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Remobilization of nitrogen for wheat grain formation as affected by temperature and drought

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Abstract Collection of eleven cultivars of winter wheat plants were cultivated in field experiments in Prague-Ruzyně in growing season 2004/2005 and 2005/2006. Two levels of N fertilization (110 and 160 kg N per ha) were used. At three terms during the period of grain formation (beginning of flowering, 15 and 25 day after flowering) wheat plants were harvested and flag leaves were analyzed for contents of chlorophyll, soluble protein, total N content and activity of nitrate reductase. Higher temperature and lack of precipitation during phase of grain filling (2006) induced earlier senescence of flag leaves. In all cultivars this process was succeeded by rapid lowering of nitrate reductase activity, decrease in total nitrogen content, soluble protein content and faster chlorophyll degradation.

Key words: *wheat, grain formation, nitrogen remobilization*

Introduction

Nitrogen use for wheat yield and its quality depends on many endogenous factors and environmental conditions. It is known, that yield of grain crops integrates two main components: grain number per m^2 and average grain weight. Fisher (1985) showed that critical period determination of grain number spans about 20 days before anthesis. Next studies extended this critical period from ca. 20 days before anthesis to ca. 10 days after anthesis. On the other hand it has been generally accepted that grain weight is determined in the period between anthesis and physiological maturity when grains actually grow (for review see Urgarte et al. 2007). Main sources of assimilates for grain formation are originated from metabolic activity of leaves, temporal reserves in uppermost part of stem and remobilization from senescing leaves. It is known, that about 80 % of the total N content accumulates at anthesis and it accounts for 50 – 100 % of the total N content of the wheat grain (Gebbing, Schnyder 1999). In vegetative organs, including wheat flag leaves, the period of grain filling is characterized by transition from sink to source status (Kichey et al., 2006). Stress conditions, high temperature or water deficit, during the grain filling period show many negative effects such as inhibition photosynthetic process and acceleration of leaf senescence. Both heat and water stresses increase the rate of grain filling but they shorten the duration of this period (Tahir, Nakata, 2005). Moreover, increased grain-filling rate at mild water deficit can be attributed to the enhanced sink activity by regulating key enzymes involved in sucrose-to-starch conversion (Yang et al., 2004).

Material and methods

Two collections of selected early and late common wheat (*Triticum aestivum* L.) cultivars were grown in field experiments in growing season 2004/2005 and 2005/2006. Chosen registered cultivars were sown at Crop Research Institute (Praha – Ruzyně, Czech republic; luvisoil, average year temperature 8.4°C, average year precipitation amount 478 mm). High N - level plots received 160 kg N ha^{-1} in three applications (60 kg at tillering, 60 kg at the beginning stem elongation and 40 kg at the second-node stage). Low N – level plots received 110 kg N ha^{-1} (50 kg at tillering and 60 kg at the beginning stem elongation). Fungicide, herbicide and insecticide were used to achieve good control of parasites.

In 2005 growing season was rather warm with high precipitation, the year 2006 was characterized by unusually hot and dry weather during period of grain formation (table 1, Fig. 1).

At the beginning of flowering wheat plants in same development phase (first anther are just visible) were marked.
Flag leaves of marked plants were harvested at three terms during grain formation (beginning of flowering, 15 and 25 day after flowering). They were weight, dried at 70°C and/or stored at -80°C. Flag leaves were analyzed for contents of chlorophyll, soluble protein, nitrate and total N concentration and activity of nitrate reductase.

