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Food Chemistry 52 (1995) 427–432
Elsevier Science Limited
Printed in Great Britain.
0308-8146/95/$9.50

Effect of germination on the ribonucleic acids (RNA) of some legume seeds (*Vicia faba, Cicer arietinum* and *Lupinus termes*)

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(Received 3 March 1993; revised version received and accepted 31 August 1993)

The present study was carried out in order to evaluate some Egyptian legume seeds (*Vicia faba, Cicer arietinum* and *Lupinus termes*) as raw and germinated foods, as sources of plant proteins. The work was extended to study the changes of nucleic acids (28S RNA, 18S RNA, 16S RNA, 5·8S RNA, 5S RNA, 4.5S RNA and tRNA) content and distribution during 1 week of germination using HPLC and electrophoresis techniques. The results are summarised as follows. (1) The RNAs from the first day of germination of seeds and roots were separated into five major types: 28/18S RNA, 16S RNA, 5·8S RNA, 5S RNA and tRNA. (2) The RNAs at the seventh day of germination in both seeds and roots are 16S RNA, 5S RNA, 4·5S RNA and tRNA, in addition to two degradation regions in the range of 28/18S RNA and tRNA. Also, appearance of new RNA (mainly 4·5S RNA, a chloroplast RNA) and disappearance of 5·8S RNA, which was present at the first day of germination, were as expected after 7 days of germination. (3) The 24 h germinated seeds have a mature and complete set of the RNA species which means that the synthesis of these RNA species already occurred during the first 24 h of imbibition. (4) A new buffer system for separating the total RNA into their species, 28/18S RNA, 5S RNA and tRNA by the HPLC technique, is also reported.

INTRODUCTION

In Egypt, it is common to germinate certain legume seeds which are rich in protein (20–50%), such as termes (*Lupinus termes*), broad bean (*Vicia faba*) and chick-pea (*Cicer arietinum*) before direct eating, cooking or using in salads.

Legume seed species including lupin, chick-pea and broad bean contain an average nucleic acid nitrogen of 23·4 mg/g nitrogen or 1·05 mg/g seed for all species (Holt & Sosulski, 1981).

Early RNA synthesis of germinated faba bean seeds and their embryos increased with a low RNase decrease from 15 to 20 h of soaking. This RNA synthesis could be essential for protein synthesis. RNA synthesis occurs as a wave very similar to that of DNA synthesis but it is hard to conclude an absolute parallelism between the two nucleic acids; the water uptake by the root tips might be the factor which limits the onset of synthesis of both nucleic acids. This suggests that the enzymic apparatus necessary for nucleic acid synthesis already exists in the dry meristem and merely requires activation (Jakob & Florence, 1969).

At approximately 32 h after the beginning of imbibition of *Vicia faba* seeds, the RNA synthesis precedes a maximum DNA replication and the embryos synthesise all types of rRNA, heterodisperse RNA, and 4·5S RNA before and also during the phase of DNA replication (Fukuei *et al.*, 1977). Fukuei *et al.* (1978) found that the first DNA replication in germinating *Vicia faba* embryos takes place after a lag period and synthesis of RNA and proteins precedes the DNA replication during germination; the maximum stage in the synthesis of ribosomal proteins coincides with that of rRNA synthesis, and histone synthesis.

Newly synthesised heterogeneous nuclear RNA and possibly mRNA, tRNA, 5S rRNA and precursor of rRNA are detectable 3 h after the start of imbibition of the decoated embryo and before completion of initial

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water uptake. Thus, synthesis of all major species of RNA is simultaneously initiated in the radicle of the germinating embryo. A complete maturation of the rRNA is only observed after 12 h (Van de Walle *et al.*, 1976, 1983).

During the first 15 days of germination of faba bean cotyledons, 73% and 80% of RNA and protein but only 20% of DNA were lost, as compared with dormant cotyledons (Dhillon & Miksche, 1983).

