TARTU RIIKLIKU ÜLIKOOLI
TOIMETISED

УЧЕНЫЕ ЗАПИСКИ
ТАРТУСКОГО ГОСУДАРСТВЕННОГО УНИВЕРСИТЕТА
ACTA ET COMMENTATIONES UNIVERSITATIS TARTUENSIS

845

PUBLICATIONS IN PLANT PHYSIOLOGY AND PLANT BIOCHEMISTRY

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The formation of lignin in wheat plants and its connection with mineral nutrition

TARTU 1989

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ALUSTATUD 1893.a. VIHIK 845 ВЫПУСК ОСНОВАНЫ В 1893.г

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Published by the order of the Publications Advisory Committee of the Tartu University

Editorial Board:

H. Miidla (chairman), J. Simisker, T. Alamäe

Editor-in-Chief: E. Padu

Revised by Ü. Türk

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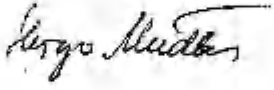


PROLOGUE

This issue of the Department of Plant Physiology and Plant Biochemistry University of Tartu is dedicated to Professor HEIGO MIIDLA on the occasion of his 70th birthday. His former students and research associates, together with some of his closer colleagues, have contributed papers written under his supervision to express their respect and gratitude.

His pupils respect his astonishingly broad knowledge not only in different fields of plant physiology, plant growing and horticulture, but also in cultural life, and admire his fairminded and liberal attitude to his students and co-workers. Especially thankful are Estonian plant physiologist to prof. H. Miidla for writing an original textbook on Plant Physiology (Tallinn, 1984, for students of higher educational establishments).

His former and present students, research associates and close colleagues wish prof. HEIGO MIIDLA a HAPPY BIRTHDAY, and further good health and prosperity for the coming years.

Prof. HEIGO MIIDLA, D. Sc. (Biology)
(till y. 1939 Herald Mittenbritt)

Curriculum Vitae

Born on 29 December 1919 in Kloostri district, Estonia.
Married, 2 sons, 4 grandsons.

1927-1937	Progymnasium
1938-1940	Nõmme Humanitarian Gymnasium
1940-1946	Student, Faculty of Agriculture, University of Tartu
1946-1949	Research worker, Institute of Agriculture, Academy of Sciences Estonian SSR
1950-1953	Scientific Assistant, Agricultural Faculty, University of Tartu
1954-1955	Agronomist of horticulture, Tartu
1956-1962	Senior lecturer, Faculty of Biology-Geography, University of Tartu
1959	Habilitation, degree of candidate of biological sciences, University of Tartu
1961-1964	Dean, Faculty of Biology-Geography, University of Tartu
1963-1973	Assistant Professor, Faculty of Biology-Geography, University of Tartu
1970	Habilitation, degree of doctor of biological sciences, University of Kiev
1974-	Professor, Department of Plant Physiology and Plant Biochemistry, University of Tartu
1975	Visiting Professor, University of Warsaw
1976-	Chairman, Department of Plant Physiology and Plant Biochemistry, University of Tartu

Participation in International Congresses

1975 XII Botanical Congress, Leningrad, USSR

1977 Regulation Growth and Development of Plants, Halle, DDR

1978 Growth Regulators of Plants, Liblitz, Czechoslovakia

1979 XI Biochemical Congress, Toronto, Canada

1983 15th FEBS Meeting, Brüssel, Belgium

1985 XIII Biochemical Congress, Amsterdam, Netherlands

1987 XIV Botanical Congress, West-Berlin

Research interests

Prof. H. Miidla was the first scientist in Estonia and in the Soviet Union who recognized the central role of the process of lignification (phenylpropanoid metabolism - hydroxycinnamic acids and activity of different forms of peroxidases, lignin, OCH_3-groups, aldehydes - after nitrobenzene oxidation) in woody plants (grape) in connection with their acclimatization and elaborated the physiological and biochemical bases of the relationship between the processes of lignification and frost resistance (in apple-tree).

His present work deals with clarifying the mechanism of lodging resistance in cereals (wheat).

Tartu, December 1988 E. Padu

PREFACE

It is widely known that main mineral nutrients (N,P,K) have a great influence on the primary metabolism of plants. **Nitrogen** is of extreme importance for plants because it is a constituent of proteins, nucleic acids, and many other important substances. It does not, however, appear to have any specific catalytic or electrochemical roles apart from the fact that it is structurally involved in most catalytic molecules.

Plants respond to a high or low nitrogen supply in a variety of ways. Overabundant nitrogen often causes a great proliferation of stalks and leaves but a reduction in fruit crop of plants. A slightly reduced nitrogen supply (but not a critical shortage), in relation to a potassium and phosphorous supply, usually results in the most effective seed and fruit production of agricultural crops.

Phosphorus, like nitrogen, is an extremely important structural part of a number of compounds, notably of nucleic acids and phospholipids. In addition, phosphorus plays an indispensable part in energy metabolism, the high energy of hydrolysis of pyrophosphate and various organic phosphate bonds being used to drive chemical reactions.

Phosphorus deficiency affects all aspects of plant metabolism. Phosphorus deficient plants develop slowly and are often stunted in growth. Soluble carbohydrates may accumulate in phosphorus deficient plants. One of the characteristics of phosphorus deficiency is a striking increase in the activity of the enzyme phosphatase. This may be related to the mobilization and reuse of the available phosphate that take place under these conditions.

Potassium seems to have no structural role in plants but it fulfils a number of catalytic roles. These are mostly not clearly defined, and the exact nature of much of the large potassium requirement is unknown. Many enzymes, for example several involved in protein synthesis, do not act efficiently in the absence of potassium, although it does not seem to bind to them in the usual way. It may affect protein conformation causing exposure of active sites.

Potassium deficiency is often manifested by rosette or bushy habit of growth. Other consequences are the reduction in stalk growth, weakening of the stalk, and lowered resistance to pathogens, so that potassium

deficient plants, especially cereals, lodge easily (are knocked down by weather) and are susceptible to diseases. Because of the reduction in protein synthesis and impairment of respiration, low-molecular-weight compounds such as amino acids and sugars tend to accumulate to unusually high levels in potassium deficient plants while the content of proteins and polysaccharides is reduced.

But the influence of the abovementioned mineral elements on, and their interaction with the secondary metabolism of plants have recieved relatively little attention. The little information available on this subject in literature is controversial.

The articles mentioned below led us to the hypothesis that exogenous mineral nutrients (N, P, K) might influence the metabolism of phenolpropanoids in wheat plants and especially in their cell walls. It is a well-known fact that grasses lodge on account of high doses of nitrogen nutrients and, therefore, the crop harvest decreases by about 40 %. Lodging is the result of several factors interacting in a complex and intricate manner. Plants may lodge because of a disease and insect damage, of low mechanical stalk strength, or of the interrelationship of these and other factors. The low mechanical stalk strength may be associated with high yield levels, poor cultural practices, early maturity, unfavourable weather conditions, inherent weaknesses, or an improper nutrient balance. It is suggested that lodging in cereals might not be dependent on physical attributes alone, and that the chemical nature of stem constituents could be of importance in determining variational behavior and treatment responses. The intensive lignification of cell walls has been suggested to be one component of lodging-resistance mechanism in plants. The lignin content and composition in plants can be manipulated by different methods. The activation of phenolic biosynthesis involving the induction of PAL and other enzymes of the phenylpropanoid pathway leads to the formation of polymeric phenolic compounds. One of the terminal enzymes catalyzing oxidative condensation is peroxidase.

Our previous papers have shown that hydroxycinnamic acids, particularly *p*-coumaric and ferulic acids, are generally found to be esters rather than free acids which are released on alkaline hydrolysis in the ethanol-insoluble fraction and can be used as precursors in the biosynthesis of lignin in cell walls. Lignin oxidation products with nitrobenzene give C_6 - C_1-aldehydes - vanillin and syringaldehyde - under alkaline conditions. The last compound shows a level of methylation, especially the molar ratio of vanillin/syringaldehyde. The abovementioned indicators may characterize the degree of lignification from the biochemical standpoint.

The aim of the following papers is to show the

results of scientific experiments carried out in the laboratory of plant physiology of the department of plant physiology and plant biochemistry at the University of Tartu. The results characterize the physiological and biochemical indicators and the lodging-resistance mechanism in wheat stalks, and show how, with different mineral nutrients (N, K) given at different times and in different doses, it is possible to regulate the processes of lignification in cell walls. The authors are sure that it is possible to grow large-yielding and not lodging-resistant cultivars of cereals when the farmers take advantage of the right regime of mineral nutrients. The following physiological and biochemical indicators are used to characterize lignification: 1. The biosynthesis of lignin in wheat; 2. the parameters of growth and development of wheat; 3. the contents of ester-linked alkaline phenolic acids; 4. the content and chemical composition of lignin; 5. the polymerization process catalyzing enzyme peroxidase activity and its isoenzyme composition, and 6. the activity of PAL.

Materials and general methods

Plant material: Spring wheat *(Triticum aestivum L.)* cv 'Leningradka' was planted on May 7, 1986 in the field of the Estonian Agricultural Academy*: Plots were 3 x 10 m in four repetitions. The standard cultural and management pratices were followed for planting and control of diseases and insects. Phenological stages were marked throughout the study. The plant material was sampled from the middle of the plots to avoid the effect of the edges. Samples of stalks and leaves were collected after 5 and 10 days from the beginning of the phase of stalking. For the structural analysis, the length and diameter, and the fresh and dry mass of the stalks, area of the fourth and the flag leaves and their fresh and dry mass, and the height of 120 plants were measured. At the harvest the structural analysis of ears was made and the content of protein was determined. For finding the content of phenolic acids and lignin, the stalks were fixed at 105 °C and milled. Enzyme activities were determined from the material frozen in liquid nitrogen.

Fertilization was carried out in three varieties: var. 1 - N_{50} P_{60} K_{60} kg/ha given on sowing (control), var. 2 - like Var. 1 + N_{100} at the beginning of tille-

* Acknowledgements: Our thanks are due do the head of the department of agriculture of the Estonian Agricultural Academy prof. H. Vipper and assistant lecturer T. Tiidt for their technical help.

ring (N plants), var. 3 - like var. 1 + N_{100} + K_{80} at the beginning of tillering + K_{100} at the beginning of stalking (N, K plants).

The experiments were organized on the eutric podzoluvisol. The content of moving elements of the 100 g of soil was: P - 14...17 mg and K - 23...30 mg and the content of humus was 2.1 ... 2.5 %, pH in soil was about 5. The preliminary crop was barley.

The results were processed statistically and given as arithmetical averages: n = 3 (by biochemical analyses); n = 50 (by measuring and weighing vegetative organs of plants); p = 0.05. The statistical evaluation refers to plants of different sets of each treatment.

Here we confine ourselves mainly to the results of the field experiments of the year 1986 as explaining the results of all tree years of the experiment (1984... 1986) would make the presentation of data much too complicated. It must be mentioned, however, that the sowing times of seed were similar in the abovementioned years. The greatest dislocations were in the undergoing of phenophases depending on the conditions of weather. So in the year 1984 the yield was harvested on August 28, in 1985 - on August 30, and in 1986 - on August 13.

In some articles the data of the experiments in the year 1987 have also been used. In that year the growing season was widely divergent - the temperature was low, there was little sunshine and especially much rainfall.

H. Miidla

BIOCHEMICTRY OF LIGNIN FORMATION

H. Miidla

Although secondary products are formed by animals, fungi and bacteria, it is estimated that over 80 % of the known secondary products are formed in higher plants. These compounds are secondary in the sense that they cannot have an essential role of primary life processes of plant cells because they are not present in all plants. The phenolic compounds are one group of secondary products which are almost universally present in higher plants. These compounds are aromatic in character and possess one or more phenolic hydroxyl groups.

The pathway of phenolic compounds' biosynthesis is affected by a wide range of environmental, hormonal and nutritional factors. In some systems phenolic biosynthesis is activated by light of various qualities, by a range of stress factors such as wounding, infection and low temperature stress, by growth substances such as ethylene, and, in some cases, by the transfer of culture cells into media with low auxin content and in the conditions of N, P and K deficiency or excess.

As regards the functions of phenolic compounds, the almost universal presence of the phenylpropanoid pathway is related largely to the formation of lignin which is a significant factor in the mechanical structure of the aerial parts of terrestrial plants. Phenolics protect wounded and infected plants. Many of plant pigments are flavonoids and, in the process of pollination, give a range of flower colours. The colour, taste and flavour of fruit are affected by phenolic compounds. Many phenolic compounds are implicated as such allelopathic agents and are phytotoxic.

During the last few decades, our knowledge of the secondary plant metabolism has increased greatly. Progress has been made in many fields, not only in its structural elucidation but also in the understanding of biosynthesis, enzymology, compartmentalization, regulation and ecological significance of natural products: One of the most important crossroads of phenylpropanoid metabolism is the metabolism of cinnamic acids and their involvement in the formation of lignin.

Nearly one and a half century has passed since Anselme Payen (SCHULZE, 1865) first used the term

"lignin" in reference to the "incrusting material" which he removed from wood by the application of acidic and alkaline reagents.

Lignin demands attention for its sheer quantity as about a quarter to a third of most wood is lignin and it ranks next to cellulose as the most abundant natural product. Lignin constitutes about 20 - 35 % of the cell walls of leaf wood or of conifers, the other part is composed of cellulose (~ 40 %) and hemicelluloses (~ 30 %). In the phase of dough ripeness, graminae (wheat) contain about 20 % lignin.

Lignification is the incrustation of lignin into the fibrous network of cellulose. This process has been compared to the formation of reinforced concrete because it forms rigid cell walls by combining the tensile strength of cellulose with the pressure strength of lignin. The ability of plants to form lignin must have been a decisive factor in the evolution of water to land plants. Only lignified cell walls made it possible to build the rigid stems of woody plants and trees and the conductive cell elements for water transport. FREUDENBERG (1964) has shown that lignin is an amorphous, optically inactive heteropolymer of substituted cinnamyl alcohols.

Analytical data, model experiments, biosynthetic investigations and recent structural studies of lignin with the aid of ^{13}C nuclear magnetic responce (NMR) spectroscopy have confirmed the structure of lignin. Fig. 1 shows the structure of beech lignin as suggested from degradative studies and ^{13}C-NMR spectroscopy (WEISSENBÖCK, 1976). (The scheme consists of 25 C_9 - units of which 6 can be partially substituted by the bracketed dilignolunits. The sheme shows a representative pattern from about 10-20 times larger "molekule" of the beech wood lignin, in which 10 connecting links of monomeres are random distributed. The constitution is explicable through the oxidative association of the mixture from 14 molecules coniferylalcohol, 10 molecules of sinapalcohol and 1 molecule of *p*-coumaralcohol, where are 59 hydrogenatoms removed and 11 molecules of water added. This constitutional structure is suitable for the angiosperms and for the coniferlignin too.)

The residues of 4-coumaryl alcohol (1), coniferyl alcohol (2) and sinapyl alcohol (3) can be recognized in this structure (fig. 2).

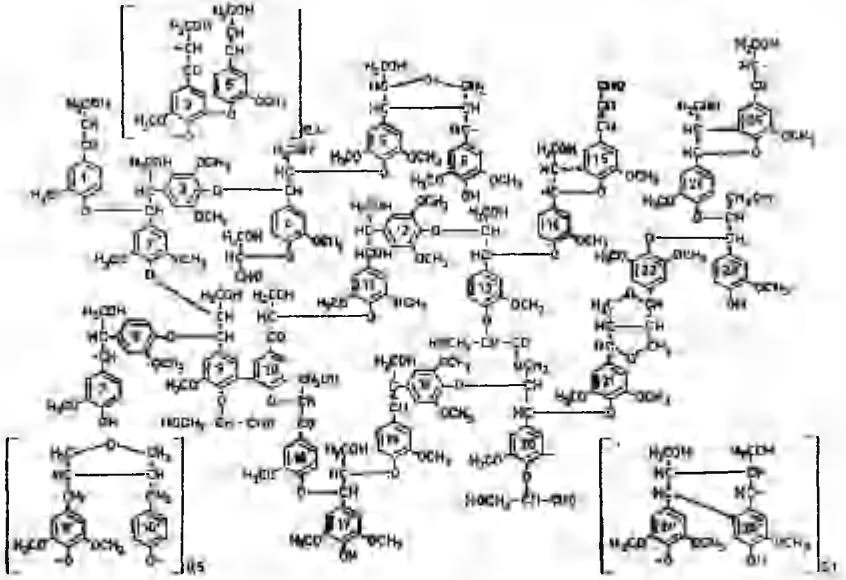
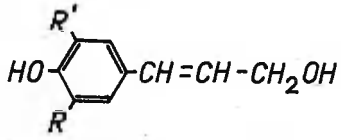


Fig. 1. A constitutional scheme of structure of beech wood lignin (WEISSENBÖCK, 1976).



Structure of lignin monomers

1. R = R' = H
2. R = OCH_3 R' = H
3. R = R' = OCH_3

Fig. 2. Structure of lignin monomers

Coniferin lignin has the same general structure but differs from angiosperm lignin by its high content of coniferyl units. Grass lignin has a great proportion of 4-coumaryl residues. However, this can be ascribed to *p*-coumaric acid esterified with lignin. Therefore it is probable that there is very little fundamental difference between grass and hardwood lignins (NAKAMURA et al., 1974).

Tracer studies on the biosynthesis of lignin have confirmed that cinnamyl alcohols are the primary building stones of lignin (FREUDENBERG, 1968). The present article focuses on recent progress in understanding the process of lignification.

Formation of phenylalanine and tyrosine

Before the reactions of lignification in plants can be carried out, the plants must assimilate carbon dioxide and convert it to carbohydrates from which, in some way, a benzene ring is synthesized, then hydroxylated or methoxylated in appropriate positions, and, after that, a three-carbon side-chain is introduced. Only then can polymerization to lignins occur.

DAVIS (1955) has pointed out the key role of shikimic acid pathway (fig. 3) in aromatic ring synthesis by bacteria and shown that shikimic acid is a good precursor of lignin in several species. HIGUCHI and SHIMANDA (1967) have demonstrated that the same shikimic route that has so well been documented in bacteria also operates in plants.

Phoshoenolpyruvate and erythrose-4-phosphate condense to form a seven-carbon intermediate (a heptulsonic acid phosphate). Cyclization follows and further condensations and rearrangements lead to prearomatic metabolite prephenic acid. As shown in fig. 3, the latter can be decarboxylated (with the formation of an

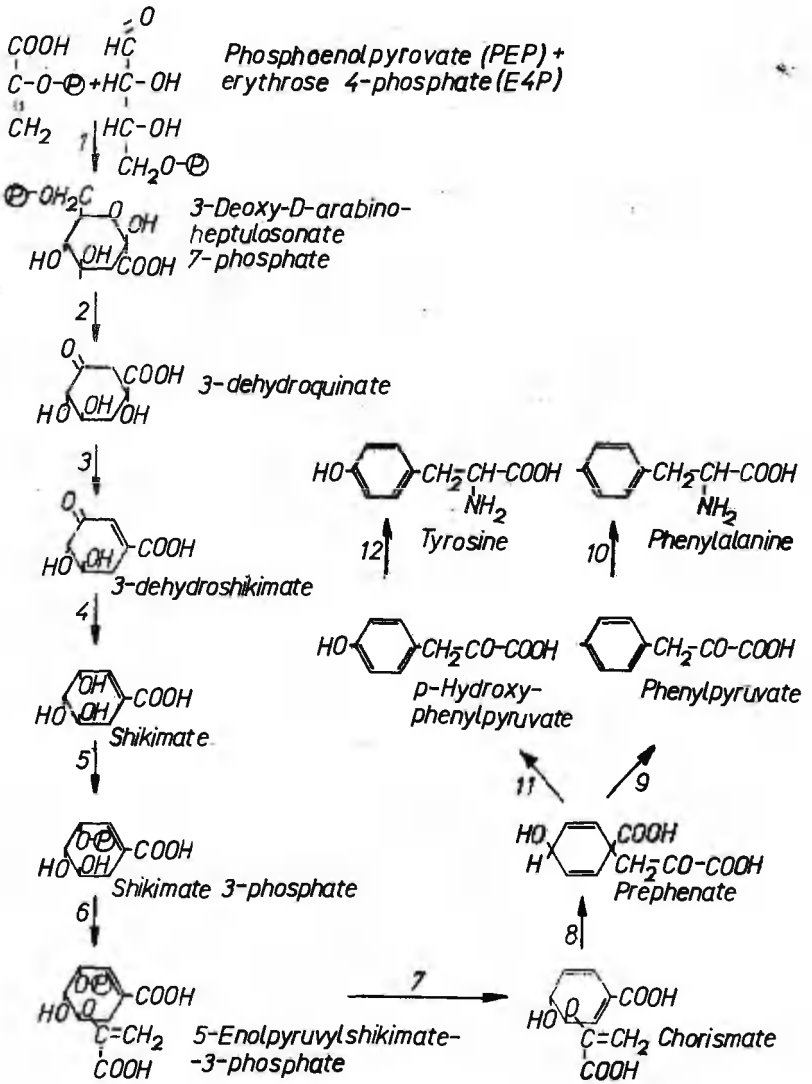


Fig. 3. Biosynthetic pathways to phenylalanine and tyrosine.

(1) 3-Deoxy-D-arabino-heptulosonate 7-phosphate synthase, (2) 3-Dehydroquinate synthase, (3) Dehydroquinate hydro-lyase, (4) Shikimate dehydrogenase, (5) Shikimate kinase, (6) 5-Enolpyruvylshikimate-3-phosphate synthase, (7) Chorismate synthase, (8) Chorismate mutase, (9) Prephenate dehydratase, (10) Phenylalanine amino transferase, (11) Prephenate dehydrogenase, (12) Tyrosine aminotransferase.

aromatic compound) by two different routes, only one of them involving the retention of the ring hydroxyl. Thus, phenylpyruvic acid and its 4-hydroxy derivative are the first fully aromatic compounds formed from shikimic acid. They are readily transaminated by plant aminotransferases to phenylalanine and tyrosine respectively.

The general phenylpropanoid metabolism

The general phenylpropanoid pathway is defined as the sequence of reactions involved in the conversion of *L*-phenylalanine to activated cinnamic acids (fig. 4).

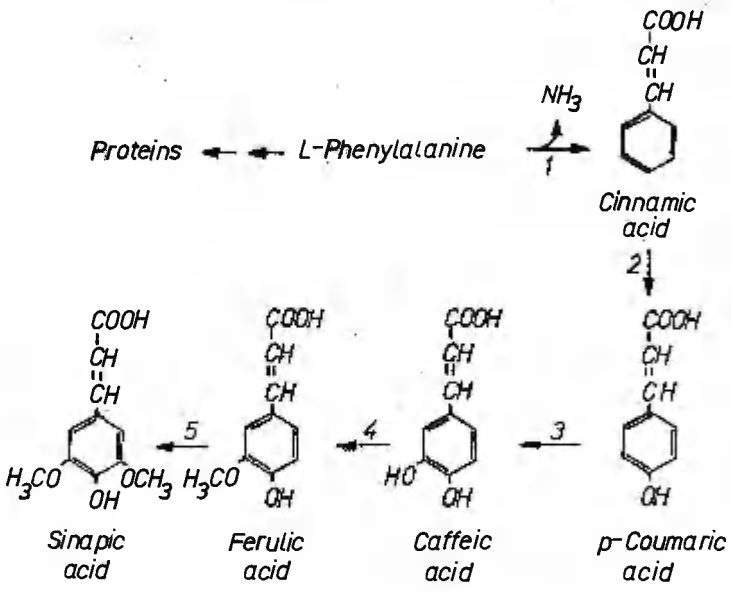


Fig. 4. 1 - Phenylalaline ammonia lyase (PAL),
2 - Cinnamic acid 4-hydroxylase,
3 - *p*-coumaric acid 3-hydroxylase,
4 - catechol *O*-methyltransferase,
5 - ferulic acid 5-hydrxylase.

These activated acids can then enter different biosynthetic pathways leading not only to lignin but also to flavonoids, stilbenses, benzoic acids and other compounds. The reaction sequence shown in fig. 3 does

not imply that only one set of enzymes is present in a particular plant. Parallel pathways could occur in different cell compartments and metabolic channelling would be possible in the presence of multienzyme complexes or isoenzymes (GRISEBACH, 1977).

The first enzyme of this pathway is phenylalanine ammonialyase - EC 4.3.1.5 (1) - which catalyzes the trans elimination of ammonia from *L*-phenylalanine to *trans*-cinnamic acid. KOUKOL and CONN (1961) were the first to show this reaction. At about the same time, NEISH (1961) established the presence in grasses of an analogous enzyme, tyrosine ammonia-lyase deaminating tyrosine to *p*-hydroxycinnamic (*p*-coumaric) acid. Later BROWN (1969) showed that the efficient utilization of tyrosine for lignin formation in grasses was two times smaller than that of phenylalanine. Cinnamic acid is then converted by a sequence of hydroxylation and methylation reactions to a number of substituted acids that can be activated to the corresponding coenzyme- A esters. Hydroxylation is mediated by cinnamic acid 4-hydroxylase - EC 1.14.13.11 (2).

Further, 4-coumaric acid can be hydroxylated by the action of *p*-coumaric acid 3-hydroxylase - EC 6.2.1.12 (3) followed by the methylation of the 3-hydroxyl group to ferulic acid. The reaction is catalyzed by catechol *O*-methyltransferase - EC 2.1.1 (4) - with *S*-adenosylmethionine as methyl donor. It is probable that ferulic acid-5-hydroxylase - EC 2.1.1.6 (5) or *O*-methyltransferase catalyze the methylation of ferulic acid to sinapic acid.

By now, the ferulic 5-hydroxylase has been characterized as a cytochrome P-450-dependent microsomal enzyme (GRAND, 1984).

Formation of cinnamoyl-CoA esters and their reduction to cinnamyl alcohols

The final step in the general phenylpropanoid metabolism is the activation of cinnamic acids to form coenzyme A thioesters according to the following equation:

Cinnamic acid + CoASH + ATP = cinnamoyl CoA + AMP + PP_1

The activation of cinnamic acid is catalyzed by 4-coumaroyl - CoA synthase (4-coumaric acid: CoA ligase). Two isoenzymes with different substrate specificities are present. It is postulated that synthase one belongs to the lignin and synthase two to the flavonoid pathway. The best substrates with the highest V/K_m ratios for synthase 1 are 4-coumaric, ferulic and sinapic acids.

MANSELL et al. (1972) obtained the reduction of ferulic acid to coniferyl alcohol *via* coniferaldehyde,

and described the formation of coenzyme-A-esters of cinnamic acids in a cell-free system from higher plants. It was shown that the reduction requires two enzymes, (cinnamoyl-CoA reductase EC 1.1.1 and cinnamyl alcohol dehydrogenase EC 1.1.2), fig. 5.

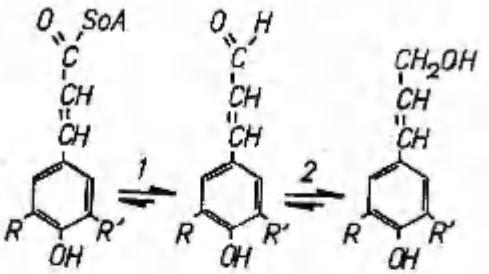


Fig. 5. Reduction of cinnamoyl-CoA ester to cinnamyl alcohol *via* the aldehyde
1 - Cinnamoyl-CoA: NADPH oxydoreductase,
2 - Cinnamyl alcohol dehydrogenase.

The best substrate for both enzymes is feryloyl-CoA. 4-Coumaroyl-CoA and sinapoyl-CoA can also be reduced efficiently beside some other cinnamoyl-CoA esters. The enzymes are specific for NADPH as a cofactor and transfer the hydrogen atom of NADPH to cinnamoyl-CoA.

Two isoenzymes with quite different substrate specificities were isolated from soybean cell cultures. While isoenzyme 1 can only reduce coniferaldehyde to conyferyl alcohol (or oxidize coniferyl alcohol), isoenzyme 2 is specific for the reduction of a number of cinnamaldehydes including 4-coumaraldehyde, coniferaldehyde, and sinapaldehyde (GRISEBACH, 1977).

Polymerization of cinnamyl alcohols to lignin

FREUDENBERG and SCHLÜTER (1955) showed that coniferyl alcohol is enzymically dehydrogenated by peroxidase (EC 1.11.1.7) in the position of *p*-hydroxyl forming the free radical of coniferyl alcohol (fig. 6).

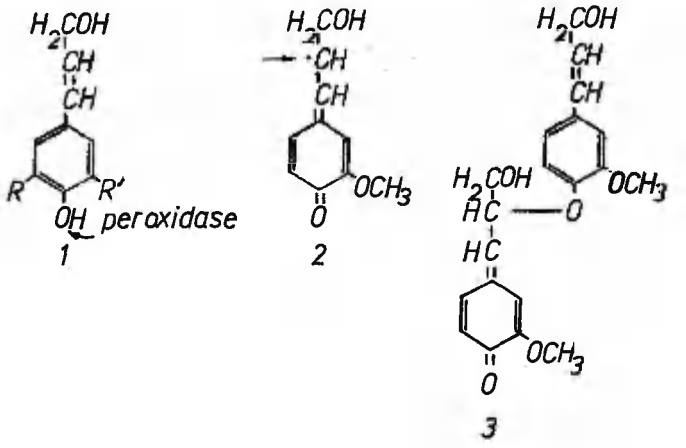


Fig. 6. Formation of mesomeric-free radical of coniferyl alcohol

1 - Coniferyl alcohol
2 - Free radical of dehydrogenated coniferyl alcohol
3 - Quinone methide.

