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CHLOROPHYLL AND CAROTENOID CONTENT IN WHEAT CULTIVARS AS A FUNCTION OF MINERAL NUTRITION

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Abstract **-** Determination of chlorophyll content as an indirect method of estimating the productivity of vegetation represents a good way to gain an understanding of the photosynthetic regime of plants. Physiological investigations of chlorophyll and carotenoid content in the uppermost internode of several wheat cultivars were carried out at the outset of the flowering phase. The dependence of chlorophyll and carotenoid content on the fertilization variant was established at that time. The tested wheat cultivars were grown under conditions of five fertilization variants. The content of chlorophyll ***a***, chlorophyll ***b***, and total chlorophyll (Chl ***a***+***b***) was measured and carotenoid content was determined on each variant. The results indicate that chlorophyll and carotenoid content depended on the presence and ratio of mineral elements in the substrate. This is demonstrated by the variant with unfertilized soil, where chlorophyll and carotenoid content in all cultivars was lowest. The variant of fertilization with N and P turned out to be most favorable. The next most favorable variant was the one with nitrogen alone, and it was followed by the variant with N and K.

Key words: Chlorophyll, carotenoid, wheat cultivars, mineral nutrition

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INTRODUCTION

Direct and indirect methods can be used to investigate primary organic production. Indirect methods are often used in practice for an approximate estimate of the value of organic production because it is fairly difficult to employ direct methods in plant communities. As indirect methods, it is possible to monitor and measure all phenomena and processes correlated with productivity (A r a n a y a *et. al.* 2003; R a y n o l d s *et al*. 2000; K o f *et al*. 2004). Chlorophyll content is one of the indices of photosynthetic activity (L a r c h e r , 1995). It is of particular significance to precision agriculture as on indicator of photosynthetic activity.

Leaf chlorophyll content varies within wide limits (from 0.05 to 0.30% of fresh matter). According to the majority of investigators, the ratio between chlorophylls ***a*** and ***b*** is 3:1. These values vary as a function of plant growth and development, the cultivar of plant in question and a number of environmental factors. The greatest chlorophyll content in plants occurs at the outset of the flowering phase, and chlorophyll is believed to take part in the process of organoganesis (S i m o v a *et al.* 2001). There is usually 4-5 mg of chlorophyll per unit of leaf surface. It should also be stressed that color of the leaves of certain cultivars and varieties is not always directly correlated with chlorophyll concentration.

Carotenoids have a very important role in photosynthesis. Biosynthesis of carotenoids in plants is a genetic characteristic, but environmental conditions also have an essential role.

Many authors have established that chlorophyll synthesis is dependent upon mineral nutrition. Mineral nutrition significantly affects the dynamics of leaf surface formation and the extent of leaf surface, which is reflected in the sum total of leaf surface, the photosynthetic potential, and pure productivity of photosynthesis. Of all macrometabolic elements, the gretest influence on development of plants in general and their leaf surface is exerted by nitrogen, whose effect is enhanced by phosphorus and to a lesser extent by potassium.

Nitrogen concentration in green vegetation is related to chlorophyll content, and therefore indirectly to one of the basic plant physiological processes: photosynthesis (Haboudane, 2002; Amaliotis *et al.* 2004; Lelyveld *et al.* 2004; Cabrera, 2004). Nitrogen is an essential element for plant growth and is frequently the major limiting nutrient in most agricultural soils (Daughtry, 2000). Phosphorus is involved in many metabolic processes essential for normal growth, such as photosynthesis. This element exert influence on stability of the chlorophyll molecule. Potassium is also essential for photosynthesis because it activates many enzymes involved in this process (Ray Tucker, 2004).

MATERIALS AND METHODS

Complex physiological investigations were carried out on material taken from a test plot of the Small Grains Research Center in Kragujevac. Used as material in the present study were organs of the uppermost internode (terminal leaf, uppermost part of stem and spike) of five wheat cultivars. The cultivars investigated were as follows: Lazarica, Studenica, Matica, KG 56 and KG 100. The material was collected in mid-May, at the outset of the flowering phase.

Experimental conditions

For analysis of chlorophyll content, samples were taken from five basic variants of soil fertilization (Table 1).