For *Cicer arietinum* the specific activity of RNA was found to increase three-fold within 2 h and five-fold within 10 h of germination (Ashok *et al.*, 1975). rRNA and mRNA synthesis occurred during 24 h of germination and were dependent on the embryo (Matilla *et al.*, 1982). Net synthesis of both DNA and RNA was initiated at 18 h after the onset of germination (Rodriguez *et al.*, 1982).

Germination of yellow lupin (*Lupinus termes*) seeds for 48 h indicated that nucleic acids in the germ increased especially in the first 6–12 h of growth, the gradual decrease of RNA in cotyledons was related to their transport in the germ and reduction by nucleases (Vecher *et al.*, 1970). The nucleic acid content increased in the germs and decreased in cotyledons, during the initial 48 h of germination (Vecher & Golynskaya, 1971).

The structure and possible function of some ribosomal RNAs have been studied by many investigators. Pieler *et al.* (1984) reported that the large ribosoal subunit of eukaryotic cells contains a small ribosomal RNA designed as 5S rRNA which is 120 nucleotides long and has a molecular weight of approximately 40 kDa (Erdmann *et al.*, 1987). The suggested function of 5S rRNA is the recognition of, and interaction with, other ribosomal components or molecules involved in protein biosynthesis and, more speculatively, its possible interaction with tRNAs, 16S rRNA and 23S rRNA (Pieler *et al.*, 1984; Erdmann *et al.*, 1987). Hariharan *et al.* (1987) found that the 48 h germinated embryos contain another species of 5S rRNA which differs by three nucleotides from the ungerminated embryos, thereby showing the expression of two 5S rRNA genes during germination.

The eukaryotic 60S ribosomal subunits contain a second small-sized 5·8S ribosomal RNA, which is 155 nucleotides in length and hardly modified. Sequence comparison show that this RNA contains significant homologies to the 5' end of prokaryotic 23S rRNAs (Pieter *et al.*, 1984) which is an RNA polymerase III transcript (Kiss *et al.*, 1987).

The third small-sized ribosomal RNA found is the large subunit of chloroplast 4·5S rRNA; it is approximately 105 nucleotides in length and does not contain modified nucleotides. Little is known about its function (Kiss *et al.*, 1987).

The aim of this investigation is to study the changes in RNAs during 1 week of germination in the seeds and roots of the following legume seeds: *Cicer arietinum, Vicia faba* and *Lupinus termes*. Two different methods were used, namely HPLC and polyacrylamide gel electrophoresis (including 8 M urea).

MATERIALS AND METHODS

Source of samples

Three different types of legume seeds (*Vicia faba*, Giza 402; *Lupinus termes*, Giza 2 and *Cicer arietinum*, Giza 2) were obtained from the Field Crops Research Institute, Agricultural Research Centre, Giza, Egypt.

Germination

Seeds were washed with distilled water, then surface-sterilised by washing for 60 s in 0·01% NaN_3 and germinated at 25°C in an aired and dark incubator according to Stegemann *et al.* (1987). Germinated samples were frozen and kept at –20°C.

RNA extraction

RNAs were extracted using cold phenol according to the method of Pieler (1984).

Ethanol precipitation

RNAs were precipitated by ethanol; the pellet was freeze-dried and dissolved in a small volume of distilled water. Optical density (OD) was measured at A_{260} according to the method of Pieler (1984).

Microdialysis

Desalting (microdialysis) was done according to the method of Marusyk and Sergeant (1980). The samples were carefully taken by a micropipette, and concentrated by freeze-drying. The OD was measured at A_{260} and kept at –20°C until use.

Separation of the RNA by HPLC

The RNA samples and RNA marker (tRNA/5sRNA) were separated using a column of silica-based aqueous gel, TSK-GEL G 3000 SW according to the method of Uchiyama *et al.* (1981). The run was done by using a computer program (Gold System, LKB, Germany) loaded on an IBM computer in connection with the HPLC from LKB.

Separation on urea–PAGE

Concentrated RNAs of the first and seventh day of germination of seeds and roots were applied with tRNA/5S RNA as a marker (from Serva, Heidelberg) to 10% 8 M urea–PAGE running at 14 W according to the method of Stegemann *et al.* (1987).

