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Ribulose-1,5-bisphosphate carboxylase/oxygenase content and degradation in diploid, tetraploid, and hexaploid wheat species during monocarpic senescence

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Abstract

Wheat provides a unique genetic system in which variable sink size is available across the ploidies. We characterized monocarpic senescence in diploid, tetraploid, and hexaploid wheat species in flag leaf from anthesis up to full grain maturity at regular intervals. *Triticum tauschii* Acc. cv. EC-331751 showed the fastest rate of senescence among the species studied and the rate of loss per day was highest in terms of photosynthesis rate, ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO) content, and flag leaf N content coupled with a higher rate of gain in grain N content. Cultivars Kundan and HD 4530 maintained high flag leaf N content throughout grain filling as compared to the diploids and showed a slower rate of senescence. RuBPCO content was higher in the diploids as compared to Kundan and HD 4530 at anthesis. However, the rate of decline in RuBPCO content per day was also higher in the diploids. This degradation in RuBPCO was mediated by high endoproteolytic activities in the diploids which in turn supported its higher rate of N mobilization as compared to the tetraploid and hexaploid wheat. Acidic endopeptidases were responsible for the mobilization of flag leaf nitrogen in wheat across ploidy levels ($r = –0.582$, $p<0.01$).

Additional key words: chlorophyll; endopeptidase; endoproteolytic activity; flag leaf; grain; leaf area; net photosynthetic rate; ploidy; protein.

Introduction

Leaf cells undergo a genetically programmed degradation of cellular organelles involving the loss in photosynthetic activities and hydrolysis of macromolecules such as proteins, lipids, *etc*. during grain development, a phenomenon known as monocarpic senescence (Noodén *et al*. 1997). During this degenerative phase, there is an increase in the massive remobilization of hydrolyzed compounds to meet the demands of the growing sink.

The nitrogen demand of the growing sink is fulfilled mainly by the degradation of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO) which is also the prime enzyme involved in CO_2 fixation. RuBPCO is rapidly and selectively degraded during senescence (Moreno *et al*. 1995). This degradation is mediated by the action of proteases, which in turn meets the nitrogen demand of the growing sink. Acidic endoproteases are responsible for the loss in flag leaf nitrogen in hexaploid wheat (Dalling *et al*. 1976). The regulation of proteolytic activity may be useful in improving crop productivity (Vierstra 1996). Hence, a basic knowledge about the activity of the proteases involved in RuBPCO degradation during monocarpic senescence in different crops is necessary.

There has been considerable interest in breeding crop cultivars with delayed senescence in the hope of increasing crop productivity (Nam 1997). The growing sink regulates leaf senescence (Gan and Amasino 1997). Due to the potential benefits of delayed leaf senescence in agriculture, various studies have been conducted on the source-sink manipulation in different crops of which wheat has been a favourite target (Patterson and Brun 1980, Mackown and Van Sanford 1988, Mackown *et al*. 1989, 1992, Guitman *et al*. 1991, Srivalli and Khanna-Chopra 1998, Cruz-Aquado *et al*. 1999). Physical manipulation

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Abbreviations: Chl, chlorophyll; DAA, days after anthesis; P_N, net photosynthetic rate; PAGE, polyacrylamide gel electrophoresis; PMSF, phenyl methyl sulfonyl fluoride; PVPP, polyvinyl polypyrrolidone; RuBPCO, ribulose-1,5-bisphosphate carboxylase/oxygenase; SDS, sodium dodecylsulphate; TCA, trichloroacetic acid; β-ME, 2-mercaptoethanol.
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of the sink by removal of some spikelets or by excision of 50 % ears resulted in a better N mobilization to the ear. This was due to the increased N availability to the ear as a result of an increased source-sink ratio. The removal of the ears also led to delayed senescence in wheat plants (Srivalli and Khanna-Chopra 1998).