A free radical mechanism has been suggested for the formation of these condensation products. The central intermediate in the formation of coniferyl-type polymers is a quinone methide (fig. 6) where one ring is again aromatic. Now a third molecule, water, enters the picture. A hydroxyl ion of water nucleophillically attacks the carbon adjacent to the quinone methide ring introducing a hydroxyl substitute in that position and causing aromatization of the ring. Oxidation of coniferyl alcohol is an enzyme-catalyzed process but once the radicals have been formed their coupling is believed to be spontaneous and to require no enzyme mediation. There are numerous possibilities for the formation of branched structures during polymerization, and lignin is indeed regarded as a branched polymer in which the aldehydes or the alcohols coniferyl, *p*-hydroxycinnamyl and sinapyl can polymerize "head" and "tail" positions (fig. 1). Although laccase is known to occur in some higher plants, it does not appear to be widely distributed. A better candidate for the job is peroxidase which, acting in conjunction with hydroperoxide, is known to occur widely in the plant kingdom (GASPAR, 1985).

MÄDER et al. (1980) have detected three groups of peroxidase isoenzymes in tobacco. One group has been found to be localized only in cell walls yielding maximal polymerization rates for coniferyl and *p*-coumaryl alcohols. Two other isoenzyme groups have been found to

be localized in protoplasts showing lower rates. MIIDLA et al. (1987) have shown that old and already lignified internodes which have stopped growing are characterized by the high activity of anodic peroxidases and do not react to the mechanical stimulus. The activity of all types of peroxidases increases basipetally along with the growth cessation and lignification of internodes.

Bound intermediates in lignification

It is well known that hydroxycinnamic acids such as *p*-coumaric, caffeic, ferulic and sinapic acids are widely distributed in vascular plants where they are generally found as esters rather than as free acids. Already EL-BASYOUNI (1964), BARDINSKAYA (1964) and MIIDLA (1970) showed that insoluble esters may be more directly involved than soluble esters in lignin biosynthesis. The cellwall bound cinnamic acid moieties may act as free-radical initiation sites for the oxidative polymerization of cinnamyl units into lignin (FRY, 1982).

Our experiments (see p. 37) show that the content of bound ferulic and *p*-coumaric acids, as precursors of lignin, increases in the first internode of wheat stalk parallel with the activity of PAL in the growing season up to the cessation of growth and then begins to decrease.

We suggest, and the same results have been obtained by several other investigators (FUKUDA, KOMAMINE, 1982; MILLER et al., 1985; STRACK et al., 1987), that the initial increase in the bound hydroxycinnamic acids was due to the synthesis of $C_6 - C_3$ lignin precursors, while the subsequent decrease in phenolics was due to their polymerization when the level of PAL is at its minimum. These data are contrary to the results obtained by GLASS and BOHM (1972) who estimated PAL's maximum in wheat to be within 10 days of germination, at the time when lignification was at its minimum and, therefore, it is difficult to imagine how this enzyme could function in a regulatory capacity. But we must not forget that cinnamic acids are one of the most important crossroads of phenylpropanoid metabolism and are involved in the formation of lignins, flavonoids, coumarins and other kinds of compounds. The key enzyme in this pathway is PAL.

Regulation of lignification

The conversion of phenylalanine to cinnamic acid is the entry point into phenylpropanoid metabolism. Since phenylalanine is also an essential amino acid for protein biosynthesis, it can be expected that the

enzyme phenylalanine ammonia-lyase is under regulatory control. Numerous internal factors, e.g. substrates, products, light, mineral nutrients, hormones can affect the synthesis or activity of this enzyme. But CUNHA (1987) shows that standard methods for the determination of PAL activity lead to measuring the activities of *L*-phenylalanine ammonia lyase (PAL, EC 4.3.1.5) and of *L*-phenylalanine amino-transferase (PAT, EC 2.6.1.1) together. The ratio of PAL to PAT activity is found to be 1:9. The incorporation of *L*-phenylalanine into *t*-cinnamic acid is little when compared with that of *L*-phenylalanine into *L*-phenylpyruvic acid and, therefore, the results concerning PAL activities are non-linear with respect to time and protein concentration. The author suggests estimating PAL activity alone. To do so, a specific inhibitor of PAT activity - *L*-asparatic acid - must be used to inhibit PAT.

The activation of cinnamic acids is another potential control point. The regulative enzyme here is cinnamic acid CoA ligase.

The regulation of the reduction of cinnamoyl-CoA esters to cinnamoyl alcohols through the activity of cinnamoyl-CoA reductases is yet the third control point.

To alter the structure of lignin, the process of methylation can be regulated by the activation of *O*-methyltransferase.

STAFFORD (1981) has discussed the possible role of multienzyme complexes in lignin biosynthesis.

For the regulation of lignification, it is also important that the enzymes involved in the biosynthesis of lignin precursors are tissue-specific and predominantly or exclusively located in the xylem in which lignification occurs. Xylem tissue seems, therefore, to be autonomous for lignin biosynthesis.

The transport of lignin precursors into the extraplasmatic space remains an open question. It is probable that it is mediated by vesicles (GROSS, 1979). There is a correlation between lignification and the formation of the secondary wall.

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MORPHO-PHYSIOLOGICAL PARAMETERS OF LODGING IN SPRING WHEAT PLANTS

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Introduction

The growth and development of plants is an excellent coordination of numerous phenomena at different levels, from biophysical and biochemical to organismal, which results in creating a whole organism.

When the deficit or excess of environmental factors damages the equilibrium in a culture plant, its growth and development do not proceed normally any more. One of the possible deviations is the lodging of cereals. Therefore it is interesting to know what kind of qualities must the stem of a plant or the stalk of a cereal have so that they were standing-resistant.

Lodging may be a result of the low content of dry mass per unit length of stalk and a reduced content of lignin (BLOMQUIST et al., 1973, KUMAKOV, 1980).

YAP and HARVEY (1972) obtained significant positive correlation coefficients between the basal stalk diameter, the plant height and the internodes' length in barley on lodging.

ATKINS (1948), PINTHUS (1973), KUPERMAN et al. (1973) and LAMAN et al. (1984) have all found that the mass per unit length, the total mass of culm, the plant height, the diameter of culm near the base of the plant, the length of the lower internode, the mass of the head and the length of the head are the most important indices for studying the lodging-resistance of cereals.

PRUSSAKOVA (1975), CHIZHOVA (1978) and LAMAN et al. (1984) accentuated the formation of lodging-resistance in the basal zone of cereals in sowing seeds. The sowing density is of special importance, for the morphogenesis of the basal part of the stalk - the length of the lower internode etc. - depend on it.

There are contradictory opinions as to the importance of the flag leaves of wheat for its lodging-resistance. FOWLER and RASMUSSON (1969) established that cereals with large flag-leaves are not lodging-resistant, for the large flag-leaf prevents the head from receiving maximum light energy for photosynthesis. HERZOG (1980) suggested that the effect of the flag leaf depends mainly on the longevity of the leaves, and so the importance of the flag leaves of wheat during the grain filling period and their contribution to the

final yield of crops has been widely assessed. It is apparent that grain yield in corn and the metabolism of all the plants have a functional relationship to the flag leaf area and to the total chlorophyll content - the ratio of chlorophyll a/ chlorophyll b.

The main objective of the present morpho-physiological studies has been to see how the different mineral nutrients, given at different times and in different doses, influence the abovementioned morpho-physiological characteristics which might control the lodging-resistance of spring wheat plants.

Materials and methods

Field experiments were performed with spring wheat (see p. 9).

For the weighing and measuring of each index, samples from 50 stalks and leaves were always used. The mass of the stalk internodes, of the leaves, and of the grains were determined with an electron-scale sensitivity of 0.001 g. The length of stalks was measured in centimetres, the diameter of stalks - with a micrometer. The flag leaf blade area (cm^2) was obtained by multiplying the leaf length by the maximum flag leaf width, by 0.67 (FLOWER, RASMUSSON, 1969). The chlorophyll content was assayed by the method of WINTERMANS and DE MOTS (1965). Simple correlation coefficients were computed.

The results were given as arithmetical means and processed statistically: $n = 50$, $p = 0.05$, $\Delta x \% = 24.4$.

Results

Table 1 gives the phenophases and the dates of analyses of the control plants in the years 1986 and 1987. Comparing the data of the control plants with those of N- and NK-plants, we find very few differences, only in the length of phenophases.

But the growing seasons of the two years differed in month temperature (15.3 heat units in 1986 and 13.3 heat units in 1987), and especially in rainfall (92.7 cm in 1986 and 109.2 cm in 1987). The pattern of rainfall was undoubtedly more important than the amount of temperature. The summer of 1987 was very rainy and therefore the vegetative growth was higher than in 1986 (tab. 2). Wet weather led to tardy ripening. It is therefore not surprising that gene action in the two years differed for a number of traits.

Table 2 shows the morpho-physiological parameters for lodging-resistance of the three experimental varieties of mineral nutrients. It is interesting to note that the indices that are characteristic of lodging-re-

Table 1

The growth and development of the spring wheat plants cv 'Leningradka' (control-variety)

Phenophases*	Date of phenophases		Age of plants in phenophase (in days)		Length of phenophase in days		Data of analysis		Age of plants in analysis (in days)	
	1986	1987	1986	1987	1986	1987	1986	1987	1986	1987
Sowing	07.05.	08.05.								
Sprouting	15.05.	17.05.	8	9	8					
Tillering	07.06.	06.06.	30	30	15	30	07.06.	08.06.	30	32
Stalking	12.06.	29.06	37	53	14	23	12.06.	12.06.	35	36
Booting	27.06.	09.07.	49	63	12	10	17.06.	18.06.	40	42
Flowering	07.07.	20.07	60	74	11	11	22.06.	24.06.	45	48
Milk ripeness	28.07.	12.08.	81	97	21	23	27.06.	29.06.	50	53
Dough ripeness	13.08.	20.08.	97	105	16	6	02.07.	06.07.	55	60
							07.07.	09.07.	60	63
							12.07.	20.07.	65	74
							17.07.	31.07.	70	85
							28.07.	12.08.	81	97
							13.08.	25.08.	97	110

* Dates of phenophases show that 50 % of plants have got abovementioned phases

Table 2

Effect of mineral nutrients on the morpho-physiological parameters of the spring wheat plants cv 'Leningradka' (in the phase of milk ripeness)

N	Variable	Varieties						*'Arkas'	
		I		II		III			
		1986	1987	1985	1987	1986	1987	1986	1987
1.	Stalk length mm	662.1	942.0	706.4	1046.2	832.5	1014.6	542.2	668.0
2.	First internode length mm	32.9	24.1	34.3	39.0	39.4	36.9	31.6	29.0
3.	Second internode length mm	65.7	-	104.2	-	123.4	-	72.8	-
4.	First internode diameter mm	2.54	2.75	2.64	2.81	2.71	2.93	2.39	2.48
5.	Second internode diameter mm	2.82	-	2.83	-	2.99	-	2.43	-
6.	First internode dry mass mg	74.3	55.2	78.6	86.0	96.7	94.8	54.0	89.6
7.	Second internode dry mass mg	140.0	-	160.3	-	202.4	-	97.0	-
8.	First internode dry mass $mg \cdot cm^{-1}$	22.4	23.2	22.3	21.6	24.3	28.7	27.0	30.9
9.	Second internode dry mass $mg \cdot cm^{-1}$	21.3	-	15.3	-	16.3	-	13.3	-
10.	Underhead node length mm	324.2	399.2	290.4	396.4	324.6	432.0	266.0	321.0
11.	Underhead node diameter mm	2.40	2.32	2.15	2.68	2.32	2.75	2.31	2.10
12.	Underhead node dry mass $mg \cdot cm^{-1}$	10.31	-	11.6	-	12.0	-	11.4	-

* 'Arkas' is a short-stalk and lodging resistant cultivar. Here its morpho-physiological parameters are given as a comparison with the long-stalk and lodging susceptible cv 'Leningradka'

sistant plants, such as the diameter of the first internode, dry mass and dry mass per unit length ($mg \cdot cm^{-1}$), are high in NK-plants (Var. 3). On the other hand, the same plants have also high indicators for the height of plants and for the length of internodes, which are characteristic of lodging-susceptible plants. At the same time, all the reproductive indices of NK-plants, such as the crop of grain (ts/ha), the mass of 1000 grains, the number of grains in the main sprout, the number of productive sprouts per plant and the protein content of the grain, are maximal (tab. 3).

The morpho-physiological indices are the lowest for N-plants, while the control plants have intermediate indices.

The interrelationship of some characteristics measured was determined by means of correlation coefficients as shown in table 4. A study of correlation coefficients indicates that many of these characteristics are associated with one another to some extent at least. The dry mass per unit length ($mg \cdot cm^{-1}$) was correlated with the total dry mass of culm and the diameter of culm and with the length of the first internode in control plants ($r = 0.53$). With other characteristics, similar correlations were not found.

In case of NK-plants, we find almost the same correlations but in some instances the coefficients of correlation increase, e.g. between the total dry mass and the first internode length ($r = 0.61$) and the first internode dry mass $mg \cdot cm^{-1}$ ($r = 0.62$). Between the first internode diameter and the first internode dry mass per unit, a negative correlation is found ($r = -0.62$). In case of N-plants the value of correlation coefficients decreases.

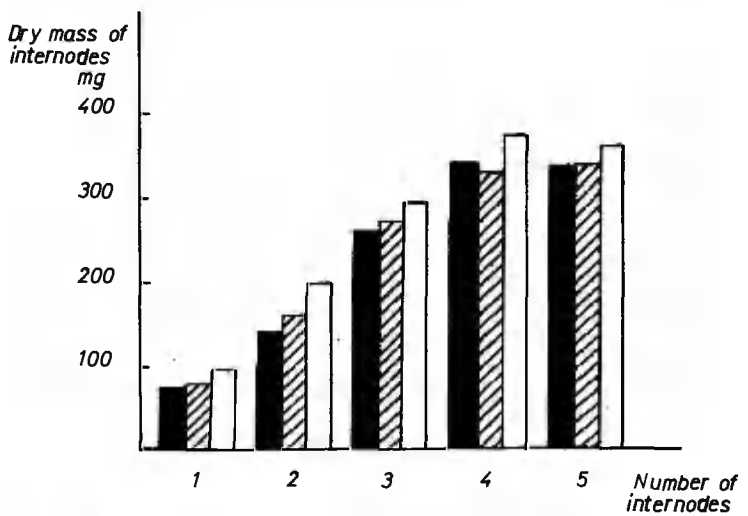

Fig. 1. The content of dry mass (mg) in the internodes of stalks in the phase of milk ripeness

■ - I var., ▨ - II var., □ - III var.

Table 3

Some parameters of the generative and vegetative organs of spring wheat cv 'Leningradka'

Var.	Crop of grain ts/ha	Protein content of grain %	Mass of 1000 grains g	Grains in main sprout per plant	Productive sprouts per plant	Height of the plant cm	Mass of stalk internodes mg		Fresh mass per leaf mg		Dry mass per leaf mg	
							fresh	dry	4. leaf	flag leaf	4. leaf	flag leaf
I												
$N_{50}P_{60}K_{60}$	21.2	12.3	43.6	39.4	1.5	66.2	1633	720	197	94	45	32
II												
$N_{150}P_{60}K_{60}$	29.2	14.3	39.1	37.8	1.9	70.6	2287	952	219	146	48	49
III												
$N_{150}P_{60}K_{240}$	42.3	14.8	49.5	40.4	3.0	83.2	3001	1281	225	197	48	65

Table 4

A matrix of simple correlation coefficients for 9 variables of 3 varieties of mineral nutrients

Variable	Variable number								
	2	3	4	5	6	7	8	9	Var.
1. Stalk length	0.413	0.325	0.548	0.567	-0.324	-6.161	-0.092	0.460	I
	-0.293	0.401	-0.294	0.513	-0.365	0.074	-0.029	0.382	II
	0.149	-0.191	0.379	0.371	0.476	-0.286	0.285	0.571	III
2. First internode length		-0.111	00.437	0.526	0.367	-0.476	-0.224	0.523	I
		-0.096	0.399	0.479	0.309	-0.493	-0.010	0.345	II
		-0.590	0.166	0.601	0.051	0.327	0.078	0.611	III
3. First internode diameter			0.225	0.587	-0.603	80.220	0.193	0.443	I
			0.247	0.353	-0.036	-0.000	-0.022	0.344	II
			0.426	-0.605	-0.176	0.046	0.158	0.533	III
4. First internode dry mass				0.466	-0.114	0.098	-0.190	0.446	I
				0.195	0.446	-0.054	0.087	0.421	II
				0.538	0.288	0.048	-0.134	0.522	III
5. First internode dry mass $mg \cdot cm^{-1}$					-0.686	0.015	-0.190	0.576	I
					0.205	-0.172	-0.313	0.424	II
					0.101	0.225	0.288	0.621	III
6. Underhead node length						0.495	0.530	0.341	I
						0.348	0.341	0.293	II
						0.500	0.444	0.295	III
7. Underhead node diameter							-0.064	0.081	I
							0.051	0.080	II
							0.042	0.071	III
8. Underhead node dry mass $mg \cdot cm^{-1}$								0.527	I
								0.462	II
								0.555	III
9. Total stalk dry mass									

Figure 1 shows the trends of dry mass accumulation in the internodes of stalks.

In NK-plants, the flag leaves are about twice as great as in control plants (fig. 2) and similar correlation is to be seen in case of chlorophyll a and in the ratio of chl a/ chl b (tab. 5).

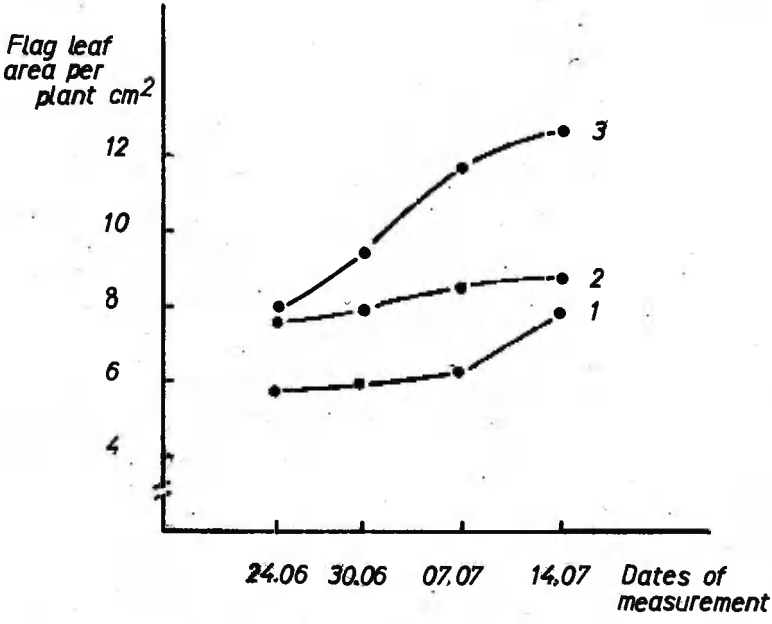

Fig. 2. Flag leaf area per plant cm^2

1 - I var.; 2 - II var.; 3 - III var.

Table 5

Content of chlorophylls in the flag-leaves of spring wheat cv 'Leningradka' in the phase of flowering mg·g^{-1} dry mass (07.07.86)

Chl a	Chl b	ΣChl	Chl a/ Chl b
	Var. I		
5.10	4.10	9.20	1.2
	Var. II		
8.21	4.90	13.11	1.6
	Var. III		
9.08	4.61	13.69	1.9

Discussion

Such morpho-physiological parameters as the dry mass per unit length, the dry mass of the stalk and the diameter of the first internode are in correlation with lodging-resistance. These indicators have the highest correlation coefficients (tab. 4). These coefficients indicate that the morpho-physiological method may be used for estimating the lodging of cereals. Our data coincide with the results achieved by ATKINS (1948), BLOMQUIST et al. (1973) and KUPERMAN et al. (1977) who showed that the abovementioned indices could be useful for classifying wheats and soybean lines for lodging-resistance. Mineral nutrients (N, K) given at different times and in different doses to long-stalk wheat promote the production of the strong straw and increase the abovementioned indices, e.g. the diameter of the stalk (tab. 2). This trait might result in a greater movement of water and nutrients to and from the flag leaf and might, in turn, be associated with a slower rate of senescence of the flag leaf. In addition, such plants ought to be more resistant to lodging.

NK-plants accumulated 15 % more dry mass than control plants and 8 % more than N-plants (fig. 1).

In the phase of flowering, when the wheat plant goes from the vegetative phase to the generative, control plants accumulate 7.4 % of the total dry mass of the plants, N-plants - 7.5 % and NK-plants - 8.4 % (fig. 3). The same tendency is noticeable in the phase of milk ripeness. Then the dry mass of the first internode constitutes 6.4 % of the total dry mass of the stalk in control plants, 6.2 % - in N-plants, and 7.0 %

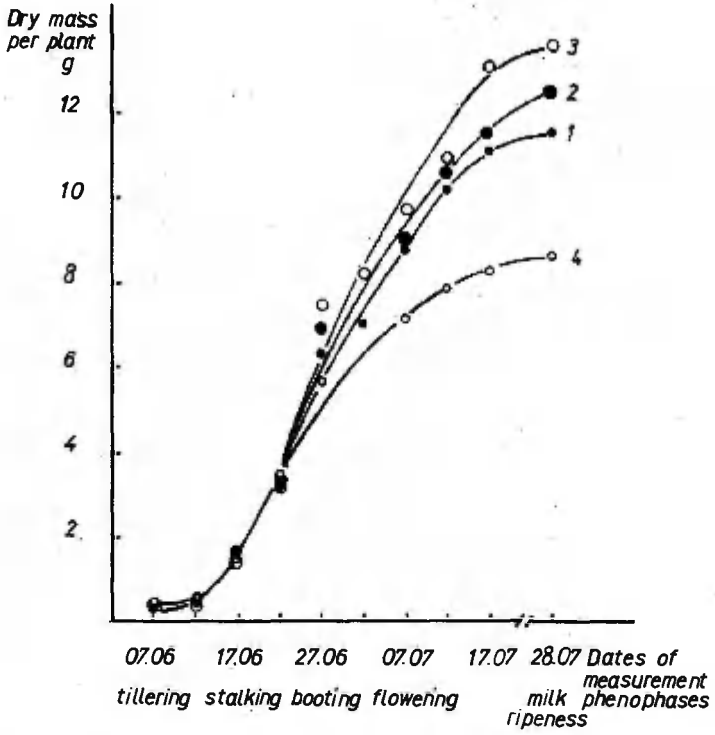

Fig. 3. Changes of dry mass of the stalk (all internodes) per plant
1 - control, 2 - N plants, 3 - N, K plants 4 - 'Arkas'

- in NK-plants (fig. 1). It seems that the deposition of dry mass in cell walls is characteristic of the lodging-resistant wheat plants. So, according to HANWAY and WEBER (1971), KUMAKOV (1983), and our data, lodging may result from the content of dry mass, especially from the content of dry mass per unit length of stalk of the first internode (tab. 2).

But the content of dry mass per unit length ($mg \cdot cm^{-1}$) in the second internode, from where the cereals often break and what some scientists (KUMAKOV, 1986) suggest to use for making the morpho-physiological analysis, did not show any correlation with the lodging-resistance of wheat in our experiments (tab. 2). It should also be mentioned that the short-stalk cultivar of wheat 'Arkas' contains less dry mass per unit length of stalk in the second internode (tab. 2) than the long-stalk cultivar 'Leningradka'. From our point of view the abovementioned characteristic has significance only within a cultivar and for the first (not the second) internode.

The comparison of fig. 2 and tab. 3 reveals that the flag leaf area of spring wheat is closely associated with high grain yields. BERDAHL et al. (1972) stated that the flag leaf of the large-leaf lines produces nearly twice as much photosynthate as the flag leaf of the small-leaf lines. They postulated that small leaf area favors the development of larger numbers of culm while large leaf area favors higher kernel mass.

As regards the relationship of the area of leaves with lodging, it seemed that the larger the area of a leaf is the more intensive the photosynthesis is and the more the leaf accumulates organic compounds. From those, young plants store glucosides of phenolic alcohols and derivatives of cinnamic acids that are later converted to coniferyl alcohol, a component of lignin. Of course, different mineral nutrients must be in definite proportion. According to our experiments, the main role here is played by potassium, with nitrogen given at different times and in different doses.

The content of chlorophyll a (tab. 5) fluctuated around 5.10 $mg \cdot g^{-1}$d.m. in control plants, 8.22 $mg \cdot g^{-1}$d.m. in N-plants and 9.08 in KN-plants. Ammonium fertilizers stimulate the accumulation of chlorophyll b instead of chlorophyll a. The chl a/chl b index increases in plants grown with K, N fertilizers. Similar results were obtained in the experiments with *'Lolium perenne'* carried out by BERGARECHE and SIMON (1986).

To sum up our results we may say that morpho-physiological parameters are of importance for determining the characteristics of lodging.

Such indices as the total dry mass, the dry mass per unit length ($mg \cdot cm^{-1}$) and the diameter of culm are in a positive correlation with lodging. The last-

mentioned indices with photosynthetic areas of flag leaves and with the content of chlorophyll make a greater contribution to the yield of grain.

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METABOLISM OF PHENOLIC ACIDS IN WHEAT PLANTS

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Introduction

It is a well-established fact that hydroxycinnamic acids such as *p*-coumaric, ferulic, caffeic and sinapic acids are widely distributed in vascular plants where they are generally found as esters rather than as phenolic glycosides and free acids. EL-BASYOUNI et al. (1964), TCHIRGIN et al. (1973) and MIIDLA et al. (1975) show that relatively large amounts of hydroxycinnamic acids, particulary *p*-coumaric and ferulic acids, are released on the alkaline hydrolysis of the insoluble residue remaining from an ethanol extraction of wheat stalks. They suggest that these esters are not inert storage products but may be active intermediates in phenylpropanoid metabolism. HARTLEY and JONES (1977) showed that ferulic and *p*-coumaric acids bound to cell walls are lignin precursors. SCALBERT et al. (1985) showed that 93 % of *p*-coumaric acid is alkali label and thus linked as ester-bond fraction but ferulic acid is more acid label and linked as glycoside-bond one in 35-75 %. The authors point out that parallel increase in lignin, in *p*-coumaric and ferulic acid esters has been observed in wheat coleoptile cell wall during the cessation of growth between the 5th and 9th days of development. These esters establish cross links with lignin and in this way restrict growth and contribute to the rigidity of the cell wall by a mechanism similar to the peroxidase-mediated dimerization.

Besides light and water, mineral nutrients determine growth and development of higher plants. In the agricultural practice, the mineral nutrition factor is manipulated to increase yield and to control the quality of crops. Nitrogen, phosphorus, and potassium (N, P, K) are quantitatively the most important mineral elements. The significance of these nutrients to the yield of crops and the underlying physiological processes on the level of primary metabolism has been intensively studied (CLARKSON and HANSON, 1980). It has been found that photosynthesis is strongly influenced by the nutrient status, especially the nitrogen level, of cereal seedlings (DALE, 1972; OSMAN et al., 1977).

But there are very few data concerning the effect of different mineral nutrients on the secondary metabolism.

MULDER, (1954) showed that nitrogen reduced the amount of lignin in the stem and roots so influencing the lodging.

PHILLIPS (1971) pointed out a negative connection between the high level of nitrogen nutrients and the synthesis of phenolic compounds in tomato plants.

MIIDLA (1970), MIIDLA et al. (1970) explained nitrogen influence on the secondary metabolism in apple-tree with the increase in the level of phenylalanine. As a result, a competition between the metabolism of phenolic compounds and proteins will occur in cells.

MILLER and ANDERSON (1965) found that phosphorus increased the incidence of lodging but failed to suggest a mechanism which might account for this effect.

MENGEL et al. (1981) mentioned that potassium strengthened the cell walls of oat, and showed that higher K supply resulted in an increased content of soluble amino acids in wheat.

HOJATTI and MALEKI (1972) reported that K increased the methionine content in wheat during the vegetative growth of spring wheat. *L*-methionine may be a precursor of $-OCH_3$ groups by the process of methylation in lignin formation.

PENEL (1986) showed that induction with Ca^{2+} ions increased the activity of peroxidase and promoted the content of lignin.

MERVARY (1985) reported that calcium deficiency increased the content of phenolic acids in wheat.

We may conclude that the few data available on the effect of mineral nutrients on the biosynthesis of phenolic compounds are contradictory.

So the aim of the present paper, first and foremost, is to show changes in the content of most prevailing C_6 - C_3-acids (ferulic and *p*-coumaric acids) as precursors in the biosynthesis of lignin in wheat cell walls in the ontogeny of wheat plants. We also want to indicate the balance between free, ester and glycoside fractions in ethanol soluble and ethanol insoluble compounds which release hydroxycinnamic acids on alkaline and acid hydrolyses.

Secondly, from the standpoint of practice, it is very important to know how, with different mineral nutrients given at different times and in different doses, it is possible to regulate the metabolism of hydroxycinnamic acids as precursors of lignin formation in wheat.