Gel staining and destaining

Staining and destaining of the gel were carried out according to the method of Pieler (1984).

HPLC buffer modification

The RNA samples were separated using the method of Uchiyama *et al.* (1981) except that another elution buffer was used (50 mM Tris, 20 mM $MgCl_2$, pH 7·0) instead of the (200 mM sodium phosphate, pH 7·0, containing 0·1% SDS).

RESULTS AND DISCUSSION

HPLC RNA separation

The total RNA extract of seeds and roots of the three investigated legume seeds from the first and seventh day of germination were subjected to HPLC analysis using silica gel TSK-GEL G 3000 column at A_{254}. The results are shown in Fig.1 for *Cicer arietinum*, Fig. 2 for *Lupinus termes* and Fig. 3 for *Vicia faba*, respectively.

At the first day of germination, the RNAs of seeds (1S) and roots (1R) in *Lupinus termes* consisted of three major peaks appearing at 6, 12·5 and 15 min for 28/18S RNA (peak a), 5S rRNA (peak b), and tRNA (peak c), respectively. tRNA was the most pronounced RNA in comparison with 28/18S RNA and 5S rRNA, respectively, while in both *Cicer arietinum*, and *Vicia faba* the 28/18S RNA is the most predominant RNA in comparison with the other RNAs, 1S and 1R. These results are in agreement with those of Uchiyama *et al.* (1981) using phosphate buffer pH 7·0.

On the other hand, the profile of the RNA distribution at seventh day of germination of *Cicer arietinum* (Fig. 1), *Lupinus termes* (Fig. 2) and *Vicia faba* (Fig. 3) seeds (7S) and roots (7R) gave additional small peaks 1, 2 and 3, at 10, 19 and 30 min, respectively, except for *Cicer arietinum* seeds (7S) which showed the first peak (1) at 17 min.

The results revealed that the small peaks 1, 2 and 3 may be considered as degradation products of 28/18S RNA and tRNA. For instance, peak 1 appeared after peak 28/18S RNA in all cases of 7S and 7R except that of *Cicer arietinum*, which means that the peak 1 molecular weight is lower than those of 5S rRNA and tRNA, while in the other cases of 7S and 7R peak 1 molecular weight is the same as 16S RNA. Peak 2 always appears after and close to the RNA peak (c) so this peak might be a result of degradation of tRNA. The very small RNA molecular weight of peak 3 appears far from this group; this peak might result from peak (a) by its relative size, and it seems to be 2S RNA.

The previous data first showed the possibility of degradation of RNA according to the type of germination (the period) and, second, showed a relationship between the three new peaks with the mother peaks, i.e. the 28/18S RNA peak (a) as mother peak and a new peak (3) and also the tRNA mother peak (c) and new peak (2). The new peaks arise from the degradation of the mother peaks.

Urea–PAGE RNA separation

The electrophoretic separation of total RNAs was only carried out after the first day (Fig. 4A) and the seventh

