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Regrowth dynamics of *Calamagrostis epigejos* after defoliation as affected by nitrogen availability

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Abstract

Young plants of a rhizomatous grass *Calamagrostis epigejos* (L.) Roth were grown from seed in nutrient solutions containing nitrogen in concentrations 0.1, 1.0, and 10 mM. After six weeks of cultivation the plants were defoliated and changes in growth parameters and in content of storage compounds were measured in the course of regrowth under highly reduced nitrogen availability. Plants grown at higher nitrogen supply before defoliation had higher amount of all types of nitrogen storage compounds (nitrates, free amino acids, soluble proteins), which was beneficial for their regrowth rate, in spite of lower content of storage saccharides. Amino acids and soluble proteins from roots and stubble bases were the most important sources of storage compounds for regrowth of the shoot. Faster growth of plants with higher N content was mediated by greater leaf area expansion and greater number of leaves. In plants with lower contents of N compounds number of green leaves decreased after defoliation significantly and senescing leaves presumably served as N source for other growing organs. Results suggest that internal N reserves can support regrowth of plants after defoliation even under fluctuating external N availability. Faster regrowth of *C. epigejos* with more reserves was mediated mainly by changes in plant morphogenesis.

Additional key words: amino acids, nitrates, nitrogen storage compounds, non-structural saccharides, soluble proteins.

Introduction

Defoliation induces major changes in carbon and nitrogen metabolism of plants (Richards 1984, Volenec *et al.* 1996). The uptake and assimilation of carbon as well as nitrogen are usually severely reduced due to loss of photosynthetic tissue and concurrent down-regulation of nitrogen uptake (Macduff and Jackson 1992). Since the rapid development of new photosynthesizing area of leaf blades is crucial for survival of plants after defoliation, immediate shoot regrowth is often supported by compounds mobilized in remaining organs (Ourry *et al.* 1988, 1989, De Visser *et al.* 1997). The ability of plants to mobilize reserves has been accepted as an important part of adaptation to defoliation (Volenec *et al.* 1996).

Nitrogen availability in the soil significantly affects the amount of nitrogen compounds available for mobilization in remaining organs after defoliation. Higher contents of nitrates, free amino acids and soluble proteins were frequently observed in plants grown under high N availability (Morvan-Bertrand *et al.* 1999a, Louahlia *et al.* 1999, Gloser 2002). Although the high content of soluble nitrogen compounds is not necessarily connected with rapid plant regeneration, the magnitude and dynamics of mobilization of nitrogen reserves after defoliation usually depends on the plant N status at the time of defoliation (Ourry *et al.* 1989, Louahlia *et al.* 1999). Dependence of defoliated plants on nitrogen and carbon reserves also varies throughout regrowth (De Visser *et al.* 1997, Morvan-Bertrand *et al.* 1999a).

Calamagrostis epigejos is a perennial grass with an excellent adaptability to a wide spectrum of climatic and edaphic conditions, and with a remarkable ability to colonize rapidly disturbed segments of both the natural and the man-made landscapes (Rebele and Lehmann 2001). Management practices such as cutting, ploughing or milling are used to reduce abundance of this species but they frequently have little or no effect on plant growth and abundance (Rebele and Lehmann 2001 and references therein). The underlying mechanisms of resistance of this species to mechanical destruction of above-ground organs are, however, largely unknown. Maintenance of an extensive pool of storage compounds and their efficient use after defoliation could be one of

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the putative mechanisms.

The most important organs for storage of reserves in grasses are frequently stubble and root, where considerable mobilization of both carbon and nitrogen during plant regrowth has been observed (Ourry *et al.* 1988, Louahlia *et al.* 1999, Morvan-Bertrand *et al.* 1999b). On the other hand, little is known about the role of rhizomes of rhizomatous grasses in plant regeneration after defoliation. The total amount of N available in spring for mobilization in rhizomes of *C. epigejos*, a typical representative of rhizomatous grasses, indicated only marginal function of this organ in nitrogen storage (Gloser 2002). No information was available on the role of storage compounds in rhizomes during regrowth after defoliation.

