Fractions of nucleic acids in seedlings and leaves of winter wheat in the vegetative and generative development phases

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Abstract

The composition of nucleic acid preparations isolated from seedlings and leaves of plants grown from vernalized and nonvernalized winter wheat grain was chromatographically investigated on a methylated albumin kieselguhr column (MAK column). The intensity of synthesis of the particular nucleic acid fractions was compared by the ^{32}P -incorporation technique and the nucleotide composition of the newly synthesized nucleic acid fractions was determined. It was observed that the rate of ^{32}P incorporation into the sRNA, rRNA+mRNA and TB-RNA fractions of the leaves of vernalized plants was about two times higher as compared with that in the nonvernalized control.

INTRODUCTION

The hypotheses concerning the important role of nucleic acids in the mechanism of thermic induction of flowering, i.e. vernalization were advanced mainly on the basis of results of experiments in which, for vernalization of cold-requiring plants, treatment with nucleic acid solutions and their components was substituted. On the other hand, vernalization could be abolished by means of nucleic acids antimetabolites. Positive results in this respect were obtained among other authors by Evans (1959), Hess (1959), Tomita (1964) and Suge and Yamada (1963, 1965).

Direct investigations of nucleic acids in plants in connection with the vernalization process have been undertaken within a relatively narrow scope. They consisted mainly in determination of changes in nucleic acid content (Konarev, 1954; Finch and Carr, 1956; Sechet, 1962; Stoletov et al., 1965; Shvedskaya and Kruzhi-

lin, 1968 and Markowski and Madej, 1968). In general, no major differences in the DNA and RNA contents in plant material were found, specific for the process of induction of flowering under conditions of low temperature. Neither did the investigations of Markowski et al. (1968) dealing with the nucleotide composition of wheat germ RNA and DNA show any marked changes in this respect.

The results of studies on the role of nucleolytic enzymes in processes of vernalization inducing flowering reported by Devay (1965, 1966), Markowski and Piskornik (1970 a, b) and Markowski and Dubert (1972) are not quite concordant and the investigations are continued.

The present study was undertaken to compare: (a) the contribution of various nucleic acid fractions in preparations from plant material previously vernalized or not, (b) the intensity of synthesis of various nucleic acids' fractions by way of determination of the rate of incorporation into them of the radioactive phosphorus isotope ^{32}P and (c) the nucleotide composition of the newly synthesised fractions of these objects. Similar methods find more and more applications in the study of biochemical and molecular mechanisms controlling various physiological processes.

MATERIAL AND METHODS

The investigations involved leaves and etiolated seedlings of winter wheat Ostka Złotokłosa grown from vernalized and nonvernalized grain. The grains were vernalized under conditions of sterility after Markowski (1962). Grain sterilization was achieved by means of 5 per cent aqueous solution of calcium hypochloritechloride CaOCl_2 . Grain vernalization was achieved in special containers in the dark at $+1$ to $+2^\circ\text{C}$ with constant flow of sterile air for a period of 63—70 days. The grain for growing the control plants was pregerminated at 20° in darkness for a time so adjusted as to obtain similar vernalized and control seedlings. Etiolated seedlings vernalized or not were taken for investigation of their nucleic acids (in this case without application of ^{32}P), or set in pots with sand and a nutrient solution. The plants were cultured in vegetation chambers at 20° for about 40 days under 14—16 h of daylight in 24 h, light intensity at the height of the leaves was 5,000 lx. At this phase of development (beginning of shooting of vernalized plants) leaves were collected for examination from the entire plants. Damaged leaf blades, yellow ones etc. were discarded. Five-gram leaf portions were thoroughly washed in distilled and sterilized water, cut in pieces (Munsche and Wollgiehn, 1970; Wollgiehn and Munsche, 1970). The samples were incubated exposed to light on Petri dishes in 90—120 ml

sterile solution containing 10^{-4} citric acid, 1 per cent sucrose and carrier-free H^{32}PO_4 (Cherry et al., 1965). Specific radioactivity of the solution was 1 mCi per 30 ml. The solution pH was adjusted to 6.0 with NH_4OH . The preparations were incubated for 1 h at 25° . As shown by tests, addition of antibiotics to the incubation medium proved unnecessary, as reported by other authors.

Isolation of nucleic acids from plant material was done by the detergent-phenol method used by Cherry et al. (1965, 1967), but with further purification of the preparations according to Ralph and Bellamy's method (1964) with the use of cetyltrimethylammonium bromide (CTA-Br).