Modern days' wheat provides a classical example of evolution through allopolyploidy (Wendel 2000) and thus provides a unique genetic system in which variable sink size is available across the ploidies. Net photosynthetic rate (P_N) and RuBPCO contents are considerably influenced by source-sink relationships, which are different in wheat across ploidies. Several studies done on the photosynthetic capacity of the various ploidy levels of wheat have shown that P_N per unit leaf area basis declined with increase in ploidy, *i.e.* from diploid to hexaploid species (Evans and Dunstone 1970, Dunstone *et al*. 1973, Austin *et al*. 1982, Carver *et al*. 1989), whereas P_N per unit cell basis increased with increase in ploidy (Jellings and Leech 1984, Warner and Edwards 1993). This was due to the larger cell size of hexaploid wheat, which resulted in a lower number of cells and chloroplasts under a unit leaf area causing a lower P_N per unit leaf area.

The present study was initiated to characterize monocarpic senescence in diploid, tetraploid, and hexaploid wheat species, which differ considerably in grain sink strength. Endopeptidase activity was also measured across ploidy levels to see if the relationship between acidic endoproteases and nitrogen mobilization holds true across different ploidy levels.

Materials and methods

Plants: Five genotypes of wheat were used in this study (Table 1). Seeds were obtained from the Division of Genetics, IARI, New Delhi, India and were sown in 60 kg pots in the net house of Water Technology Centre, IARI, New Delhi. The soil was of sandy loam type. Eight seeds were sown initially which were later thinned down and four plants were maintained per pot during the duration of the experiment. Commercial fertilizers were applied at the rate of 6 : 4 : 4 g m^{-2} of N, P, and K, respectively, as a basal dose. Diploid species were given long day treatment from 20th day after germination to spike emergence by using 60 W incandescent bulbs from a height of 1.5 m.

Sampling of the flag leaf was done for the various biochemical analyses from anthesis up to full maturity at regular intervals depending on the duration of the grain growth period (Table 1). This was at 7-d interval for the cultivated genotypes *Triticum aestivum* cv. Kundan and *T. durum* cv. HD 4530, at 6-d interval for *T. monococcum* and *Aegilops speltoides,* and at 5-d interval for *T. tauschii*.

Table 1. Details of the five genotypes of wheat used in the present study.

	Species	Cultivar	Grain growth] duration [d]	Genome	Classification
Diploid (2x = 14)	*T. monococcum* ssp. *aegilopoides* (=*boeoticum*) (Link) Thell.	ASP4	24	A	Wild
	Aegilops speltoides Tausch.	I-95-1	24	B=S	Wild
	T. tauschii (=*Ae. squarossa*) Coss.	EC-331751	20	D	Wild
Tetraploid (2x = 28)	*T. turgidum* ssp. *durum* (Desf.) Husn. Husn.	HD4530	35	BA	Pasta Wheat
Hexaploid (2x = 42)	*T. aestivum* L.	Kundan	35	BAD	Bread Wheat

Green flag leaf area was measured using non-destructive sampling. Measurement on 12 plants constituted a set and there were four such sets.

***P*$_N$** of the flag leaves was measured in the morning at 10:00–11:00 using *Licor-6200* portable photosynthesis instrument (*Licor*, USA). Three leaves constituted a sample set and there were four such sets.

For the biochemical assays, leaves were cut into small pieces after their weighing. Three replicates were used for all measurements. The leaf material was dried in the oven at 80 °C to obtain dry matter (DM). For a set of leaves, fresh matters (FM) were taken after measuring the leaf area. Contents of chlorophyll (Chl), total soluble proteins, and RuBPCO were expressed per unit leaf area.

Contents of Chl and total soluble proteins were measured according to Arnon (1949) and Lowry *et al*. (1951), respectively.

