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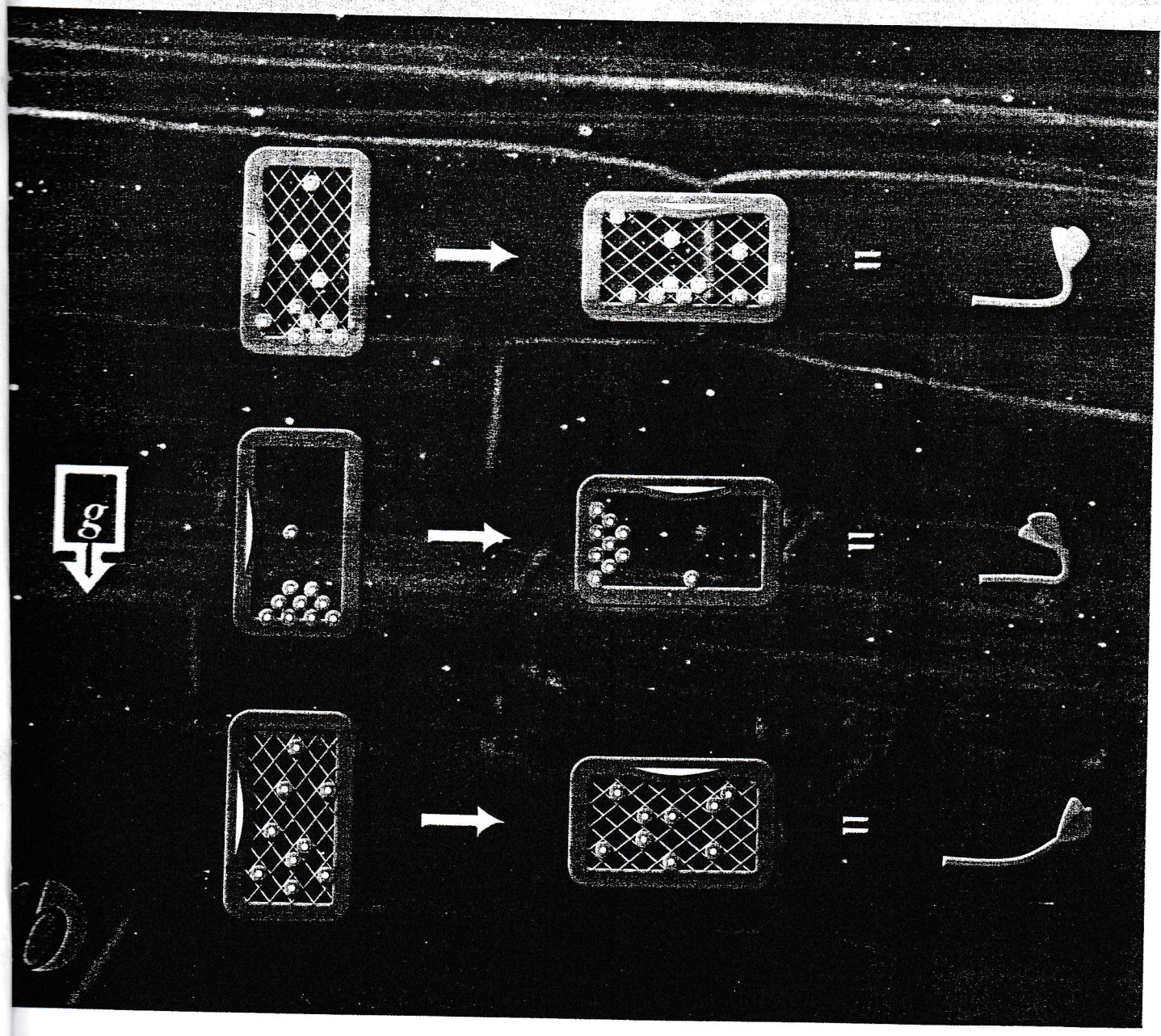

Physiologia Plantarum

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Cover illustration: Palmieri et al. (pp 613-626) investigated if amyloplasts in the statocytes of *Arabidopsis* hypocotyls are dependent on myosin motor proteins for movement before and during gravitropic stimulation by reorientation. In the control (top) the reoriented hypocotyl exhibits a normal curvature response. After disruption of the actin cytoskeleton with latrunculin B (middle), reoriented hypocotyls overshoot the vertical. After inhibition of myosin ATPase activity with 2, 3-butanedione monoxime (bottom), reoriented hypocotyls curve less in response to gravity. Gravity vector = *g*; arrow. Background: Confocal microscopy image of a Pt-GFP *Arabidopsis* hypocotyl. GFP signal from amyloplasts (green) is visible in the endodermal cells.

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Leaf senescence and N uptake parameters as selection traits for nitrogen efficiency of oilseed rape cultivars

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The cultivation of N-efficient oilseed rape cultivars could contribute to a reduction of the large N balance surpluses of this crop. To facilitate the breeding process of N-efficient cultivars, the identification of secondary plant traits correlating with N efficiency is necessary. The objectives of this study were to investigate leaf senescence and N uptake parameters of oilseed rape cultivars and doubled haploid (DH) lines with contrasting N efficiency in a short-term nutrient solution experiment and to relate these results to their performance in field experiments. In the nutrient solution experiment, genotypes differed in leaf senescence of fully expanded leaves and maximum N uptake rate per unit root length under low N supply. A high maximum N uptake rate seemed to have contributed to delayed leaf senescence by enhancing N accumulation in leaves. Also in the field experiments, genotypes differed in leaf senescence after flowering at limiting N supply. Additionally, the most N-efficient DH line was able to adapt leaf photosynthetic capacity to the low-light conditions in the canopy during flowering. N efficiency (grain yield at limiting N supply) was positively correlated with delayed leaf senescence both in nutrient solution and field experiments. It is concluded that important leaf and root traits of N-efficient cultivars are expressed in short-term nutrient solution experiments, which may facilitate the selection of N-efficient cultivars.

Introduction

Nitrogen (N) is the element required in largest quantities for growth and development of crops. However, when high amounts are given, environmental pollution like nitrate leaching into the ground water and nitrous oxide emission into the atmosphere may occur. Often, less than 50% of the fertilizer N is recovered by the crop (Raun and Johnson 1999, Schjoerring et al. 1995). A more efficient use of fertilizer N could be achieved by decreasing the N rate. To avoid negative impacts on grain yield, the use of N-efficient cultivars is necessary, which are defined as giving above-average yields under reduced N fertilizer rates (Craswell and Godwin 1984, Sattelmacher et al. 1994).

