

Effects of Alternative Partial Root-zone Irrigation and Nitrogen Fertilizer on *Plukenetia volubilis* Seedlings

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Abstract This study was aimed to investigate the effects of alternative partial root-zone irrigation and nitrogen fertilizer on the potted seedlings of *Plukenetia volubilis*. A total of 7 treatments were designed with three factors, i.e., irrigation amount, irrigation mode and nitrogen fertilizer. The growth, photosynthesis and water use efficiency were analyzed. The results showed that compared with those under full irrigation, the biomass and water consumption under alternative partial root-zone irrigation were reduced by 5% and 75%, respectively, and the water use efficiency was increased by 60%. Under severe drought conditions, the root cap ratio in the nitrogen fertilizer treatment group was increased by 30%; the leaf area index, photosynthetic rate and biomass under alternative partial root-zone irrigation were reduced by 38%, 9% and 18%, respectively. It indicates that under severe drought conditions, alternative partial root-zone irrigation is not suitable to be matched with application of nitrogen fertilizer. In short, under moderate drought conditions, alternative partial root-zone irrigation could reduce transpiration and improve water use efficiency, and it is an effective water-saving irrigation technology for the plantation of *P. volubilis* plants.

Key words *Plukenetia volubilis* L.; Alternative partial root-zone irrigation; Nitrogen fertilizer; Growth; Water-use efficiency

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Plukenetia volubilis L. (Euphorbiaceae) is a perennial woody vine. The seeds of *P. volubilis* are rich in fat (45%–60%), protein (27%–33%), vitamins, sterols and other biologically active substances. Among multi-unsaturated fatty acid, Omega fatty acid is the dominant component^[1–2]. The oil of *P. volubilis* is considered one of the world's best edible vegetable oils^[3–5]. *P. volubilis* was introduced to Xishuangbanna Tropical Botanical Garden in 2006. Nowadays, it has a certain scale of planting in Xishuangbanna and Laos.

Due to monsoon climate, annual rainfall distribution is usually uneven in tropical and subtropical regions with a long dry season (November to April next year). In the dry season, plant

growth is inevitably affected by water stress. Previous studies have shown that compared with those under full irrigation, the female flowers, male flowers, fruits and source-sink ratio of *P. volubilis* plants under natural drought stress were significantly reduced, and the abortion rate of fruits was increased in Xishuangbanna, a tropical region in southwest China^[6]. The developed fruits in the late dry season are probably to be aborted when they enter rainy season, resulting in low fruit yield in early rainy season^[6]. Therefore, dry-season irrigation is necessary for the yield improvement of *P. volubilis* plants. Southwest China is short of water resources, especially in the last several years when serious drought appeared. It's an inevitable

分根区灌溉和氮肥处理对星油藤幼苗的影响

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摘要 探讨分根区灌溉和氮肥对盆栽星油藤幼苗生长的影响。试验设置3个因素, 灌水量、灌溉方式和氮肥, 共7个处理, 测量了幼苗生长、光合和水分利用效率等指标。结果表明: 与充分灌溉相比, 分根区灌溉的处理, 生物量降低5%, 节水75%, 水分利用率高出60%, 表现出极大的节水效益。重度干旱条件下, 施氮肥的处理, 根冠比增加达30%, 但分根区灌溉处理叶面积指数下降38%, 光合速率下降9%, 生物量下降18%, 分根区灌溉在严重干旱条件下不适宜施氮肥。总之, 在适度干旱条件下, 分根区灌溉降低蒸腾, 提高水分利用率, 是一种有效的节水灌溉技术。

关键词 星油藤; 分根区灌溉; 氮肥; 水分利用效率

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development tendency for modern agriculture to improve water use efficiency and yield with developed water-saving irrigation technologies.

Alternative partial root-zone irrigation (partial root-zone drying) is a new water-saving technology, which has attracted much attention [7]. Based on the theory of root-sourced signaling, alternative partial root-zone irrigation could control dried roots, so it has been widely promoted and applied in maize, soybeans and other field crops, and grapes and other fruit trees [8–12]. However, there has been no report on application of alternative partial root-zone irrigation in *P. volubilis* plants. Although alternative partial root-zone irrigation has been widely used in arid and semi-arid regions, there are few researches in tropical and subtropical regions [13]. Based on indoor pot experiment, the effects of alternative partial root-zone irrigation on growth and physiology of young *P. volubilis* seedlings were investigated in this paper, thereby providing theoretical basis for reasonable irrigation of *P. volubilis* plants in the field during the dry season.

