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The dynamics of acid-soluble phosphorus compounds in the course of winter and spring wheat germination under various thermic conditions

Part II. Labile phosphorus after hydrolysis of the acid-soluble fraction

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Abstract:

The changes in labile phosphorus compounds content during germination of wheat were investigated. These compounds were determined in acid-soluble germ extracts separated into fractions according to the solubility of their barium salts. Low germination temperature was found to raise the labile phosphorus content in the fraction of insoluble barium salts. If we assume that labile P of this fraction consisted mainly of adenosinedi- and triphosphates, it would seem that the rise in the ATP and ADP level under the influence of low temperature may be essential for initiating flowering in winter varieties.

INTRODUCTION

In the previous paper (1971) the results of studies on the changes in acid-soluble phosphorus compounds content in the process of vernalization of winter wheat were described. Fractionation of the acid extract by the precipitation method allowed to obtain a fraction of barium--insoluble compounds, including phytin and inorganic phosphorus which constitute the bulk of the acid-soluble fraction and mask the changes in other compounds. Thus the barium-soluble alcohol-insoluble fraction, after elimination of phytin, revealed the dynamics of phosphorylated saccharides — the first metabolites of glycolysis. The investigations gave support to the conclusion that glycolysis is an essential component of the process of vernalization.

Further investigations on acid-soluble phosphorus compounds in the course of vernalization, and particularly the changes in polyphosphate

ADP and ATP compounds, are reported here. Fractionation based on the varying solubility of barium salts made possible, namely, the separation of these nucleotides from other labile phosphorus compounds present in the acid-soluble extract. The labile phosphorus of the fraction of barium--insoluble salts, obtained by hydrolysis with hydrochloric acid, could be, therefore, the measure of the adenosinedi — and — triphosphate content. Additional determinations of labile phosphorus of the barium-soluble alcohol-insoluble fraction of the germ extract also aimed at veryfying the assumption advanced in the previous paper (Barbaro 1971) that this fraction reflects the variations of glycolysis metabolites in the course of wheat seed germination.

The aim of the present investigations was to determine the labile phosphorus content particularly of ADP and ATP during germination of winter and spring wheat at low vernalizing temperatures and at higher ones excluding vernalization. This study seemed of interest in view of the important role of the adenosine system in the coordination of many physiological processes, and on the other hand, the paucity of data on the dynamics of these compounds in the course of vernalization.

MATERIAL AND METHODS

The investigations were made on the same experimental material as was used in part I of the present study (Barbaro 1971). The object examined were germs of winter 'Ostka Złotokłosa' wheat, and as control served those of the spring variety 'Ostka Chłopicka', separated from the endosperm after various germination periods: 0 - 70 days at 1.5°C and 20 - 96 h at 22°. All details concerning the plant material, extraction conditions and fractionation are given in part I of the present work on the dynamics of acid-soluble phosphorus compounds.

Essential in the method was the maintenance of 0° temperature during the entire analytical procedure with the exception of a 20-min. period at 20° indispensable for full development of the blue colour in the reaction with the molybdate reagent. In this way the abstraction of the phosphate groups from the labile ester compounds could be avoided, which in the Fiske - Subbarov method may lead to excessive values in the determination of inorganic phosphorus, at the cost of organic P (Lindberg, Ernster 1956).

Extraction and fractionation were conducted simultaneously for four samples differing in germination time, in order to eliminate to some extent the influence of the conditions of analysis on the results of phosphorus determination. The analyses were performed in 3 - 8 replications.

Hydrolysis. In the course of fractionation, labile phosphorus of the barium-insoluble phosphorus compounds (fraction I) and of the fraction of barium-soluble alcohol-insoluble salts (fraction II) was determined. The samples of fraction I were hydrolysed in 1 N HCl on a water bath at 100° for 7, 15, 30, 60 and 180 min., and of fraction II only for 60 min. Hydrolysis was arrested by placing the samples in an ice bath.