One of the crops with the highest N balance surpluses within European agriculture is oilseed rape (Gäth 1997). The high N balance surpluses are mainly caused by two processes, a low N recovery from the soil and an incomplete retranslocation from the source organs, leaving high soil mineral N contents and high N amounts in crop residues in the field (Aufhammer et al. 1994, Lickfett et al. 2001). On the basis of these results, two

Abbreviations – DAT, days after treatment; DH, doubled haploid.

possible ideotypes of N-efficient rape cultivars have been hypothesized (Wiesler et al. 2001): an "improved traditional ideotype" with vigorous growth and high N uptake until flowering and efficient N retranslocation into the seeds during reproductive growth and an "alternative ideotype" with comparatively slow growth and N uptake rates until flowering, which, however, continue during reproductive growth. It has been found that N-efficient rape cultivars can be assigned to the "alternative ideotype" because positive correlations of seed yield with N uptake during reproductive growth under limiting N supply have been found, but not with N uptake up to flowering (Behrens 2002, Wiesler et al. 2001).

Also in maize, N uptake after flowering is an important trait for N efficiency (Akintoye et al. 1999), which is accompanied by delayed leaf senescence in the N-efficient cultivars (Mi et al. 2003, Rajcan and Tollenaar 1999, Worku 2005). A delayed leaf senescence leads to a better assimilate supply to the roots, so that a high root activity and N uptake is maintained and, in turn, a longer lifespan of leaves (Osaki 1995). By an enhanced N uptake, stay-green cultivars are able to compensate for the lower N remobilization from the leaves (Ta and Weiland 1992). For a high N efficiency, delayed leaf senescence with a subsequent rapid N remobilization to the seeds is most desirable (Heuberger 1998). Arabidopsis lines with delayed leaf senescence were especially efficient in N remobilization (Diaz et al. 2005) suggesting that stay green and efficient N remobilization can be combined. However, the causal relationship between leaf senescence and the N uptake capacity of the roots in N-efficient cultivars has not yet been clarified (Ma and Dwyer 1998). Cultivars may also differ in root activity and N uptake, which then causes a delay in leaf senescence.

Differences in leaf senescence under limiting N supply have also been found for oilseed rape cultivars. The N-efficient cultivar 'Apex' had a higher number of leaves, chlorophyll content and a higher photosynthetic capacity at the end of flowering than the inefficient cultivar 'Capitol' (Wiesler et al. 2001). Like in maize, the causal relationship between leaf senescence, N uptake during reproductive growth and root activity remains to be clarified.

The objective of the present study was to investigate leaf senescence and N uptake parameters of oilseed rape cultivars and doubled haploid (DH) lines with contrasting N efficiency in a short-term nutrient solution experiment and to relate these results to their performance in the field. This approach will help to identify plant traits that are suitable for the selection of N-efficient cultivars and may also contribute to the clarification of the causal relationship between leaf senescence and N uptake.

Materials and methods

Nutrient solution experiment

Plant material and growing conditions

Plants were grown in a greenhouse at the Institute of Plant Nutrition, University of Hannover, Germany from 30 August to 15 October 2002. Two winter oilseed rape cultivars and four DH lines were used in the study. The DH lines were derived from a cross between the cultivars 'Apex' and 'Mohican'. Seeds were germinated in quartz sand and moistened with 1 m*M* $CaSO_4$ solution. After germination, four seedlings of each genotype were transplanted into continuously aerated nutrient solution in 5-l plastic pots. The composition of the nutrient solution was 500 μM K_2SO_4, 250 μM KH_2PO_4, 325 μM $MgSO_4$, 50 μM NaCl, 8 μM H_3BO_3, 0.4 μM $MnSO_4$, 0.4 μM $ZnSO_4$, 0.4 μM $CuSO_4$, 0.1 μM $MoNa_2O_4$ and 40 μM Fe-ethylenediamine-di-o-hydroxyphenylacetate. $Ca(NO_3)_2$ was used as nitrogen source. The plants were precultured at high N supply (2 m*M*) for 24 days. Thereafter, the N concentration for half of the pots was set to 0.1 m*M* N, while the other half remained at 2 m*M* (start of the treatment). The nutrient solution was replaced when the N concentration of the nutrient solution in the 2 m*M* N rate pots fell below 0.5 m*M*, as measured daily with nitrate test strips (Merck, Darmstadt, Germany). A second set of pots with two seedlings per 5-l pot was set up for the determination of the N uptake kinetic parameters. These plants were treated in the same way, but because only two plants per pot were cultured, N rates of 1 and 0.05 m*M* N after a pretreatment at 1 m*M* N were chosen. The experiment was completely randomized with five replications.

Measurements and plant analysis

One plant per pot was harvested 7, 14 and 21 days after treatment (DAT). Plants were separated into shoot and roots. The youngest fully expanded leaf as well as the next two older leaves were harvested separately. Leaf area of all leaves was measured by a leaf-area meter (LI-3100, LI-COR, Lincoln, NE). DW of the plant fractions was measured after drying the samples at 70°C until they attained a constant weight. N concentrations of the dried and ground plant fractions were determined using an elemental analyzer (Vario EL, Elementar Analysensysteme, Hanau, Germany).

Prior to harvest, non-destructive measurements of the chlorophyll contents of the leaves (SPAD values) were taken by a portable chlorophyll meter (SPAD-502, Minolta, Japan). Three readings per leaf were taken. The chlorophyll meter measures leaf transmittance in two

wavelength regions of maximum light absorbance by chlorophyll *a* and *b* (Konica Minolta Sensing, Inc. 2003). It reflects leaf chlorophyll contents for a range of species and environments (Yadava 1986). Photosynthesis rates of the same leaves were measured using a portable gas exchange system (LI-6400, LI-COR) with a photosynthetic photon flux density of 1000 μmol m^{-2} s^{-1} and an incoming CO_2 concentration of 360 μmol mol^{-1}. For one plant per pot, which was kept until the end of the experiment, SPAD values on the youngest fully expanded leaf were measured from 12 DAT (when the oldest leaves of the plants grown under low N supply started to show N deficiency symptoms) and repeated every 3–4 days, until the end of the experiment.

N uptake kinetics were determined 22 DAT according to Claassen and Barber (1974). The plant roots were washed in distilled water and set into 10 m*M* $CaSO_4$ solution for 10 min. Thereafter, the plants were transferred to 1-l pots filled with nutrient solution containing 150 μ*M* $Ca(NO_3)_2$ for 4 h. Two-milliliter samples of nutrient solution were removed after 5, 10, 15 and 30 min and thereafter every 30 min. Nitrate concentrations of the samples were measured using an autoanalyzer (Technicon Autoanalyzer II, Bran + Lübbe, Norderstedt, Germany). The depletion of nitrate (Q) over time (t) is proportional to the nitrate uptake rate I (influx rate) (pmol cm^{-1} s^{-1}) and inversely proportional to the root length L (cm). The influx rate is dependent on the ion concentration in the solution and can be described by Michaelis–Menten kinetics using the following three parameters: I_{max} (pmol cm^{-1} s^{-1}), K_M (μ*M*) and E (pmol cm^{-1} s^{-1}). I_{max} describes the maximum uptake rate under saturating nutrient concentration, K_M is the nutrient concentration where the actual uptake equals half of I_{max} and E is the efflux, the movement of ions out of the roots into the external solution. Thus, the following equation can be used to describe the ion depletion from the solution with a constant volume (v):

$$\frac{dQ}{dt} = -L\left[\frac{I_{max} \cdot Q/v}{K_m + Q/v} - E\right] \quad (1)$$

Equation (1) was integrated numerically, and I_{max} and K_M were obtained using a Newton iteration scheme by the SAS program (D. Seidel and L. Hothorn, personal communication).

