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Physiological and metabolic profiles of common reed provide insights into plant adaptation to low nitrogen conditions

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ABSTRACT

One of the most distinct features of the common reed (*Phragmites australis*) is its ability to survive under extremely low nitrogen conditions. To explore the regulation mechanisms of reed to adapt to nitrogen deficiency, we treated reed seedlings under long-term extremely low nitrogen conditions and profiled the physiological and metabolic features of photosynthesis, metabolism, growth, nutrient balance, and enzyme activities. Unexpectedly, the photosynthesis, biomass and carbon content were still maintained at high levels in reed under N-deficient conditions regardless of the decreased content of chlorophyll and nitrogenous compounds. Using mass spectrometry, we profiled metabolism of 627 metabolites and found the concentrations of lactic acid and galactinol were accumulated under the treatment. The development of underground organs and nutrient accumulation (B, P, Zn and Na) were also enhanced under the condition. Unlike the positive correlation of nitrate reductases and N levels in other plants, we found the catalytic activities of nitrate reductases were dramatically elevated in roots under the N-deficient condition, which may increase the intracellular NO_3^- and NH_4^+ levels. Our experiments characterized the unique features of reed under extreme nitrogen deficiency conditions and also provided valuable information for other corps to develop the cultivars with high yield under low nitrogen input.

1. Introduction

Nitrogen (N), is a constituent of many macronutrients that are vital for plant growth, development, reproduction, and stress-responses; it is an essential building block of numerous important compounds, including amino acids, proteins (enzymes), nucleic acids, chlorophyll, and plant hormones (Comadira et al., 2015; Duan et al., 2007). Accordingly, the amount of soil N is considered to be an important factor determining plant growth and crop productivity. As a consequence, large amounts of N fertilizers are applied to fields to maximize crop productivity (O'Brien et al., 2016). However, plants generally only require a limited amount of the N fertilizer applied and most is lost into the ground water, causing environmental problems (Pratelli and Pilot, 2014). Additionally, nitrogen application is a major economic cost for crop producers. Finally, increase in N levels in surface soil may also contribute to global climate changes (Niu et al., 2016). Thus, planting crop cultivars with a high efficiency of nitrogen usage in low N soil will be essential for agricultural sustainability. However, as already mentioned, farmers inevitably choose to apply N to maximize crop yields. As a result, after domestication or long-term acclimatization under high N condition, crops are not usually able to use N efficiently (Rao et al., 2016). Therefore, investigation of wild plant species with higher N usage efficiency may provide useful information for improving crop cultivars.

Plants can respond to low N stress by changing root lengths and increased root branching relative to plants under stress-free conditions (Amtmann and Armengaud, 2009; O'Brien et al., 2016; Rao et al., 2016). As documented by previous studies, low N stress increases the ratio of plant roots to shoots (Kiba and Krapp, 2016; Niu et al., 1995). In addition, changes to the uptake of other elements, such as P, Mg, and Ca were also observed in plants under N stress conditions (Amtmann and Armengaud, 2009).

N exists in the soil in the form of NO_3^- and NH_4^+. Plants utilize NO_3^- by reducing them to nitrites using nitrate reductase (NR) and then to ammonium (NH_4^+) with nitrite reductase (Chamizo-Ampudia et al., 2017). Eventually, NH_4^+ from both NO_3^- reduction

