BIOLOGIA PLANTARUM 53 (1): 171-174, 2009

BRIEF COMMUNICATION

Nitrate reduction in ramets of a clonal plant *Eichhornia crassipes* responding to nitrate availability during clonal growth stage

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Abstract

Effect of nitrate availability on nitrate reduction was examined in inter-connected ramets of invasive clonal plant *Eichhornia crassipes* grown with two nitrate supply regimes during different clonal growth stage. Increase of nitrate availability accelerated nitrate reductase activity (NRA) in parent and offspring ramets of *E. crassipes*, and there was greatly different pattern in inter-connected ramets during clonal growth stage. Leaf NRA was lower in offspring than that in parent ramets in phase 1, while significantly higher leaf NRA in offspring ramets was detected during phase 2. The results indicated NRA in inter-connected ramets of *E. crassipes* was highly dependent on nitrate availability and growth stage.

Additional key words: inter-connected ramets, nitrate reductase activity, relative growth rate.

Nitrate is the main inorganic nitrogen resource for higher plants in many habitats, and its uptake, translocation, and assimilation by plant dramatically affects plant growth and development. It is widely accepted that nitrogen input to an ecosystem increases invasion of some plant species, which may proliferate at expense of more native species under eutrophic environment (Burns 2004, Bidwell *et al*. 2006). Establishment and spread of clonal invasive plant species mainly depend on fast clonal growth *via* vegetative fragments (Duarte and Sand-Jensen 1996). One of the significant characteristics of clonal plant is architecture of inter-connected ramets, which allows nutrient to transfer from established ramets to developing ramets within clone fragment, termed physiological integration (Alpert and Mooney 1986). During the clonal growth of offspring ramets, nitrogen demand of the initiated offspring ramets mainly depends on inter-connected parent ramets by translocation nitrate from parent to offspring ramets at the first stage (phase 1). Once offspring ramets developed at the second stage (phase 2), horizontal growth of offspring ramets becomes a relatively independent unit of nitrate uptake and assimilation. Thus, pattern of nitrate allocation and reduction in inter-connected ramets within clone fragment probably varies during establishment and spread, which is greatly different from that in non-clonal plant species (Jónsdóttir and Callaghan 1991). Therefore, investigating nitrate reduction in ramets of invasive clonal plant during different growth stage is critical not only for understanding the adaptive mechanism of invasion but also for design and implementation of the potential control strategies.

Eichhornia crassipes is considered as one of the worst invasive weeds. After establishment, the invader rapidly spread by generation of the ramets and occupy broad range of habitats in a short time (Howard and Harley 1998, Li *et al*. 2006). Large numbers of data, including biomass production, population dynamics, and phenotypic plasticity showed the advantage of nitrogen utilization in *E. crassipes* (Center and Spencer 1981, Watson and Cook 1987, Xie *et al*. 2004, Li and Wang 2007). However, the nitrate reduction in physiologically integrated ramets respond to nitrogen availability remains unknown.

Received 4 February 2008, *accepted* 20 July 2008.
Abbreviations: NRA - nitrate reductase activity; RGR - relative growth rate; R/S - root/shoot ratio.
Acknowledgements: The authors are grateful to Mr. Tao Zhang for his technical assistance. This work was carried out with the financial support from the National Natural Science Foundation of China (No. 30521004, 30170177) and PCSIRT.
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The objective of the present study is to investigate tissue NRA in inter-connected parent and offspring ramet responding to nitrate availability. We also examine the patterns of nitrate reduction in parent and offspring ramet during different growth stage.

The clone fragments of *Eichhornia crassipes* (Mart.) Solms with similar size, which consisted of inter-connected parent and initiated offspring ramet, were grown in homogeneous or heterogeneous nitrate treatments. In heterogeneous nitrate treatments, we mimicked the growth condition of new generated offspring ramets during early establishment (phase 1). Parent ramets received steady nitrate supply while connected offspring ramets were grown in Petri dishes containing N-free nutrient solution. In order to avoid mixing nutrient solution (N-free and nitrate solution), the Petri dishes were carefully placed on polystyrene foam floating on nitrogen nutrient solution. In homogeneous nitrate treatments, we mimicked the condition of phase 2, and both parent and offspring ramets were kept in full nutrient solutions. The full nutrient solution contained 0.5 or 5 mM nitrate given as $Ca(NO_3)_2$ with pH value kept at 5.8. The plants were grown in greenhouse (Wuhan University) with 12-h photoperiod (irradiance of 450 μmol m^{-2} s^{-1}), day/night air temperature 30/22 °C and 70 % relative humidity. Each treatment was replicated six times.

