A Cytochemical Study of Nucleic Acids in Plant Cells

IV. Isolation and estimation of nucleic acids from corn root tip by Schmidt and Thannhauser's method

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Useful method for determining the deoxyribonucleic acid (DNA) and pentose nucleic acid (PNA) contents of animal tissues has been reported by several authors (Schmidt and Thannhauser 1945; Schneider 1945), while Ogur and Rosen (1950) reported a procedure of differential extraction of nucleic acids with perchloric acid (PCA) from plant tissues, and the contents of nucleic acids of corn root tip were thus determined.

Among them, Schneider's method is disadvantageous since the DNA and PNA are extracted together by hot trichloroacetic acid (TCA). Contrarily, an advantage of Ogur and Rosen's method, which was originally designed for plant tissues, is that DNA and PNA are isolated differentially with PCA, though the condition of isolation is not easy.

Schmidt and Thannhauser's method, on the other hand, has following advantages: 1) Condition of extraction of nucleic acids with warm dilute alkali can be controlled easily, 2) DNA and PNA can be separated independently without difficulty.

In the present experiments, a modified Schmidt and Thannhauser's method was used for isolation of nucleic acids and other phosphorus containing substances from corn root tip, and these substances were determined by an estimation procedure based on the phosphate. The contents of nucleic acids of corn root tip were determined by Ogur and Rosen (1950) who carried out the differential extraction with perchloric acid. The comparison between the result obtained by Ogur and Rosen (1950) and that from the present study was also given in this paper.

In the present experiments, the nucleic acids isolated were especially determined by several estimation methods based on the three nucleic acid components, such as sugars, phosphorus and purines and pyrimidines.

Material and methods

1) Preparation of corn root tip

Seeds of *Zea Mays* (Kōshu-ki) were soaked in tap water for 15 hours at 30°C, then they were germinated at 30°C in the dark for 48 hours.

After germination, seeds having 1.5–3.0 cm length of primary root were selected, and apical 3 mm segments of primary root were cut out and used in this experiment. Total number and weight of segments used were 1400 and 3.311 g in fresh weight.

2) Fractionation by a modified Schmidt and Thannhauser's method

Corn root tips were ground and suspended in approximately 20 volumes of ice-cold 7% trichloroacetic acid (TCA) per g of fresh weight, and the suspension was cooled in ice-water for 20 minutes. Then the suspension was centrifuged at 5000 r.p.m. about 10 minutes. Then the precipitates were resuspended in ice-cold 7% TCA. These treatments were repeated, until the supernatant obtained finally by centrifugation did not give positive tests for pentose with orcinol reaction and for inorganic-P by PVM-method (Sugiyama, Shinke and Ishida 1954). The precipitate was washed with 20 volumes of ice-cold 1% TCA solution and then with ice-cold water. All supernatants obtained by centrifugation were combined in a flask as 'acid soluble fraction'. The supernatant contains acid soluble P-containing substances.

Then the precipitate was suspended in absolute ethanol for dehydration, and then the suspension was centrifuged. This treatment was repeated twice. The precipitate was suspended in about 30 volumes of ethanol-ether (3:1) mixture and boiled for 7 minutes in a water bath. After boiling, the suspension was cooled in ice-water and centrifuged. The precipitate was then suspended in about 30 volumes of methanol-chloroform (1:1) mixture and boiled for 40 minutes and centrifuged. The precipitate was washed with ether and dried at room temperature. All supernatant solutions obtained by centrifugation were combined in a flask as 'lipid fraction' containing phospholipids.

The residue dried from ether was suspended in N-KOH solution and kept for 15 hours at 37°C. After this treatment, the suspension was cooled and centrifuged. The supernatant solution contains DNA, depolymerized PNA, and inorganic phosphates derived from phosphoproteins. The precipitated residue was washed with small amount of warm N-KOH solution and centrifuged. The supernatant solution was then mixed with the above extraction solution.

For the separation of DNA, 0.2 volume of 6 N-HCl and one volume of 5% TCA were added to one volume of the KOH extraction solution, and the acidified mixture was kept in ice-cold water. White precipitate containing DNA, a fairly large amount of proteins and a very small amount of PNA were formed. After 30 minutes, the suspension was centrifuged. The supernatant contains PNA and phosphoprotein-P ('PNA-phosphoprotein-P fraction'). For removal of the PNA contamination from the precipitate, the precipitate was washed carefully with a suitable amount of ice-cold mixture of N-HCl and 5% TCA. After centrifugation, the supernatant solution was

added to above 'PNA-phosphoprotein-P fraction'. The precipitate or 'DNA fraction' was obtained.

