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The Effect of 2-Thiouracil on RNA Synthesis in Pollen Tubes of *Nicotiana alata*

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Abstract. The level of RNA in pollen is approximately 20 mg g^{-1} and remains constant during 6 h pollen germination *in vitro* also in the presence of 2-thiouracil which stimulates pollen tube elongation. The synthesis of RNA in pollen tubes was investigated according to the incorporation of the label from uracil-2-^{14}C, 2-thiouracil-2-^{14}C, orotic acid-5-^{3}H, fructose-U-^{14}C and from $^{32}PO_4^{3-}$ into RNA fractions separated by methylated albumine kieselguhr chromatography. The distribution of radioactivity on elution profiles was different according to the radioactivity source, however it was not changed by the presence of 2-thiouracil in cultivation medium. 2-Thiouracil incorporates into pollen tube RNA at about 50% the rate of uracil. It inhibited the incorporation of orotic acid, of fructose and of phosphate into all RNA fractions. It is suggested that the analogue inhibits the enzymes involved in RNA synthesis essentially as 2-thiouridine-5'-phosphate.

2-thiouracil and some other analogues or nucleic acid bases greatly stimulate pollen tube growth *in vitro* (Tupý *et al.* 1965). In stimulated pollen tubes protein synthesis is enhanced, however the synthesis of RNA, as determined by incorporation of phosphate, of uracil and of orotic acid into the total RNA fraction, was found to be inhibited (Tupý 1966). These results arouse an interest to see how 2-thiouracil affects the synthesis of different types of pollen tube RNA.

Most RNA formed in pollen tube culture is polydisperse in size (Mascarenhas and Bell 1970) and its base composition resembles that of DNA (Tano and Takanahashi 1964). For its fractionation we made use of chromatography on MAK columns which proved to be suitable for the separation of DNA-like RNA (*e.g.* Oravec and Sourkes 1974).

Material and Methods

The pollen of *Nicotiana alata* Link et Otto was collected shortly after its release from the anthers and stored at about —10 °C. As cultivation medium 0.3 M sucrose solution in 0.001% boric acid with or without thiouracil was used. The concentration of thiouracil was 0.1 mg ml^{-1} which stimulates pollen tube growth by about 100% (Tupý *et al.* 1965). The volume of 1.5 ml was pipetted into Petri dishes of 5 cm in diameter and 50 mg of pollen was sown on the surface

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of the solution. The Petri dishes were then kept in moist chambers at 26 °C. The growth of pollen tubes was stopped by addition of potassium acetate to the concentration of 2% and of 2.5 volumes of ethylalcohol.

Before RNA extraction the samples were left at least overnight at —10 °C.

The total RNA was determined according to the method described earlier (SÜSS 1972). The 10% NaCl solution used for RNA extraction contained acetate buffer pH 5.2 at 0.1 M concentration. Also the phenol method of RNA extraction and the MAK chromatography were the same as already described (TUPÝ and RANGASWAMY 1973).

To determine the radioactivity in the fractions from the columns, the nucleic acids were precipitated by trichloracetic acid after adding albumine as carrier; the precipitate was dissolved in hyamine hydroxide and mixed with the scintillation solution of PPO in toluene-methanol. The ^{32}P-labelled samples were measured directly as aqueous solutions. All measurements were carried out in a Nuclear Chicago Mark I Model spectrometer. The values obtained were corrected on isotope disintegration and on efficiency of measurement which was 80%, 25% and 26% respectively for ^{14}C, ^{3}H and ^{32}P.

Results and Discussion

The Level of Total Pollen RNA During Pollen Tube Growth

In agreement with earlier estimations (SÜSS 1972) the total amount of RNA in *Nicotiana alata* pollen was found to be approximately 20 mg g^{-1} (Table 1). This level remains practically constant during the period of 6 h

TABLE 1

The level of RNA in pollen during 6 h of germination. Means of 3 experiments and of 6 samples. The small differences between the values are insignificant

Cultivation [h]	mg RNA g^{-1} pollen	
	Control	2-TU
0	20.6	20.9
2	20.9	21.8
4	20.6	21.5
6	21.1	20.9

of cultivation and is not affected by thiouracil. Similar results were obtained in experiments in which the pollen was cultivated under shaking. These results together with the earlier finding on inhibitory effect of thiouracil on RNA synthesis (TUPÝ 1966) show that the RNA formed in germinating pollen represents a very small fraction of the total RNA. They are consistent with the conclusion of MASCARENHAS and BELL (1970) that rRNA and tRNA required for pollen tube growth are synthesized before the pollen is released from the anther.