RuBPCO content: Leaf samples frozen in liquid nitrogen were ground in a mortar and a pestle with liquid nitrogen and then extracted [3 cm^3 per 0.25 g(FM)] in 30 mM Tris buffer, pH 7.8, containing 1 mM ascorbic acid, 1 mM EDTA, 5 mM $MgCl_2$, 1 mM dithiothreitol, and 0.5 mM phenyl methyl sulfonyl fluoride (Zivy *et al*. 1983). Polyvinyl polypyrrolidone (PVPP) was added at the time of grinding [0.05 kg kg^{-1}(FM)]. The extracted samples were passed through cheesecloth and centrifuged at 10 000×*g* for 20 min. To the supernatant, eight volumes of acetone were added and the protein precipitated overnight. The samples were again centrifuged at

10 000×*g* for 20 min. To the pellet, electrophoresis sample solution [125 mM Tris-Cl buffer, pH 6.8, 10 % glycerol, 5 % 2-mercaptoethanol (β-ME), and 2 % SDS] was added and boiled for 4 min. Aliquots of 20 mm^3 of the protein samples were subjected to electrophoresis on a 10 % SDS-PAGE (Laemmli 1970). In all cases 30 μg of total protein was loaded. After electrophoresis, the gel was stained with Coomassie Brilliant Blue G-250 (*Sigma Chemical Co*., St. Louis, USA) and de-stained with a methanol (40 %) and glacial acetic acid (10 %) solution. RuBPCO was quantified by scanning the gels using a laser densitometer *Ultroscan XL* (*Pharmacia LKB*).

Endopeptidase activity was measured following the modified version of Peoples *et al*. (1983). Samples frozen in liquid nitrogen were ground in a mortar using liquid nitrogen and after that suspended in 250 mM Tris-Cl buffer, pH 7.0 containing 10 mM β-ME [4 cm^3 per g(FM)]. During homogenisation, PVPP (20 kg m^{-3} final concentration) was added and the extract was centrifuged at 10 000×*g* for 20 min at 4 °C. The supernatant was collected and passed through three layers of cheesecloth. The supernatant was dialysed overnight against 25 mM Tris-Cl buffer, pH 7.0 containing 10 mM β-ME. The reaction mixture contained 100 mm^3 of crude extract, 250 $mm^3$of 250 mM sodium acetate buffer, pH 4.8 containing 10 mM β-ME and 150 mm^{-3} of RuBPCO as a substrate. After incubation at 50 °C for 1 h, the reaction was stopped by adding 1 cm^3 10 % trichloroacetic acid (TCA) solution and incubated at 4 °C for 1 h. After centrifugation at 25 000×*g* for 10 min, TCA-soluble peptides generated during the reaction were estimated at A_{340} of the supernatants. Controls were kept for zero time and without substrate. One unit of proteolytic activity was defined as an increment of 0.01 in A_{340} in 1 h.

Total nitrogen content in the flag leaves and grains was determined in dried samples by *Kjeltec 1030 Autoanalyzer* (*Tecator*, USA) after digestion in sulphuric acid.

Statistical analysis: ANOVA and mean comparisons (CRD, 2-factor analysis) were conducted using the *MSTAT* statistical analysis package (*CIMMYT*, Mexico). Differences referred to as significant represent significance at the 5 % level.

Results

The grain dry matter for the tetraploid HD 4530 was the highest followed by the hexaploid Kundan (Table 2). Amongst the diploids, *T. monococcum* had the highest grain dry matter and grain number per spike and *Ae. speltoides* had the least, with *T. tauschii* having intermediate levels (Table 2). Kundan and HD 4530 also maintained a high flag leaf N content throughout grain filling (Table 2, Fig. 1*A*). Amongst the diploids, flag leaf and grain N contents were the highest in *T. tauschii* (Fig. 1). The grain N content was lower in the diploids as compared to HD4530 and Kundan due to lower grain DM per spike (Table 2). *Ae. speltoides* had the lowest grain N content amongst all the species. The maximum N mobilisation was observed in *T. tauschii*, the flag leaf contributed almost 72 % to the spike followed *by Ae. speltoides* (Table 2). *T. monococcum,* which had the highest sink strength in terms of grain number and grain dry matter per spike amongst the diploids, showed the least N mobilised from the flag leaf (Table 2).