Inorganic phosphates, derived from phosphoproteins, in 'PNA-phosphoprotein-P fraction', were separated from this fraction by Delory's method (Delory, 1938) modified by us (Sugiyama, Shinke and Ishida 1954). In this modified method, a 50% liquid ammonium hydroxide was added to 30 volumes of 'PNA-phosphoprotein-P fraction' until the mixture showed alkaline color with phenolphthalein as an indicator. The each 2 ml of 2% CaCl_2 and 0.5% MgCO_3 were added to this mixture. After 30 minutes, the mixture was centrifuged. The supernatant solution contains PNA ('PNA fraction') and the precipitate contains phosphoprotein-P ('phosphoprotein fraction').

3) Estimation of phosphorus

a) *Decomposition of organic phosphorus*

Organic phosphorus must be converted into inorganic phosphates by combustion, since organic phosphorus can not be determined by PVM-method. Details of the procedure were reported by Sugiyama, Shinke and Ishida (1954). An outline of the procedure is given below: A suitable aliquot of each fraction, which contains P-containing organic substances, is decomposed with 70% perchloric acid (PCA) in test tubes over microburner (King 1932). The combustion is proceeded until the solutions with PCA are clarified. The organic phosphorus are converted completely into inorganic phosphates by this procedure. Results of our experiments show that the procedure using PCA gives a better result than H_2SO_4 or HNO_3 (Sugiyama, Shinke and Ishida 1954).

b) *Estimation of inorganic phosphates by phosphovanado-molybdate (PVM) method*

After combustion with PCA, inorganic phosphates converted from organic phosphorus, are determined colorimetrically by PVM-method. Detail procedure of the PVM-method was reported by Sugiyama, Shinke and Ishida (1954). Outline of this procedure is: Suitable amount of distilled water is added to the solution in test tubes after combustion. Then following reagents are added to 5 ml of the solution containing inorganic-P. 1) 0.6 ml of 50% HNO_3 2) 1 ml of 10% ammonium molybdate solution 3) 3.4 ml of ammonium vanadate solution (1.175 g of ammonium metavanadate dissolved in 10 ml of conc. HNO_3 and 460 ml of distilled water added). After addition of the reagents, the mixtures are kept in room temperature. Yellow color develops, and color developed is very stable as seen in Fig. 1. Intensity of the color developed is measured at 410 $\text{m}\mu$ by a Beckman's spectrophotometer.

Effects of some inorganic salts on the intensity of the color developed have been investigated, and results are shown in Table 1 and Fig. 1. It is seen in Table 1 that the presence of salts, except silicates which though affects to a certain extent, does not give significant influence on the reaction

color development:

Fig. 1 shows effects of some salts on the stability of the colors developed after addition of the reagents. In this figure, it is seen that the reaction

Fig. 1. Shows effect of salts on stability of the color developed with phosphate by PVM-method. Equal volume of 10 mg/ml salt solution and 20 μ g/ml KH_2PO_4 solution was mixed. 10 μ g/ml KH_2PO_4 solution was used as a control. —○—, KH_2PO_4 ; —×—, $\text{MgSO}_4 + \text{KH}_2\text{PO}_4$; —●—, $\text{K}_2\text{SO}_4 + \text{KH}_2\text{PO}_4$; —△—, Sodium silicates + KH_2PO_4 . In cases of addition of other salts, as mentioned in Table 1, intensities of the color developed were also very stable, but increased gradually in the presence of silicates.

colors developed are very stable even though such salts are present in the color reaction mixtures.

The intensity of the color is, however, affected by the amount of PCA in reaction mixture. For instance, when a concentration of PCA in reaction mixture is 30%, the intensity of the color is

Table 1. Effect of salts on the intensity of the color developed with inorganic phosphate (KH_2PO_4) by PVM-method

Salts added	Absorbance	%
K_2SO_4	0.179	98.9
MgSO_4	0.177	97.8
Na_2CO_3	0.181	100.0
NaCl	0.179	98.9
KCl	0.179	98.9
$\text{Ca}(\text{NO}_3)_2$	0.182	101.1
KNO_3	0.178	98.5
KClO_4	0.180	99.4
Sodium silicates*	0.217	119.9
KH_2PO_4 (Control)	0.181	100.0

5 ml of 10 mg/ml salt solutions were added to 5 ml of 10 μ g/ml KH_2PO_4 solution. 5 μ g/ml KH_2PO_4 solution was used as a control.

