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RESEARCH ARTICLE

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Foliar Carbon Isotope Composition (δ^{13}C) and Water Use Efficiency of Different *Populus deltoids* Clones Under Water Stress

Abstract Foliar carbon isotope composition (δ^{13}C), total dry biomass, and long-term water use efficiency (WUE_L) of 12 *Populus deltoids* clones were studied under water stress in a greenhouse. Total dry biomass of clones decreased greatly, while δ^{13}C increased. Single-element variance analysis in the same water treatment indicated that WUE_L difference among clones was significant. Clones J_2, J_6, J_7, J_8, and J_9 were excellent with high WUE_L. Extremely significant δ^{13}C differences among water treatments and clones were revealed by two-element variance analysis. Water proved to be the primary factor affecting δ^{13}C under water stress. It showed that there was a good positive correlation between δ^{13}C and WUE_L in the same water treatment, and that a high WUE_L always coincided with a high δ^{13}C. δ^{13}C might be a reliable indirect index to estimate WUE_L among *P. deltoids* clones.

Keywords carbon isotope composition (δ^{13}C), long-term water use efficiency (WUE_L), water stress, total dry biomass, *Populus deltoids*

1 Introduction

Populus deltoids is one of the most ideal tree species used for industrial timber production in midlatitude areas. In the past few years, many elite *P. deltoids* clones have been introduced into China. Great economic benefits have been achieved after the popularization of *P. deltoids* in China. Aside from being fast-growing, *P. deltoids* also attracts public concern for its large water consumption. Thus, it is urgent for forest tree breeders to select *P. deltoids* of high water use efficiency (WUE) to counteract water deficiency worldwide. However, how to precisely measure WUE is a puzzling problem that scientists have been facing for a long time. Generally, there are two methods used to determine WUE directly. One method is individual or group long-term water use efficiency (WUE_L), which is the ratio of total dry biomass to water consumption during growth and is usually expressed as milligrams of dry biomass per gram of water. WUE_L is relatively accurate and meets practical needs, but it is time-consuming and laborious. The other method is single-leaf WUE (WUE_I), also called instantaneous WUE, which is often expressed as the ratio of net photosynthetic rate (P_n) to transpiration rate (T_r) (μmol CO_2/mmol H_2O). This method is simpler than WUE_L, but it only reflects instantaneous leaf behavior during a specific period and lacks close correlation with WUE_L [1].

At present, a common method used by scientists, which indirectly estimates WUE, is derived from carbon isotope composition (δ^{13}C) technology. Based on a substantial number of experiments on various plants, Farquhar et al. [2] and Farquhar and Richards [3] found that there was a positive relationship between δ^{13}C and WUE; this theory was first proven in *Triticum aestivum*. This indirect method measures the general WUE of plants by analyzing δ^{13}C accumulated in leaves or other organic tissues for a long time and yields a more accurate result than WUE_I. In addition, the measurement of δ^{13}C is not restricted by time and season because the δ^{13}C of organic tissues will not change any more once the tissue has been sampled and dried [3]. Scientists from different countries have performed experiments on a variety of crops, such as *T. aestivum* [4], *Arachis hypogaea* [5], *Gossypium hirsutum* [6], and *Hordeum vulgare* [7]. Most results proved that there was a positive relationship between δ^{13}C and WUE, while some results showed negative or no relationship [8]. In recent years, relationships between the δ^{13}C and WUE of different genotypes had been extensively studied, many of which

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focused on tree species used in artificial intensive forestry, such as *Picea glauca* [9] and *Eucalyptus globules* [10]. It was reported that a high δ^{13}C could be regarded as a reliable index of clones with high WUE in various water treatments. A study on *Picea mariana* by Osorio and Pereira [11] showed that the positive correlation between δ^{13}C and WUE only occurred under water stress. The study on δ^{13}C started relatively late in China and focused mainly on crops such as soy, wheat [12], and peanuts [13]. All the results proved that δ^{13}C was a good index in selecting high-WUE clones. Related studies on tree species in China only involved δ^{13}C differences among species [14,15], as well as in different environments [16]. The results showed that there were marked δ^{13}C differences among tree species. It was also found that various environmental factors had impacts on δ^{13}C, and that the WUE of trees increased under water stress [17]. The present study attempted to study the foliar δ^{13}C, total dry biomass, and WUE_L of different *Populus deltoids* clones under different water stresses and to analyze their interrelationships in an effort to provide a theoretical basis and methods for the selection of high-WUE clones.