Fertilization variants	Nutrition N	Elements P_2O_5	[kg/h] K_2O
0	0	0	0
N	150	0	0
NPK	150	80	100
NP	150	80	0
NK	150	0	100

Table 1. Treatments in a long-term fertilizer experiment: 0 - unfertilized soil; N - soil fertilized with nitrogen only; NPK - completely fertilized soil; NP - soil fertilized with nitrogen and phosphorus; NK - soil fertilized with nitrogen and potassium.

Each parcel was given the same amounts of potassium ammonium nitrate (27 %), superphosphate (18 or 45 % P_2O_5) and potassium chloride (60 % KCl). Unfertilized soil belonged to the smonitza type in the process of degradation and had a weak acidic reaction with pH of 6.03 to 6.10 in water and 4.76 to 4.84 in KC1. The content of total nitrogen ranged from 0.11 to 0.15 %, while the levels of accessible phosphorus and potassium were less than 1 and from 10.3 to 11.1 mg/100 g of soil respectively (Jelić, 1994).

Processing of material

The material was processed in the fresh state immediately after collection. After fine chopping, portions weighing 0.5 g were measured off on an analytical balance. The measured-off material was then homogenized in a homogenizer with the addition of 10 ml of 80 % acetone. A primary acetone extract containing all chloroplast pigments was obtained in this way. The extract was then centrifuged at 2500 rpm for 5 min. Since the concentration of pigments was in most cases too great for reading to be performed on a spectrophotometer, the obtained extract was diluted by adding 9 ml of 80% acetone per ml of extract. The extract produced in this way was subjected to reading on a spectrophotometer. Chlorophyll content was calculated according to Wellburn (1994).

RESULTS

The content of chlorophyll *a*, that of chlorophyll *b*, and total chlorophyll content were measured and the ratio of chlorophylls *a* and *b* determined in all five wheat cultivars as a function of mineral nutrition at the outset of flowering.

Chlorophyll content in the terminal leaf

Fig. 1 presents the results of measuring chlorophyll content in the terminal leaf of wheat. The content of chlorophyll *a* is significantly greater than that of chlorophyll *b* on all variants of soil fertilization. The concentration of chlorophyll *b* is virtually equal in all cultivars regardless of the fertilization variant and fluctuates around 0.5 mg/g. The concentration of chlorophyll *a* exhibits significantly greater variation in relation to presence or absence of mineral elements in the soil.

As was expected, the lowest chlorophyll content in all cultivars was recorded on unfertilized soil (where it did not exceed 1.5 mg/g). The greatest chlorophyll content was measured on the soil fertilized with nitrogen and phosphorus (NP variant). The Lazarica, Studenica, and Matica cultivars had maximal values of chlorophyll concentration on this soil (from 2.4 to 3.2 mg/g). Among the remaning fertilization variants, the N variant was the most favorable. On this variant, the KG 56 cultivar had