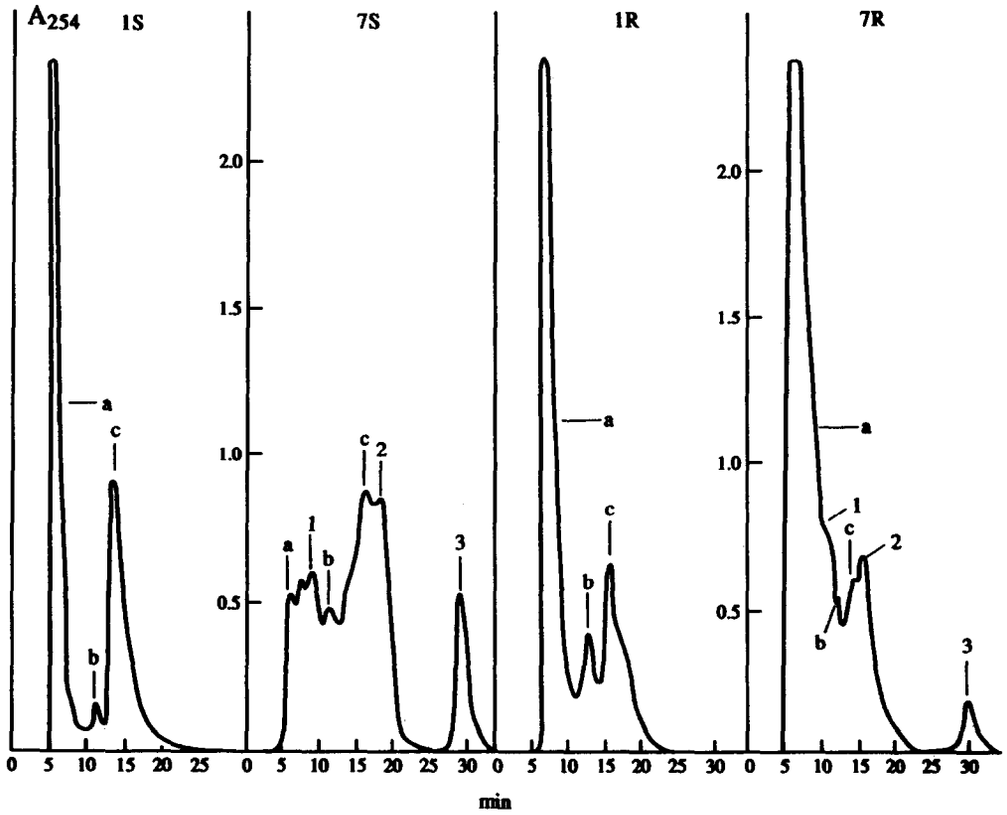

Fig. 1. HPLC chart of total RNA of *Cicer arietinum* seeds and roots at the first (1S and 1R) and seventh (7S and 7R) day of germination. a,b,c — 28S + 18S RNA, 5S rRNA and tRNA, respectively. 1,2,3 — New peaks appear at the seventh day of germination.