The purified nucleic acid preparations were fractionated on MAK (methylated albumin-kieselguhr) columns according to the method of Mandell and Hershey (1960) with the introduction of a modification similar to that described by Yamane and Sueoka (1963). A glass chromatographic column, inner diameter 1.5 cm, with a water jacket was used. For forming the layer in the column 12 g of kieselguhr, Celite-Hyflo, mixed with 100 ml 0.1 M NaCl in 0.05 M phosphate buffer, pH 6.7, and 5 ml of 1 per cent aqueous solution of methylated albumin from bovine blood, fraction V were used. This layer was covered with another protective one consisting of 1 g kieselguhr without albumin. Each separation was run on a newly prepared column. The nucleic acids (50—80 optical density units) were separated in a linear gradient of sodium chloride concentration from 0.3 to about 1.1 M in 0.05 M phosphate buffer, pH 6.7. Filling of the column and chromatography were performed at 25° . The tenaciously bound RNA (TB-RNA) was recovered by the procedure described by Blinov and Seyts (1970) with the use of 1.4 M NaCl at 90° . The nucleic acid fractions were eluted from the column at a flow rate of about 60 ml/h. MAK profiles illustrating the changes in optical density at 260 nm and the radioactivity of the effluents from the column were obtained.

The nucleotide composition of the newly synthesised (^{32}P containing) RNA fraction was determined as follows: the solutions desalted by dialysis, containing the sRNA, rRNA, mRNA and TB-RNA fractions were evaporated to dryness and hydrolyzed (together with 3 mg commercial RNA preparation added as carrier) in 1 ml of 0.3 N KOH for 18 h at 37° (Tanimoto and Masuda, 1969). After neutralization of the hydrolysate with HClO_4 , the nucleotide composition of ^{32}P -RNA was determined by paper chromatography after Lane (1963) combined with radioactivity determination. Chromatographic filter paper Whatman No. 1 impregnated with ammonium sulphate was used. The chromatograms were developed with 75 per cent ethanol for about 20 h by the descending technique.

RESULTS AND DISCUSSION

Chromatography on MAK columns of nucleic acid preparations from wheat leaves either vernalized or not showed on the profiles peaks corresponding to the following fractions: low molecular weight compounds, sRNA-I (soluble RNA 4S-tRNA), sRNA-II (soluble RNA 5S), "sRNA-III", DNA-RNA, rRNA-I, rRNA-II and rRNA-III (Figs 1 and 2). The fraction rRNA-I, rRNA-II and rRNA-III were jointly determined as rRNA+mRNA in view of the reports of various authors indicating that, within these fractions, and particularly within what is known as the heavy ribosomal RNA fractions, messenger RNA fractions may be localised during chromatography on MAK columns. As seen (Table 1), the composition of the nucleic acid preparations from both objects was almost identical. Neither were major differences observed in the amount of nucleic acids in these objects.

In the chromatographic profiles of the nucleic acid preparations obtained from etiolated seedlings both vernalized and nonvernalized there were no traces of the presence of the "sRNA-III" fraction (Figs 3

Fig. 1. MAK-chromatography elution pattern of nucleic acids isolated from leaves of vernalized winter wheat, after 1-hr incubation in a solution containing ^{32}P .

Fig. 2. MAK-chromatography elution pattern of nucleic acids isolated from leaves of unvernallized winter wheat, after 1-hr incubation in a solution containing ^{32}P .

Table 1

Composition of nucleic acid preparations isolated from seedlings and leaves of nonvernallized and vernallized winter wheat (relative units)

Objects		sRNA	DNA-RNA	rRNA+ +mRNA	sRNA + DNA-RNA + rRNA+ +mRNA	rRNA+mRNA sRNA
Seedlings	After verna- lization*)	13,34	25,33	61,33	100,00	4,60
	Control*)	14,38	20,93	64,69	100,00	4,50
Leaves	After verna- lization**)	13,40 $\pm$ 0,97	34,27 $\pm$ 2,36	52,33 $\pm$ 2,05	100,00	3,91
	Control**)	13,60 $\pm$ 0,82	33,86 $\pm$ 1,54	52,54 $\pm$ 2,01	100,00	3,86

*) Mean values from three determinations

**) Mean values from eight determinations $\pm$ mean error of arithmetic mean

and 4). The proportions of the particular fractions in preparations from both objects were similar (Table 1). It results from the data in this table that differences in the composition of nucleic acids were noted between