Table 1 Average temperature and precipitation amount in years 2005, 2006 and in long-time average

month	1	2	3	4	5	6	7	8	9	10	11	12	year
temperature (°C)													
average	**-1.2**	**-0.2**	**3.8**	**7.9**	**13.3**	**16.2**	**18.1**	**18.1**	**13.7**	**8.6**	**3.0**	**0.4**	**8.5**
2005	1.2	-2.5	2.6	10.5	14.4	17.3	19.2	17.3	15.7	10.5	3.3	0.6	9.2
2006	-4.3	-0.9	2.2	9.8	14.2	18.1	22.9	16.6	17.4	11.3	6.6	3.6	9.8
precipitation (mm)													
average	**21.7**	**19.8**	**27.7**	**29.0**	**63.4**	**66.9**	**67.8**	**61.8**	**36.0**	**29.0**	**30.0**	**24.0**	**477.1**
2005	26.4	31.4	11.6	12.2	90.4	68.2	139.6	66.2	42.2	12.2	10.8	21.8	533.0
2006	7.0	14.2	35.6	67.8	99.0	92.8	19.6	99.4	6.6	31.2	10.2	13.4	496.8

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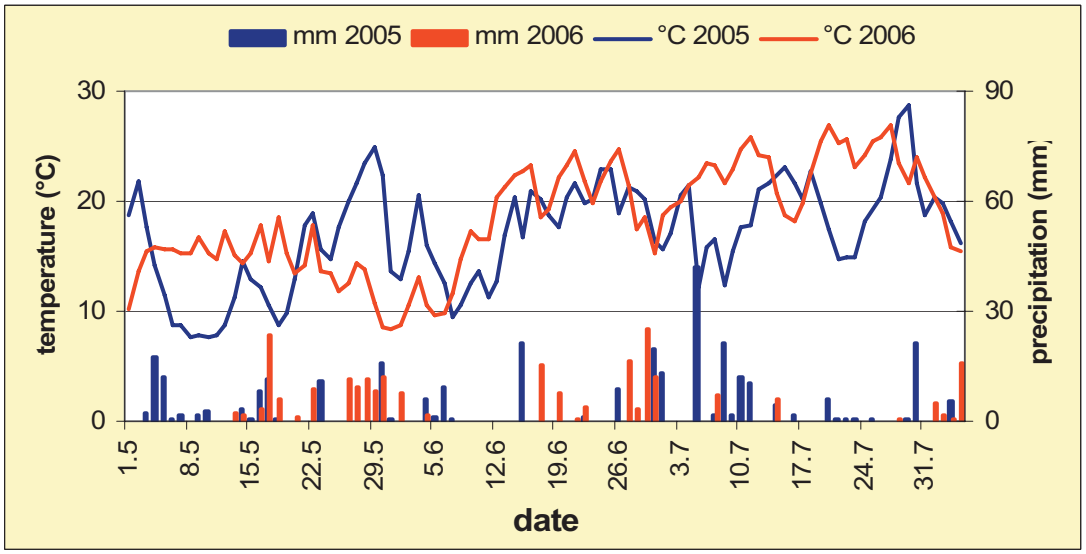

Fig .1 Average temperatures and precipitation amount in during stage of grain formation in 2005 and 2006

Determination of chlorophyll content
Chlorophyll content in frozen leaves (mg g^{-1} FW) was estimated spectrophotometrically using method No 942.04 AOAC Official methods of analysis (1990).

Determination of nitrate reductase activity
Leaf samples (1 g FW) were homogenized in liquid nitrogen and extracted in 5 ml of 50 mM Tris-HCl buffer (pH 8.0) containing 3% (w/v) bovine serum albumine at 4°C for 30 min. Insoluble material was removed by centrifugation (15000 g, 30 min). The 0.9 ml reaction mixture consisted from 0.5 ml 0.1 M phosphate buffer pH 7.5, 0.1 M KNO_3, 0.15 ml enzyme extract, and 0.15 ml 0.2 % (w/v) $NADH^+$. After incubation for 10 min at 25°C the reaction was terminated by addition of 0.1 ml 0.03M oxaloacetic acid. Formation of azo dye was measured at 540 nm. NR activity was expressed as the rate of generated NO_2^- (µmol g^{-1}FW min^{-1}).

Determination of total nitrogen content

Dried ground samples of plant tissues were mineralised with sulphuric acid and selenium as catalyst. Total nitrogen content was determined spectrophotemetrically using SKALAR San Plus System analyzer and Bertholet reaction.

