# CHEMICAL INVESTIGATION OF WHEAT

## IV. Dynamics of Various Forms of Phosphorus in Wheat during Its Ontogenesis[1]

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**ABSTRACT**

The content of phosphorus in desoxyribonucleic acid and ribonucleic acid and the resulting ratio of the two acids in the gluten of various low-yielding and high-yielding wheat varieties has been investigated. From the results obtained it was concluded that the assumption of Bourdet and Herard about a possible correlation between the content of nucleic acids in wheat flour and the biological and technological properties of a wheat variety is not justified for, nor applicable to, wheat samples cultivated under ordinary crop production (uncontrolled climatic conditions, undefined soil characteristics, variable agrotechnical measures, etc.). Moreover, it was demonstrated that the content of ribonucleic acid in wheat grain depended not only on the variety, but also on applied agrotechnical measures, i.e., on the use of nitrogen, phosphorus, and potassium fertilizers, and that, therefore, the ratio of nucleic acids in the grain could not be specific for a wheat variety, since it is not constant. At the same time, the observed variations in the accumulation of ribonucleic acid in wheat grain have confirmed that the biosyntheses of ribonucleic acid and proteins in the grain are, at least partly, coupled.

Previous investigations have shown that the amount of phosphorus in ribonucleic acid (RNA) of ripe wheat grain varied according to the wheat variety, in the range of 5.9 to 9.95 mg. % (1). In the same report the difficulties which are encountered in the separation of RNA[3] and desoxyribonucleic acid (DNA) were emphasized. The spectrophotometric method recently developed for direct determination of nucleic acids (NA) in cereal grains on the basis of purines present in the hydrolysate (2) is not specific for individual NA. Owing to the presence of a large amount of starch in wheat grain, the older methods for the determination of RNA and DNA (3,4,5) are not applicable, since they give unreliable results. That is probably the main reason why the content of DNA in wheat grain has not yet been investigated.

However, if starch is removed from whole-meal flour by washing with water, then the remaining gluten still represents a material con-

[1]Manuscript received July 10, 1963. Contribution from the Faculty of Science, Department of Chemistry, Beograd, from the Faculty of Forestry, Beograd, and from the Faculty of Technology, Novi Sad (Yugoslavia). The third paper in the series is cited in reference 1 herein.

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[3]Abbreviations used throughout the text: RNA, ribonucleic acid; DNA, desoxyribonucleic acid; NA, nucleic acids; RNA-P, DNA-P, NA-P, phosphorus in the corresponding acids; N, P, and K fertilizers, nitrogen-, phosphorus- and potassium-containing fertilizers.

venient for the determination of individual NA with satisfactory accuracy and without methodological difficulties. In this way, by separating uridylic acid and thymine derivatives, Bourdet and Herard (6) have recently identified the presence of both acids (RNA and DNA) in the nucleic acid extract of some French types of flour. The results of the quantitative investigation of these acids have suggested the possibility that the ratio DNA/RNA might represent a value characteristic for a wheat variety, i.e., for its gluten. This problem, although of considerable theoretical and practical significance for contemporary crop production, selection requirements, and milling industry in general, has not been hitherto studied.

Therefore, the present study was undertaken with a view to investigating: (a) the qualitative and quantitative differences and possible correlations existing between DNA and RNA, i.e., DNA-P and RNA-P, in the gluten of some high-yielding and low-yielding wheat varieties; (b) the constancy of the ratio DNA/RNA and the possibility of utilizing this ratio as a value which would be characteristic for a given wheat variety; (c) whether increasing levels of N fertilizer can influence the coupled biosyntheses of proteins and RNA in wheat grain, in the sense of increasing the amount of RNA, and if so, what are the variations in the content of this acid in a given wheat variety, and how is the ratio DNA/RNA affected by these changes; (d) whether variable levels of inorganic fertilizers, i.e. N, P, and K fertilizers, which have been shown to influence the amount of phytic acid phosphorus (7), will also change, and to what extent, the contents of nucleic and nucleoproteid phosphorus in wheat grain.

## Materials and Methods

For the investigation of gluten DNA and RNA, the following wheat varieties were used: high-yielding varieties – Abbondanza, Autonomia, Elia, Fortunato, Funo, Leonardo, Leone, Mara, Patricio, Produttore, San Pastore, San Marino (all Italian varieties), and Etoile de Choisy (French variety); low-yielding (Yugoslav) varieties – Bankut 1205, Novosadska 1446, Novosadska 1993, and Rumska crvenka.