Material and method

In the year 1984, phenolic acids for quantitative analyses were determined in wheat plants grown in lysis in the Botanical Gardens of the University of Tartu, and in 1986 - in plants grown in field experiments of mineral nutrients (see p. 9). Wheat seeds *Triticum aestivum* L. cv 'Leningradka' in lysis were planted in a composted soil mixture. The plants received no addi-

tional nutrients. Phenolic acids were established from fixed air-dried and milled material of the leaves and of the first internodes of wheat-stalks by paperchromatography (MIIDLA et al, 1975) using an ascending two-dimensional method: 2 % acetic acid in the first and n-butanol, acetic acid, water (4 : 1 : 5 - upper phase) in the second direction after spectrophotometrically determining their respective absorbance maxima - ferulic acid 311 nm and *p*-coumaric acid 289 nm.

The results were calculated using the following formula:

$$C_{\mu g \cdot g^{-1}} = \frac{E.M.d}{\varepsilon .1} \quad \text{where}$$

C - concentration $\mu g \cdot g^{-1}$ dry mass
E - extinction
M - molar mass of the determined matter
d - dilution
ε - extinction coefficient: ferulic acid - 14.250, *p*-coumaric acid - 16.380.

In the ethanol soluble fraction free acids from ethanol extraction and ethanol "soluble esters", acids which release on alkaline and "soluble glycosides", acids which release on acid hydrolysis were determined. In the ethanol insoluble fraction from the residue "soluble esters" and "soluble glycosides" were determined in the same way.

Results

Leaves and stalks of spring wheat contain hydroxycinnamic acids: ferulic, *p*-coumaric and caffeic acids, the two first ones being prevalent. Of the derivatives of hydroxybenzoic acids wheat contains syringic, *p*-hydroxybenzoic and vanillic acids.

During the period of vegetation, the general amount of ferulic acid in wheat leaves is about 4...5 times greater than that of *p*-coumaric acid (tab. 1). The content of ferulic acid in the leaves of spring wheat is on the average 2309 $\mu g \cdot g^{-1}$ dry mass and that of *p*-coumaric acid - 577 $\mu g \cdot g^{-1}$ dry mass.

In the ethanol insoluble fraction, 8.03 % of ferulic acid is in glycoside and 37.70 % in esterbond forms. In ethanol soluble fraction the percentages are 10.63 and 38.28 respectively, 5.36 % of ferulic acid being in the form of free acid.

p-Coumaric acid is characterized by the following percentages: in the ethanol insoluble fraction 17.20 and 31.20 % respectively, and in the ethanol soluble fraction 14.91, 30.11 and 6.58 % respectively.

Stalks contain different fractions of ferulic and *p*-coumaric acids in almost equal amounts (tab. 2).

The ratio of ferulic acid to *p*-coumaric acid is higher in leaves (5.25 on the average) - in mesophyll

Table 1

The content of phenolic acids in the leaves of lysis-grown wheat ($\mu g.g^{-1}$ dry mass)

Date and phenophase	Ferulic acid						*p*-Coumaric acid						Ratio insol. est. f. / *p*-coum.	Ratio Σ f. / Σ *p*-coum.
	Ethanol soluble			Ethanol insoluble		Σ	Ethanol soluble			Ethanol insoluble		Σ		
	Free	Esters	Glycosides	Esters	Glycosides		Free	Esters	Glycosides	Esters	Glycosides			
27.06. booting	95	742	206	702	195	1940	5	134	92	142	81	454		
	4.9	38.3	10.6	36.1	10.1		1.1	29.5	20.3	31.3	17.8		4.34	4.27
10.07. flowering	125	805	197	690	189	1804	47	112	72	121	92	444		
	6.8	33.5	10.9	38.3	10.5		10.6	25.2	16.2	27.2	20.7		5.76	4.06
01.08.	115	842	188	796	175	2118	43	132	34	138	87	432		
	5.4	39.8	8.9	37.7	8.2		9.9	30.6	78.7	31.5	20.1		5.07	4.90
11.08. milk ripeness	115	907	241	841	160	2264	35	147	79	152	81	494		
	5.1	40.1	10.6	37.2	7.0		2.1	29.8	16.0	31.7	16.4		5.63	4.59
25.08. dough ripeness	111	899	277	903	119	2309	28	198	31	198	72	577		
	4.8	38.9	12.0	39.1	5.2		4.9	44.3	14.4	34.3	12.5		4.56	4.00
Σ	559	3995	1109	3934	838	10435	158	723	358	748	413	2401	5.28	4.35
%	5.4	38.3	10.6	37.7	8.0		6.6	30.1	14.9	31.2	17.2			

Table 2

The content of phenolic acids in the first internodes of lysis-grown wheat-stalks ($\mu g \cdot g^{-1}$ dry mass)

Date and pheno-phase	Ferulic acid						p-Coumaric acid						Ratio insol. est. f.	Ratio Σ f.
	Ethanol soluble			Ethanol insoluble			Ethanol soluble			Ethanol insoluble				
	Free	Esters	Glycosides	Esters	Glycosides	Σ	Free	Esters	Glycosides	Esters	Glycosides	Σ	p-coum.	p-coum.
27.06. booting	10	187	97	187	105	616	10	182	145	130	132	659		
	6.5	30.4	15.8	30.7	16.5		1.5	27.0	22.0	20.8	20.1		0.58	0.33
10.07. flowering	47	162	120	202	107	628	45	147	132	193	78	598		
	7.5	24.2	19.1	32.2	17.0		7.5	24.0	22.1	32.6	13.2		1.04	1.04
01.08.	30	206	[illegible]	252	92	878	37	257	137	324	123	918		
	4.1	24.8	17.2	47.9	11.0		4.0	28.0	27.4	35.3	10.3		1.13	1.30
11.08. milk ripeness	31	207	87	318	97	735	33	181	175	352	149	690		
	4.2	28.3	11.8	42.5	13.2		3.7	20.3	19.7	39.6	16.7		0.89	0.43
25.08. dough ripeness	25	197	87	280	96	682	37	742	191	327	115	912		
	3.6	28.5	18.3	41.9	12.7		4.1	26.5	20.9	35.9	12.6		0.89	0.76
Σ	177	918	533	1347	483	3459	262	2035	830	1360	586	3977		
%	5.1	27.1	15.4	38.9	13.9		4.1	25.5	22.8	34.9	14.8		0.97	1.01

cells - (tab. 1) than in stalks (0,97) - in non-mesophyll ones (tab. 1, 2)

Different mineral nutrients brought about quantitative changes in the content of *p*-coumaric and ferulic acids during the period of vegetation (tab. 3). Significant ($p \leq 0.05$) changes are only in the content of the ethanol insoluble fractions while the content of the other fractions (free and ethanol soluble) shows no noticeable changes. The plants of Var. 3 (N, K plants) of a high level of N and K given at different times and in different doses accumulate the highest content of ethanol in-soluble potential precursors for lignin synthesis - ferulic and *p*-coumaric acids. Stalks of the lowest nutrient level (var. 1 - control) show the lowest phenolic content with the plants of var. 2 (N plants) occupying the medium position.

The ratio of ferulic acid to *p*-coumaric acid in different varieties of mineral nutrients is not similar. High ratios are found in the plants of var. 1 and var. 2 but N, K plants (var. 3) have a low ratio. All the ratios have a tendency to decrease towards the end of the vegetation period.

Discussion

The fact that the leaves of wheat plants contain 4...5 times more ferulic acid than *p*-coumaric acid (tab. 1, 2) shows that *p*-coumaric acid is confined mainly to lignified cell walls. HARRIS et al. (1980) suggested that non-mesophyll cell walls contained much larger amounts of ferulic acid than mesophyll ones. VANCE and SHERWOOD (1976) showed a higher incorporation of *p*-coumaric acid into lignin of *Phalaris arundinacea*. It is well documented that *p*-coumaric acid esters are incorporated into lignin in the *Gramineae* to a larger extent than in other cinnamic acids (EL-BASYOUNI et al., 1964; SCALBERT et al., 1985).

These two monomeric phenolic acids are mainly esterfied with wheat cell wall polymers. It is probable that *p*-coumaric acid is exclusively associated with lignin but ferulic acids, on the other hand, are mainly esterified with hemicelluloses and have been identified in many non-lignified tissues.

Since these phenolic acids are bifunctional they are able to form ester or glycoside linkages by the reaction of their carboxyl or phenolic groups respectively. These phenolic acids, free or bound forms, can copolymerisize with lignin, and in different fractions of wheat stalk lignin, they can be isolated on mild alkaline and mild acid hydrolyses.

One of the main functions of the cinnamic esters is to supply units for the biosynthesis of lignin. Reduction to the corresponding alcohols can give the substrates needed for dehydropolymerization to lignin.

Table 3

Effect of mineral nutrients on the content of phenolic acids (μg.cm^{-1}) in the first internodes of field-grown wheat-stalks 1986

Date and pheno-phase	I var.							II var.							III var.						
	Ferulic acid			p-Coumaric acid			Ratio of EIS f/p-c	Ferulic acid			p-Coumaric acid			Ratio of EIS f/p-c	Ferulic acid			p-Coumaric acid			Ratio of EIS f/p-c
	F	ES	EIS	F	ES	EIS		F	ES	EIS	F	ES	EIS		F	ES	EIS	F	ES	EIS	
11.06. stalking	0.27	2.46	22.57	1.69	3.67	36.43	0.76	0.34	1.33	36.07	2.03	9.68	36.92	0.93	0.88	3.62	40.57	1.44	3.30	62.68	0.56
28.06. booting	1.03	4.16	50.29	4.50	7.07	94.60	0.63	0.37	5.06	52.19	4.35	10.69	107.57	0.49	0.95	3.01	75.08	4.41	4.01	120.96	0.64
12.07. flowering	1.29	3.02	59.76	5.31	7.76	107.30	0.56	0.87	4.77	54.08	5.07	10.36	117.5	0.45	1.17	4.16	74.76	4.09	10.51	104.72	0.46
26.07. milk ripeness	1.59	3.57	46.50	2.82	5.45	86.05	0.54	0.61	2.90	60.45	2.39	7.55	116.0	0.55	0.71	3.60	70.20	2.53	5.77	102.81	0.96
25.08. dough ripeness	0.55	0.79	25.70	2.56	3.17	80.03	0.31	0.53	3.65	58.5	2.16	6.31	106.5	0.55	0.69	3.16	60.87	2.29	5.32	122.71	0.50
Σ	3.79	14.00	213.97	16.65	25.88	405.19		2.82	18.71	261.27	17.13	40.60	487.7		4.72	15.83	327.42	14.56	34.6	406.56	
% of ΣΣ	0.55	2.19	30.37	2.42	3.77	58.07		0.86	2.37	31.73	2.08	4.94	59.52		0.90	14.97	29.86	1.01	3.28	69.31	

F – free acids
ES – ethanol soluble
EIS – ethanol insoluble

There is a nearly parallel increase in lignin and in the content of *p*-coumaric and ferulic acid esters (fig. 1, 2) during the cessation of growth in the first period of lignification in the first half of the vegetation period before flowering.

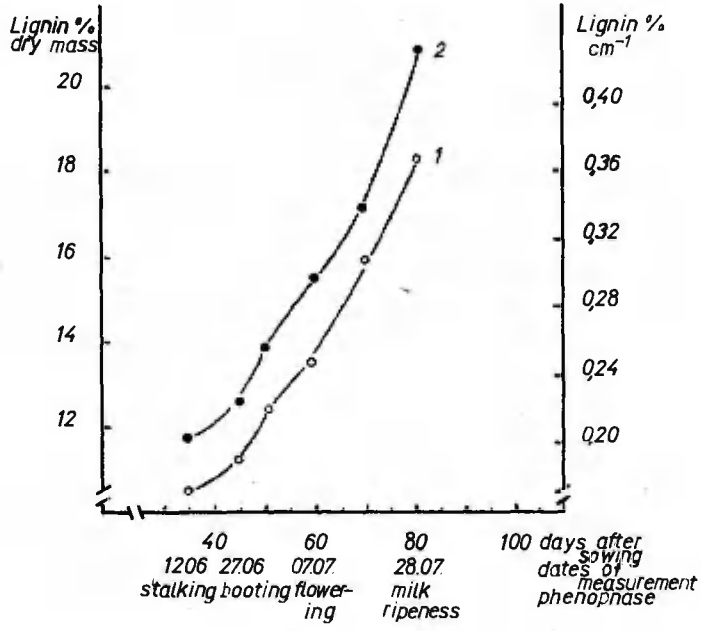

Fig. 1. Dynamics of lignin in the first internode of wheat
1 - lignin % cm^{-1}
2 - lignin % dry mass

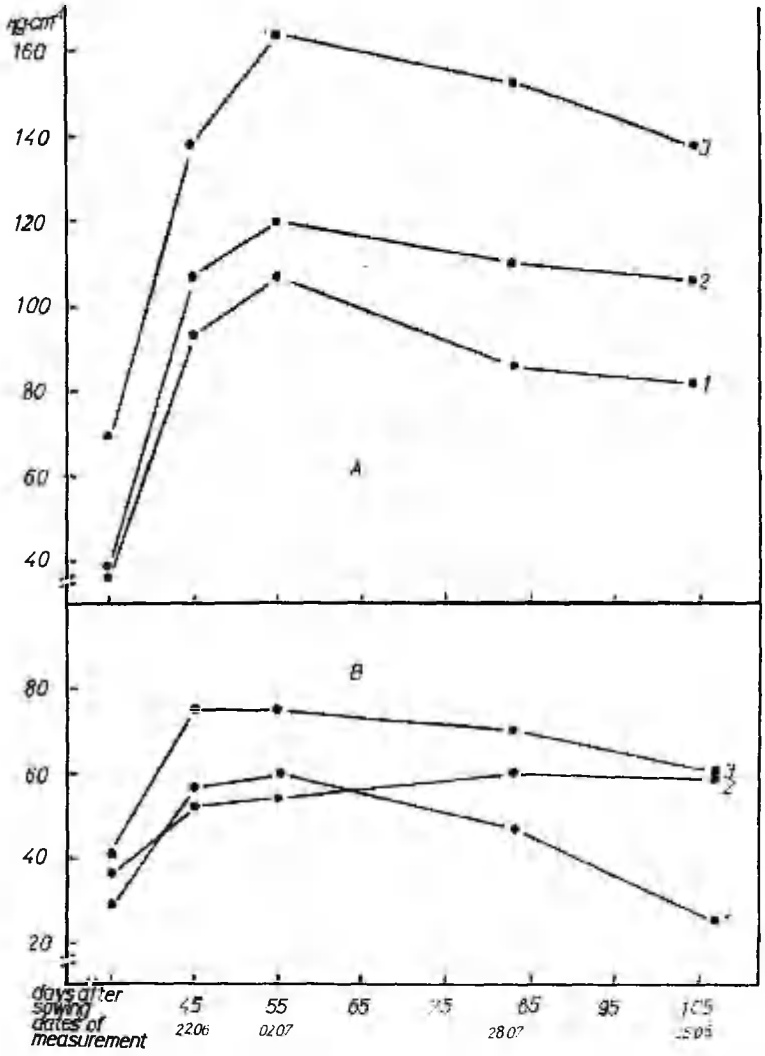

Fig. 2. The content of in ethanol insoluble esters (µg.cm $^{-1}$) in the first internode grown in different mineral nutrients

A – *p*-coumaric acid
B – ferulic acid

1 – I var.
2 – II var.
3 – III var.

The contents of ferulic and *p*-coumaric acids increase dynamically and reach their maximum in flowering (fig. 2). In the phase of flowering, the wheat plant passes from the vegetative phase to the generative one and evidently the metabolism of the plant is reorganized. Ferulic and *p*-coumaric acids inhibit the lengthgrowth of the plant (fig. 3). Later, after flowering, the content of the two compounds decreases.

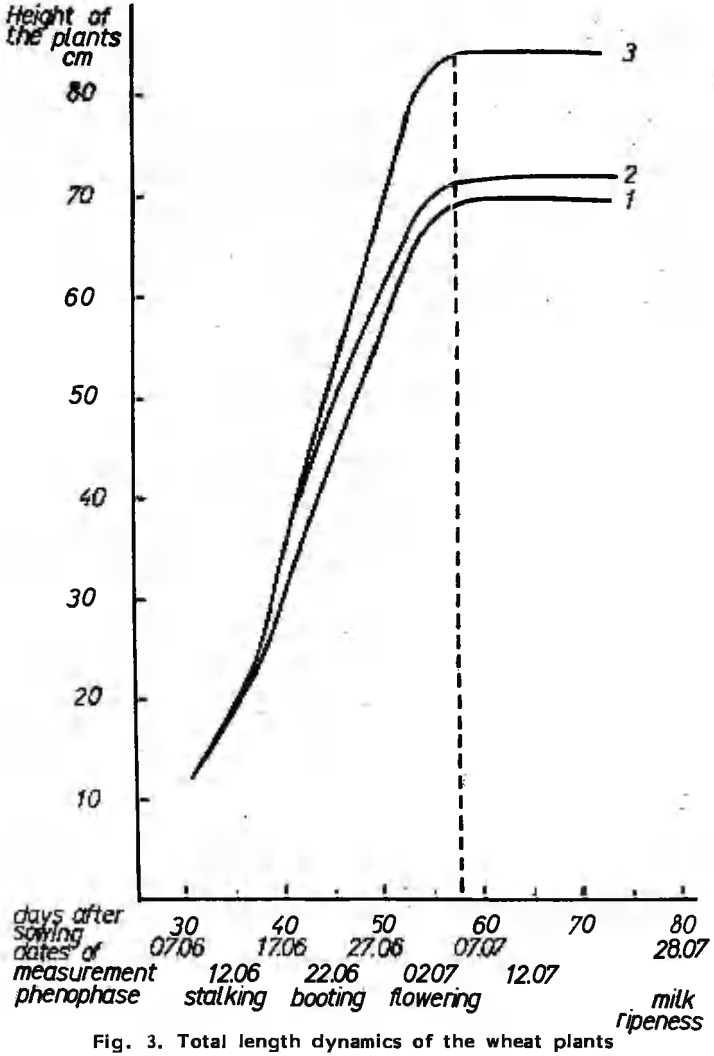

Fig. 3. Total length dynamics of the wheat plants

It is probable that with the inhibition of the length-growth the second period of lignification begins where the cinnamic acids as precursors of lignification are utilized for the biosynthesis of lignin. MILLER et al. (1985) also show that soluble phenolic acids increase concomitant with the first phase of lignification in *Lactua sativa* and then decrease during the second phase.

FRY (1979) showed that ferulic esters esterified to hemicelluloses in young primary cell walls would thus establish cross links with lignin and in this way might restrict the growth and contribute to the rigidity of the cell wall by a mechanism similar to the peroxidase-mediated dimerization.

Our data (p. 44) show that the process of lignification proceeds very quickly, so on the 36th day of growth in the phase of stalking 11.8 % of lignin in the first internode is already formed (fig. 1). It is about 56 % of the maximum content of lignin.

Our experiments with quantitative chromatography of phenolic acids from the cell walls of all the parts of spring wheat have shown that the amounts of *trans*-isomer of ferulic or *p*-coumaric acids are greater than those for the corresponding *cis*-isomer.

And so the experiments of wheat coleoptile elongation bioassays (MIIDLA, HALDRE, 1977) showed (fig. 4)

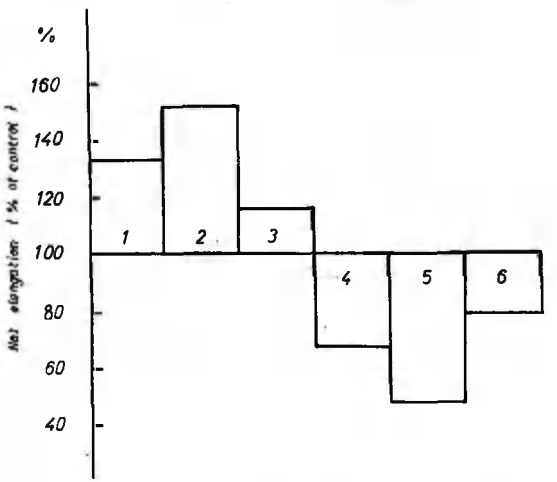

Fig. 4. Histogram - interaction of native phenolic acids, isolated from the leaves of the wheat plants, on auxin-induced growth of wheat coleoptiles. Initial length of sections 4 mm. Incubation medium: sucrose (1 %), IAA (10^{-5}M), phosphate buffer (pH 6). 1 - caffeic acid *(trans)* - $4.8.10^{-4}$M; 2 - *p*-coumaric acid *(cis)* - $3.8.10^{-4}$M; 3 - ferulic acid *(cis)* - 2.10^{-4}M; 4 - *p*-OH-benzoic acid - $8.3.10^{-4}$M; 5 - *p*-coumaric acid *(trans)* - $6.7.10^{-4}$M; 6 - ferulic acid *(trans)* - 4.10^{-4}

that native *cis*-forms of phenolic acids isolated from the leaves of wheat plants act synergistically with IAA promoting straight growth of wheat coleoptile sections. The results with other phenolic acids and their isomers are negative.

Phenolic acids have been shown to increase the destruction of IAA by IAA oxidase *in vitro*: *p*-coumaric acid and *p*-hydroxybenzoic acids; and to decrease the destruction - caffeic acid (ZENK and MÜLLER, 1963). Contradictory results have been obtained with ferulic acid by SACHER (1963) who obtained an increased rate of destruction of IAA by IAA oxidase in the presence of ferulic acid. Our data (MIIDLA, HALDRE, 1977) show (fig. 5) that IAA oxidase activity was stimulated by

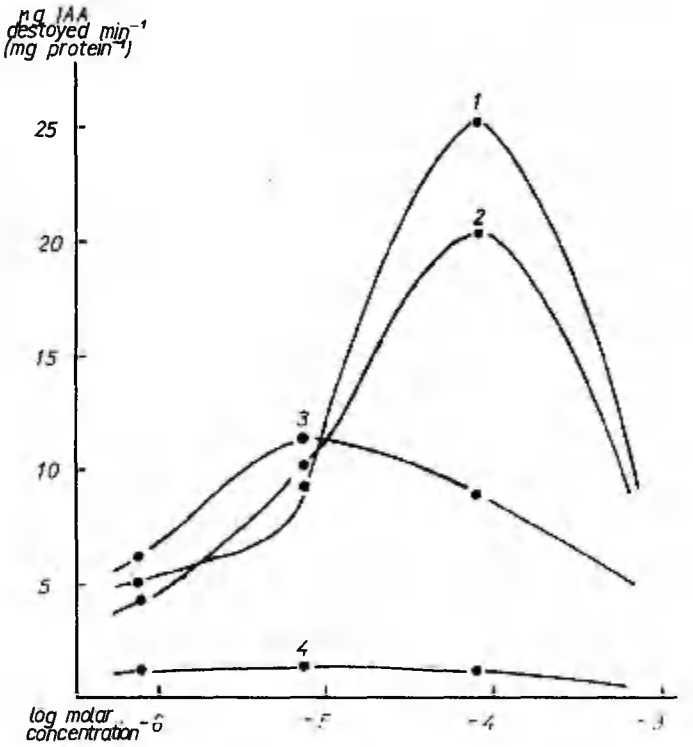

Fig. 5. Effect of native phenolic acids (NPA) on the destruction of IAA. The system consisted of: 1 ml enzyme solution, 250 µg IAA, 1 ml NPA and phosphate buffer pH 4,0 in a total volume, of 10 ml. IAA was determined with Salkowski reaction. 1 - *p*-coum., 2 - *p*-OH-bz., 3 - fer., 4 - caf.

low levels of ferulic acid while at higher levels it acted as an enzyme inhibitor.

So these acids, before they are activated for the polymerization of lignin, interact with auxin-induced growth.

The contents of phenolic acids in the first internodes of wheat stalks are strongly influenced by the nutrient content (N, P, K) of the substrate (tab. 3). The phenolic contents are higher in stalks of wheat plants growing with an elevated nutrient supply (N, K plants, Var. 3) - the mineral elements being given at different times and in different doses (N_{100} + K_{80} at the beginning of tillering + K_{100} at the beginning of stalking).

Our laboratory data (see the article in our abstract book p. 62) show that the intensity of lignin biosynthesis is in positive correlation with the intensity of growth at the beginning of the growth period. In accordance with the view that the biosynthesis of lignin and the lengthgrowth of plants proceed unidirectionally our numerical results of the content of phenolic acids have been calculated for the lengthunit of stalk (1 cm).

It is already known that in N deficiency many phenolic compounds accumulate in plants, therefore the biosynthesis of protein decreases and phenylalanine is formed. The latter is utilized favourably in the biosynthesis of phenolic compounds but in the excess of nitrogen nutrition the protein synthesis prevails (MEI, THIMANN, 1984).

The elements P and K, especially potassium, increase the biosynthesis of phenolic compounds (KLINGUER et al., 1986).

In our experiments comparing control plants (Var. 1, tab. 3) with N plants (var. 2) and with N, K plants (var. 3), we see that in N, K plants the content of cell wall bound (ethanol-insoluble) phenolic acids increases significantly ($p \leq 0.05$).

The same correlation is also established in the lengthgrowth of wheat plants (fig. 3).

From these facts we can conclude that single nutrient elements have an unidirectional influence, but if they are given together at different times and in different doses their effect is synergical (see the parameters of growth and development p. 27). That is natural because no single elements of mineral nutrients may be in deficiency. Mineral elements given at different times and in different doses influence first of all the primary metabolism in plants, increase the photosynthetic capacity and the content of carbohydrates and proteins - on what the secondary metabolism depends. MENGEL (1976) suggests in his monograph giving high doses of nitrogen together with high doses of potassium. In this case the minerals influence synergically, cell walls develop and the lodging resistance

of cereals increases.

The ratio of ethanol insoluble ferulic to *p*-coumaric acid is on the average the smallest in N and K plants (0.49) compared with plants of other varieties (var. 1 - 0.56 and var. 2 - 0.60 (tab. 3)). The small ratio shows that the amount of *p*-coumaric acid incorporated in lignin is greater, i.e. cell walls are more lignified. A similar conclusion can be drawn from the work by HARTLEY (1972) who showed that as ***Lolium perenne*** plants matured, the amount of ferulic acid relative to *p*-coumaric acid in their cell walls decreased and, at the same time, the lignin content of the cell walls increased.

In this way, we think, we have found a good indicator for estimating the process of lignification in cell walls.

We conclude from the results obtained that intermediates in lignin biosynthesis in wheat are esters of hydroxycinnamic acids which are not soluble in ethanol.

In high nitrogen and potassium conditions plants assimilate carbon dioxide by photosynthesis and use carbohydrates thus obtained for the synthesis of phenylalanine or tyrosine by the shikimic acid pathway. In addition to contributing to protein synthesis these amino acids can undergo loss of nitrogen to form corresponding cinnamic acid derivatives. These acids lead to the formation of lignin.

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REGULATION OF LIGNIN FORMATION IN WHEAT STALKS

H. Miidla, E. Padu, A. Tänav

Introduction

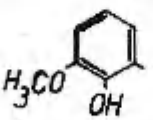
The stiff-strawed lodge-resistant varieties of wheat (*Triticum aestivum* L.) were characterized by a high lignin content per unit length of stalk (see our previous article p. 44).

Factors that tended to increase lodging (high nitrogen and low potassium content, shading, etc.) also reduced the lignin content of stalks (LAUDE, PAULI, 1956).

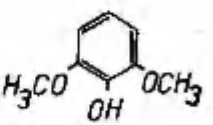
The work of PHILLIPS et al. (1949) showed that wheat, oat and barley seedlings contained a small amount of lignin and that a rather rapid increase in it took place in about 40 days after the plants had emerged from the soil. In all the cases, the young lignin had lower methoxyl content and the abovementioned authors concluded that this was definite evidence against Klason's hypothesis that plants synthesize lignin from coniferyl alcohol (or aldehyde) by a process of polymerization, for it was agreed that Klason's theory would require a constant methoxyl content at all the stages of phytochemical synthesis.

Cereal straws are angiosperms and the lignin is consequently made up of *p*-hydroxyphenyl (I), guaiacyl (II) and syringyl (III) units.

I



II



III

A change in the ratio of these building units during the growth of the plant could account for any variations in methoxyl content found in the lignin and does not eliminate the possibility that coniferyl alcohol played a part in the formation of II.

In the previous article we pointed out (see p. 37) that the primary precursors of lignin are the alcohols of *p*-coumaric, ferulic and sinapic acids. The lignin formed in wheat is composed of guaiacyl (containing

mainly *p*-coumaryl residues), syringyl (consisting mainly of ferulyl residues) and *p*-hydroxyphenyl units. Therefore, an alkaline nitrobenzene oxidation carried out on plants at various stages of growth, followed by the quantitative determination of vanillin, syringaldehyde and *p*-hydroxybenzaldehyde so formed, should provide evidence as to the relative amounts of the guaiacyl, syringyl and *p*-hydroxyphenyl units present in lignin. The amount of aldehyde derivable from the plant should also provide a good indication of the amount of lignin present.

Lignin is a polymer synthesized *via* the shikimic acid pathway from erythrose-4-phosphate and phosphoenol pyruvate (BROWN, 1969). Two major products of the shikimic acid pathway are phenylalanine and tyrosine. Both these amino acids can be deaminated to phenolic alcohols by the respective ammonia lyases (NORTHCOTE, 1969).

Phenylalanine ammonia lyase (EC 4.3.1.5, PAL) is present as an inducible enzyme (ZUCKER, 1968) that catalyzes the first reaction in a series leading to the formation of phenolic alcohols (NEISH, 1961).

Peroxidases (EC 1.11.1.7) catalyze the formation of free radicals, from the phenolic alcohols, that spontaneously condense to form the lignin polymer (BROWN, 1969). FREUDENBERG (1959) is of the opinion that young plants store glucosides of phenolic alcohols and cinnamic acid derivates that are later converted to coniferyl alcohol, a component of lignin. Therefore, peroxidases might control the rate or time of lignin formation.