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and the soil is incorporated into organic molecules by glutamine synthetase (GS), glutamate synthase (Fd-GOGAT and NADH-GOGAT), or glutamate dehydrogenase, which are mainly expressed in chloroplasts and mediate assimilation of NH_4^+ from photorespiration (O'Brien et al., 2016). In principle, low N stress may strongly influence photosynthesis and limit the growth of plants (Pinto et al., 2014). Photosynthesis and N metabolism interact in the chloroplasts. To achieve efficient photosynthesis, prevention of NH_4^+ toxicity is mediated through the GOGAT/GS cycle during photorespiration. However, photosynthesis can also supply the GOGAT/GS cycle with reducing activity through ATP and NADPH. Thus, low N conditions may directly affect photorespiration in chloroplasts through the GOGAT/GS cycle and eventually affect the efficiency of photosynthesis and plant growth. Plants can store nitrogen in large amounts within enzymes involved in carbon fixation; for example, leaf Rubisco and phosphoenolpyruvate (PEP) carboxylase are involved in the C4 carbon concentrating mechanism and CO_2 fixation (Fischer et al., 2013). As a consequence, low N conditions can reduce the capacity of these key enzymes and result in inefficient photosynthesis and, thereby, slow plant growth (Niu et al., 1995).

The common reed is a rhizomatous perennial graminoid grass and is a common species of wetlands throughout the world. It is known for its adaptability to extreme environments, such as high salinity, drought, heavy metal stress, and low nutrition. This adaptability suggests its genome may include valuable gene resources related to stress tolerance, such as to low N conditions. In the present study, we investigated the effects of low N conditions on photosynthesis, N metabolism, metabolomics, growth, and nutrient balance in the common reed to elucidate its adaptive mechanisms to N deficiency. Our analyses indicated that the common reed shows physical and metabolomic changes in response to low N conditions, and we suggest two possible mechanisms for these responses.

2. Materials and methods

2.1. Plant materials, growth conditions and treatments

Seeds of the common reed were collected from Jilin Province, China. The seeds were sown in washed sand in 34-cm plastic pots with drainage holes at the bottom. After germination, each pot contained five seedlings, which were watered to saturation with half-strength (0.5×) Hoagland nutrient solution. All plants were grown under 16 h light/8 h dark at 26 °C during the day and 19 °C at night. The seedlings were grown under these conditions for 30 days. Then, 24 pots of uniformly sized seedlings were randomly assigned to 2 sets, i.e., 12 pots in each set. Each pot was considered as a single replicate. One set was used as the untreated control group. The other set served as low N stress treatment group. Control plants were watered with 0.5 Hoagland nutrient solution (5 mM N) at 17:00–18:00 each day. The low N treatment group was watered thoroughly once per day at the same time with 0.5 Hoagland nutrient solution containing 0.1 mM N and other nutrients. The low N treatment lasted 60 days.

2.2. Physiological measurements

Chlorophyll fluorescence parameters, net photosynthetic rate (P_N), stomatal conductance (g_s), internal CO2 concentration (Ci), transpiration rate (E), PSII efficiency (Φ_{PSII}), efficiency of excitation capture by open PSII centers (F_v'/F_m'), minimal fluorescences (Fo), maximal fluorescences (Fm), maximum quantum yield of photosystem II (F_v/F_m), chlorophyll-*a* (Chl-*a*) and chlorophyll-*b* (Chl-*b*) were measured using the portable open flow gas exchange system LI-6400 (LICOR, USA), between 8:30 to 10:30 a.m. on fully expanded leaf blades of the sampled plants. A photosynthetic active radiation (PAR) wavelength of 1200 μmol m^{-2} s^{-1} was used.

2.3. GS and NR activity measurement

GS and NR activities were analyzed using the previous described methods (Luo et al., 2013). For GS activity measurement, fresh samples were homogenized at 4 °C in 50 mM Tris-HCl extraction solution (pH 8.0) containing 2 mM $MgCl_2$, and 2 mM DTT. The assay solution contained 0.35 ml of 40 mM ATP and 0.8 ml of 0.1 M Tris-HCl buffer (pH 7.4) with 20 mM Na-glutamate, 80 mM $MgSO_4$, 20 mM cysteine, 2 mM EGTA, and 80 mM NH_2OH. After adding the enzyme extract to the assay solution, the mixture was incubated at

Table 1
Effects of low N on photosynthesis of common reed plants.