After the measurement, roots were harvested for root length determination. The fresh roots were cut into 1- to 2-cm pieces and carefully spread in a thin layer of water (2 mm) on a transparent tray, avoiding an overlapping of individual pieces, and scanned. Root length was estimated by the software WINRHIZO-2003 (Regent Instruments, Quebec, Canada).

Field experiments

A field experiment was performed in 2002 and 2003 on a luvisol in Göttingen, Germany. In 2002, the same six winter oilseed rape genotypes as used in the nutrient solution study were investigated under low (unfertilized) and high N supply (240 kg ha^{-1}, including soil mineral N content). The experiment was laid out in a split-plot design with N rate as main plot and cultivar as subplot in four replications. In 2003, 25 cultivars and DH lines were cultivated at the same N rates in a split-plot design with two replications. Soil mineral N content in early spring accounted to 13 kg ha^{-1} in 2002 and 23 kg ha^{-1} in 2003. The N fertilization was applied in two doses at early spring (March) and at the beginning of shooting (April). The plot size was 11.25 m^2 with six rows and a planting density of 32 plants m^{-2}.

Grain yield was recorded for all cultivars on July 16 (low N) and July 25 (high N) in 2002 and on July 15 in 2003. In 2002, chlorophyll contents of all green main stem leaves of one plant per plot were measured at the end of flowering (May 30) by a portable chlorophyll meter (SPAD-502, Minolta, Japan). Photosynthesis rates of the same leaves were measured with a portable gas exchange system (LI-6400, LI-COR) at a photosynthetic photon flux density of 1000 μmol m^{-2} s^{-1} and an incoming CO_2 concentration of 360 μmol mol^{-1}. In 2003, chlorophyll contents and photosynthesis rates were measured 1 week after flowering (June 2) on all main stem leaves of four plants in one replication for the genotypes 'Apex', 'Capitol', 'DH 4' and 'DH 42'. After cessation of vegetative growth at the beginning of flowering, 10 plants in the two middle rows of all plots with the genotypes 'Apex', 'Capitol', 'DH 4' and 'DH 42' were marked, and the number of green main stem leaves was recorded weekly until maturity. For 'Apex', 'Capitol' and 'DH 4', light curves were measured on one leaf not showing signs of senescence from five plants after the end of flowering (June 3) in one replication within the low N rate. The light curves were measured using the above-mentioned gas exchange system, and the light intensities used were 0, 100, 200, 500, 1000 and 1500 μmol PAR m^{-2} s^{-1}. The relationship between photosynthesis rate (P; μmol m^{-2} s^{-1}) and light intensity (I_L; μmol PAR m^{-2} s^{-1}) was described using the formula of Johnson and Thornley (1984):

$$P = \left[\left(P_m + \phi I_L\right) - \left\{\left(P_m + \phi I_L\right)^2 - 4\phi\theta P_m I_L\right\}^{1/2}\right] / \left(2\theta\right) \quad (2)$$

where P_m is the photosynthesis rate under light saturation, ϕ, the initial slope of the curve and θ, a dimensionless parameter indicating the convexity of the curve.

Statistical analysis

Statistical analysis of the nutrient solution data was performed using the PROC GLM procedure of SAS (SAS Institute 2003) followed by the LSMEANS/pdiff statement for multiple comparison of the means within N rates. The field data were analyzed by the PROC MIXED procedure, and multiple comparisons were also performed with the LSMEANS/pdiff statement. Pearson correlation coefficients were calculated using the PROC CORR procedure of SAS. Curve fitting was performed using SIGMA PLOT version 8.

Results

Nutrient solution experiment

Genotypes differed significantly in shoot dry matter production as well as in shoot N uptake at the end of the experiment (Table 1). The ranking of the genotypes differed depending on the N rate. Apex and Capitol had the highest and DH 4 the lowest shoot dry matter and N uptake under low N supply, while DH 4 had the highest N uptake and also a comparatively high shoot dry matter production under high N supply. The genotypes did not differ in N concentrations at low N supply. Therefore, differences in N uptake were mainly related to differences in shoot dry matter production. Genotypic differences in leaf area were very similar to the differences in shoot dry matter under both N rates. Root dry matter did not differ between the N rates. The genotype ranking in root dry matter was not the same as for shoot dry matter, leading to significant genotype differences in root:shoot ratio at low N supply. DH 15 had the highest root:shoot ratio and Apex the lowest.

Root length was greater for the plants grown under high N supply compared with low N supply (Table 2). Apex displayed a higher root length than DH 15 at high N supply, but no genotypic differences were detected at low N. I_{max} was higher for plants grown under low N compared with high N supply. Genotypic differences in I_{max} could be found under low N, but not under high N supply. DH 4 had a significantly higher I_{max} than all other genotypes except Apex. Thus, the low total N uptake of this line (Table 1) was apparently because of a low shoot dry matter production, not of a low N uptake capacity. DH 28 had the lowest I_{max}. For K_M, the variation between individual plants was greater than variations caused by N rate or genotype.

The SPAD values of the youngest fully developed leaves dropped from an average of 33.6 at the beginning of the measurements to 24.3 at low N and to 30.4 at high N supply within the investigated period (Fig. 1). Between 12 and 16 DAT, a drop in SPAD took place under both low

Table 1. Mean shoot dry matter, shoot nitrogen, shoot nitrogen concentration, leaf area, root dry matter and root:shoot ratio of six oilseed rape genotypes grown under low (0.1 m*M* N) and high (2 m*M* N) N supply in nutrient solution at 21 DAT. Values denoted by different letters (lower and upper case letters for low and high N supply, respectively) are significantly different between genotypes within columns at $P < 0.05$. ns, non-significant. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$.