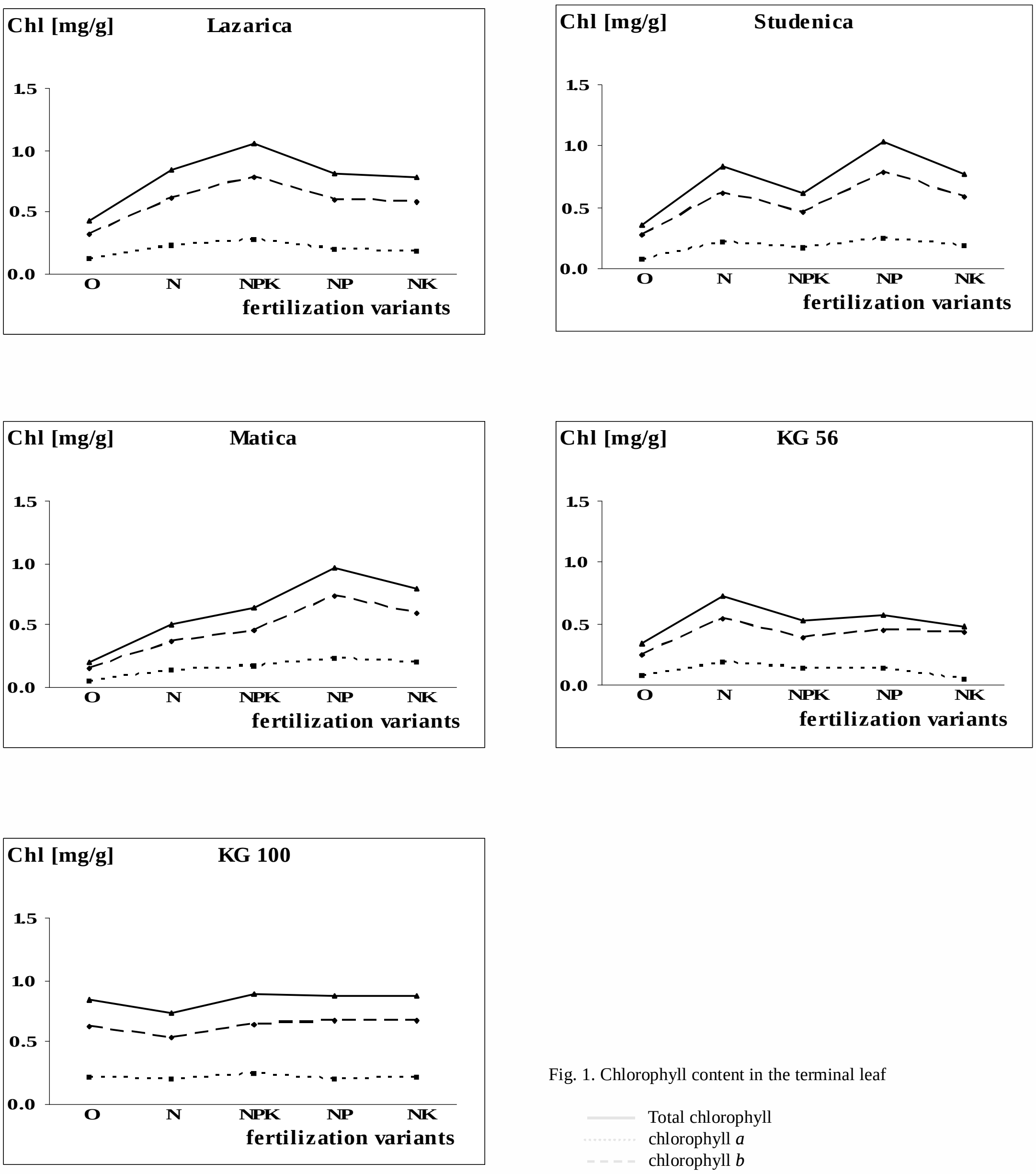


Fig. 1. Chlorophyll content in the terminal leaf

the highest value of chlorophyll content (3.0 mg/g). Except on unfertilized soil, the KG 100 cultivar had approximately equal leaf chlorophyll content on all variants of soil fertilization (2.1-2.2 mg/g).

Chlorophyll content in the uppermost part of the stem

As can be seen from the graphs presented in Fig. 2, chlorophyll content in the uppermost part of the stem at the outset flowering is significantly lower than in the terminal leaf (maximal values fluctuate around 1 mg/g). The lowest chlorophyll content in this plant organ too was measured on unfertilized soil, the investigated cultivars attaining the greatest stem chlorophyll concentration on different variants of soil fertilization. The Studenica and Matica cultivars had the greatest chlorophyll content (1.0 mg/g) on the NP variant of fertilization. In the Lazarica and KG 56 cultivars, the greatest chlorophyll content was recorded on the NPK variant and on soil fertilized with nitrogen only, respectively. The KG 100 cultivar proved