Phosphorus determination was done by the Fiske-Subbarov method in Müller's modification, immediately after obtaining the fraction and after hydrolysis. The difference in inorganic phosphorus in the fraction before and after acid hydrolysis was considered as the labile fraction. Inorganic phosphorus was determined after hydrolysis by comparing the colour of the samples analysed with that of the standard samples with the same amount of HCl added.

Classification of phosphate compounds on the basis of the lability of their bonds was adopted according to Leloir and Cardini (1957). In the separation od phosphorus compounds to the particular fractions after LePage and Umbreit (1948), it was assumed that in the fraction of barium-insoluble salts (fraction I) inorganic phosphates would be the extralabile compounds, and inositol phosphates and phosphoglyceric acid — the stable compounds. To the labile ones would belong adenosinetri- and adenosinediphosphate releasing after 7-min. hydrolysis 67 and 50 per cent of phosphate groups, respectively, as well as fructose-1,6-diphosphate which hydrolyses in as little as 26 per cent and constitutes usually only a very small part of the phosphorus of this fraction.

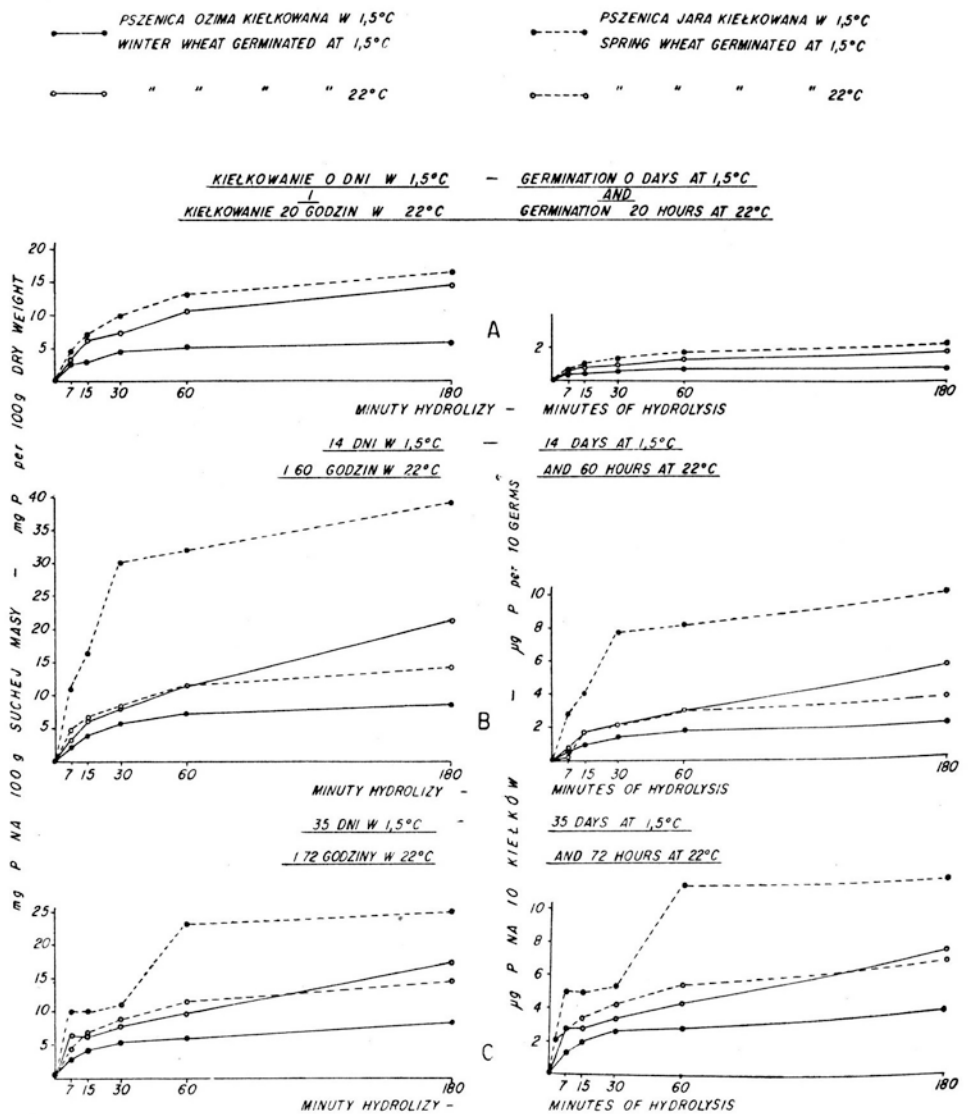
In the fraction of barium-soluble alcohol-insoluble salts (fraction II), extralabile would be ribose- and desoxyribose-1-phosphates determined as inorganic phosphorus of this fraction, and phosphopyruvic acid, glucose-6-phosphate, pentose-3,5-phosphates and pyrimidine nucleotides would be the stable compounds. To the labile compounds would belong glucose-1-phosphate unstable in 100 per cent in 7-min. hydrolysis and fructose-1-phosphate unstable in 70 per cent as well as uridinediphosphate occurring as a rude in negligible quantities.