Fig. 3. MAK-chromatography elution pattern of nucleic acids isolated from etiolated, vernalized winter wheat seedlings.

the preparations from leaves and those from young wheat seedlings. Particularly in preparations from leaves the contribution of the DNA-RNA fraction was higher than in seedlings, whereas the contribution of the rRNA+mRNA fractions was lower. Since the differences were of a similar degree in the vernalized and nonvernalized objects, it may be assumed that they are characteristic for various developmental phases of wheat, they are not, however, related with the thermic induction of generative development. Similar differences in the composition of the nucleic acid preparations were observed by Lynn and Pillay (1971) in chromatographic analysis on MAK columns of nucleic acids isolated from soybean leaves of various ages.

As seen in figs 1 and 2, in the chromatographic profiles of nucleic acid preparations from wheat leaves incubated in solution containing ^{32}P the following peaks of radioactivity were noted: 1 - one identical with that of optical density of low molecular weight compounds; 2 - a peak common to sRNA-I; sRNA-II and "sRNA-III"; 3 - a small peak within the DNA-RNA fraction; 4 - a peak within the ribosomal RNA fraction

closest to that of rRNA-II and with only slight signs of the radioactivity of fraction rRNA-I; 5 - a radioactivity peak specific according to various authors (Chroboczek and Cherry 1965; Cherry and Huys-tee, 1965; Ingle and Key, 1965; Ingle et al., 1965; Yoshida

Fig. 4. MAK-chromatography elution pattern of nucleic acids isolated from etiolated, nonvernalized winter wheat seedlings.

et al., 1967, Tanimoto and Masuda 1969) for the messenger RNA fraction; this peak is distinctly shifted from that of rRNA-II optical density towards the heavy rRNA fractions; and 6 - a peak specific for what is known as "tenaciously bound RNA fractions" (TB-RNA). This peak was traced only on the basis of radioactivity changes in the effluents from columns, because of the high contamination of this fraction with albumin released from the columns during recovery of this fraction (Tanimoto and Masuda, 1969). The above enumerated radioactivity peaks appeared during chromatography of nucleic acid preparations obtained from the leaves of both vernalized (Fig. 1) and nonvernalized plants (Fig. 2). The distribution of the phosphorus isotope in the particular fractions expressed in relative units (Table 2) was very similar in both

Table 2

Distribution of radioactive phosphorus in nucleic acid fractions isolated from leaves of vernalized and nonvernalized winter wheat, incubated for 1 h in solution containing ^{32}P (relative units)

Objects	sRNA	DNA-RNA	rRNA+ +mRNA	TB-RNA	sRNA +DNA-RNA +rRNA+mRNA +TB-RNA	rRNA+mRNA sRNA
After vernaliza- tion	23,28 $\pm$ 1,82	4,76 $\pm$ 0,24	56,13 $\pm$ 1,89	15,83 $\pm$ 1,76	100,00	2,41
Control	22,10 $\pm$ 2,63	5,09 $\pm$ 0,62	56,48 $\pm$ 3,36	16,33 $\pm$ 1,61	100,00	2,55

The results are mean values from six determinations $\pm$ mean error of arithmetic mean

Table 3

Specific radioactivity of nucleic acid fractions from leaves of vernalized and nonvernalized winter wheat, incubated for 1 h in solution containing ^{32}P

Objects	Units	Low molecu- lar weight compounds	sRNA	DNA-RNA	rRNA+ +mRNA	TB--RNA*)
After vernaliza- tion	Imp./1 u. opt. dens. x 1 min relative	536 237 2,33 $\pm$ 0,34	11 887 1,85 $\pm$ 0,15	1 098 1,77 $\pm$ 0,29	7 815 1,81 $\pm$ 0,20	3 326 1,75 $\pm$ 0,17
Control	Imp./1 m. opt. dens. x 1 min. relative	230 146 1,00	6 426 1,00	620 1,00	4 318 1,00	1 900 1,00

*) Specific radioactivity of fraction TB-RNA was conventionally calculated (Tanimoto and Masuda, 1969) from ratio of sum of radioactivity of this fraction and number of optical density units of DNA-RNA fraction, in view of considerable contamination of fraction TB-RNA with albumin released from MAK column during recovery of this fraction