	Shoot				Root	Root:shoot
Genotype	Dry matter (g plant^{-1})	Nitrogen uptake (mg plant^{-1})	Nitrogen concentration (%)	Leaf area (cm^2 plant^{-1})	Dry matter (g plant^{-1})	(g g^{-1})
0.1 m*M* N						
Apex	4.5 a	77 a	1.7 a	644 a	0.78 ab	0.18 c
Capitol	3.8 a	78 a	2.0 a	633 a	1.01 a	0.27 b
DH 15	2.4 bc	48 bc	2.0 a	487 ab	0.81 ab	0.34 a
DH 28	3.4 ab	73 ab	2.1 a	617 ab	0.91 a	0.27 b
DH 4	2.0 c	44 c	2.2 a	327 b	0.46 b	0.23 bc
DH 42	3.9 a	71 ab	1.8 a	658 a	0.85 ab	0.22 bc
2 m*M* N						
Apex	6.3 AB	313 AB	4.9 AB	1302 B	0.77 A	0.12 A
Capitol	6.0 AB	303 AB	5.0 AB	1232 B	0.76 A	0.13 A
DH 15	5.3 B	272 B	5.2 AB	1296 B	0.64 A	0.12 A
DH 28	7.1 A	336 AB	4.7 AB	1546 AB	0.90 A	0.12 A
DH 4	6.6 AB	375 A	5.7 A	1518 AB	0.58 A	0.09 A
DH 42	7.4 A	321 AB	4.4 B	1596 A	1.00 A	0.14 A
Genotype	***	*	**	***	***	***
N rate	***	***	***	***	ns	***
Genotype × N	***	*	ns	***	ns	***

Table 2. Mean root length, maximum nitrate influx (I_{max}) and Michaelis–Menten constant (K_M) of six oilseed rape genotypes grown under low (0.1 m*M* N) and high (2 m*M* N) N supply in nutrient solution at 22 DAT. Values denoted by different letters (lower and upper case letters for low and high N supply, respectively) are significantly different between genotypes within columns at $P < 0.05$. ns, non-significant, $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$.

Genotype	Root length (m plant^{-1})	I_{max} (pmol cm^{-1} s^{-1})	K_M (μM)
0.1 m*M* N			
Apex	269 a	0.57 ab	7.4
Capitol	271 a	0.55 bc	3.8
DH 15	399 a	0.45 bc	5.9
DH 28	299 a	0.36 c	11.8
DH 4	211 a	0.73 a	3.3
DH 42	313 a	0.42 bc	9.5
2 m*M* N			
Apex	547 A	0.42 A	26.1
Capitol	361 AB	0.46 A	3.3
DH 15	333 B	0.72 A	8.1
DH 28	374 AB	0.43 A	5.5
DH 4	355 AB	0.39 A	1.8
DH 42	486 AB	0.39 A	34.0
Genotype	*	**	ns
N rate	***	*	ns
Genotype × N	**	***	ns

and high N supply, which may have been because of the fact that the leaves were still expanding during this period. Thereafter, SPAD decreased further under low N, while under high N supply the SPAD values remained nearly constant. At 12 DAT, no significant differences between N rates were detected for the youngest expanded leaves, but genotypes differed significantly (Table 3). Apex and Capitol had higher SPAD values than DH 42, DH 28 and DH 15. A different development of genotypic differences in SPAD under low and high N supply became visible from 16 DAT. After 22 DAT, the differences in genotype ranking under low and high N supply (Genotype × N interaction) became so important that no genotypic differences over N rates could be detected any more. Capitol had the most distinct SPAD loss under low N supply (Fig. 1), while the other genotypes showed a more constant SPAD value up to 22 DAT. Under high N supply, the drop in SPAD was not significant for all genotypes.

SPAD values and photosynthesis rates were significantly related under both low and high N supply (Fig. 2A). The relationship was closer for leaves grown under low N supply because the leaves showed a higher variation in SPAD values. The relationship between photosynthesis rate and leaf N content was in the same range as for photosynthesis and SPAD under low N supply

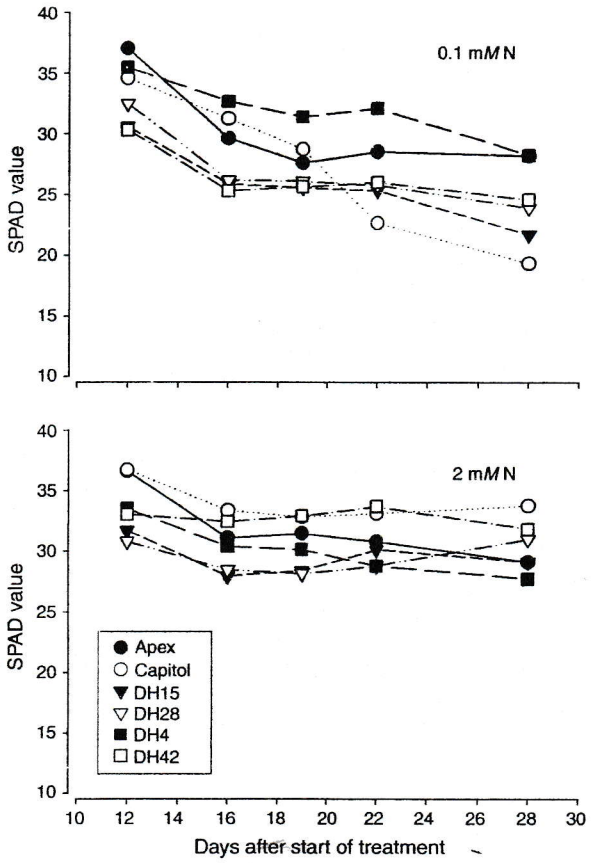

Fig. 1. Development of SPAD values of the youngest fully expanded leaf at 12 DAT over time for oilseed rape six genotypes grown under low (0.1 m*M* N) and high (2 m*M* N) N supply in nutrient solution.

(Fig. 2B). Under high N supply, photosynthesis was not as clearly related to leaf N content as to SPAD values, but still a clear positive relationship was found. No clear genotypic differences in the dependence of photosynthesis rate on leaf N content were found over the three leaf positions. However, young leaves of the genotypes DH 15 and DH 42 had a higher photosynthetic nitrogen-use efficiency than DH 28 irrespective of the N supply. The relationships between SPAD values and leaf N contents were not linear, but could be described by exponential

Table 3. *F*-test for the SPAD values of the youngest fully expanded leaf between 12 and 28 DAT for six oilseed rape genotypes grown under high (2 m*M* N) and low (0.1 m*M* N) N supply in nutrient solution. ns, non-significant, $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$.