The cultivated genotypes Kundan and HD 4530 had a higher leaf area and longer leaf area duration as compared to the diploids (Fig. 2*A*, Table 1). There was a sharp decline in the green flag leaf area of Kundan and HD 4530 21 DAA (d after anthesis). Among the diploids, *T. monococcum* had the maximum green leaf area throughout grain development while *Ae. speltoides* had the least green leaf area amongst all the genotypes. The rate of loss in the green flag leaf area was maximum in Kundan (0.838 cm^2 per day) and minimum in *Ae. speltoides* (0.302 cm^2 per day).

Monocarpic senescence was characterized by measuring contents of Chl and total soluble proteins: Kundan maintained higher content of Chl from 14 DAA (Fig. 2*B*). The rate of Chl loss was the highest in both *Ae. speltoides* and *T. tauschii* (0.012 g m^{-2} d^{-1}). The mean leaf protein contents per unit leaf area of *Ae. speltoides* and

Table 2. Grain matter, grain number per ear, grain N at full grain maturity, flag leaf N at anthesis, and full grain maturity and loss in flag leaf N in the different wheat species of different ploidy levels expressed as mean (±SE).

Species	Grain dry matter [mg per spike]	Grain no. per spike	Grain N [mg per spike]	Flag leaf N [mg] Anthesis	Harvest	Loss in N
T. monococcum Acc. ASP4	632.0 (±61.0)	47.20 (±3.43)	13.60 (±0.79)	4.478 (±0.070)	0.204 (±0.010)	4.275 (±0.070)
Ae. speltoides Acc. I-95-1	108.0 (±10.0)	12.30 (±1.46)	2.60 (±0.11)	1.739 (±0.020)	0.225 (±0.010)	1.513 (±0.010)
T. tauschii Acc. EC-331751	144.0 (±13.3)	19.40 (±1.00)	3.34 (±0.13)	2.562 (±0.170)	0.168 (±0.010)	2.393 (±0.170)
T. durum cv. HD4530	1840.0 (±123.3)	44.83 (±4.00)	35.84 (±0.19)	4.915 (±0.040)	0.364 (±0.020)	4.551 (±0.030)
T. aestivum L. cv. Kundan	1760.0 (±53.3)	35.33 (±0.74)	32.56 (±1.24)	5.499 (±0.020)	0.391 (±0.060)	5.109 (±0.060)

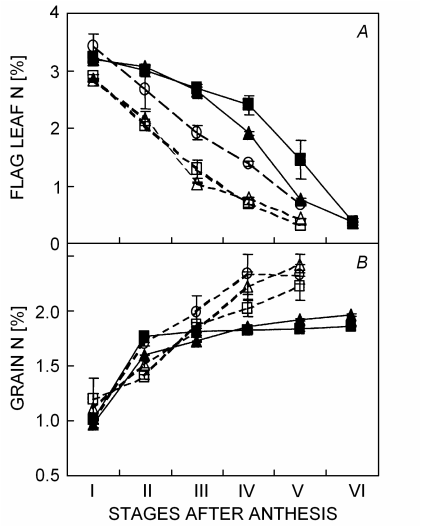

Fig. 1. Effect of grain development on flag leaf N [%] (*A*) and grain N [%] (*B*). Stages represent days after anthesis [DAA] on which sampling has been done and which vary for different ploidies as described in Materials and methods. Kundan (–■–); HD4530 (–▲–); *T. monococcum* (- □ -); *Ae. speltoides* (- Δ -); *T. tauschii* (- ○ -). *Vertical bars* indicate SE ($n = 3$). LSD value for flag leaf N (genotypes×GG stages) = 0.291, $p<0.05$. LSD value for grain N (genotypes×GG stages) = 0.179, $p<0.05$.