The influence of variable levels of N, P, and K fertilizers on the accumulation of RNA in wheat grain was studied on the high-yielding variety San Pastore. The details of field experiments, performed with increasing levels of inorganic fertilizers by a random-block system on chernozem soil type, were described previously (7,8).

Total phosphorus of grain and gluten was determined according to known procedures (8). Separation and determination of RNA from ground wheat were carried out essentially as reported previously (1).

Gluten was obtained in the usual way, by washing whole-meal flour with water to remove starch. Moist gluten was dried by rinsing with acetone and ether; the rest of the lipid was removed with methanol-chloroform (1:2 v/v). Nucleotides, orthophosphate, and other acid-soluble phosphorus compounds were eliminated from gluten by means of 0.2*N* $HClO_4$ at 0°C.

Gluten NA was extracted with 0.5*N* $HClO_4$ at 70°C. (3), and in the resulting extract NA was determined either spectrophotometrically or by decomposition of the extract with 1 ml. of concentrated $HClO_4$ and a few drops of concentrated $HNO_3$ in a Kjeldahl microflask (9), followed by determination of NA-P through reduced heteropolymolybdophosphoric acid, according to Harris and Popat (9). Results obtained by these two methods were in good agreement. In the original perchloric extract DNA was determined by Burton's modification of the Dische reagent (10) and by the micromethod of Ceriotti (11), both procedures affording results which agreed satisfactorily. The content of gluten RNA-P was calculated by subtracting DNA-P from total NA-P.

The absorbances of NA were recorded in the UV region at 220–300 mμ, on a quartz Beckman spectrophotometer, Model DU.

Wheat grain proteins were determined by a Kjeldahl semimicro method, in the presence of a selenium catalyst; liberated ammonia was distilled in a 2% solution of boric acid (apparatus according to Markham, 12).

## Results and Discussion

As evident from Table I, the highest contents of gluten DNA-P were observed in the high-yielding varieties. However, even in this group of wheats the differences are considerable, ranging from 24.3 (Produttore) to 65.3 mg. % DNA-P (gluten basis) (Autonomia), with a maximum difference of 41 mg. %. The low-yielding varieties had a lower content of DNA-P, varying in more narrow limits from 16.9 to 36.4 mg. % (maximum difference 19.5 mg. %).

Similarly to DNA-P, the content of gluten RNA-P also varied significantly in the high-yielding varieties, from 20.9 (Mara) to 60.6 mg. % (Patricio), with a maximum difference of 39.7 mg. %. In the low-yielding varieties this difference was smaller (25.3 mg. %), but, as compared to DNA-P, the contents of RNA-P varied in a wider range, the highest content being found in the variety Novosadska 1993 (47.6 mg. % RNA-P).

If total NA-P (DNA-P + RNA-P) of both wheat groups is compared, then it can be seen, with the exception of the varieties Mara and Produttore, that gluten of the high-yielding varieties contains more NA-P

TABLE I

CONTENTS OF DNA-P, RNA-P, DNA, AND RNA IN THE GLUTEN OBTAINED FROM THE GRAIN OF SOME ITALIAN HIGH-YIELDING AND YUGOSLAV LOW-YIELDING WHEAT VARIETIES

(mg. per 100 g. dry gluten)

| WHEAT VARIETY | DNA-P | RNA-P | DNA | RNA | DNA/RNA |
|---|---|---|---|---|---|
| | *mg.* | *mg.* | *mg.* | *mg.* | *mg.* |
| High-yielding varieties | | | | | |
| Abbondanza | 52.6 | 24.1 | 554.0 | 243.5 | 2.28 |
| Autonomia | 65.3 | 22.5 | 687.3 | 227.2 | 3.03 |
| Elia | 28.8 | 56.0 | 303.1 | 566.0 | 0.54 |
| Etoile de Choisy[a] | 34.6 | 50.1 | 364.2 | 506.0 | 0.72 |
| Fortunato | 56.1 | 45.5 | 590.7 | 459.5 | 1.29 |
| Funo | 62.8 | 35.9 | 660.1 | 365.2 | 1.81 |
| Leonardo | 63.1 | 57.5 | 665.0 | 580.5 | 1.15 |
| Leone | 59.1 | 46.9 | 622.0 | 474.0 | 1.31 |
| Mara | 36.3 | 20.9 | 382.0 | 211.1 | 1.81 |
| Patricio | 47.5 | 60.6 | 500.0 | 612.1 | 0.82 |
| Produttore | 24.3 | 31.2 | 261.5 | 315.0 | 0.83 |
| San Pastore | 31.6 | 45.9 | 335.0 | 464.0 | 0.72 |
| San Marino | 42.5 | 57.3 | 447.3 | 479.0 | 0.93 |
| Low-yielding varieties | | | | | |
| Bankut 1205 | 26.5 | 39.9 | 279.0 | 406.0 | 0.69 |
| Novosadska 1446 | 24.0 | 29.8 | 250.5 | 302.0 | 0.83 |
| Novosadska 1993 | 16.9 | 47.6 | 178.5 | 482.0 | 0.37 |
| Rumska crvenka | 36.4 | 22.3 | 383.1 | 225.0 | 1.70 |