This paper contributes to elucidation of the role of carbon and nitrogen storage compounds in organs of *C. epigejos* during regrowth after defoliation. We tested the hypothesis that the regrowth potential of *C. epigejos* is dependent on the content of storage compounds in remaining organs at the time of defoliation. We also investigated if the size of internal reserves may affect the regrowth dynamics and final yield of biomass that is an important factor underlying high competitive ability of this species.

Materials and methods

Cultivation of plants: Caryopses of *Calamagrostis epigejos* (L.) Roth. were germinated on the quartz sand moistened by half diluted nutrient solution (Gloser 2002). After three weeks of cultivation in semi-controlled conditions in greenhouse, single plant seedlings were planted into plastic containers filled with purely inorganic substrate [quartz sand and *Zeolite* (*Clinoptiolite*, *Zeocem*, Bystré, Slovakia), 1:1]. Containers were randomly divided into three groups each representing one level of nitrogen availability. Three times a week all containers received modified Hoagland nutrient solution (100 cm^3 per container) containing nitrogen in the form of NH_4NO_3 and in concentration 0.1, 1.0 and 10 mM resulting in L, M and H experimental treatments. Plants grew in containers six weeks from May till July in air-conditioned greenhouse, air temperature 22 ± 4 °C, natural irradiance, photoperiod approximately 16 h and mean air humidity 75 %. Then all shoot parts above 5 cm were removed by cutting. Two hours before defoliation took place substrate in each container was washed thoroughly by 2 dm^3 of nitrogen-free nutrient solution. Plants in all nitrogen treatments were during 12-d long regrowth period supplied only by nitrogen-free nutrient solution.

Harvests and measurements: There were two harvests of experimental plants. One was done few hours before defoliation whereas the second took place after 12 d of regrowth. Six plants from each treatment were divided into leaves, stubbles, stubble bases, rhizomes and roots. Leaf area of harvested plants was measured by computer scanner and simple program for image analysis. The samples of biomass were then frozen in liquid nitrogen, and freeze-dried for 48 h. Ground biomass of two plants was pooled and used as one replicate for chemical analyses.

The rates of leaf elongation [mm d^{-1}] and leaf area expansion [mm^2 d^{-1}] of regrowing plants were calculated separately for four time intervals within the regrowth period (days 0 - 3, 3 - 6, 6 - 9 and 9 - 12). The calculations were based on the distance measured from the lowest part of stubble base to the tip of the longest leaf and area of all green leaf blades on pertinent days. The area of individual leaves was estimated on intact plants from their linear dimensions (length multiplied by width and by a coefficient derived from direct planimetric and biometric measurements on sets of leaves detached from reference plants). Leaf area of the whole plant was calculated as a sum of areas of all green leaves. All non-destructive measurements on plants regrowing in dark were conducted as fast as possible in a room with photosynthetic flux density < 5 μmol m^{-2} s^{-1}.

Chemical analyses: The extract of 40 mg of plant material in 2 × 1 cm^3 of 0.05 M phosphate buffer (pH 7.5) was used for all chemical analyses of nitrogen storage compounds. Nitrate was determined after reduction to nitrite (Cataldo *et al.* 1975) and the content of soluble proteins by the staining with the Coomassie Brilliant Blue (Bradford 1976) using bovine serum albumin as a standard. Content of total free amino acids was estimated with ninhydrin (Rosen 1957) using leucine as a standard. The proteins were removed from extract prior the analysis of amino acids by precipitation with 5 % (final concentration) sulfosalicylic acid followed by centrifugation (14 000 *g* for 20 min) because of the possible interference with assay.

Total non-structural saccharides were extracted from 20 mg of biomass using 1.5 cm^3 HCl (3 %; v/v). Microtubes were mixed for 10 min and then heated to 125 °C for 3 h. Majority of non-structural poly-saccharides and oligosaccharides were decomposed to reducing monosaccharides during this treatment (Butler and Bailey 1973). Content of monosaccharides was determined in extracts after hydrolysis and centrifugation by anthron method (Scherz and Bonn 1998).

