Study on nitrogen mobilization of senescence leaves under different nitrogen rate in winter rapeseed (*B. napus* L.)

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Abstract

Leaves of rapeseed are not only a main photosynthetic organ before flowering, but also a organ for N storing. Data from field experiments conducted on a fertile sandy-loam soil in rapeseed growing season of 2001/2002 and 2002/2003 at experimental farm of Yangzhou university in the People's Republic of China were used to analyze the changes of nitrogen and soluble protein concentration in senescence leaves in winter rapeseed (*B. napus* L). The N concentrations per unit area of long-stalk, short-stalk and non-stalk leaves and soluble protein concentrations per unit area of short-stalk and non-stalk leaves were measured respectively. The main results were as follows. (1) The concentration of nitrogen (N) and soluble protein in leaves decreased gradually as the senescence of leaves. The changes of N concentration per unit area and the days after flowering in senescence leaves could be expressed as cubic curve. (2) The ratio of soluble protein N to total N increased gradually as the senescence of leave. (3) The concentration of N and soluble protein and the amounts of N mobilized increased, but the proportion of N mobilized to the total N of leaves decreased (from 83.21% to 76.73% in short-stalk leaves and from 73.49% to 62.21% in non-stalk leaves respectively) as the increasing of N fertilizer application (from 0 to 360kgN/ha). (4) The amounts of N mobilized in long-stalk, short-stalk and non-stalk leaves were about 85%, 80% and 70% respectively during their senescence.

Key words: Leaf senescence, N concentration, N mobilization, *Brassica napus*, Soluble protein

Introduction

Before flowering, leaves are the main photosynthetic organs in rapeseed, but after flowering, pods become the main photosynthetic orzgans. The seed yield of rapeseed mainly comes from the photosynthate of pods (Inaga, 1986 et al.; Leng et al., 1992). The alternate period of these two kinds of photosynthetic organs is at the flowering stage. During this stage, the leaves shed fast. From the start to end of flowering, the leaf area index usually decreases about 70-80% at high yielding cultivation trial (Leng, 2002) with a largely different from other field crops, e.g. wheat, rice, cotton etc. It has been reported that the main reason of leaf senescence accelerated during flowering time of rapeseed is the shading of flowers and pods (Gabrielle el al., 1998). The increasing flower canopy intensifies photon reflectivity and absorption to 60-65% of incoming radiation (Bilsborrow and Norton, 1984; Yates and Steven, 1987; Leach et al., 1989). Small petals or apetalous flowers may improve light absorption by the green canopy (Mendham et al., 1981; Yates and Steven, 1987; Rao et al., 1991). Leaves senescence also occurs in the vegetative phase because of N deficiency in leaf tissues, as observed in rapeseed by Triboi-Blondel(1988). In a Lucerne canopy, Lemaire et al. (1991) found a close relationship between the vertical distributions of light and specific leaf N (SLN), with an abrupt decrease of SLN beneath the layer of leaves receiving less than 15% of the incoming radiation. They hypothesized that during their growth cycle, leaves were gradually shaded by newly generated leaves, and supplied N to these new leaves until their specific N dropped below a threshold value which marked the onset of senescence and abscission. At the end of the growing season the N of senescing vegetative tissues can be stored to meet the demands of next seasons' re-growth in perennial species. The re-sorption of the N before leaf fall is crucial to reduce N losses and contributes significantly to the annual N economy of plants (Aerts and Chapin, 2000).

The N concentration in rapeseed leaves is higher before flowering and decreases gradually after flowering. N concentrations in whole shoots and vegetative organs declined during the season (Rood et al., 1984; Barraclogh, 1989; Hocking et al., 1997). 52% of the N content of the mature plants was accumulated before flowering and maximum N contents for leaves occurred at the beginning of flowering (Hocking et al., 1997). The N concentration and total N amount in different organs were affected by N fertilizer application significantly and the N is distributed mainly in leaves before flowering and in pods and seeds after the end of flowering (Shan et al., 1996). N for grain filling mostly occurs through mobilization of N derived from vegetative tissues (Rossato et al., 2001), and leaves, stems and pods walls each contribute about one-third of the final seed N content in plants with high N application (Schjoerring et al., 1995). A glasshouse study of rapeseed using ^{15}N-urea indicated that N could be mobilized from fruiting branches and upper leaves, and translocated to developing pods (Zhang et al., 1991). 60-65% of the N was apparently mobilized from the leaves after flowering and the mobilization from leaves could have contributed to about 32.5% (mean of all treatments) of the N accumulated by seeds (Hocking et al., 1997). N accumulated in pod walls can be mobilized and transported to seeds and provided 25-33% of the N content of the seeds (Hocking and Mason, 1993; Zhang et al., 1993; Schjoerring et al., 1995).