Materials and Methods

Materials

The test was carried out in the greenhouse of Xishuangbanna Tropical Botanical Garden (21°56' N, 101° 1' E; altitude 560 m) during November 20, 2013 to January 11, 2014. There were fly nets around the greenhouse, and the average temperature inside the greenhouse was about 22 °C. The mature seeds of *P. volubilis* were

nursed in sandy soil. The growth-uniform young seedlings of *P. volubilis*, in height of about 20 cm, were transplanted to pots (inner diameter 26 cm, height 23 cm) with 7 kg soil. The basic physical and chemical properties of the tested soil were as follows: organic matter 18.39 g/kg, available nitrogen 110 mg/kg, available phosphorus 5.98 mg/kg, available potassium 98 mg/kg.

Methods

Experimental design

A total of three factors were designed, *i.e.*, irrigation mode, irrigation amount and fertilization. The irrigation modes included conventional irrigation and alternative partial root-zone irrigation (RD). The irrigation amount covered three levels, *i.e.*, normal supply (NS, 100% of transpiration amount; transpiration amount referred to the weight difference of every pot every two days), mild drought (MD, 75% of transpiration amount) and severe drought (SD, 50% of transpiration amount). For fertilization, nitrogen fertilization (N, along with urea, 5 g/plant) and no fertilization treatments were used. A total of seven treatments (Table 1) were designed with 20 pots for every treatment. In the RD treatment group, the pot was equally divided into two parts with a plastic plate, and the roots of every *P. volubilis* plant were equally divided into the two parts; the roots in the two parts were irrigated once alternatively every two weeks. After 10-d adaptation with full irrigation, the measurement was started. The pots in all the treatment groups were covered with plastic film to re-

duce soil evaporation. The water supply was strictly controlled, and the irrigation was carried out by hand. The pots were weighed and irrigated once every two days. The irrigation amount of every pot was recorded every time. Total irrigation amount (I) of every pot referred to the sum of irrigation amounts during the test. The nitrogen fertilizer was dissolved in irrigation water and applied diagonally uniformly around the seedlings. Throughout the experimental period, the nitrogen fertilizer was applied four times.

Measurement

When the test began, total five young seedlings were selected randomly for every treatment. They were dried at 105 °C for 30 min, and then at 70 °C to constant weight (W_1). Before the test ended, the net photosynthetic rate (P_n), transpiration rate (T_r), stomatal conductance (G_s) and intercellular CO_2 concentration (C_i), of fully expanded mature leaves of *P. volubilis* were measured with Li-6400 portable photosynthesis system during 8:30–11:00 of a sunny day. The instantaneous water use efficiency (WUE_i , P_n/T_r) was calculated. When the test ended, total five plants were sampled randomly for every treatment. The fresh blades were scanned with CanoScan4400F scanner, and their leaf area indexes were calculated using ImageJ software. The five plants in every treatment were divided into roots, stems and leaves, which were dried at 105 °C for 30 min and then at 70 °C to constant weights (W_2 , W_3 and W_4). The nitrogen content (N, %) in dried leaves was measured with TOC/TN analyzer.

Root cap ratio (g/g) = $W_2/(W_3 + W_4)$;

Photosynthetic N utilization efficiency ($\mu\text{mol}/(\text{mol}^2 \cdot \text{s} \cdot \text{N}) = P_n/N$;

Irrigation water use efficiency (WUE , g/L) = $(W_2 + W_3 + W_4 - W_1)/I$.

Data statistics and analysis

The data were analyzed using SPSS 13.0. ANOVA tests were conducted for the no fertilization treatments, and the multiple comparisons were conducted with least significant difference (LSD) method ($\alpha=0.05$). Two-way ANOVA test was conducted for the severe drought treatment. All figures were drawn using SigmaPlot.

Table 1 Irrigation treatments of *P. volubilis* seedlings

Treatment/%	Times	Irrigation mode	Fertilization	Total irrigation amount/ml
NS	26	Full irrigation	–	3 372
MD	26	Full irrigation	–	1 680
MD+RD	26	Alternative partial root-zone irrigation	–	1 315
SD	26	Full irrigation	–	684
SD+N	26	Full irrigation	✓	695
SD+RD	26	Alternative partial root-zone irrigation	–	350
SD+RD+N	26	Alternative partial root-zone irrigation	✓	325

NS, normal supply, the irrigation amount is the same with the transpiration; MD, mild drought, the irrigation amount is 75% of the transpiration; MD+RD, mild drought and alternative partial root-zone irrigation (RD); SD, severe drought, the irrigation amount is 50% of the transpiration; SD+N, severe drought and N fertilizer (N); SD+RD, severe drought and alternative partial root-zone irrigation; SD+RD+N, severe drought, alternative partial root-zone irrigation and N fertilizer.