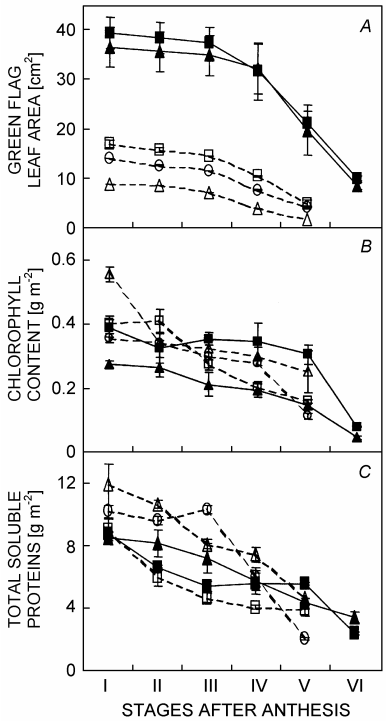

Fig. 2. Effect of grain development on green flag leaf area (*A*), chlorophyll content (*B*), and content of total soluble proteins (*C*). Symbols as in Fig. 1. *Vertical bars* indicate SE (*A*: $n = 4$; *B, C*: $n = 3$). LSD values (genotypes×GG stages): for green flag leaf area = 6.344, $p<0.05$; for chlorophyll content = 0.054, $p<0.05$; for total soluble proteins = 1.367, $p<0.05$.

T. tauschii were higher than in HD 4530 and Kundan during most of the grain development period (Fig. 2*C*). The rate of loss in the mean protein content was maximum in *T. tauschii* at the rate of 0.406 g m^{-2} d^{-1} and minimum in HD 4530 (0.145 g m^{-2} d^{-1}).

Kundan maintained a high P_N throughout grain development (Fig. 3*A*). *Ae. speltoides* had the least P_N as compared to other genotypes. The daily rate of loss in P_N was, however, maximum in *T. tauschii* at the rate of 0.671 μmol(CO_2) m^{-2} s^{-1}. As in the case of protein, RuBPCO content was higher in the diploids as compared to HD 4530 and Kundan at anthesis (Fig. 3*B*). Although *T. tauschii* had a high RuBPCO content at anthesis, the rate of decline was rapid and it had the lowest RuBPCO content at grain maturity.

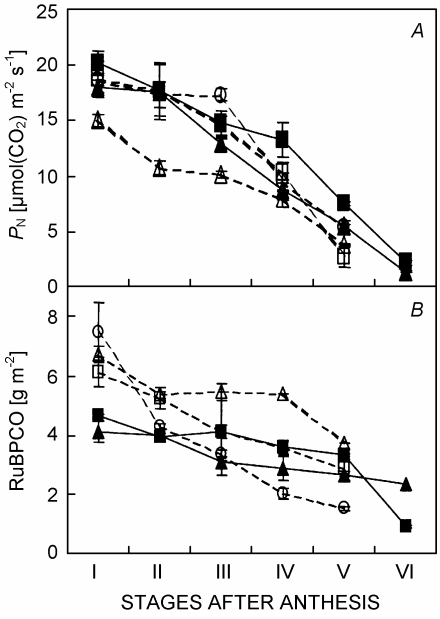

Fig. 3. Effect of grain development on net photosynthetic rate, P_N (*A*) and RuBPCO content (*B*). Symbols as in Fig. 1. *Vertical bars* indicate SE (*A*: $n = 4$; *B*: $n = 3$). LSD values (genotypes×GG stages) for P_N 3.12, $p<0.05$, for RuBPCO content 0.835, $p<0.05$.

The diploids showed a higher endopeptidase activity than HD 4530 and Kundan (Fig. 4*A*). *Ae. speltoides* showed the highest endopeptidase activity amongst all the genotypes during most of the grain development period. The hexaploid Kundan showed a bimodal pattern of activity with high endopeptidase activity at 14 and 28 DAA. The endopeptidase activity measured using RuBPCO as a substrate was responsible for the N mobilisation from the flag leaf across different ploidy levels ($r = –0.582$, $p<0.01$) (Fig. 4*B*).

