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Contribution to the Estimation of Nucleic Acids in Wheat Roots

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Abstract. Several methods of NA isolation and estimation were examined in order to determine RNA in the whole root systems of young wheat plants. It was found out that during the hydrolysis of roots with perchloric acid according to OGUR and ROSEN (1950), SPIRIN (1958) and HEITEFUSS (1965) a considerable amount of orcine-positive compounds is released which cannot be adequate to the RNA content. Therefore the separate RNA determination in the presence of DNA was excluded even after the NA fractionation by hydrolysis at various temperature and perchloric acid concentration. Besides NA hydrolysates contained a high amount of other compounds absorbing in the UV-region. Compounds interfering with both these methods were present especially in the basal parts of roots.

Using the method of KERN (1960) a nucleoprotein fraction was isolated containing about 75 per cent of the total NA content in the tissue. This fraction consisted of more than 90 per cent RNA combined with proteins, namely the ribosomal RNA. After the hydrolysis it could be estimated spectrophotometrically for the hydrolysate did not contain other compounds absorbing in the UV-region. In the root tips the content of this fraction was high in comparison with the basal parts of roots and it changed with the kind of nutrition.

The NA estimation in plant material involves a series of problems to which several extensive comprehensive publications are devoted. Their authors (STAFFORD 1951, MARTIN and MARTON 1956, DE DEKEN-GRENSON and DE DEKEN 1959, SMILLIE and KROTKOV 1960, KERN 1960, HUTCHINSON and MUNRO 1961, CHERRY 1962, HOLDGATE and GOODWIN 1965 and oth.) besides criticizing the procedures hitherto used, tried in some cases also to improve them. For example, when cleaning the NA hydrolysate be means of adsorbents or ion-exchangers, not only compounds interfering with NA estimation are removed but a considerable amount of NA is lost as well (up to 40 per cent according to HOLDGATE and GOODWIN 1965). Such methods need a certain standardization but in any case they give relative res ults therefore they are not quantitative.

On the other hand there are methods by means of which the subcellular structure is at first separated from the tissue (MARTIN and MARTON 1956,

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KERN 1960, and others). From this structure NA can be released in a fairly pure state, e.g. by hydrolysis. Compounds interfering with the NA determination remain in the tissue residue. Neither does this procedure represent the total NA estimation but it can be useful from the physiological point of view.

The number of modifications of the original three classical methods of NA isolation and estimation (SCHNEIDER 1945, SCHMIDT and THANNHAUSER 1945, OGUR and ROSEN 1950), almost equals that of papers published nowadays on plant NA estimation; this indicates how their use is limited not only to a certain species but also to a certain tissue (CHERRY 1962). Besides this, when analysing plant tissues it is necessary to take into account that the physical properties of NA, as solubility or hydrolysability and oth. can change with the kind of nutrition as shown by LINDBLAD (1959). But this could be true also for the quality and quantity of interfering substances.

With regard to the aim of our further work which should have been devoted to the study of influence of humic acid in the form of Na-humate on the NA metabolism, it was necessary to determine RNA, especially the ribosomal RNA. Its quantity is in connection with the growth rate which is considerably influenced by Na-humate. For the study of this connection it would be useful to analyse the growth zone of roots. Owing to technical difficulties connected with the cutting of a great number of wheat root tips (more than one thousand per one analysis) and further owing to the fact that during this procedure a partial degradation of NA can occur, it was necessary to analyse the whole root systems. We intended to isolate RNA for analysis in such pure state which would permit us to determine it spectrophotometrically. The procedure should have been simple enough to enable the simultaneous analysis of a great number of samples.

Material and Methods

Experimental plant and its cultivation

Seedlings of wheat *Triticum aestivum* L., cv. Pyšelka served as experimental plants.

Analyses were carried out in both roots of seedlings cultivated for 48 hours in Petri dishes in dark at 25° C and roots of seedlings cultivated further (till 7 days) in water cultures (lots of 20 plants in 300 ml cultivation solution) under artificial conditions. The temperature was about 20° C, the relative air humidity 50—60 per cent. The intensity of the 16 hour illumination was 900 lx on the level of leaves. Ten 40 W white fluorescent tubes were used for this purpose. The wheat grains were allowed to germinate and plants were cultivated in various solutions, namely in a nutrient solution, in distilled water and in a solution of Na-humate with regard to the intention of the following work.

The more detailed characteristic of the analysed material is given in the description of the procedure to be examined. The procedure according to

HEITEFUSS (1965) was examined in this author's laboratory using 7 day-old plants cultivated in sand cultures with a nutrient solution under the conditions given by this author.

