

Differential Effects of Ammonium and Nitrate on Growth Performance of *Glechoma longituba* under Heterogeneous Cd Stress

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Abstract: Water, minerals, nutrients, etc., can be shared by physiological integration among inter-connected ramets of clonal plants. Nitrogen plays an important role in alleviating cadmium (Cd) stress for clonal plants. But how different forms of nitrogen affect growth performance of clonal plants subjected to heterogeneous Cd stress still remains poorly understood. A pot experiment was conducted to investigate the differential effects of ammonium and nitrate on growth performance of *Glechoma longituba* under heterogeneous Cd stress. In the experiment, parent ramets of *Glechoma longituba* clonal fragments were respectively supplied with modified Hoagland solution containing 7.5 mM ammonium, 7.5 mM nitrate or the same volume of nutrient solution without nitrogen. Cd solution with different concentrations (0, 0.1 or 2.0 mM) was applied to offspring ramets of the clonal fragments. Compared with control (N-free), nitrogen addition to parent ramets, especially ammonium, significantly improved antioxidant capacity [glutathione (GSH), proline (Pro), peroxidase (POD), superoxide dismutase (SOD) and catalase (CAT)], PSII activity [maximum quantum yield of PSII (F_v/F_m) and effective quantum yield of PSII (Φ PSII)], chlorophyll content and biomass accumulation of the offspring ramets suffering from Cd stress. In addition, negative effects of nitrate on growth performance of whole clonal fragments were observed under Cd stress with high concentration (2.0 mM). Transportation or sharing of nitrogen, especially ammonium, can improve growth performance of clonal plants under heterogeneous Cd stress. The experiment provides insight into transmission mechanism of nitrogen among ramets of clonal plants suffering from heterogeneous nutrient supply. Physiological integration might be an important ecological strategy for clonal plants adapting to heterogeneous environment stress conditions.

Keywords: Clonal plant; physiological integration; nitrogen form; antioxidant capacity; chlorophyll fluorescence



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1 Introduction

One of the most striking attributes of clonal plants is their capacity for physiological integration, which enables the inter-connected ramets to share water, carbohydrates, minerals and nutrients as well as signal substance [1–4]. Resources sharing among inter-connected ramets could buffer the negative influences of heterogeneous distribution of resources [2,5–7] and heterogeneous environment stress, such as wind erosion [8], water stress [6,9] and heavy metals stress [10].

Cadmium (Cd) is an extremely harmful pollutant in the environment, which seriously affects a variety of physiological processes of plants [11–13]. Proline content and antioxidant enzymes (peroxidase [POD] superoxide dismutase [SOD] and catalase [CAT]) play very important roles in maintaining redox homeostasis [14,15] and scavenging free radicals [16,17] and ROS for plants [13,18–19]. Cd stress generally induces nitrogen and carbon metabolism changes [11,20,21], water and mineral nutrition malabsorption [22,23], enzyme activities inhibition [21,24], reactive oxygen species (ROS) accumulation [24,25] and membrane damage [11,26]. Cd disrupts cellular redox homeostasis and generates the overproduction of reactive oxygen species (ROS) such as superoxide anion (O_2^-), hydroxyl radical (OH^\cdot) and hydrogen peroxide (H_2O_2) [13,24,27]. Overproduction of ROS causes lipid peroxidation, electrolyte leakage, DNA and proteins damages in plants [28,29]. The phytotoxicities of Cd stress may result in poor growth, low biomass accumulation and death of plants [20,21].

Nitrogen addition may alleviate Cd stress for plants [30,31]. Ammonium (NH_4^+) and nitrate (NO_3^-) ions are the two major forms of nitrogen taken up by the root of plant. Ammonium is usually incorporated into amino acids (glutamine/glutamate) by the GS/GOGAT pathway [6,32]. Meanwhile, nitrate reduced and stored in the vacuoles. In addition, it is translocated to the shoot for reduction and vacuolar storage [32]. Compared with nitrate, ammonium is more effective on improving foliar water content, transpiration rate, stomatal conductance and photosynthetic activity of plants suffering from Cd stress [20,21,33]. When ammonium is taken up by plants, rhizosphere soil pH is decreased [33,34]. Meanwhile, ammonium competes with Cd^{2+} for absorption sites in roots [35–37], depolarizes membrane potential of root cells [33,38]. The decrease of rhizosphere soil pH enhances the absorption of Cd^{2+} [33,34], but the competitive absorption of ammonium and Cd^{2+} may inhibits Cd^{2+} uptake in plants as well as the change of membrane [33–37]. Conversely, when nitrate is taken up by plants, rhizosphere soil pH is increased, which diminishes the absorption of Cd^{2+} [33,39]. As a signal substance in plants, nitrate can up-regulate IRT_1 expression, facilitating the uptake of metal cation including Cd^{2+} [12,39]. How ammonium and nitrate application affect growth performance of clonal plants subjected to heterogeneous Cd stress still remains poorly understood.

