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Nitrogen remobilization in shoots of *Paris polyphylla* is altered by gibberellic acid application during senescence

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Abstract

Nitrogen remobilization during senescence has been studied in perennial herb *Paris polyphylla.* We analyzed changes in N content, amino acids, N-remobilization enzymes and effects of gibberellic acid (GA) during natural senescence. There was a gradual decrease in the content of N, chlorophyll and soluble proteins and activities of glutamine synthetase (GS; EC 6.3.1.2) and glutamate dehydrogenase (GLDH; EC 1.4.1.2). Activity staining and Western blots showed that GS2 activity decreased, whereas GS1 activity was relatively stable over time. In contrast, the C/N ratio and total amino acid content increased. Among individual amino acids, the proportions of glutamine (Gln) and asparagine (Asn) increased, and proportions of arginine, aspartate and glycine decreased. Treatment with GA slowed the senescence and retarded decreases in the activities of GS and GLDH and the content of N, chlorophyll and soluble proteins. Conversely, this treatment slowed increases in the C/N ratio, total free amino acid content, and proportions of Gln and Asn. We conclude that low N resorption efficiency during senescence of *P. polyphylla* results from a sharp decrease in N remobilization enzyme activity.

Additional key words: C/N ratio, glutamate dehydrogenase, glutamine synthetase**,** nitrogen resorption efficiency.

Introduction

Nitrogen is one of the most important plant nutrients and is essential for plant growth. To reduce N fertilizer inputs for the benefit of the environment, it is important to improve the efficiency of N use. N mobilization from senescing leaves and reuse by other parts of plants may enhance N use efficiency (Masclaux-Daubresse *et al.* 2010). Senescence is a highly regulated, genetically controlled process that results in the death of plant parts or the whole organism (Noodén *et al.* 1997, Munné-Bosch 2008). The induction of a series of metabolic changes during this process leads to remobilization and recycling of nutrients, and helps plants resume growth when climate conditions change from adverse to mild (Lim *et al.* 2007, Munné-Bosch 2008). Senescence is thought to be a developmental process, shaped by natural selection, whereby plant cells undergo a highly ordered disassembly through cellular metabolic processes and degeneration of cellular structures, and the key products of this disassembly are nutrients that are exported to younger, reproductive, or storage organs (Gan and Amasino 1997, Lim *et al.* 2007).

The processes of N remobilization during leaf senescence have been investigated in many plant species, including *Arabidopsis* (Diaz *et al.* 2008), tobacco (Masclaux *et al.* 2000), sunflower (Cabello *et al.* 2006), and maize (Martin *et al.* 2005). The glutamine synthetase (GS; EC 6.3.1.2)/glutamate synthetase (EC 1.4.1.14) pathway is the primary mechanism of inorganic N assimilation. Glutamate dehydrogenase (GLDH; EC 1.4.1.2) catalyzes the incorporation of ammonium into glutamate

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Abbreviations: Arg - arginine; Asn - asparagine; Asp - aspartate; GA - gibberellic acid; GLDH - glutamate dehydrogenase; Gln - glutamine; Glu - glutamate; Gly - glycine; GS - glutamine synthetase; LSU - Rubisco large subunit; PSP - percentage of senescent shoots; SSU - Rubisco small subunit.

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by reversible reductive amination of 2-oxoglurate (Forde and Lea 2007). The activity of the chloroplastic GS2 isoform, which is the primary N-assimilating enzyme in rapidly photosynthesizing leaves, decreases during senescence, whereas the activity of the cytoplasmic GS1 isoform, which synthesizes glutamine from ammonium provided by GLDH, increases during senescence (Masclaux-Daubresse *et al*. 2010).