[a] French high-yielding variety.

(maximum value 120.6 mg. %) than gluten of the low-yielding varieties (maximum content 66.4 mg. %).

Similar results were obtained for gluten DNA and RNA.

As expected, the ratio DNA/RNA was variable but was not characteristic for a given wheat variety, since identical or similar ratios were found for the gluten of two or more varieties (see Table I) having widely different varietal characteristics, particularly biological potentials. Therefore, data for the contents of DNA and RNA, when considered separately and expressed as percentage of gluten, cannot be used for obtaining numerical constants which would be specific for an individual wheat variety, although their sum might be useful for yield-group indication.

To get more information on this point, the content of gluten NA-P based on gluten total phosphorus, grain total phosphorus, and gluten present in 100 g. of grain was next investigated. The data obtained are presented in Table II.

As can be seen from Table II, the content of total gluten phosphorus was high in both wheat groups and, with a few exceptions, varied between 350.8 and 560.2 mg. % phosphorus. These results are not unexpected, since it was recently found that practically all phospholipids in the grain of high- and low-yielding wheat varieties were bound to gluten proteins and represented up to 20% of the total phos-

TABLE II

GLUTEN NA-P, BASED ON TOTAL GRAIN AND GLUTEN PHOSPHORUS, OF SOME HIGH-YIELDING AND LOW-YIELDING WHEAT VARIETIES

| WHEAT VARIETY | TOTAL GRAIN P | TOTAL GLUTEN P | GLUTEN P | GLUTEN P | GLUTEN DNA-P | GLUTEN DNA-P | GLUTEN DNA-P | GLUTEN RNA-P | GLUTEN RNA-P | GLUTEN RNA-P | DNA-P/ RNA-P |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | *mg./100 g. grain* | *mg./100 g. gluten* | *mg./100 g. grain* | *% of total grain P* | *mg./100 g. grain* | *% of total grain P* | *% of total gluten P* | *mg./100 g. grain* | *% of total grain P* | *% of total gluten P* | |
| High-yielding varieties | | | | | | | | | | | |
| Abbondanza | 420.3 | 440.1 | 38.1 | 9.1 | 4.6 | 1.1 | 11.9 | 2.1 | 0.5 | 5.5 | 2.18 |
| Autonomia | 453.9 | 360.2 | 29.1 | 6.4 | 5.3 | 1.2 | 18.1 | 1.8 | 0.4 | 6.2 | 2.90 |
| Elia | 516.4 | 390.7 | 49.2 | 9.5 | 3.6 | 0.7 | 7.4 | 7.0 | 1.4 | 14.3 | 0.51 |
| Etoile de Choisy | 433.7 | 240.2 | 18.2 | 4.2 | 2.6 | 0.6 | 14.4 | 3.8 | 0.9 | 20.9 | 0.69 |
| Fortunato | 402.3 | 409.5 | 26.5 | 6.6 | 3.6 | 0.9 | 13.7 | 2.9 | 0.7 | 11.1 | 1.23 |
| Funo | 489.6 | 494.0 | 56.2 | 11.5 | 7.1 | 1.5 | 12.7 | 4.1 | 0.8 | 7.3 | 1.75 |
| Leonardo | 417.1 | 423.8 | 51.7 | 12.4 | 7.7 | 1.8 | 14.9 | 7.0 | 1.7 | 13.6 | 1.10 |
| Leone | 416.1 | 518.2 | 60.6 | 14.6 | 6.9 | 1.7 | 11.4 | 5.5 | 1.3 | 9.1 | 1.26 |
| Mara | 478.4 | 391.5 | 24.6 | 5.1 | 2.5 | 0.5 | 9.3 | 1.4 | 0.3 | 5.3 | 1.74 |
| Patricio | 500.0 | 350.8 | 26.8 | 5.4 | 3.6 | 0.7 | 13.5 | 4.6 | 0.9 | 17.3 | 0.78 |
| Produttore | 401.8 | 297.0 | 16.1 | 4.0 | 1.3 | 0.3 | 8.2 | 1.7 | 0.4 | 10.5 | 0.78 |
| San Pastore | 455.5 | 279.6 | 20.4 | 4.5 | 2.3 | 0.5 | 11.3 | 3.4 | 0.7 | 16.4 | 0.69 |
| San Marino | 432.5 | 256.3 | 13.8 | 3.0 | 2.3 | 0.5 | 16.6 | 3.1 | 0.7 | 22.4 | 0.74 |
| Low-yielding varieties | | | | | | | | | | | |
| Bankut 1205 | 504.1 | 451.6 | 56.2 | 11.1 | 4.7 | 0.9 | 5.9 | 5.0 | 1.0 | 8.8 | 0.66 |
| Novosadska 1446 | 530.4 | 560.2 | 79.3 | 14.9 | 3.7 | 0.2 | 4.3 | 4.6 | 0.9 | 5.3 | 0.81 |
| Novosadska 1993 | 468.6 | 462.8 | 86.6 | 18.5 | 3.2 | 0.7 | 3.7 | 8.9 | 1.9 | 10.3 | 0.36 |
| Rumska crvenka | 461.3 | 318.0 | 14.0 | 3.0 | 1.8 | 0.4 | 11.4 | 1.1 | 0.2 | 0.7 | 1.63 |