Because the N concentration of leaves changes among different leaves in plant and usually increases as the increasing of leaf order during rosette stage (Leng, et al., 2001) the leaf numbers in plant varies at different growing stages, the N fertilizer

application affects N concentration significantly, therefore the changes of N amount of whole leaves per plant can not accurately indicate the mobilized N amount of senescence leaf. The purpose of this study is to determine the total N amount mobilized during leaves senescence under different rates of N application.

Material and methods

Plant material and growth conditions

The experiment took place in 2001/2002 and 2002/2003 on experimental farm of Yangzhou University (latitude:32°30′N; longitude:118°50′E) on a sandy-loam soil with total N concentration of 1.12g/kg, available N 99.76mg/kg, available phosphorus 34.76mg/kg, available potassium 92.01mg/kg. Winter rapeseed variety Yangyou No.4 (*Brassica napus* L.) was sown on Sept. 19, 2001 and Sept. 18, 2002. A fully randomized design with three replications within treatments was employed in 2002/2003. Field plots ($20m^2$each one) were transplanted on Oct.25, 2001 and Oct.23, 2002. There were four nitrogen fertilizer treatments (only one N fertilizer treatment in 2001/2002, $240kgN/hm^2$) of 0 (N0), 120(N1), 240 (N2) and 360kgN/ha (N3) as urea. The proportion of pre-transplanting fertilizer (applying before transplanting), seedling fertilizer (applying 15days after seedling transplanting) and bolting fertilizer (stem length at 10cm) was 5:2:3. P ($150kgP_2O_5/hm^2$ as single superphosphate) and K ($188kgK_2O$/ha as KCl) were applied before transplanting. Density was 12×10^4plant/hm^2 with a row spacing of 40cm.

Analysis

On Mar. 24, 2002 (16 days before the beginning of flowering) and Apr.7, 2003 (five days after the beginning of flowering), 30 plants per plot were labeled. Two pieces of little round leaves (diameter: 1.22cm) near the middle vein (avoiding bigger side vein) of leaf were sampled on 5th long-stalk leaves (from up to bottom, 2002), 3rd short-stalk leaves (2002 and 2003) and 3rd non-stalk leaves (2003) in five days intervals. Total 60 pieces of little round leaves per plot were dried at 75°C and weighed for N analyze. Total N concentration was determined by Kjeldahl digestion and soluble protein was determined by the protein-dye binding reaction of Bradford. The concentration of N in soluble proteins was calculated assuming a ratio of 1:6.25 between N and other constituents of the protein molecules.

Results

Effect of N fertilizer application on N concentration of leaves

The treads of N concentration of these leaves were shown in Tab.1 and Tab.2. In the growing season of 2001/2002, the N concentration of long-stalk leaves decreased gradually and the N concentration of short-stalk leaves increased from Mar. 24 to Apr.3 (6 days before the start of flowering) and then decreased gradually. In 2002/2003, the N concentration of short-stalk leaves and non-stalk leaves decreased gradually. With the exception of N2 and N3 treatments in non-stalk leaves, the N concentration increased from Apr. 7 to Apr.12 (10 days after flowering) and then decreased. The N concentrations of both short-stalk leaves and non-stalk leaves increased as the increasing of N fertilizer application. The average N concentrations measured at six sampling times of short-stalk leaves were $6.83mgdm^{-2}$ in control (N0), and 7.68, 8.61 and $9.70mgdm^{-2}$ in N1, N2 and N3 treatments respectively. The average N concentrations measured at seven sampling times of non-stalk leaves were $9.14mgdm^{-2}$ in control (N0), and 10.07, 12.19 and $13.34mgdm^{-2}$ in N1, N2 and N3 treatments respectively. Compared with the control, the N concentrations of N1, N2 and N3 treatments increased 12.46%, 25.97% and 41.90% in short-stalk leaves and 10.15%, 33.31% and 45.85% in non-stalk leaves respectively. It indicated that the N fertilizer application could increase N concentration of leaves in rapeseed and the more the N fertilizer applied, the higher the N concentration of leaves.