Results and Analysis

Growth and biomass

Biomass, as one of the most important indicators, could intuitively reflect the growth of plants. As shown in Fig.1-A, no significant differences were found in biomass of young *P. volubilis* seedlings among the no fertilization treatment groups ($P>0.05$). With the decreased irrigation amount, the biomass of young seedlings was reduced. The biomass of young seedlings in the NS treatment group was highest, and it was 0.31 g (27%) higher than that all in RD treatments. Water shortage affected the biomass accumulation in young *P. volubilis* seedlings. When the irrigation amount was equivalent, the biomass of young seedlings under alternative partial root-zone irrigation was higher than that under conventional irrigation. Compared with that in the MD treatment, the biomass in the MD +RD treatment was higher by 15%, and that in the SD+RD was higher by 18%. Besides, there was no significant difference among NS, MD+RD and SD+RD treatments, and the seedling biomass in the NS treatment was higher than those in the MD +RD and SD +RD treatments by 5% in average. In the case of limited irrigation water, alternative partial root-zone irrigation could alleviate water stress to some extent, and ensure biomass accumulation. Under severe drought, there was no significant difference in seedling biomass between irrigation mode and fertilization ($P>0.05$). It suggested that under severe drought condition, irrigation mode and N fertilization showed little effect on biomass accumulation in *P. volubilis* seedlings. The seedlings biomass in the SD +RD +N treatment

group was 7% higher than that under severe drought, but was 12% lower than that in the SD +RD treatment group. It indicated that under the condition of severe drought, N fertilization affected the biomass accumulation in young *P. volubilis* seedlings.

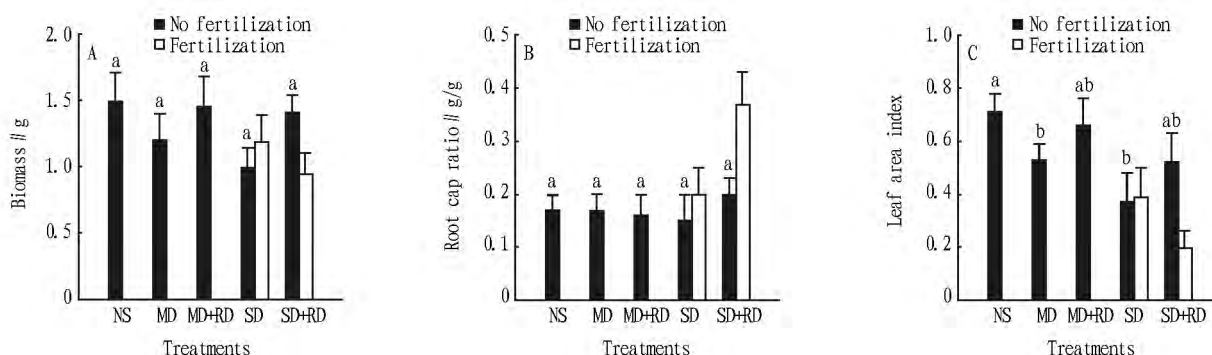
Root is the main vegetative organ of plants to absorb water and nutrients. Root cap ratio reflects the distribution of biomass between ground and underground parts of plants. The variance analysis (Fig.1-B) showed that there were no significant differences in root cap ratio among the no fertilization treatment groups ($P>0.05$). The root cap ratio under severe drought was a little higher. It indicated that under the condition of severe drought, biomass was preferentially allocated to the roots of plants. Under severe drought, significant difference was found in root cap ratio between irrigation mode and fertilization ($P<0.05$), but their interaction was insignificant ($P>0.05$). In average, the root cap ratio under N fertilization was about 30% higher than that when no fertilizer was applied. Under serious water shortage, alternative partial root-zone irrigation and N fertilization significantly promoted the transport of nutrients to seedling roots, increased root cap ratio and root biomass, and increased root ability to absorb nutrients and water, thus improving drought resistance.