MAK Chromatography of RNA Synthesized in Pollen Tubes

In preliminary experiments the rate of utilization of exogenous phosphate was found to be the highest at the beginning of pollen germination, most probably as a result of the great uptake of water solutes due to pollen swelling and initial rapid tube growth. Based on these observations, the pollen tubes

Abbreviations used: MAK, methylated albumine kieselguhr; 2-TU, 2-thiouracil; PPO, 2,5-difenyloxazol.

were grown in further experiments in the radioactive solution for a period of 4 h from the time of pollen sowing. Several radioactive compounds were used as RNA precursors. In general, the MAK elution profiles showed one or two radioactivity peaks in the region of sRNA (tRNA + 5 S RNA) and two eluted together with rRNA (Fig. 1). However, the radioactivity did not

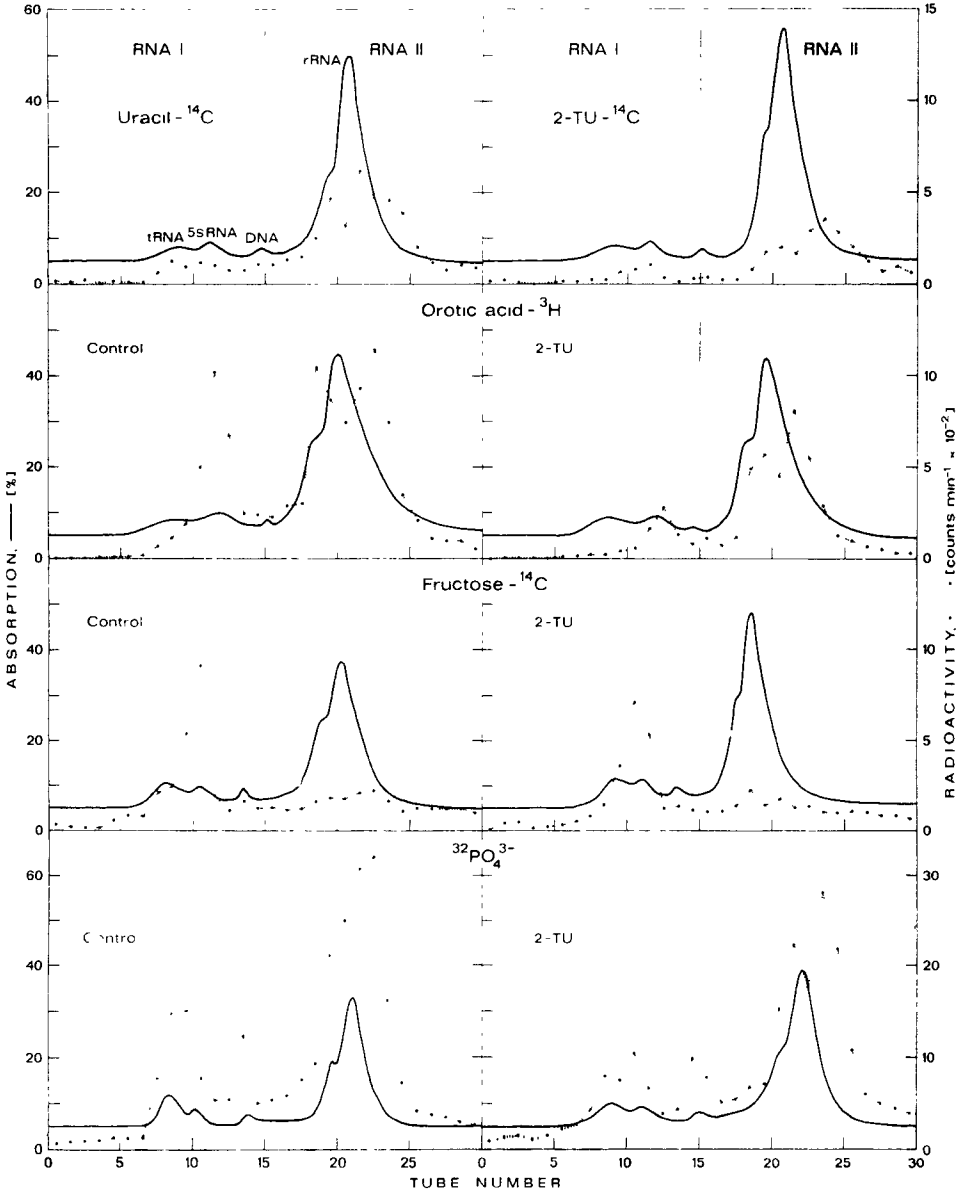

Fig. 1. MAK chromatography of RNA formed during 4 h of pollen germination. Uracil-2-^{14}C (30 mCi mmol^{-1}), 2-thiouracil-2-^{14}C (30 mCi mmol^{-1}), orotic acid-5-^{3}H (15.6 Ci mmol^{-1}, D-fructose-U-^{14}C (100 mCi mmol^{-1}) or carrier free $^{32}PO_4^{3-}$ were added to the activity of 10 µCi ml^{-1} at the time of pollen sowing. Linear gradient from 75 ml each of 0.3 M and 1.1 M NaCl.

follow the absorption curve, similarly as in experiments of STEFFENSEN on *Lilium* pollen (1966). This is especially the case of the RNA eluted at concentrations higher than 0.7 N NaCl which indicates that the radioactive RNA is not rRNA. We were able to confirme this by sucrose density gradient centrifugation (unpublished results). The fact that no rRNA is synthesized in pollen tubes was already demonstrated by MASCARENHAS and BELL (1970).

For better comparison of RNA labelling in different samples, the total A_{260} absorbance and radioactivity were measured in pooled aliquots of fractions obtained from MAK columns (Table 2). The material eluted before and after DNA was pooled separately and because the two combined fractions contain several types of RNA they are called RNA I and RNA II. Essentially, the RNA I corresponds to low-molecular-weight RNA and RNA II to high-molecular-weight RNA. Nevertheless, the specific radioactivity from the total absorbancy and the total counts was calculated to eliminate the effect of the variation in the amount of the separated RNA.