Discussion

The rate of senescence and the remobilization of leaf nitrogen are related to the source/sink relationship (Crafts-Brandner *et al*. 1998, Ono *et al*. 1999). The wild genotypes of wheat tend to have small leaves with high N content and the cultivated types have larger leaves with lower N content (Champigny and Moyse 1979). This was not the case in Kundan and HD 4530, which maintained high flag leaf nitrogen throughout grain filling (Table 2, Fig. 1*A*). Thus, the relationships drawn for leaves at the vegetative stage may not always hold for leaves at the reproductive stage.

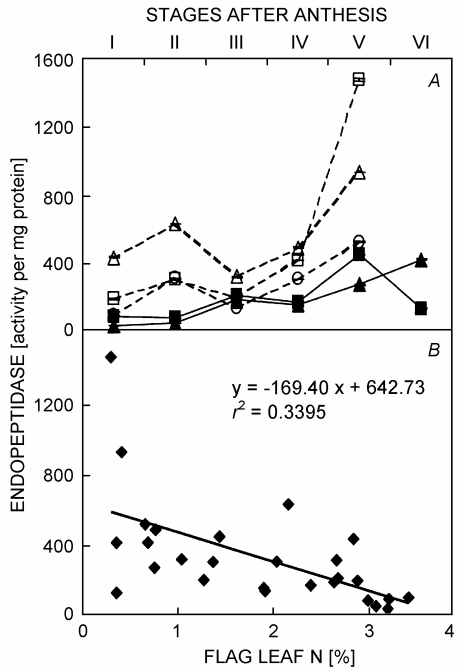

Fig. 4. (*A*) Effect of grain development on endopeptidase activity. Symbols as in Fig. 1. *Vertical bars* indicate SE ($n = 3$). LSD value for RuBPCO content (genotypes×GG stages) 3.776, $p<0.05$. (*B*) Relationship between endopeptidase activity and the loss in flag leaf N, regression significant at $p<0.01$.

Senescence is an active process, which is characterized by the transition from nutrient assimilation to nutrient remobilization (Masclaux *et al*. 2000, Hörtensteiner and Feller 2002). The maximum nitrogen mobilization was observed in *T. tauschii* flag leaf which contributed almost 72 % to the spike followed *by Ae. speltoides* (Table 2). This indicated that during the course of evolution, the flag leaf ceased to be the main source of nitrogen to the ear in the tetraploid and hexaploid.

Maintenance of active photosynthesis especially by the flag leaf during grain development is a major requirement for high grain yield (Haour-Lurton and Planchon 1985, del Blanco *et al*. 2000). Kundan maintained high P_N throughout grain development (Fig. 3*A*). The high P_N of Kundan indicated that the source of the D genome, responsible usually for the depression of photosynthesis in a polyploid, could be from an accession of *T. tauschii* having high P_N. The P_N of a polyploid can vary depending on the source of the D genome (Watanabe *et al*. 1997).

RuBPCO content was higher in the diploids as compared to HD 4530 and Kundan at anthesis (Fig. 3*B*). During senescence, the degradation of RuBPCO leads to an early decline of P_N and is a factor limiting productivity. The complete hydrolysis of proteins to free amino acids depends on the action of both endopeptidases and exopeptidases (Brouquisse *et al*. 2001). Endopeptidases are essential for the cleavage of peptide bonds in a protein and, therefore, also for the catabolism of this protein. *Ae. speltoides* showed the highest endopeptidase activity amongst all the genotypes during most of the grain development period (Fig. 4*A*). The diploids in general showed a higher endopeptidase activity than HD 4530 and Kundan. Hexaploid wheat shows a bimodal pattern of proteolytic activity during grain development (Dalling *et al*. 1976, Srivalli and Khanna-Chopra 1998). Endopeptidase activity has not been characterized in other wheat species so far. We found that acidic endopeptidases were responsible for the N remobilization from the flag leaf of diploid, tetraploid, and hexaploid wheat ($r = –0.582$, $p<0.01$) (Fig. 4*B*).