phorus present in gluten (6,13). On the other hand, Courtois and Barré described precipitation reactions between phytic acid and proteins (14,15). Without discussing in which form phytic acid is present in gluten, Bourdet and Herard stated that wheat gluten contained a high percentage of phytic acid phosphorus, i.e. from 30 to 46% of total gluten phosphorus (6).

As is evident from Table II, of total gluten phosphorus only a small percentage is represented by DNA-P and RNA-P. In contrast to the results given in Table I, DNA-P expressed in this way shows smaller variations, particularly in the high-yielding varieties. In the low-yielding varieties this content was lower, and ranged from 3.7 to 11.4% DNA-P.

Gluten RNA-P, expressed as percentage of gluten total phosphorus, varied in a somewhat wider range (from 5.3 to 22.4%) in high-yielding varieties; the differences in low-yielding varieties were slightly lower (5.3–10.3%), as compared to gluten DNA-P. Total NA-P (DNA-P + RNA-P), expressed in the same way, was also variable and ranged from 39.0% (San Marino) to 14.6% (Mara) for high-yielding varieties, and from 9.6 to 18.4% for low-yielding varieties.

The ratio DNA-P/RNA-P obtained from the above-mentioned results had values higher and lower than 1, which varied between 0.36 and 2.90. These ratios were not characteristic for a given wheat variety, two or more varieties having identical or very similar ratios.

From all the facts described above, it appears that no definite correlation exists between, on one hand, the contents of DNA and RNA, and on the other, the biological-varietal properties and the known technological characteristics of the investigated pure wheat varieties cultivated under ordinary conditions of crop production (uncontrolled climate conditions, undefined soil characteristics, variable agrotechnical measures, etc.). Therefore, under these conditions, the ratio DNA-P/RNA-P, based on gluten total phosphorus (or grain total phosphorus), cannot be considered as a constant which could determine a given variety, nor can it be approximately used for the identification of wheat varieties.

Because of these results, it seemed of interest to investigate whether the unspecificity of the ratio DNA/RNA was perhaps due to the variation of RNA within a given wheat variety. This problem is of particular significance from the aspect of high levels of N and P fertilizer treatment and in the frame of contemporary agrotechnical measures, since N and P are elements which enter into the composition of RNA.

According to recent investigations on the biosynthesis of RNA in wheat (16–21), one might expect a certain variation in the course of

this synthesis and therefore also changes in accumulation of RNA in wheat grain, under the influence of variable levels of P and particularly N fertilizers. The presence of $NH_4^+$-ions in excess can cause an increase in the conversion of alpha-ketoglutaric and oxalacetic acids from the Krebs cycle to the corresponding amino acids (glutamic and aspartic acids), while an increase in the process of glycolysis would furnish glycine, the third amino acid essential for the biosynthesis of RNA.

On the other hand, that increasing levels of N and K fertilizers will enhance the biosynthesis of amino acids and therefore the accumulation of proteins in grain is a known fact. Since the biosyntheses of proteins and RNA are coupled (22), an increase in the content of RNA in wheat grain should be expected under these conditions.