Table1 Changes of N concentration (mg/dm^2) of senescence leaves (2001/2002)

Date(m/d)	3/24	3/29	4/3	4/8	4/13	4/18	4/23	4/28	5/3
Long-stalk leaves	8.66±0.49	7.69±0.41	6.32±0.38	5.40±0.52	4.52±0.36	4.06±0.29	3.40±0.21		
Short-stalk leaves	13.08±0.51	15.33±0.63	15.49±0.55	14.03±0.75	11.95±0.58	9.41±0.41	6.65±0.31	4.24±0.36	3.44±0.28

The treads of N concentration of leaves did not decrease linearly. The changing curves between N concentrations (Y, $mgdm^{-2}$) and the days after flowering (X, days) could be expressed as cubic equation (R^2 = 0.9859 ~0.9999, significant at P=0.01). It could be calculated that the maximum (maximum N concentrations of leaves) and minimum values (N residues of the senescence leaves) of different leaves with different N fertilizer level (Tab.3) from the cubic equation. In 2001/2002, the proportion of mobilized N to the total N in long-stalk leaves and short-stalk leaves was 83.85% and 78.78% respectively.

The maximum N concentrations and N residues in leaves increased as the increasing of N level. Compared with the control, the maximum N concentrations in short-stalk leaves of N1, N2, and N3 increased 4.71%, 8.58% and 10.82% respectively, but the N residues in short-stalk leaves of N1, N2, and N3 treatments increased 12.83%, 44.45% and 53.59% respectively. Therefore, the proportion of mobilized N to the total N in short-stalk leaves decreased as the increasing of N level. About 80% (83.21-76.73%, decreasing with the increasing of N level) of the total N was mobilized during senescence of short-stalk leaves. The changing treads of maximum N concentrations, N residues and the proportion of mobilized N to the total N in non-stalk leaves were similar to short-stalk leaves, but the maximum and minimum values were larger, and the proportions of mobilized N to the total N were smaller. About 70% (73.49-62.21%, decreasing with the increasing of N

fertilizer application) of the total N could be mobilized during the senescence of non-stalk leaves.

Table2 Effect of N rates on N concentration (mg/dm^2) of senescence leaves (2002/2003)

	Date (m/d)	N0	N1	N2	N3	$LSD_{0.05}$
Short-stalk leaves	4/7	$12.07^{a}\pm0.49$	$13.50^{b}\pm0.39$	$15.02^{c}\pm0.65$	$15.73^{c}\pm0.52$	1.12
	4/12	$9.85^{a}\pm0.47$	$11.28^{b}\pm0.56$	12.94 ± 0.56^{c}	$13.71^{c}\pm0.61$	1.24
	4/17	$7.53^{a}\pm0.47$	$8.30^{ab}\pm0.28$	$9.11^{b}\pm0.22$	$10.71^{c}\pm0.58$	0.95
	4/22	$5.30^{a}\pm0.30$	$6.18^{b}\pm0.39$	$6.52^{b}\pm0.24$	$8.40^{c}\pm0.28$	0.71
	4/27	$3.61^{a}\pm0.33$	$3.91^{ab}\pm0.37$	$4.42^{b}\pm0.19$	$5.44^{c}\pm0.35$	0.71
	5/2	$2.62^{a}\pm0.28$	$2.94^{a}\pm0.24$	$3.63^{b}\pm0.40$	$4.18^{c}\pm0.12$	0.52
Non-stalk leaves	4/7	$15.29^{a}\pm0.38$	$16.04^{ab}\pm0.50$	$17.07^{bc}\pm0.58$	$17.92^{c}\pm0.66$	1.21
	4/12	$13.94^{a}\pm0.62$	$14.50^{a}\pm0.50$	$18.06^{b}\pm0.53$	$19.30^{c}\pm0.57$	1.16
	4/17	$10.79^{a}\pm0.50$	$11.93^{b}\pm0.51$	$15.06^{c}\pm0.50$	$16.74^{d}\pm0.43$	1.08
	4/22	$7.78^{a}\pm0.45$	$9.43^{b}\pm0.34$	$11.31^{c}\pm0.89$	$12.92^{d}\pm0.60$	1.30
	4/27	$6.50^{a}\pm0.34$	$7.76^{b}\pm0.43$	$9.93^{c}\pm0.15$	$11.22^{d}\pm0.45$	0.74
	5/2	$5.20^{a}\pm0.30$	$5.85^{a}\pm0.40$	$7.24^{b}\pm0.50$	$8.38^{c}\pm0.41$	0.76
	5/7	$4.47^{a}\pm0.48$	$4.99^{a}\pm0.30$	$6.33^{b}\pm0.38$	$6.88^{b}\pm0.40$	0.87