* The concentration of sodium silicates is 0.1 mg/ml. The compositions of silicates are: Na_2SiO_3 , $\text{Na}_6\text{Si}_2\text{O}_7$ and $\text{Na}_2\text{Si}_3\text{O}_7$; they being dissolved in distilled water by heating.

decreased about 50% than that of control solution without PCA¹.

¹ Details of this experiment will be published by us later.

4) Estimations of nucleic acids by ultraviolet (UV) absorption and by color reaction of sugars

a) *Estimation of DNA*

'DNA fraction', obtained by the method described in the preceding page of this paper, contains a fairly large amount of proteins. The proteins must, therefore, be removed before the estimations of DNA because the proteins affect the UV-absorption measurement and color development of deoxyribose with Dische's and Feulgen's methods (Ishida 1956, 1959).

In the present experiment, isolation of DNA from 'DNA fraction' using PCA solution has been carried out by Ogur and Rosen's method (1950). The procedure is given below: 0.5 N-PCA solution is added to 'DNA fraction', and heated for 20 minutes at 70°C in a water bath. After heating, the solution is cooled and centrifuged. The precipitate obtained contains proteins, while supernatant contains DNA. These procedures of heating with PCA solution and centrifugation are repeated twice. The supernatants obtained by centrifugations are combined, and the amount of DNA in supernatant is determined by UV-absorption, Dische's and Feulgen's methods.

UV-absorption method: An absorption curve of DNA obtained by above procedure is shown in Fig. 2. It is seen in this figure that the wavelength of the absorption maximum of the DNA isolated by heating with 0.5 N-PCA locates at 268 m μ , that is, heating with PCA shifts the maximum from 260 m μ to 268 m μ . Similar phenomena were also observed on the DNAs from the same material by Ogur *et al* (1950) and from calf thymus and some plant tissues by Ishida (unpubl.).

The 0.1 N-KOH solution of 'DNA fraction', without heating with PCA, does not show the characteristic UV-absorption curve (Fig. 2-A). It is assumed that this phenomenon perhaps

Fig. 2. A) UV-absorption curve of 'DNA fraction' obtained by modified Schmidt and Thannhauser's method. 'DNA fraction' was dissolved in 0.1 N-KOH solution. B) UV-absorption curve of DNA extracted from 'DNA fraction' with hot PCA. The wavelength of the absorption maximum was found at 268 m μ .

is due to the presence of a large amount of proteins in this solution.

Optical density of DNA solution isolated with hot PCA, is measured at $268\text{ m}\mu$ by a Beckman's spectrophotometer. Calf thymus DNA is also heated with 0.5 N-PCA solution and used as a standard for the estimation by photometry.

Dische's method: Deoxyribose of DNA is estimated by this method. A modification of Dische's method was reported in the previous paper (Ishida 1954, 1956). Outline of the modified procedure is described below: DNA solution from 'DNA fraction' with hot PCA is neutralized by an addition of dilute KOH solution. Two volumes of Dische's diphenylamine reagent are added to one volume of the neutralized DNA solution, and heated at 90°C for 20 minutes in a water bath. Blue color develops. Intensity of the color developed is measured at $600\text{ m}\mu$. Calf thymus DNA treated throughout the same process is used as a standard.

Feulgen's method: An estimation of deoxyribose of DNA with Feulgen's reaction *in vitro* has been reported in the previous paper (Ishida 1959). The PCA solution of DNA isolated from 'DNA fraction' with hot PCA, is neutralized by an addition of dilute KOH solution. Then one volume of 0.2 N-HCl is added to one volume of the DNA solution, and heated at 90°C for 20 minutes. After hydrolysis of DNA with HCl, pH of this solution is adjusted at 2.28^1 by an addition of glycine-NaCl solution (Sørensen's buffer solution), and the solution is kept in a water bath at 10°C . One volume of Schiff's reagent is then added to nine volumes of the solution. Reddish purple color develops. Intensity of the color developed at 10°C is measured at $565\text{ m}\mu$ by a Beckman's spectrophotometer. Calf thymus DNA is used as a standard.