	12 DAT	16 DAT	19 DAT	22 DAT	28 DAT
Genotype	***	***	**	ns	ns
N rate	ns	**	***	***	***
Genotype × N	ns	*	*	**	**

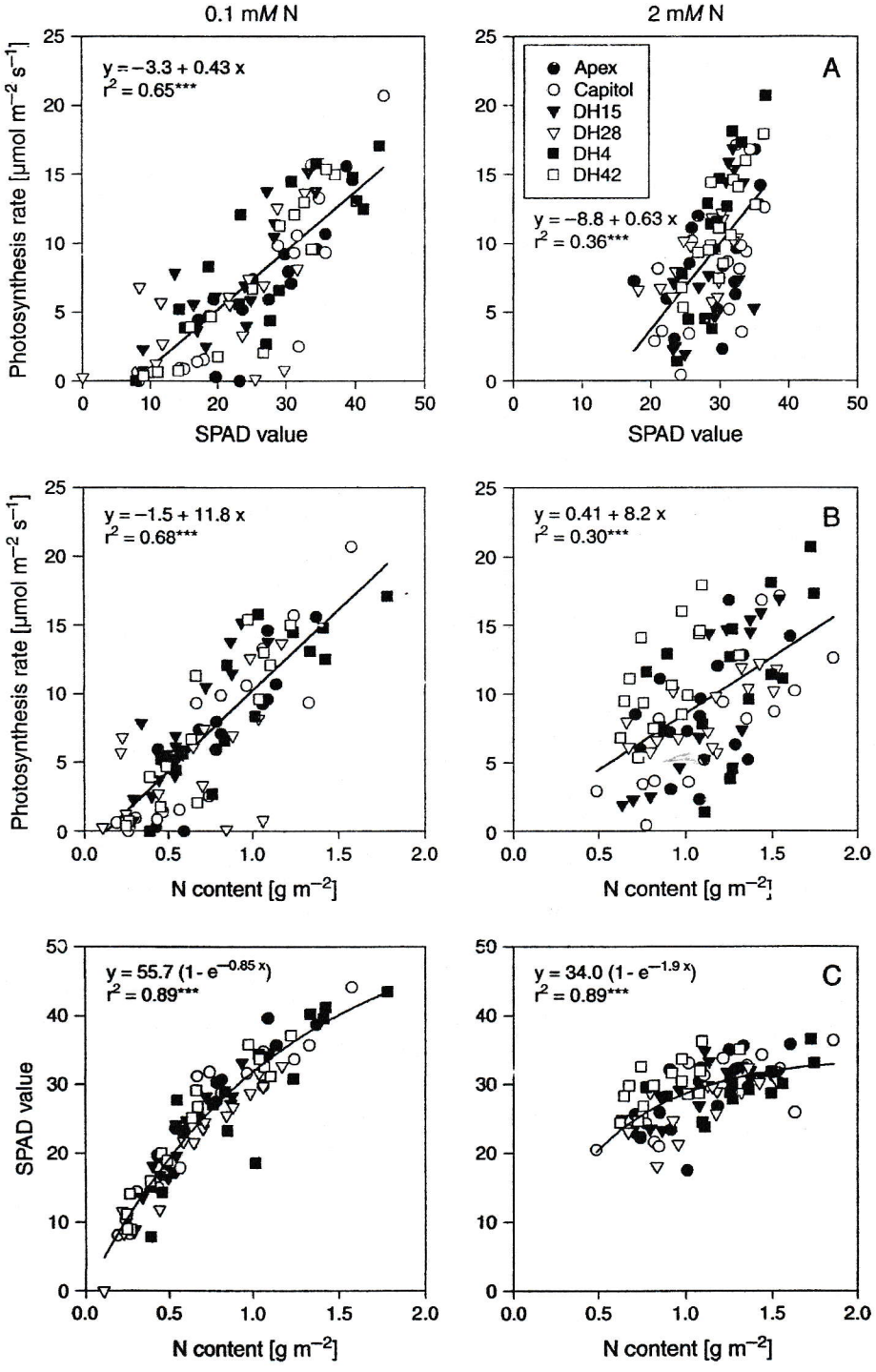

Fig. 2. Relationships between photosynthesis rates and SPAD values (A), photosynthesis rates and N contents (B) and SPAD values and N contents for three different leaf positions (youngest fully expanded leaf and the next two older leaf positions) of six oilseed rape genotypes grown under low (0.1 m*M* N) and high (2 m*M* N) N supply in nutrient solution.

functions (Fig. 2C). With increasing leaf N contents, the increase in SPAD became smaller. While SPAD values reflected leaf N contents very well under low N supply, this relationship was looser under high N supply.

Field experiments

Significant genotypic differences in grain yield under limiting and non-limiting N supply were found in both years (Table 4). DH 4 was the most N-efficient genotype with the highest grain yield under limiting N supply, followed by Apex. Capitol in both years and DH 42 in 2003 had the lowest grain yields. Under high N supply, the genotype rankings were different from the low N rate (significant Genotype × N interaction), indicating genotypic differences in susceptibility to limiting N supply.

For the evaluation of leaf longevity, the number of green leaves on the main stem, as well as their chlorophyll contents and photosynthetic capacity, were recorded at the end of flowering (Tables 5–7). In 2002, the leaf number at this developmental stage was significantly lower at low N compared with high N supply, but significant genotypic differences could not be detected (Table 5). In 2003, when the measurements were recorded 1 week after the end of flowering, the leaf number differed between the N rates and also between the cultivars. DH 4 had a significantly higher leaf number than DH 42 under low N supply, and also Apex tended to have a higher leaf number than Capitol and DH 42.

Table 4. Grain yield (t ha^{-1}) of six oilseed rape genotypes grown under low (N0: without fertilization) and high (N2: 240 kg N ha^{-1} including N_{min}) N supply in field experiments in Göttingen in 2002 and 2003. Values denoted by different letters (lower and upper case letters for low and high N supply, respectively) are significantly different between genotypes within years at $P < 0.05$. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$.

Genotype	2002	2003
N0		
Apex	3.1 ab	2.8 b
Capitol	2.7 c	2.6 b
DH 15	2.8 bc	2.9 ab
DH 28	2.8 bc	2.7 b
DH 4	3.3 a	3.3 a
DH 42	2.9 bc	2.4 b
N2		
Apex	4.2 B	3.6 B
Capitol	4.2 B	5.0 A
DH 15	3.8 C	4.8 A
DH 28	3.6 C	4.9 A
DH 4	4.6 A	5.0 A
DH 42	3.8 C	4.2 B
Genotype	***	***
N rate	***	*
Genotype × N	**	***

Table 5. Mean number of photosynthetically active leaves per plant of six oilseed rape genotypes grown under low (N0: without fertilization) and high (N2: 240 kg N ha^{-1} including N_{min}) N supply in field experiments in Göttingen in 2002 and 2003. Values denoted by different letters (lower and upper case letters for low and high N supply, respectively) are significantly different between genotypes within years at $P < 0.05$. ns, non-significant. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.05$.

Genotype	2002	2003
N0		
Apex	3.0	2.0 ab
Capitol	2.3	0.8 ab
DH 15	2.8	—
DH 28	2.0	—
DH 4	2.8	2.3 a
DH 42	3.3	0.0 b
N2		
Apex	6.0	4.0 B
Capitol	5.8	7.0 A
DH 15	5.3	—
DH 28	4.8	—
DH 4	6.0	3.5 B
DH 42	6.5	4.5 B
Genotype	ns	*
N rate	**	***
Genotype × N	ns	***

Contrary to this, Capitol had the highest leaf number under high N supply. The leaf longevity of DH 15 and DH 28 were not recorded in that year.