Correlation of the level of enzyme activities or lignin, aldehyde and methoxyl contents with lodging could be useful for studying lodging resistance.

From the theoretical and, especially, from the practical standpoint it is very interesting to know how mineral nutrients (N, P, K) activate the metabolism of phenylpropanoid and, particularly, how they influence lignin formation in cell walls in connection with the lodging of wheat plants.

The biochemical mechanism of lodging and the particular influence of mineral nutrients on this process have not been thoroughly studied and are still badly understood.

In the present study we report the effects of elevated N, K levels on the process of lignification: the accumulation and structure of lignin and $-OCH_3$ groups.

Material and methods

Material: see the materials and general methods p. 9.

The extraction and spectrophotometric determina-

tion of lignin content were carried out using the technique of JOHNSON et al. (1961) with our modifications.

After the removal of interfering phenolics and proteins, the lignin content of dried grasses can be measured by dissolving the residue in 25 % acetyl bromide in acetic acid and determining the absorption at 280 nm.

All the samples of stalk internodes for determining the content of lignin, aldehydes and $-OCH_3$ groups were fixed in a thermostat at 105 °C for 30 min, then dried at 60 °C for 12 h and milled to an 0.5 mm screen.

The dried internodes (1.0 g) were first extracted with distilled water (100 ml) at 60 to 65 °C for 30 min, then with 1 M NaCl at room temperature for 24 h with occasional shaking. The residue was washed thoroughly with water, ethanol, acetone and diethyl ether until no futher colour appeared in the filtrate and the preparation was dried at 50 °C overnight. The volumes of the reagents used for washing varied depending on the state of maturity of the material but did not exceed 100 ml for any (see the contents of protein in plants depending on their average, fig. 1).

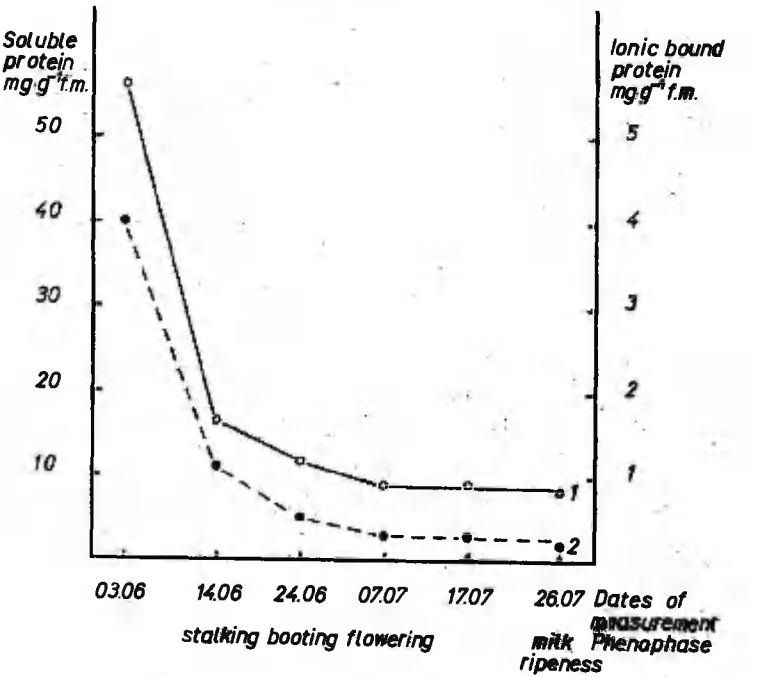


Fig. 1. Protein content as a function of age

The residue was extracted in Soxlet apparatus with ethanol-benzene (1:2, v/v) for 18 h. After that, the alkaline hydrolysis of dried residue was carried out with 2M NaOH in the atmosphere of argon for 1 h, washed with diethyl ether and dried at 50 °C. From the final residue, 10 mg of dry powder were weighed and the lignin was solubilized by heating at 70 ± 0.1 °C for 30 min in glass-stoppered test tubes with 5 ml of 25 % acetyl bromide in glacial acetic acid. After cooling to 20 °C, the material was transferred to a 100 ml volumetric flask containing 4.5 ml of 2M sodium hydroxide in 10 ml of acetic acid. Acetic acid was used to wash the residue from the tube and, to bring the volume up to 100 ml, 0.5 ml of 7.5M-hydroxylamine hydrochloride was added. The purpose of the hydroxylamine hydrochloride is to remove the bromine and polybromide formed during the reaction. Bromine itself is only a weak absorber at 280 nm but the polybromide ion which forms readily when bromine and bromide are mixed is a very strong absorber at 280 nm. The contents of the flask were brought up to the mark with acetic acid, shaken and allowed to stand for at least 1 h to let the protein sediment settle before reading the extinction of the solution at 280 nm. A reagent blank was run with every set of estimations. Fresh reagents were always prepared. Results were expressed as a percentage of the final dry mass according to the formula:

$$\text{Lignin } \% = \frac{E \cdot d \cdot 100}{\varepsilon \cdot \text{final dry mass (g)}}$$

where ε = 21.8, coefficient of wheat dioxan lignin standard determined in our laboratory,
d - delusion
E - extinction.

Aldehydes, the products of alkaline nitrobenzene oxidation of lignin were determined according to EL-BASYOUNI et al. (1964) with our modifications.

After the extraction and hydrolysis, (see the preparation of samples for the determination of lignin p. 55), 0.23 ml of nitrobenzene and 4 ml of 2M NaOH were added to the cell wall material (0.1 g) in a stainless steel bomb (microautoclave) which was then kept at 160 °C for 2.5 h under continuous shaking in oil bath. After this, the bomb was plunged into an ice bath to cool, its contents were transferred to a separatory funnel and extracted by shaking with four 100-ml portions of ether. The extracts were discarded and the aqueous layer was acidified (pH 5.5) and continuously extracted with ether for 20 h. This ether extract contained phenolic aldehydes resulting from the oxidation of lignin. The phenolic aldehydes were separated using the benzene:acetic acid:water (10:7:3) solvent for the first direction and n-butanol saturated with 3 % NH_4OH (upper phase) in the second di-

rection in thinlayer chromatogram (Silofol UV_{254}).

The quantitative determination of aldehydes was carried out spectrophotometrically and their amount was calculated according to Lambert-Beer law (see p. 39). Extinctions were measured at 280 mm for vanillin and *p*-hydroxybenzaldehyde and at 305 nm for syringaldehyde.

Phenolic aldehydes were eluted from chromatograms with absolute ethanol.

OCH_3-content was determined with the iodine-metric method according to KREUTZBERG and GRABOVSKI (1962) with our modifications. The principle of this method lies in the separation of methoxyl groups from the substrate in the form of CH_3I after being influenced with HI. CH_3I is a vaporizing matter and it is caught with glacial acedic acid in Br. During the reaction lasting for an hour at 50 °C, iodine escapes and is titrated with $Na_2S_2O_3$. The reaction proceeded as follows:

$$ROCH_3 \rightarrow CH_3I \rightarrow IBr \rightarrow HIO_3 \rightarrow I_2 \rightarrow 6Na_2S_2O_3.$$

Accordingly, 1 g/mol $-OCH_3$ corresponds to 6 g/mol $Na_2S_2O_3$.

The content of OCH_3-groups was calculated in percentages:

$$-OCH_3\ \% = \frac{a \cdot n \cdot B \cdot 100}{A \cdot 6}$$

where a - 0.1M $Na_2S_2O_3$ ml for titration

n - normality of $Na_2S_2O_3$

B - molar mass

A - dry mass (mg).

Each of the abovementioned sets of experiments was repeated at least three times with different series of plants. n = 3, p = 0.05, $\Delta x\ \% = 1$ for the determination of lignin and OCH_3-groups and $\Delta x = 6$ for the determination of aldehydes.

The methods used for determining peroxidases are given on p. 70, for PAL - p. 86 and for protein - p. 70.

Results

Fig. 2 shows the analytical values for the lignin and $-OCH_3$ content of plants in the ontogeny and the effect of mineral nutrients on the abovementioned compounds.

The percentage of lignin is low in young plants but rises rapidly and obtains 11.8 %, i.e. 56 % of the maximum value, in the phase of stalking and then increases more slowly as the plant reaches the final stages of maturity.

The same tendency can be seen in the content of OCH_3-groups but only 29 % of methoxyl groups are formed

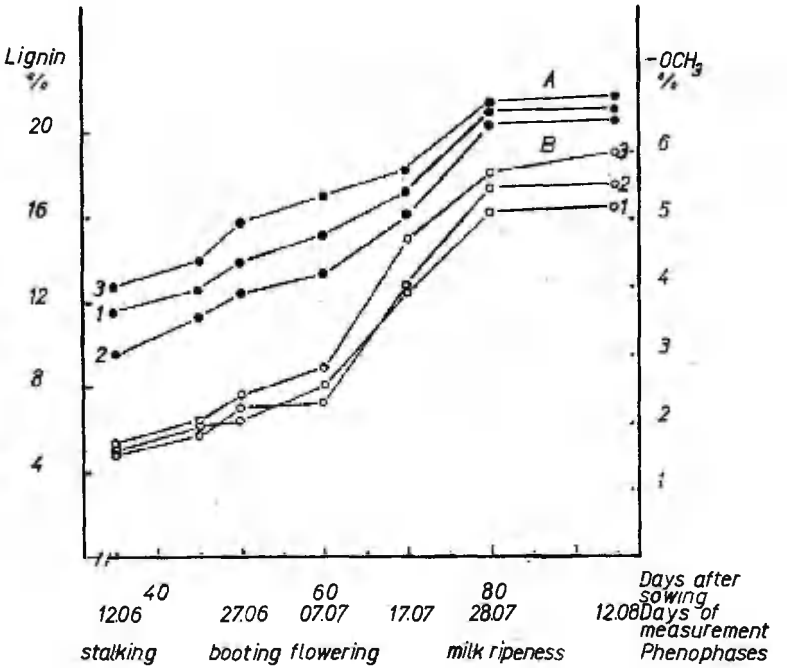


Fig. 2. The effect of mineral nutrients on the content of lignin (A) and $-OCH_3$ groups (B) (in dry mass)
1 - var. 1
2 - var. 2
3 - var. 3

in the phase of stalking. It means that the process of methylation increases at the end of maturity.

Table 1

The effect of mineral nutrients on the content of aldehydes (mg.g^{-1} d.m.) and -OCH_3 groups (% d.m.) of fully extracted wheat plants 1986 y.

Variants and phenophases	Dates of measurement	Vanillin	Syringaldehyde	Aldehydes	Ratio v/s	-OCH_3 groups
var. I						
stalking	12.06	7.19	5.83	13.02	1.23	1.54
booting	27.06	7.50	7.10	14.60	1.05	2.02
milk ripeness	28.07	8.24	9.89	18.13	0.83	5.15
var. II						
stalking	12.06	8.37	6.28	15.15	1.33	1.62
booting	27.06	8.91	8.30	17.21	1.07	2.28
milk ripeness	28.07	9.86	11.56	21.42	0.85	5.46
var. III						
stalking	12.06	9.12	9.06	18.12	1.00	1.64
booting	27.06	9.18	11.17	20.17	0.82	2.40
milk ripeness	28.07	9.26	13.40	22.66	0.68	6.69

Table 1 shows the results of alkaline nitrobenzene oxidation which was carried out not on the isolated lignin itself but on the plant material which had been extracted with alcohol-benzene. There are several noteworthy points in connection with fig. 2 and fig. 5. First, the percentage of vanillin and syringaldehyde increases rapidly in the stage of growth where lignification takes place, resembling the methoxyl content in this respect. The ratios of vanillin and syringaldehyde obtained by oxidation change with the growth of the plant. In the early stages, the order of abundance is as above, whereas in the maturity the order is reversed, syringaldehyde now being predominant. This may be taken to mean that there is, indeed, a basic difference in the nature of lignin at various growth stages, and the early predominance of aldehyde with one methoxyl group followed by the later predominance of a

methoxylated unit is qualitatively in agreement with the increase in the methoxyl content of lignin.

The difference in the ratio of the two aldehydes (vanillin and syringaldehyde) at various stages shows that there are two types - the guaiacyl and the syringyl types - of lignin, each giving rise to a certain percentage of vanillin and syringaldehyde upon oxidation.

When comparing the content of aldehydes in the fully extracted plants with that in the original, unextracted material (fig. 3), we can see that the extraction process removes a large percentage of supposedly non-lignin material and so the amount of aldehydes and methoxyl rises.

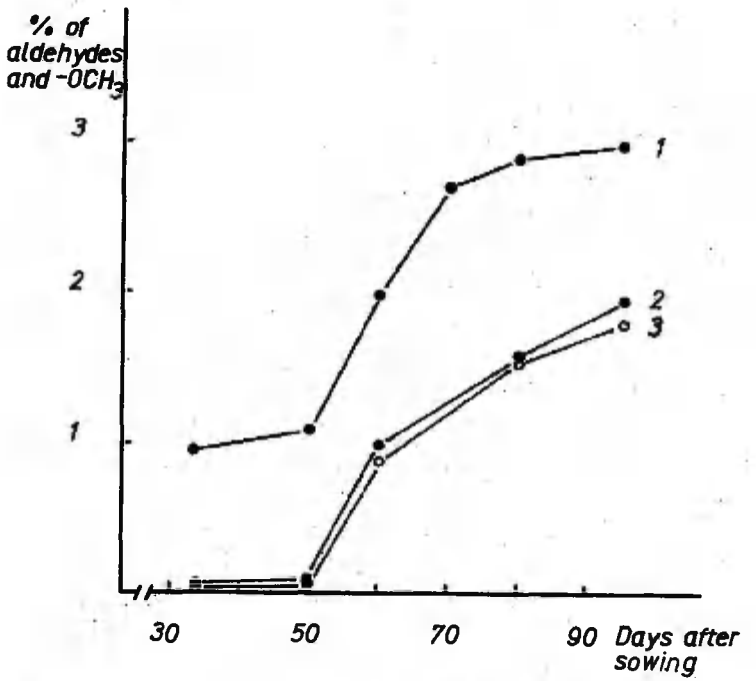


Fig. 3. Methoxyl content (% d.m.) and aldehyde yield (mg.g.$^{-1}$d.m.) of unextracted whole control plants
1 - methoxyl
2 - vanillin
3 - syringaldehyde

As regards the effect of mineral nutrients on the abovementioned compounds, we can see that elements N and K given together at different times and in different dozes have the greatest influence. At the end of vegetation period (28.07.), the content of $-OCH_3$ groups in N, K plants increased by 14 %, the content of lignin dry mass - by 4 % (fig. 2), the content of lignin (%.cm^{-1}) - by 29 % (fig. 4) and the total content of aldehydes - by 24 %, (fig. 2), when compared with control plants.

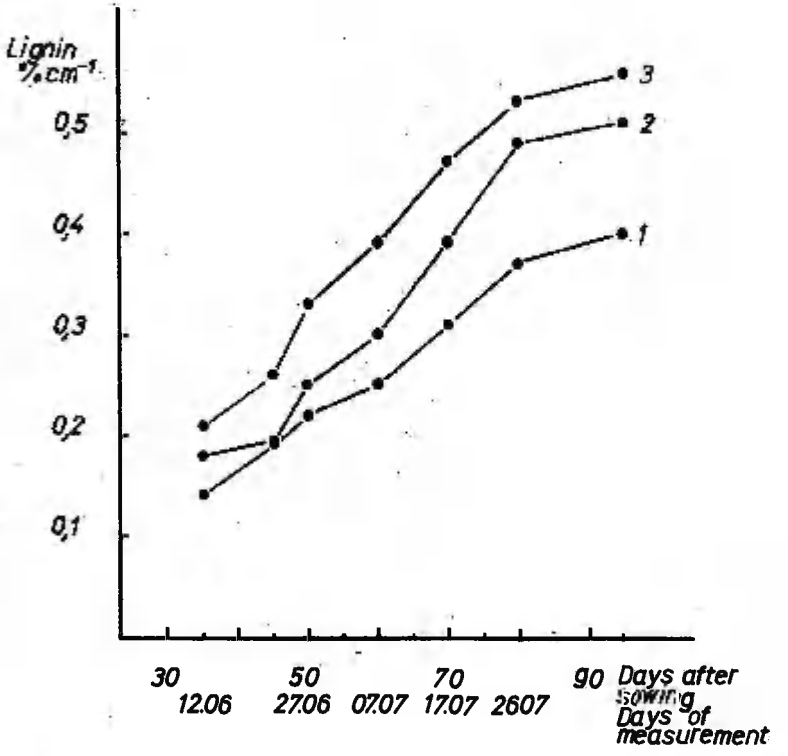


Fig. 4. The effect of mineral nutrients on the content of lignin (% cm^{-1})
1 - I var.
2 - II var.
3 - III var.

Changes in the structure of lignin and in the molar ratio of vanillin to syringaldehyde in milk ripeness are 0.68 in N, K plants (compare with control plants - 0.83, tab. 1). In N, K plants the number of $-OCH_3$ units is larger and the molar ratio is smaller than in control plants (tab. 1).

Fig. 5 shows the dynamics in the internode growth

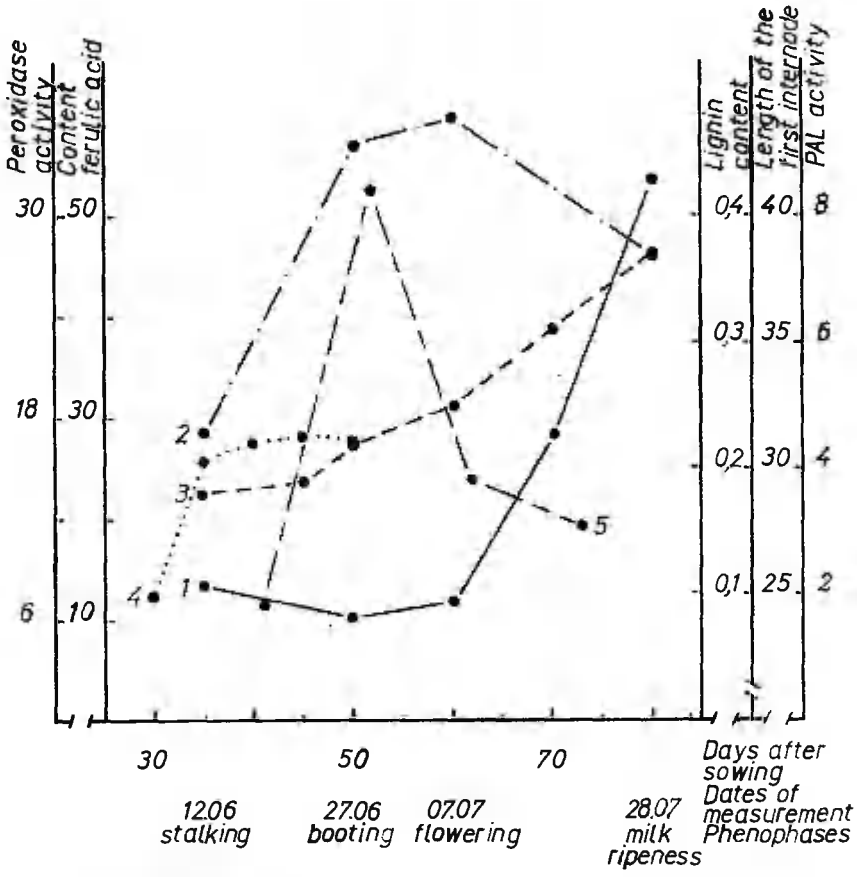


Fig. 5. The activities of enzymes and the polymerisation of lignin in control plants

1 - specific activity of ionically bound peroxidase (μ mol min^{-1} . mg^{-1} protein)
2 - content of ester-linked bound ferulic acid ($g.cm^{-1}$ internode)
3 - content of lignin (% cm^{-1} internode)
4 - growth dynamics of first internode (mm)
5 - activity of PAL-(cinnamic acid $g.g^{-1}f.m.$)

of control plants, the dynamics in the accumulated lignin and phenolic acid per 1 cm of stalk and the activities of peroxidases and phenylalanines ammonia lyase (PAL). After the cessation of the first internode growth (50th day, booting phase), the specific activity of ionically bound peroxidase begins to increase parallel to the dynamics of lignin biosynthesis. After this phase, the content of phenolic begins to decrease. The highest degree of the activity of ionically bound peroxidase is found at the end of full maturity. The curve of PAL activity is correlated with the curve of ester-linked bound ferulic acid.

Discussion

Fig. 5 shows that the content of ferulic acid as precursor of lignin increases in the first internode of wheat stalk till the cessation of growth and then begins to decrease. MILLER et al. (1985) suggested that the initial increase in phenolics was due to the synthesis of $C_6 - C_3$ lignin precursors, while the subsequent decrease in phenolics was due to their polymerization. FUKUDA and KOMAMINE (1982) failed to show a relationship between phenolic changes and lignification.

PAL activity peaked in the phase of stalking and declined in the period of flowering and grain-filling (fig. 5). It seems that there is a competition between the two metabolic pathways - proteins and phenolics (compare fig. 1 and fig. 5).

The initial rise in the production of phenolic acids is correlated with PAL activity. GUERRA et al. (1985) suggest that the high PAL activity is related to lignin synthesis, and cinnamic acid derivates can be oxidized by peroxidases.

The high activity of the terminal enzyme of lignification - peroxidase - is in an inverse correlation with the dynamics of the first internode growth and with the dynamics of lignin in it until the end of the cessation of the first internode growth. In this period, during the anthesis, the activity of peroxidases is the smallest and the plant goes from the phase of vegetative growth to the phase of generative growth. It seems as if there were two phases of lignification in wheat plants. In the vegetative phase (I phase), peroxidase cells predisposed to the lignification process *per se*, namely cells in the localized regions wherein lignification subsequently occurred, i.e. the cells which had become noticeably elongated, had a more intense peroxidase-staining reaction (phloroglucinol + HCl) than the neighbouring non-differentiating cells.

In the generative phase (II phase) when internodes (tissues) have ended their growth, the rapid

process of polymerization begins and the activity of peroxidases, especially the activity of ionically bound wall peroxidase, increases (fig. 5) and is correlated with the dynamics of lignin. The activity of some of the isoenzymes present enhances and some new ones appear (see p. 73). In this phase peroxidase generates H_2O_2 necessary for the oxidation and polymerization of cinnamyl alcohols. Our previous papers (MIIDLA et al., 1985, 1987 a, b, c) also show that activities of peroxidases are in close correlation with the lignification only in the final phase of the vegetation period and they show further that the wall peroxidase activity is high even in young tissues just undergoing lignification.

As regards the dynamics of lignin calculated in percentages of dry matter or unit of length, we can see that the content of lignin increases linearly with time course (fig. 2, 4). Our data are at variance with the results obtained by some other investigators such as PALEEV (1953) and LIASKOVSKY and KALININ (1973) who show a delignification of wheat stalks. It is difficult to understand the use of lignin during the period of vegetation. We can only suggest that it has something to do with carbohydrates in the nucleus of benzene, that they actually decrease, not lignin itself.

The high K supply resulted in an increased content of soluble amino acids in grain (MENGEL et al. 1981). This finding indicates that K probably promotes availability of amino acids for grain protein synthesis (see the previous article p. 29, tab. 3). Such an explanation is in good agreement with the favourable effect of K on phloem transport. K promotes the flow of carbohydrates and amino acids towards the grain. Phloem loading with amino acids is an energy dependent process which requires ATP. Since ATP synthesis is promoted by K, the favourable effect of K on phloem loading may result from the higher availability of ATP.

Increased sucrose concentration has been reported to stimulate the accumulation of phenolics. Carbohydrates are presumably required as precursor materials for phenolics biosynthesis and for the regulation of tracheary element differentation (PHILLIPS, HENSHAW, 1977).

The aromatic amino acid - phenylalanine - is a common precursor of both proteins and phenolics; it can either be incorporated directly into the growing polypeptide chain in protein synthesis or converted by the enzyme PAL into *trans*-cinnamic acid, a key intermediate in the phenylpropanoid metabolism. There will be a competition between the two metabolic pathways for the common precursor (MIIDLA, 1970). It is probable that the level of carbohydrates and the activity of PAL in K, N plants are high which leads to the rapid phenolic accumulation and so increases lodging resistance.

HOJATTI and MALCKI (1972) have reported that K

increases the methionine content of spring wheat during its vegetative growth. KREUTZBERG and GRABOVSKI (1962), giving labelled ^{14}C-methionine in the nutrient solution of oat, point out that methyl groups are carried from methionine to lignin. In their experiment they showed that more than 90 % of the radioactivity of lignin was in the carbon of the $-OCH_3$ group. So *L*-methionine may be a precursor in the formation of $-OCH_3$ groups catalyzed by methyltransferase in plant lignin. It means that K increases the donor of $-CH_3$ groups - S-adenosyl methionine. MILLER and ROBERTS (1982) showed that the addition of *L*-methionine to a xylogenic culture medium strongly stimulated tracheary element differentiation in explants of lettuce pith.

Our experiment shows that the optimum K supply with N given at different times (before sowing, at the beginning of tillering and stalking) is required not only for the maximum production of vegetative organs and cereal grains and for the formation of seed with high single grain mass (see p. 29, tab. 3) but the influence of K is expressed in the process of lignification and methylation (fig. 2). The latter process proceeds through the biosynthesis of the sulphur-containing amino acid methionine as a donor of $-CH_3$ groups.

In the process of methylation, a very important part is played by *O*-methyltransferase (OMT) catalyzing phenolic compounds in the presence of S-adenosyl-L-methionine which acts as a donor of methyl groups. So the activity of OMT is not an indicator of the intensity of the lignification process but, as NIKOLAEVA and ZAPROMIOTOV (1987) noted, its activity influences the structural quality of lignin. FUKUDA and KOMAMINE (1982) suggested that the OMT activity is related not to the lignin synthesis but to the synthesis of soluble phenolics.

Our data show that in N, K plants synthesized lignin contains a much higher proportion of syringyl than of guaiacyl units (tab. 1) when compared with plants of other varieties. The synthesized polymer has a different structure, i.e. different molar ratio of vanillin to syringaldehyde and different content of $-OCH_3$ groups.

The ratios of vanillin to syringaldehyde did not remain constant in plants of different ages and with a different level of mineral nutrients, the percentage of syringaldehyde being lower than that of vanillin in young and N plants and higher in more mature and N, K plants. This is qualitatively in agreement with an increase in the methoxyl content of lignin (MIIDLA, PADU 1987 a; MIIDLA et al. 1987 b; NIKOLAEVA, ZAPROMIOTOV 1987).

In our experiments mainly two oxidative nitrobenzene aldehydes - vanillin and syringaldehyde - were established because *p*-hydroxybenzaldehyde occurs only

in very small amounts, often as traces. GLAZENER (1982) points out that if proteins from wall-bound enzymes are still present in the alkaline insoluble cell wall material when it is subjected to alkaline nitrobenzene oxidation, *p*-hydroxybenzaldehyde will result as a degradation product of tyrosine. So it occurred to the present authors that the source of the *p*-hydroxybenzaldehyde obtained on alkaline nitrobenzene oxidation of wheat stalks might be not lignin but tyrosine associated with protein. Therefore, the oxidative nitrobenzene degradation is not specific enough for *p*-hydroxybenzaldehyde either to conclude that the degradation products originate from lignin alone.

To sum up the results we should like to point out that using different mineral elements we can influence phenylpropanoid metabolism which is connected with lignification on which the lodging resistance of cereals depends. The alterations in the ratios of nitrobenzene oxidation products and changes in the ionization difference spectrum were found to be of the greatest significance. The synthesized polymer influenced by mineral nutrients is shown to have a different structure, i.e. the ratio of vanillin to syringaldehyde is different. Considering all this we reached the conclusion that the smaller the ratio of vanillin to syringaldehyde is the more methylated the lignin contained in the plant and the more lodging resistant the stalk of the plant are. The quantitative content of lignin is not the only indicator of lodging resistance.

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THE ACTIVITY AND ISOENZYME PATTERNS OF CYTOPLASMIC AND WALL-BOUND PEROXIDASES IN WHEAT

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Peroxidase (EC 1.1.1.7), a wide-spread plant enzyme, is always found in a great number of isoforms. The existence of different molecular forms makes the control of metabolism possible. The isolation and characterization of separate isoperoxidases has repeatedly been attempted (ZMRHAL and MACHAČKOVA, 1978; KIM et al., 1980) but detailed knowledge of the *in vivo* functions of individual isoenzymes of peroxidase is still lacking. Different subcellular localizations of various isoenzymes together with their different catalytic properties point to differences in their functions.

The object of the present study was to observe changes in the activity and isoenzyme patterns of the peroxidase located in cytoplasm and bound to cell walls by ionic and covalent bonds in order to determine possible differences in the significance of different isoforms of peroxidase to the biosynthesis of lignin.

Materials and methods

Experiments were performed with the two cultivars ('Leningradka' and 'Arkas') of the spring wheat *Triticum aestivum* L. Plants were grown in the field as described above (p. 9). Experiments were carried out with control plants grown under the mineral nutrition conditions of $N_{60}P_{60}K_{60}$ kg ha^{-1} in 1985 and of $N_{50}P_{60}K_{60}$ kg ha^{-1} in 1986. For biochemical analyses two weighings (2 g each) per the average test of 30 plants were used. Enzyme activity for both the weighings was determined in three repetitions.