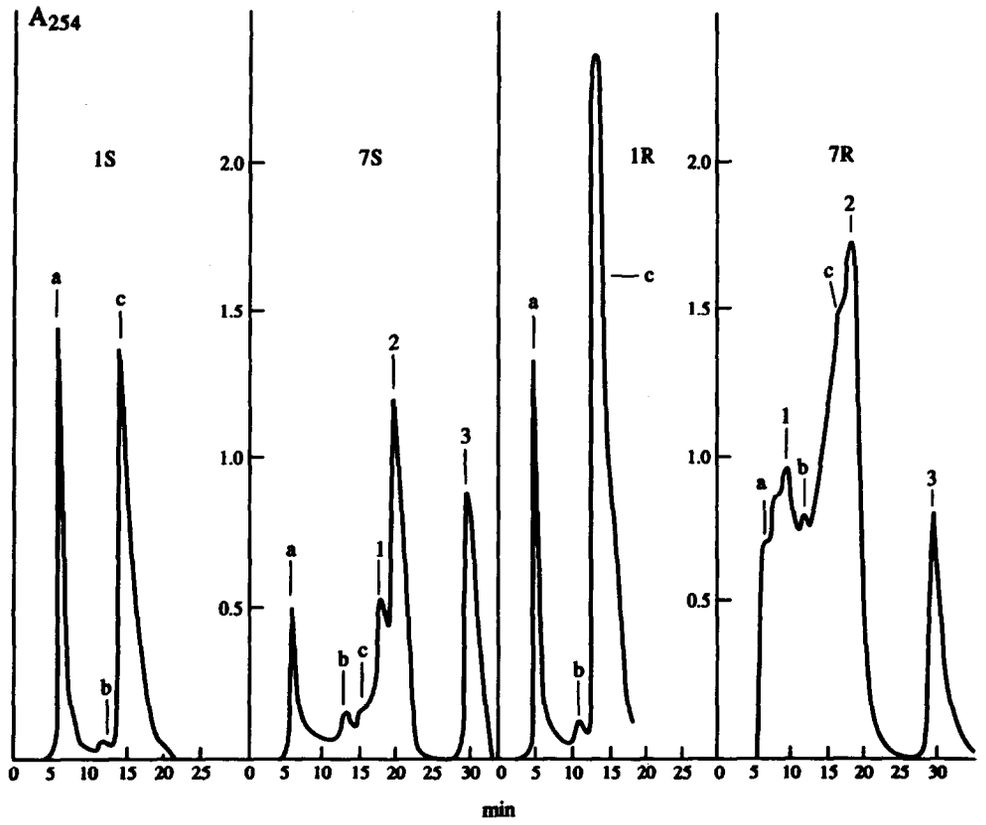

Fig. 2. HPLC chart of total RNA of *Lupinus termes* seeds and roots at the first (1S and 1R) and seventh (7S and 7R) day of germination. a,b,c — 28S + 18S RNA, 5S rRNA and tRNA, respectively. 1,2,3 — New peaks appear at the seventh day of germination.

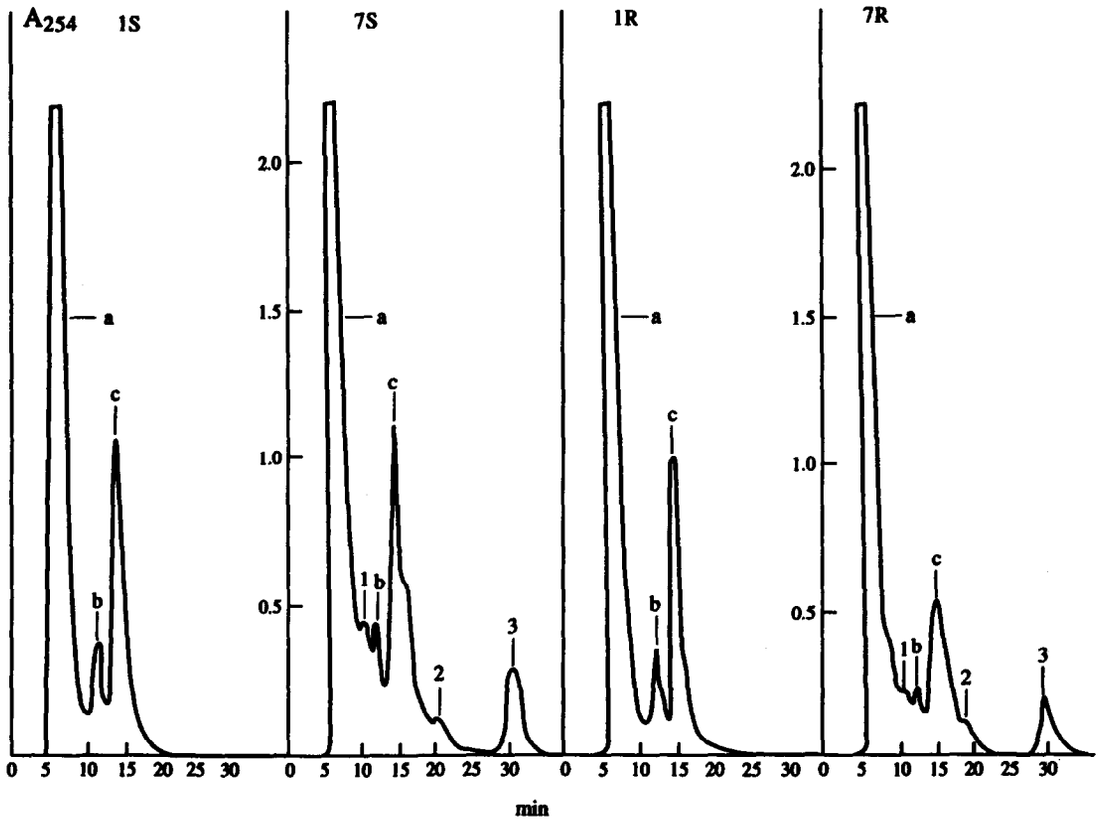

Fig. 3. HPLC chart of total RNA of *Vicia faba* seeds and roots at the first (1S and 1R) and seventh (7S and 7R) day of germination. a,b,c — 28S + 18S RNA, 5S rRNA and tRNA, respectively. 1,2,3 — New peaks appear at the seventh day of germination.