Statistics: The effects of experimental factors were tested by the analysis of variance either as one-way or as multifactorial design with repeated measures (elongation and expansion rates) or as completely randomized design. *Statistica v. 6* (*StatSoft* Inc., Tulsa, USA) was used to evaluate the results. The multiple comparisons of means

were based on the method of LSD contrasts. The homogeneity of variances was checked by Bartlet's and Cochran's tests, and heterogeneous sets of data were transformed appropriately.

Results and discussion

Effect of N availability before defoliation: Reduced biomass production has frequently been observed in plants with low N supply in comparison to those with non-limited N availability (Robinson and Rorison 1988, Poorter *et al.* 1995). Moreover, plant biomass allocation usually changes under low N supply in favour of allocation to roots (Kuiper *et al.* 1988). Improved root growth may increase potential for uptake of nutrients, as well as enlarge plant capacity for storage of reserves. In the presented experiments lower nitrogen availability resulted also in greater roots growth and decreased shoot/root (S/R) ratio in plants under limited N supply (Table 1). The number of tillers increased with the level of N supply from 6 when grown on the 0.1 mM N to about 10 when grown with the 10 mM N. Shoot height, leaf area and number of green leaves were not affected by nitrogen treatment (Table 1).

The content of nitrogen compounds in all plant organs usually reflects the availability of N in substrate (Heilmeier and Monson 1991, Poorter *et al.* 1995). Indeed, the content of amino acids, soluble protein and nitrate in all examined organs of *C. epigejos* plants in our experiments increased with N supply (Table 1). In contrast, high accumulation of saccharides namely in plants in L treatment suggests that a mild limitation of growth due to low N supply probably occurred. The excess of carbon from photosynthesis was then accumulated as non-structural saccharides. This effect has previously been observed also in some other species (Gloser 1993, Den Hertog *et al.* 1998).

Dynamics of plant regrowth and plant morphology: It has been well established that plant nutrient status at the time of defoliation is an important determinant of subsequent regrowth (Ourry *et al.* 1989, Thornton and Millard 1996, Volenec *et al.* 1996, Louahlia *et al.* 1999). Regrowth dynamics of *C. epigejos* plants in our experiment fits well with previous findings. The rate of leaf area expansion was strongly stimulated by greater nitrogen reserves ($P < 0.01$) and increased over time ($P < 0.001$). The leaf area expansion rate was stimulated namely in H treatment (Fig. 1). On the other hand, the axial shoot elongation rate was not affected by the nitrogen treatment (Fig. 1, $P > 0.994$).

Table 1. Structural characteristics (dry mass of a whole plant [mg], shoot to root dry mass ratio (S/R), number of green leaves per plant, whole plant leaf area [cm^2], and maximum shoot height [cm]) and the contents [mg g^{-1}(DM)] of nonstructural saccharides, nitrate, free amino acids and soluble protein in different organs of *C. epigejos* plants immediately before and 12 d after defoliation. Plants were grown in three levels of nitrogen availability in substrate (0.1, 1.0, 10 mM for L, M and H treatment, respectively) before cut. During regrowth the external supply of N was stopped and plants relied mainly on their internal reserves. Means ± SE, $n = 3$ - 6. Dissimilar letters denote significant differences between means within respective parameter ($P < 0.05$). In rhizomes saccharides were not detected.