Our current understanding of senescence comes mostly from work on annual plants, and senescence in perennials has been little studied (Munné-Bosch 2008). It is still unclear whether N remobilization in herbaceous perennials during senescence is similar to the process in annual plants. *Paris polyphylla* is an herbaceous perennial of the *Trilliaceae* family. Mature *P. polyphylla* plants are usually 30 - 100 cm tall, with 5 - 9 verticillate leaves. The plants emerge in April and May, and grow vigorously between June and October. Shoots enter senescence and begin progressive wilting in November. The apical buds of the rhizome are fully developed in winter and are prepared for emergence the following spring (Li 1998). During senescence, N is remobilized from the shoots (source) and translocated to rhizomes (sink). This relatively clear source/sink relationship in *P. polyphylla* facilitates the study of N remobilization during senescence.

We previously examined the effects of gibberellic acid (GA) on the senescence of *P. polyphylla* shoots and found that the application of this hormone can prolong the growth phase for 4 weeks, resulting in improved rhizome yield and quality (Yu *et al*. 2009a,b, Li *et al*. 2010). Here, we extend our earlier work by the study of N remobilization during senescence.

Materials and methods

Paris polyphylla Smith var. *yunnanensis* (Franch.) plants were grown in a greenhouse under natural solar radiation reduced to 89 % of full intensity. The maximum photosynthetically active radiation was from 190 to 230 μmol(photons) m^{-2} s^{-1}. Field experiments were conducted in a completely randomized block design (three replications of each treatment combination) with *ca*. 190 plants in each block. All experiments were conducted from early November, when shoots were about to enter senescence, to the end of December. A solution of 100 mg dm^{-3} GA or water (control) was sprayed weekly onto plant leaves. Plant leaves from at least 15 individuals in each block were harvested weekly in the morning (about 65 h after GA treatment), washed with water, surface dried with paper towels, and immediately frozen in liquid N until analyses. Week 1 began following the first GA treatment.

Leaves were considered senescent when > 50 % of the leaf area was yellow or dead. The percentage of senescent shoots (PSP) was calculated using the following formula: PSP [%] = (number of senescent shoots/total number of plants) × 100 (Yu *et al*. 2009b).

Chlorophyll was measured in 80 % acetone extracts following the method of Lichtenthaler and Wellburn (1983). The soluble protein content in the extracts used for testing GS activity was determined according to Bradford's (1976) protocol, with bovine serum albumin as the standard.

Frozen leaf tissue was ground and oven-dried at 60 °C to constant mass. Total N and C contents were measured with an elemental analyzer (*Vario EL III*, *Elementar Analysensysteme*, Hanau, Germany).

Amino acids were extracted in 80 % (v/v) ethanol. Total amino acid content was determined by the Rosen (1957) reaction, using Gln as the reference. Individual amino acid composition was determined by reverse-phase HPLC of *o*-phthaldialdehyde derivatives, as described by Puiatti and Sodek (1999).

To extract GS and GLDH, frozen leaves (1 g) were ground in extraction buffer containing 50 mM Tris-HCl (pH 7.6), 1.0 mM EDTA, 1.0 mM $MgCl_2 \cdot 6H_2O$, and 10 mM β-mercaptoethanol. The homogenates were centrifuged at 19 000 *g* for 30 min at 4 °C. GS activity was measured according to Rhodes *et al.* (1975), with hydroxylamine as the substrate. The reaction was carried out at 37 °C, and one unit of GS activity was defined as the amount of enzyme catalyzing the formation of 1 μmol of γ-glutamylhydroxamate min^{-1} at 540 nm. GLDH activity was determined according to Loulakakis and Roubelakis-Angelakis (1990), using both the aminating (NADH-GLDH) and deaminating (NAD-GLDH) directions, by following changes in absorbance at 340 nm. The reaction was run at 37 °C, and one unit of GLDH activity was defined as the reduction or oxidation of 1 μmol of coenzyme (NAD or NADH) min^{-1}.