The cultivation solutions

1. Knop nutrient solution containing in millimoles: 4·87 Ca^{2+}; 4·79 K^{+}; 0·81 Mg^{2+}; 1·47 $H_2PO_4^{-}$; 11·72 NO_3^{-}; 1·53 Cl^{-}; 0·81 SO_4^{2-}; 0·062 Fe^{3+} per liter distilled water. Chemicals purchased by the firm "Lachema" were used in this work. The nutrient solution was used diluted with water in the ratio 1 part of the solution to 3 parts of water.
2. Glass-distilled water. The electrical conductivity was in the range of values $4{\cdot}5-6{\cdot}4 \times 10^{-6}\ \Omega^{-1}\ cm^{-1}$.
3. Solution of Na-humate in distilled water (100 mg humic acid dissolved in 2 ml 0·2 N NaOH and made up with water to 1000 ml). The humic acid originated from the preparation "Humussäure", Riedel & de Haën, Hannover. The solution contained 4×10^{-4} M sodium.

Preparation of the experimental material for analysis

Gathered plants were measured, rinsed with distilled water, dried, roots were separated from shoots, weighed, cut into 5 mm. parts, frozen in an aluminium foil to −10° C and ground in a mortar cooled in an ice bath with NaCl to −5° C. This procedure was used on examining the method of OGUR and ROSEN (1950) and that of SPIRIN (1958). In the case of KERN'S method (1960) the cut roots were frozen in an aluminium foil with solid CO_2 and ground for 20—30 min. in a mortar cooled in a bath prepared from a mixture of ethylalcohol and solid CO_2. This procedure was quicker and the homogenization was better. Examining the procedure of HEITEFUSS (1965) fresh tissues were processed.

Methods of NA isolation and estimation

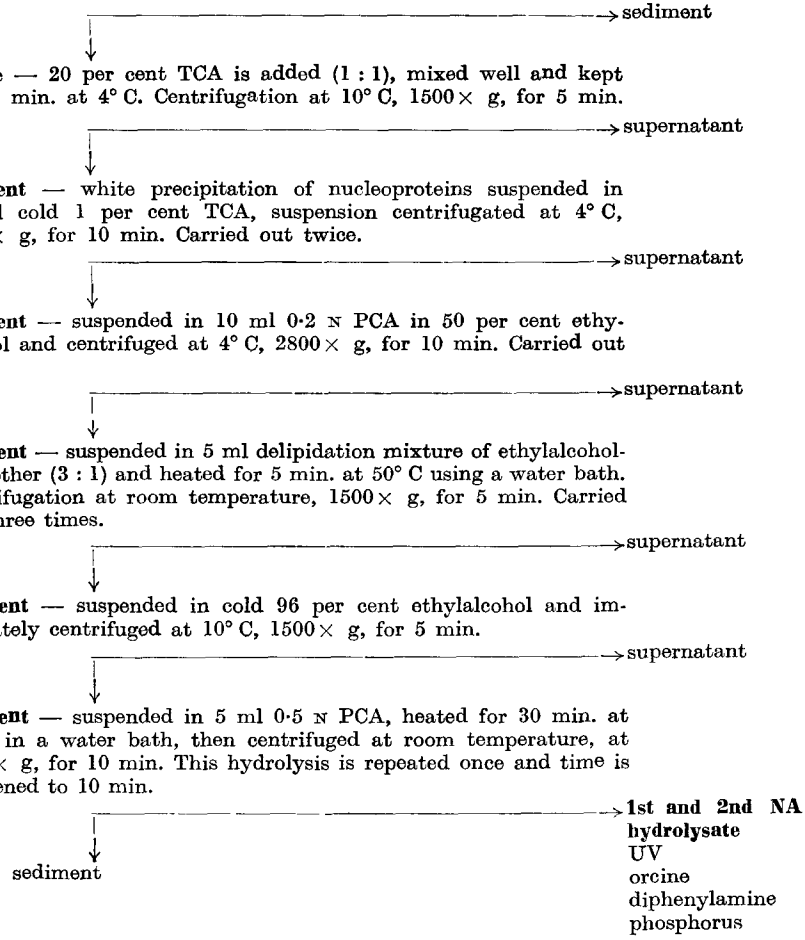
The method of OGUR and ROSEN (1950) was examined using roots of plants cultivated for 7 days in distilled water and in a nutrient solution. We used the procedure referred to by KEIL and ŠORMOVÁ (1959). After removing low molecular phosphorus compounds and lipids, NA's were hydrolysed in two fractions, i.e. with 1 N perchloric acid (PCA) at 4° C (RNA) and with 0·5N PCA at 75° C (DNA). The absorbance of hydrolysates was measured in the UV-region and their content of orcine- and diphenylamine-positive substances was estimated.