RESULTS

Labile phosphorus determination in insoluble barium salts of fraction I shows an interaction between germination temperature and the variety. The Lohmann curves (Leloir, Cardini 1957), plotted on the basis of changes in inorganic phosphorus content after hydrolysis in 1 N HCl of organic phosphorus extract, obtained at 100° have a different course in dependence on the variety, temperature and length of the

germination period (Fig. 1). The characteristic relations are best seen in Fig. 2 where variations in labile P over the entire wheat germination period are plotted. These relations expressed as per cent of labile P released after 7 min. of hydrolysis in the dry weight of germs are as follows:

— in the winter variety at 1.5°, at the beginning of germination, labile P content is the lowest as compared with the other combinations, and distinctly rises towards the end of germination;



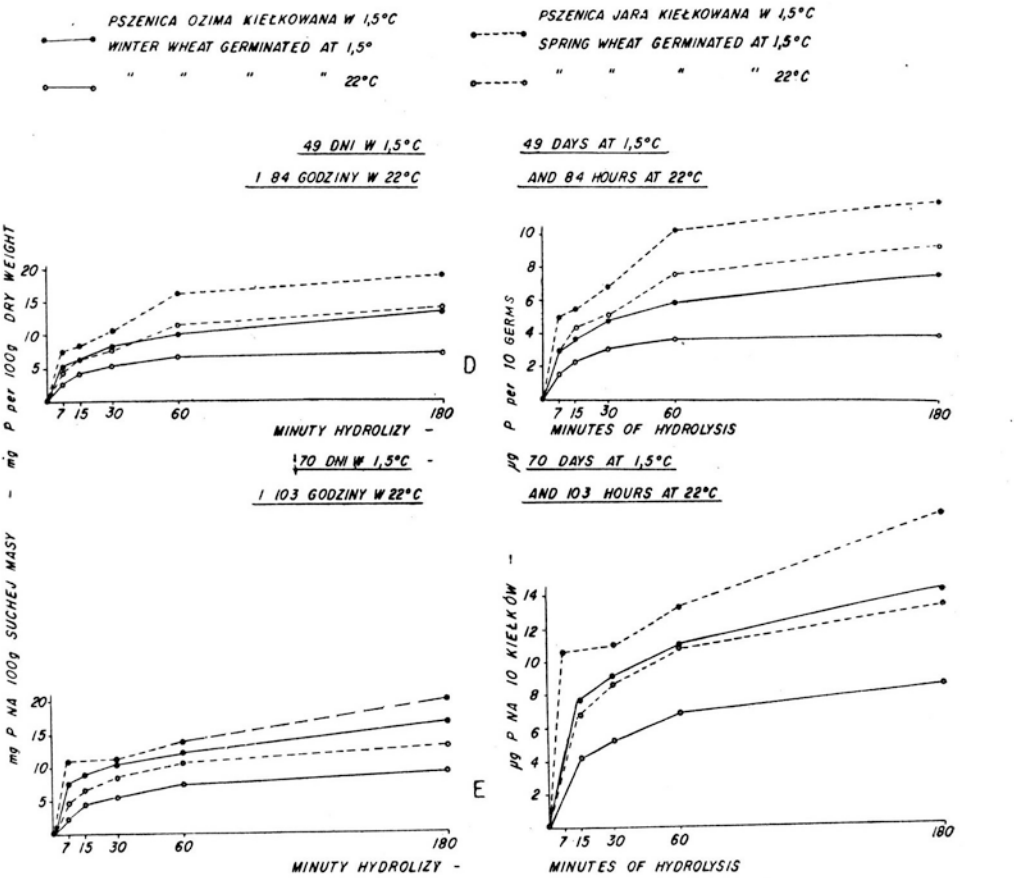


Fig. 1 (cont.)

Fig. 1. Phosphorus of the labile phosphates in the barium-insoluble fraction. Hydrolysis curves after various times of germination (A—E) of winter and spring wheat germs at 1.5 and 22°. Ordinates: amount of phosphorus released after hydrolysis in 1 N HCl at 100°; abscissae: time of hydrolysis