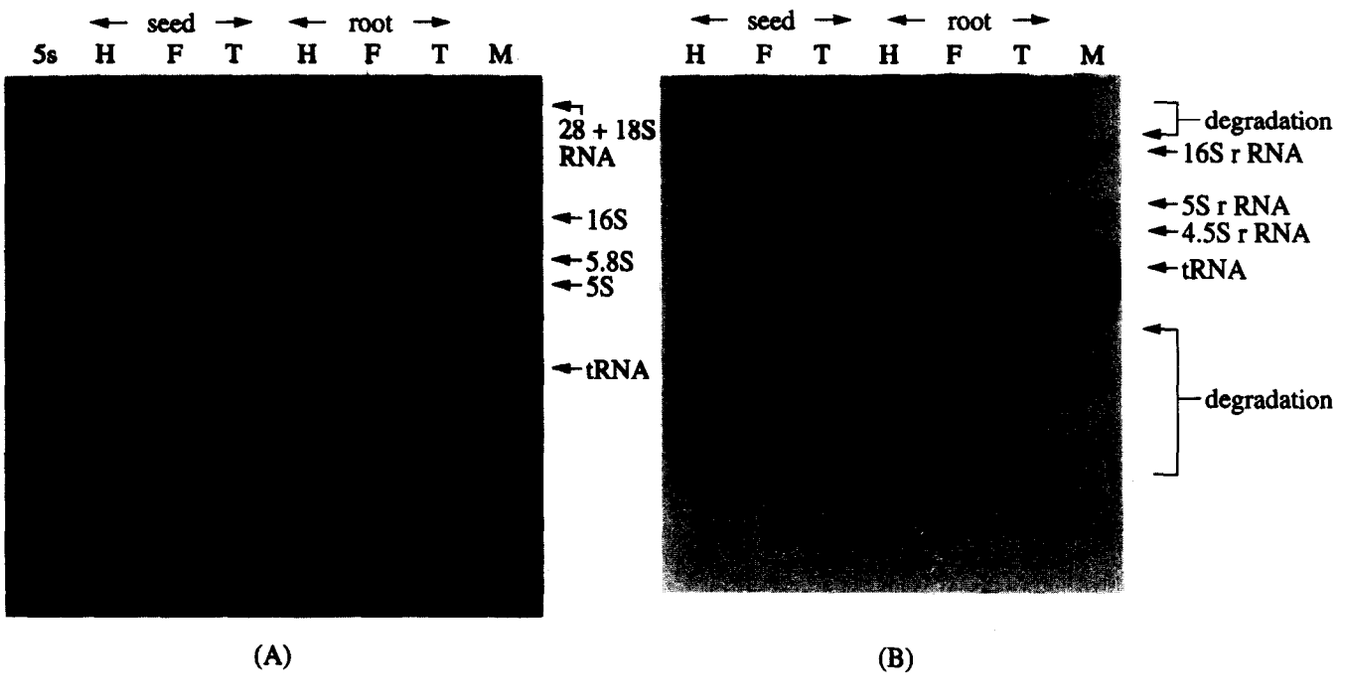

Fig. 4. Comparison between the total phenol extract RNA at (A) the first, (B) the seventh days of germination. T, *Lupinus termes*, F, *Vicia faba*, H, *Cicer arietinum*. 5S is the 5S rRNA isolated from non-germinated *Vicia faba* seed and used here as a marker. M is a tRNA marker containing 5S and 16S rRNA from yeast.

day (Fig. 4B) of germination, for seeds and roots from the three legumes. The results in Fig. 4(A) show that the RNAs from the first day of germination in seeds and roots are separated into five major types: 28/18S RNA, 16S RNA, 5·8S RNA, 5S RNA and tRNA. The following are observed:

(1) 28/18S RNA remains at the upper part of the gel and looks like waves but not like bands in any samples because of its high molecular weight;
(2) tRNA consists of two heavy bands in all samples; and
(3) all samples contain a sharp band upper and close to 5S RNA; this band was identified as 5·8S RNA.