The SPAD values of the selected leaves were significantly lower at low compared with high N supply (Table 6). This applies for the youngest leaves (leaf 1) on the main stem and even more clearly for the comparatively older leaves (leaves 2 and 3). While the SPAD values decreased with leaf age under low N supply, this was not the case under high N supply. Significant genotypic differences were mainly found for the younger leaves because the variation of SPAD values of older leaves between individual plants was high. Under low N supply, Apex had the highest SPAD value for the youngest leaf in both years and also displayed high values for the older leaves. DH 4 had a low SPAD value for the youngest leaf, but also showed a low SPAD loss in the older leaves, so that the SPAD values of the older leaves were comparatively high. DH 42 showed a very different performance of SPAD values in 2002 and 2003. While the SPAD values especially of the older leaves were high in 2002, in 2003 all leaves had been lost already 1 week after the end of flowering in 2003. At high N supply, Capitol had the highest SPAD values in 2002, but no genotypic differences were found in 2003.

The photosynthesis rates of the main stem leaves were measured at a light intensity near light saturation and thus reflect the photosynthetic capacity of the leaves. The

Table 6. Mean chlorophyll content (SPAD values) of the youngest, the second and third youngest main stem leaf (leaf 1, 2 and 3, respectively) at the end of flowering of six oilseed rape genotypes grown under low (N0: without fertilization) and high (N2: 240 kg N ha^{-1} including N_{min}) N supply in field experiments in Göttingen in 2002 and 2003. Values denoted by different letters (lower and upper case letters for low and high N supply, respectively) are significantly different within columns at $P < 0.05$. ns, non-significant. $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$.

	2002			2003		
	Leaf 1	Leaf 2	Leaf 3	Leaf 1	Leaf 2	Leaf 3
N0						
Apex	41.2 a	24.7	17.3	36.1 a	15.2 ab	15.1
Capitol	35.5 abc	34.0	7.1	22.2 b	0.0 b	0.0
DH 15	36.9 abc	25.7	9.8	—	—	—
DH 28	31.2 bc	14.2	7.7	—	—	—
DH 4	29.4 c	20.0	14.2	26.1 ab	26.6 a	10.5
DH 42	38.0 ab	32.6	21.5	0.0 c	0.0 b	0.0
N2						
Apex	53.1 BC	52.4	47.6	44.1 A	48.5 A	46.8
Capitol	62.3 A	53.1	53.3	40.4 A	38.0 A	45.5
DH 15	46.1 C	41.7	35.5	—	—	—
DH 28	52.0 BC	46.2	43.0	—	—	—
DH 4	52.5 BC	54.9	50.6	39.2 A	42.3 A	45.7
DH 42	57.1 AB	50.0	49.8	37.1 A	38.4 A	43.4
Genotype	*	ns	ns	***	*	ns
N rate	**	**	**	***	***	***
Genotype × N	*	ns	ns	*	ns	ns

photosynthetic capacity of the youngest (leaf 1) and second youngest (leaf 2) main stem leaves did not differ between the N rates (Table 7). Only the older leaves (leaf 3) had a higher photosynthetic capacity under high N supply. Like the SPAD values, the photosynthetic capacity decreased for older leaves under low N supply but not under high N supply. The cultivar differences in photosynthetic capacity showed the same pattern as for SPAD values. SPAD values and photosynthetic capacities were more closely positively correlated under low N supply ($r = 0.90^{***}$ in 2002 and 0.92^{***} in 2003) than under high N supply ($r = 0.37^{**}$ in 2002 and 0.35^{*} in 2003).

From the beginning of flowering on, the light intensities within the canopy were considerably below the light intensity used for the measurements. The daytime mean of light intensity at the height of the main stem leaves in the week of photosynthesis measurements in 2003 was 209 µmol PAR m^{-2} s^{-1}. To take this into consideration, gas exchange was measured at different light intensities on leaves of the genotypes Apex, Capitol and DH 4 under low N supply (Fig. 3). The comparable leaves of different plants differed considerably in their photosynthesis rates at high light intensities probably reflecting differences in chlorophyll contents of the individual leaves. At light intensities below 200 µmol PAR m^{-2} s^{-1} all leaves showed similar photosynthesis rates. However, at 1500 µmol PAR m^{-2} s^{-1}, the mean photosynthesis rate of Capitol was 20% higher than of DH 4, whereas the mean photosynthesis rate at 200 µmol PAR m^{-2} s^{-1} was 5% lower. Apex had a 22% and 44% higher photosynthesis rate than Capitol at 1500 and 200 µmol PAR m^{-2} s^{-1}, respectively.

Table 7. Mean photosynthesis rate (µmol m^{-2} s^{-1}) of the youngest, the second and third youngest main stem leaf (leaf 1, 2 and 3, respectively) at the end of flowering of six oilseed rape genotypes grown under low (N0: without fertilization) and high (N2: 240 kg N ha^{-1} including N_{min}) N supply in field experiments in Göttingen in 2002 and 2003. Values denoted by different letters (lower and upper case letters for low and high N supply, respectively) are significantly different at $P < 0.05$. ns = non-significant, $*P < 0.05$ and $**P < 0.01$.

	2002			2003		
	Leaf 1	Leaf 2	Leaf 3	Leaf 1	Leaf 2	Leaf 3
N0						
Apex	14.7	7.5	5.8	10.7 a	4.8 a	4.1
Capitol	11.2	10.8	1.5	8.0 ab	0.0 b	0.0
DH 15	10.6	7.1	3.7	—	—	—
DH 28	10.3	3.5	1.5	—	—	—
DH 4	8.4	6.5	4.9	4.2 b	5.6 a	3.4
DH 42	14.7	13.0	8.4	0.0 c	0.0 b	0.0
N2						
Apex	11.1	12.9	11.1	3.6 A	5.6 A	4.3
Capitol	11.1	12.5	12.3	6.3 A	4.4 A	6.4
DH 15	7.0	9.0	6.7	—	—	—
DH 28	10.6	8.0	11.4	—	—	—
DH 4	11.8	11.5	8.0	6.7 A	6.0 A	8.5
DH 42	13.2	9.4	12.6	3.3 A	5.6 A	5.6
Genotype	ns	ns	ns	*	ns	ns
N rate	ns	ns	*	ns	*	**
Genotype × N	ns	ns	ns	*	ns	ns