— in the spring variety at 1.5° the labile P content is the highest from the beginning, it rises twice soon after germination starts, and *remains at a high level during the entire period of germination;*

— the labile P content in the winter variety decreased during germination at 22°;

— when the spring variety was germinated at 22° it remained constant at a relatively low level during the period of germination.

If we take the labile P content of the spring variety germinated at 22° as reference value, it may be said that it was reached in the winter wheat germinated at 1.5° towards the end of germination; in the course

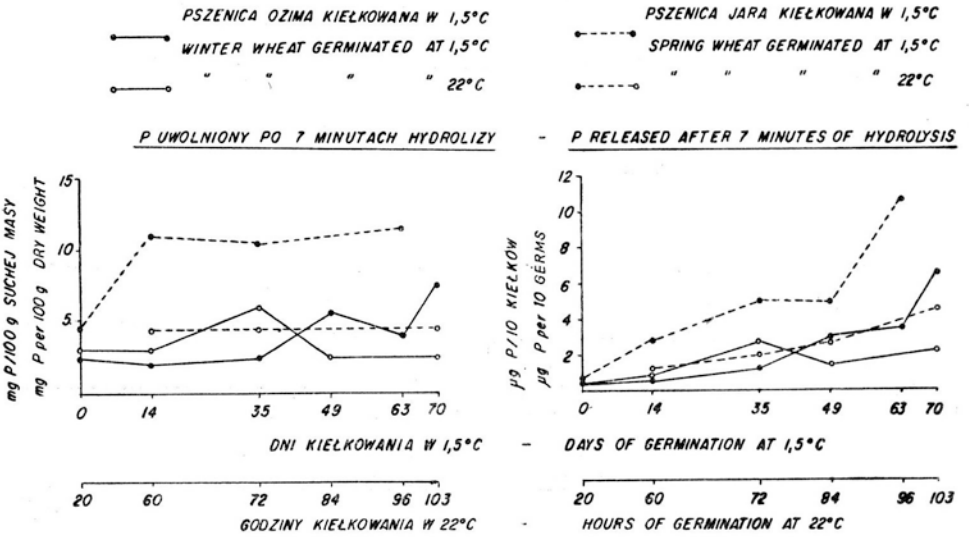


Fig. 2. Changes in labile phosphates content in the course of germination of winter and spring wheat germs in terms of P released after 7 min. of hydrolysis of the barium-insoluble fraction

of germination of the winter wheat at 22° it first remained at the same level, and towards the end of germination was twice lower than at the beginning. In the spring variety germinated at 1.5° it reached values twice higher than in the other cases. In relation to one plant, labile phosphorus content increased during germination in all the four combinations (Figs. 1 and 2 — right side). The interaction between germination temperature and variety is also noticeable in this presentation.