The results in Fig. 4(B) reveal that the main types of RNA at the seventh day of germination in both seeds and roots are 16S RN, 5S RNA, 4·5S RNA and tRNA, in addition to two degraded regions at the upper part of the gel in the range of 28/18S RNA and down to the lower gel for tRNA. From the data the following can be observed:

(1) 28/18S RNA at the upper part of the gel was degraded by germination for 7 days into several bands very close to each other;
(2) tRNA appears as only one band instead of two as shown at the first day of germination; this may be due to its degradation;
(3) a new RNA band appears lower and close to the 5S RNA which may be 4·5S RNA (chloroplast RNA); and
(4) the band of 5·8S RNA which was present at the first day of germination disappeared at the seventh day of germination.

From the previous results, it was clear that the seeds germinated for 24 h have a mature and complete set of the RNAs species, which means that the synthesis of these RNA species already occurred during the first 24 h of imbibition. These results are in line with those observed by Van de Walle *et al.* (1976, 1983), Jakob and Florence (1969) and Morales *et al.* (1987), who found that a complete maturation of the RNA occurred after 12 h of germination, meaning that water uptake by the root tips might be the factor limiting the onset of synthesis of the nucleic acids. This would suggest that the enzymic apparatus necessary for nucleic acid synthesis already existed in the dry meristem and merely required activation.

New buffer for RNA separation by HPLC

The method of Uchiyama *et al.* (1981) was used to separate the RNA species from all RNA samples but with modified elution buffer containing Tris–HCl and $MgCl_2$ at pH 7·0. The separated peaks were then subjected to 10% PAGE containing 8 M urea. The results are shown in Figs 5(A) and 5(B). The figure presents sharp peaks and good separation of the RNA species 28/18S RNA, 5S rRNA and tRNA. The separation seems to be better than that of Figs 1 and 2 in which the original method of Uchiyama *et al.* (1981) with phosphate buffer pH 7·0 was used for elution. It is also clear that by using Tris–HCl/$MgCl_2$ buffer the peaks are far from each other and sharper. The only disadvantage is the time of separation, about 80 min, which was only 30 min when the phosphate buffer was used.

Figure 5(A) explains and confirms the results of Fig. 5(B), where the separated peaks are plotted on the gel, and give sharp bands of 5S rRNA (lane 3) and tRNA

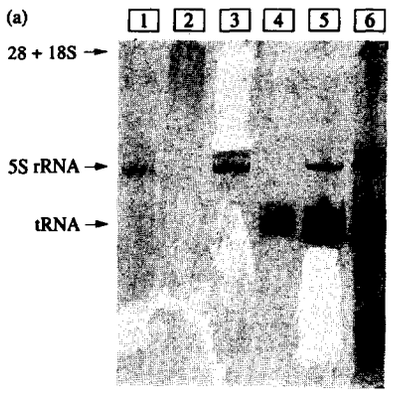

Fig. 5(a). Urea gel of separated RNA from faba bean by HPLC with a modified buffer. 1 and 3, 5S rRNA (two concentrations); 2, 28S + 18S RNAs; 4, tRNA; 5, tRNA/5S RNA marker from yeast; 6, Total RNAs of faba bean.

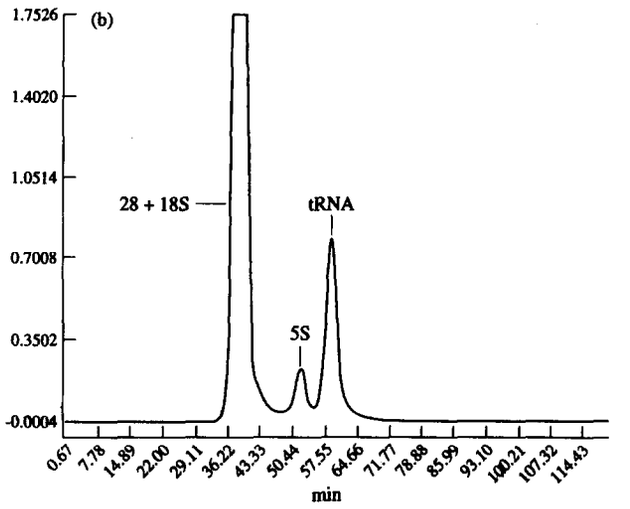

Fig. 5(b). HPLC chart the separation of RNAs from faba bean with a modified buffer.