Preparation of enzyme extracts

The method of RIDGE and OSBORNE (1970) with some changes was used for separating differently bound forms of peroxidase. The material fixed with liquid nitrogen was homogenized in the mill. The soluble peroxidase was extracted from 2 g of the homogenized material of the phosphate buffer of Sörensen (pH 6.2) using glass filters with the sample size of 100. After four ex-

tractions, practically the whole soluble fraction was separated. The volume of the extract was brought up to 25 ml.

In order to separate the cell-membrane-bound peroxidase, the precipitate was twice (for 15 min each) treated with the solution of 2 % triton X-100. The mixture of the volume of triton X-100 extracts and twice extracted precipitate with phosphate buffer was brought up to 15 ml.

In order to separate the ionically bound peroxidase, the precipitate was twice (in the course of an hour) treated with 1M NaCl and then twice with the phosphate buffer (pH 6.2). The filtrates were joined together and the volume of the extract was brought up to 10 ml. Before the activity was determined, the extracts of both soluble and ionically bound peroxidases were dialysed for 24 h in relation to the phosphate buffer (pH 6.2).

In order to determine the activity of the covalently bound peroxidase, reaction mixture was added to the precipitate, homogenized, and, in a minute, the homogenate was filtrated through a glass filter, and, at once, the extinction of the reaction mixture was determined.

Determination of peroxidase activity

The activity was determined after Pütter (BERGMEYER, 1970) using some modifications. The reaction mixture (with the volume of 3 ml) contained 4.6 mM of guaiacol, 0.12 mM of H_2O_2, 0.1 ml of enzyme extract, the phosphate buffer of Sörensen (pH 6.2). The extinction of the reaction mixture was determined spectrophotometrically at 436 nm with the intervals of 15 sec from the moment H_2O_2 was added to the mixture. Enzyme activity was expressed as the amount of substrate micromoles per 1 g of fresh mass (total activity) or protein (specific activity) oxidized in a minute using the extinction coefficient of the reaction product - octadehydrotetraguaiacol - 25.5 cm^2M^{-1}.

When syringaldazine was used as a substrate upon the determination of peroxidase activity, the reaction mixture contained 0.018 mM of syringaldazine, 0.5 mM of H_2O_2, the phosphate buffer of Sörensen (ph 7.5), 0.1 ml of enzyme extract (GRISON and PILET, 1985). The change in extinction was determined at the wavelength of 530 nm in every 15 min beginning from the moment H_2O_2 was added to the mixture. Enzyme activity was expressed as the change in extinction per 1 g of fresh mass within a minute.

Protein content was determined using the method of Lowry (LOWRY et al., 1951) with some modifications (JAASKA, 1968).

To analyze the isoforms of peroxidase, the

vertical electrophoresis in polyacryl gel was used (DAVIS, 1964).

To develop peroxidase histochemically, Nadi reaction was used in the reaction mixture of 1 mM of *O*-dianisidine-dihydrochloride, 1 mM of pyrocatehol, 2.1 mM of H_2O_2 and in 0.2 M of acetate buffer (pH 4.0).

The content of hydrogen peroxide in plants was determined after MONDAL and CHOUDHUR (1981) using $TiSO_4$.

The content of lignin was determined according to Johnson et al. as described above (see MIIDLA et al., p. 55).

Results

The activity of different forms of peroxidases was determined in leaves and in the lower and upper internodes of stalks. In leaves, the activity of the soluble peroxidase turned out to be the highest - 87 - - 93 % of the total enzyme activity. The ionically bound cell-wall peroxidase constituted 5 - 6 % and the covalently bound one - 0.3 - 5.2 % of the total peroxidase activity (tab. 1). Stalks also contained more cytoplasmatic (soluble) peroxidase (62 - 83 % of the total amount of the enzyme) than any other peroxidase variety. Ionically bound peroxidase constituted 5-20 %, covalently bound one - 1.2 - 13 %, membrane-bound one 1.0 - 9 % from the total amount of the enzyme, thus cell-wall-bound peroxidase (ionic + covalent) constituted 10 - 29 % from the total amount of the enzyme (tab. 1). Cultivar dependent differences in the content of different peroxidase varieties were not observed in wheat stalks.

During the vegetative period, considerable changes in the total activity of peroxidase as well as in the content of its different varieties took place. These changes turned out to be both organ specific and of one type in 1985 and 1986. In leaves, an increase in the total peroxidase activity as well as in the soluble PO activity was observed, though in the phases of stalking and booting the activities were practically on a level. When plants were undergoing booting, the activity of ionically bound peroxidase in the leaves decreased. In the phase of stalking, the peroxidase activity in the lower internode of the stalk was high in the case of both the cultivars. When the plant was passing into the generative phase, the enzyme activity decreased sharply and was at its lowest in the phase of booting. Beginning with flowering, the peroxidase activity increased constantly up to the end of the vegetative period. Analogically to the lower internode, in the upper internode (formed by the phase of booting) enzyme activity increased constantly and reached its maximum value by the phase of dough ripeness (tab. 1).

Table 1

The activity of peroxidase in wheat plants during the ontogeny (total act. μmol g^{-1} f.m., specific act. μmol mg^{-1} protein)

Year Cv.	Organ	Phenophase	Days after sprouting	Σ act. of total PO	Total act of sol PO	Spec. act of sol. PO	Total act. of ionic PO	Spec. act of ionic PO	Total act of co-v. PO	Total act.of Trit.-s PO	Act. sol. PO (% of act.)	Act. bound PO (% of act.)	Act. ionic bound PO (% of act.)	Act. co-v. bound PO (% of act.)	Cons. sol. prot. (mg g^{-1} f.m.)	Cont. ionic bound prot. (mg g^{-1} f.m.
1985 'Leningradka'	Leaves	Tillering	20	151	130±0	7,1±1.0	9,3±2.3	58,1±4.0	7.9±0,8	3,6±1.8	86	11.2	6	5,2	17.0	0.16
		Stalking	34	264	240	10,0	16.0	72,7	0.8	7,2	91	6,3	6	0.3	24.0	0,22
		Booting	47	288	258	10.0	14 0	25.0	7.7	16.0	87	7.7	5	2,7	23,0	0,56
		Flowering	53	496	460	27,1	35,0	61,0	11,0	-	93	7.2	5	2,2	17.0	0,49
	I internode	Stalking	34	802	35±7	18,9±7	15,0±4	100,0±5	1.3±0,4	2,4±1,3	83	14,2	13	1,2	4.5±1.8	0,13±0.0
		Booting	47	27	25±2	9,1±2	5,3±1.2	53.0±5	1,7±0,1	0.4±0.1	74	26,3	20	6,3	2.2±0,2	0,10±0.0
		Flowering	53	42	35±1	14,9±4	1,9±1.0	21,1±13	2.0±0,2	2,9	83	9,8	5	4.8	2.4±0.2	0,09±0.1
		Dough ripen.	95	170	99	58,2	14,0±3	116,7±13	1.9±0.0	11.0±1.0	79	12,5	11	1,5	1.7	0,12±0.0
	IV internode	Booting	46	31	22	5,2	5,6	15,1	1,7	1,6	71	23,5	18	5,5	6.8	0,37±0.1
		Flowering	52	39	32±12	5,4±1.3	3,5±1.0	8,9	1,8±0.1	3,2	80	10.6	6	4,6	5,9±0.9	0,28
		Dough ripen.	94	71	57±3	71,3±9	9,0±0	120,0±1	1,7±0,1	6,8±0.2	79	11.4	9	2.4	0,8±0,1	0,05±0.0
1986 'Leningradka'	I internode	Tillering	19	151	113±4	2,8±0.0	77,4±1.3	2,0±0.4	4,6±0,3	6,0±0.4	75	21	18	3	40,9±1.8	5,6±0.0
		Stalking	30	97	67±7	4,0±0.4	13,3±0.7	8,2±0.5	3,3±0,2	3,6±0.0	77	19	15	4	10,6±0.4	1,1±0.0
		Booting	40	30	20±2	1,7±0,1	4,6±0.3	6,6±0.5	3,1±0,3	1,9±0.1	67	26	15	11	11,7±0.5	0,5±0.0
		Flowering	53	41	32±1	3,5±0,1	3,4±0.2	7,4±0.7	3,0±0,1	2,6±0.1	78	15	8	7	9,2±0,4	0,3±0.0
		Milk	67	59	44±2	4,3±0,1	7,0±0.4	17,1±0.1	3,1±0,1	5,2±0.3	75	17	12	5	10,1±0,7	0,3±0.0
		ripeness	74	86	65±0	3,7±0,1	9,7±0.2	32,6±1.2	4,3±0.2	6,6±0.2	76	14	11	5	8,4±0,2	0,2±0.0
	IV internode	Booting	40	24	13±2	0,7±0,1	3,4±0.1	2,0±0.1	2,8±0.2	1,7±0.0	62	22	16	13	18,0±0,1	1,1±0.0
		Flowering	53	25	18±0,8	0,9±0,0	1,5±0,1	2,8±0,2	2,1±0.0	1,3±0.0	78	16	7	9	19,7±0,7	0,4±0.0
		Milk	63	31	24±0,6	1,5±0,1	2,4±0.1	5,0±0,2	2,2±0,1	2,4±0,1	77	15	8	17	16,3±0,5	0 ,1±0.1
		ripeness	74	48	38±1	3,4±0,1	3,5±0.1	8,5±0.2	3,2±0,1	3,2±0,1	79	14	7	7	11,1±0,7	0,1±0.0
(Urkos)	I internode	Tillering	19	152	109±6	2,6±0,2	25,7±1,0	2,8±0,1	6,7±0,4	0,5±0,2	72	21	17	4	42,7±0,2	6,0±0,0
		Booting	40	33	23±3	1,8±0,1	6,0±0.1	8,9±0,5	2,9±0,1	2,5±0,1	67	27	18	9	12,3±0,1	0,3±0,0
		Milk ripen.	74	87	66±2	8,7±0,3	19,1±0,1	31,6±0,7	4,5±0,2	6,7±0,1	76	11	12	5	7,6±0,0	0,7±0,0
	IV internode	Booting	40	27	18±1	1,1±0,1	3,6±0,2	3,6±0,1	3,3±0,1	1,1±0,0	68	26	14	12	16,6±0,3	0,1±0,1
		Milk ripen.	74	50	39±4	4,3±0,0	9,0±0,1	8,1±0,2	3,2±0,0	3,3±0,1	78	16	10	6	9,0±0,2	0,1±0.0

The upper internode peroxidase activity was lower than the lower internode one at practically all the moments of analysis.

The activity dynamics of the ionically bound peroxidase generally coincided with the changes in the activity of the soluble peroxidase - the activity was high at the beginning and at the end of the vegetative period. But unlike the soluble peroxidase, the total activity of the ionically bound peroxidase was at its lowest in the phase of flowering, not in that of booting (tab. 1).

In stalks, the activity of the covalently bound peroxidase was stable and uniformly low (1.3 - 2.0 μM min g^{-1}) during the vegetative period of 1985. In 1986, its activity was somewhat higher (2.4 - 4.3 μM min g^{-1}) but likewise stable in all the phenophases observed.

The relative content of bound peroxidases (in most cases exceeding 20 % from the total amount of peroxidases) in stalks was high in the first stages of vegetation - in the phases of sprouting, stalking and booting, evidently in connection with the high activity of the ionically bound peroxidase during this period. The relative content of the covalently bound peroxidase was at its highest in the phase of booting.

Experiments were carried out to study the isoforms of peroxidases of upper and lower internodes of stalks in the cultivars 'Leningradka' and 'Arkas'. The content of molecular forms in both the cultivars turned out to be identical, organ specific and underwent changes during the vegetative period (fig. 1).

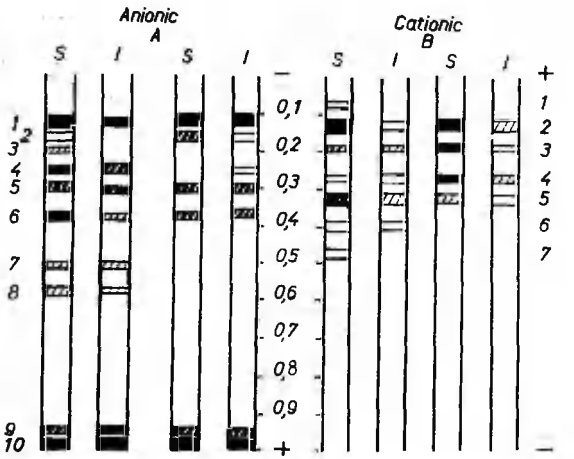


Fig. 1. Isoperoxidase patterns in wheat stalk (in increasing order of activity - blanck, narrow striped, wide striped, black).
A - first internode; B - fourth internode;
S - soluble isoperoxidases; I - ionic bound peroxidases.

In the lower internode 10 and in the upper internode 6 anodic isoforms of soluble peroxidase were found. In the lower internode, the highest activity was characteristic of slow isoforms 1, 4, 5, 6, and of rapid isoforms 9 and 10. During the period of vegetation, the activity of isoforms 1, 4, 5 decreased. Isoform 2 was distinguishable from the generative phase (booting, flowering) till the end of the vegetative period. Isoforms 7 and 8 were formed in the phases of milk and dough ripeness.

Peculiar to the isoenzymic content of the soluble PO of the upper internode was the continuous existence of isoform 2 and the absence of isoforms 3, 4, 7, 8 (fig. 1). During the vegetative period, the activity of isoforms, 1, 2 and 5 decreased while that of isoform 6 increased.

Isoforms lacking in the soluble peroxidase were also absent from the ionically bound peroxidase. Isoforms 2 and 3, being of low activity in the soluble fraction of the lower internode, were not found in the ionically bound PO. In the upper internode, isoenzyme 2 had continuously high activity and it was also found in the fractions of the ionically bound PO. It is of some interest that isoform 4, lacking from the soluble fraction of the upper internode, was present in the bound fractions of both varieties but its activity decreased as the plants grew older while the activity of isoform 6 increased. In the lower internode, there was a decrease in the activity of isoforms 1 and 4 (which disappeared when plants reached the phase of ripeness) and also of isoform 5 of the ionically bound isoperoxidases. In the phase of dough ripeness, ionic isoforms 6, 7 and 8 were activated, isoforms 7 and 8 only in the lower internode.

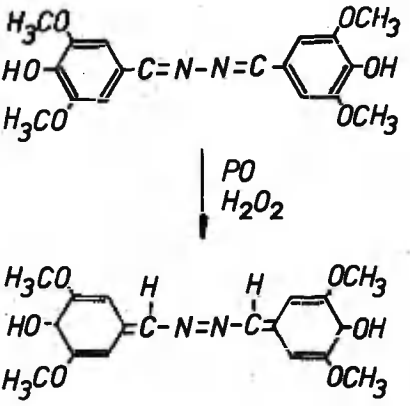
4 active cationic isoforms of peroxidase were found in both - the lower (isoforms 2, 3, 5, 6) and the upper (isoforms 2, 3, 4, 5) internodes. In the first stages of ontogeny (sprouting, stalking) as well as at the end of it (in the phases of flowering and ripeness), rapid isoform 7 was sometimes found in the lower internode, too. All the more active cationic isoforms of the soluble PO were also represented in the ionic fraction.

During the ontogeny, the weakening of soluble isoforms 2 and 3 could be observed in both the upper and the lower internodes, isoforms 6 and 5 reached their maximal activity in the phases of flowering and dough ripeness. The cationic isoform complex as a whole had its highest activity in the soluble fraction in the phase of flowering while the ionically bound isoform complex had a high activity in the phase of stalking, too.

Experiments were also carried out to study the substrate specificity of wheat peroxidase with two substrates - guaiacol (a widely used not a natural

substrate for determining peroxidase activity) and syringaldazine (a substrate resembling in its chemical structure the precursors of peroxidatic oxidation of lignin).

The formula of syringaldazine:



When guaiacol was used as a substrate, acidic PO zones 1 and 4 appeared in foreogrammes. We did not succeed in colouring histochemically peroxidase isoforms with syringaldazine.

Table 2

Activity of cytoplasmic and cell wall bound peroxidase (E min^{-1}, g^{-1} fresh mass)* with different substrates

Phenophase	The activity of peroxidases					
	Guaiacol			Syringaldazine		
	Cyto-plasmic	Ionic	Cova-lent	Cyto-plasmic	Ionic	Covalent
Stalking	0,30	0,30	0,30	0,05	0,09	0,04
Booting	0,20	0,20	0,20	0,04	0,06	0,04

* Activity of all forms of peroxidase towards guaiacol is reduced to similar activity.

Table 2 gives the results of the two experiments in which the activity of soluble and bound peroxidases was determined from the lower internodes of plants in the phases of stalking and booting using two substrates. Before the enzyme activity was determined using syringaldazine, the activity of soluble, ionically and covalently bound peroxidase extracts had been brought to

the same level in relation to guaiacol. According to the results obtained, ionically bound peroxidase had a higher activity with syringaldazine than the peroxidase located in cytoplasm did.. The covalently bound peroxidase was not found to have a higher activity in relation to syringaldazine than the soluble peroxidase.

It is also worth mentioning that by the end of flowering, when the peroxidase activity began to increase, the intensity of plant growth decreased and reached a plateau (fig. 2).

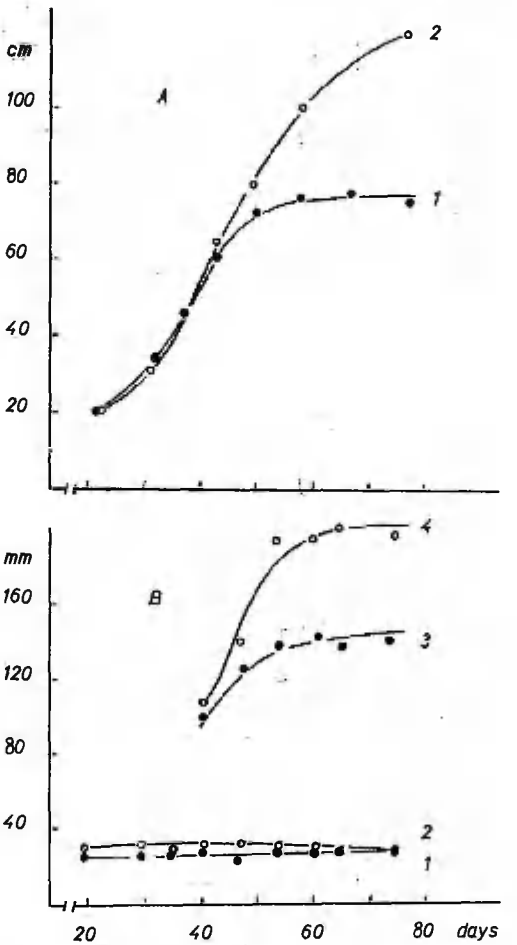


Fig. 2. The growth of wheat plants
A - total length 1985; B - length of first internode (1, 2) and fourth internode (3, 4) 1986.
1, 3 - 'Arkas'; 2, 4 - 'Leningradka'

During the generative phase, plants grew mainly on account of the lengthening of the fourth internode and of the underhead stalk. In both the lower and the upper internodes, the activity of the ionically bound PO was found to be at its minimum in the phase of flowering.

Discussion

According to the literature consulted, plant peroxidase is mainly located in cytoplasm in its soluble form but it is also found in almost all the organelles, in vacuole and in cell walls in its bound form (GASPAR et al., 1982). The present study has confirmed that in the wheat cultivars 'Leningradka' and 'Arkas', the soluble form accounts for the majority of the summaric peroxidase activity in both leaves and stalks but in stalks the bound forms play a greater part than in leaves (10-30 % and 5-11 % respectively). The peroxidase located in organelles accounts for 1-9 % of the summaric enzyme activity. According to the literature consulted, the ratio of the activity of free to bound forms differs in different plant varieties and in different tissues and organs. In carrot roots and tobacco pith practically all the peroxidase is cell-wall bound (BIRECKA and MILLER, 1974). There are few data available concerning monocotyledons, only the composition of differently bound peroxidase forms in leaves has been practically studied. For instance, in maize leaves, the bound forms of peroxidase account for 20 % (BIRECKA and CATALFAMO, 1975) and in *Phalaris arundinaceae* L. - for 30 % (VANCE et al., 1976) of the total enzyme activity.

Our studies have shown that in wheat the activity of the ionically bound peroxidase (5-20 % of the total activity) is higher than that of the covalently bound peroxidase (1-13 % of the total activity). According to the data published, this ratio is also subject to wide-range variations depending on the plant variety, tissue and organ. In horse-radish *(Armoracia rusticana* L.) roots practically all the peroxidase is ionically bound to cell walls (LIU and LAMPORT, 1974) but in pea roots the activities of ionically and covalently bound forms are practically equal (FIELDING and HALL, 1978).

It should be pointed out that our studies have established that in laboratory conditions when plants were grown in the phytotron (lighting 24 W m^{-2}, t° - 22 °C and 8 h of darkness with t° 15 °C), the activity of the covalently bound peroxidase in stalks was higher than that of the ionically bound peroxidase. Differences between the results obtained in the laboratory and those obtained in the field may have resulted not only from differences in the conditions of growth but also from the fact that from the plants grown in the

phytotron the covalently bound peroxidase was separated enzymatically, using cellulase (Onozuka) 3 mg ml^{-1} and pectinase (Fluka) 17 mg ml^{-1}. The determination of the activity of the covalently bound peroxidase by reducing it to its free form using cellulolytic and pectinolytic enzymes yields heightened results evidently for the reason that the enzymatic reaction carried out directly in cell walls prevents the substrate molecules from reaching all the enzyme molecules (enzyme activity depends on the degree of fineness of the material).

In addition to the variety and organ specificity of the activity and relative amounts of different peroxidase forms, the activity, relative content and isoenzymes of all the peroxidase forms varied during the ontogeny depending on the phase of development. In the lower internode, the activity of soluble and bound peroxidase was at its highest in the phase of stalking when the majority of the possible amount of lignin in stalk was also formed (in the field experiments of 1985 - 59 % and of 1986 - 56 % of the maximal possible amount of lignin - tab. 3). In the initial period of

Table 3

The content of lignin in wheat stalk (cv. 'Leningradka') during the period of vegetation

Organ	Phenophase	Lignin content			
		1985		1986	
		% of dry mass	% of lignin from dough-ripeness	% of dry mass	% of lignin from milk ripeness
First internode	Stalking	12.1	59	11.8	56
	Booting	17.2	84	14.0	67
	Flowering	17.7	86	-	-
	Milk ripeness	-	-	21.0	100
	Dough-ripeness	20.4	100	-	-
Fourth internode	Booting	16.0	96	-	-
	Flowering	16.5	99.3	-	-
	Dough-ripeness	16.6	100	-	-

the ontogeny, the highest content of bound forms also decreased (tab. 1).

According to WEISSENBÖCK (1976), in the differentiating xylem of araucaria, the biosynthesis of

lignin begins in the period of high peroxidase activity and the decrease in the intensity of lignin biosynthesis is accompanied by the decrease in peroxidase activity.

During the period of intensive lignin synthesis - in the phase of stalking-, the specific activity of ionically bound peroxidase in the lower internode (where a greater amount of lignin is synthesized) is higher than that in the upper internode.

Our experiments performed to study the substrate specificity of soluble and bound peroxidases, have shown that the peroxidase ionically bound to cell walls and catalyzing the last stage in the biosynthesis of lignin in cell walls has a higher activity towards syringaldazine than the soluble peroxidase (tab. 2). On analysing the data presented in table 2, we came to the conclusion that the covalently bound peroxidase does not participate in the biosynthesis of lignin as effectively as the ionically bound form of the enzyme does. This may be due to the fact that, as has been shown in literature, the ionically bound peroxidase is located in the middle lamellae where the process of lignification begins, the covalently bound peroxidase, on the other hand, is bound to pectin and hemicellulose (FIELDING and HALL, 1978).

Differences in the quantitative composition between the ionic and soluble forms of peroxidase may account for the fact that the ionic form is more active towards syringaldazine than the soluble one. In the fraction of bound peroxidase, there were no isoenzymes not present in the composition of soluble peroxidase but the anodic isoformas 2 and 3, present in the soluble fraction, were missing. In the lower internode, the ionically bound peroxidase was represented by the largest number of most active isoforms at the beginning of ontogeny - in the phases of sprouting and stalking - when the high-activity slow anodic isoforms 1, 4, 5 and the rapidly moving isoforms 9, 10 were bound to cell walls. All the cationic isoforms characteristic of the soluble peroxidase were also present in cell walls in anodically bound forms but their activity differed greatly during the two vegetative periods - in 1985, the ionic cationic peroxidase proteins had a very low activity throughout the vegetative period but in 1986, cationic isoforms had the maximal activity in the phases of stalking and dough ripeness.

The activity of slow anodic isoforms decreased when plants were passing from stalking to the generative phase, isoform 5 had disappeared by the phase of flowering in the summers of both 1985 and 1986, isoform 4 - in the summer of 1985 (in 1986 it weakened greatly).

The results of our experiments have shown that there is a correlation between the intensity of the biosynthesis of lignin and the activity of the slow

anodic isoforms of peroxidase.

Quite a number of researchers have established differences in the substrate specificity of different peroxidase isoforms but there are few data concerning the metabolism of phenolic compounds and especially the biosynthesis of lignin. Anodic isoperoxidases, bound to the cell walls of the callus tissue of tobacco, show a greater affinity for coniferyl alcohol in the formation of dehydrogenisation polymer than soluble peroxidases (MÄDER et al., 1977). The cell wall bound peroxidase A1 is supposed to participate in the biosynthesis of lignin for the reason that it is located in the sklerenhyme of xylem (BARTOŠOVA et al., 1982).

There is a correlation between the intensity of the biosynthesis of lignin and the ability to oxidize syringaldazine (KEVERS and GASPAR, 1985). GOLBERG together with his colleagues (1981) has shown that the oxidation of syringaldazine occurs only in the tissues where the biosynthesis of lignin takes place. In addition, it has been shown that in the cell culture of tobacco only the acidic forms of peroxidase are secreted into cell walls through the medium of the Golgi complex (MÄDER and WALTER, 1986).

A result of ours deserving special notice is the fact that the activity of the soluble as well as the bound peroxidase is at its lowest in both the lower and the upper internodes in the phases of booting and flowering. As to the physiological significance of the low peroxidase activity in the generative phase, it has been suggested that there is a connection between the decrease in the enzyme activity and the necessary increase in the concentration of indole-3-acetic acid when passing into the phase of flowering (GASPAR et al., 1985). Our experiments have shown that in wheat the ability to oxidize auxin is mainly exhibited by the cationic isoforms of peroxidase (PADU et al., 1983). During the vegetative period, the alkaline isoforms 2 and 3 were those that weakened noticeably in the soluble fraction of both the lower and the upper internodes, the activity of other isoforms remained relatively stable and might even increase during the flowering. In case of ionically bound cationic isoforms (whose participation in the regulation of the content of endogenous IAA is, however, not very probable), the whole complex reached its maximal activity in the phase of stalking, isoform 5 in the lower internode - in the phase of dough ripeness, too.

The high activity of peroxidase at the end of ontogeny in the phase of dough ripeness when stalks were turning yellow, had lost their leaves and the content of dry matter reached 36 %, was to some extent unexpected for us. An increase (though not so marked) in the peroxidase activity has also been observed in winter wheat (BARTOŠOVA et al., 1982). Since the content of both bound and soluble proteins in wheat stalks

was continuously decreasing during the period of vegetation, an increase in the total and specific activity of peroxidase probably shows a considerable increase in the content of peroxidase protein in the total content of protein. An increase in the peroxidase activity has been regarded as an indicator of plant aging (BIRECKA et al., 1979; MONDAL, CHOUDHURI, 1981; MIIDLA et al., 1987). Physiologically, an increase in the enzyme activity may be of significance for the final formation of cell wall structure. It is quite well known that peroxidase participates in the formation of bonds between the residues of ferulic acid located in cell walls (FRY, 1979) and in the binding of phenolic compounds to the saccharidic and proteinaceous components of cell walls (WHITMORE, 1976, 1978). Since the activity of catalase is decreasing, peroxidase is also necessary for decomposing the hydrogen peroxide accumulating in aging plants (BIRECKA et al., 1979). Our studies of wheat plants have also shown an increase in the content of hydrogen peroxide during the ontogeny. 15-day-old plants contain 2.2 μmoles of hydrogen peroxide per 1 g of dry mass of leaves, the leaves of 25--day-old plants - 5,4 μmoles. It should be mentioned that by the phase of dough ripeness, two new acidic isoforms (7 and 8) have been formed in the soluble fraction of peroxidase in the lower internode of both cultivars (and also in the upper internode of 'Arkas'). They may supply another reason (in addition to the activation of old isoforms) for an increase in the PO activity in the phase of dough ripeness, and indicate a change or an improvement in the function of peroxidase in old plants.

An increase in the peroxidase activity of aging plants coincides with the cessation of growth. A number of researchers have observed an inverse relation of peroxidase to growth (EVANS, ALLDRIDGE, 1965; CHIBBAR et al., 1989).

Thus, we may conclude that the different phases in the development of wheat plants are characterized by the different activity, different relative content and different isoenzyme composition of free and bound peroxidases. The high activity of slow isoforms, supposedly catalyzing the oxidation of cinnamoyl alcohols by the biosynthesis of lignin, is typical of the initial stages of wheat stalk differentiation. In the generative phase, the peroxidase activity is low with, the cationic isoforms of auxin oxidase activity having the low activity. In the phase of ripening, the activity of both the bound and the soluble peroxidase increases, probably guaranteeing the final formation of cell wall structure and enabling to decompose the hydrogen peroxide accumulating in aging plants. In the plants which have reached the phase of ripeness, two new acidic isoforms of peroxidase are formed. Consequently, the last stages of cell wall formation and of

the biosynthesis of lignin are also characterized by the regulation on the level of peroxidase isoforms, which has already been demonstrated in case of other enzymes of secondary metabolism (EBEL et al., 1974; HAHLBROCK and GRISEBACH, 1979; GROSS, 1979).