A pot experiment was conducted by using *Glechoma longituba* clonal fragments under heterogeneous Cd stress. Each clonal fragment selected in the experiment consisted of parent and offspring ramets. The offspring ramets were treated with Cd solution with different concentrations (0, 0.1 and 2.0 mM), whereas the parent ramets were supplied with modified Hoagland solution containing ammonium or nitrate as the sole nitrogen source. In the control, parent ramets were supplied with the same volume of nutrient solution without nitrogen. Stolon between two successive ramets remained intact. In the experiment, we tested the following specific hypotheses. (1) The negative effects of Cd stress on growth performance of offspring ramets will be alleviated by the transportation or sharing of nitrogen from their connected parent ramets. (2) Ammonium will be more effective in buffering the negative effects of Cd stress on growth performance of offspring ramets than nitrate.

2 Materials and Methods

2.1 Plant Material and Treatment

Glechoma longituba (Nakai) Kupr is a stoloniferous perennial herb. A genet or fragment consists of a number of ramets connected by stolons. Ramets can develop on all stolon nodes. Each ramet has two

opposite single leaves originating from a stolon node. Every leaf axil bears one bud that may grow into a secondary stolon. The plant is generally found in forests, on roadsides or close to creeks and is distributed all over China except for the Northwest [40].

On June 15th 2015, clonal fragments of *Glechoma longituba* were selected from the plant stock with uniform size. Each fragment consisted of parent and offspring ramets. Parent and offspring ramets of each pair were rooted individually in 0.5 L plastic pots filled with acid washed sand. The experimental design consisted of two crossed factors: ‘nitrogen addition’ (N-free, ammonium and nitrate) and ‘Cd stress’ (0, 0.1 and 2.0 mM Cd). Parent ramets were supplied with 50 mL modified Hoagland solution containing 7.5 mM ammonium or 7.5 mM nitrate as the sole nitrogen source (Tab. 1). Meanwhile, 50 mL Cd solution with different concentration (0, 0.1 and 2.0 mM) was applied to offspring ramets. Stolon between two successive ramets remained intact (Please see Fig. 1). Modified Hoagland solution and Cd solution were applied to parent and offspring ramets respectively every three days. To avoid nutrient or Cd build-up, all pots were watered every two days [6]. The experiment was performed in a greenhouse for 45 days. During the experiment, the mean temperature in the glasshouse was $28.5 \pm 1.4^\circ\text{C}$ and the relative humidity $70.5 \pm 2.5\%$. The minimum and maximum photosynthetic photon flux density (PPFD) in the glasshouse were 136.2 and 325.1 $\mu\text{mol m}^{-2} \text{s}^{-1}$, respectively. Each treatment was replicated 8 times. No ramet died during the experiment.

Table 1: The composition of modified Hoagland solution containing different forms of nitrogen or without nitrogen

Nutrient composition	Ammonium treatment (μM)	Nitrate treatment (μM)	N-free (μM)
$(\text{NH}_4)_2\text{SO}_4$	3,750	0	0
KNO_3	0	7,500	0
K_2SO_4	3,750	0	3,750
Na_2SO_4	0	7,500	3,750
NaH_2PO_4	3,750	3,750	3,750
MgSO_4	2,500	2,500	2,500
CaCl_2	5,000	5,000	5,000
H_3BO_3	50	50	50
MnSO_4	2.5	2.5	2.5
ZnSO_4	2.5	2.5	2.5
CuSO_4	0.5	0.5	0.5
Na_2MoO_4	1	1	1
Fe-EDTA	100	100	100