Table3 The equation and parameters of different leaves and N rate

		Regression equation	Max. value (mgdm^{-1})	Min. value (mgdm^{-1})	Down (%)
2001/2002 Long-stalk leaves		$y = 0.000038\ x^3 + 0.003073\ x^2 - 0.178106\ x + 5.243830$	19.86	3.21	83.85
2001/2002 Short-stalk leaves		$y = 0.000668\ x^3 - 0.017328\ x^2 - 0.404092\ x + 13.803851$	15.59	3.31	78.78
2002/2003 Short-stalk leaves	N0	$y = 0.000399\ x^3 - 0.014726\ x^2 - 0.291161\ x + 13.847033$	15.03	2.52	83.21
	N1	$y = 0.000545\ x^3 - 0.022131\ x^2 - 0.235228\ x + 15.183133$	15.74	2.85	81.91
	N2	$y = 0.000964\ x^3 - 0.041069\ x^2 - 0.056255\ x + 16.305867$	16.32	3.65	77.66
	N3	$y = 0.000694\ x^3 - 0.033617\ x^2 - 0.033514\ x + 16.653200$	16.66	3.88	76.73
2002/2003 Non-stalk leaves	N0	$y = 0.000498\ x^3 - 0.022352\ x^2 - 0.178647\ x + 16.920543$	17.25	4.57	73.49
	N1	$y = 0.000395\ x^3 - 0.019711\ x^2 - 0.144584\ x + 17.292657$	17.54	4.96	71.71
	N2	$y = 0.001160\ x^3 - 0.071762\ x^2 + 0.853909\ x + 14.751771$	17.61	6.42	63.55
	N3	$y = 0.001197\ x^3 - 0.077293\ x^2 + 1.013965\ x + 14.951257$	18.73	7.08	62.21

Effects of N fertilizer application on soluble protein concentration in leaves

The soluble protein concentration in short-stalk leaves and non-stalk leaves on main stem of rapeseed (15 days after flowering) decreased strongly as the formation and development of pods and seeds (Tab.4). There were significant differences among N level at the same sampling time. The soluble protein concentrations of both short-stalk leaves and non-stalk leaves increased as the increasing of N level. The average soluble protein concentrations measured at four sampling times of short-stalk leaves were15.09, 17.31, 19.39 and 23.99mgdm^{-2} in N0, N1, N2 and N3 respectively. The average soluble protein concentrations measured at five sampling times of non-stalk leaves were 21.26, 13.14, 26.45 and 28.22mgdm^{-2} in N0, N1, N2 and N3 treatments respectively. Compared with the N0, the soluble protein concentrations of N1, N2 and N3 increased 14.77%, 28.54% and 59.00% in short-stalk leaves and 8.80%, 24.38% and 32.69% in non-stalk leaves respectively. Compared with non-stalk leaves, the soluble protein concentrations of short-stalk leaves were lower at same sampling time in same treatment and decreased faster as the leaves senescence. The average soluble protein concentrations of different treatments on Apr. 17 and May 2 were 25.92 and 12.89 mgdm^{-2} and decreased 50.26% in short-stalk leaves and 31.42 and 21.46 mgdm^{-2} and decreased 31.70% in non-stalk leaves respectively.

Table4 Effect of N rate on soluble protein concentration in leaves (mg/dm)

	Date (m/d)	N0	N1	N2	N3	$LSD_{0.05}$
Short-stalk leaves	4/17	$22.60^{a}\pm1.10$	$24.46^{ab}\pm0.90$	$26.57^{b}\pm0.98$	$30.07^{c}\pm0.94$	2.26
	4/22	$16.84^{a}\pm1.05$	$19.64^{b}\pm0.99$	$20.80^{b}\pm0.93$	$26.91^{c}\pm0.63$	2.07
	4/27	$12.11^{a}\pm1.18$	$13.91^{ab}\pm1.34$	$16.00^{b}\pm0.53$	$21.70^{c}\pm0.84$	2.28
	5/2	$8.84^{a}\pm0.96$	$11.27^{b}\pm0.39$	$14.19^{c}\pm0.74$	$17.27^{d}\pm1.11$	1.77
Non-stalk leaves	4/17	$27.43^{a}\pm1.21$	$29.21^{a}\pm1.05$	$33.95^{b}\pm0.94$	$35.12^{b}\pm1.21$	2.09
	4/22	$24.41^{a}\pm0.68$	$26.17^{ab}\pm0.87$	$28.32^{bc}\pm1.34$	$29.40^{c}\pm1.02$	2.30
	4/27	$22.83^{a}\pm1.38$	$24.87^{b}\pm0.90$	$26.16^{b}\pm0.73$	$28.64^{c}\pm0.99$	1.90
	5/2	$17.53^{a}\pm0.61$	$19.04^{a}\pm0.83$	$23.37^{b}\pm0.92$	$25.94^{c}\pm0.70$	1.68
	5/7	$15.14^{a}\pm0.46$	$16.39^{a}\pm0.88$	$20.47^{b}\pm0.37$	$22.01^{c}\pm0.84$	1.38