Extracted proteins were also used for both denaturing and native polyacrylamide gel electrophoresis (PAGE). For denaturing SDS-PAGE, 50 μg of protein extract was loaded into each lane of a 15 % polyacrylamide gel, and the protein bands were visualized by staining with Coomassie Brilliant Blue R-250. For native PAGE, 50 μg of protein extract were loaded into each lane of a 12 % (m/v) native gel, and the protein bands were visualized following the methodology of Zhang *et al.* (1997). After native PAGE, the separated proteins were electrophoretically transferred to a nitrocellulose membrane, and the GS protein band was detected using polyclonal antiserum against rice root GSrb (Lin *et al.* 2000). Immunoreactive bands were visualized by incubation with an alkaline phosphatase-conjugated secondary antibody.

Differences between plant groups were analyzed with Student's *t*-test. All statistical analyses were performed using the *SPSS* package (*SPSS Inc*., Chicago, IL, USA).

Results

PSP increased progressively over time in both the treated and control groups (Table 1). Senescence of the control plants proceeded rapidly, and PSP was significantly higher in the controls compared with the treated group after 3 weeks. Similar results were obtained for chlorophyll content, which was significantly lower in control plants than in GA-treated plants (Table 1). Total N content decreased throughout the senescence in both plant groups, but the decrease was greater in control than GA-treated plants (Table 1). Total C content was relatively stable, leading to increasing C/N ratio (Table 1).

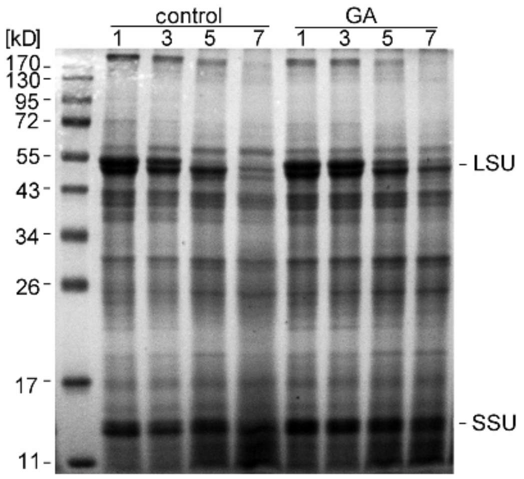

Fig. 1. Changes in Rubisco large subunit (LSU) and small subunit (SSU) content as revealed by Coomassie Blue-stained gels in leaves of control and GA-treated plants for 1, 3, 5 and 7 weeks. 50 µg of total soluble protein was loaded in each lane.

At the beginning of the experiment, the soluble proteins content was similar in GA-treated and control plants, but the protein content was significantly higher in the GA-treated plants from week 4 to week 7, indicating a lower protein degradation in the treatment group (Table 1). Total amino acid content increased in both plant groups during senescence, but the increase was greater in the controls than the GA-treated plants (Table 1). Rubisco large subunit (LSU) and small subunit (SSU) proteins gave bands at ~50 and ~15 kDa, respectively. The Rubisco content decreased with senescence in both groups, but the rate was slower in the GA-treated plants (Fig. 1).

The proportions of Gln and Asn increased in both groups during senescence, but the rate of increase was slower in the GA-treated plants (Table 2). The proportions of Arg, Asp, and Gly followed a reverse trend over time, but the rate of decrease was slower in the GA-treated plants, especially in the late stage of the experiment when significantly higher proportions of Arg, Asp, and Gly occurred in the control plants (Table 2). The proportion of Glu did not differ significantly between GA-treated and control plants (Table 2).

GS activity decreased throughout the senescence in both groups, but the GA-treated plants retained more GS activity (Table 3). Significant differences in GS activity occurred after week 3. Both NADH-dependent and NAD^+-dependent GLDH activities were higher in GA-treated plants than in controls, and the differences were significant after weeks 3 and 4 (Table 3).

We measured the abundance of GS isoenzymes (GS1 and GS2) by gel activity staining and immunoreactivity

Table 1. Changes in the percentage of senescent shoots (PSP), chlorophyll, N and C contents, C/N ratio, soluble protein content and total amino acids content in the leaves of control and GA-treated plants. Values are means ± SE, *n* = 3. Significant difference (Student's *t*-tests, $P < 0.05$) between control and GA-treated plants are indicated by an *asterisk*.