Leaves are the main photosynthetic organ, and leaf area index reflects the growth conditions of leaves of young seedlings. The variance analysis (Fig.1-C) showed that water supply significantly affected seedling leaf area index ($P<0.05$). The leaf area index in the NS treatment was 25% and 42% higher than those in the MD and

RD treatments respectively, but was only 7% and 27% higher than those in the MD +RD and SD +RD treatment groups. It indicated that drought stress inhibited leaf growth, but alternative partial root-zone irrigation alleviated the adverse effects of drought stress on the growth of seedling leaves. There was significant difference in leaf area index between irrigation mode and fertilization ($P<0.05$), but their interaction was insignificant ($P>0.05$). The leaf area index in the SD +RD treatment group was 38% higher than that in the SD+RD+N treatment group. Under the condition of alternative partial root-zone irrigation, fertilization was not conducive to the leaf growth of *P. volubilis* under severe water stress.

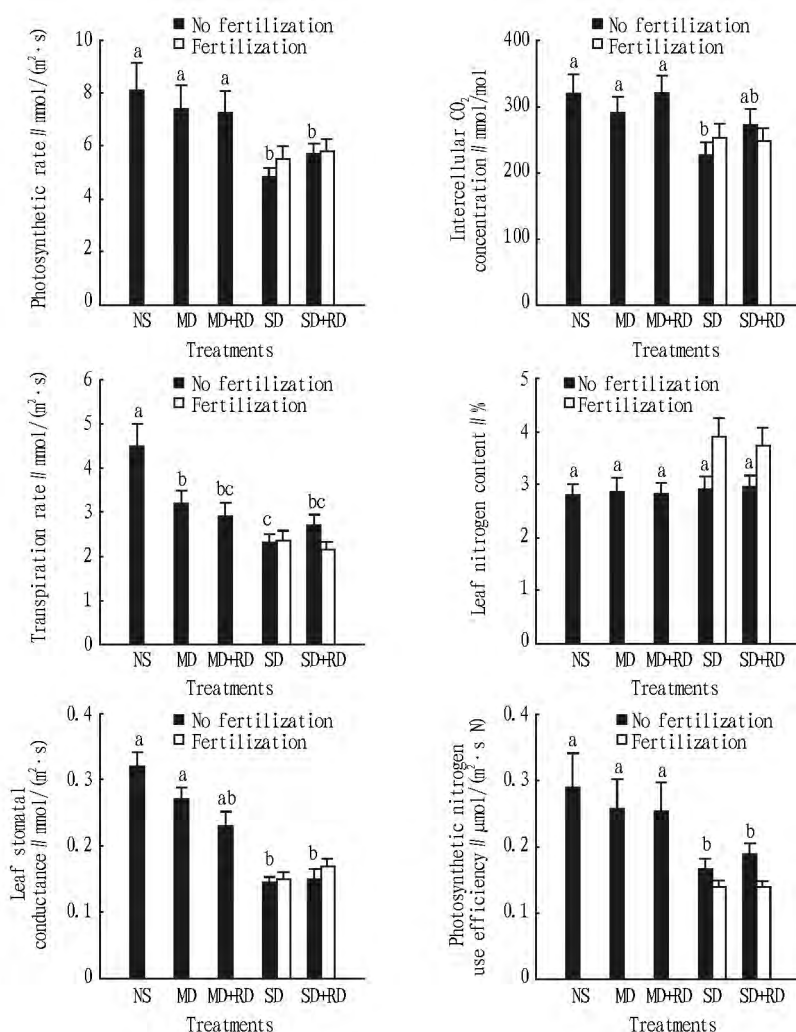
Photosynthetic physiology

As shown in Fig.2, there was no significant difference in photosynthetic rate ($P>0.05$), but there were significant differences in transpiration rate and stomatal conductance ($P<0.05$) between normal water supply and mild drought treatments. The transpiration rate and stomatal conductance in the MD +RD treatment group were 33% and 36% lower than those under normal water supply, and were 9% and 18% lower than those under mild drought. It indicated that with decreased irrigation amount, transpiration rate and stomatal conductance were reduced as well. However, under the condition of mild drought, alternative partial root-zone irrigation significantly reduced stomatal conductance and transpiration rate, instead of photosynthetic rate. The photosynthetic indexes in the SD+RD treatment group were significantly lower than in the other irrigation treatments. The variance analysis showed that irrigation mode