2-Thiouracil-2-^{14}C is incorporated into pollen tube RNA at a rate of about 50% as compared with uracil-2-^{14}C. The distribution of radioactivity on MAK chromatograms is similar in both cases, being very low in the region of sRNA. On the other hand, a high specific radioactivity peak appears in the position of 5 S RNA when orotic acid-5-^{3}H is used as RNA precursor. The synthesis of this fraction is strongly inhibited by thiouracil. The compound also inhibits orotic acid incorporation into RNA II fraction.

In further work we tested the effect of thiouracil on the overall metabolism of RNA synthesis from the basic sugar source. For this purpose we used fructose-U-^{14}C because of its rapid utilization in pollen tube respiration (TUPÝ 1962). Incorporation of the label into RNA II fraction was relatively low and again was inhibited by thiouracil by about 35%. The high peak of radioactivity which appears on MAK chromatograms between tRNA and 5 S RNA was only slightly lower when thiouracil was present. Thiouracil also inhibits the incorporation of $^{32}PO_4^{3-}$ into all RNA fractions separated on the MAK column. The radioactivity peak in the position of DNA belongs to polyphosphate (*e.g.* RICHTER 1966) and was not included into the pooled RNA fractions.

From the results obtained we can conclude that the formation of at least most RNA species in pollen tubes is inhibited by thiouracil. It is of interest that in spite of this inhibition thiouracil stimulates pollen tube growth. It indicates that RNA synthesized is not important at least for their initial growth. This also follows from the observation of MASCARENHAS (1966) on *Tradescantia* and of DEXHEIMER (1968) on several pollen species that in the presence of actinomycin D germination and early pollen tube elongation are not inhibited.