The method according to SPIRIN (1958) was examined using twice 2 g. fresh weight (f.w.) of roots of plants grown for 7 days in distilled water and in a nutrient solution. In contrast to SPIRIN who analysed nucleoproteins, we investigated roots containing substances with the NA-like physico-chemical properties and therefore we used the same procedure as in the method of OGUR and ROSEN in order to remove them. NA's were then hydrolysed according to SPIRIN with 0·5 N PCA (10 ml/2 g. f.w.) either for 20 min. at 100° C or for 15 min. at 70° C. The absorbance of the hydrolysates was measured in the UV-region.

The method of HEITEFUSS (1965) was examined using wheat plants cultivated for 7 days in a nutrient solution. Both leaves and roots were analysed simultaneously to consider differences in the NA estimation in these organs. Fresh tissue was ground under cooling with 10 per cent PCA and after removing the low molecular phosphorus compounds and lipids NA's were hydrolysed with 1 N PCA in two fractions, i.e. at 20° C (RNA) and at 37° C (DNA). The absorbance of the hydrolysates was measured in the UV-region and their content of orcine- and diphenylamine-positive compounds and phosphorus was estimated.

The method of KERN (1960) was examined using roots of seedlings which were allowed to germinate in distilled water and in a solution of Na-humate for 2 days. In both cases a portion of 7·7 g. f.w. was analysed. The procedure is demonstrated in Scheme 1. The homogenate was divided into three sam-

Scheme 1. A portion of 2—3 g. of frozen roots is gently ground in a mortar under cooling, then ground with 0·01 M Na-tetraborate (without coffeinanhydride) in the ratio 10 ml to 1 g. f.w. The tissue homogenate is filtred through a close silon fabric.



ples of about 2·5 g. which were analysed individually. The absorbance of the hydrolysates was measured in the UV-region.

Content of UV-light-absorbing substances in hydrolysates was estimated using the following spectrophotometers: Unicam with automatic registration (for preliminary establishment of spectra character — see Figures), SF 4 (for serial determinations of E_{260} — see Tables) and Zeiss (when using the procedure of HEITEFUSS).

The diphenylamine-positive compounds were estimated according to BURTON (1956) using all chemicals of a high degree of purity (pro analysi), diphenylamine was recrystallized from ethylalcohol. The reaction mixture contained 1 ml hydrolysate and 2·5 ml BURTON'S solution. The standard curve was prepared by measuring thymus DNA in the concentration range 20—200 μg. for each estimation.

The orcine-positive compounds were estimated according to MEJBAUM (1939) using p.a. chemicals; orcine was purified by means of charcoal and recrystallized from a water solution. The reaction mixture contained 0·05 ml hydrolysate, 1 ml 0·5 N PCA, 3 ml 0·1 per cent $FeCl_3$ in concentrated HCl and 0·3 ml 10 per cent orcine in 96 per cent ethylalcohol. For the preparation of the standard curve yeast RNA was used in the concentration range of 10—300 μg. for each estimation.

The phosphorus content in hydrolysates was determined either according to LOWRY and LOPEZ (1946), or according to ALLEN (1940) when using HEITEFUSS' procedure.

The colorimetric measurements were carried out using Hilger's photocolorimeter.

The NA bases were separated by circular paper chromatography on Whatman paper No. 1, using the mixture of n-butylalcohol—acetic acid—water (4 : 1 : 5) as a solvent system. The bases were detected in UV-light and they were estimated after elution with 4 ml 0·1 N HCl at 20° C for 12 hours.

Results and Discussion

1. Results of examined methods of NA isolation and estimation

Acid hydrolysates obtained by the method of OGUR and ROSEN (1950) when measured spectrophotometrically gave spectra (Fig. 1, 2) which indicate the presence of a considerable amount of UV-absorbing substances different from NA. The orcine- and diphenylamine-positive substances were present both in the fraction "RNA" (I.), and in the fraction "DNA" (II.) as seen from Table 1 and 2. The high content of the orcine-positive compounds in the fraction "DNA" proves that the NA fractionation to RNA and DNA cannot be carried out in this way or that the reaction of RNA ribose is not involved. Results of the estimation of diphenylamine-positive substances were variable. In the series of further repetitions differences as high as 400 per cent were found. According to these results the given method does not permit the estimation of NA, particularly RNA

Fig. 1.

Fig. 2.

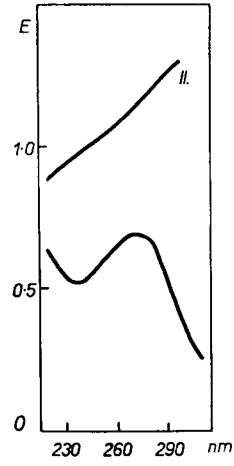


Fig. 1. Absorption spectra of substances hydrolysed with 1 N PCA at 4° C (I) and 0·5 N PCA at 75° C (II) according to OGUR and ROSEN from roots of wheat plants cultivated in water for 7 days.