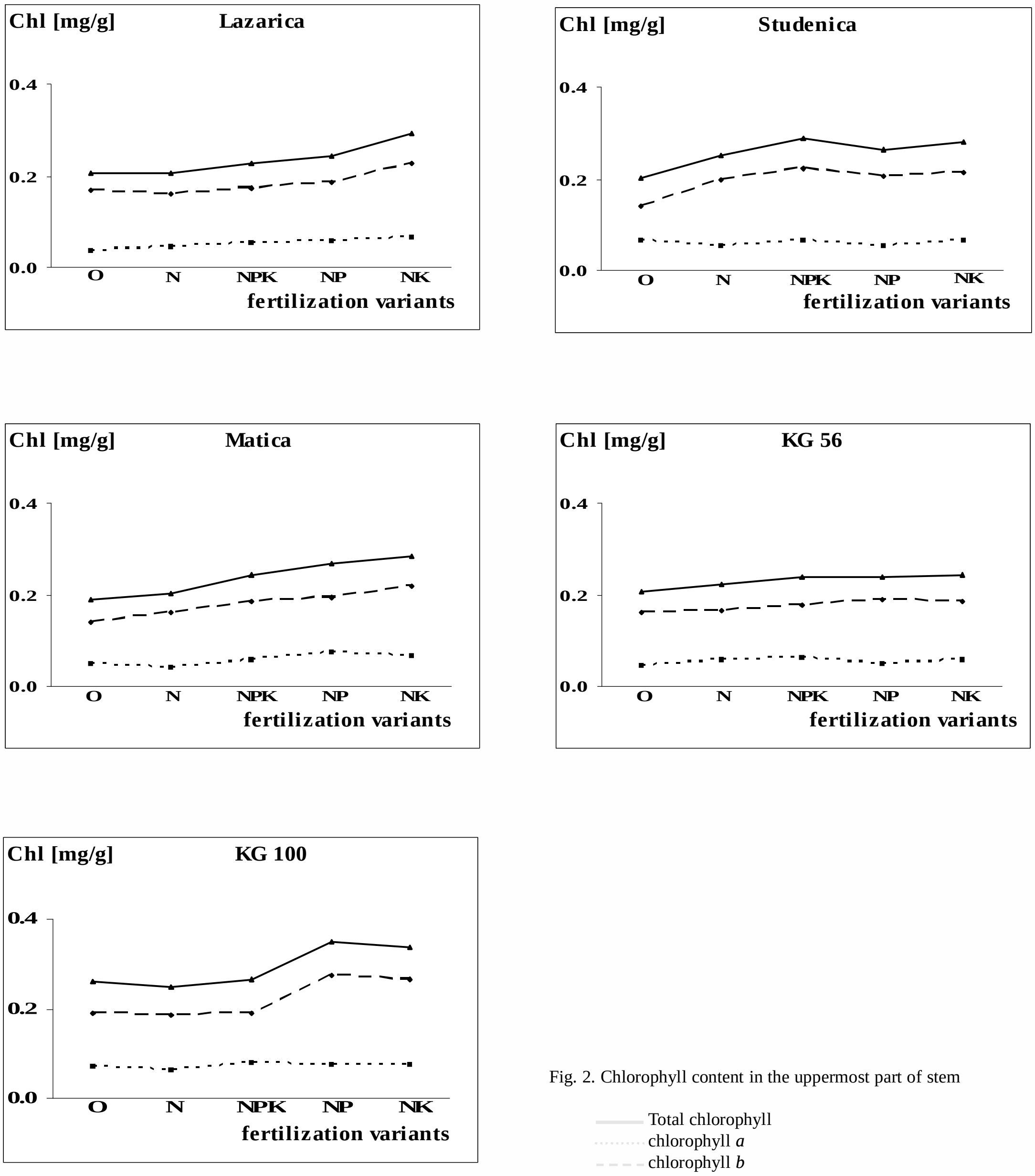


Fig. 2. Chlorophyll content in the uppermost part of stem

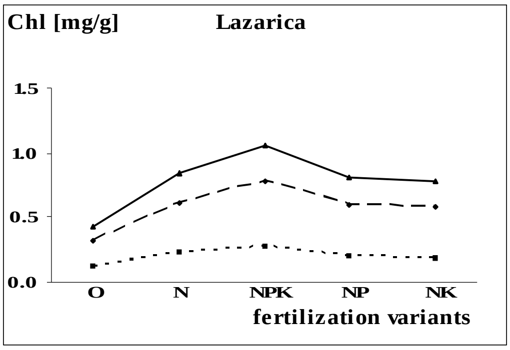
to be especially interesting in this part of the experiment, since it had approximately equal stem chlorophyll content on all variants of soil fertilization (around 0.8 mg/g).