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THE ACTIVITY OF *L*-PHENYLALANINE AMMONIA-LYASE AND PEROXIDASE, AND THE BIOSYNTHESIS OF PHENOLIC COMPOUNDS IN WHEAT UNDER DIFFERENT CONDITION OF MINERAL NUTRITION

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Introduction

After a long period of intensive research, the main outlines of the composition and structure of lignin have been elucidated. Plant materials exhibit a great heterogenity in the concentration and composition of lignins. Taxonomic differences in the content of lignin in angiosperms, gymnosperms and grasses are widely known (WEISSENBÖCK, 1977). In mature wood of angiosperms and gymnosperms the lignin concentrations vary from 15 to 36 % of the dry mass (SARKANEN and HERGERT, 1971), during the development of plants the amounts of lignin increase and the polymer becomes richer in siringyl units (GRAND and RANJEVA, 1979). It is also well known that lignin is formed on contamination by pathogenes (VANCE and SHERWOOD, 1976). Variations in the amount and content of lignin in nature indicate that artificial influencing of the biosynthesis of lignin is also possible, for that purpose treatment with growth substances(BRYANT, 1976) and herbicides (ENGELSMA, 1973) has been used. The possibility of influencing secondary metabolism by changing the mineral nutrition of plants has been relatively little investigated although it would be one of the more easily practicable ways of directing the biosynthesis of lignin and other phenolic compounds (HAHLBROCK, 1975; PHILLIPS and HENSHAW, 1977; MIIDLA et al., 1970).

The aim of the present study was to elucidate the effect of the heightened amounts and different administering times of potassium and nitrogen fertilizers on *L*-phenylalanine ammonia-lyase (PAL, EC 4.3.1.5) activity, the latter being the first enzyme in the pathway leading to phenylpropanoid biosynthesis. In addition, the influence of mineral nutrition on the peroxidase (PO - EC 1.1.1.7) activity was also investigated (this enzyme catalyzes the last phase in the biosynthesis of lignin - the dehydrogenative polymerization of cinnamyl alcohols in cell walls) to substantiate biochemically, by changes in the properities of enzymes, changes in secondary metabolism, especially in the biosynthesis

of lignin in plants grown under different conditions of mineral nutrition.

Material and methods

Field experiments were performed with spring wheat *Triticum aestivum* L. cv-s 'Leningradka' and 'Arkas', laboratory experiments - with the sprouts of cv 'Leningradka'.

The conditions of growth of plants

In the laboratory experiments, seeds soaked in water germinated for 24 h at room temperature were in 20ies planted into pots filled with soil, and placed into a phytotron. Knop's nutrient solution served as a nutrient mixture. It was poured on the convettes into which the pots with sprouts had been placed. Plants were grown under long-day conditions of 16 h of light of 93 W m^{-2} at the temperature of 22 °C, and of 8 h of darkness at the temperature of 18 °C.

In the field experiments, plants were grown in plots of 3 x 10 m in four repetitions in the field of Estonian Agricultural Academy. In 1984, the seeds were sown on May 8 and they sprouted on May 15. They were grown in two different soil conditions of nitrogen content - 50 kg N ha^{-1} and 150 kg N ha^{-1}. In the spring of 1987, the seeds were also sown on May 8 but they sprouted a day later - on May 16. The plants of cv 'Leningradka' were grown in three different conditions of mineral nutrition (look materials and general methods p. 9).

The plants of cv 'Arkas' were grown under the conditions identical to those of the control plants (Var. 1) of cv 'Leningradka'.

The determination of the *L*-phenylalanine ammonia-lyase activity

The fresh material (0.5 g) fixed in liquid nitrogen was homogenized in 5 ml 0.05 M Tris-HCl buffer (pH 8.7). The homogenate was centrifuged at the temperature of 4 °C for 15 min. at the rate of 12.000 g min^{-1}. The supernatant was passed through the Sephadex G-25 (medium) column with 0.05 M Tris-HCl buffer (pH 3.7) to remove phenolic compounds. The PAL activity was determined in combined protein fractions. The formation of cinnamic acid from *L*-phenylalanine was measured spectrophotometrically (ZUCKER, 1965). The reaction mixture used contained 5.0 ml of 0.05 M Tris-HCl buffer (pH 8.7), 1.0 ml of enzyme extract and 0.2 ml of 0.1 M *L*- phenylalanine. The amount of the cinnamic acid formed was measured at 290 nm at the temperature of 37 °C at 30-minute intervals in the course of 90 minutes. Under those conditions, the PAL

activity was linear in time for at least two hours. The control mixture contained, instead of the enzyme extract, the same amount of buffer. The activity of PAL was expressed in the amount of µg cinnamic acid formed in 1 min per 1 g of fresh mass (in tables - PAL total) or in the amount of µg cinnamic acid per 1 mg of protein (PAL specific).

The same method was adapted for the assay of tyrosine ammonia-lyase, with controlled pH and temperature - the absorbance of both tyrosine and *p*-coumarate is affected by a temperature-sensitive phenolic ionization. On determining the TAL activity, the reaction mixture contained 4.9 ml of 0.05 M Tris-HCl buffer (pH 8.7), 1.0 ml of enzyme extract and 0.3 ml of the saturated solution of tyrosine (Reanal) in 0.05 M Tris-HCl buffer (pH 8.7). The TAL activity was assayed by measuring at 308 nm the amount of µg *p*-coumarate formed per 1 gram of fresh mass or per 1 mg of protein. The activity of peroxidase was determined as described elsewhere in the present collection of papers (p. 70).

The determination of the content of soluble phenolic compounds

In the laboratory experiments, 2 g of fresh material was homogenized with 10 ml of 96 % ethanol, then extracted for 30 min and centrifuged for 15 min at the rate of 8.000 g min^{-1}. The precipitate was, in addition, twice extracted with 5 ml of 96 % ethanol. Supernatants were gathered and the extract of phenolic compounds was brought up to 25 ml by adding 96 % ethanol to it.

In the field experiments, phenolic compounds were extracted from dry material. Plants were fixed in a thermostat at the temperature of 105 °C for 2 hours and then at 60 °C for 24 hours. 0.5 g of dry material was three times (for 2 hours each) extracted with 20 ml of 85 % ethanol in a flask with a vertical cooler over the boiling water-bath. The extracts were combined and brought up to 50 ml by adding 85 % ethanol to them.

In the extracts obtained, the content of phenolic compounds was determined with the Folin-Denise reagent as equivalents of ferulic acid in milligrams per 1 g of fresh or dry mass. The content of ethanol-soluble phenolic compounds was determined from two weighings, both in three repetitions, the content of bound phenolic compounds was determined from one weighing in two repetitions. The error in determining the content of bound phenolic compounds did not exceed 8.5 %.

The content of lignin was determined in dry material by the method of Johnson as described above (MIIDLA et al., p. 55).

The content of protein, was determined after LOWRY et al., 1951. The protein was precipitated with 5 % trichloroacetic acid.

Results

The properties of *L*-phenylalanine ammonia-lyase of wheat

The optimum activity of the enzyme in wheat leaves at the temperature of 37 °C in the Tris-HCl buffer was found under the conditions of pH 8.7 (fig. 1)

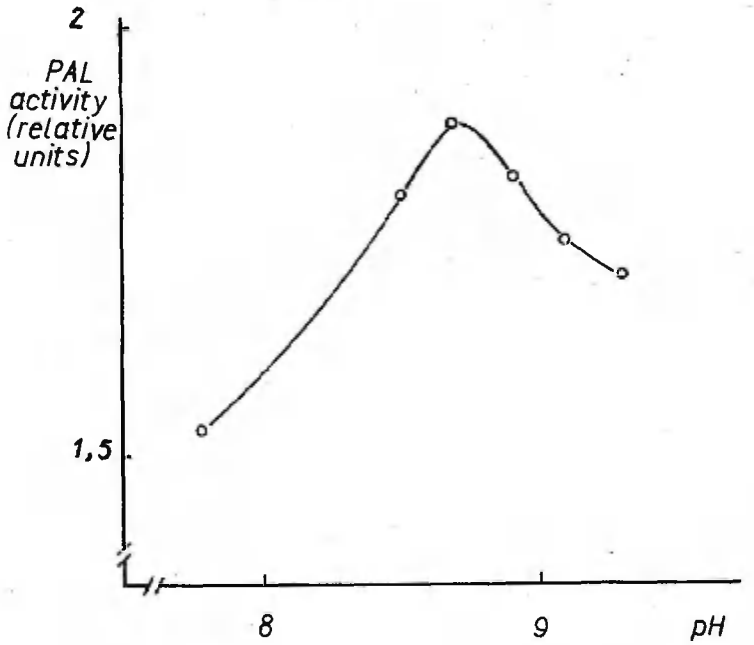

Fig. 1. The connection of the activity of PAL with pH

at the substrate concentration of 2 mM (1.5 - 2.5 mM) (fig. 2). The activity of tyrosine ammonia-lyase (TAL)

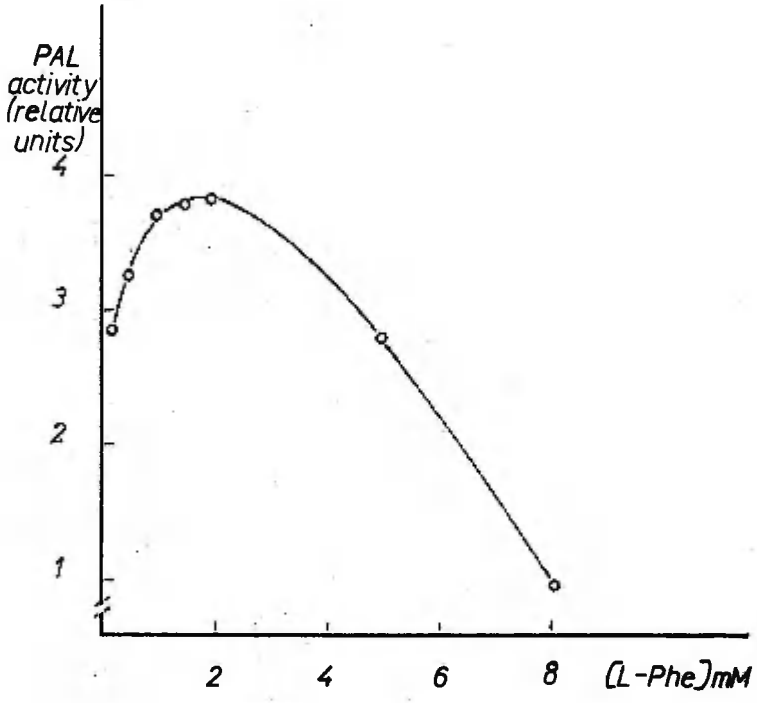

Fig. 2. The connection of the activity of PAL with the concentration of *L*-phenylalanine

in wheat was also found. The ratio of the activity of PAL to that of TAL varied to a large extent - from 5.0 to 12.8 (tab. 1) - in the leaves of the plants of different age and grown under different conditions of mineral nutrients, but in all the plants analyzed the activity of PAL was considerably higher than that of TAL.

Table 1

The total activity of PAL and TAL in the leaves of wheat ($\mu g.min^{-1}g^{-1}f.m.$)

Variant	Days after germinating	PAL	TAL	PAL/TAL
Var. I	14	2.14	0.32	6.7
Var. II	14	2.15	0.29	7.4
Var. I	20	2.94	0.23	12.8
Var. II	20	2.66	0.51	5.2

Var. I - plants grown in the Knop solution
Var. II - plants grown in the Knop solution with a twice higher content of N

The activity of PAL and the content of phenolic compounds during the germination of wheat

In ungerminated seeds, the activity of PAL was practically nonexistent but in the seeds which had germinated for 24 h, the activity of the enzyme was quite noticeable, and after five days of germination, the enzyme was observed in the first leaves of the plants (tab. 2). When the growth of leaves ended, the activity of the enzyme decreased. The content of ethanol-

Table 2

The activity of PAL (total-$\mu g.min^{-1}g^{-1}$ f.m; specific-$\mu g.min^{-1}mg^{-1}$ protein), content of ethanol soluble phenolic compounds ($mg.g^{-1}$ f.m.) and content of protein ($mg\ g^{-1}$ f.m.) in case of wheat germination*

Days after germinating	PAL		Phenols content	Protein content	Dry mass 1st leaves
	Total act.	Spec. act.			
0	0.02	-	1.66	-	
1	1.75	-	1.72	-	
5	8.79±0.13	2.00	2.81±0.09	4.39	5.9
10	10.20±0.16	2.97	1.44±0.03	3.43	7.6
15	7.34±0.82	4.95	1.25±0.01	1.48	7.5
20	1.50±0.05	1.06	1.06±0.02	1.42	7.2

* Beginning with the 5th day of growth, all the analyses were made from the 1st leave, before that, the embryo as a whole was used for the experimental material. The average from 30 leaves was used for determining the mass of the leaves.

soluble phenolic compounds at first increased during the germination of wheat reaching its maximum on the 5th day of germination, and, thereafter, decreased continually for the whole 20-day period investigated.

The ontogenetic variability of the activity of PAL in wheat

The activity of the enzyme was analyzed in the upper (fourth) and lower (first) internodes of the stalk and in leaves of wheat at different stages of plant development.

In leaves, the activity of PAL decreased from the phase of sprouting up to the phase of booting and then remained practically stable throughout the phase of flowering (tab. 3). The activity of PAL in leaves was lower than in stalks in all the phenophases.

According to the data obtained during the vegetation period of 1984, the total activity of PAL in both the upper and the lower internodes decreased continually but the specific activity of the enzyme in the lower internode was maximal in the phase of booting and decreased then until the plant reached dough ripeness. In the upper internode, both the total and the specific activities of PAL decreased continually together with the increase in the age of the plant. In the upper internode, the activity of PAL was higher than in the lower internode (tab. 3).

According to the data of the year 1987, both the total and the specific activities of PAL in the lower internode of the stalk increased beginning with the phase of sprouting, reached their maxima at the end of the phase of stalking after which the activity of the enzyme decreased until the end of the vegetation period. In the fourth internode, both the total and the specific activities of PAL were considerably lower in the phase of flowering than in the phase of booting. In the fourth internode, the total activity of PAL was noticeably higher than in the first internode. All the above-mentioned regularities hold good in case of cv 'Leningradka' (Tab. 4) as well as cv 'Arkas' (tab. 5). In case of the more lodging-resistant cultivar 'Arkas', the total and the specific activities of PAL in the lower internode were higher from the end of the phase of stalking than in cv 'Leningradka'. In the upper internode, reversely, the activity of PAL was higher in cv 'Leningradka' than in cv 'Arkas'.

Table 4

The activity of PAL (total - μg min^{-1} g^{-1} f.m.; specific - μg min^{-1} mg^{-1} protein) and content of protein (mg g^{-1} f.m.) in wheat plants of cv 'Leningradka' during the ontogenesis 1987

Organ	Phenophase	Days after sprout.	PAL activity						Protein content		
			I var.		II var.		III var.		I var.	II var.	III var.
			Total act.	Spec. act.	Total act.	Spec. act.	Total act.	Spec. act.			
1st internode	Tillering	23	0.38±0.00	0.01±0.00	—	—	—	—	32.58±0.42	—	—
	Tillering	32	2.06±0.08	0.18±0.00	2.02±0.01	0.17±0.00	0.42±0.00	0.03±0.00	11.67±0.09	11.70±0.06	9.80±0.11
	Stalking	42	1.83±0.08	0.23±0.00	7.61±0.05	0.83±0.00	5.23±0.03	0.59±0.00	7.98±0.18	9.12±0.01	8.88±0.06
	Stalking	53	8.43±0.01	2.58±0.00	17.05±0.03	5.17±0.00	18.93±0.28	6.13±0.03	3.27±0.02	3.30±0.01	3.09±0.03
	Booting	63	3.80±0.03	1.55±0.01	17.46±0.01	5.49±0.00	9.12±0.28	3.90±0.05	2.46±0.00	3.21±0.00	2.34±0.01
	Flowering	74	2.89±0.01	1.38±0.00	6.69±0.60	2.27±0.00	5.85±0.01	2.42±0.00	2.16±0.00	3.03±0.02	2.25±0.01
4th internode	Booting	63	35.94±0.01	24.71±0.01	47.58±1.21	4.13±0.01	24.84±0.04	3.39±0.00	7.05±0.01	11.52±0.00	7.35±0.00
	Flowering	74	16.91±0.06	23.49±0.11	(9.0)±0.02	2.01±0.00	15.68±0.01	2.94±0.00	0.72±0.00	9.48±0.02	5.67±0.02

Table 3

The activity of PAL (total – μg min^{-1} g^{-1} f.m.; specific– μg min^{-1} mg^{-1} protein) and content of protein (mg g^{-1} f.m.) in wheat plants of cv 'Leningradka' during the ontogenesis 1984

Organ	Phenophase	Days after sprouting	PAL activity				Protein content	
			I var.		II var.		I var.	II var.
			Total	Spec.	Total	Spec.		
Leaves	Tillering	23	4.03±0.6	0.25±0.02	5.44±0.20	0.44±0.02	16.0	14.0
	Stalking	30	2.43±0.09	0.12±0.01	5.05±0.15	0.24±0.01	21.0	24.0
	Booting	51	1.90±0.05	0.05±0.01	2.10±0.05	0.08±0.01	35.0	33.0
	Flowering	58	2.04±0.08	0.06±0.03	2.62±0.09	0.08±0.00	37.0	39.0
1st internode	Stalking	30	6.06±0.25	0.39±0.01	10.40±0.23	0.48±0.01	15.5	21.2
	Booting	51	3.60±0.05	0.58±0.03	10.21±0.15	1.62±0.02	6.3	16.3
	Flowering	58	3.01±0.06	0.37±0.01	4.62±0.07	0.36±0.01	8.2	14.3
	Dough-ripeness	95	1.16±0.04	-	1.74±0.08	-	-	-
4th internode	Booting	51	11.57±0.32	0.77±0.04	12.15±0.39	0.40±0.01	15.0	30.5
	Flowering	58	8.17±0.17	0.50±0.07	9.62±0.14	0.59±0.02	16.4	20.5
	Dough-ripeness	95	3.35±0.06	-	5.10±0.18	-	-	-

Table 5

The activity of PAL (total - μg min^{-1} g^{-1} f.m.; specific - μg min^{-1} mg^{-1} protein) and content of protein (mg g^{-1} f.m.) cv 'Arkas' during the ontogenesis 1987

Organ	Phenophase	Days after sprouting	PAL activity Total	Spec.	Protein content
1st internode	Tillering	23	0.06±0.00	0.00	23.46±0.00
	Tillering	32	2.38±0.01	0.20±0.00	15.12±0.00
	Stalking	42	2.29±0.06	0.17±0.00	13.14±0.23
	Stalking	53	17.10±0.10	5.43±0.01	3.15±0.07
	Booting	63	5.23± 0.00	1.82	2.88±0.01
	Flowering	74	3.99±0.01	1.58±0.00	2.52±0.00
4th internode	Booting	63	23.93±0.48	2.12±0.00	11.31±0.03
	Flowering	74	6.23±0.01	1.49±0.00	4.17±0.03

The dynamics of phenolic compounds and the content of protein during the period of vegetation

According to the data obtained, in the lower internode of the stalk, the content of ethanol-soluble phenolic compounds increased slowly from the phase of stalking (2.28 mg g^{-1} dry mass) up to that of milk ripeness (5.88 mg) in case of cv 'Leningradka'. In case of cv 'Arkas', the content of phenolic compounds showed a similar tendency to increase - from 2.35 mg g^{-1} dry mass in the phase of stalking up to 6.8 mg in the phase of milk ripeness (tab. 6). In the upper internode, contrary to the lower one, the content of soluble phenolic compounds decreased during the last phases of ontogeny.

The content of lignin was determined only from the stalks of cv 'Leningradka'. According to the data obtained, the content of lignin increased during the ontogeny (tab. 6). In the lower internode, lignin constituted 55 mg g^{-1} of dry mass at the beginning of the phase of stalking. During the phases of stalking and booting, lignin was intensively deposited in cell walls - in the phase of booting lignin constituted 209 mg g^{-1} of dry mass and in the phase of ripening its content reached 226 mg g^{-1} of dry mass, i.e. lignin constituted 23 % of dry mass. The content of lignin in the upper internode was lower than in the lower internode - in the phase of

Table 6

The content of lignin and in ethanol soluble phenolic compounds (mg g^{-1} dry mass) in wheat stalk in case of different mineral nutrients

Cv Var.	Phenophase	Days after sprouting	1st internode		4th internode	
			Lignin	Soluble phenolic compounds	Lignin	Soluble phenolic compounds
'Leningradka' I	Tillering	32	-	2.28	-	-
	Stalking	42	55	2.41	-	-
	Stalking	53	70	3.67	-	-
	Booting	63	209	4.12	-	5.09
	Flowering	74	244	4.60	94	7.90
	Ripening	97	266	5.88	116	5.10
II	Tillering	32	-	4.50	-	-
	Stalking	42	69	5.20	-	-
	Stalking	53	89	5.58	-	-
	Booting	63	182	5.76	-	7.20
	Flowering	74	166	5.90	110	4.88
	Ripening	97	241	7.91	161	4.30
III	Tillering	32	-	3.50	-	-
	Stalking	42	175	4.71	-	-
	Stalking	53	182	4.91	-	-
	Booting	63	184	5.29	-	6.50
	Flowering	74	202	5.30	100	6.20
	Ripening	97	228	10.44	236	4.40
'Arkas'	Tillering	32	-	2.35	-	-
	Stalking	42	-	3.13	-	-
	Stalking	53	-	3.82	-	-
	Booting	63	-	3.77	-	5.90
	Flowering	74	-	4.70	-	4.85
	Ripening	97	-	6.80	-	3.10

ripening lignin constituted 116 mg g^{-1} of dry mass, i.e. 12 % of the dry mass of the upper internode.

According to the data obtained during the vegetation period of 1987, the content of protein in stalks decreased continually together with an increase in the age of plants (tab. 4, 5). (The data obtained in 1984 were not so definite - see tab. 3). In the upper internode, the content of protein was higher than in the lower internode. In leaves, the content of lignin increased from the phase of sprouting up to the phase of flowering (tab. 3).

The influence of mineral nutrition on the activities of PAL and PO and on the content of phenolic compounds in wheat plants

The results of the experiments of both 1984 and 1987 indicate that the heightened nitrogen concentration in the nutrient medium (as indicated in "Methods" of the present paper) increased the total activity of PAL in the leaves and stalks of wheat plants (tab. 3, 4, fig. 3). In the conditions of the increased nitrogen content, the activity of PAL per unit of protein also increased. The experiment of 1987 constituted an exception - the activity of PAL per unit of protein in the upper internode was higher in the unfertilized variety. The reason may have been an exceptionally low protein content of the upper internode of the unfertilized variety (tab. 4). The heightened nitrogen concentration in the nutrient medium also lengthened the period of the high PAL activity in plants - in case of var. 1 and 3, and of cv 'Arkas' (tab. 5), the activity of PAL in the lower internode had already markedly decreased by the phase of booting when in var. 2 the enzyme still retained its maximal activity. The additional amounts of potassium in the nutrient medium (var. 3) also increased the total activity of PAL (tab. 4, fig. 3) but only in the lower internode.

As regards soluble PO, its total activity and the activity per unit of protein increased in the lower internodes of the plants of var. 2 and 3 in all the phenophases investigated (tab. 7), but in var. 3 the increase in its activity per unit of protein was considerably smaller than in var. 2. In the upper internode, its total activity increased in the plants of both var. 2 and 3 but its activity per unit of protein remained practically stable.

As to ionically bound PO, its total activity in the lower internode increased in the plants receiving additional amounts of nitrogen and potassium but its activity per unit of protein was in var. 3 higher than in var. 2. In the upper internode, its total activity increased, but the activity per unit of protein remained stable or even decreased.

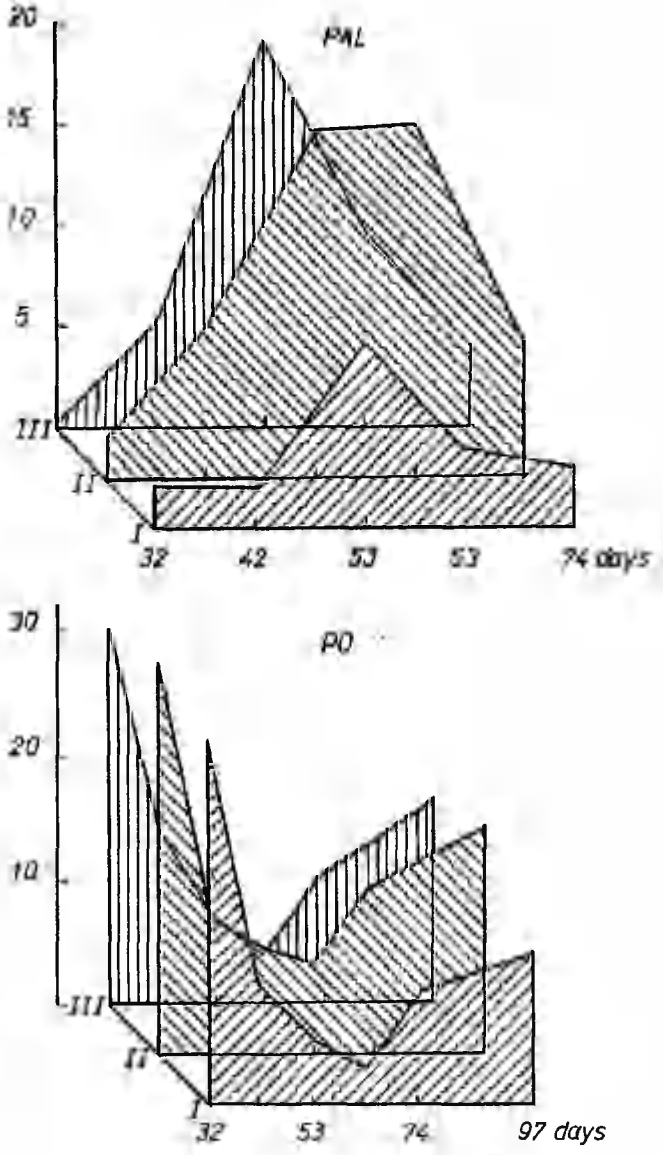

Fig. 3. The activity of PAL (μg.min^{-1} g^{-1} f.m.) and the summary activity of PO (μmol min^{-1} g^{-1} f.m.) in the first internode of wheat grown in different conditions of mineral nutrients

Table 7

The activity of PO (total $\mu mol^{-1} g^{-1}$ f.m.; spec. – $\mu mol\ min^{-1} mg^{-1}$ protein) in wheat plants of cv 'Leningradka' during the ontogenesis 1987

Organ	Phenophases	Days after sprouting	I var.		II var.		III var.	
			Total act.	Spec. act.	Total act.	Spec. act.	Total act.	Spec. act.
			Soluble PO					
1st internode	Tillering	32	17.83±0.19	2.72±0.25	21.38±0.26	2.94±0.31	19.53±0.45	2.79±0.48
	Stalking	42	7.43±0.18	1.33±0.27	8.05±0.24	1.60±0.28	10.35±0.31	1.50±0.37
	Stalking	53	4.15±0.11	0.98±0.17	6.58±0.04	1.38±0.17	5.91±0.25	1.27±0.3
	Booting	63	2.12±0.11	0.58±0.19	5.82±0.18	1.38±0.23	2.68±0.00	0.69±0.19
	Flowering	74	7.96±0.26	2.56±0.30	11.64±0.17	3.23±0.23	8.43±0.08	2.56±0.20
	Ripening	97	9.16±0.11	2.00±0.19	13.90±0.04	3.10±0.20	13.00±0.18	3.03±0.24
4th internode	Booting	63	2.90±0.04	0.62±0.18	3.76±0.01	0.66±0.21	3.08±0.08	0.57±0.20
	Flowering	74	3.10±0.15	0.69±0.22	4.65±0.87	0.87±0.24	3.18±0.04	0.64±0.20
	Ripening	97	4.51±0.06	2.19±0.19	5.34±0.16	1.75±0.23	4.86±0.14	2.48±0.20
			Ionically bound PO					
1st internode	Tillering	32	8.28±0.35	11.80±0.37	6.85±0.24	7.25±0.24	7.22±0.25	8.29±0.28
	Stalking	42	1.75±0.19	3.57±0.22	2.27±0.18	4.00±0.20	3.08±0.15	4.97±0.18
	Stalking	53	0.74±0.28	2.24±0.40	1.40±0.12	2.42±0.17	0.72±0.09	2.88±0.14
	Booting	63	0.61±0.08	0.77±0.15	0.83±0.06	1.66±0.15	1.09±0.06	2.42±0.14
	Flowering	74	0.80±0.04	2.95±0.12	1.25±0.04	3.05±0.13	1.51±0.12	4.48±0.08
	Ripening	97	2.46±0.18	11.18±0.21	3.43±3.15	11.43±0.18	2.98±0.11	12.96±0.15
4th internode	Booting	63	0.51±0.04	1.30±0.10	0.62±0.08	1.23±0.13	0.54±0.06	1.21±0.13
	Flowering	74	1.14±0.09	4.97±0.13	1.51±0.06	6.04±0.15	1.32±0.28	4.47±0.18
	Ripening	97	1.75±0.11	10.97±0.17	2.23±0.10	9.38±0.15	2.06±0.15	8.97±0.18

During the vegetation period there was no change in the dynamics of the PO activity in the plants grown under different conditions of mineral nutrition (fig. 3).