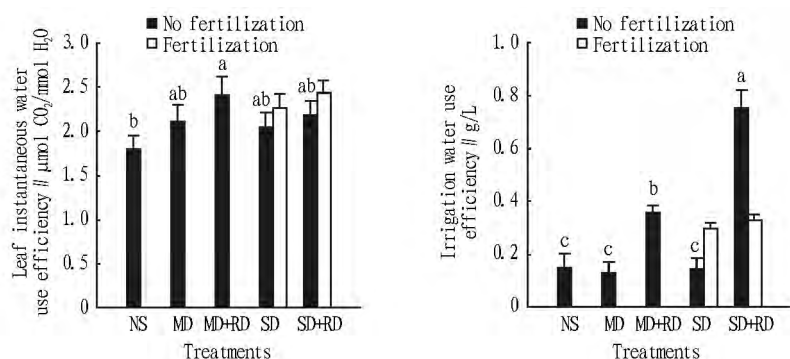
Different lowercase letters indicate significant differences at the 0.05 level.

Fig.1 Comparisons of biomass, root cap ratio and leaf area index of *P. volubilis* seedlings among different treatment groups



Different lowercase letters indicate significant differences at the 0.05 level.

Fig.2 Comparisons of photosynthetic physiology and N use efficiency of young *P. volubilis* seedlings among different treatment groups



Different lowercase letters indicate significant differences at the 0.05 level.

Fig.3 Comparison of water use efficiency in young *P. volubilis* seedlings among different treatment groups

and fertilization showed no significant effects on photosynthesis of *P. volubilis* ($P>0.05$). It suggested that water stress significantly inhibited the photosynthesis process, and its effect on *P. volubilis* seedlings was greater than those of irrigation mode and fertilization.

There were no significant differences in leaf N content among the no

fertilizer treatment groups ($P>0.05$), but the average leaf N content in the fertilization treatment groups was 10% in average higher than that in the no fertilizer treatment groups. The absorption of water and N fertilizer was independent for plants, and drought stress did not affect the absorption and utilization of N fertilizer in *P. volubilis*

seedlings. Instantaneous nitrogen use efficiency reflects (*PNUE*) the status of N physiological utilization in crop blades, and it is the main factor determining plant growth and leaf nitrogen productivity. The variance analysis showed that the *PNUE* under severe drought was significantly lower than those in the other treatments ($P<0.05$), indicating water shortage affecting *PNUE*. Under severe drought condition, fertilization had a significant effect on N photosynthetic rate ($P<0.05$), however, the interaction between irrigation mode and fertilization was insignificant ($P>0.05$). The average *PNUE* of the fertilization treatment groups was 22% lower than that of no fertilizer treatment groups. The *PNUE* was negatively related to leaf nitrogen content.

Leaf instantaneous water use efficiency (*WUEi*) only reflects the status of water utilization in leaves within a certain time, and the relationship between the results of instantaneous and long-term overall measurement is unclear^[14]. Therefore, *WUEi* is not suitable for reflecting water use efficiency throughout the test period.

As shown in Fig.3, there were no significant differences in *WUEi* among the irrigation treatments except MD + RD and NS treatment [the *WUEi* in the MD + RD treatment group was about 25% higher than that in the NS treatment group]. For MD+RD treatment, it reduced the transpiration efficiency without reducing photosynthetic rate. This might be because that drought stress force *P. volubilis* close the stomas, and its luxury transpiration was reduced, thus the *WUEi* was improved. In the SD treatment, the interactions between irrigation mode and fertilization on *WUEi* was insignificant ($P>0.05$). However, the *WUEi* in the fertilization treatment groups was higher, which might be due to fertilization's improving leaf nitrogen content and photosynthetic rate.

Irrigation water use efficiency

Fig.3 showed that there were significant differences in water use efficiency (*WUE*) of young *P. volubilis* seedlings among different irrigation treatments ($P<0.05$). With the decrease of irrigation amount, the *WUE* of young seedlings increased signifi-

cantly. The *WUE* in the SD+RD treatment group was highest, and it was about 60% higher than that under normal water supply. Maybe *P. volubilis* seedlings had small demand for water or alternative partial root-zone irrigation contributed to improving the *WUE*, especially under severe drought condition. Irrigation mode had significant effect on *WUE* in *P. volubilis* seedlings ($P < 0.05$), but the interaction between irrigation mode and fertilization was insignificant ($P > 0.05$). The *WUE* in the SD+N treatment group was higher than that in the SD+RD+N treatment group. It indicated that under severe drought stress condition, the mode of alternative partial root-zone irrigation could reduce transpiration without affecting photosynthesis, thereby maximizing the *WUE*. However, the factor of N fertilization failed to show the affect.