Fig. 3. A) UV-absorption curve of 'PNA fraction' obtained by the modified Schmidt and Thannhauser's method is shown. B) shows UV-absorption curve of PNA extracted from 'PNA fraction' with PCA at room temperature. The absorption maximum locates at $260\text{ m}\mu$.

The 'PNA fraction' solution itself does not show the typical UV-

¹ Determined by a Beckman's pH-meter.

b) Estimation of PNA

absorption curve, as seen in Fig. 3-A. It seems to present some amount of proteins. For removal of the proteins, 'PNA fraction' is neutralized, and dried on a warm water bath. Then a suitable amount of N-PCA is added to the dry 'PNA fraction' and kept for 5 hours in room temperature. PNA is extracted in N-PCA. PCA solution of PNA shows a characteristic absorption curve having the maximum at 260 $m\mu$ as seen in Fig. 3-B.

UV-absorption method: Optical density of the solution of PNA, obtained by the above procedure, is measured at 260 $m\mu$. PNA¹ from yeast is treated simultaneously and used as a standard for the photometry.

Orcinol method: Procedure of orcinol reaction for estimation of pentose sugar is carried out according to Mejbaum's method (Mejbaum 1939). The PNA solution isolated from 'PNA fraction' with N-PCA is neutralized by an addition of suitable amount of dilute KOH solution. The orcinol reagent is then added to the neutralized PNA solution and heated for 20 minutes in a boiling water bath. The absorption maximum of the reaction color developed lies at 656 $m\mu$. The color intensity is measured at this wavelength. Yeast PNA¹ is used as a standard.

Results

1) Contents of nucleic acids and other P-containing substances in apical 3 mm segments of corn root tip

P-contents of each fraction, obtained by a modified Schmidt and Thannhauser's method, were estimated by PVM-method and were summarised in Table 2. It is seen in this table that phosphorus content of 'PNA fraction' shows the highest value among all fractions and that phosphoprotein-P is

Table 2. Amounts of phosphorus in each fraction obtained from 3 mm segments of corn root tip by modified Schmidt and Thannhauser's method

Fractions	Phosphorus contents			per cent to total-P
	$\mu\text{g/g}$ fresh weight	$\mu\text{g/g}$ dry weight	$\mu\text{g/3}$ segment	
Acid soluble	709.8	5245.8	1.679	36.7%
Lipid	377.5	2790.2	0.893	19.5
DNA	111.1	821.2	0.263	5.7
PNA	721.5	5332.4	1.706	37.3
Phosphoprotein	14.5	107.1	0.034	0.8
Total sum of each fraction	1934.4	14296.7	4.575	100.0

Dry weight was determined after drying 200 apical 3 mm root tip segments at 60°C for 48 hours. Water content of these materials were 86.5%, and when the root tip segments had also been dried in the condition of 110°C for 2 hours, the water content being found the same as that of above condition.

¹ Obtained from Merck.

negligibly low. 'Acid soluble fraction', which contains many kinds of substances such as inorganic salts, sugars, nucleosides, nucleotides and acid soluble proteins, etc., shows a high value as well as in 'PNA fraction'.

In Table 3, the results obtained by Ogur *et al* (1950) and the present experiment on the contents of nucleic acids and nucleic acid-P are compared. As seen in this table, the contents of both nucleic acids per mg of dry weight, are a little higher than the values obtained by Ogur and Rosen (1950), but the values per mg of fresh weight and 3 mm segment, are a little lower than the values determined by Ogur *et al*. The ratio of PNA to DNA in corn root tip is about 6.5 and is approximately equal to the value calculated by Ogur *et al*.

Table 3. Amounts of nucleic acids and nucleic acid phosphorus in the apical 3 mm segments of corn root tip

Nucleic acids	$\mu\text{g}/\text{mg}$ fresh weight		$\mu\text{g}/\text{mg}$ dry weight		$\mu\text{g}/3$ mm segment	
	present experiment	Ogur and Rosen	present experiment	Ogur and Rosen	present experiment	Ogur and Rosen
DNA-P	0.111	0.132	0.821	0.735	0.263	0.301
DNA	1.12	1.33	8.29	7.42	2.66	3.04
PNA-P	0.722	0.794	5.33	4.42	1.706	1.81
PNA	7.58	8.35	56.0	46.3	17.9	19.0

DNA-P and PNA-P were determined by PVM-method for an estimation of inorganic-P. In Ogur and Rosen's data, P-estimation was made by the method of Fiske and SubbaRow (1925). Amounts of nucleic acids were calculated from Schneider's factor; DNA-P was converted to DNA and PNA-P to PNA by dividing by 0.095 and 0.099 respectively (cf. Schneider, 1945).