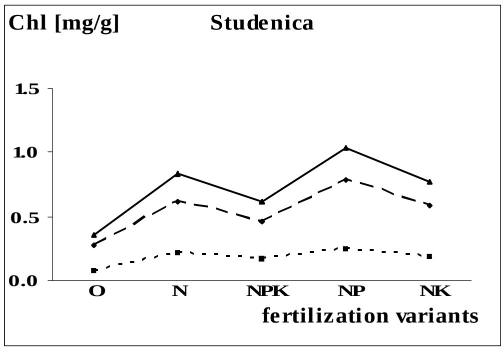
Chlorophyll content in the spike

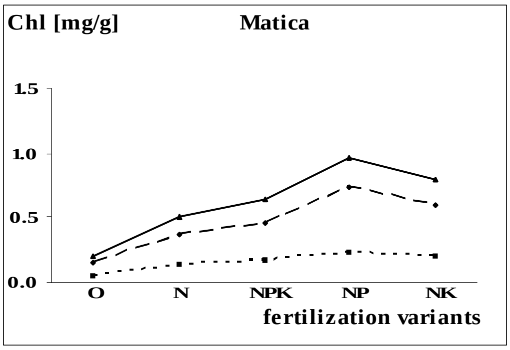
Chlorophyll content in the spike, even in the initial phase of flowering, was very low in relation to the terminal leaf and uppermost part of stem and did not exceed 0.4 mg/g (Fig. 3). Spikes of the Lazarica, Matica and KG 56 cultivars had equal chlorophyll content on all fertilization variants. Somewhat greater spike chlorophyll content was recorded on the NP variant of fertilization. On this variant the KG 100 cultivar had the greatest concentration of chlorophyll.

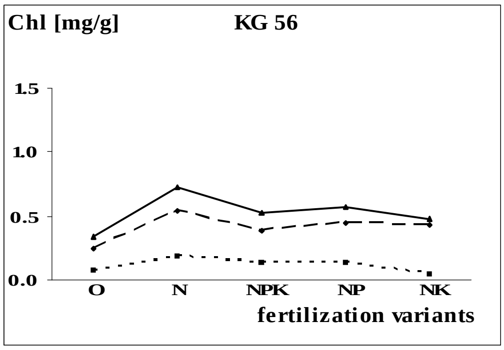
Carotenoid content

Fig. 4 shows carotenoid content in the leaf, uppermost part of the stem, and spike. The greatest values of carotenoid content were recorded in the leaf, but these values were considerably lower than values of chlorophyll









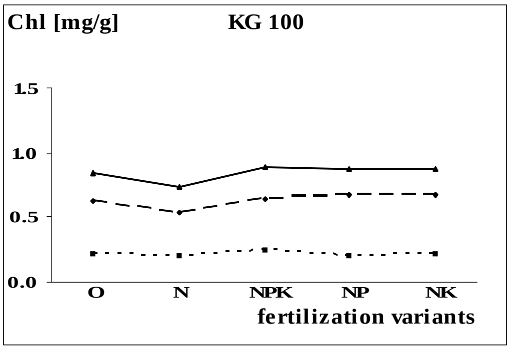


Figure 3. Chlorophyll content in the spike

Total chlorophyll
chlorophyll *a*
chlorophyll *b*

content.

The lowest carotenoid content in all cultivars was measured on unfertilized soil. On the remaining fertilization variants carotenoid content showed little variation as a function mineral nutrition. Thus, the Lazarica, Studenica, and (especially) KG 100 cultivars had approximately equal carotenoid content on all fertilization variants (between 0.4 and 0.5 mg/g). The Matica cultivar had a very high value on the NK variant (above 0.6 mg/g), while in KG 56 the greatest carotenoid content was measured on the NPK and N variants.

Carotenoid content in the stem and especially in the spike was very low (below 0.2 and 0.1 mg/g, respectively).

DISCUSSION

The greatest part of the yield of cultivated plants is known to result from work of the photosynthetic apparatus, in which the chlorophyll molecule occupies a key place. In the present study, photosynthetic pigments content was investigated in organs of the uppermost internode of wheat because wheat during grain formation and plumping is supplied with carbohydrates of current photosynthesis created for the most part by activity in organs of the given internode (the terminal leaf, spike, and