Parameters	Treatments	1	2	3	4	5	6	7 weeks
PSP	control	1.44±0.21	9.15±1.02	23.13±2.79*	30.13±3.34*	47.26±1.09*	69.49±2.40*	89.40±1.43*
[%]	GA	1.87±0.79	4.99±0.86	8.28±2.03	9.58±1.92	14.98±2.10	21.23±1.01	54.78±2.39
Chlorophyll	control	13.59±0.30	14.47±1.62	11.42±0.59*	11.33±0.72*	10.22±0.93*	5.82±0.14*	4.24±0.32*
[mg g^{-1}(d.m.)]	GA	14.66±0.42	14.10±1.35	13.55±0.57	12.98±0.86	12.71±0.84	10.62±0.12	8.66±1.24
N	control	3.25±0.07	3.50±0.14	3.01±0.06*	2.84±0.08*	2.79±0.06*	2.38±0.12*	2.12±0.09*
[%]	GA	3.42±0.12	3.43±0.13	3.47±0.07	3.13±0.07	2.93±0.10	2.83±0.10	2.56±0.08
C	control	44.83±0.36	45.26±0.49	43.66±0.40	44.01±0.19	44.02±0.48	44.33±0.25	44.53±0.27
[%]	GA	44.58±0.06	44.52±0.07	44.21±0.22	44.39±0.49	43.55±0.26	44.12±0.09	43.87±0.20
C/N	control	13.81±0.25	13.02±0.39	14.55±0.23*	15.54±0.45*	15.76±0.25*	18.85±0.84*	21.22±0.88*
	GA	13.10±0.41	13.06±0.49	12.76±0.23	14.21±0.36	14.27±0.25	15.67±0.53	17.22±0.54
Soluble protein	control	61.45±3.73	55.99±1.87	49.78±3.06	31.70±1.66*	30.31±0.95*	23.71±1.62*	11.52±2.34*
[mg g^{-1}(d.m.)]	GA	58.50±1.04	59.15±2.23	54.04±2.76	52.56±4.25	54.88±6.76	46.28±1.98	30.86±1.42
Total amino acid	control	2.25±0.09	2.71±0.07*	2.47±0.08*	2.69±0.17*	3.83±0.11*	4.20±0.43*	4.52±0.19*
[mg g^{-1}(d.m.)]	GA	2.19±0.15	2.43±0.05	1.86±0.11	1.87±0.16	2.40±0.10	2.59±0.09	2.56±0.05

Table 2. Changes in the proportions of the amino acids glutamine (Gln), asparagine (Asn), arginine (Arg), aspartate (Asp), glycine (Gly), and glutamate (Glu) in leaves of control and GA$_3$-treated plants. Values are means ± SE, $n = 3$. Significant difference ($P < 0.05$) between control and GA-treated plants are indicated by an *asterisk*.

Parameters	Treatments	1	2	3	4	5	6	7 weeks
Glutamine	control	6.49±0.21	10.40±0.60*	7.65±0.48	15.03±1.97*	17.77±1.66*	18.49±0.28*	23.14±2.95*
[%]	GA	5.18±0.44	6.83±0.38	6.57±1.15	6.89±0.81	8.81±0.27	11.30±1.27	10.18±0.56
Asparagine	control	4.34±0.04*	6.97±1.20	6.49±0.65*	5.70±1.01	7.92±1.68*	14.69±2.48*	16.94±0.20*
[%]	GA	3.67±0.16	3.17±0.88	2.75±0.27	2.85±0.91	2.67±0.48	4.68±0.66	8.01±1.57
Arginine	control	28.74±4.20	20.72±4.29	18.06±3.24	17.57±4.55	11.61±0.96*	8.24±1.29*	7.18±0.32*
[%]	GA	19.68±2.67	22.28±3.16	23.10±3.51	18.56±2.75	18.17±1.97	22.18±3.82	20.74±2.17
Aspartate	control	9.54±0.27	10.91±2.47	8.22±1.42	7.44±1.44	7.63±0.86	4.39±1.31*	3.22±0.35*
[%]	GA	11.48±1.50	10.72±0.90	11.06±1.32	6.60±0.13	7.76±1.05	10.08±0.97	10.31±1.79
Glycine	control	6.29±0.63	4.73±0.56	4.90±0.26	5.38±0.25	3.85±0.11	3.94±0.26*	2.64±0.46*
[%]	GA	7.70±3.43	5.32±0.70	7.36±1.52	5.66±0.57	4.57±0.57	4.87±0.16	4.63±0.52
Glutamate	control	14.18±1.76	17.78±1.92	18.80±1.84	21.91±2.15	19.98±1.30	18.56±3.72	13.31±0.63
[%]	GA	17.54±3.17	14.52±1.11	21.76±2.61	15.46±1.02	15.11±2.13	18.37±2.43	18.85±2.65