2.2 Determination of GSH and Pro Contents

Total glutathione (GSH) content was measured according to the spectrophotometric method [41,42]. This method is based on the reduction of oxidized glutathione (GSSG) to reduced GSH using the GSH-reductase recycling procedure. With 50 $\text{g}\cdot\text{L}^{-1}$ tri-chloroacetic acid (TCA), 1.0 g fresh leaves of offspring ramets were ground and centrifuged at $12,000 \times g$ for 20 min. The supernatant was used for GSH assay. In the presence of glutathione reductase (GR) and NADPH, total GSH (GSH + GSSG) was determined by the colorimetric reaction of DTNB [5, 5'-dithio-bis (2-nitrobenzoic acid)] with GSH to form TNB

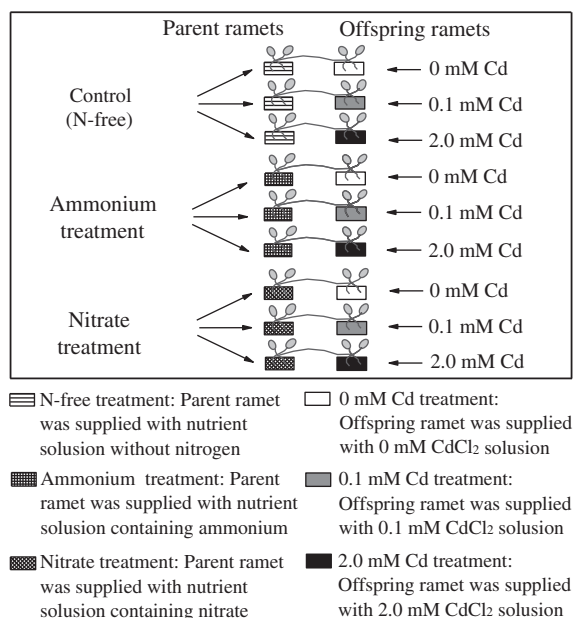


Figure 1: Schematic representation of the experimental design by using *Glechoma longituba* clonal fragments

(5-thio-2 nitrobenzoic acid). The rate of TNB formation, which was proportional to the total GSH concentration, was measured spectrophotometrically at 412 nm.

Proline was determined according to the procedure described by Bates et al. [43]. Fresh leaves (0.5 g) of offspring ramets were used to extract Pro with 3% aqueous 5-sulphosalicylic acid, centrifuged at $10,000 \times g$. The sample of the supernatant was used for the Pro assay and measured at 520 nm.

2.3 Antioxidant Enzymes Extraction and Assays

Two days before harvest, in a mortar, 0.5 g fresh leaves of offspring ramets were ground to a fine powder with liquid nitrogen. Then, the fine powder was extracted in 3 ml of 50 mM sodium phosphate buffer (pH 7.8) including 1.0 mM EDTA and 2% (w/v) polyvinylpyrrolidone (PVPP). The homogenate was centrifuged at $10000 \times g$ for 30 min and the supernatant was stored at -75°C for SOD, POD and CAT activity analyses.

SOD activity was determined according to the method of Beauchamp et al. [44]. The reaction mixture (3 mL) contained 50 mM phosphate buffer (pH 7.8), 0.1 mM EDTA, 13 mM methionine, 75 μM nitroblue tetrazolium (NBT), 2 μM riboflavin and 0.2 mL of the enzyme extract. Riboflavin was added last and the reaction was initiated by placing the tubes under two 20 W fluorescent lamps. The reaction was terminated after 10 min by removing the reaction tubes from the light source. Non-illuminated and illuminated reactions without supernatant served as calibration standards. The photoreduction of NBT (production of blue formazan) was measured at A_{560} . One unit of SOD was defined as the enzyme activity that inhibited the photoreduction of NBT to blue formazan by 50%, and SOD activity of the extracts was expressed as SOD units per g of fresh plant weight.

POD activity was determined according to the method of Zhang et al. [13]. In the presence of H_2O_2 , POD catalyzes the transformation of guaiacol to tetraguaiacol (brown product). The oxidation of guaiacol was measured by the increase in absorbance at 470 nm for 1 min ($\epsilon = 26.6 \text{ L}\cdot\text{mmol}^{-1} \text{ cm}^{-1}$). The reaction mixture contained 50 μL of 20 mM guaiacol, 2.7 mL of 10 mM phosphate buffer (pH 7.0), and 0.2 mL enzyme extract. The reaction was started with 20 μL of 40 mM H_2O_2 . One unit of POD activity was expressed as POD units per min and g of fresh plant weight.