2) Estimations of nucleic acids by UV-absorption procedure and some color reactions of deoxyribose and pentose sugars.

The amounts of nucleic acids estimated by several estimation methods are summarized in Table 4. It is seen in this table that the value of DNA, calculated from the value obtained by estimation of nucleic acid-P by PVM-

Table 4. Nucleic acid contents ($\mu\text{g}/\text{mg}$ of fresh weight) of 3 mm segments of corn root tip determined by several estimation methods

Nucleic acids	Estimation methods				
	P. V. M. phosphorus	U. V. purine and pyrimidine	Dische's deoxyribose sugar	Feulgen's deoxyribose sugar	Orcinol pentose sugar
DNA	1.12 ¹ (100.0%)	1.31 (116.9%)	1.15 (102.7%)	1.27 (113.4%)	— (—)
PNA	7.58 ¹ (100.0%)	7.89 (104.1%)	— (—)	— (—)	7.71 (101.7%)

¹ Amounts of nucleic acids were calculated from Schneider's factor after the estimation of nucleic acid phosphorus by PVM-method.

method, is less than the values obtained by the procedures of UV-absorptions and color reactions of sugars. Table 4 also shows that the value of DNA determined by UV-absorption methods is higher than that by other procedures of estimation.

The ratios (%) in round brackets are expressed as relative value, assuming that each value of DNA and PNA obtained by PVM-method is 100.0%. Here we find good agreements among the values of PNA obtained by three estimation methods, while in DNA, some variations are found among four values determined by independent estimation procedures.

Discussion and conclusion

1) Isolation and estimation procedures of nucleic acids

A modified method of Schmidt and Thannhauser's method (cf. Sugiyama, Shinke and Ishida 1954) was used in this study. The results of a biochemical study of the root tips of *Vicia faba* showed that the 'DNA fraction' obtained by the modified Schmidt and Thannhauser's method contained a fairly large amount of proteins (unpubl.). It is highly probable, therefore, to consider that the 'DNA fraction' obtained from the root tips of *Zea Mays* has a certain amount of proteins as a contaminant. This assumption is quite in accord with the fact that the absorption curve of the 'DNA fraction' does not show the typical UV-absorption curve. These co-existing proteins affect the estimation of nucleic acids by procedures of UV-absorption and some color reactions of sugars. Hence it is necessary to remove these proteins from both nucleic acid fractions before estimation. For this purpose, Ogur and Rosen's method using PCA (Ogur and Rosen 1950) has been employed in this study, that is, DNA was extracted from 'DNA fraction' by the treatment of hot PCA while PNA being extracted from 'PNA fraction' with PCA at room temperature. The UV-absorption curves of both nucleic acids are shown in Fig. 2-B and 3-B respectively.

It is seen in Fig. 2-B that the wavelength of the maximum of absorption curve of DNA extracted with hot PCA locates at $268\text{ m}\mu$, that is, heating of DNA with PCA shifts the absorption maximum from $260\text{ m}\mu$ to $268\text{ m}\mu$ as shown also by Ogur and Rosen (1950). Moreover, optical density of DNA is affected by this treatment to a certain extent. Similar phenomenon is also observed in the DNAs extracted from calf thymus and *Marchantia polymorpha* with hot PCA (Ishida unpubl.). It is assumed that these effects of heating with PCA on the intensity of UV-absorption and shifting of maximum wavelength of UV-absorption, are caused by an optical resonance of the molecules of the degradation products of DNA (cf. Tamm *et al* 1955).

On the other hand, optical density of PNA at $260\text{ m}\mu$ is increased when it is treated with cold PCA. Similar result was observed by Ogur and Rosen (1950). This phenomenon has been reported as 'hyperchromic effect' on nucleic acids (Magasanik *et al* 1951; Tamm *et al* 1952), that is, the de-

composition of highly polymerized nucleic acids is accompanied by emancipation of the suppression of certain chromophores. When the estimations of nucleic acids are carried out, DNA and PNA which are used as standards must, therefore, be treated in the same way with nucleic acids to be assayed before estimation.