		Before defoliation			After defoliation		
		L	M	H	L	M	H
Plant	plant DM	772.2 ± 65.0x	912.6 ± 73.0x	813.0 ± 100x	432.9 ± 42.7c	366.1 ± 49.8bc	559.5 ± 43.1d
	S/R	2.8 ± 0.3x	3.7 ± 0.2y	4.0 ± 0.2y	1.2 ± 0.1c	1.4 ± 0.1d	1.5 ± 0.1e
	leaf number	17.5 ± 1.5x	20.2 ± 2.2x	21.0 ± 2.3x	16.4 ± 2.7b	14.0 ± 2.8b	22.9 ± 2.9c
	leaf area	163.3 ± 11.0x	188.5 ± 13.0x	181.4 ± 2.0x	50.5 ± 5.7b	45.8 ± 5.4b	77.6 ± 9.7c
	shoot height	64.4 ± 3.1x	66.1 ± 2.9x	61.4 ± 1.9x	285.2 ± 18.0c	274.8 ± 7.1c	277.0 ± 15.0c
Root	saccharides	107.5 ± 16.9cd	95.1 ± 18.1c	84.0 ± 14.8abc	59.7 ± 3.1ab	56.4 ± 4.2a	53.4 ± 3.2a
	nitrate	9.2 ± 1.6bc	16.0 ± 0.7ef	23.9 ± 2.8h	9.1 ± 1.7bc	17.9 ± 0.3fg	21.7 ± 2.4gh
	amino acids	62.6 ± 5.3ab	80.6 ± 9.3abc	89.7 ± 8.8abc	57.3 ± 18.0a	69.1 ± 9.2abc	67.6 ± 11.0ab
	soluble protein	17.3 ± 1.2fg	23.7 ± 2.3ij	20.7 ± 1.0hi	12.0 ± 0.6cde	11.7 ± 0.5bcde	14.3 ± 1.6ef
Rhizome	nitrate	3.7 ± 0.8a	6.5 ± 0.6ab	10.9 ± 1.3cd	4.3 ± 0.2a	9.2 ± 0.3bc	12.4 ± 2.9cde
	amino acids	113.0 ± 13.4cd	256.1 ± 10.8gh	290.0 ± 17.0h	202.8 ± 14.0fg	255.4 ± 15.0gh	298.8 ± 33.0h
	soluble protein	7.6 ± 0.2a	9.0 ± 0.4abc	8.7 ± 0.2ab	10.7 ± 0.2abcd	13.1 ± 0.5de	12.5 ± 0.6de
Stubble base	saccharides	133.3 ± 36.0d	110.7 ± 20.4cd	15.6 ± 33.0cd	86.5 ± 5.6abc	91.3 ± 4.5bc	110.6 ± 7.3cd
	nitrate	4.5 ± 0.5a	9.0 ± 0.2bc	10.7 ± 0.3cd	6.1 ± 0.7ab	13.8 ± 1.9de	15.0 ± 1.3ef
	amino acids	69.9 ± 0.6abc	162.0 ± 18.1ef	224.4 ± 20.0gh	71.8 ± 8.8 abc	106.7 ± 5.1bcd	135.1 ± 24.0de
	soluble protein	17.3 ± 0.5fg	18.6 ± 1.1gh	20.5 ± 1.0h	24.9 ± 1.9j	29.7 ± 1.2k	35.6 ± 1.4l

Strong discrepancy between effects of N on shoot elongation and leaf area expansion was driven by two mechanisms. The most likely explanation of faster growth of leaf area in H treatment is connected with the more robust stubble of these plants, containing meristem of bigger size. This meristem was able to produce greater number of cells in horizontal layer that later expanded to broader leaf blade. With similar elongation rates H plats then produced bigger leaf area. Alternative or concurred mechanism is the stimulatory effect of high nitrogen reserves on cell division in leaf growth zone and on orientation of the subsequent cell elongation (Volenec and Nelson 1984).

Number of regrowing leaves and leaf area was significantly greater in H treatment than in M and L treatments (Fig. 2). Two processes contributed to this result. First, reduction in number of remaining green leaves within 3 d after defoliation was smaller in H plants than in other two treatments. Mobilization of N from older leaves was observed in plants under N deficiency (Vouillot and Devienne-Barret 1999, Gloser 2005). Nitrogen from senescing leaves is then remobilized to support developing organs (Vouillot and Devienne-Barret 1999, Bausenwein *et al*. 2001). Role of green leaves as source of mobilizable N in spring regrowth of *C. epigejos* after overwintering has been proposed (Gloser 2005). Second, leaf appearance rate was stimulated 10 d after the cut in H treatment. Possible mechanism of this effect was suggested in experiments with *Lolium temulentum* where the high N supply increased the leaf appearance rate by decreasing the duration of leaf extension (Thomas 1983). Results in the present paper show that high content of nitrogen reserves can help plants maintain greater leaf area after defoliation and, consequently, stimulate faster initiation and development of new leaves. As a consequence of greater leaf area, total dry mass of plants in H treatment was about 40 % greater than those in M and L treatments after 12 days of regrowth (Table 1). Stimulated growth of all plant organs contributed to this effect (data not shown).