CAT activity was measured according the method of Aebi [45], with minor modifications. The reaction mixture (1.5 mL) consisted of 100 mM phosphate buffer (pH 7.0), 0.1 mM EDTA, 20 mM H₂O₂ and 50 µL enzyme extract. The reaction was started by addition of the extract. The decrease of H₂O₂ was monitored at 240 nm and quantified by its molar extinction coefficient (36 L·mmol⁻¹ cm⁻¹) and the results expressed as CAT units per min and g of fresh plant weight.

2.4 Measurement of Chlorophyll Fluorescence Parameters and Chlorophyll Content

Chlorophyll fluorescence parameters were measured by the saturation pulse method [6,46] with a portable chlorophyll fluorometer (PAM-2100, Walz, Effeltrich, Germany). A pulse of saturating light (>4000 µmol photons m⁻² s⁻¹, 0.8 s pulse length, actinic white light) was applied through an optical fibre at an angle of 60° relative to the sample and a distance of 12 mm from the leaf. Measurements were made on the upper surface of the two leaves of each original offspring ramet two days before harvest. The maximum quantum yield of PSII (F_v/F_m) and effective quantum yield of PSII (Φ_{PSII}) were determined. We calculated the maximum quantum yield of PSII as the ratio $F_v/F_m = (F_m - F_0)/F_m$ [47], where F_0 and F_m are the minimal and maximal fluorescence yields of a dark-adapted sample, respectively, with all PSII reaction centers fully open (i.e., all primary acceptors oxidized). These parameters were measured after dark adaptation for 30 min. The F_v/F_m ratio provides an estimate of the efficiency of excitation energy capture by open PSII reaction centers [48]. We calculated Φ_{PSII} as $(F'_m - F_t)/F'_m$ [49], where F'_m is the maximal fluorescence yield reached in a pulse of saturating light with an illuminated sample and F_t is the fluorescence yield of the leaf at a given photosynthetic photon flux density. Φ_{PSII} is a measure of the fraction of the light absorbed by chlorophyll that is photochemically converted into PSII [50].

Total chlorophyll was extracted from 0.5 g fresh leaves of offspring ramets using 80% (v/v) acetone. Absorbance was measured at 663 and 645 nm by a spectrophotometer (UV-2450, Shimadzu Corporation, Japan). Extinction coefficients and equations reported by [51] were used to calculate the amounts of total chlorophyll content.

2.5 Determination of Biomass Accumulation

At the end of the experiment, parent and offspring ramets were harvested separately and dried to constant weight at 70°C to determine dry mass. The newly produced stolons and new ramets were assigned to the ramets (initial parent or offspring ramets) producing them.

2.6 Statistical Analyses

Prior to analysis, Pro content, SOD activity and POD activity were square root-transformed to meet the requirements of normality and homoscedasticity for ANOVA. Differences among treatments for antioxidant capacity, PSII activity, total chlorophyll content and biomass accumulation were evaluated by two-way ANOVA with 'nitrogen addition' and 'Cd stress' as the main factors. Fisher's least-significant difference test (LSD) was employed to compare the means in all measured characters (SPSS 23.0).

3 Results

3.1 Antioxidant Capacity of Offspring Ramets

Foliar antioxidant enzyme (SOD, POD and CAT) activities of offspring ramets were significantly affected by nitrogen addition, Cd stress and their interaction as well as contents of GSH and Pro (Tab. 2). Compared with control, ammonium addition to parent ramets significantly increased foliar GSH content, SOD, POD and CAT activity by 14.9%, 11.9%, 22.0% and 257.5% in offspring ramets subjected to low concentration (0.1 mM) Cd stress (Figs. 2A, 2C–2E). Nitrate addition to parent ramets significantly increased foliar Pro content, SOD, POD and CAT activity by 85.0%, 5.1%, 27.4% and 46.9% in offspring ramets subjected to low concentration (0.1 mM) Cd stress (Figs. 2B–2E). But it significantly reduced foliar GSH content of offspring ramets (Fig. 2A). Compared with nitrate, GSH content SOD and CAT

Table 2: Effects of nitrogen addition, Cd stress and their interaction on antioxidant capacity of offspring ramets

Effect	df	Foliar GSH content	Foliar Pro content	Foliar SOD activity	Foliar POD activity	Foliar CAT activity
Nitrogen addition (N)	2	<i>F</i> 1725.444 <i>P</i> <0.001	279.269 <0.001	1065.097 <0.001	160.063 <0.001	1027.349 <0.001
Cadmium stress (Cd)	2	<i>F</i> 6987.554 <i>P</i> <0.001	739.841 <0.001	733.385 <0.001	841.315 <0.001	395.711 <0.001
N × Cd	4	<i>F</i> 1102.832 <i>P</i> <0.001	229.241 <0.001	47.994 <0.001	154.181 <0.001	257.841 <0.001
Error	63					
Total	71					

Values are in bold where $P < 0.05$.