Fig. 2. Absorption spectra of substances hydrolysed with 1 N PCA at 4° C (I) and 0·5 N PCA at 75° C (II) according to OGUR and ROSEN from roots of wheat plants cultivated in a nutrient solution for 7 days.

from the whole root systems for the same reasons which were found in analysing other plant material (STAFFORD 1951, MARTIN and MARTON 1956, DE DEKEN-GRENSON and DE DEKEN 1959, HOLDGATE and GOODWIN 1965 and others).

Table 1

Content of orcine-positive substances (in RNA equivalent) and diphenylamine-positive substances (in DNA equivalent) in acid hydrolysates obtained according to OGUR and ROSEN from roots of wheat plants cultivated for 7 days in water (in μg. per 100 mg. dry weight of residue after hydrolysis)

Sample	Fraction "RNA" 1 N PCA 4° C		Fraction "DNA" 0·5 N PCA 70° C	
	RNA	DNA	RNA	DNA
1	36·6	5·0	416	12·3
2	35·2	5·3	429	7·8
3	37·1	9·1	425	3·3
4	44·6	16·5	428	3·5
Average	38·8	8·9	424	6·7

The kind of nutrition had no influence on the quality of the results (compare with LINDBLAD 1959).

Hydrolysates obtained by SPIRIN's method (1958) contain besides NA a considerable amount of other UV-light-absorbing substances as well

Table 2

Content of orcine-positive substances (in RNA equivalent) and diphenylamine-positive substances (in DNA equivalent) in acid hydrolysates obtained according to OGUR and ROSEN from roots of wheat plants cultivated in a nutrient solution for 7 days (in μg. per 100 mg. dry weight after hydrolysis)

Sample	Fraction "RNA" 1 N PCA 4° C		Fraction "DNA" 0·5 N PCA 70° C	
	RNA	DNA	RNA	DNA
1	223	6·4	524	18·1
2	252	12·7	610	22·2
3	236	10·2	574	20·3
Average	237	9·7	569	20·2

(Fig. 3). The concentration of these substances increased with the temperature during hydrolyses. (Tab. 3). With regard to the character of spectra it was not possible to evaluate the NA content from the difference between E_{270} and E_{290} as suggested by SPIRIN.

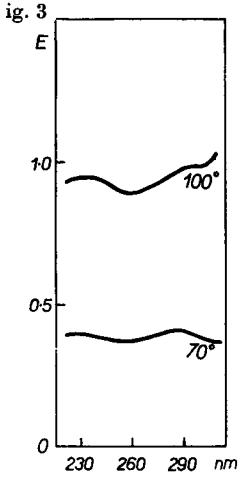


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Table 3

Relative content of UV-absorbing substances (from E_{260}) in acid hydrolysates obtained at various temperatures according to SPIRIN from roots of wheat plants cultivated for 7 days in water or in a nutrient solution (per 2 g. initial fresh weight)

Cultivation Solution	Sample	Temperature during Hydrolysis	E_{260}
H_2O	1	100 °C	1·160
	2	70 °C	0·302
Nutrient Solution	3	100 °C	1·180
	4	70 °C	0·302

Fig. 3. Absorption spectra of substances hydrolysed with 0·5 N PCA at 70° C and 100° C according to SPIRIN from roots of wheat plants cultivatedin water for 7 days.

As follows from the spectrophotometric measurements of hydrolysates obtained according to the procedure of HEITEFUSS (1965) at 20° C a considerable amount of UV-absorbing substances ("RNA") is hydrolysed from leaves and they are identical with NA in a very pure state (Fig. 4). Far less UV-light-absorbing substances are hydrolysed at 37° C ("DNA"), and among them compounds different from NA prevail. Owing to the NA content in the first fraction (20° C) its proportion in the second fraction (37° C) will probably be small as one can presume from the character of the absorption spectrum and from the phosphorus content (Tab. 4).

Just the opposite was true for the analysis of roots. Owing to the fact that the first fraction of NA is not so pure (according to the character of the

spectrum) as it was in the analysis of leaves, and further that at both 20° C and 37° C about the same amount of UV-absorbing substances was released, the second fraction (37° C) could have still a relatively high NA content. It means that in the analysis of roots it is necessary to take a greater error into account than in the analysis of leaves and above all for the reason

Fig. 4

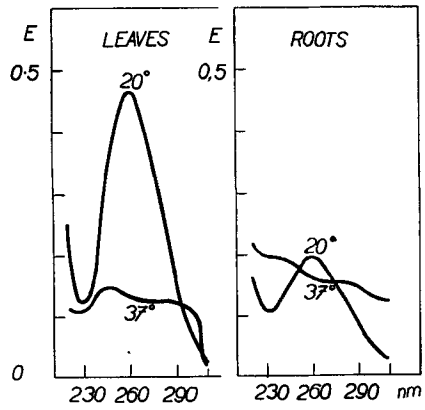

Fig. 4. Absorption spectra of substances hydrolysed with 1 N PCA at 20° C and 37° C according to HEITEFUSS from leaves and roots of wheat plants cultivated in a nutrient solution for 7 days.