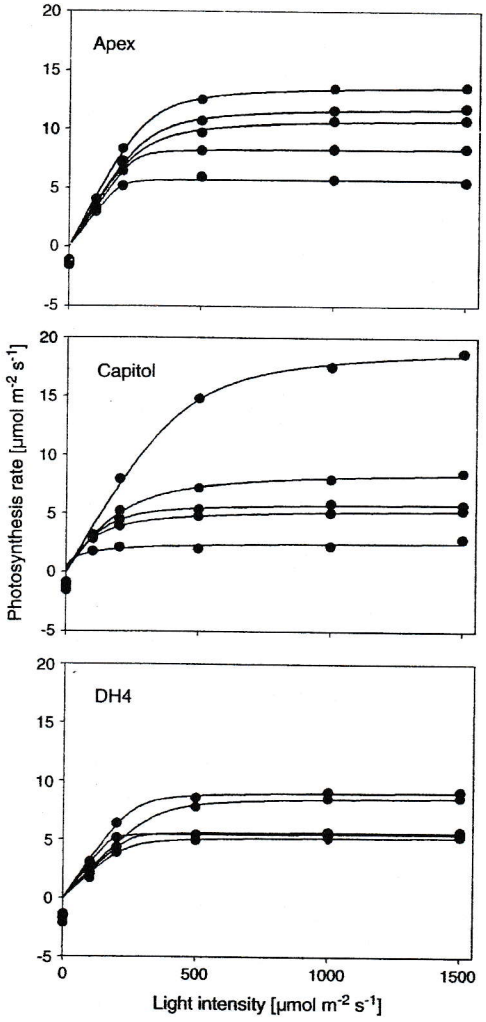

Fig. 3. Photosynthesis rate as affected by light intensity at the end of flowering for one young leaf of five plants of the oilseed rape cultivars Apex and Capitol and the DH line 4 grown without N fertilization in a field experiment in Göttingen in 2003.

In 2003, the ratio of green leaves on the main stem was determined as an additional measure of leaf longevity (Table 8). High rates of leaf loss were detected from mid of flowering on for both N rates. The leaf loss was greater at low compared with high N supply after the end of flowering. Genotype Capitol had the highest green leaf percentages under both N rates up to the end of flowering. After flowering, however, Apex and DH 4 had higher green leaf percentages than Capitol under low N supply. DH 42 lost its main stem leaves already earlier than the other genotypes under both N rates, so that it had the lowest green leaf percentages of all genotypes already during flowering.

Table 8. Percentage of green leaves in relation to all main stem leaves at the beginning of flowering of two oilseed rape genotypes grown under low (N0: without fertilization) and high (N2: 240 kg N ha^{-1} including N_{min}) N supply in a field experiment in Göttingen in 2003. Values denoted by different letters (lower and upper case letters for low and high N supply, respectively) are significantly different at $P < 0.05$. ns, non-significant. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$.

	Middle of flowering	End of flowering	1 week after end of flowering	2 weeks after end of flowering
N0				
Apex	80.7 b	33.7 b	16.7 a	2.9 a
Capitol	84.1 a	41.8 a	10.3 b	0.5 a
DH 4	82.6 ab	37.8 ab	17.2 a	1.5 a
DH 42	79.4 b	31.6 c	11.8 ab	0.8 a
N2				
Apex	78.3 B	49.4 B	33.3 AB	19.2 A
Capitol	84.6 A	62.4 A	37.8 A	23.5 A
DH 4	82.6 AB	51.1 B	38.0 A	22.4 A
DH 42	73.9 C	43.7 C	30.9 B	12.6 B
Genotype	***	***	*	**
N rate	ns	ns	ns	*
Genotype × N	*	ns	ns	**

Relationships between nutrient solution and field experiment results

Generally, positive relationships were found between leaf chlorophyll content of the selected leaf grown under low N supply in nutrient solution and grain yield under low N supply in the field experiments (Table 9). Significant correlations were found between the SPAD values measured at 22 and 28 DAT, when N deficiency started to have a significant influence on the cultivar differences in the leaf chlorophyll content (Fig. 1, Table 3) and the grain yield in 2002. In 2003, this correlation was significant only at the $P > 0.1$ level at 22 DAT. Besides grain yield, also the percentage of green leaves 1 week after the end of flowering at low N supply in 2003 was reflected by the SPAD values at 22 and 28 DAT in nutrient solution. For the data recorded at high N supply, the correlation coefficients were mostly negative, and no significant relationships were found between nutrient solution and field results.

No relationship existed between grain yield and root length in nutrient solution. However, the maximum nitrate uptake rate (I_{max}) under low N supply was positively correlated with grain yield under low N supply in the field experiments ($r = 0.77^{+}$ for 2002 and $r = 0.75^{+}$ for 2003), but no relationships were found between I_{max} and grain yield under high N supply (results not shown).

Table 9. Correlation coefficients between SPAD values from the nutrient solution experiment and grain yield (2002 and 2003, n = 6) and the percentage of green leaves 1 week after the end of flowering (2003, n = 4) of the same oilseed rape genotypes in field experiments in Göttingen. $^{+}P < 0.1$, $*P < 0.05$ and $***P < 0.001$.

	12 DAT	16 DAT	19 DAT	22 DAT	28 DAT
0.1 m*M* and N0					
Grain yield 2002	0.57	0.56	0.69	0.98***	0.91*
Grain yield 2003	0.45	0.59	0.70	0.80^{+}	0.54
Green leaves 2003	0.63	0.40	0.49	0.93^{+}	0.94^{+}
2 m*M* and N2					
Grain yield 2002	−0.33	−0.10	0.32	0.38	0.62
Grain yield 2003	0.15	−0.32	−0.39	−0.24	−0.40
Green leaves 2003	0.27	−0.11	−0.44	−0.49	−0.08

Discussion

The SPAD values under low-N conditions at the beginning of leaf senescence (22 DAT) reflected very well the N efficiency of the genotypes (Table 9). This suggests that this trait may be suited as a selection criterion for N-efficient cultivars. Also in maize, leaf senescence has been found to be suited as a secondary plant trait for the selection of N-efficient cultivars (Bänziger and Lafitte 1997), and cultivar differences in leaf senescence related to N efficiency could be reproduced in a short-term nutrient solution experiment (Schulte auf'm Erley et al. 2007). Delayed leaf senescence during grain filling enhances the photosynthetic capacity of the plants, especially under stress conditions (Wolfe et al. 1988). Many modern maize hybrids show delayed leaf senescence (stay green), which is connected to high rates of dry matter accumulation during reproductive growth and high yields (Tollenaar 1991, Tollenaar and Aguilera 1992). Also, the root activity is enhanced for cultivars with delayed leaf senescence (Tollenaar and Wu 1999) as well as the N uptake during reproductive growth (Mi et al. 2003, Osaki 1995). Different from maize, for oilseed rape the pods play an important role in supplying carbohydrates to the kernels (Diepenbrock 2000). However, model calculations revealed that a longer leaf-area duration and higher leaf N contents during flowering would be highly effective in enhancing total canopy photosynthesis, especially under low N (Dreccer et al. 2000b). This will also increase yield, which is frequently limited by assimilate availability during reproductive growth (Dreccer et al. 2000a, Habekotté 1993).