The results of such investigations on the high-yielding variety San Pastore are listed in Table III and shown in Figs. 1, 2, and 3.

TABLE III

CONTENT OF GRAIN RNA-P OF THE WHEAT VARIETY SAN PASTORE, AS AFFECTED BY INCREASING LEVELS OF N, P, AND K FERTILIZERS; MEAN VALUES OBTAINED FROM THREE PLOTS

| FERTILIZER | GRAIN RNA-P | | FERTILIZER | GRAIN RNA-P | |
|---|---|---|---|---|---|
| | *mg./100 g. grain* | *% of total grain P* | | *mg./100 g. grain* | *% of total grain P* |
| PK | 4.15 (0.071)[a] | 0.50 (0.012) | $NK\text{-}P_{144}$ | 4.72 (0.169) | 0.53 (0.024) |
| $PK\text{-}N_{60}$ | 4.83 (0.198) | 0.52 (0.027) | $NK\text{-}P_{252}$ | 6.20 (0.126) | 0.63 (0.007) |
| $PK\text{-}N_{100}$ | 3.69 (0.122) | 0.39 (0.012) | NP | 5.64 (0.129) | 0.61 (0.022) |
| $PK\text{-}N_{160}$ | 6.26 (0.032) | 0.69 (0.032) | $NP\text{-}K_{80}$ | 5.32 (0.080) | 0.58 (0.004) |
| NK | 3.61 (0.158) | 0.42 (0.020) | $NP\text{-}K_{160}$ | 4.53 (0.224) | 0.52 (0.025) |
| $NK\text{-}P_{72}$ | 4.45 (0.249) | 0.55 (0.029) | $NP\text{-}K_{240}$ | 3.95 (0.060) | 0.45 (0.010) |

[a] Mean deviations in parentheses.

With increasing levels of N fertilizer (from $N_0$ to $N_{160}$ kg./hectare) the content of grain RNA-P increased steadily, with the exception of the level $PK+N_{100}$, from 4.15 to 6.26 mg. % of grain (or expressed in percentage of grain total phosphorus, from 0.50 to 0.69%). These results confirm in part the theoretical assumptions described above. If the accumulation of RNA is compared with the accumulation of proteins in grain, it can be seen that both contents are similarly affected by increasing levels of N fertilizer (Fig. 1). This finding is in accordance with the Brachet hypothesis (22), but does not agree with the experiments of Semenenko (21), who found that by the addition of nutritive solutions containing $Ca(NO_3)_2$ and $NH_4Cl$, the content of purine bases in wheat remained constant while total nitrogen increased. How difficult it is to explain these facts is shown also by the example of the level $N_{100}$ (Table III, Fig. 1). For as yet unknown reasons this combination, in all plots, decreased the content of grain proteins to 11.58%

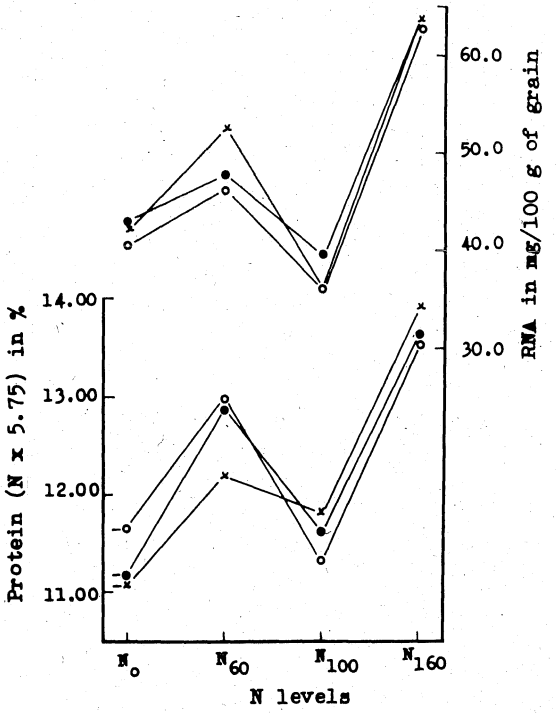


Fig. 1. Protein and RNA contents in the grain of the wheat variety San Pastore, as affected by increasing levels of N fertilizer (P and K constant). The curves with the corresponding marks (x, solid circles, and open circles) represent results obtained from replicate plots. (The same applies to Figs. 2 and 3.)

(mean value), but, accordingly, the content of RNA was also lowered to its minimum value: 37.1 mg. % RNA (Fig. 1) or 3.69 mg. % RNA-P (average amounts based on grain) (Table III).