Discussion

Present study showed that alternation of pods and leaves was not only the photosynthetic organs, but the N and other elements in rapeseed (*Brassica napus*). It could be seen from field experiments that when planting density was very low the bottom and middle leaves could receive enough sunlight and but they were also senescence fast, especially under lower rate of N fertilizer application. Flowers and pods removal could delay the senescence of leaves (Noquet, et al., 2004). Rossato et al. (2001) reported that during flowering and pod development stages, the capacity for N uptake declined significantly. In fact, the formation and development of flowers, pods and seeds during this stage were very vigorous demanding a large amount of N and other nutrient elements. Tab5 showed that during flowering period the amounts of N in pods increased 477.36, 498.18, 626.54 and 723.10mgplant^{-1}in N0, N1, N2 and N3 respectively, accounting for about 40% (36.68-41.40%)of their final N. The N amounts increased slightly in roots and increased largely in stem and branches especially at the higher N level treatment. But it decreased largely in leaves during this stage. Although the N amounts reduced in leaves was increasing as the increasing N level, the proportions of N amounts reduced to the total N amounts in leaves decreased as the increasing of N level. Under lower N level, the flowers and pods were less and the light received by leaves should be better than that in higher Nlevel, but the leaf senescence was faster in higher N level than that in lower N level. It could be inferred that the main reason of leaves senescence accelerated during flowering stage was not the shading of flowers and pods. Leaves senescence was perhaps the initiative proceeding in order to meet the demands of the N and other nutrient elements in the formation and development of flowers, pods and seeds. The N in leaves was probably the main N source for pods formation. Diepenbrock (2000) emphasized that dry matter (mainly of leaves) produced in early spring was important for later pod growth in oilseed rape by mobilizing the transiently stored substances. The mobilization of N from the leaves could contribute about 32.5% of the N accumulated totally in seeds (Hocking et al., 1997). Therefore it is beneficial to form proper pod numbers by promoting the growth of vegetative organs, especially the leaves before flowering.

Table5 Amounts of N accumulating indifferent organs during flowering period (mg/plant)

	N0	N1	N2	N3
Roots	3.74	4.6	0.24	9.86
Stem and branches	47.78	70.88	236.32	338.39
Leaves	-381.15	-387.06	-427.27	-471.77
Pods	477.36	498.18	626.54	723.1

The decreasing of N concentration per unit area can reflect the N amount mobilized during leaves senescence. The changing curves between N concentration and the days after flowering can be expressed as cubic equation. In long-salk leaves, the proportion of mobilized N to their maximum value was about 85%. The N amount mobilized during short-stalk leaves and non-stalk leaves senescence increased but the proportion N amounts mobilized to the maximum values decreased as the increasing of N fertilizer application. About 80% and 70% N in short-stalk leaves and non-stalk leaves transported to other organs (mainly pods) during the senescence of these leaves. Therefore, the average percentage of N mobilized of all leaves was approximately 80%, including transported to new leaves at rosette stage. Hocking et al. (1997) found the net mobilization of N in leaves was63.4%. The percentage apparent mobilization of total N from leaves was calculated as : {(Maximum amount of N recorded in leaves - Amount of N in leaves at maturity)/Maximum amount of N in leaves}×100. Because the maximum amount of N recorded and amount of N at maturity of leaves included the N of dropped leaves before flowering, therefore the value (63.4%) was under-estimates.

Compared to cereals, rapeseed requires a higher amount of nitrogen, and available nitrogen frequently limits seed yield (Rathke et al., 2005). Oilseed rape has a higher critical N demand for biomass formation than wheat. To produce 0.1t of seeds, the whole crop accumulates approximately 8-10kg (Holmes,1980; Schjoerring et al., 1995; Taylor et al., 1991), 6kg of N (Colnenne et al., 1998), 4.3-4.7kg (Hocking et al., 1997; Smith et al., 1988) and it increases from 5kg to 6kg as the increasing of N fertilizer application (Shan et al., 1996). Although rapeseed absorbs higher amount of N during its life cycle, a lot of N absorbed can return to the soil as the falling of leaves and other organs. The leaves shed before flowering contain a significant amount of N, usually exceeding 2% of their dry weight (Rossato et al., 2001). Amounts of N lost in shed leaves ranged from 10-30kgha^{-1} (Hocking et al., 1997). In this study, about 20% N in leaves returns to the soil as the falling of leaves. Therefore the rapeseed is benefit to the following crops (Prew et al., 1986; Christen et al., 1992; Sieling, 2000; Christen, 2001).

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