As to soluble phenolic compounds, their content in the lower internodes of the plants of var. 2 and 3 appeared to be higher than that of the plants of var. 1 grown without additional fertilization in all the stages of development investigated (tab. 6). In the upper internodes of the plants of var. 2 and 3, their content was higher only in the phase of booting, in the phases of flowering and dough ripeness it was lower than in the plants of var. 1.

The content of lignin in the lower internodes of plants given additional amounts of nitrogen was somewhat higher in the phase of stalking, but lower in the phases of booting and flowering than in the plants of var. 1. The results of the analyses indicate that potassium promotes the quick deposition of lignin already in the early stages of the development of the stalk in the phase of stalking (tab. 6). By the phase of ripening, the content of lignin in all the varieties reached practically the same level. In the upper internode, the content of lignin in the plants of var. 2 and 3 was higher than in the plants of var. 1 in the phase of ripening.

The protein content in the leaves and stalks of the plants given additional amounts of nitrogen was higher than in var. 1 (tab. 3, 4). The upper internodes of the plants given additional amounts of potassium also contained more protein than those of var. 1 plants but the lower internodes of the plants of var. 1 and 3 contained practically equal amounts of protein (tab. 4).

The growth of plants was more intensive on the soil given additional amounts of potassium and especially of nitrogen than on the soil without additional fertilization - the height of plants, dry mass per plant and the diameter of stalks all increased (tab. 8). In all the conditions of mineral nutrition, plants grew intensively until they reached the phase of flowering. Than the growth in height practically stopped (fig. 4).

Table 8

The growth of wheat plants during the vegetation period 1987

Cv var.	Phenophase	Days after sprouting	Dry mass per plant (g)	1st internode		4th internode	
				Length (mm)	Diameter (mm)	Length (mm)	Diameter (mm)
'Leningradka'	Tillering	32	0.072				
	Stalking	42	0.219	40 ± 2	2.5 ± 0.1		
	Stalking	53	0.578	42 ± 5	2.7 ± 0.1	110 ± 10	2.4 ± 0.2
	Booting	63	1.233	51 ± 5	2.9 ± 0.1	173 ± 9	3.4 ±
	Flowering	74	1.770	59 ± 6	2.9 ± 0.1	299 ± 18	2.7 ± 0.2
	Ripening	97	2.200	57 ± 3	2.8 ± 0.1	245 ± 7	3.0 ± 0.1
II	Tillering	32	0.076				
	Stalking	42	0.258	36 ± 2	3.3 ± 0.2		
	Stalking	53	1.572	36 ± 4	3.1 ± 0.1	111 ± 10	2.6 ± 0.1
	Booting	63	1.572	36 ± 5	3.1 ± 0.1	163 ± 15	3.8 ± 0.1
	Flowering	74	2.527	37 ± 3	3.0 ± 0.1	248 ± 10	3.8 ± 0.1
	Ripening	97	2.719	39 ± 4	3.1 ± 0.1	246 ± 6	3.6 ± 0.1
III	Tillering	32	0.062				
	Stalking	42	0.258	29 ± 2	3.1 ± 0.6		
	Stalking	53	0.703	53 ± 6	3.1 ± 0.1	91 ± 17	3.0 ± 0.2
	Booting	63	1.351	44 ± 5	3.0 ± 0.1	139 ± 7	3.6 ± 0.1
	Flowering	74	1.956	31 ± 4	2.8 ± 0.1	287 ± 10	3.3 ± 0.1
	Ripening	97	2.880	28 ± 3	3.0 ± 0.1	277 ± 6	3.2 ± 0.1
'Arkas'	Tillering	32	0.073				
	Stalking	42	0.284	24 ± 2	2.9 ± 0.6		
	Stalking	53	0.772	26 ± 2	3.1 ± 0.1	89 ± 26	2.8 ± 0.4
	Booting	63	1.155	30 ± 3	2.8 ± 0.1	159 ± 6	3.3 ± 0.9
	Flowering	74	1.793	28 ± 2	2.6 ± 0.1	168 ± 4	3.0 ± 0.1
	Ripening	97	2.119	27 ± 1	2.6 ± 0.1	180 ± 2	3.0 ± 0.1

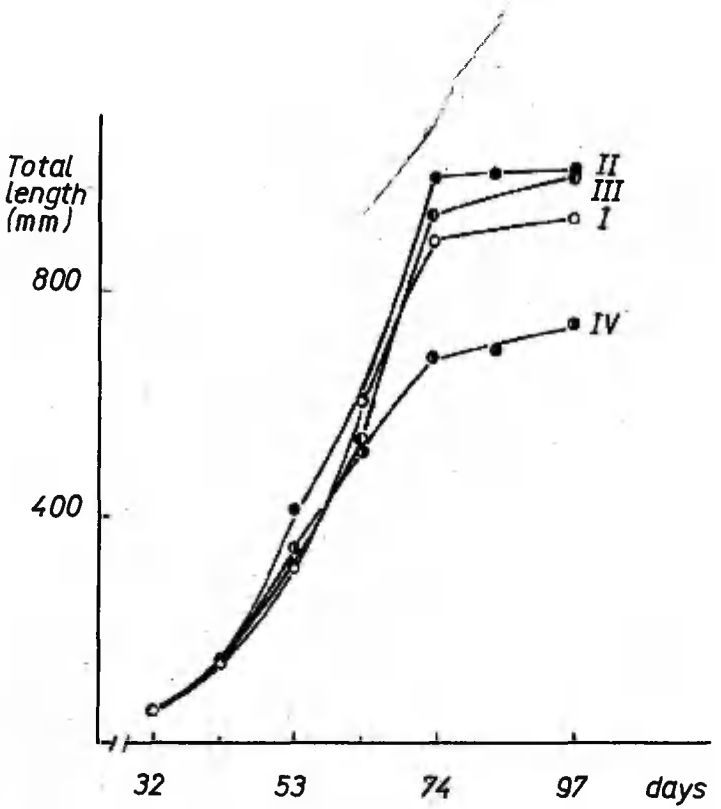

Fig. 4. The total length of wheat plants in different conditions of mineral nutrients (var. I - III cv 'Leningradka', var. IV cv 'Arkas')

Discussion

To discuss the regulation of metabolic flux in the phenylpropanoid pathway, the knowledge of the kinetic properties of the enzymes of this pathway is required. The pH optimum of the activity of PAL - the key enzyme in the phenylpropanoid pathway - in wheat was established in the alkaline medium at pH 8.7. According to the literature, the optimum of the PAL activity in other plants has also been found in the alkaline medium within the pH range of 8.0 - 8.6 (O'NEAL and KELLER, 1970). No shift in the pH optimum was observed with *L*-tyrosine.

The experiments we carried out indicated that in the conditions of an excess of substrate, the activity of the enzyme decreased - the concentration optimum of *L*-Phe was located in the interval of 1.5 - 2.5 mM *L*-Phe. According to the literature, the negative co-operative

effect with the given substrate has previously also been observed (JONES, 1984). The decrease in the activity of the enzyme at high concentrations of the substrate may be caused by the inhibition with the reaction product - cinnamic acid - and with other compounds of similar structure (*p*-coumaric acid, sinapic acid, caffeic acid) formed in the phenylpropanoid pathway (KUDAKASSERIL and MINOSHA, 1984).

In our experiments, we also determined the activity of TAL. The activity of TAL has been found in a number of grasses and an opinion has been expressed that the activity of TAL in monocotyledons may account for a large *p*-coumaric alcohol content in the lignin of these plants (WEISSENBÖCK, 1976). *L*-tyrosine which is not converted to lignin in gymnosperms and most angiosperms is by grasses efficiently transformed into hydroxyphenylguaiacyl and -syringyl units of lignin and also into the esterified *p*-coumaric acid (HIGUCHI et al., 1967). It has not been ascertained whether PAL in wheat is an enzyme with a wide substrate specificity so that a single active site can act on both Phe and Tyr as has been established for the maize enzyme (HANSON and HAVIR, 1981) or whether PAL and TAL are two different proteins. Neither can the results of our experiments clarify the matter. In the laboratory experiments, the ratio of activities (PAL/TAL) varied from 5.2 to 12.8. According to the literature, different assays may give different ratios (EMES and VINING, 1970) - the absorbance of both tyrosine and *p*-coumarate is affected by temperature sensitive phenolic ionization. In addition, it has also been established that although the activity of PAL is usually higher than that of TAL, the ratio of PAL/TAL depends on the light conditions of plants, the type of tissue and the age of plants (GUERRA et al., 1985).

The results obtained from our experiments indicate that the activity of PAL during the ontogeny of wheat changes greatly. In ungerminated seeds, the activity of PAL is practically nonexistent. The appearance of the activity of PAL on germination has been accounted for by the induction with a functionally active form of phytochrome formed in light which increases the expression of the PAL gene (MOORE, 1979) while the PAL synthesis *de novo* considerably exceeds its degradation (FOURCROY, 1980). An increase in the PAL activity on germination has also been explained by the activation of the inactive molecules of the enzyme on the decomposition of the specific proteinous inhibitor (ZUCKER, 1971).

Changes in the activity of PAL in the lower internode of the wheat stalk during the period of vegetation appeared to be to a certain extent analogous with changes in the activity of the enzyme during germination. Before stalking, the activity of the enzyme in leaf sheats was low, during the phase of stalking the

activity increased, reached its maximum by the end of the phase of stalking and then began to decrease. GUERRA et al (1985) have also demonstrated that the activity of PAL in wheat plants increases until the plants reach the age of 40-50 days and then begins to decrease. The quick increase in the activity at the beginning of the development of plants and the decrease in it during the senescence of plants are characteristic of anabolic enzymes. The opinions of the authors about the reasons for the active decrease in the activity of PAL in older plants differ: some consider it the result of the quickened degradation of enzymatic protein (SACHER, TOWERS, 1972), some think that the slowing-down in the synthesis of the enzyme also contributes to it (SHIELDS et al., 1982).

To interpret the changes in the activity of PAL that occur during the growth and development of plants under different conditions of mineral nutrition, the content of ethanol-soluble phenolic compounds and of lignin were determined in our experiments. During germination, parallel to an increase in the activity of PAL, the content of soluble phenolic compounds also increased. These data agree with those published in literature about the positive correlation between the changes in the activity of PAL and the formation of phenolic compounds (SHAH, METHA, 1980; WELLMANN, 1974; DAVIES, 1972; WESTCOTT and HENSHAW, 1976).

According to the literature, it may be stated that the activity of PAL is relatively high in lignifying tissues of various plant stems (RUBERY and NORTHCOTE, 1968) and that its rise and growth in stalks may be regarded as an indicator of the formation of the elements of xylem and of the lignification of cell walls (GROSS, 1981). Our experiments showed the high activity of PAL in stalks during the phase of stalking and especially at the end of it, and the same period saw the most intensive deposition of lignin in stalks. Several authors (KEVERS and GASPAR, 1975; VANCE, 1976; GUERRA et al., 1985) have demonstrated an increase in the activity of PAL in lignifying tissues.

The contamination of the plants, characterized by the intensive biosynthesis of lignin, with pathogenes is accompanied by the activation of PAL and other enzymes of the phenylpropanoid pathway (MAULE, RIDE, 1976; VANCE, SHERWOOD, 1976). Our data show that in wheat leaves where the amount of lignin deposited is smaller than in stalks, the activity of PAL is also lower. In the upper internode it turned out to be higher than in the lower internode. This regularity may be connected with the fact that the fourth internode is formed only in the phase of booting and, therefore, the biosynthesis of lignin in it must proceed more quickly than in the first internode.

When the activity of PAL and the speed of growth

were compared, it turned out that the growth of plants slowed down in the phase of flowering - consequently, the activity of PAL decreased already in plants still growing intensively.

The regulation of the biosynthesis of phenolic compounds and especially that of lignification *in vivo* is still practically unknown. The lignin content and composition in early and late wood and in the middle lamella of fibers and vessels differ (SCOTT et al., 1969). The results of our experiments indicate that the conditions of mineral nutrients influence the content of phenolic compounds and the activity of PAL and PO - enzymes participating in their biosynthesis. In the stalks of plants additionally fertilized with nitrogen and potassium, the activity of PAL and PO were higher than in the stalks of the plants of var. 1. Not only the total activity of the enzymes but also their activity per unit of protein increased, therefore, we may suppose that an increase in the number of enzyme molecules takes place in the total amount of protein.

It has been established that the activity of the enzymes of the biosynthesis series of phenylpropanoids (such as PAL, cinnamyl CoA ligase, cinnamyl 4-hydrolyase etc.) changes co-ordinately (EBEL et al., 1974; HAHLBROCK, GRISEBACH, 1979; RHODES et al., 1976) and *in vivo* these enzymes are found as complexes (STAFFORD, 1981). Therefore, we may suppose that in the stalks of plants grown in the conditions of heightened mineral nutrition, the activity of other enzymes of the biosynthesis of phenylpropanoids also occurs. The higher activity of PAL in plants grown in these conditions was associated with the higher content of soluble phenolic compounds.

The combinations of mineral fertilizers used did not influence materially the amount of lignin deposited in the cell walls of the first internode by the phase of dough ripeness, but they did influence the speed of the lignin deposition which is considered essential for increasing the lodging resistance of wheat plants (LYASKOVSKI, KALININ, 1977). The additionally fertilized wheat plants had a higher activity of PAL and more lignin deposited in the phase of stalking than the plants of var. 1. WEISSENBÖCK (1976) shows that the maximum activity of PAL does not correlate with the amount of lignin but with the intensity of the process of lignification. In the plants with the heightened content of potassium, the quick process of lignification at the beginning of ontogeny is accompanied by the high activity of the ionically bound PO in both the lower and the upper internodes.

Few data can be found in the literature concerning the influence of mineral nutrition on secondary metabolism and on the formation of cell walls. MENGEL (1965) shows that potassium promotes the lignification of cell walls and increases their diameters. The heightened

concentration of nitrogen in the environment increases the protein content and decreases the content of soluble phenolic compounds (HAHLBROCK, 1975; SHAH, METHA, 1978; LYASHENKO, 1983). The results of our experiments did not show an inverse correlation between the protein content and the content of phenolic compounds.

The reason why our data differ from those published in literature may rise from the fact that the plants analyzed were undergoing different stages of development. MILLER et al. (1985) argue that in lettuce plants two phases of lignification can be distinguished. In the first phase, the differentiation of cells and an increase in the content of phenolic compounds take place. In the second phase, an intensive xylogenesis and deposition of lignin occur and the content of soluble phenolic compounds decreases because they are used up in the biosynthesis of lignin. The majority of scientific papers does not take into consideration the number of phenolic units deposited in lignin, although during the biosynthesis of lignin greater part of the phenolic compounds synthesized is used up. Neither can we exclude the possibility that by the biosynthesis of protein and phenols, different pools of amino acids exist (Phe, Tyr) (MARGNA, 1977). Recently it has also appeared that standard methods for the determination of PAL lead to the measurement of both *L*-phenylalanine amino-transferase (EC 2.6.1.1 PAT) and PAL activities together (DA CUNHA, 1987).

Consequently, further systematic investigations are needed on the regulating mechanism of enzyme synthesis, on the compartmentation and the genetic controls of phenylpropanoid metabolism during differentiation and on the influence of the environment on plant tissues.

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HISTOCHEMICAL METHODS IN THE DETERMINATION OF THE LIGNIFICATION PROCESS

H. Miidla, A. Hellenurme, A. Tänav, R. Tänav

Introduction

When studying the process of lignification by biochemical methods use can be made of many histochemical ones which show the location where processes are detected and simultaneously their presence is noticeable. The histochemical method is much more convenient and easy in that it is based on colour reactions and shows the process directly *in situ* in the structural tissues.

The classical histochemical method used for the determination of lignification in cell walls *in situ* is the phloroglucinol in HCl manipulation. The lignin and lignin-like compounds detected with phloroglucinol/HCl give a raspberry-red coloration after reaction with *p*-hydroxycinnamyl aldehyde moieties in the lignin polymer (ADLER et al., 1948; ROBERTSEN, 1986).

Using the polarizing microscopy we can better determine and measure the structural elements of the tissues. It is well-known that the lignified cell walls are more polarized (ALKIN, BURDIC, 1975).

Some authors (BRABER, 1980; CATESSON et al., 1986) agree that the lignification of foliar tissue is accompanied by an activation of peroxidase.

The aim of the present study is to demonstrate the localization of the activity of peroxidase, the histochemical reaction of phloroglucinol in HCl and the intensity of polarization of the cell walls in the first internodes of wheat stalks in connection with the process of lignification to explain the importance of histochemical methods in our work.

Materials and methods

All the material for the examination was taken from the midregion of the first internode of the spring wheat 'Leningradka', grown in the field in normal conditions in the year 1986.

Peroxidase activity was determined from the fresh material. Small tissue pieces (cá 30 µm) were cut by hand with razor blades and transferred into the fresh-made incubation medium. The incubation medium consisted

of 1 ml of saturated NH_4Cl, 1 ml of 5 % EDTA, 9 ml of saturated guiacol and 1 drop of 3 % H_2O_2. Floating sections were incubated in the medium for 5 min., then twice washed in 0.06 M phosphate buffer (pH 5.6) and finally placed into the same solution on glass slides of a light microscope. Staining orange intensity was assessed visually in a 5-grade system. Control sections were devoid of H_2O_2.

For phloroglucinol reaction and polarizing microscopy the material was fixed in 4 % formalin. 1 cm internodes were dehydrated through an ethanol series, embedded in paraffin, sectioned transversely at 20 μm on a sledge microtome and mounted on glass slides and then were deparaffinized with xylol, ethanol and distilled water.

The sections were stained for lignified cell walls with acid phloroglucinol. This stain reacts with lignin, contributes to the measurements of cell walls in which secondary wall formation had occured. Aldehydes react with phloroglucinol, and lignified parts stain raspberry red. Control segments were incubated in water. Staining intensity was processed visually in a 5-grade system under light microscope and preparations were photographed.

Deparaffinized preparations were examined under polarizing microscope. Areas of the tissues were estimated from lengths, widths, diameters and were photographed for producing direct positive images.

Results

Table 1

Dimensions of the structural elements of wheat stalk ($x.10^{-2}$ mm)

Date of analysis	First internode				Fourth internode		
Structure element	18.06	09.07	20.07	12.08	09.07	20.07	12.08
ø of vascular bundles	19	20	21	22	17	15	16
number of vascular bundles on 10 mm²	4	5	4.5	4.5	3.5	3.5	3
ø of large vascular bundles	4	4	4.5	4.5	4.5	3	3
ø of mechanical ring	8	11	14	15	6.5	8	8

Date of analysis	First internode				Fourth internode		
Structure element	18.06	09.07	20.07	12.08	09.07	20.07	12.08
number of parenhymal cells	17	24	23	23	31	30	32
cell wall thickness of parenhymal cells	1.12	1.12	-	1.52	0.72	-	1.32
cell-wall thickness of mechanical tissues	1.12	1.40	-	1.83	-	-	-

Table 1 shows the dimensions of structural elements of the internodes of wheat-stalks. It seems that the elements of the fourth internodes do not obtain such dimensions as those of the first internode. It means that under the weight of the head they might break. The number of parenhymal cells is greater than in the tissues of the first internodes.

The polarizing microscope shows that the mechanical ring of the first internode (fig. 1) is thicker

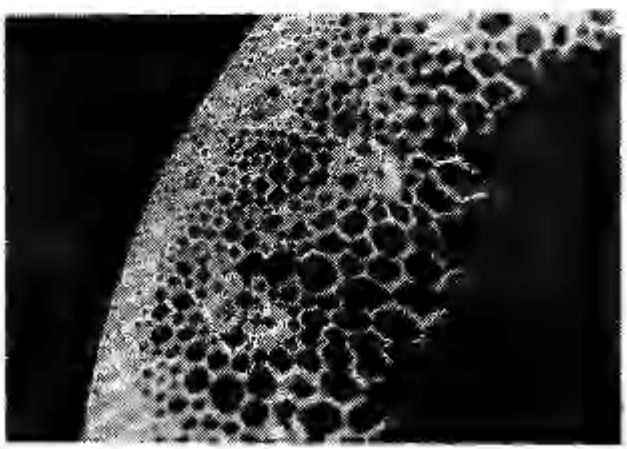

Fig. 1. Polarizing micrograph of the first internode cross section of a wheat stalk (x 100)

Fig. 2. Polarizing micrograph of the fourth internode cross section of a wheat stalk (x 100)

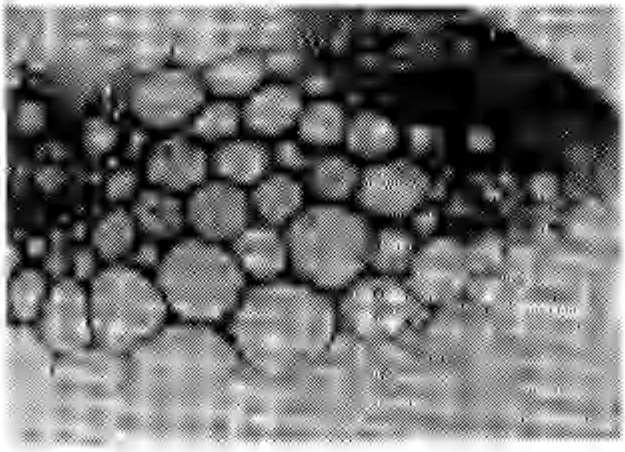

Fig. 3. Light micrograph of a cross section of the first internode of a wheat stalk in the phase of full ripeness stained with phloroglucinol in HCl (x 600)

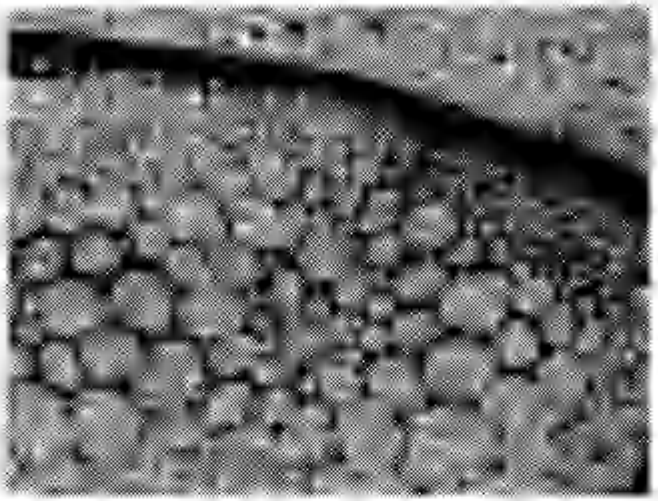

Fig. 4. Light micrograph of a cross section of the first internode of a wheat stalk in the phase of stalking (stained with phloroglucinol in HCl) (x 600)

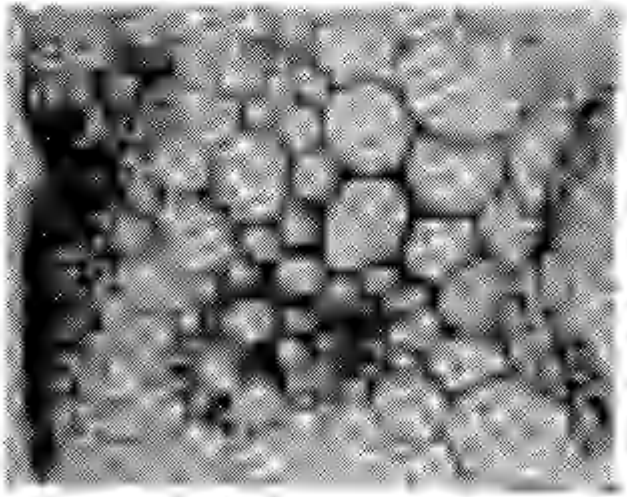

Fig. 5. Visualization of peroxidatic activities of the first internode cross section of a wheat stalk in the phase of full ripeness (Substrate guaiacol $+H_2O_2$ x 600)

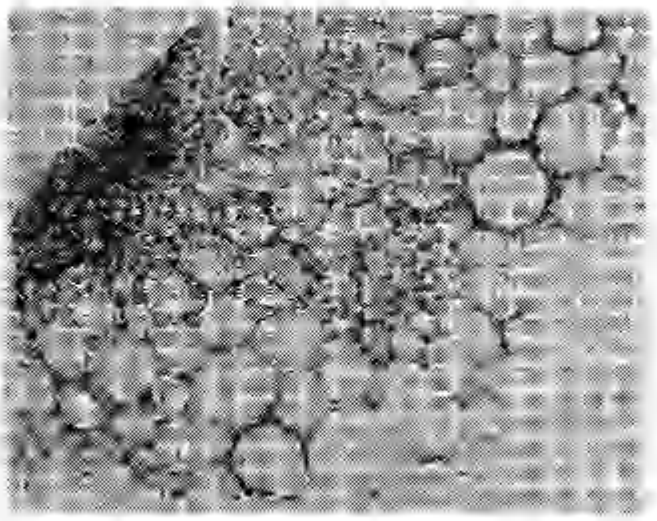

Fig. 6. Visualization of peroxidatic activities of the fourth internode cross section of a wheat stalk in the phase of stalking (Substrate guaiacol $+H_2O_2$, x 600)

than that in the fourth one (fig. 2). In the fourth internode there are sets of unlignified parenhymal cells that are able to photosynthesize.

Histochemical tests for lignin with phloroglucinol in HCl, a test for cinnamaldehyde end groups, gave a positive raspberry-red coloration indicating the presence of lignin.

The strongest reaction was given by the cell walls of wheat stalks in the phase of full ripeness (25.08., fig. 3) and the smallest in the phase of stalking (14.06., fig. 4).

Histochemical demonstration of peroxidase activities occurred only in the walls of differentiating and lignifying cells. There was no reaction when guaiacol was used alone without H_2O_2. The guaiacol staining of the cell walls was much more observed in differentiating cell walls (fig. 5) than in the little-differentiated walls (fig. 6, compare also fig. 3 and 4).

Discussion

The results presented here show that the final step of lignin biosynthesis takes place in the apoplast. This confirms the findings of GOLDBERG et al., 1985.

They found a good correlation between the distribution of peroxidases involved in the oxidative polymerization of lignin and that of the hydrogen peroxide generating system: both were restricted to lignifying tissues.

We, in our experiments, could find correlation between histological localization and biochemical assays of peroxidases (compare our papers 4 and 6) only in the final stage, but not in the first step of lignification (compare fig. 3-6).

Peroxidases were observed in the earlier stages and as shown here, they were localized in the inner wall layers. The activity of the peroxidase in the cell wall specifically suggests its role in cell wall formation.

HADDON and NORTHCOTE (1976) also showed, that wall peroxidase activity was high even in tissues not undergoing lignification, or at least did not show the classical phloroglucinol-lignin reaction (compare fig. 3-6).

To sum up the histochemical methods are of good evidence in comparison with the determination of lignification with biochemical methods. It must be mentioned that histochemical activities of peroxidase in the cell walls of young plants do not correlate with the results of biochemical analyses of the activity of peroxidase. But in the cell walls of mature plant (in lignified cell walls) there exists a strong correlation between histochemical activities of soluble peroxidase. Phloroglucinol reaction is in a positive correlation with the results of biochemical analyses in the determination of lignin.

The presence of peroxidases in unlignified parts of vessel walls is still unexplained. Otherwise, enzyme activity in xylem and fiber walls is in accordance with its role in lignification processes. Lignin content is higher in the primary wall where peroxidase activity is also the most important and the most lasting. According to the current hypothesis (VAN HUYSTEE and CAIRNS, 1982) the enzymes are probably synthesized on endoplasmatic rediculum ribosomes and then proceed *via* the Golgli apparatus. In this case they would discharge either into the vacuoles or into the periplasmic space.

In wheat vessels, a strong reaction was seen in the whole secondary wall.

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THE INFLUENCE OF THE CONDITIONS OF GROWTH ON THE DEVELOPMENT OF BARLEY

T. Tiidt

In connection with the development of intensive land cultivation in Estonia, more attention must be paid to the environmental protection and economical utilization of energy than has been done hitherto. Studies of individual agricultural methods are of little efficiency and, therefore, multifarious researches into their complex influence on the growth and development of plants are of primary importance. Regardless of the fact that complex experiments with many factors are rather labour-consuming, there seem to be no better ways for obtaining data on production conditions.

In 1982, the first complex experiments were begun at Eerika, in the Estonian SSR, in the experimental fields of the Department of Soil Cultivation of the Estonian Agricultural Academy, with the aim of clarifying the influence of crop rotation and various methods of soil treatment (common and minimized) and complex weed control on the physical, chemical and biological properties of soil, its weediness, yield structure, chemical composition, plant diseases, etc.

Estonian scientists LAURINGSON and VIPPER (1986) have shown that deeper ploughing makes the air and water regime in soil more favourable to the growth and development of plants, and the use of herbicides with variants ploughed with a disk harrow adds to the yield. Our experiments of the year 1982 showed that deeper ploughing (the depth of 21...22 and 24...25 cm) had a positive effect on the yield of grain crops (an increase in yield - 15.5...23.5 % compared to that of the ploughing depth of 16...17 cm).

The highest and quantitative yield of winter rye (4.2 t/ha) was secured in the rotation of crops after black fallow, after field grass fallow the yield was 3.7 t/ha, and after early barley fallow - 3.4 t/ha. The grains of rye cultivated on black fallow contained 1.2...1.4 % more protein than these grown on field grass and barley fallows.