that the spectrophotometric measurements cannot be reliably completed with the ribose or deoxyribose estimation. As follows from the orcine and diphenylamine tests (Tab. 4) the substances with a positive reaction are released at both 20° C and 37° C, both from roots and leaves. This proves that not even this procedure is reliable for fractionating NA to RNA and DNA or that the orcine and diphenylamine tests are not specific for pentoses, particularly for the pentoses of NA. Results of the phosphorus

Table 4

Content of orcine- and diphenylamine-positive substances and phosphorus in acid hydrolysates obtained according to HEITEFUSS from roots and leaves of wheat plants cultivated in a nutrient solution for 7 days (in μg. per 1 g. of initial fresh weight)

	Leaves		Roots	
	Fraction ‚RNA' 20° C	Fraction ‚DNA' 37° C	Fraction ‚RNA' 20° C	Fraction ‚DNA' 37° C
Orcine-positive substances in RNA equivalents	830	1956	1242	2200
Diphenylamine-positive substances in DNA equivalents	36	84	72	48
Phosphorus	144	33	54	24

content estimation indicate that NA's in the fraction obtained at 37° C make about 1/3 of the total NA content of both fractions. But one must presuppose that still a certain part of it is represented by RNA, as it is not probable that its hydrolysis at 20° C is complete. But this portion cannot be estimated and thus neither can the total RNA content in roots. KERN'S method (1960) gave a result substantially different from the preceeding three

Fig. 5.

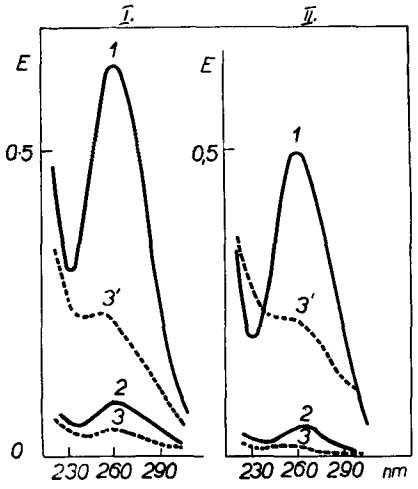

Fig. 6.

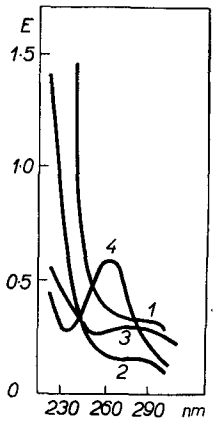

Fig. 5. Absorption spectra of substances obtained in three subsequent hydrolyses (1, 2, 3) with 5 ml 0·5 N PCA (30, 10, 10 min. resp.) from the nucleoprotein fraction isolated and analysed according to KERN from roots of wheat plants which were allowed to germinate for 2 days in water (I) and in Na-humate (II). 3'-spectrum of substances in the third hydrolysate at a higher concentration.

Fig. 6. Absorption spectra of substances extracted from the nucleoprotein fraction with 1 per cent TCA (1), with 50 per cent ethylalcohol with 0·2 N PCA (2), with a mixture of ethylalcohol and ethylether (3) and NA hydrolysed with 0·5 N PCA (4).

methods. As shown by spectrophotometric measurements, NA in a very pure state can be isolated using this procedure (Fig. 5). This is indicated not only by the character of spectra but also by the value of the ratio E_{260}/E_{235} which is higher than 2. As stated by KERN such values are obtained with preparations of a pure NA. The kind of nutrition had no effect on the character of the absorption spectrum (compare spectrum I and II in Fig. 5).

Conditions for the quantitative NA release were found using three successive hydrolyses of the same sample (every time using 5 ml 0·5 N PCA per 2—3 g. of the initial f.w.) and each hydrolysate was measured separately. The duration of the first two hydrolyses equaled that given in Scheme 1; the third hydrolysis took 10 min. It was verified (Fig. 5) that a substantial amount of NA is released as early as during the first hydrolysis (spectrum 1). In spite of the fact that during the second hydrolysis (spectrum 2) only a small amount of NA was released it is necessary to repeat the hydrolysis once more. On the other hand during the third hydrolysis a very small

amount of UV-light absorbing substances was released (spectrum 3). Their absorption maximum, more expressive at a higher concentration (spectrum 3'), was shifted to 250 nm so that probably the presence of NA cannot be taken into consideration.