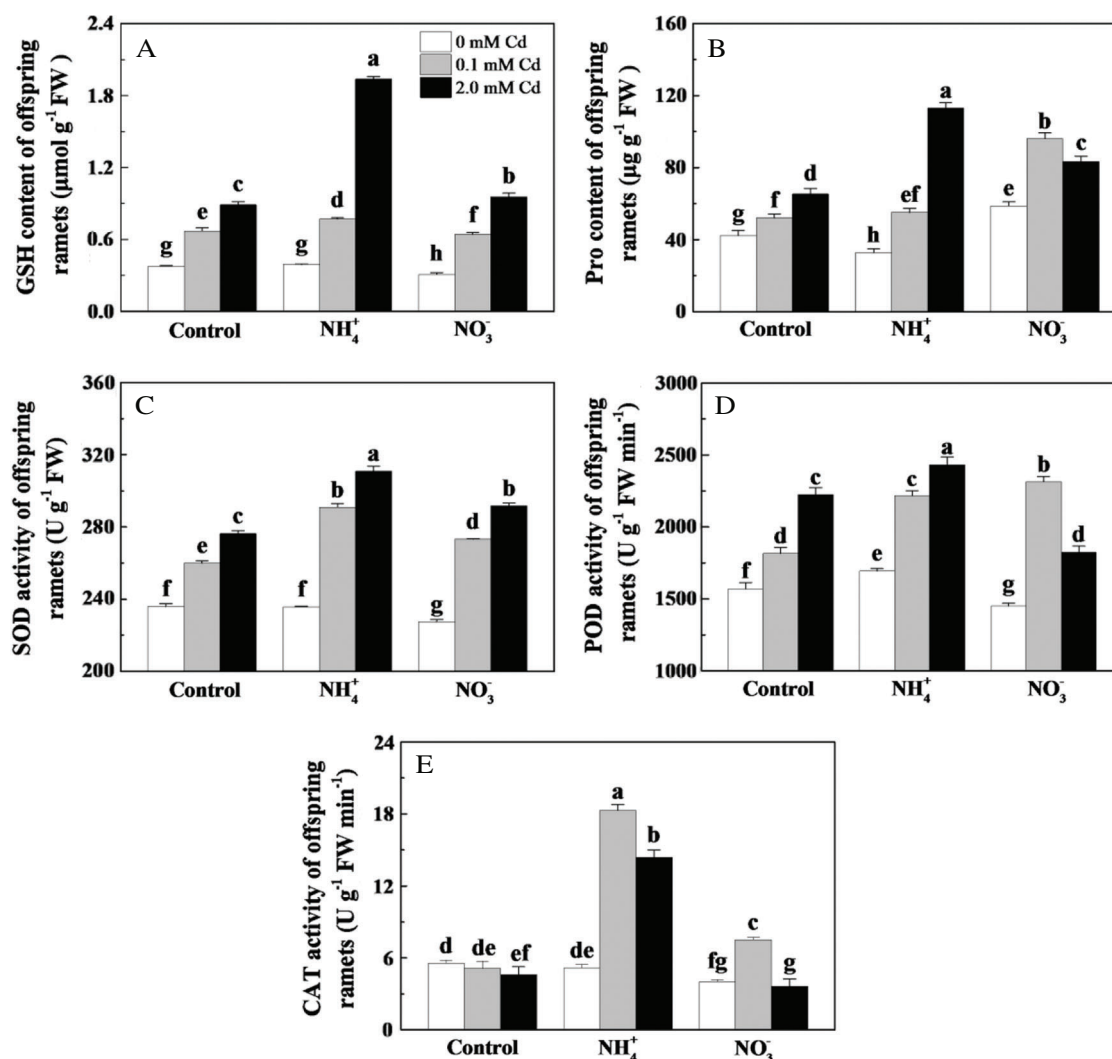


Figure 2: Foliar GSH content (A), Pro content (B), SOD activity (C), POD activity (D) and CAT activity (E) of offspring ramets suffering from different concentrations (0, 0.1 and 2.0 mM) of Cd stress in the control and nitrogen (ammonium and nitrate) addition treatments. Bars marked with different lowercase letters are significantly different at $p \leq 0.05$

activity of offspring ramets in ammonium treatments were significantly higher by 14.9%, 11.9% and 143.5%, respectively (Figs. 2A, 2C, 2E); but Pro content and POD activity of offspring ramets were lower, respectively by 84.3% and 4.4% (Figs. 2B, 2D).