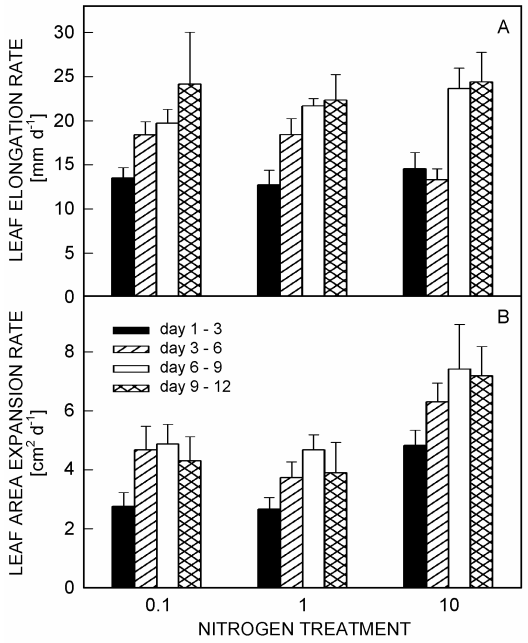

Fig. 1. Leaf elongation rate (*A*) and leaf area expansion rate (*B*) during regrowth of *C. epigejos* plants after defoliation. Rates were calculated separately for four time intervals during 12 d of regrowth after defoliation. Leaf area was calculated from linear dimensions of leaves on intact plants. Several weeks before defoliation plants in respective nitrogen treatments were regularly fertilized by nutrient solutions containing 0.1, 1.0 or 10 mM nitrogen to induce different levels of storage compounds. Most of N was washed out from the substrate at the time of defoliation. Means ± SE, $n = 6$.

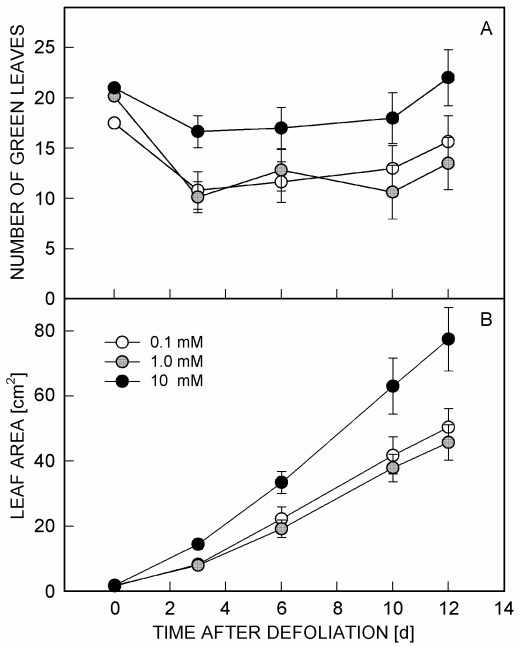

Fig. 2. Number of green leaves (*A*) and whole plant leaf area (*B*) during regrowth of *C. epigejos* plants after defoliation. Several weeks before defoliation plants in respective nitrogen treatments were regularly fertilized by nutrient solutions containing 0.1, 1.0 or 10 mM nitrogen to induce different contents of storage compounds. Most of N was washed out from the substrate at the time of defoliation. Means ± SE, $n = 6$.