The biological activity of soil (the separation of CO_2 from the soil) was the highest (472.7...518.3 mg/m^3 per hour) in case of rotation 2 in the year 1985 (see the rotation of crops in Material and Methods) when the number of earthworms was also the highest (771 worms/ha).

As in literature few data can be found concerning the influence of longtime crop rotation on the physiological and biochemical indices of cereals, the aim of the present paper is to offer such information based on the results of our experiments of the years 1982 to 1985.

Material and methods

The experiment included the following rotations of crops:

Year / Rotation	1982	1983	1984	1985	1986
R-1	mixed crop	early barley	rye	barley	oats
R-2	field grass	black fallow	rye	barley	oats
R-3	early barley	black fallow	rye	barley	oats
R-4	potato	potato	spring wheat	barley	potato
R-5	potato	early barley	rye	barley	potato

In each rotation the following methods of soil cultivation were used: in autumn, shallow ploughing with a heavy disk harrow in the depth of 10...12 cm, in spring, the pre-showing treatment - smoothing + harrowing in the depth of 3...4 cm and cultivating twice in the depth of 3...4 cm. In all the fields of rotations three depths of ploughing were used: 16...17, 21.. 22, and 24...25 cm.

The area of the experimental field was 67.5 m^2 in four replications. In spring, the complex fertilizer $N_{80}P_{80}K_{80}$ kg/ha as an active substance was given to the soil. The sowing with the rate of 280 kg/ha was carried out on May 7. The plot was rolled. The depth of ploughing was 25 cm and the content of humus 2.1... 2.5 %. The bulk density of soil was 1.35...1.40 g/cm^3 and the content of moving elements in 100 g of soil was P 6.1 and K 20 mg.

In May, the precipitation was 79 mm, temperature exceeded the norm by 3...4 °C. June was cool, precipitation 115 mm. The period of vegetation proceeded under the conditions of sufficient or even excessive moisture.

Examinations and analyses were made with the cultivar of barley 'Julia' in the year 1985.

The plant material was taken from 100 plants (moving across the plot) before noon. An increase in

the aboveground phytomass, size of the leaf area per plant (by measuring its length and width - NITCHIPOROVITCH et al., 1961), grain yield with the moisture content of 14 % were determined. The chlorophyll content of leaves was determined photoelectrocolorimetrically (PLESHKOV, 1968). Yellow leaves were not used in analyses. The indices were determined at the full stages of tillering (June 5), stalking (June, 25) and booting (July 15). The grain yield was taken at the stage of full maturity. The results were treated mathematically: $p = 0.05$.

Results

In case of all crop rotations, a maximum ploughing depth (24...25 cm) increases the phytomass of barley at the booting stage by up to 22 % (Fig. 1) as compared to a medium (21...22 cm) and shallow (16...17 cm) ones. In most cases (except R-2) deeper ploughing also enlarges the leaf area by 44.4 % (Fig. 2), and increases the chlorophyll content of a leaf by 10.5 % (Fig. 3) as compared to shallow ploughing. The maximum ploughing depth increases the length of the leading stalk at the booting stage in case of R-4 and R-5 by 3.9...5.9 % as compared with medium ploughing. A probable increase in yield is obtained by R-1 (0.016 t/ha) as compared to the minimum depth of ploughing and by R-1 and R-5 (0.023...0.033 t/ha) as compared to the medium depth of ploughing (Fig. 4).

Discussion

To obtain maximum yields, all the factors must be optimum to meet the biological requirements of plant species. At the beginning of growth, a quick formation of the organic substance is characteristic of barley - during the first 10 days in May the dry mass constitutes 10.6 g, by the third ten-day period in June - 215.4 g (KODANEV, 1964). The dynamics of foliage formation is of great importance to the yield production. In grain crops, the assimilates leading to the development of caryopsides are mainly formed in the flag leaf, stalk or spike (MIIDLA, 1984).

The largest amounts of the aboveground phytomass of barley were obtained at the booting stage in all crop rotations: comparing deep ploughing with the shallow one, the amount of phytomass increased by 7... 29.4 %, with the medium one - by 10.8...16.1 %.

A leaf is the chief organ of photosynthesis. With the advance of the booting stage, the intensity of photosynthesis and also yield increase due to an enlarged leaf area (ALEKSEYENKO, 1968). In leaves with a small chlorophyll content, the intensity of photosynthesis grows with an increase in light intensity. The

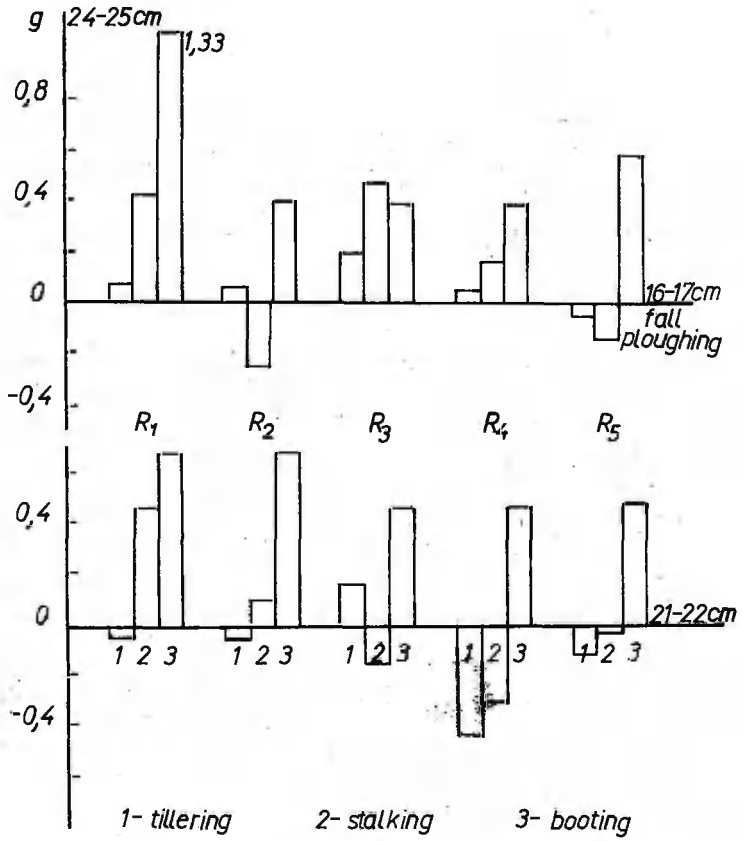

Fig. 1. Overground phytomasses of a barley plant in case of different ploughing depths and crop rotations in 1985 (comparing with the depth of ploughing 24-25cm-control).

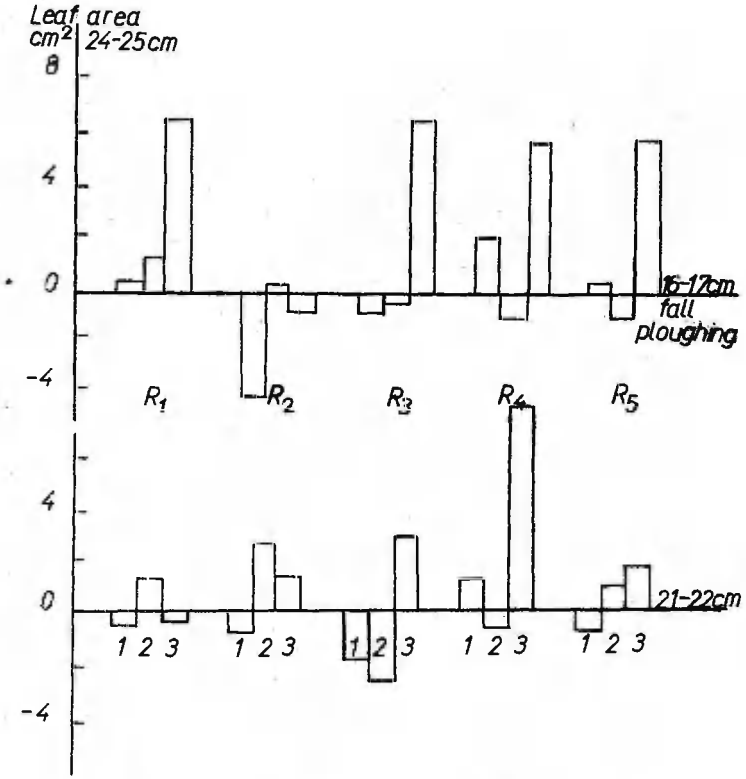

Fig. 2. Leaf areas of a barley plant in case of different ploughing depths and crop rotations in 1985 (comparing with the depth of ploughing 24-25cm-control).

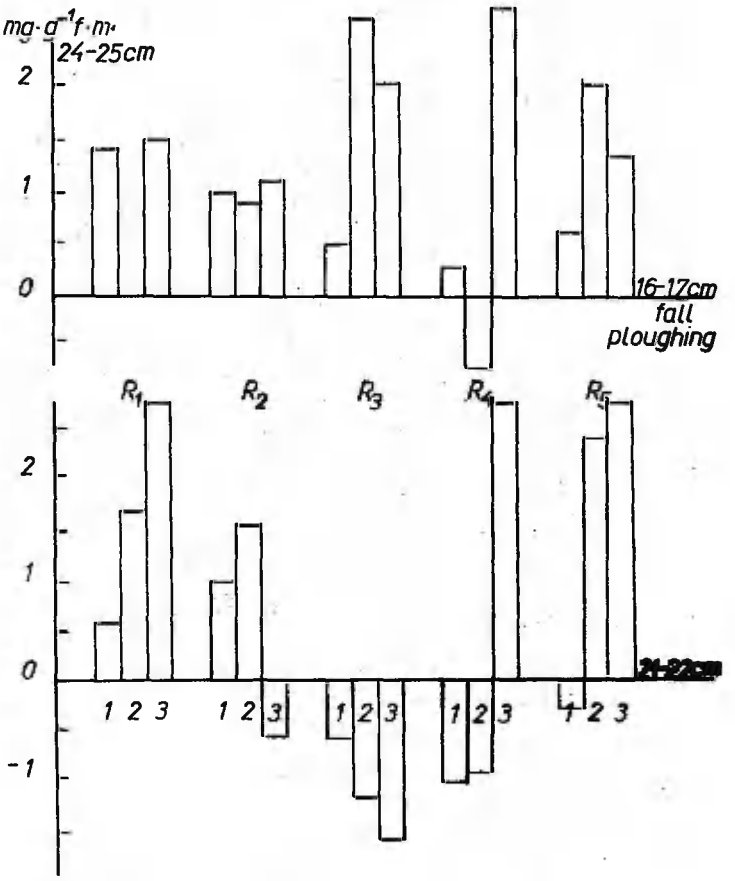

Fig. 3. The chlorophyll content of a barley leaf in case of different ploughing depths and crop rotations in 1985 (comparing with the depth of ploughing 24-25 cm-control).

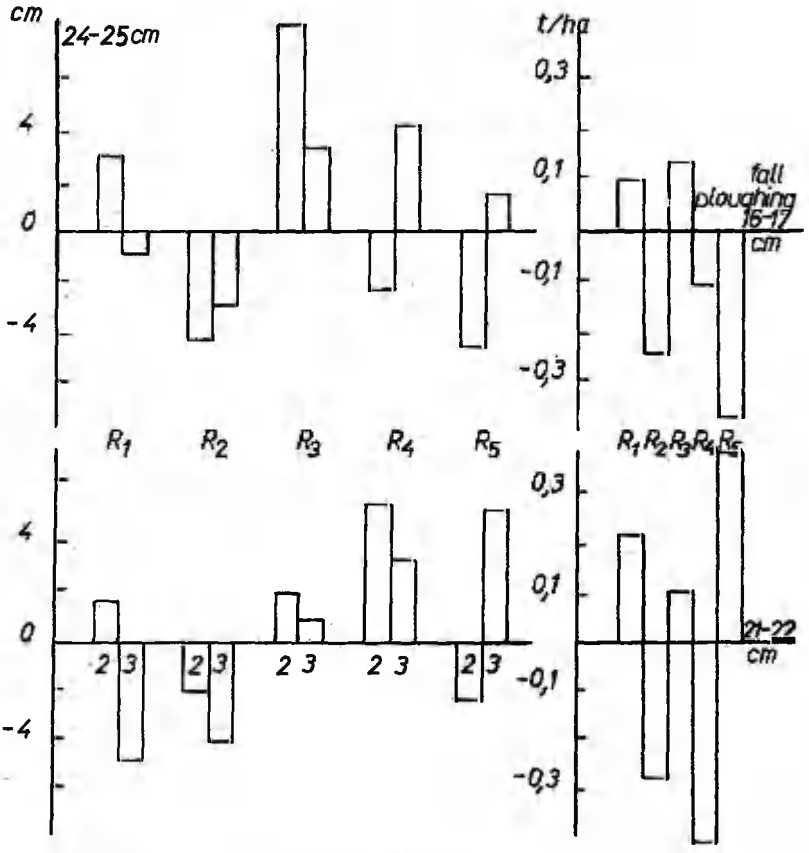

Fig. 4. The length of the leading stalk and grain yield of a barley plant in case of different ploughing depths and crop rotations in 1985 (comparing with the depth of ploughing 24-25cm-control).

largest leaf areas occur at the booting stage in all crop rotations (except R-2), comparing deep ploughing with the shallow one, the leaf area increased by 24.7...44.4 %, and in comparison with the medium one - by 36.0 % (Fig. 2) in case of R-4. Deeper ploughing also contributes to the formation of chlorophyll: under the influence of R-1, R-2 and R-4, the greatest amounts of chlorophyll (2.4...3.0 mg g^{-1} fresh mass) are formed at reaching the booting stage. In comparison with medium-deep ploughing, there is a probable increase in the formation of chlorophyll of 17.2 % in case of R-4, and of 15.4 % in case of R-5. Fig. 4 shows that deep ploughing as compared to the shallow one increases the length of the leading stalk of barley at the stalking stage under the effect of R-3 by 6.5...21.8 %, and at the booting stage under the influence of R-3, R-4 and R-5 by up to 4.1 %. Comparing the ploughing depth of 21...22 cm with that of 24...25 cm, the latter is likely to ensure an increase in the length of a stalk of 13.1 % at the stalking stage under the influence of R-4, and at the booting stage - of 5.4 % under the effect of R-4 and R-5. A probable increase in yield is obtained in case of R-1, R-3 and R-5 (0.015...0.033 t/ha) by the shallow ploughing as compared to that of 21...22 cm (Fig. 4, tab. 1, p = 0.05).

Table 1

The influence of ploughing depths on the grain yield of barley (t/ha) in different rotations in 1985

Ploughing depth cm	Rotations				
	R_1	R_2	R_3	R_4	R_5
16 - 17	3.62	4.58	4.20	3.82	4.10
21 - 22	3.50	4.59	4.32	4.26	3.33
24 - 25	3.78	4.30	4.32	3.71	3.72

p = 0.05 $K_{0.95}$ = 0.014

To sum up, the ploughing depth of 24...25 cm secures a healthy air and water regime in the soil (i.e. conditions of growth) and due to that the growth indices are also better: at the end of the vegetation period, an increase in dry mass, leaf area, chlorophyll content of a leaf, length of the main stalk and the yield of grain increase. The best conditions are ensured by R-1, R-4 and R-5. The reason is that the preceding crops - mixed crop, potato and rye well enriched with organic matter - increase the fertility of soil and, thus, favour the growth and development of barley.

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THE INFLUENCE OF AGROCOMPLEXES ON THE GROWTH OF OATS

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It should be mentioned that in Estonia, oats is a rare culture in a rotation. But at the same time, due to its fodder qualities, oats is a better feed for cattle than barley. Proceeding from the structure of grain crops cultivation, the growing area of oats should be increased on account of barley (LOKO, 1987).

To obtain a high and qualitative yield, it is of great importance that the growth conditions of oats were optimum at its every stage of growth and development. In the system soil-plant-agricultural methods, the central place is occupied by the plant. Its requirements must be met by changing its growth conditions.

To clarify the influence of different soil treatments and rotations on the growth and grain production of oats (variety 'Selma') a research into the growth dynamics of oats was carried out as a part of a long-time experiment in 1986.

Material and methods

The experiment included the following rotations:

	1982	1983	1984	1985	1986
R-1	mixed crop	early barley	rye	barley	oats
R-2	field grass	field grass	rye	barley	oats
R-3	early barley	black follow	rye	barley	oats

and variants:

1 - in autumn, shallow ploughing with a heavy disk harrow at a depth of 10...12 cm, and after that, ploughing at a depth of 16...17 cm;

2 - the same as in case of var. 1, fall ploughing at a depth of 21...22 cm;

3 - the same as in case of var. 1, fall ploughing at a depth of 24...25 cm;

4 - in autumn, shallow ploughing with a moulboard stubble breaker at a depth of 10...12 cm and fall ploughing at a depth of 16...17 cm.

The total area of leaves, the dry mass content of the aboveground phytomass, the total stalk height and the grain yield of oat plants were determined. The determinations were carried out at the tillering, stalking and booting stages. 50 plants were taken from each replication (4), thus all in all 200 plants were analyzed. The data on weather conditions during the experiment were presented in the previous paper. The results of the experiment were given in Tab. 1, 2 and 3. They have been treated mathematically with the probability of $p = 0.05$.

Results

According to the data given in Tab. 1, the moulboard stubble breaking as compared with common disk harrowing considerably increases the growth indices of oats: the leaf area increases by 2.9...17.8 cm^2 during the whole vegetation period, stalk height by 2.7...12.6 cm, dry mass yield at the booting stage by 3.8 % in case of R-1 and R-2, and grain yield by up to 0.53 t/ha (Tab. 2). Tab. 1 and 2 give results obtained by comparing the effectiveness of different ploughing depths of disk harrowing: the ploughing depth of 24...25 cm as compared with that of 16...17 cm increased the leaf area at the beginning of growth in case of R-1 and R-2 by 2.6 cm^2 and 7.6 cm^2 respectively, the dry mass yield by up to 7.0 % in case of R-2 and grain yield presumably only in case of R-3.

Tab. 3 shows a comparison of R-1 with the other rotations. In case of R-2 and R-3, we can see no essential improvement in the growth indices of oats. Our ultimate object is to ensure an increase in yield but in case of R-2, the increase in the stalk height is not enough to ensure it.

According to the experiment carried out, better growth conditions for the development of oats were secured by R-1 (barley abundant in peas, i.e. a mixed crop four years ago, then early barley, rye, barley, and, finally, oats).

It is expedient to vary the depth of fall ploughing in rotations every year. The ploughing depth of 21...23 cm is in most cases suitable for grain crops (VIPPER, 1987).

According to KOVALEVICH (1959), high yields of oats can be obtained on any soil after the cultivation of potato, maize and forage beet.

Table 1

The influence of agrocomplexes on the growth indices of oats ('Selma') in 1986

Variants	Way of soil treatment		R_1					R_2					R_3				
			Leaf area			Stalk height		Leaf area			Stalk height		Leaf area			Stalk height	
	Way of shallow ploughing	Fall ploughing cm	A	B	C	B	C	A	B	C	B	C	A	B	C	B	C
1	disk harrow	16-17	65.2	47.0	58.7	15.5	54.6	64.5	49.5	58.1	16.7	53.0	71.1	44.1	49.2	16.0	47.9
2	disk harrow	21-22	+1.5	-4.8	-8.3	+0.9	-2.9	+2.2	+2.4	+5.0	-1.4	+2.9	-5.3	+5.1	-0.7	+0.2	-0.1
3	disk harrow	24-25	+2.6	+1.1	+6.6	+2.4	-3.6	-5.8	+7.6	+8.1	-2.1	+2.4	-3.3	+12.1	+7.6	-1.1	+0.2
4	mouldboard stubble breaker	16-17	+2.9	+6.0	+17.8	+3.5	+8.6	-2.9	+4.2	+13.2	+2.7	+12.6	-0.5	+4.1	+8.6	+4.4	+4.7
	p = 0.05;	$K_{0.95}$ =	2.1	3.6	6.2	2.9	3.4										

A - tillering
B - stalking
C - booting

Table 2

The influence of agricomplexes on the dry mass dynamics and grain yield of oats in 1986

Variants	Way of soil treatment		R_1				R_2				R_3			
	Way of shallow ploughing	Fall ploughing cm	of dry mass % A	B	C	grain yield t/ha	of dry mass % A	B	C	grain yield t/ha	of dry mass % A	B	C	grain yield t/ha
1	disk harrow	16-17	12.5	19.2	34.5	3.56	12.2	13.8	32.8	4.09	11.6	18.3	33.7	3.47
2	disk harrow	21-22	-0.1	-0.8	+0.7	+0.03	+0.8	+3.4	+1.4	-0.14	-0.9	-1.0	+0.4	+0.24
3	disk harrow	24-25	+1.0	-2.7	+0.3	-0.04	-1.0	+7.0	+2.9	-0.18	+1.8	+2.1	+1.9	+0.59
4	mouldboard stubble breakes	16-17	+1.0	-2.8	+2.7	+0.53	-1.9	+6.5	+3.8	+0.04	+0.3	-1.1	+1.5	+0.11
	$p = 0.05$; $K_{0.95}$ =		0.9	2.2	1.9	0.40								

Table 3

The influence of agrocomplexes on the growth indices of oats at the heading stages in different rotations in 1986

Variants	Physiological indices	Rotation		
		R_1 (control)	R_2	R_3
	Dry mass %	34.5	-2.2	-0.8
	Stalk height cm	54.6	-1.6	-6.7
1	Leaf area cm²	58.7	-0.6	-9.5
	Grain yield t/ha	3.56	+0.53	-0.09
	Dry mass %	35.2	+1.5	-1.1
	Stalk height cm	51.7	+4.2	-3.9
2	Leaf area cm²	52.4	+10.7	-3.9
	Grain yield t/ha	3.59	+0.36	+0.12
	Dry mass %	34.8	+0.4	+0.8
	Stalk height cm	51.0	+4.4	-2.9
3	Leaf area cm²	52.1	+6.1	+4.7
	Grain yield t/ha	3.52	+0.39	+4.7
	Dry mass %	37.2	-1.1	-2.0
	Stalk height	63.2	+2.4	-10.6
4	Leaf area cm²	76.5	-5.2	-18.7
	Grain yield t/ha	4.09	+0.04	-0.51

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Name index

УДК 581.19

БИОХИМИЯ ОБРАЗОВАНИЯ ЛИГНИНА

Х. Мийдла. // Уч. зап. Тарт. ун-та,
вып. 845. Тарту, 1989, с. 11-23.

В статье дается краткая общая характеристика биосинтеза лигнина в высших растениях. Описываются промежуточные продукты и ферменты шикиматного пути, механизм активации фенолкарбоновых кислот и восстановления активированных соединений до соответствующих спиртов с последующей полимеризацией в лигнин.

Илл. 6. Библ. 28. На английском языке.

УДК 581.1+581.44.445

МОРФОФИЗИОЛОГИЧЕСКИЕ ПАРАМЕТРЫ ПОЛЕГАНИЯ ПШЕНИЦЫ

Х. Мийдла, Э. Паду, К. Праакли, А. Тянав. //
Уч. зап. Тарт. ун-та, вып. 845.
Тарту, 1989, с. 24-36.

Приводятся ряд биометрических параметров роста (общая длина стеблей, длина отдельных междоузлий, площадь листьев, общая масса растения) в связи с различным минеральным питанием растений.

Илл. 3. Таб. 5. Библ. 16. На английском языке.

УДК 581.19+581.131.

МЕТАБОЛИЗМ ФЕНОЛКАРБОНОВЫХ КИСЛОТ В ПШЕНИЦЕ

Х. Мийдла, М. Лийнамяэ, Х. Хелленурме. //
Уч.зап. Тарт. ун-та, вып. 845.
Тарту, 1989, с. 37-52.

Дается краткий обзор современных представлений о физиологическом значении фенолкарбоновых кислот в высших растениях. Рассматривается метаболизм фенолкарбоновых кислот в различных условиях минерального питания в связи с полеганием пшеницы.

Илл. 5. Таб. 3. Библ. 31. На английском языке.

УДК 581.19+581.131

РЕГУЛЯЦИЯ ОБРАЗОВАНИЯ ЛИГНИНА В СТЕБЛЯХ ПШЕНИЦЫ

Х. Мийдла, Э. Паду, А. Тянав. //
Уч. зап. Тарт. ун-та, вып. 845.
Тарту, 1989, с. 53-68.

В статье приводятся данные о регулирующем влиянии минеральных элементов на образование лигнина и на его химический состав - количество ванилина и сиреневого альдегида и степень метоксилирования отдельных компонентов лигнина.

Илл. 5. Таб. 1. Библ. 26. На английском языке.

УДК 581.19+577.152.193.

АКТИВНОСТЬ И ИЗОФЕРМЕНТНЫЙ СОСТАВ
РАСТВОРИМОЙ И СВЯЗАННОЙ ПЕРОКСИДАЗЫ В ПШЕНИЦЕ

Э. Паду, Х. Мийдла, М. Саллум. //
Уч. зап. Тарт. ун-та, вып. 845.
Тарту, 1989, с. 69-84.

Приводятся данные об активности и изоферментном составе растворимой, ионно- и ковалентносвязанной пероксидазы в стеблях пшеницы. Характеризуется изменение активности и изоферментного состава свободной и связанной пероксидазы в течение вегетационного периода в нижнем и верхнем междоузлиях стебля пшеницы. Выдвигается предположение о различной функциональной роли различных изоферментов пероксидазы в регуляции метаболизма в растениях пшеницы.

Илл. 2. Таб. 3. Библ. 36. На английском языке.

УДК 581.19+577.152.193

АКТИВНОСТЬ ФЕНИЛАЛАНИН АММОНИАК-ЛИАЗЫ И ПЕРОКСИДАЗЫ И НАКОПЛЕНИЕ ФЕНОЛЬНЫХ СОЕДИНЕНИЙ В ПШЕНИЦЕ В РАЗЛИЧНЫХ УСЛОВИЯХ МИНЕРАЛЬНОГО ПИТАНИЯ

Э. Паду, Л. Мейнер, Р. Сельгис. //
Уч. зап. Тарт. ун-та, вып. 845.
Тарту, 1989, с. 85-108.

Приводятся данные об изменении активности фенилаланин аммониак-лиазы и свободной и связанных форм пероксидазы в стеблях пшеницы в течение онтогенеза растений. Характеризуется содержание этанолрастворимых фенольных соединений и лигнина в растениях, выращенных в разных условиях минерального питания. Обсуждается роль фенилаланин аммониак-лиазы и пероксидазы в биосинтезе растворимых и локализованных в клеточных стенках фенольных соединений.

Илл. 4. Таб. 8. Библ. 40. На английском языке.

УДК 581.1+581.4

ГИСТОХИМИЧЕСКИЕ МЕТОДЫ ПРИ ОПРЕДЕЛЕНИИ ПРОЦЕССА ЛИГНИФИКАЦИИ

Х. Мийдла, А. Хеллемурме, А. Тянав, Р. Тянав. //
Уч. зап. Тарт. ун-та, вып. 845.
Тарту, 1989, с. 109-116.

Описываются гистохимические методы определения локализации лигнификации в анатомических срезах стебля пшеницы в связи с минеральным питанием и полеганием.

Илл. 6. Таб. 1. Библ. 8. На английском языке.

УДК 581.1+581.14.143

ВЛИЯНИЕ УСЛОВИЙ РОСТА НА РАЗВИТИЕ ЯЧМЕНЯ

Т. Тийдт. // Уч. зап. Тарт. ун-та, вып. 845.
Тарту, 1989, с. 117-125.

Характеризуется влияние ротации полевых культур при помощи биометрических и физиологических показателей роста и развития растений ячменя.

Илл. 4. Таб. 1. Библ. 6. На английском языке.

УДК 581.1+581.14.143

ВЛИЯНИЕ АГРОКОМПЛЕКСОВ НА РОСТ РАСТЕНИЙ ОВСА

Т. Тийдт. // Уч. зап. Тарт. ун-та, вып. 845. Тарту, 1989, с. 126-131.

Характеризуется влияние агрокомплексов на различные биометрические и физиологические показатели растений овса.

Таб. 3. Библ. 3. На английском языке.

УДК 581.1+581.19.

БИБЛИОГРАФИЯ ПЕЧАТНЫХ ТРУДОВ ПРОФЕССОРА ХЕИГО МИЙДЛА

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Печатные работы проф. Х. Мийдла охватывают широкий круг научных проблем. Большинство научных трудов посвящено метаболизму вторичных соединений высших растений.

На эстонском, русском, немецком и английском языках.

Ученые записки Тартуского государственного университета.
Выпуск 845.
ОБРАЗОВАНИЕ ЛИГНИНА В РАСТЕНИЯХ ПШЕНИЦЫ И ЕГО СВЯЗЬ С МИНЕРАЛЬНЫМ ПИТАНИЕМ.
Труды по физиологии и биохимии растений V.
На английском языке.
Тартуский государственный университет.
ЭССР, 202400, г. Тарту, ул. Юликооли, 18.
Vastutav toimetaja E. Padu.
Korrektor Ü. Türk.
Paljundamisele antud 04.04.1989.
MB 01444.
Formaat 60x90/16.
Kirjutuspaber.
Masinakiri. Rotaprint.
Arvestuspoognaid 10,16. Trükipoognaid 9,75.
Trükiarv 500.
Tell. nr. 39.
Hind 2 rbl.
TRÜ trükikoda. ENSV, 202400 Tartu, Tiigi tn. 78.