlings) grown out of doors. Extraction by PCA as described above. Adsorption on Dowex 1, X-10, 200—400 mesh, 1 × 20 cm, formate form, flow rate 1 ml per min. The extinction was measured by means of a spectrophometer, Zeiss type PMQ II.

The peaks are identified as follows: A:CMP; B:DPN; C:AMP; D:GMP; E:UMP; F:ADP; G:UDPx; H:ATP; I:UTP; J:GTP. UDPx is a sugar derivative of uridine diphosphate.

The present work establishes the occurrence, in the beans, of all the four ribonucleotides in the form of the acid soluble 5′-nucleotides at different levels of phosphorylation. Nearly 30 % of the acid soluble nucleotides consists of cytidine and adenosine derivatives, the cytidine derivatives predominating. The acid extract of the seeds showed that CMP corresponds to the highest peak in the whole spectrum, next to CMP come UMP, DNP, AMP and ATP. The other five nucleotides are present to lesser extents. The same picture is found in the seedlings which are one week old with the exception that CMP is half its amount in the seeds. Another slightly different picture was observed in the second week but still CMP is present in the highest amount. Next in abundance are UTP, ATP, DNP, UDPx and UMP. GMP, GTP and ADP are present at levels which are nearly equal for the three nucleotides. At the end of the third week the acid extract showed another picture where almost all the nucleotides are present in more or less equal quantities with the exception that the amount of CMP is still high.

In the fourth week chromatogram, before fruiting, the picture was exactly the same as that previously obtained [1]. Experiments carried out with pods revealed a very low nucleotide yield.

From the preceding discussion one can conclude that the best material for obtaining good yield of the different nucleotides is the second week seedlings, see Fig. 1. Not only are the nucleotides present in good amounts, but they can also be well separated.

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