Labile phosphorus content of the soluble barium salts precipitated by alcohol (fraction II) exhibited similar variations as the whole fraction II; the winter variety when germinated at a higher temperature showed — in contrast to the other three combinations — a constant phosphorus level in the dry weight of germs over the entire period investigated. The rise in phosphorus level was most pronounced in spring wheat at 1.5°, winter wheat at the same temperature ranked second.

DISCUSSION

Labile phosphorus of fraction II. The labile phosphorus of the fraction of the soluble barium salts precipitated with alcohol may originate — according to the classification of Leloire — in the first place from fructose-1 and glucose-1 phosphates, thus, from

compounds which may be the first metabolites of glycolysis. The results obtained in the present study seem to indicate that the variations in labile phosphorus reflect those of the entire fraction II observed in the course of germination of winter and spring wheat under various thermic conditions (Barbaro 1971); they seem to support the assumption of the author in the previous paper claiming that the phosphorus content in fraction II is the measure of the amount of glycolysis metabolites in the germs.

Labile phosphorus of fraction I. The fraction of phosphorus compounds precipitated by barium when subjected to acid hydrolysis supplied some very interesting observations. The amount of labile phosphorus compounds in the course of germination of spring wheat at higher temperature remained during this entire period at the same level. In the germs of the winter variety, on the contrary, it increased distinctly as germination progressed at low temperature, and decreased at elevated germination temperature. It would result therefrom that low temperature enabled the winter form to accumulate labile phosphorus compounds in such amounts as were found in the spring variety. Higher temperature, on the contrary, caused a rapid utilisation of the initially present amount of these compounds and prevented their further accumulation.

The labile phosphorus compounds of this fraction consisted — beside ATP and ADP — of fructose diphosphate. It would seem, however, that the course of the curves (Fig. 2) was determined by the variations in free nucleotides content, since their amount, and the lability of these compounds as compared with diphosphates are as a rule much higher (Leloir, Cardini 1957; LePage Umbreit 1948). It should be mentioned here, that in view of the small amounts of the labile phosphorus detected, of the order of less than 20 mg in 100 g dry weight of germs, as compared with several hundred milligram of organic phosphorus in the same fraction, measurement errors cannot be excluded. However, the distinct tendency of the changes seems to confirm that the means obtained are representative. Also the agreement between the changes in labile phosphorus compounds and those of fraction II (Barbaro 1971) observed under various germination conditions for both wheat finds confirmation in the commonly observed interrelation between the course of glycolysis and the ATP level. This level regulates glycolysis and is dependent on it, as confirmed by the more refined studies of Brever (1969) who demonstrated that inhibition of hexokinase activity by phosphoglyceric acid is abolished by ATP in proportion to the concentration of the latter.

The different — from the point of view of labile compounds — phosphorus metabolism in winter varieties at low temperature making

vernalization possible, and at a higher one excluding it, gives reason to believe that high-energy compounds play a key role in the vernalization process. It would seem that the action of low temperature in vernalization consists in producing conditions in which the ATP in the winter plant reaches a certain level which determines its further generative development. This hypothesis, however, requires further confirmation.

The postulated necessity of reaching a definite value of the ratio ATP synthesis/ATP utilisation seems to find support in the constant level of free nucleotides in certain phases of ontogenesis, observed by other authors. Cherry and Hagemann (1961) noted a rise in the di- and triphosphate nucleotide level only in the first three days of maize germination, and Grzelczak and Buchowicz (1967) a rise of adenine and adenosine content at the very beginning of spring wheat germination, later, at further germination periods, this value remained unchanged. In both the above quoted papers, the order of the P values calculated from the nucleotides determined is the same as that of the here determined labile phosphorus. The constancy of the adenosine phosphate ratio is confirmed also by the studies of Okuncov et al. (1967) who found similar values for the ATP and ADP synthesis coefficients in wheat and pea leaves under various light conditions.