Contents of storage compounds after regrowth: Defoliation significantly altered contents of storage compounds in majority of examined organs (Table 1). We observed the most important changes in roots (frequently source of mobilized reserves in grasses, Ourry *et al*. 1988, Volenec *et al*. 1996, Kavanová and Gloser 2005) and stubble base that acts predominantly as sink for mobilized compounds during regrowth. The content of saccharides and soluble protein decreased in roots after defoliation to the similar levels in all N treatments (Table 1). Decline of protein content in roots of *C. epigejos* during regrowth after defoliation has been shown previously, although no specialized vegetative storage proteins were found (Kavanová and Gloser 2005).

Our results indicate that protein content in roots declined to similar levels in all N treatments irrespective to its content at the time of defoliation (Table 1). On the other hand, the content of amino acids was similar in roots and stubble of experimental plants in all treatments probably due to balanced rates of protein decomposition in roots, transport and their use in regrowing leaves.

In contrast to roots, we found increased protein content in stubble of plants in all treatments (Table 1). The increase of protein content in stubble of regrowing plants corresponds well with high rates of cell production and leaf area expansion since the meristems of grasses are located predominantly there. Similar mechanism was probably responsible for increase of protein content in rhizomes. Rhizomes often develop new shoots at their ends especially when N supply is plentiful (Gloser 2005).

We found increased nitrate content in stubble especially in H treatment (Table 1). Nitrate may serve as osmolyte in elongating cells (Walch-Liu *et al*. 2000), especially when carbon supply is limited after defoliation. Indeed, we found significantly decreased contents of saccharides in stubble base after defoliation (Table 1).

Regrowth of plants in the field usually occurs after site disturbance, which usually results in increased nitrogen availability in the soil. Results of the present experiments suggest that internal N reserves can support regrowth of plants after defoliation even under fluctuating external N availability. Faster regrowth of *C. epigejos* was mediated namely by changes in morphogenetical processes (leading to faster leaf area development), which were most likely induced by higher content of soluble N compounds. We also suggest that remaining lower parts of leaf blades after cutting may serve as an important source of N for growing organs namely in plants with lower levels of N reserves.

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Dutta Gupta, S., Ibaraki, Y. (ed.): **Plant Tissue Culture Engineering.** - Springer, Dordrecht 2006. 480 pp. EUR 149.80. ISBN 1-4020-3594-2.

This book is the sixth volume of the series "Focus on Biotechnology" edited by M. Hofman and J. Anzé.

In contrast to many previous books focused on basic methods and applications of plant tissue and cell cultures, this book combines plant science with engineering principles. This new direction is a prerequisite for development of efficient, cost effective and large scale applications of plant *in vitro* cultures.

The book is divided into five parts. Part 1 deals with new approaches for monitoring of a quality of *in vitro* grown plants. Its four chapters comprise image analysis of chlorophyll fluorescence for determination of photosynthetic capacity of *in vitro* grown plants, methods for control transient and stable gene expression, simulations of metabolism of plants *in vitro* using network technology, and evaluation of plant suspension cultures by texture analysis. The largest Part 2 containing eight chapters reviews bioreactor technologies and possibilities of their application in micropropagation, cultivation of transgenic plants, or production of proteins and pharmacologically important compounds. Possibilities of different innovations (*e.g.* airlift, thin films of liquid media, nutrient mist, temporary immersion, wave-induced agitation) for improvement in bioreactor efficiency are discussed. Two chapters of the Part 3 show ways for reduction of labour costs by mechanization of micropropagation (automation technologies and robotics). Additional methods and technologies important in large scale plant propagation are surveyed in Part 4 (closed transplant production systems using minimum resources, engineering aspects of vessel design, determination and effects of gel firmness, role of dissolved oxygen concentration, commercialized photoautotrophic micro-propagation, and temperature gradients in culture vessels). The last Part 5 with 4 chapters covers rather special techniques such as electrical control of plant morphogenesis, the use of ultrasound in tissue cultures, acoustic characteristics of *in vitro* plants, and perspectives of *in vitro* plant cryopreservation.

This comprehensive book with many tables and figures can serve not only as up-to date source of information for researchers and teachers in the area of plant biotechnology but it also open up new vistas in this field.

J. POSPÍŠILOVÁ (*Praha*)