Table 3. Changes in the activities [μmol g^{-1}(d.m.) min^{-1}] and specific activities [μmol g^{-1}(protein) min^{-1}] of GS, NADH-GLDH, and NAD^{+}-GLDH in leaves of control and GA-treated plants. Values are means ± SE, $n = 3$. Significant difference ($P < 0.05$) between control and GA-treated plants are indicated by an *asterisk*.

Parameter	Treatments	1	2	3	4	5	6	7 weeks
GS activity	control	5.00±0.42	4.36±0.18	3.76±0.20*	1.61±0.20*	1.71±0.24*	1.47±0.23*	0.76±0.05*
	GA	5.15±0.13	4.59±0.16	4.52±0.05	3.90±0.25	4.15±0.67	4.16±0.16	3.52±0.24
NADH-GLDH	control	1.90±0.11	1.98±0.17	1.50±0.15*	1.24±0.12*	1.22±0.12*	0.54±0.07*	0.20±0.06*
activity	GA	2.11±0.08	2.13±0.30	2.21±0.04	1.89±0.17	1.84±0.17	1.71±0.19	1.23±0.25
NAD^{+}-GLDH	control	0.95±0.05	0.99±0.02	0.97±0.10	0.83±0.04*	0.82±0.03*	0.55±0.01*	0.46±0.05*
activity	GA	0.98±0.06	0.96±0.08	1.07±0.13	1.07±0.06	1.11±0.02	0.91±0.04	0.86±0.08
GS specific	control	82.36±10.0	77.94±3.58	76.34±7.20	50.53±4.37*	63.62±1.12*	55.93±4.53*	54.34±3.23*
activity	GA	88.07±3.57	77.61±0.68	84.15±5.21	74.38±2.05	75.02±2.79	90.34±5.43	113.88±2.38
NADH-GLDH	control	93.12±3.98	106.29±10.1	102.04±2.80	128.90±23.0	137.47±15.3	69.45±4.41*	51.98±11.1*
specific activity	GA	108.54±5.99	109.03±18.3	123.24±7.91	113.20±15.8	103.54±10.6	115.39±14.5	122.40±15.9
NAD^{+}-GLDH	control	26.10±1.84	32.62±1.91	36.46±4.72	40.39±6.18	55.21±2.33*	55.49±3.46*	76.84±6.38*
specific activity	GA	31.06±2.33	30.38±3.51	36.07±3.79	37.25±5.74	38.48±3.75	37.00±3.14	51.73±1.77

(Fig. 2). Thermostability experiments were performed to identify the positions of GS1 and GS2, as GS1 has greater thermostability than GS2 (Hirel and Gadal 1981, Cabello *et al.* 1994, Ghosh 2004). Thus, the lower band was considered to be GS1 because it was more thermostable than the upper band (Fig. 2*A*). GS2 activity decreased during senescence in the control plants, whereas GS1 activity was maintained at a relatively high level (Fig. 2*B*). Furthermore, GS1 activity increased slightly in both plant groups. The patterns of GS1 and GS2 obtained from Western blots were similar to those of the activity staining (Fig. 2*C*).