Determination of soluble protein content

Leaf samples (0.5 g FW) were homogenized in liquid nitrogen and extracted in 5 ml of 50 mM Tris-HCl buffer (pH 7.5) at 4°C. Insoluble material was removed by centrifugation (15000 g, 30 min). Protein content in leaf extracts was estimated using Comasie brilliant blue (Bradford 1976).

Results

At more favourable weather in 2005 the beginning of flowering of early cultivars was observed on the 1st June, flowering of the late cultivars started six - seven days later. In 2006 wheat flowering started two weeks later (13th June) and it followed after dry period (Fig. 1). Under unusually hot weather (maximum temperature near 30 °C) the difference in anthesis between early and late cultivars was only three days.

Chlorophyll content

In many cases chlorophyll content of flag leaf was the highest 15 days after anthesis. More significant differences in chlorophyll content between years 2005 and 2006 were observed at low nitrogen treatment (LN) in early wheat varieties. In 2006 at two nitrogen rate chlorophyll content of both early and late cultivars decreased quickly between 15th and 25th day after anthesis.

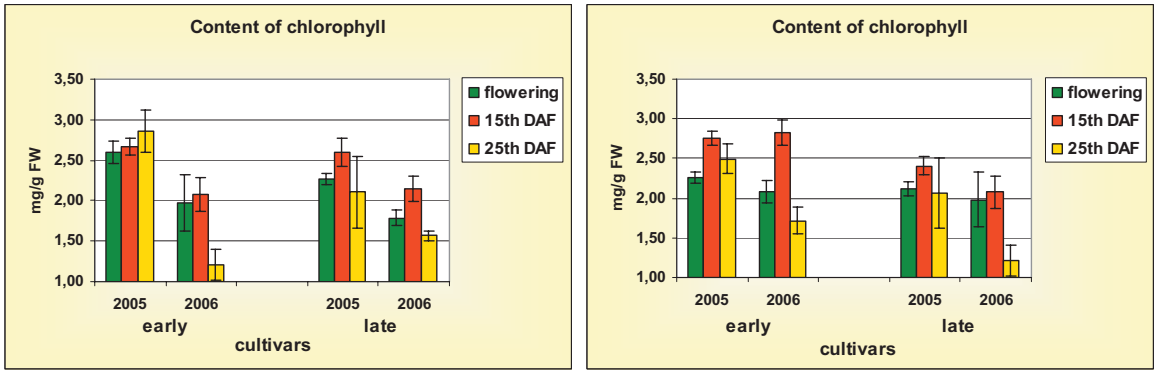

Fig. 2 Average chlorophyll content in flag leaf of wheat cultivars in two growing season
DAF: days after beginning of flowering, left part: LN – lower rate of nitrogen fertilization, right part: HN – higher rate of nitrogen fertilization