Under high concentration (2.0 mM) Cd stress, foliar GSH and Pro contents, SOD, POD and CAT activities of offspring ramets were increased, respectively by 117.9%, 73.1%, 12.5%, 9.3% and 210.4% in ammonium addition as compared to control (Figs. 2A–2E). In nitrate treatment, foliar GSH and Pro contents, and SOD activity of offspring ramets were significantly increased, respectively by 7.6%, 27.5% and 5.6% as compared to control (Figs. 2A–2C). However, foliar POD and CAT activities of offspring ramets was significantly decreased 21.9% and 26.8% in nitrate treatment (Figs. 2D, 2E). Compared with nitrate, GSH and Pro contents, SOD, POD and CAT activities were higher, respectively by 45.8%, 35.8%, 6.5%, 33% and 294% (Figs. 2A–2E).

3.2 PSII Activity and Total Chlorophyll Content of Offspring Ramets

Foliar PSII activity (F_v/F_m and Φ PSII) and total chlorophyll content of offspring ramets were significantly affected by nitrogen addition, Cd stress and their interaction (Tab. 3). Compared with control, foliar F_v/F_m , Φ PSII and total chlorophyll content of offspring ramets subjected to low concentration (0.1 mM) Cd stress were significantly increased, respectively by 14.3%, 43.7% and 66.7% when ammonium was added to parent ramets; Nitrate addition to parent ramets significantly increased foliar F_v/F_m , and Φ PSII of offspring ramets suffering from low concentration (0.1 mM) Cd stress, respectively by 5.9% and 18.7%. Meanwhile, F_v/F_m , Φ PSII and total chlorophyll content of offspring ramets in ammonium treatments were 7.5%, 21.1% and 58.3% higher, respectively, than those in nitrate treatment (Figs. 3A–3C).

Table 3: Effects of nitrogen addition, Cd stress and their interaction on PSII activity and total chlorophyll content of offspring ramets

Effect	df		F_v/F_m	Φ PSII	Total chlorophyll content
Nitrogen addition (N)	2	<i>F</i>	35.977	19.610	251.770
		<i>p</i>	<0.001	<0.001	<0.001
Cadmium stress (Cd)	2	<i>F</i>	161.531	56.779	107.005
		<i>p</i>	<0.001	<0.001	<0.001
N × Cd	4	<i>F</i>	10.391	3.240	40.698
		<i>p</i>	<0.001	0.020	<0.001
Error	63				
Total	71				

Values are in bold where $P < 0.05$.

When offspring ramets subjected to high concentration (2.0 mM) Cd stress, ammonium addition to parent ramets significantly increased foliar F_v/F_m , Φ PSII and total chlorophyll content of offspring ramets, respectively by 11.5%, 29% and 22.6%, as compared with control (Figs. 3A–3C); however, similar patterns were not observed in nitrate treatment (Figs. 3A–3C). Compared with nitrate, foliar F_v/F_m , Φ PSII and total chlorophyll content of offspring ramets in ammonium treatments were significantly higher by 15.3%, 29% and 30%, respectively (Figs. 3A–3C).

3.3 Biomass Accumulation of Clonal Fragments

Biomass accumulation of clonal fragments were significantly affected by nitrogen addition and Cd stress as well as parent and offspring ramets. Meanwhile, significant interaction of nitrogen addition and Cd stress on biomass accumulation of offspring ramets and clonal fragments was detected (Tab. 4). Compared with