(lane 4), while the high molecular weight 28/18S RNA is difficult to resolve in 10% gel concentration, and appears as a wave in the upper part of the gel (lane 2).

REFERENCES

Ashok, K. & Azhar, S. & Krishna Murti, C. R. (1975). *Phytochemistry*, **14**, 903–7.

Dhillon, S. S. & Miksche, J. P. (1983). *Histochem. J.*, **15**(1), 21–37.

Erdmann, V. A., Walters, J., Pieler, T., Degweed, M., Specht, T. & Ulbrich, N. (1987). *Endocytobiology III* (Vol. 503), pp. 103–24.

Fukuei, K., Sakamaki, T., Takahashi, N., Takaiwa, F. & Tanifuji, S. (1977). *Plant cell Physiol.*, **18**(1), 173–80.

Fukuei, K., Yakura, K. & Tanifuji, S. (1978). *Biochim. Biophys. Acta*, **518**, 390–400.

Hariharan, N., Reddy, P. S. & Padayatt, J. D. (1987). *Plant Molec. Biol.*, **9**, 443–51.

Holt, N. W. & Sosulski, F. W. (1981). *Can. J. Plant Sci.*, **61**(3), 515–23.

Jakob, K. M. & Florence, B. (1969). *Expr. Cell Res.*, **54**, 118–26.

Kiss, T., Antal, M. & Solymosy, F. (1987). *Nucleic Acid Res.*, **15**(2), 543–60.

Marusyk, R. & Sergeant, A. (1980). *Anal. Biochem.*, **105**, 403–4.

Matilla, A., Nicolas, G. & Sierra, J. M. (1982). *Plant Sci. Lett.*, **25**(2), 209–17.

Morales, C., Cusido, R. M. & Seranno, M. (1987). *Rev. Esp. Fisiol.*, **43**(1), 87–93.

Pieler, T. (1984). Ribosomale 5S RNS — ein Modell-system für RNS (RNA) Strukture und Funkionsuntersuchungen. PhD thesis from Dept. of Chemistry. FU Thielallee, Berlin, Germany.

Pieler, T., Digweed, M. & Erdmann, V. A. (1984). The structure and function of ribosomal 5S rRNAs. *Gene Expression* (Alfred Benzon Symposium 19), ed. F. C. Brian. Clark & Hans Uffem Munksgaard, Copenhagen.

Rodriguez, D., Nicolas, G. & Matilla, A. (1982). *Acta Physiol. Plant.*, **4**(3–4), 97–102.

Stegemann, H., Burgermeister, W., Francksen, H. & Kröger-reclenfort, E. (1987). PANTA-PHOR (and MONO-PHOR). In *Gel Electrophoresis and Isoelectric Focusing* (Manual book, revised edn).

Uchiyama, S., Imamura, T., Nagai, S. & Konishi, K. (1981). *J. Biochem.*, **90**, 643–8.

Van de Walle, C., Forgeur, G., Bernier, G., Deltour, R. & Bornchart, R. (1976). *Arch. Int. Physiol. Biochem.*, **84**(3), 676–7.

Van de Walle, C., Deltour, R. & Forgeur, G. (1983). *Physiol. Plant.*, **57**(2), 181–8.

Vecher, A. S. & Golynskaya, L. A. (1971). Change in the nucleic acid and ribonuclease activity of germinating lupin seeds. *Dokl. Akad. Nauk Belorussia, SSR*, **15**(9), 78–87.

Vecher, A. S., Bulko, O. P. & Golynskaya, L. A. (1970). *Dokl. Akad. Nauk Belorussia, SSR*, **19**(5), 471–4.