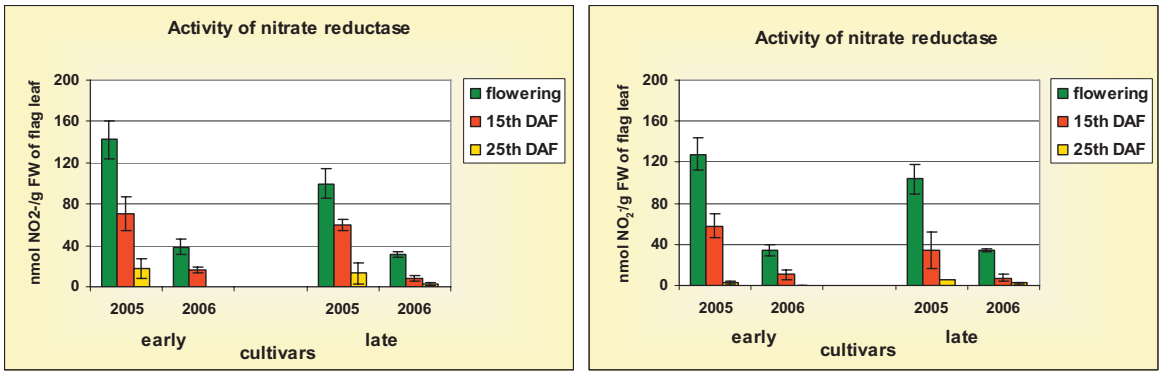

Fig. 3 Activity of nitrate reductase in fresh flag leaf of wheat in two growing season
DAF: days after beginning of flowering, left part: LN – lower rate of nitrogen fertilization, right part: HN – higher rate of nitrogen fertilization

Activity of nitrate reductase

The most significant differences were found in nitrate raductase activity of flag leaves (Fig.3). Unusually high enzyme activity at anthesis in 2005 was conditioned by sufficient supply of mineral nitrogen and water in soil (data not shown). In comparison with year 2006 it was over twice higher and detectable activity was observed 25 days after beginning of flowering, High nitrate reductase activity of early cutivars was characterized by faster decreasing during grain formation. At late phase of grain filling in both years there were found great differences within both early cultivars group and late cultivars group.

Content of soluble proteins

In LN treatment content of soluble protein in flag leaf (Fig. 4) was similar in 2005 and 2006 years, at the anthesis it ranged between 35 – 40 mg per g of fresh leaf (early cultivars) or approached to 30 mg (late cultivars). It more quickly decreased during period of grain formation in season 2006. In HN treatments at more favourable conditions (2005) initial soluble protein content of both early and late cultivars slowly increased up to15th day after beginning of flowering (DAF).

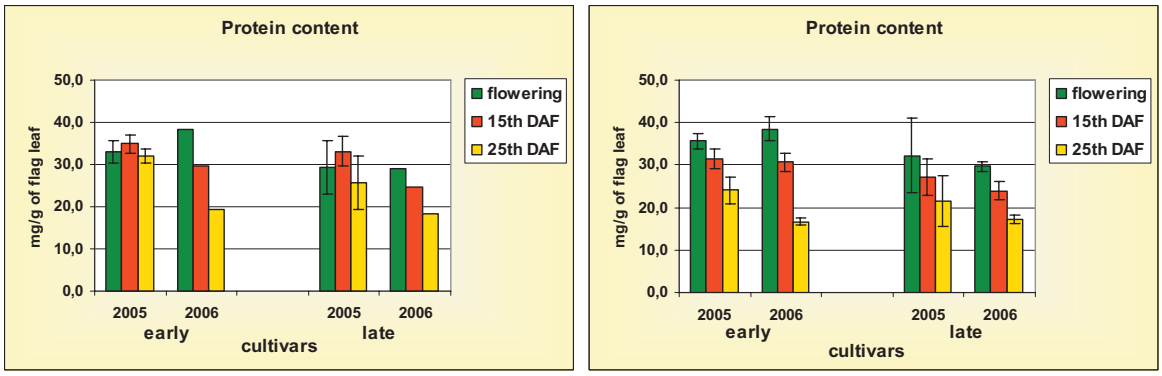

Fig. 4 Average content of soluble proteins in fresh flag leaf of wheat in two growing season
DAF: days after beginning of flowering, left part: LN – lower rate of nitrogen fertilization, right part: HN – higher rate of nitrogen fertilization