The whole clones were harvested at 14 d after transferring pairs of clone fragment to growing medium. The harvested clone fragments were separated into leaves and roots of parent and offspring ramets, then six individuals from each treatment were dried at 85 °C for 24 h to measure relative growth rate (RGR) of whole clone fragments (Hunt 1982) and root/shoot ratio (R/S) of parent and offspring ramets. In the same samples nitrate accumulation in tissues was determined using salicylic acid method according to Cataldo *et al.* (1975). The youngest fully expanded leaves and new-formed roots were used to detect NRA in parent and offspring ramets of *E. crassipes*, and *in vitro* NRA (maximum enzyme activity) assays were performed by a modification method of Reda and Klobus (2006). Each data point was the mean of six replicates, and all data were analyzed using one-way *ANOVA* followed by Tukey multiple tests at $P \leq 0.05$ significance level using *SPSS* software package (release *11.5*, *SPSS Inc.*, USA).

Nitrate availability significantly simulated growth of *E. crassipes* during different growth stage (Table 1). At 5 mM nitrate, the RGR of *E. crassipes* increased by 18.6 and 20.5 % during phase 1 and phase 2, respectively. In terms of biomass allocation between roots and shoots, R/S of both ramets and whole clone fragments significantly decreased with increase in nitrate availability except for R/S of offspring ramets during phase 1 (Table 1). Previous studies also reported that R/S ratio of *E. crassipes* decreased continually during the experimental period when nutrient concentration was regularly increased (Center and Spencer 1981, Xie *et al.* 2004，Li and Wang 2007). When whole clone fragment received heterogeneous nitrate, R/S of offspring ramets showed no significant difference with change of nitrate availability. Under heterogeneous nitrate condition, no significant response in ramets to local poor nitrate availability has been found in many other clonal plant species (Dong *et al.* 2002). The independent response to nitrate supply of parent ramets might be beneficial to increase the survival of the parent ramets especially in the severe resource-deficit conditions (Stuefer *et al*. 1994, Wijiesinghe and Handel 1994, Roiloa and Retuerto 2006).

Table 1. Growth and root/shoot ratio of ramets in *E. crassipes* grown with two nitrate supply regimes during different growth stages. Means ± SE, $n = 6$, values followed by different letters indicate significant differences at $P \leq 0.05$.

Stage	Nitrate [mM]	RGR [g g^{-1} d^{-1}]	R/S whole clone	R/S parent ramets	R/S offspring ramets
1	0.5	0.059 ± 0.005c	0.42 ± 0.05a	0.52 ± 0.07a	0.28 ± 0.05b
	5.0	0.070 ± 0.007b	0.27 ± 0.02b	0.31 ± 0.04b	0.22 ± 0.03b
2	0.5	0.073 ± 0.005b	0.48 ± 0.05a	0.47 ± 0.05a	0.49 ± 0.07a
	5.0	0.088 ± 0.007a	0.29 ± 0.03b	0.28 ± 0.04b	0.29 ± 0.03b

Table 2. Effect of nitrate availability on nitrate content [μmol g^{-1}(d.m.)] and NRA [μmol g^{-1}(d.m.) h^{-1}] in parent and offspring ramets of *E. crassipes* at different growth stages. Means ± SE, $n = 6$, values followed by different letters indicate significant differences at $P \leq 0.05$.