Among diploids, *T. tauschii* commonly known as goat grass showed the fastest rate of senescence whereas *T. monococcum* showed the slowest rate of senescence associated with a high sink strength. In *T. tauschii* the rate of loss per day was highest in terms of Chl, total soluble proteins, P_N, RuBPCO content, and flag leaf N coupled with a higher rate of gain in grain nitrogen.

In conclusion, the study revealed that diploid species had higher RuBPCO content which was degraded at a faster rate due to higher endoproteolytic activities as compared to the tetraploid and hexaploid wheat. High endoproteolytic activities in the diploids were responsible for the higher rate of N mobilization as compared to the tetraploid and hexaploid wheat, which led to the hastening in the senescence rate. Slower senescence rate has evolved during the development of genotypes in order to support higher sink size in tetraploid and hexaploid wheat in comparison to the diploids.

References

Arnon, D.I.: Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. – Plant Physiol. **24**: 1-15, 1949.

Austin, R.B., Morgan, C.L., Ford, M.A. Bhagwat, S.G.: Flag leaf photosynthesis of *Triticum aestivum* and related diploid and tetraploid species. – Ann. Bot. **49**: 177-189, 1982.

Brouquisse, R., Masclaux, C., Feller, U., Raymond, P.: Protein hydrolysis and nitrogen remobilization in plant life and senescence. – In: Lea, P.J., Morot-Gaudry, J.-F. (ed.): Plant Nitrogen. Pp. 275-293. Springer-Verlag, Berlin 2001.

Carver, B.F., Johnson, R.C., Rayburn, A.L.: Genetic analysis of photosynthetic variation in hexaploid and tetraploid wheat and their interspecific hybrids. – Photosynth. Res. **20**: 105-118, 1989.

Champigny, M.-L., Moyse, A.: Photosynthetic carbon metabolism in wild, primitive and cultivated forms of wheat at three levels of ploidy: Role of the glycolate pathway. – Plant Cell Physiol. **20**: 1167-1178, 1979.

Crafts-Brandner, S.J., Hölzer, R., Feller, U.: Influence of nitrogen deficiency on senescence and the amounts of RNA and proteins in wheat leaves. – Physiol. Plant. **102**: 192-200, 1998.

Cruz-Aquado, J.A., Reyes, F., Rodes, R., Perez, I., Dorado, M.: Effect of source-to-sink ratio on partitioning of dry matter and ^{14}C-photoassimilates in wheat during grain filling. – Ann. Bot. **83**: 655-665, 1999.

Dalling, M.J., Boland, G., Wilson, J.H.: Relation between acid proteinase activity and redistribution of nitrogen during grain development in wheat. – Aust. J. Plant Physiol. **3**: 721-730, 1976.

del Blanco, A., Rajaram, S., Kronstad, W.E., Reynolds, M.P.: Physiological performance of synthetic hexaploid wheat-derived populations. – Crop Sci. **40**: 1257-1263, 2000.

Dunstone, R.L., Gifford, R.M., Evans, L.T.: Photosynthetic characteristics of modern and primitive wheat species in relation to ontogeny and adaptation to light. – Aust. J. biol. Sci. **26**: 295-307, 1973.

Evans, L.T., Dunstone, R.L.: Some physiological aspects of evolution in wheat. – Aust. J. biol. Sci. **23**: 725-741, 1970.

Gan, S., Amasino, R.M.: Making sense of senescence. Molecular genetic regulation and manipulation of leaf senescence. – Plant Physiol. **113**: 313-319, 1997.

Guitman, M.R., Arnozis, P.A., Barneix, A.J.: Effect of source-sink relations and nitrogen nutrition on senescence and N remobilization in the flag leaf of wheat. – Physiol. Plant. **82**: 278-284, 1991.