When washing the precipitation of nucleoproteins before the hydrolysis no undesired hydrolysis takes place and thus not even their lost (Fig. 6). With regard to the fact that KERN's method gave a good result and that the NA fraction concerned represents more than 90 per cent RNA (according to KERN) combined with proteins, i.e. the ribosomal RNA, this method was considered to be suitable for the intention of our further work. At first it was necessary to investigate the reproducibility of this method and then the distribution of RNA between the apical and basal parts of the root in order to decide whether or not the whole roots can be analysed.

2. Content of the NA fraction (isolated according to KERN) in the apical and basal parts of the root

Root tips 5—7 mm long were analysed separately from the remaining basal root parts. Three hundred plants cultivated in water for 5 days gave 1410 root tips (2·2 g. f.w.) and 17 g. basal root parts. From 270 plants cultivated in a solution of Na-humate the yield was 1040 tips (1·5 g.) and 20 g. basal root parts. After the homogenisation larger lots were divided into smaller portions corresponding to 2—3 g. of the initial f.w. Experiments were repeated three times using the same number of plants. We kept the procedure given in Scheme 1. Both hydrolysates were combined and made up (with not more than 0·2—0·3 ml 0·5 N PCA) to 10 ml. For the spectrophotometric measurement the combined hydrolysates were diluted with nine parts of 0·5 N PCA to one part of the hydrolysate. The absorption maximum usually was in the range of extinctions 0·3—0·6. In one of the series of parallel samples the whole spectrum was always measured, E_{260} in

Table 5

Content of NA hydrolysed according to KERN from apical and basal parts of roots of wheat plants cultivated in water and in Na-humate for 5 days (in μg. per plant; n = 3)

Material analysed	H_2O $\bar{x} \pm s_{\bar{x}}$	$s_{\bar{x}}$ in % $\bar{x}$	Na-humate $\bar{x} \pm s_{\bar{x}}$	$s_{\bar{x}}$ in % $\bar{x}$	$\bar{d}$ % to H_2O	Significance	P %
Root tips	15·2 ± 0·152	1·0	20·0 ± 0·498	2·3	+31·3	+++	9·10
Basal part	6·9 ± 1·060	14·5	8·8 ± 0·807	9·1	+27·5	—	1·40
Whole root	22·1 ± 1·100	5·0	28·8 ± 0·340	1·2	+29·4	++	6·16

— = insignificant P < 5%
\+ = on the level of significance P > 5%
++ = significant P > 1%
+++ = highly significant P > 0·1%

other samples. The NA content was calculated from this value according to CHARGRAFF (referred by KEIL and ŠORMOVÁ 1959, p. 541). For the molar extinction coefficient εP the average value was introduced from εP given by the authors mentioned for RNA and DNA, i.e. 775, because the specific phosphorus content of NA in the investigated nucleoprotein fraction of wheat roots was not known. The calculated amount of NA was expressed per 1 plant and evaluated statistically. The reproducibility of the estimation of this NA fraction was good (Tab. 5) especially in the analysis of the root tips; the estimation was repeated with about 2 per cent error. When analysing the basal root parts the error was almost 15 per cent. The amount of NA estimated per one root tip (15—20 μg.) was of the same order as that given by WOODSTOCK and SKOOG (1962) for the total NA content in 5 mm maize root tips (30—40 μg.). In the root tips the content of the studied NA fraction was expressive enough to be apparent even when analysing the whole roots. It was highly influenced by the kind of nutrition.

3. The participation of the NA fraction (isolated according to KERN) in the total NA content in the root tissue

In order to give further precision to the applicability of KERN'S procedure it was useful to find out which part of the total NA content, particularly RNA, goes to nucleoproteins isolated according to this procedure. For this purpose parallelly with the nucleoprotein fraction the tissue remainder after the separation of this fraction was analysed as well. It means that after the filtration of the Na-tetraborate solution containing the nucleoprotein fraction the tissue residue placed on a silon fabric was suspended in 0·2 N PCA in 50 per cent ethylalcohol and further washed up and hydrolysed according to the procedure given in Scheme 1. An analysis was carried out of 15 g. amount of roots of wheat seedlings cultivated in Petri dishes in distilled water for 2 days. The absorbance of the hydrolysates from the nucleoprotein fraction and from the tissue residue was measured in the UV-region and their content of orcine- and diphenylamine-positive substances and phosphorus was es-

Table 6

Content of UV-light absorbing substances (from E_{260}) phosphorus, orcine- and diphenylamine-positive substances in acid hydrolysates from nucleoproteins and tissue residues and dry weights after hydrolysis (per 1 g. of initial fresh weight). Percentage values indicate the relative distribution of determined substances between both fractions analysed

Material analysed	E_{260}		NA from E_{260}	Phosphorus		Orcine-positive substances in RNA equiv.		Diphenylamine-positive substances in DNA equiv.		% DNA of 100% NA (from E_{260})	Dry weight of tissue residue after hydrolysis	
		%	μg	μg	%	mg	%	μg	%	%	mg	%
Nucleoproteins	0·745	43	304	38·7	75	1·24	14	23	26	7·7	4·85	13
Tissue residue	1.000	57	—	12.7	25	8.00	86	66	74	—	32.50	87

timated. In the hydrolysate from the tissue residue the presence of purines and pyrimidines and their content were established in addition by means of the paper chromatography.