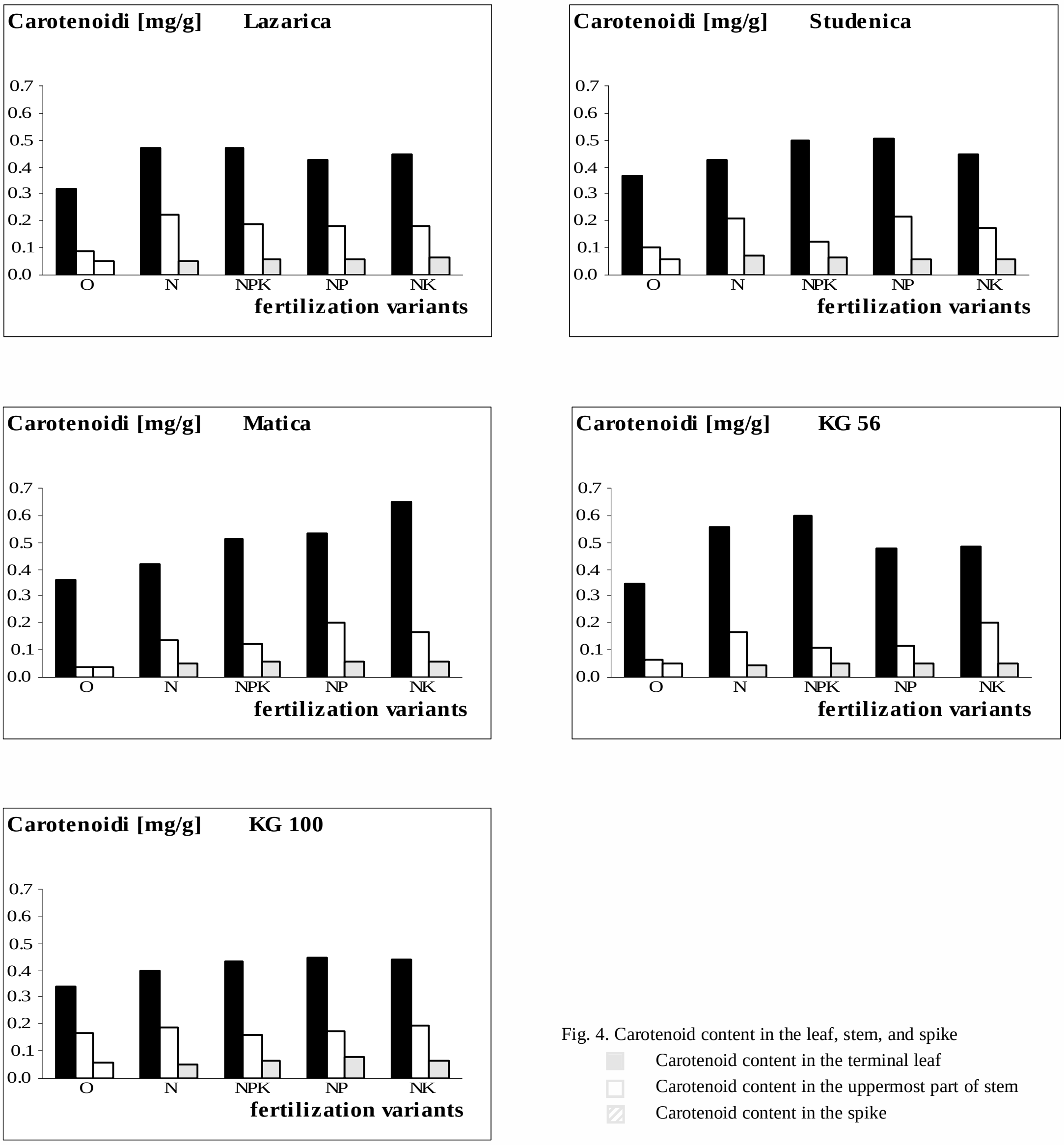


Fig. 4. Carotenoid content in the leaf, stem, and spike
Carotenoid content in the terminal leaf
Carotenoid content in the uppermost part of stem
Carotenoid content in the spike

uppermost part of the stem). The leading role in these processes is ascribed to the terminal or flag leaf (Đ o k i ć , 1999).

Winter wheat consumes relatively large amounts of mineral elements in the course of its vegetation. Of all macrometabolic elements, wheat consumes nitrogen in the greatest quantities, potassium in somewhat smaller amounts, and phosphorus in considerably smaller amounts. Of all these elements, soil lacks nitrogen and phosphorus in greater measure and potassium to a lesser extent. Sufficiency of these elements for wheat is virtually non-existent today. An especially small share of the main nutritive elements is in forms readily accessible to the plant. For this reason, wheat responds very positively to application of mineral fertilizers. In the last years, an enormous body of experimental data has been accumulated on the mineral nutrition of wheat (Evans, 1989; N e m e t h *et al.* 2000; P e p o , 2000; A m a l i o t i s , 2004; C e c c h i n , 2004; R a y T u c k e r , 2004).