Increasing levels of phosphorus fertilizer ($P_0$—$P_{252}$ kg./hectare) caused a regular increase in the biosynthesis of RNA, in accordance with theoretical considerations (see above) (Table III, Fig. 2). The content of RNA-P changed from 3.61 ($P_0$) to 6.20 mg. % of grain ($P_{252}$), or, if expressed as percentage of total grain phosphorus, from 0.42 to 0.63%, these values being comparable to the amounts of RNA-P found when increasing levels of N fertilizer were used (Table III). At the same time, the content of grain proteins increased also (Fig. 2), but to a considerably lower extent as compared to the amount of proteins observed when increasing levels of N fertilizer were applied (Fig. 1). Here also, one level ($P_{144}$) lowered the protein content to its minimum value, but, in contrast to the N fertilizer, did not decrease the amount of RNA. From these results it appears that when increasing levels of P

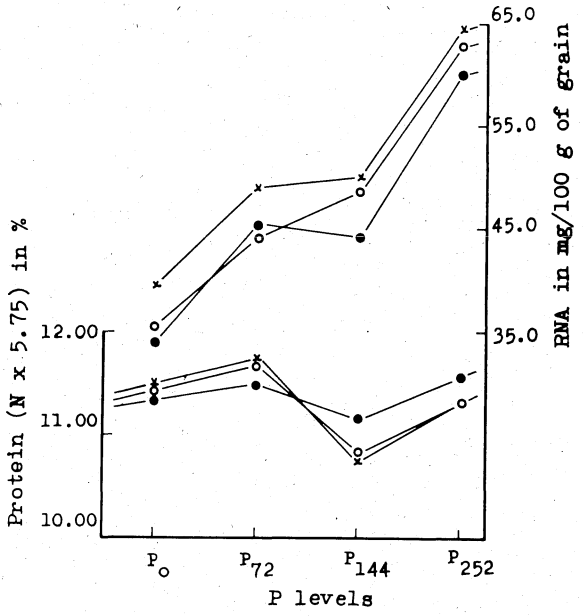


Fig. 2. Protein and RNA contents in the grain of San Pastore wheat, as affected by increasing levels of P fertilizer (N and K constant).

fertilizer are used, the biosynthesis of RNA in wheat grain might be partially independent of the coupled process of RNA and protein biosynthesis.

With increasing levels of K fertilizer ($K_0$ —$K_{240}$ kg./hectare) the content of grain RNA, i.e. RNA-P, slowly and continuously decreased (Table III, Fig. 3). However, the accumulation of proteins in grain gave quite opposite results. Here, the first three levels of K fertilizer greatly enhanced the protein content, but the highest level stopped this increase (Fig. 3). These unexpected findings, in contradiction to the Brachet hypothesis (22), suggest that increasing levels of K fertilizer increase the biosynthesis of proteins by pathways which are not connected to the coupled biosynthesis of proteins and RNA.

Values for the content of RNA (RNA-P) given in Figs. 1 to 3 and Table III were obtained by extraction of RNA with cold perchloric acid (1). However, under these conditions only 60.3% of the total RNA was extracted from gluten of the wheat variety San Pastore (Table IV); the rest remained in the gluten, as an insoluble nucleoproteid, and could be removed with perchloric acid only at higher temperatures. The gluten of other varieties behaves similarly (Table IV).

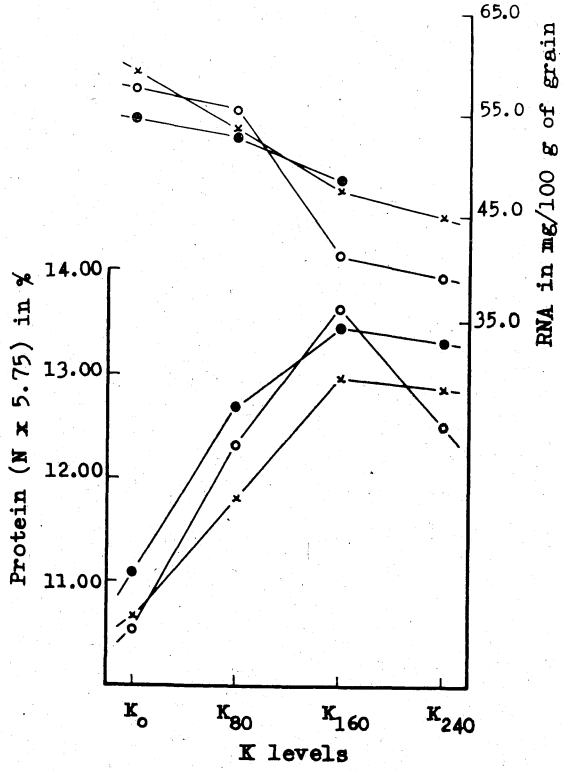


Fig. 3. Protein and RNA contents in the grain of San Pastore wheat, as affected by increasing levels of K fertilizer (N and P constant).