Both the initial SPAD values of the young leaves and the timing of the beginning of SPAD loss were decisive for the SPAD values under low N at 22 DAT (Fig. 1). Capitol showed a significant loss in SPAD already earlier than the other genotypes, which may reflect a higher susceptibility of this cultivar in the induction of leaf senescence by N deficiency. Apex and the DH lines derived from Apex had a comparatively delayed start of SPAD loss, irrespective of their N efficiency. For these genotypes, the differences in SPAD of the young leaves were more decisive for the SPAD values at 22 DAT. The SPAD values at low N were closely related to the N content of the leaves (Fig. 2C). A high N accumulation in young leaves will also contribute to delayed leaf senescence because a higher N amount can be remobilized from that leaf before senescence is induced. Because leaves with high SPAD values had N contents above average (Fig. 2C), significant N remobilization may take place without a decrease in SPAD. Also in maize, N remobilization and leaf senescence were found to be controlled independently (Martin et al. 2005). Thus, it can be concluded that SPAD at 22 DAT reflected well the beginning of leaf senescence for the genotypes studied.

Although leaf chlorophyll content is usually a good biomarker for leaf senescence (Masclaux et al. 2000), the maintenance of chlorophyll concentrations may not reflect a high photosynthetic capacity (Thomas and Smart 1993) or a low decrease in total N content (Hirel et al. 2005). Therefore, leaf N contents and photosynthesis rate of different leaf positions were also measured. SPAD values and N contents of the leaves were closely related under low N supply (Fig. 2C). This shows that the SPAD value reflected well the N status of the leaf at lower leaf N contents also when different genotypes were compared. The SPAD values were also positively related to the photosynthesis rate of the leaves, both for young and senescing leaves (Fig. 2A). The maintenance of high leaf greenness thus also reflected the maintenance of a high photosynthetic capacity. Monitoring SPAD values, therefore, seems to be an appropriate measure of leaf senescence for the investigated rape genotypes.

Plant growth and N uptake in the nutrient solution experiment did not differ systematically between N-efficient and inefficient genotypes (Table 1). Also, the initial SPAD values of young leaves and their development over time (Fig. 1) did not seem to have been related to plant growth or N uptake. A comparison of shoot dry matter production and N uptake from the nutrient solution experiment with unpublished data from field experiments did not reveal relationships between cultivar performance in nutrient solution and in the field (data not shown). Obviously, seedling growth is not decisive for vegetative growth at later growing stages or when interactions between cultivars and the environmental conditions occurred.

Beside the SPAD values at the beginning of leaf senescence, the maximum nitrate uptake rate of the roots (I_{max}) under low N supply (Table 2) was also related to the N efficiency of the genotypes ($r = 0.77^{+}$ for 2002 and

$r = 0.75^{+}$ for 2003). For barley and wheat, no differences in I_{max} were found between the cultivars, and a clear overcapacity of the measured I_{max} values for total N uptake and growth under low N supply was found (Mattson et al. 1991, Oscarson et al. 1995) suggesting that genotypic differences in I_{max} are not decisive for the N uptake in the field. However, in the nutrient solution experiment, I_{max} under low N was significantly positively related to the SPAD values of young fully expanded leaves ($r = 0.91^{*}$ for 16 DAT and $r = 0.93^{**}$ for 19 DAT). Because the carbon supply to the roots may limit nitrate uptake during leaf expansion (Tolley-Henry et al. 1988), high I_{max} values for nitrate may reflect a better assimilate supply to the roots of these genotypes and thus a higher N uptake during leaf growth. This will contribute to an enhanced N accumulation in young leaves maintaining a higher photosynthetic capacity and delaying leaf senescence.

From the nutrient solution experiment, it can be concluded that genotypic differences in leaf senescence exist. Corresponding genotypic differences in leaf senescence were found also in the field where a loose positive relationship was found between the percentage of green leaves and grain yield at low N supply for the genotypes investigated in 2003. The percentage of green leaves was also positively related to the dry matter increment after the end of flowering ($r = 0.94^{+}$). Leaf chlorophyll contents and the photosynthetic capacity also tended to be higher for the N-efficient compared with the inefficient genotypes (Tables 6 and 7). Exceptions were the unexpectedly low chlorophyll content and photosynthetic capacity of the N-efficient genotype DH 4 for the younger leaves at the end of flowering, although the number of green leaves and chlorophyll contents and photosynthetic capacity of older leaves were comparatively high. The low chlorophyll content and photosynthetic capacity of the younger leaves may represent an adaptation of this genotype to the low light intensity in the canopy at flowering. The measured light intensity curves showed that a high chlorophyll content and a high photosynthetic capacity could not be fully utilized under the given light conditions (Fig. 3). Under such circumstances, low chlorophyll contents and a low photosynthetic capacity may be advantageous because the N amount required in the leaves will be lower. Thus, N may be remobilized during flowering without a loss in the actual photosynthesis rate of the leaves, leading to an enhanced photosynthetic nitrogen-use efficiency. A low N requirement for photosynthesis in older shaded leaves was highlighted by Field (1983), who hypothesized an advantage of a remobilization of N to younger, better illuminated leaves for total plant photosynthesis. A gradient of leaf N contents in the plant canopy that caused a higher total plant carbon gain was indeed found for various plant species (Connor et al. 1995, Hirose and Werger 1987, Pons et al. 1989). In the case of oilseed rape, the remobilized N from the leaves may be used to increase the photosynthesis rate of the pods, which contribute significantly to carbon gain of oilseed rape after flowering (Gammelvind et al. 1996) or to avoid a reduction in pod number that is clearly enhanced by N deficiency (Hocking et al. 1997) and appears to be related to N remobilization from vegetative plant parts (Schjoerring et al. 1995). Thus, this trait might have contributed to the high N efficiency of DH 4. In maize, a stay-green phenotype caused by a mutagenesis in a protease gene was described, which showed an improved N reallocation to the cobs and a high yield under limiting N supply (Donnison et al. 2007). This suggests that a repression of leaf senescence at later senescence stages may not inhibit N remobilization. Thus, a combination of stay green and high N retranslocation seems to be possible, which may be most effective in enhancing N efficiency.

In conclusion, the results suggest that for a high grain-yielding capacity of oilseed rape in the field at limiting N supply (N efficiency), three factors are most important: (1) a high efficiency of the N uptake system until flowering, allowing a high N accumulation in young leaves; (2) a delayed induction of leaf senescence and (3) a higher photosynthetic N efficiency under low-light conditions during reproductive growth. If this hypothesis is true, studying both root and leaf traits is necessary to select cultivars with a high N efficiency. However, it appears that secondary plant traits contributing to N efficiency are expressed in short-term nutrient solution experiments. A better understanding of the physiological/molecular mechanisms controlling the relationships between N uptake, uptake parameters and leaf traits contributing to delayed leaf senescence is necessary to develop quick screening methods, simplifying the selection for N efficiency in breeding programs.

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