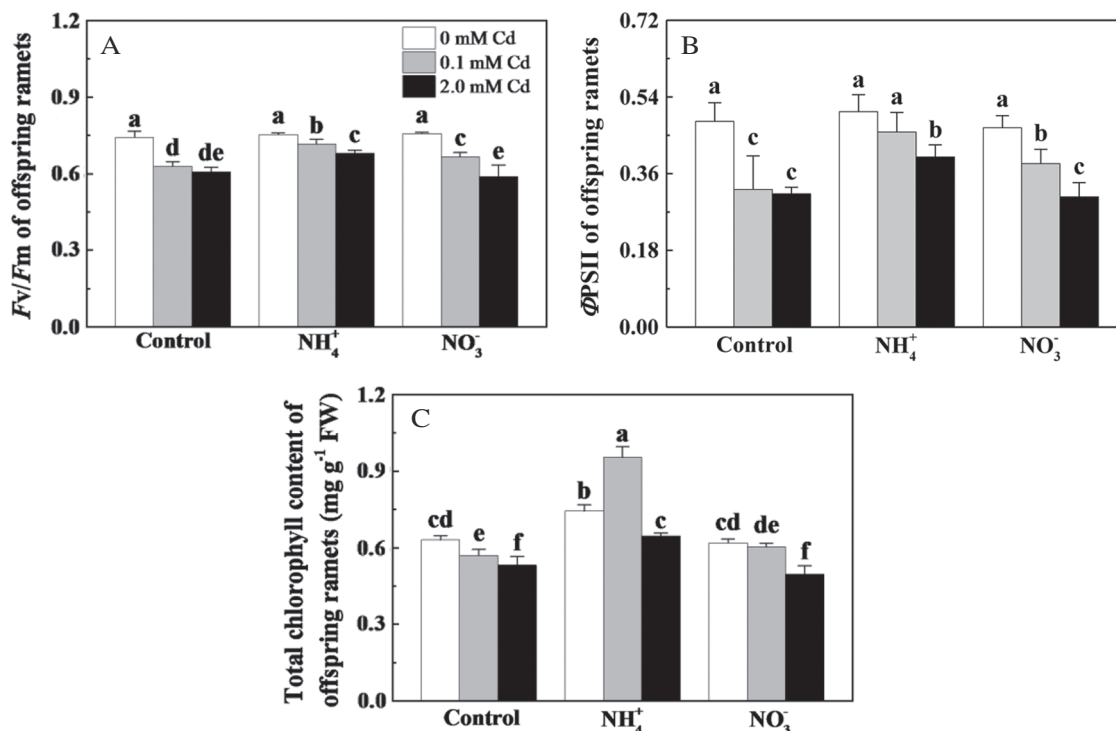


Figure 3: Foliar F_v/F_m (A), Φ PSII (B) and total chlorophyll content (C) of offspring ramets suffering from different concentrations (0, 0.1 and 2.0 mM) of Cd stress in the control and nitrogen (ammonium and nitrate) addition treatments. Bars marked with different lowercase letters are significantly different at $p \leq 0.05$

Table 4: Effects of nitrogen addition, Cd stress and their interaction on biomass accumulation of parent ramets, offspring ramets and clonal fragment

Effect	df		Parent ramets	Offspring ramets	Clonal fragment
Nitrogen addition (N)	2	<i>F</i>	53.373	33.624	175.343
		<i>p</i>	<0.001	<0.001	<0.001
Cadmium stress (Cd)	2	<i>F</i>	32.002	90.518	248.483
		<i>p</i>	<0.001	<0.001	<0.001
N × Cd	4	<i>F</i>	2.384	9.843	22.613
		<i>p</i>	0.076	<0.001	<0.001
Error	63				
Total	71				

Values are in bold where $P < 0.05$.

control, biomass accumulation of clonal fragments, parent and offspring ramets were significantly increased 71.8%, 66.6% and 77.9% in ammonium and 41.4% 50.7% and 28.8% in nitrate treatments, respectively, when offspring ramets suffering from low concentration Cd stress (0.1 mM) (Fig. 4). Biomass accumulation of clonal fragments and offspring ramets in ammonium treatments were 21.5% and 38.1% higher, respectively, than those in nitrate treatment.