Discussion

The ability to store, remobilize, and recycle N resources is a prerequisite for both annual and perennial plant life (Cooke and Weih 2005). In perennial plants, seasonal N cycling is a process by which remobilized N is translocated to growing tissues during the spring flush, and nitrogenous substances are also remobilized and translocated from dying tissues to storage organs during autumn senescence (Cooke and Weih 2005). Senescence involves well-organized recycling of N, with GS1 assimilating ammonium from the catabolism of amino acids and GLDH deaminating ammonium products in mitochondria, concomitantly with the provision of carbon in the form of 2-oxoglutarate to the tricarboxylic acid cycle (Bernard and Habash 2009). In the present study, we tracked N recycling and remobilization during senescence of shoots of the herbaceous perennial, *P. polyphylla*, and determined the effects of exogenous GA on this process.

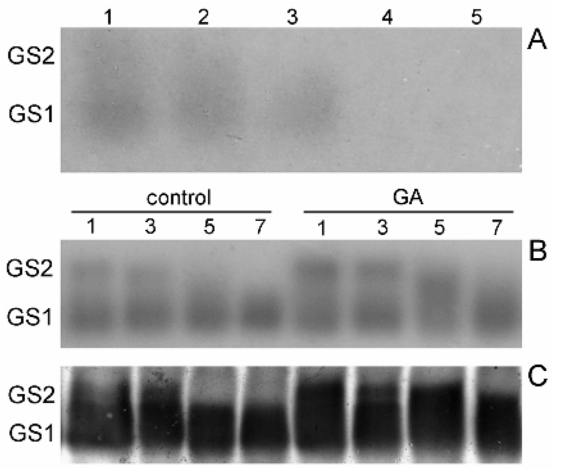

Fig. 2. Analysis of *P. polyphylla* GS isoenzymes. *A* - thermostability of GS1 and GS2. *Lanes* 1, 2, 3, 4, and 5 represents controls after 1.5 h incubation at 25 °C, 30 min incubation at 37 °C, 30 min incubation at 45 °C, and 15 min incubation at 55 °C. *B* - Non-denaturing gel stained for GS1 and GS2 activities. *C* - Western blots showing the expression of GS2 and GS1. Leaves of control and GA-treated plants for 1, 3, 5 and 7 weeks.

We used PSP, a direct indicator of senescence (Yu *et al*. 2009a), and chlorophyll degradation as markers for the onset and progression of senescence in leaves (Li *et al*. 2010). The role of GA in retarding plant senescence has been described previously for many species (Aharoni and Richmond 1978, Kappers *et al*. 1998, Rosenvasser *et al*. 2006, Yu *et al*. 2009a), and our results are in agreement with earlier reports. Both soluble proteins and amino acids are pools of remobilized N (Diaz *et al*. 2008). Total amino acids increased gradually, consistently with progressive degradation of soluble proteins (Table 1, Fig. 1). Rubisco represents as much as 50 % of leaf protein and provides a considerable N resource for future growth when broken down during leaf senescence (Hörtensteiner and Feller 2002, Cooke and Weih 2005). In our study, Rubisco was degraded dramatically in control plants, and GA treatment delayed this process. Moreover, the high C/N ratio that we observed in the late stages of the experiment may cause reductions in photosynthesis and degradation of photosynthetic proteins, as reported by other researchers (Pourtau *et al*. 2006, Wingler *et al*. 2006). The higher content of total N, soluble protein, and total amino acids in GA-treated plants compared to control indicates that N remobilization was retarded during the delayed senescence.

We also showed that the content of individual amino acids changed differently between GA-treated and control plants. Gln, Asn, Arg, Asp, Gly, and Glu are the major amino acids shown to fluctuate during senescence in other plants (Diaz *et al*. 2005, Masclaux-Daubresse *et al.* 2010). This suggests that not all amino acids are equally important in N remobilization during senescence. In the control plants, Gln and Asn were accumulated to a much greater extent than Arg, Asp, and Gly, which were gradually degraded (Table 2). Thus, Gln and Asn may be usable N pools during senescence of *P. polyphylla* shoots. Gln and Asn in senescing leaves are interconvertible and are exported efficiently to the storage rhizome. They are recognized as major amino acids in translocation processes (Masclaux-Daubresse *et al*. 2010). Our results corroborate this view, and shows that GA treatment delayed these patterns of amino acids, which is probably because of slower interconversion of amino acids.