The results in this table are means from six determinations $\pm$ mean error of arithmetic mean

objects. The intensity of radioactive phosphorus incorporation into all the RNA fractions examined, evaluated in terms of specific radioactivity (Table 3) was about two times higher in the vernalized object than in the nonvernalized control. If it is assumed that the properties of leaf tissues and cells of vernalized and nonvernalized plants, which decide of the infiltration rate of radioactive phosphorus, are similar, the above reported results may be considered as data testifying to a markedly more intensive synthesis of nucleic acids in wheat leaves in the generative phase of development as compared with that in plants remaining

Table 4

Nucleotide composition of RNA fractions synthesized during incubation of leaves of vernalized and nonvernalized winter wheat in solution containing ^{32}P

RNA fractions	Material (leaves)	CMP	GMP	AMP	UMP	$\frac{\text{GMP} + \text{CMP}}{\text{AMP} + \text{UMP}}$	$\frac{\text{AMP}}{\text{UMP}}$	$\frac{\text{CMP} + \text{GMP}}{\text{AMP} + \text{UMP}}$	$\frac{\text{GMP} + \text{AMP}}{\text{CMP} + \text{UMP}}$
sRNA	Vernalization	29,57 $\pm 0,83$	34,57 $\pm 0,67$	18,10 $\pm 0,26$	17,76 $\pm 0,20$	64,14 $\pm 0,36$	1,02	1,79	1,11
	Control	30,64 $\pm 0,98$	33,41 $\pm 0,39$	18,90 $\pm 0,41$	17,05 $\pm 0,28$	64,05 $\pm 0,48$	1,11	1,78	1,10
rRNA	Vernalization	24,19 $\pm 0,42$	29,76 $\pm 0,38$	25,58 $\pm 0,45$	20,48 $\pm 0,35$	53,95 $\pm 0,28$	1,25	1,17	1,24
	Control	25,02 $\pm 0,33$	29,95 $\pm 0,47$	24,48 $\pm 0,48$	20,56 $\pm 0,35$	54,97 $\pm 0,55$	1,19	1,22	1,19
mRNA	Vernalization	24,82 $\pm 0,40$	27,89 $\pm 0,34$	25,32 $\pm 0,24$	21,97 $\pm 0,23$	52,71 $\pm 0,33$	1,15	1,11	1,14
	Control	24,59 $\pm 0,36$	28,08 $\pm 0,30$	25,58 $\pm 0,31$	21,75 $\pm 0,33$	52,67 $\pm 0,41$	1,18	1,11	1,16
TB-RNA	Vernalization	24,11 $\pm 0,24$	27,32 $\pm 0,25$	26,09 $\pm 0,22$	22,48 $\pm 0,18$	51,43 $\pm 0,20$	1,16	1,06	1,15
	Control	21,18 $\pm 0,78$	29,05 $\pm 0,55$	27,01 $\pm 0,53$	22,75 $\pm 0,35$	50,23 $\pm 0,41$	1,19	1,01	1,28

The results are means from 12-16 determination $\pm$ mean error or arithmetic mean

in the vegetative phase (nonvernalized). These results do not agree with the communication of Hess (1961) who observed a somewhat lower intensity of ^{32}P incorporation into the RNA of *Streptocarpus vendlandii* leaves after thermic induction of flowering, as compared to incorporation into the control not subjected to induction. It is possible that this state occurs only transitorily immediately after induction of flowering. More recent investigations, on the other hand, indicate, that induction of flowering enhances in general the intensity of metabolism in plants. Myczkowski (1970 a) observed an increase in proteolytic activity of extracts from winter wheat germs under the influence of vernalization. The same author (1970 b) also found an increase in proteolytic activity in extracts of winter wheat leaves to be characteristic for stages of generative development of growing points. An enhancement of the intensity of nucleic acids synthesis—as results of vernalization—was demonstrated in the investigations of Stassevskaya and Kalinin (1971). These authors observed a two to three times higher rate of ^{32}P incorporation into nucleic acids in embryos of vernalized winter wheat in relation to the nonvernalized control. This enhanced intensity involved in a similar extent all the RNA fractions examined by these authors and separated by way of gradual isolation from plant material and DNA. The similarity of these observations and those obtained in the present study suggests that, as a result of vernalization, an intensification of nucleic acids metabolism, and particularly of their synthesis occurs and that this intensity of synthesis of particular RNA fractions remains higher in vernalized plants as compared with that in nonvernalized ones in further stages of vegetation. This might point to an important influence of thermic induction of generative development on the metabolism of nucleic acids in plants.