Haour-Lurton, B., Planchon, C.: Role of D-genome chromosomes in photosynthesis expression in wheats. – Theor. appl. Genet. **69**: 443-446, 1985.

Hörtensteiner, S., Feller, U.: Nitrogen metabolism and remobilization during senescence. – J. exp. Bot. **53**: 927-937, 2002.

Jellings, A.J., Leech, R.M.: Anatomical variation in first leaves of nine *Triticum* genotypes, and its relationship to photosynthetic capacity. – New Phytol. **96**: 371-382, 1984.

Laemmli, U.K.: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. – Nature **227**: 680-685, 1970.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J.: Protein measurement with the Folin phenol reagent. – J. biol. Chem. **193**: 265-275, 1951.

Mackown, C.T., Van Sanford, D.A.: Nitrogen allocation with altered sink demand in wheat. – Crop Sci. **28**: 133-136, 1988.

Mackown, C.T., Van Sanford, D.A., Ma, Y.-Z.: Main stem sink manipulation in wheat. Effects on nitrogen allocation to tillers. – Plant Physiol. **89**: 597-601, 1989.

Mackown, C.T., Van Sanford, D.A., Zhang, N.: Wheat vegetative nitrogen compositional changes in response to reduced reproductive sink strength. – Plant Physiol. **99**: 1469-1474, 1992.

Masclaux, C., Valadier, M.-H., Brugière, N., Morot-Gaudry, J.-F., Hirel, B.: Characterization of the sink/source transition in tobacco (*Nicotiana tabacum* L.) shoots in relation to nitrogen management and leaf senescence. – Planta **211**: 510-518, 2000.

Moreno, J., Penarubbia, L., Garcia-Ferris, C.: The mechanism of redox regulation of cellular ribulose-1,5-bisphosphate carboxylase/oxygenase turnover. A hypothesis. – Plant Physiol. Biochem. **33**: 121-127, 1995.

Nam, H.G.: The molecular genetic analysis of leaf senescence. – Curr. Opin. Biotechnol. **8**: 200-207, 1997.

Noodén, L.D., Guiamét, J.J., John, I.: Senescence mechanisms. – Physiol. Plant. **101**: 746-753, 1997.

Ono, K., Ishimaru, K., Aoki, N., Ohsugi, R.: Transgenic rice with low sucrose phosphate synthase activities retain more soluble protein and chlorophyll during flag leaf senescence. – Plant Physiol. Biochem. **37**: 949-953, 1999.

Patterson, T.G., Brun, W.A.: Influence of sink removal in the senescence pattern of wheat. – Crop Sci. **20**: 19-23, 1980.

Peoples, M.B., Pate, J.S., Atkins, C.A.: Mobilization of nitrogen in fruiting plants of a cultivar of cowpea. – J. exp. Bot. **34**: 563-578, 1983.

Srivalli, B., Khanna-Chopra, R.: Flag leaf senescence in relation to reduced reproductive sink strength in wheat. – Indian J. exp. Biol. **36**: 1013-1019, 1998.

Vierstra, R.D.: Proteolysis in plants: mechanisms and functions. – Plant mol. Biol. **32**: 275-302, 1996.

Warner, D.A., Edwards, G.E.: Effects of polyploidy on photosynthesis. – Photosynth. Res. **35**: 135-147, 1993.

Watanabe, N., Kobayashi, S., Furuta, Y.: Effect of genome and ploidy on photosynthesis of wheat. – Euphytica **94**: 303-309, 1997.

Wendel, J.F.: Genome evolution in polyploids. – Plant mol. Biol. **42**: 225-249, 2000.

Zivy, M., Thiellement, H., de Vienne, D., Hofnann, J.P.: Study on nuclear and cytoplasmic genome expression in wheat by two-dimensional gel electrophoresis. I. First results on 18 alloplasmic lines. – Theor. appl. Genet. **66**: 1-7, 1983.