GS activity decreased sharply with aging, suggesting that N assimilation decreases over time (Table 3). Total GS activity is an informative marker of senescence and N remobilization (Diaz *et al*. 2008). As we observed by activity staining and Western blot analysis, the decrease in total GS activity was attributable to a decrease in GS2 activity, as GS1 activity showed little change (Fig. 2). In the GA-treated plants, GS and GLDH activities decreased much more slowly than in controls. Furthermore, GS activity in the treated plants increased when determined per unit protein basis (Table 3). Gómez-Maldonado *et al.* (2004) reported that exogenous GA was able to induce *GS1b* gene expression in pine. In our results, increased activities of GS and GLDH in GA-treated plants may indicate that GA treatments also induce the expression of GS and GLDH related genes during senescence of *P. polyphylla*.

Decreases of both GLDH aminating and deaminating activities during senescence occurred in parallel with a decrease in GS activity, in both plant groups (Table 3). However, per protein unit basis, deaminating activity increased in both plant groups, whereas aminating activity increased in GA-treated plants but decreased in control plants (Table 3). This was in contrast with some reports for annual plants (Kamachi *et al*. 1991, Mae 2004, Kichey *et al*. 2005). The functional role of GLDH in organ development, seed germination, and leaf senescence continues to be discussed (Cooke and Weih 2005). The activities of GS1 and GLDH were stimulated during senescence of tobacco leaves (Masclaux *et al.* 2000, Masclaux-Daubresse *et al*. 2002). GLDH aminating activity increased and deaminating activity decreased during wheat flag leaf senescence (Kichey *et al*. 2005). In rice, GLDH aminating activity decreased rapidly during leaf senescence (Kamachi *et al.* 1991). Increased GLDH activity indicated that GLDH was involved in amino acid degradation and N recycling during leaf senescence of *Arabidopsis* (Masclaux-Daubresse *et al.* 2006). Our observations of enhanced GLDH activity in GA-treated plants suggest that the variation in *P. polyphylla* N remobilization patterns were due to the well-known retardation of senescence by GA treatment.

Approximately 35 % of leaf N was recycled during senescence of shoots in *P. polyphylla* (Table 1). The N resorption during senescence involved remobilization and translocation of leaf N, which plays an important role in regulation of plant N economy. The processes provided substantial amounts of N to developing tissues and help plants to adapt to harsh conditions (Yasumura *et al.* 2007). As a plant survival strategy, usually roots and their meristems are protected under environmental stresses (Conran 2008, Shane *et al.* 2009). This is in accordance with our observation of N remobilization in *P. polyphylla* since minimum temperatures decreased gradually and even fell below 0 °C during the night and early morning in the middle and later stages of the experiment (Yu *et al.* 2009b). Efficient N remobilization should improve N use efficiency. However, plants cannot reuse all N in senescing tissue (Yasumura *et al.* 2007, Masclaux-Daubresse *et al.* 2010). The value of N resorption efficiency (35 %) we obtained was below the average values in other herbaceous perennials: Yuan *et al.* (2005) reported 55.1 %, Aerts (1996) 58.5 %, Carrera *et al.* (2003) 65.9 % and Quested *et al.* (2003) 75.8 %. The rather low N resorption efficiency *P. polyphylla* was probably due to accumulation of free amino acids which were not transported to the rhizome.

In conclusion, we showed that low N remobilization efficiency in *P. polyphylla* was associated with the accumulation of free amino acids and a sharp drop in N remobilization enzyme activities during senescence. The progress of senescence and N remobilization and resorption were retarded by GA treatment.

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