As seen from the results of the spectrophotometric measurements (Fig. 7) in the tissue residue after the separation of nucleoproteins a considerable amount of the UV-light absorbing compounds still remains (spectrum B) which is composed, according to the character of the spectra, of compounds different from NA. In comparison with the nucleoprotein fraction there is a high content of orcine- and diphenylamine-positive substances in this residue which cannot be due to the presence of the pentoses of NA (Tab. 6). Calculating from the phosphorus content, a considerable part of the total NA content should go to the nucleoprotein fraction, i.e. about 75 per cent. After subtracting the DNA proportion in this fraction (about 8 per cent as follows from the diphenylamine test) about 70 per cent of the total NA content in the tissues would go to RNA in this fraction. With regard to this value and in addition to the fact that RNA combines with proteins one can presuppose that a substantial amount of the ribosomal RNA could be involved (compare with the results of NEIDHARDT and MAGASANIK 1960).

On chromatographying the hydrolysate of the tissue residues (neutralized with KOH under cooling) the UV-light absorbing substances (spectrum B in Fig. 7) were separated into four components. Under UV-light the first of them (beginning from the starting point) had an intensive yellow fluorescence, the second and the third ones became evident as dark violet, the

Fig. 7.

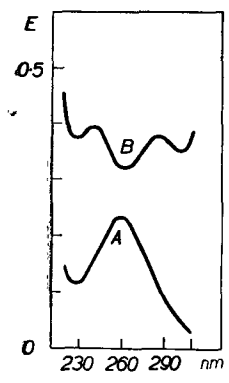

Fig. 8.

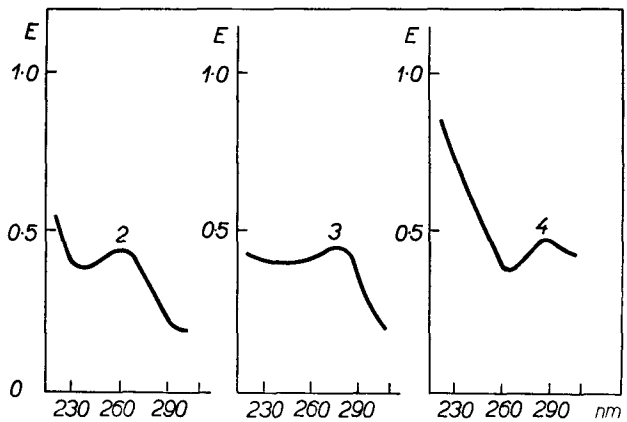

Fig. 7. Absorption spectra of substances hydrolysed with 0·5 N PCA at 70° C from the nucleoprotein fraction (A) and tissue residue (B) of roots of wheat seedlings which germinated in distilled water for 2 days.

Fig. 8. Absorption spectra of substances eluated after the chromatographic separation of the hydrolysate coming from the tissue residue. The position of spectrum 2 corresponds to standard guanine + cytosine, spectrum 3 corresponds to adenine + uracile, spectrum 4 to the blue spot.

fourth one as light blue. Measuring the eluates of the 2nd—4th spots in the UV-region (with correction for the eluates coming from a free field of the chromatogram) did not permit one to identify these substances from the character of their spectra as purines and pyrimidines (Fig. 8). Therefore their quantitative estimation could not be carried out. Apart from this it is necessary to take into account that purines and pyrimidines coming from cell-wall hemicelluloses can interfere with the estimation (SAKHAUTDINOVA 1964).

4. The problem of substances interfering with NA estimation

Comparing spectra of the hydrolysates from tissue residues of root tips with those coming from tissue residues of the basal root parts it was found that they differ in their character. Especially from the basal parts of roots under the same conditions as those in which NA's are hydrolysed, a considerable amount of substances is released which absorb UV-light and are not identical with NA (compare spectrum 2 in Fig. 9 and 10). In connection with this finding a question arose as to which organic compounds present in differentiated plant tissues can absorb UV-light. It was established that lignins have a similar absorption spectrum to NA, and they can be taken into consideration especially for the reason that interfering substances could be detected in older tissues. In order to be compared with the spectrum of compounds hydrolysed from the residue of the basal root parts (part of

Fig. 9.