	5 mmol L^{-1} N	0.1 mmol L^{-1} N
P_N [μmol (CO_2) $m^{-2}s^{-1}$]	19.45 ± 1.34	20.44 ± 1.31
g_S [mol (H_2O) $m^{-2}s^{-1}$]	0.21 ± 0.02	0.237 ± 0.002
Ci [μmol mol^{-1}]	254.89 ± 13.34	262.38 ± 6.15
E [mmol (H_2O) $m^{-2}s^{-1}$]	5.20 ± 0.31	5.68 ± 0.38
WUE (μmol $mmol^{-1}$)	2.50 ± 0.13	2.40 ± 0.01
F_v'/F_m'	0.61 ± 0.06	0.50 ± 0.05
Φ_{PSII}	0.42 ± 0.05	0.38 ± 0.02
F_o	98.85 ± 7.82	88.32 ± 20.34
F_m	471.08 ± 82.25	435.21 ± 79.80
F_v/F_m	0.79 ± 0.02	0.78 ± 0.01
Chl*a* (g kg^{-1} FM)	2.87 ± 0.13	0.77 ± 0.06*
Chl*b* (g kg^{-1} FM)	0.95 ± 0.04	0.21 ± 0.01*
Chl*a* + Chl*b* (g kg^{-1} FM)	3.82 ± 0.17	0.98 ± 0.07*
Chl*a*/Chl*b*	3.04 ± 0.01	3.69 ± 0.06

Thirty-day-old common reed seedlings were subjected to normal growth conditions and low N conditions for 60 days. Net photosynthetic rate (P_N), stomatal conductance (g_s), internal CO2 concentration (Ci), transpiration rate (E), PSII efficiency(Φ_{PSII}), efficiency of excitation capture by open PSII centers (F_v'/F_m'), minimal fluorescences (Fo), maximal fluorescences (Fm), maximum quantum yield of photosystem II (F_v/F_m), chlorophyll-*a* (Chl-*a*) and chlorophyll-*b* (Chl-*b*) are given with means (±SE) of five replicates. Statistical significance between control and treated samples was determined by *t*-tests, and significant differences were marked as * ($P < 0.05$).

Table 2
Relative concentration and fold changes of metabolites in reed leaves.

Metabolites ID	[a]Metabolites	[b]Similarity	*P*-value	Fold change
240	serine 1	937.89	0.00018	0.19
85	alanine 1	929.58	0.00294	0.33
159	valine	926.71	0.00001	0.36
60	lactic acid	921.86	0.00226	2.13
191	ethanolamine	900.79	0.00559	0.85
359	4-aminobutyric acid 1	888.86	0.01560	0.34
363	oxoproline	880.29	0.00039	0.24
824	galactinol 3	866.21	0.00072	2.47
346	aspartic acid 1	863.93	0.00397	0.18
123	4-aminobutyric acid 3	855.43	0.00321	0.40
214	proline	829.57	0.04000	0.07
345	asparagine 4	783.64	0.00798	0.01
216	glycine 2	743.14	0.00004	0.38

Thirty-day-old common reed seedlings were subjected to 0.1 mM N or 5 mM N conditions for 60 days. The relative concentration of each metabolite is the mean obtained from five biological replicates using GC-MS. Fold changes are calculated using the formula: 0.1 mM N condition/5 mM N condition. *$P < 0.05$.

[a] For the GC-Quad FiehnLib library, we named derivatives sequentially according to their retention index, e.g. serine 1, serine 2 and serine 3 (for derivatives with without TMS-groups characterizing the primary amino group).

[b] The LECO/Fiehn Metabolomics Library was used to identify compounds. It gives a similarity value for compound identification accuracy. If the similarity is > 700, metabolite identification is reliable. If the similarity is < 200, the library only uses "analyte" for the compound name. If the similarity is 200–700, the compound name is the putative annotation. We only list metabolites that showed significant differences.