TABLE IV

AMOUNTS OF RNA EXTRACTED FROM WHEAT GLUTEN BY PERCHLORIC ACID AT 0° AND 70°C.[a]

| | RNA FORMS IN GLUTEN | | |
|---|---|---|---|
| | RNA Extracted at 0°C. with 1*N* $HClO_4$ | Additional RNA Extracted at 70°C. with 0.5*N* $HClO_4$ | |
| | *mg. RNA-P/ 100 g. gluten* | *mg. RNA-P/ 100 g. gluten* | *% of total gluten RNA* |
| High-yielding varieties | | | |
| Elia | 32.0 | 24.8 | 43.7 |
| Etoile de Choisy | 29.8 | 20.3 | 40.5 |
| Funo | 32.4 | 3.5 | 9.8 |
| Leonardo | 33.4 | 24.1 | 41.9 |
| Leone | 31.4 | 15.5 | 33.0 |
| Mara | 19.2 | 2.7 | 12.3 |
| Patricio | 25.3 | 35.3 | 58.3 |
| Produttore | 16.3 | 14.9 | 47.7 |
| San Pastore | 27.7 | 18.2 | 39.7 |
| Low-yielding varieties | | | |
| Bankut 1205 | 8.9 | 31.5 | 77.8 |
| Novosadska 1993 | 14.0 | 33.5 | 70.6 |
| Rumska crvenka | 8.1 | 14.2 | 63.7 |

[a] Method of Ogur and Rosen; ref. 3.

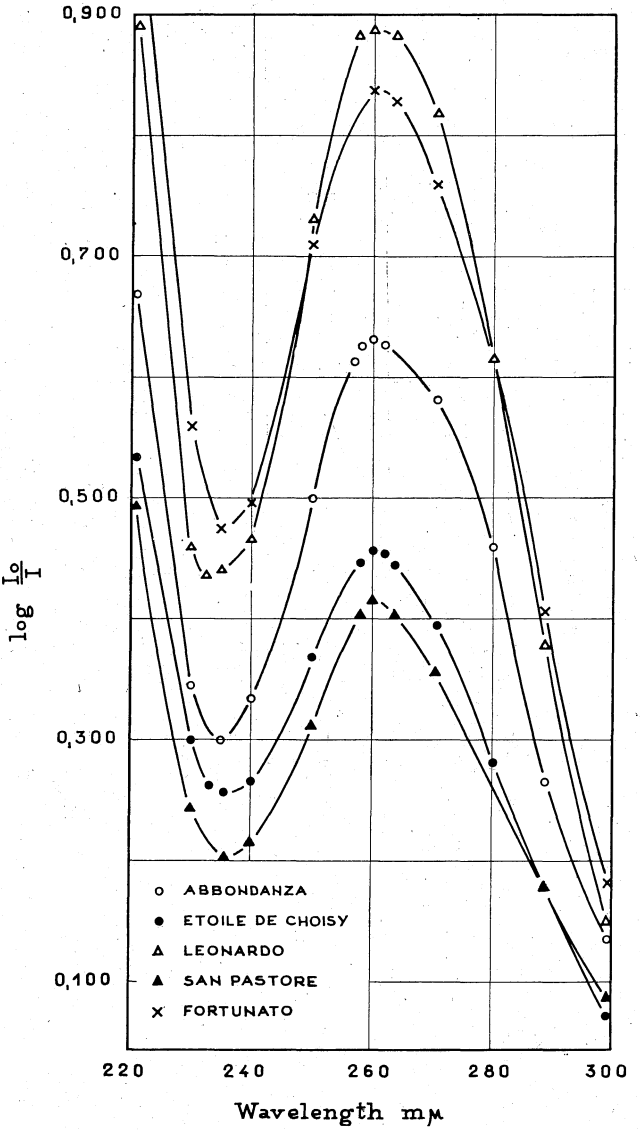


Fig. 4. UV spectra of NA isolated from gluten of some high-yielding wheat varieties.