The greatest amounts of chlorophyll and carotenoids in the flowering phase in all cultivars on all fertilization variants were recorded in the terminal leaf, a considerable smaller quantity was registered in the uppermost part of the stem, and the smallest amount was recorded in the spike. The uppermost part of the stem is an auxiliary organ in the process of photosynthesis, owing to the presence of chloroplasts in cells. However, this part has fewer chloroplasts than in leaves and (as expected) contains smaller amounts of chlorophyll and carotenoids. The main function of the spike lies in the fact that grains are formed and undergo ripening in it. However, the spike at the very outset flowering is green, which means that its cells contain chlorophyll. But the amount of chlorophyll in the spike is very small and does not exceed 0.4 mg/g.

The NP fertilization variant (soil to which nitrogen and phosphorus was added) was the most favorable variant for leaf chlorophyll content. This is in keeping with published data indicating that nitrogen and phosphorus exert the greatest influence on chlorophyll content. Nitrogen is a structural element of chlorophyll and protein molecules, and it thereby affects formation of chloroplasts and accumulation of chlorophyll in them (Ray Tucker, 2004; Daughtry, 2000). The influence of phosphorus on formation of green pigments in the leaf depends primarily on its concentration. Phosphorus affects the stability of chlorophyll in plants, especially with the advent of unfavorable weather conditions in the fall. In some cultivars, the greatest chlorophyll content was measured in leaves of plants that grew on soil fertilized with nitrogen only. Even though nitrogen is the most important mineral element in the process of chlorophyll biosynthesis, adding nitrogen to the soil can have negative as well as positive effects, since an excess of nitrogen shortens the life of leaves, increases their sensitivity, and lowers their resistance to plant diseases, which leads to decrease of leaf chlorophyll content. The NK variant was unfavorable because chlorophyll content is known to increase in the presence of a phosphorus deficit. Phosphorus deficiency inhibits plant growth and chlorophyll synthesis, which gives plants experiencing it a dark-green color. It is interesting that not one cultivar had high chlorophyll concentration on the NPK variant. This can be attributed to the fact that may be explain, Cl from KCl (which was added to the soil) can have negative effects on the photosynthetic apparatus in plants.

Thus, the content of chlorophyll content and levels of other leaf biochemical constituents can be used as indicators of crop stress under conditions of nutritional deficiencies (Tejada-Zarco, 2004). Such deficiencies leading to crop chlorosis can be allaviated through application of fertilizers, thereby improving yields and the final crop quality (Cordeiro *et al.* 1995; Chova *et al*. 2000). Increase in the yield of wheat where mineral fertilizers are used primarily results from enlargement of the photosynthetic apparatus, increase in the rate of its formation, lengthening of the plants' lifetime, and increase of photosynthetic activity. Application of mineral fertilizers also promotes better utilization of assimilates in metabolic and growth processes.

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САДРЖАЈ ХЛОРОФИЛА И КАРОТЕНОИДА КОД ПШЕНИЦЕ У ЗАВИСНОСТИ ОД МИНЕРАЛНЕ ИСХРАНЕ

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Одређивање садржаја хлорофила, као . једна од индиректних метода за оцену биљне продукције, представља добар пут за разумевање фотосинтетичкког режима биљака.

Физиолошка истраживања садржаја хлорофила и каротеноида у органима вршне интернодије код пет сорти пшенице обављена су у фази цветања. Том приликом утврђивана је зависност садржаја хлорофила и каротеноида од варијанте ђубрења земљишта, односно од минералне исхране. Испитиване сорте су гајене на пет варијанти ђубрења, при чему је на свакој варијанти мерен садржај хлорфила *a*, хлорофила *b*, укупног хлорофила и садржај каротеноида. Испитивањем су били обухваћени терминални лист, вршни део стабла и клас.

Резултати истраживања су показали да је садржај хлорофила и каротеноида значајно зависио од присуства и међусобног односа хранљивих елемената у подлози. Доказ за то је нeђубрено (контролно) земљиште, на коме је садржај фотосинтетичких пигмената био најмањи. Најповољнија је била варијанта ђубрења азотом и фосфором, на којој је већина сорти имала највећи садржај хлорофила, затим варијанта ђубрења само азотом, а онда варијанта којој је ђубрењем додаван азот и калијум. Интересантно је да потпуно ђубрена варијанта земљишта (NPK), супротно очекивањима није испољила већи ефекат на садржај фотосинтетичких пигмената.