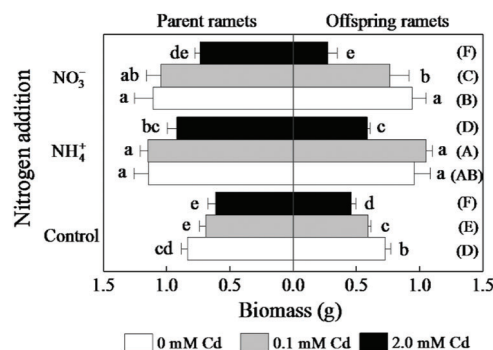


Figure 4: Biomass accumulation of parent, offspring ramets and clonal fragments in the control and nitrogen (ammonium and nitrate) addition treatments. The offspring ramets suffered from different concentrations (0, 0.1 and 2.0 mM) of Cd stress. Bars marked with different letters are significantly different at $p \leq 0.05$. Lower case letters represent parent and offspring ramets, and upper case letters represent whole clonal fragments

When offspring ramets suffering from high concentration Cd stress (2 mM), compared with control, biomass accumulation of clonal fragments, parent and offspring ramets were significantly increased 38.9%, 50.8% and 26.1% in ammonium treatments; however, biomass accumulation of offspring ramets was significantly decreased 70.2% in nitrate treatment. Compared with nitrate, biomass accumulation of clonal fragments, parent and offspring ramets in ammonium treatments were significantly higher by 48.5%, 24.3% and 114.8%, respectively.

4 Discussion

Our results supported the first hypothesis that negative effects of Cd stress on growth performance of offspring ramets could be alleviated by transportation or sharing of nitrogen from their connected parent ones. Compared with nitrate, ammonium addition to parent ramets significantly improved foliar antioxidant capacity and PSII activity of offspring ramets suffering from Cd stress as well as greater total chlorophyll content and biomass accumulation. It was concluded that ammonium could be more effective in buffering negative effects of Cd stress on growth performance of offspring ramets than nitrate. So, our results further supported the second hypothesis.

Pro, GSH and antioxidant enzymes may play an important role in alleviating Cd stress to plants [24,51,52]. Meanwhile, synthesis of GSH, Pro and antioxidant enzymes requires the participation of nitrogen [31]. Transportation or sharing of nitrogen, especially ammonium, incurred a significant increase of foliar GSH and Pro content in offspring ramets suffering from Cd stress as well as enhancement of SOD, POD and CAT activities, thereby improving their resistance to Cd stress in the experiment. This result was consistent with previous finding [20].

Cd stress causes structural changes in chloroplasts [21,53], reduces chlorophyll synthesis [54–56] and inhibits photosynthetic enzymes activities [25]. Nitrogen addition to parent ramets, especially ammonium, significantly relieved negative effects of Cd stress on PSII activity (F_v/F_m and Φ_{PSII}) and chlorophyll synthesis in offspring ramets suffering from Cd stress. A possible explanation is that transportation or sharing of nitrogen immediately promotes chlorophyll synthesis of offspring ramets suffering from Cd stress [31]. Further, increase of chlorophyll content can improve foliar efficiency to harvest light and activity of photosystem II [57].

Compared to control, nitrate addition to parent ramets significantly reduced biomass accumulation of offspring ramets suffering from Cd stress with higher concentration (2 mM). Nitrate as a signal substance, can up-regulate IRT1 expression, thereby facilitating the uptake of Cd²⁺ and developing more severe

toxic symptoms in plants [12,39]. In addition, nitrate transport across the plasma membrane results in temporary depolarized of the membrane potential in plant cell [33,58,59]. The depolarized the membrane potential may enhance the membrane transport of Cd^{2+} in plant [33,58,59]. However, uptake of ammonium reduces the membrane potential, which inhibits Cd^{2+} uptake [33,60]. In the experiment, nitrate addition to parent ramets caused higher foliar Cd content in offspring ramets than control (N-free) or ammonium (data not shown).

Transportation or sharing of nitrogen among ramets increased growth of clonal plants suffering from heterogeneous nutrient supply [61,62]. Ammonium or nitrate could be transported from parent ramets to their connected offspring ones [6]. Under heterogeneous Cd stress, differential effects of ammonium or nitrate addition to parent ramets on growth performance of offspring ramets and whole clonal fragments were observed in the experiment. However, further studies are needed to explore transmission mechanism of different nitrogen forms among ramets of clonal plants.

Our experiment provides evidence that ammonium will be more effective than nitrate in buffering negative effects of heterogeneous Cd stress on clonal plants. We tentatively conclude that preferential transportation or sharing of ammonium among ramets could be advantageous for clonal plants adapting to heterogeneous Cd stress [6]. Further studies on a wider range of species are needed to understand the generality of this pattern and to assess fully the ecological advantages afforded by these features.

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Conflicts of Interest: The authors declare that they have no conflicts of interest to report regarding the present study.

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