If the necessary correction is made, then the observed changes in RNA content of the wheat grain of the variety San Pastore, in dependence of increasing levels of inorganic fertilizers, would be: (a) mg. RNA per 100 g. of grain, (b) mg. RNA-P per 100 g. of grain; for N fertilizer, (a) 60.0–105.8, (b) 5.94–10.47; for P fertilizer, (a) 56.6–107.5,

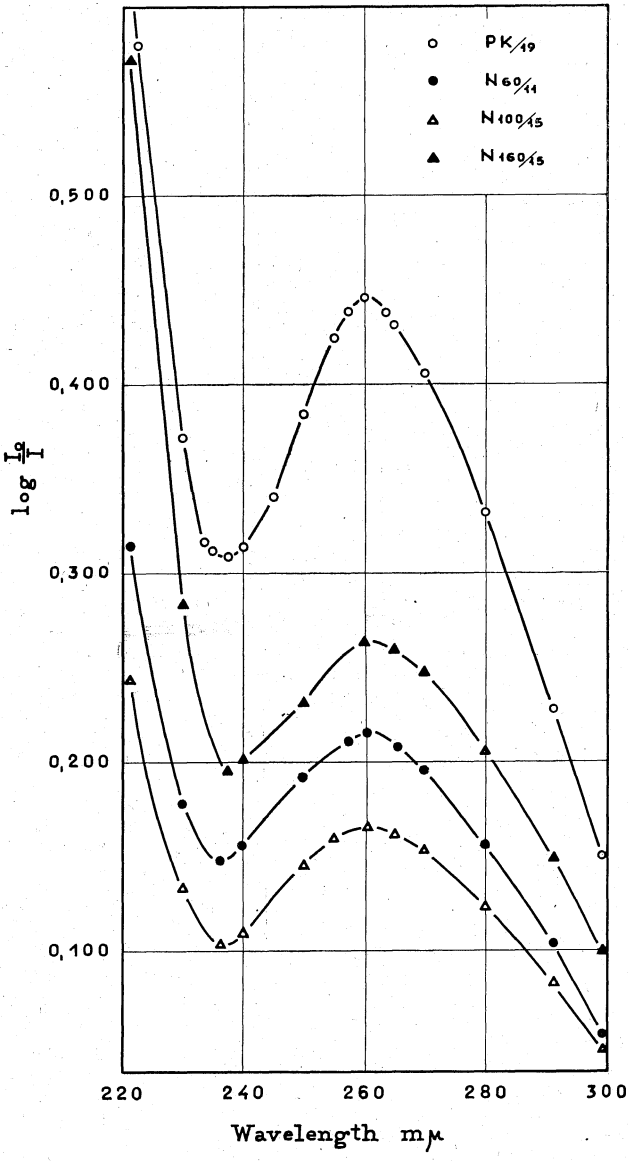


Fig. 5. UV spectra of RNA isolated from San Pastore wheat. The grain was harvested from plots treated with different levels of nitrogen fertilizer.

(b) 5.61–10.63; for K fertilizer, (a) 97.8–65.1, (b) 9.68–6.44 (compare with Figs. 1 to 3 and Table III). From these values, which are considerably higher than those ascribed so far to the RNA amount of

wheat grain, it appears that the content of grain RNA of a given wheat variety is variable and that, therefore, the ratio DNA/RNA cannot be specific for a wheat variety, since it is not constant.

Evidence that rice glutelin is a RNA-containing nucleoproteid has been reported earlier (23). The recent works of Lipshitz and Chargaff (24), Matsushita (25), Bourdet and Herard (6), and our own unpublished investigations have shown that wheat NA are also nucleoproteids. The UV spectra of gluten RNA (Fig. 4) and total grain RNA (Fig. 5) of some low-yielding and high-yielding wheat varieties, recorded in the present study, show absorbances (at 240, 260, 280 mμ, etc.) and absorbance ratios which are characteristic for the nucleoproteid nature of isolated RNA.

Our preliminary investigation on the variety San Pastore has demonstrated that globulin contains at least 2.5 times more NA (in percentage) than the nucleoproteids of gluten. Therefore, although gluten represents about 88% of grain total proteins and globulin only 8%, any major change in the ratio gluten/globulin, brought about by fertilizer treatment, might complicate the interpretation of results obtained for the contents of RNA, i.e. NA, in wheat grain. However, it was found that under such conditions of crop production the ratio gluten/globulin remained practically constant, in spite of the fact that the total content of grain proteins changed considerably. Therefore, this finding represents further evidence that the ratio DNA/RNA cannot be a specific and constant value for the whole grain or gluten of a given wheat variety.

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