Papers concerning various processes (Borzkovskaya et al. 1967; Heber, Santorius 1964; Stewart, Guinn 1969; Wilson, Huffaker 1964) report lately on the decisive role of the ATP level in the tissues. Information is, however, lacking so far as regards changes in the content of these compounds in the vernalization process (Kolli 1969, Michniewicz 1966).

On the other hand, investigations undertaken with the aim of tracing the pathways of biological oxidation in the course of vernalization are closely bound with this problem. It is possible that the inhibitory action of malonic acid on the coming into ear of winter barley observed by Hartmann and Bauschbeck (1965) was caused by the limitation of ATP synthesis by way of blocking the tricarboxylic acids cycle; the spring plants did not differ from the controls. From among the respiratory inhibitors used by Wojtaszek (1964 a, 1964 b), only sodium azide uncoupling phosphorylation, and malachite green inhibiting dehydrogenases, arrested at the same time the development of winter wheat, and remained without effect on the coming into ear of the spring variety. On the other hand, the stimulating effect of dinitrophenol on ear formation in winter wheat, observed by the same author, causing in general an uncoupling of the phosphorylation and oxidation processes may be explained by the low DNP concentration (10^{-5}) used in the experiment, which no more influenced ATP-ase activity, and, in the author's opinion, enhanced glycolysis. Krekule (1961) obtained at

higher DNP concentration a complete arrest of generative development in winter wheat.

The hypothesis of the significance of an increased ATP content during seed germination for inducing further generative development, thus the part played by this substance in the control of development, finds support in the investigations of Eizenberg and Dishler (1968) who observed the effect of ATP on the chromosomal system of pea meristems. Also Torrey (quoted after Butenko, 1964) reports on the influence of 2,4-D (known to stimulate phosphorylation) on the course of mitosis.

The part played by adenosine-phosphates in development regulation might be explained by their preponderant role as compared with the remaining nucleotides, since both de novo synthesis of purine nucleotides via inosinic acid (Davies, Giovanelli, Ress 1969) and direct nucleotide phosphorylation occur with the participation of ATP. Landin et al. (1969), in investigations on ^{32}P incorporation into the RNA of ribosome nuclei and messenger RNA, detected a much higher initial radioactivity of AMP as compared with that of other nucleotides, and a later equalisation of radioactivity which they explained by the passing of the phosphate rests to ATP. The investigations of Brown and Reichard (1969) revealed the specific role of ATP and dATP nucleotides in the regulation of the allosteric activity of enzymes. Also the studies of Kessler and Snir (1969) seem to suggest the high importance of adenine nucleotides in development. They found *in vitro* a distinct influence of gibberellins on the physical properties of DNA, but only when it was rich in A-T bonds. They suppose on the basis of the structural changes observed in DNA that the action of DNA as pattern also changes.

Konstantinova et al. (1968), after investigating the influence of dinitrophenol on the flowering of short-day plants, reached the conclusion that the dark reactions of photoperiodism depend on oxidative phosphorylation. Also Kandeler (1969), who obtained a stimulation of flowering of duckweed (*Lemna*) by exogenoulsy applied ATP, advanced the hypothesis that ATP affects flowering.

The importance and role of ATP in plants are so multiple that it is difficult to evaluate the specificity of its influence in one definite process, and in the case considered, in the bioinductive action of cold on the processes of generative development of winter plants. Considering, however, that the adenosine system is a preponderant factor coordinating numerous processes, and thus making possible a harmonius development of the plant organism, it seems greatly probable that a dynamic equilibrium in the system ATP synthesis/ATP utilisation may be the necessary condition for a full ontogenetic development of plants.