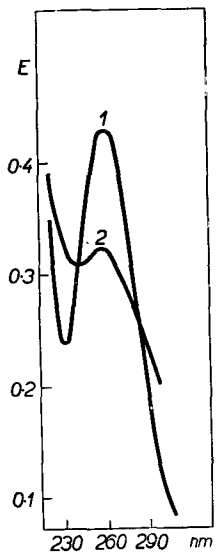

Fig. 10.

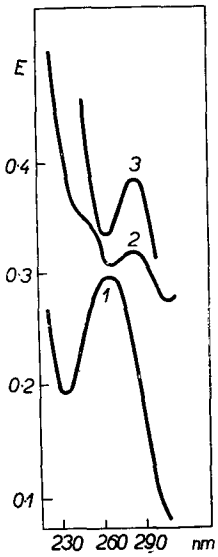

Fig. 9. Absorption spectra of substances hydrolysed from the nucleoprotein fraction (1) and from the tissue residue (2) of root tips of wheat plants grown in distilled water for 5 days.

Fig. 10. Absorption spectra of substances hydrolysed from the nucleoprotein fraction (1) and from the tissue residue (2) of the basal parts of wheat plants grown in distilled water for 5 days. Spectrum of lignin from the sugar-beet root (3).

sp ectrum 2 with the maximum at 280—290 nm — Fig. 10) the spectrum of lig nin from the sugar-beet root according to BARDINSKAYA (1964) is presented. This author found using spectrophotomertic measurements that sub stances which could be precursors of lignin were present even in 2—3 mm long root tips. In connection with lignins it is interesting that they are solub le in among other things (i.g. in methylalcohol and ethylalcohol) in hydroxides and acids and insoluble in mixtures with ethylether; they give positive reactions with diphenylamine (SCHUBERT and NORD 1950). This reaction is used in one of the classical tests for the proof of lignin. A yellow colouring of the reaction mixture results in contrast to a blue one after the reaction of diphenylamine with deoxyribose (BURTON 1956). We have verified that methyllignin (extracted with methylalcohol) from spruce also gave a positive reaction with BURTON's mixture resulting in a yellow colouring.

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Svatava Fialová, Katedra fysiologie rostlin a půdní biologie Přírodovědecké fakulty KU: **Příspěvek ke stanovení nukleových kyselin v kořenech pšenice.** — Biol. Plant. **10** : 409—423, 1968.

Prověřili jsme několik způsobů izolace a stanovení NA se záměrem stanovit RNA v celých kořenových systémech mladých rostlin pšenice. Přesvědčili jsme se, že při hydrolýze kořenů kyselinou chloristou podle Ogura a Rosenové (1950), Spirina (1958) a Heitefusse (1965) se uvolní značné množství orcinopositivních látek, které nemůže být úměrné obsahu RNA. Proto bylo vyloučeno oddělené stanovení RNA vedle DNA, a to i po frakcionaci NA hydrolýzou při různé teplotě a koncentraci kyseliny chloristé. Mimo to obsahovaly hydrolyzáty, vedle NA, výrazné množství jiných látek absorbujících UV záření. Látky interferující v obou těchto stanoveních byly přítomny zvláště v bazálních částech kořenů.

Kernovým postupem (1960) jsme izolovali frakci nukleoproteidů obsahující asi 75 % z celkového obsahu NA ve tkáni. Tuto frakci tvořila z více než 90 % RNA vázaná na bílkoviny, tedy ribosomální RNA. Po hydrolýze ji bylo možno stanovit spektrofotometricky, neboť hydrolyzát neobsahoval mimo NA jiné látky absorbující UV záření. V kořenových špičkách byl obsah této frakce výrazný, ve srovnání s bazální částí kořenů, a měnil se se způsobem výživy.

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Было проверено несколько способов выделения и определения нуклеиновых кислот с целью определить РНК во всей корневой системе молодых растений пшеницы. Мы убедились, что при гидролизе корней хлористой кислотой по Огур и Розен (1950), Спирину (1957) и Гейтефусс (1965) освобождается значительное количество орцинопозитивных веществ, которое не может быть пропорциональным содержанию РНК. Поэтому в таком случае не возможно отдельное определение РНК наряду с ДНК даже после фракционации НК гидролизом при разной температуре и концентрации хлористой кислоты. Кроме того гидролизаты содержат наряду с НК значительные количества других веществ поглощающих УФ. Вещества интерферирующие при обоих определениях находились особенно в базальных частях корней.

По Керну (1960) мы выделили фракцию нуклеопротеидов содержащую около 57 % всего содержания НК в ткани. Эта фракция состояла на более чем 90 % из РНК связанной с белками, то есть из рибозомальной РНК. После гидролиза ее можно было определить спектрофотометрически так как гидролизат не содержал кроме НК других веществ поглощающих УФ. В корневых верхушках содержание этой фракции было большим по сравнению с базальной частью корней и изменялось под влиянием условий выращивания растений.