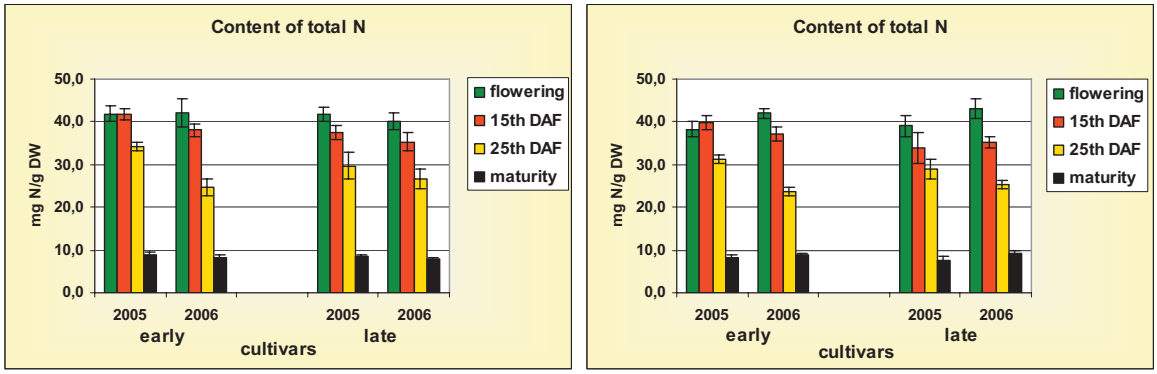

Fig. 5 Concentration of total nitrogen in dry matter of wheat flag leaf in two growing season
DAF: days after beginning of flowering, left part: LN – lower rate of nitrogen fertilization, right part: HN – higher rate of nitrogen fertilization

Total nitrogen content and N remobilization

The lowest differences were found in total nitrogen content in dry matter of flag leaf. In the most cases it reached its maximum at the beginning of flowering (Fig. 5) and slowly declined during the early phase of grain filling. The only exception was group of early cultivars in the year 2005 with almost unchanged N concentration. Main part of leaf nitrogen was remobilized at late phase of grain filling.

Discussion

Early stage of grain formation (to 10 – 12 days after pollination when cells of endosperm and plastids are differentiated) is characterized by high metabolic activity of flag leaves. *S*ynthetised assimilates are temporally stored in the last (uppermost) internode. At this time chlorophyll content increased, mainly in early cultivars. Chlorophyll degradation started more early in late cultivars; in both cultivar groups it was accelerated by higher temperature and drought. In comparison with activity of nitrate reductase or total nitrogen decrease of chlorophyll content was delayed.

Wheat plants grown in nutrient solution in controlled conditions reach the maximum of nitrate uptake capacity at the beginning of flowering. During following period of grain formation, even at sufficient nitrate supply, nitrate uptake sharply decrease (Trčková and Kamínek, 2000). In field experiment the nitrate reductase activity can be viewed as a marker of plant ability to utilize soil nitrogen (nitrate). In our experiments major dependence of nitrate reductase activity upon environmental conditions (drought and high temperature) was observed at early cultivars.

At anthesis about 45 % total nitrogen of wheat plant is localised in leaves and this amount increase to 50 % at insufficient water supply (Stehno *et al*, 2005). Main part of leaf nitrogen (or leaf protein) is represented by enzymes, in particular by Rubisco. Therefore effective remobilization of leaf nitrogen is related to leaf senescence. The leaf senescence is a programmed process representing the final phase of leaf development. In addition to chloroplast disintegration, decline in photosynthesis, proteins and nucleic acids it also includes mobilization and recycling of nutrients and organics from senescing leaves to developing seeds (Himelblau and Amasino, 2001). Like many other developmental processes senescence is, at least in part, under hormonal control. While ethylene, abscisic acid and methyl jasmonate promote leaf or senescence in many plants, cytokinins delay senescence-associated processes including degradation of chlorophyll and chloroplast proteins (Yang *et al*., 2003).

Leaf senescence can be triggered also by a high availability of carbon relative to nitrogen. This strategy is based on programmed fast translocation of metabolites from senescing leaves, where majority of relatively easily accessible N is invested to reproductive sinks (Hörtensteiner, 2006) following development of reproductive structures. Actually, most N utilized by developing seeds of wheat is mobilized from other plant parts while the plant net NO_3^- uptake capacity sharply declines after anthesis (Trčková and Kamínek, 2000, Stehno et al, 2005). Both the process of leaf senescence and rate of grain filling were accelerated by higher temperature and lack of precipitation in July 2006.

Acknowledgements

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