Contrary to the estimation by UV-absorption method, which is based on purine and pyrimidine bases of nucleic acids, amounts of nucleic acids are also determined by certain procedures such as Feulgen's, Dische's and orcinol methods based on the color reaction of pentose and deoxyribose sugars. In these cases, co-existing proteins in nucleic acid solution also affect the intensity and wavelength of absorption maximum of the color developed (Ishida 1956, 1959). These proteins must, therefore, be removed before estimations by sugar color reaction as in the case of the estimation by UV-absorption method.

2) Contents of nucleic acids and other P-containing substances in corn root tip

Table 2 shows the contents of nucleic acids and other P-containing substances in corn root tips. In this table, it is seen that values of phosphorus of the 'PNA fraction' and the 'acid soluble fraction' are higher than those of other fractions. In the last column of Table 2, the rates of phosphorus content in each fraction to total sum of phosphorus are shown in percentage. In this column, it is seen that phosphorus contents of 'acid soluble fraction' and 'PNA fraction' are high, while the ratio calculated on DNA is very low. According to Shinke and Ishida's experiment (Shinke and Ishida unpubl.), the ratios of P-content of 'acid soluble fractions' obtained from the root tip meristem and elongating zone of *Vicia faba*, show high values as in corn root tips, and also the ratio of 'PNA fraction' from the meristem shows a high value but the ratio of elongating zone is very low. The distribution of such rates among each fraction as seen in this column of Table 2 is different for tissues or individuals. Such differences on the distribution pattern are also found in other plant materials such as *Spirogyra* spp. (Ishida, unpubl.), *Oscillatoria princeps* (Shinke and Ishida, unpubl.) and *Oscillatoria limosa* (Ishida, unpubl.).

It is seen in Table 3 that DNA and PNA contents per g of fresh weight and per 3 mm segment are little lower than those obtained by Ogur and Rosen (1950) in the root tip segments of *Zea Mays* (hybrid), while both acids contents per g of dry weight obtained in this experiment are a little higher than the values obtained by them. It is assumed that the main causes for such differences between the values obtained by Ogur and Rosen (1950) and the present study are as follows: 1) Differences in strain and water content of plants used. 2) Differences of the procedures of extraction and estimation of nucleic acids.

In Table 4 the nucleic acid contents, determined by several estimation

methods, are compared. The value of DNA estimated by the PVM-method is the lowest, while the value by the UV-absorption procedure is the highest. Similar tendency is also found among the values of PNA. It is assumed that such variation among the values of particular nucleic acid estimated by different methods, are caused by differences of the chemical composition of these nucleic acids. Beaven *et al* (1955) stated the values of atomic extinction coefficient $\epsilon(P)$ will vary with the purine and pyrimidine contents of nucleic acids from different sources. It has been also shown by Laland, Overend and Webb (1952) that the values obtained by Dische's and Feulgen's methods for estimation of deoxyribose sugar of DNA are correlated with the *purine* contents of DNA.

In nucleic acid estimation, more reliable result will, therefore, be obtained by the several estimation procedures based on the different components of nucleic acids than that obtained by a method based on single component. It may also be assumed that the differences in value obtained by the different methods stated above, probably be caused by difference in compositions of the nucleic acid used as a standard and the nucleic acid to be assayed.

Summary

1) Acid soluble substances, lipids, DNA, PNA and phosphoproteins fractions from apical 3 mm segments of corn root tip meristem were obtained by a modified Schmidt and Thannhauser's method, and phosphorus contents of each fraction were estimated by PVM-method for an estimation of inorganic phosphates after combustion with PCA.

2) Amount of DNA was also estimated by UV-absorption, Dische's and Feulgen's methods after extraction with 0.5 N-PCA from 'DNA fraction' obtained by a modified Schmidt and Thannhauser's method. PNA was extracted from 'PNA fraction' with N-PCA, and estimated by the UV-absorption and orcinol reaction methods. The isolations of DNA and PNA from both 'nucleic acid fractions' were carried out according to the Ogur and Rosen's method.

3) Amounts of DNA and PNA per mg of dry weight of corn root tips were 8.29 μg and 56.0 μg respectively. The values of both acids in corn root tips obtained by Ogur and Rosen (1950) were lower, while the values per fresh weight and 3 mm segment obtained by them were higher than those obtained in this study.

4) Procedures for the estimation of nucleic acids based on their three chemical compositions were discussed in this paper.

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