Concerning the mechanism of 2-thiouracil inhibition of RNA synthesis different results were obtained with different biological material. For example, in *Escherichia coli* 2-thiouracil decreases orotic acid utilization but not that of uracil (CARDEILHAC 1967). In animal material thiouracil and its metabolites thiouridine and thiouridine-5′-phosphate were shown to inhibit competitively the utilization of normal substrates. In addition, thiouridine-5′-phosphate exerts negative feedback inhibition of orotidine-5′-phosphate decarboxylase similarly as uridine-5′-phosphate (LINDSAY and YU 1974). In pollen tubes thiouracil decreases RNA synthesis from all precursors so far tested, es-

TABLE 2

Effect of 2-thiouracil on RNA synthesis. Values for pooled fractions from MAK columns. Experiment described in Fig. 1

Substrate	RNA fraction	Absorbance units at 260 nm		Counts min^{-1}		Counts min^{-1} per absorbance unit		
		Control	2-TU	Control	2-TU	Control	2-TU	% of the control
In control:								
Uracil-2-^{14}C	RNA I	0.20	0.16	634	403	3 170	2 519	79.5
In sample with 2-TU:	RNA II	2.66	2.91	4 045	2 196	1 521	755	49.6
2-Thiouracil-2-^{14}C	Total RNA	2.86	3.07	4 679	2 599	1 636	847	51.8
Orotic acid-5-^{3}H	RNA I	0.44	0.44	3 043	781	6 916	1 775	25.7
	RNA II	2.99	2.52	7 177	3 791	2 400	1 504	62.7
	Total RNA	3.43	2.96	10 220	4 572	2 980	1 545	51.8
Fructose-U-^{14}C	RNA I	0.26	0.28	2 455	2 135	9 442	7 625	80.8
	RNA II	1.98	2.32	2 196	1 671	1 109	720	64.9
	Total RNA	2.42	2.60	4 651	3 806	2 076	1 464	70.5
$^{32}PO_4^{3-}$	RNA I	0.30	0.38	22 788	20 984	75 960	55 221	72.7
	RNA II	1.82	2.38	73 472	63 464	40 369	26 666	66.1
	Total RNA	2.12	2.76	96 260	84 448	45 406	30 597	67.4

pecially from orotic acid, the utilization of which is inhibited by about 50%. This suggests that the analogue inhibits the enzymes involved in RNA synthesis rather as thiouridine-5′-phosphate than as free base. This is further supported by the following observations. Thiouracil itself does not influence orotidine-5′-phosphate pyrophosphorylase (LINDSAY and YU 1974). The limiting factor of thiouracil metabolism in pyrimidine pathways seems to be the utilization of thiouridine-5′-phosphate (YU *et al.* 1972) which might lead to its accumulation. The growth effect of thiouracil in pollen tube culture is reverted by uridine-5′-phosphate (TUPÝ 1966).

In addition to orotidine-5′-phosphate decarboxylase, thiouridine-5′-phosphate also strongly inhibits aspartate transcarbamylase (GOODRICH and CARDEILHAC 1970). The inhibition of this enzyme may increase the level of aspartic acid and the formation of arginine from carbamylphosphate which might favour protein synthesis (CARDEILHAC 1967). In this way one could explain the enhanced protein synthesis in the presence of thiouracil and also the stimulatory effect of some other analogues on protein formation (TUPÝ 1966), as aspartate transcarbamylase is inhibited by a number of nucleotides and related compounds (LONDON and SCHMIDT 1972).

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J. Süss, J. Tupý (Praha): Vliv 2-thiouracilu na syntézu RNA v pylových láčkách *Nicotiana alata*. — Biol. Plant. **18** : 119—125, 1976.

Obsah celkové RNA v pylu je asi 20 mg g^{-1} a zůstává konstantní během 6 h klíčení *in vitro* i v přítomnosti 2-thiouracilu, který stimuluje růst pylových láček. Syntéza RNA v pylových láčkách byla sledována měřením inkorporace uracilu-2-^{14}C, 2-thiouracilu-2-^{14}C, kyseliny orotové-5-^{3}H, fruktózy-U-^{14}C a $^{32}PO_4^{3-}$ do frakcí RNA, rozdělených chromatografií na křemelině s metylovaným albumínem. Rozložení radioaktivity na chromatogramech záviselo na zdroji radioaktivity a nebylo ovlivněno přítomností 2-thiouracilu v kultivačním mediu. 2-Thiouracil se do RNA pylových láček inkorporuje s 50% intensitou vzhledem k uracilu, přičemž inhibuje inkorporaci kyseliny orotové, fruktózy a fosforečnanu do všech frakcí RNA. Předpokládá se, že analog inhibuje enzymy zúčastněné při syntéze RNA převážně jako 2-thiouridin-5'-fosfát.