The nucleotide composition of the newly synthesized sRNA, rRNA, mRNA and TB-RNA fractions is shown in table 4. As seen, there are distinct differences in the composition between the particular fractions. On the other hand, no major differences were observed in the composition of analogical fractions from vernalized and nonvernalized objects. Thus, no data were obtained which could be considered as evidence of different properties of the nucleic acid fractions from plants incorporating the phosphorus isotope, which are in the vegetative or generative phase of development.

The results presented here lead to the view that thermic induction of generative development in plants causes an increase in the rate of nucleic acids metabolism without producing major changes in the content of these substances. According to Phillips and Fletcher (1969), Gressel et al. (1970), Rejowski and Kulka (1970) and Rejowski (1971), it seems probable that there frequently exist a parallelism between the changes in the activity of enzymes breaking

down nucleic acids and the intensity of synthesis of the later. The resultant of these processes is the rate of renewal of nucleic acids which may change within wide limits with simultaneous only slight changes in the content of these substances. It would seem indicated, therefore, to apply, in further investigations on the biochemical and molecular mechanisms of vernalization processes, methods making possible the follow-up of the dynamics of metabolism of various substances, particularly nucleic acids, proteins, hormones etc.

The differences in content of the "sRNA-III" fraction observed in the investigations show no connection with the induction of generative development of the plants. This fraction was present in preparations of both vernalized and nonvernalized leaves and did not appear in preparations from etiolated seedling of both objects. These differences seem, however, interesting in view of various reports of authors who found in plants fractions of nucleic acids specific for chloroplasts. Differences in the content of the low molecular weight RNA fractions in nucleic acid preparations from various parts of vetch plants have been observed by Dyer and Leech (1968). They were widest between preparations from the leaves and those from roots. It has been demonstrated that ribosomes from cytoplasm and chloroplasts contain low molecular weight RNA fractions differing in properties between themselves. This would suggest that the "sRNA-III" fraction observed in the present study is specific for wheat chloroplasts.

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*Frakcje kwasów nukleinowych siewek i liści pszenicy ozimej
w wegetatywnej i generatywnej fazie rozwoju*

Streszczenie

Z siewek i liści roślin pszenicy ozimej, wyhodowanych z ziarniaków wernalizowanych i nie wernalizowanych izolowano kwasy nukleinowe przy pomocy metody detergentowo-fenolowej. Preparaty oczyszczano, stosując bromek cetylo-trójmetyloamoniowy. Frakcjonowanie kwasów nukleinowych przeprowadzano przy pomocy chromatografii na kolumnach MAK. Określano intensywność włączania radioaktywnego izotopu fosforu do różnych frakcji kwasów nukleinowych liści roślin wernalizowanych i nie wernalizowanych oraz skład nukleotydowy nowosyntetyzowanych frakcji RNA.

Obserwowano około dwukrotnie wyższą intensywność włączania ^{32}P do frakcji sRNA, rRNA + mRNA oraz TB-RNA w liściach pszenicy w generatywnej fazie rozwoju w stosunku do nie wernalizowanej kontroli.

Stwierdzono brak różnic w proporcjach wzajemnych pomiędzy frakcjami kwasów nukleinowych roślin w wegetatywnej i generatywnej fazie rozwoju.

W składzie nukleotydowym nowo syntetyzowanych frakcji RNA liści roślin wernalizowanych i nie wernalizowanych nie zaznaczały się wyraźne różnice.

Zaobserwowano istnienie różnych proporcji wzajemnych pomiędzy frakcjami sRNA, DNA-RNA oraz rRNA + mRNA z etiolowanych siewek i z wyrośniętych liści pszenicy. Różnice te wystąpiły zarówno w obrębie obiektów wernalizowanych, jak i nie wernalizowanych.

W preparatach kwasów nukleinowych z liści obu obiektów obserwowano występowanie frakcji „sRNA-III”, nie zaznaczającej się wyraźnie w preparatach z etiolowanych siewek. Przypuszcza się, że frakcja ta jest właściwa chloroplastom pszenicy.

Uzyskane dane, wskazujące na intensywniejszą syntezę kwasów nukleinowych w liściach roślin wernalizowanych popierają przypuszczenia, że metabolizm kwasów nukleinowych odgrywa ważną rolę w mechanizmach biochemiczno-molekularnych procesu termicznej indukcji rozwoju generatywnego.