SUMMARY AND CONCLUSIONS

The investigations here reported concern the changes in labile phosphorus content in winter and spring wheat during germination at low vernalizing temperature and higher temperature excluding vernalization. The determinations were made in the acid germ extract separated by the method of barium salts precipitation. In the author's opinion, the results demonstrate the variations in adenosinedi- and -triphosphates representing the labile phosphorus compounds of the fraction of insoluble barium salts. This leads to the conclusion that the role of ATP in the process of vernalization of winter wheat is essential.

This conclusion is based on the following observations:

1) in the spring variety, the amount of labile compounds remained constant at higher germination temperature over the entire germination period; 2) at the same temperature the content of these compounds decreased in winter wheat, but it increased distinctly at vernalization temperature; 3) under low temperatures, the labile phosphorus level reached in winter plants the level exhibited by spring plants at 22°C.

The above described variations as compared with those in adenosinetriphosphate content gave grounds to the hypothesis that the ATP level rises in the course of germination of winter varieties at low temperatures. This rise due to the influence of cold is essential in the process of vernalization for inducing flowering of the winter forms. A definite ATP synthesis/ATP utilization ratio seems to make possible further, highly endoergic reactions including the synthesis of so far unknown compounds, which may lead to morphogenetic changes in the growth apex, associated with the generative development of the plants.

The hypothesis advanced requires further investigations for confirmation, both as regards the methods of direct ATP determination and verification on more extensive plant material. The mechanism of ATP action by which it would stimulate initiation or, respectively, abolish flowering inhibition, in winter plants also remains an open question.

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Dynamika kwasorozpuszczalnych związków fosforowych w czasie kiełkowania pszenicy ozimej i jarej w różnych warunkach termicznych

Cz. II. Fosfor labilny po hydrolizie frakcji kwasorozpuszczalnej

Streszczenie

W pracy przedstawiono badania nad zmienną zawartością fosforu labilnego w czasie kiełkowania pszenicy ozimej i jarej w temperaturze niskiej jaryzującej i wyższej, wykluczającej jaryzację. Oznaczenia prowadzone w kwaśnym ekstrakcie kiełków, rozdzielonym metodą precypitacji soli barowych, pozwoliły — zdaniem autora — na prześledzenie zmienności adenozynodwu- i trójfosforanów jako fosforu labilnego frakcji nierozpuszczalnych soli barowych i na tym tle na wysunięcie wniosku o istotnej roli ATP w procesie jaryzacji pszenicy ozimej.

Powyższy wniosek oparty jest na następujących obserwacjach:

1) U odmiany jarej w wyższej temperaturze ilość labilnych połączeń pozostawała przez cały okres kiełkowania na jednakowym poziomie; 2) u odmiany ozimej w tej temperaturze obniżała się, a w niskiej jaryzującej wyraźnie wzrastała; 3) pod działaniem niskich temperatur rośliny ozime uzyskały taki poziom fosforu labilnego, jaki posiadały jare w temperaturze 22°C.

Opisana zmienność odniesiona do adenozynotrójfosforanów dała podstawę do wysunięcia hipotezy zwiększania się poziomu ATP w czasie kiełkowania odmian ozimych w niskich temperaturach jako istotnego dla procesu jaryzacji skutku działania zimna nieodzownego dla wywołania indukcji kwitnienia form ozimych. Osiągnięcie pewnej wartości stosunku synteza ATP/zużycie ATP umożliwiałoby przebieg dalszych, wysoce endoergicznych reakcji, nie wykluczając syntez nieznanych dotąd związków, które w konsekwencji mogłyby prowadzić do zmian morfogenetycznych w stożku wzrostu, związanych z rozwojem generatywnym rośliny.

Wysunięta hipoteza wymaga dalszych sprawdzających badań zarówno w zakresie metodyki bezpośredniego oznaczania ATP, jak i potwierdzenia na szerszym materiale roślinnym. Otwarty pozostaje również problem sposobu działania, poprzez który ATP stymulowałby inicjację względnie znosiłby inhibicję kwitnienia roślin ozimych.