Conclusions and Discussion

The results of this study showed that alternative partial root-zone irrigation could improve water use efficiency and alleviate drought stress to some extent by adjusting the leaf stomas, and it is an effective water-saving irrigation technology. Compared with those under full irrigation, the biomass and water consumption under alternative partial root-zone irrigation were reduced by 5% and 75%, respectively, and the water use efficiency was increased by 60%. This conclusion was consistent with the findings in rice, potatoes, corn and other field crops, and grapes, peach and other fruit trees [13,15–18]. Alternative partial root-zone irrigation can improve water use efficiency. This might be due to the stress signals generated by the root under drought stress, such as ABA. When the signals are transferred to the aboveground parts, the opening or closure of leaf stomas is regulated. Stomatal conductance shows linear relationships with transpiration water consumption, but shows non-linear relationship with photosynthetic rate. When the stomatal conductance declines gradually from the maximum, luxury transpiration is reduced significantly, but the photosynthesis is affect-

ed lightly. This difference provides a theoretical basis for improving water use efficiency of plants through regulating stomatal activities [9,19]. In this study, compared with those under full irrigation, the stomatal conductance, photosynthetic rate and transpiration rate under alternative partial root-zone irrigation were reduced by 29%, 20% and 38%, respectively, but the instantaneous water use efficiency was increased by 19%. In average, the water use efficiency in the extreme drought treatment groups was higher than that in the other treatment groups. The water use efficiency in the SD+RD treatment group was highest, and it was four times higher than that under normal water supply. This was consistent with the result in citrus [20].

Studies suggest that under drought conditions, ABA, produced by roots, is transported to shoots, and it can regulate the closure of leaf stomata, reduce the expansion rate of blades, reduce leaf growth, and allocate more assimilates to the roots, thereby promoting root growth, but inhibiting shoot growth; thus, the root cap ratio is regulated [10]. In this study, alternative partial root-zone irrigation did not significantly increase the root cap ratio, but the root cap ratio in the SD+RD treatment group was significantly higher (about 30%) than those in the other treatment groups. This conclusion was inconsistent with the findings of some previous studies [21–22], which probably be caused by the differences in experimental conditions, plant species and growth state. In addition, alternative partial root-zone irrigation could alleviate the drought to some extent. However, under the condition of long-term severe drought, alternative partial root-zone irrigation allocated more biomass to the roots, greatly reduced the leaf area index, and decreased photosynthetic indicators. Thus, the growth and development of *P. volubilis* seedlings was inevitably affected.

Under the condition of limited water, appropriate application of nitrogen can improve photosynthetic characteristics of plants, promote plant growth, and improve water use efficiency and drought tolerance of plants to some extent [6,23]. In the SD+N treatment group, the photosynthetic rate

was increased by 6%, the biomass was increased by 11%, and the water use efficiency was increased by 25%. However, the biomass in the SD+RD+N treatment group was reduced by 12% compared with that in the SD+N treatment group. Under the conditions of severe drought and alternative partial root-zone irrigation, nitrogen application adjusted the distribution of biomass and concentrated most of the biomass to roots of *P. volubilis* seedlings. Thus, the root cap ratio and root biomass were all increased by 30% in average, so as to enhance the drought tolerance of young *P. volubilis* seedlings. However, the leaf area index was reduced by 38%, the photosynthetic rate was reduced by 9%, and the total biomass was reduced by 18%. Alternative partial root-zone irrigation was not suitable to be matched with nitrogen fertilization under severe drought conditions. Photosynthetic nitrogen use efficiency (*PNUE*), as an index for evaluating the relationship between *Pn* and leaf nitrogen content, is commonly used in studies on vegetation adaptation and plant population evolution. In crops, *PNUE* is found closely related to photosynthetic characteristics, yield and nitrogen use efficiency [24–26]. In the SD+N and SD+RD+N treatment groups, the *PUNE* was negatively related to leaf nitrogen content, which was consistent with previous studies [27–28]. This might be because that the nitrogen was assigned to the leaf photosynthetic apparatus to constitute cell walls. As this experiment was conducted under the condition of pot cultivation in the greenhouse, the effect of implementation of alternative partial root-zone irrigation in the field conditions needs to be explored. In the field, the effects of heterogeneous rainfall and the duration of drought stress should also be taken into account when water-saving irrigation technologies are adopted.

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