Stage	Nitrate	Parent ramets				Offspring ramets			
	[mM]	root NO_3^-	leaf NO_3^-	root NRA	leaf NRA	root NO_3^-	leaf NO_3^-	root NRA	leaf NRA
1	0.5	57.4 ± 8.3b	67.8 ± 8.4c	4.8 ± 1.1c	11.7 ± 1.3d	10.6 ± 0.8b	14.1 ± 1.3d	28.7 ± 4.2c	52.9 ± 4.2d
	5.0	148.5 ± 12.5a	174.9 ± 13.2b	6.0 ± 0.9c	33.0 ± 3.3b	22.7 ± 3.1a	35.6 ± 2.5b	50.4 ± 3.6b	160.3 ± 13.2b
2	0.5	61.0 ± 7.3b	79.6 ± 7.1c	9.8 ± 1.5b	22.8 ± 2.2c	10.1 ± 1.4b	20.1 ± 1.9c	58.8 ± 6.2b	73.4 ± 10.0c
	5.0	158.6 ± 9.9a	281.7 ± 14.4a	20.3 ± 2.2a	51.6 ± 4.5a	20.0 ± 1.7a	45.6 ± 3.6a	157.2 ± 13.2a	272.7 ± 15.8a

Tissue NO_3^- content in both parent and offspring ramets significantly increased with increase in nitrate availability (Table 2). In general, NO_3^- content in parent ramets was higher than that in offspring ramets in both phase 1 and 2. Despite offspring ramets were supplied with N-free solution during phase 1, considerable leaf NO_3^- content and relatively lower root NO_3^- content in offspring ramets were detected. It is well established that clonal plants have the ability to share nitrate within inter-connected ramets, especially in heterogeneous nitrate environment (Alpert 1991, Saitoh *et al.* 2006), and our results were consistent with these previous studies. It was inferred that physiological integration from parent to offspring ramet should be occurred in stoloniferous herb *E. crassipes* during early growth stage. Furthermore, no significant difference of leaf NO_3^- content was found between parent and offspring ramets under 5 mM nitrate, while leaf NO_3^- content was higher in parent than in offspring ramets under 0.5 mM nitrate. These results suggested that the physiological integration was probably enhanced by nitrate availability during establishment of *E. crassipes*.

Similar to tissue NO_3^- content, root and leaf NRA in parent and offspring ramets significantly increased nitrate availability (Table 2). For example, leaf NRA in parent and offspring ramets increased by 152 and 182 %, respectively, during phase 1. Since NR is a substrate inducible enzyme, the variation of NRA was strongly correlated with changes of tissues nitrate content, similarly as reported in non-clonal plant species (Chen *et al*. 2004, Cruz *et al.* 2004). Generally, root and leaf NRA in parent and offspring ramets was greatly higher in phase 2 than that in phase 1. Compared with NRA in inter-connected ramets, quite different pattern of NRA in parent and offspring ramets were found in phase 1 and 2. Leaf NRA was higher in parent ramets than that in offspring ramets during phase 1. There was no significant difference of leaf NRA between parent and offspring ramets in phase 1, when clone fragments were supplied 5 mM nitrate. Conversely, leaf NRA in parent ramets was significantly lower than that in offspring ramets during phase 2 and at 0.5 mM nitrate higher leaf NRA in parent ramet and lower in offspring ramets during phase 1 was observed. For root NRA in parent and offspring ramets, no difference was observed in phase 2. If root of parent and offspring ramets had similar ability to absorb nitrate under homogeneous condition, the higher nitrate NRA in leaves of offspring ramets might attribute to nitrate transport from parent to offspring ramets even under homogeneous environment. This observed nitrate translocation between ramets was likely related to the maintenance of internal nutrient gradient, which was probably caused by active growth of offspring ramets. Different physiological integration was reported to take place in different clonal plant species (Alpert 1996, D'Hertefeldt and Jónsdóttir 1999), and the results in our study suggested that physiological integration within *E. crassipes* clone might mainly depend on acropetal nitrate translocation from parent to offspring ramets even in homogeneous condition. A recent study on some seagrasses species by Marbà *et al.* (2006) found that the degree of resource sharing by offspring ramets was accelerated in fast clonal growth in eutrophic environment. Similarly, the present results showed priority of nitrate allocation in offspring ramets of *E. crassipes* under high nitrate availability indicating that majority of nitrate reduction in offspring ramets should be beneficial to growth of whole clone fragment.

In short, increased availability of nitrate would favour establishment and spread of offspring ramets for invasive clonal plant *E. crassipes*. Therefore, it is concluded that the spatial distribution of nitrate reduction within *E. crassipes* clones is a particularly important mechanism to support the establishment and expansion of offspring ramets, and the management of eutrophic water ecosystems is likely effective for controlling this invasive plant.

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