BOOK REVIEWS

Gerlach, A.: **Methodische Untersuchungen zur Bestimmung der Stickstoffnettomineralisation.** — Scripta Geobotanica 5. Verlag Erich Goltze K 6, Göttingen 1973, 115 S, DM 10.

Diese Monographie aus dem Lehrstuhl für Geobotanik der Universität Göttingen setzt eine Reihe heute schon klassischer ökologischer Studien über Stickstoff als Standortsfaktor und über Stickstoffmineralisation in Waldböden von Zöttl, Ellenberg, Runge *etc.* fort. Die beschriebenen methodischen Untersuchungen wurden in Böden der im Rahmen des Internationalen Biologischen Programms studierten Probeflächen Solling und an einigen anderen Vergleichsprobeflächen durchgeführt.

Die Stickstoffmineralisation wird am meisten in Brutversuchen bestimmt und dabei ist es immer notwendig eine Reihe von Bodenproben rasch zu bearbeiten. Die Bestimmung des Ammoniumstickstoffes im Boden mit dem Conway-Verfahren und des Nitrit- und Nitratstickstoffes mit dem Xylenolverfahren ist sehr zeitraubend. Darum schlägt der Autor eine neue rasche und genaue Mikrodestillationsmethode für die fraktionierte Mineralstickstoffanalyse vor. Eine Hälfte der Arbeit ist den Untersuchungen gewidmet, die die Reproduzierbarkeit, den Konzentrationsbereich und die Genauigkeit der neuen Methode überprüfen.

In Feldversuchen wurde der Einfluss von Zerstörung der Bodenstruktur, der Mischung von Bodenmaterial aus verschiedenen Stellen eines Horizonts und Standorts auf die Stickstoffnettomineralisation mittels der Brutversuche untersucht. Weiter wurde die Auswirkung der Wurzelmasse und die Auswirkung der Erwärmung von kaltem Bodenmaterial auf die Veränderungen der N_{min}-Konzentration studiert.

Die Arbeit gibt Auskunft über eine neue, rasche und präzise Methode für Bestimmung von Mineralstickstoff in Böden, weiter macht sie auf eine Reihe von methodischen Problemen beim Studium von Stickstofftransformationen in Böden aufmerksam und schliesslich gibt sie einige wichtige Charakteristiken über Stickstoffnettomineralisation in den wichtigsten Böden der Standorte von IBP-Beständen an.

Die vorliegende Arbeit dürfte für Ökologen, Forstleute, Pedologen, Bodenmikrobiologen und für Botaniker von Interesse sein. Auch für die Landwirte kann diese Arbeit zu einem bedeutenden Beitrag bei ihrer wissenschaftlich geplanten Arbeit werden.

Blanka Úlehlová (*Brno*)

Computer Simulation of a Cotton Production System. Users Manual. ARS-S-52. Agricultural Research Service. U.S. Department of Agriculture 1975. Pp. 1—101.

The publication describes four simulation models of subsystems in the cotton production system. The work was carried out under Regional Research Project S-69 by the personnel of the Agricultural Research Service, U.S. Department of Agriculture and 10 State Agricultural Experiment Stations. The model MOIST predicts soil water profiles during the drying of soil. The model EMERGE describes the germination and emergence of the seed. SIMCOTT II is based on conservation principles that include a carbohydrate balance, a moisture balance, and a nitrogen balance for the plant. HARVISM simulates harvesting, transporting, storing, and ginning of cotton from a farm in a gin community. Each of the part contains a description of the model, its limitations, definition of terms, input/output, program setup and execution, program listing and example run. The programs are written in FORTRAN. The publication is a contribution to optimizing crop production systems.

Ingrid Tichá (*Praha*)