2 Materials and methods

2.1 Materials

The ten tested clones were hybrids of *P. deltoids* "Shanhaiguanensis" × *P. deltoids* "Harvard," which were named J_1–J_{10}, respectively. Currently, elite clones of *P. euramericana* "114/69" and *P. deltoids* "Zhonglin 46" were chosen as controls (CK_1 and CK_2, respectively).

2.2 Experimental design

Experiments were carried out in the greenhouse of the Chinese Academy of Forestry. The skylights of the greenhouse were closed only when there was rain. The greenhouse was made of glass to ensure enough natural light. From June to September, mean temperature and humidity were 26°C and 75% at 08:00 am, and 33°C and 55% at 13:00 pm, respectively. The extremely high temperature of 40°C occurred in August. Constant CO_2 concentration was approximately 380 μmol/mol. Cuttings with the same size of different clones were planted in plastic pots on April 1, 2003, with one cutting in every pot. The height of the plastic pot was 30 cm, and the inner diameter was 25 cm. The culture medium was a mixture of soil, fine sand, and grass charcoal soil, in a 10:2:1 proportion. Field moisture capacity was 20.20%, and soil bulk density was 1.274 g/cm^3. All cuttings were cultured in well-watered treatment. Water control began on June 10, when the seedlings reached 30 cm, and ended on August 20. A randomized complete block design with six repeats was adopted in the experiments. Four water treatments were designed: well-watered, slight water stress, moderate water stress, and severe water stress. The amount of irrigation water at each time was 500 mL, but the irrigation interval in different water treatments was strictly controlled. In the well-watered treatment, the seedlings of tested clones were irrigated every 2 days, and irrigation intervals were 3, 4, and 5 days in the three other water treatments. The total amounts of irrigation water were 16.5, 11.5, 8.2, and 6.7 L, respectively, by the end of the water control experiments. Soil moisture contents of different water treatments were controlled at 70%–90%, 60%–80%, 50%–70%, and 40%–60% of field moisture capacity, respectively, by means of soil sample drying and pot weighing. Seedlings were fertilized every 15 days to ensure steady nutrient supply.

2.3 Measured objectives and methods

2.3.1 Total dry biomass

The dry biomass of seedlings was measured twice (at the beginning and at the end of the experiment, on June 10 and August 20, respectively). Four seedlings of moderate size in different clones were selected after measurements of seedling height and root diameter. Selected seedlings were rinsed, baked in an oven at 105°C for 0.5 h, and then dried at 70°C for about 72 h up to a constant weight. Dry biomass was measured with a sensitivity of 1/10,000. The difference of two dry biomass results was regarded as the total dry biomass (g) accumulated during the water control period.

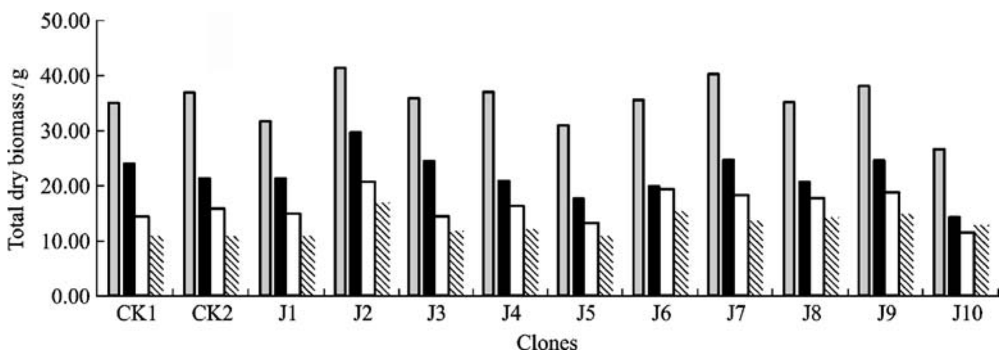

Fig. 1 Total dry biomass of different clones in different water treatments

Table 1 The WUE_L and $\delta^{13}C$ values of different clones in different water treatments

Clones	Well-watered		Slight water stress		Moderate water stress		Severe water stress	
	WUE_L /(mg·mL^{-1})	$\delta^{13}C$ /%	WUE_L /(mg·mL^{-1})	$\delta^{13}C$ /%	WUE_L /(mg·mL^{-1})	$\delta^{13}C$ /%	WUE_L /(mg·mL^{-1})	$\delta^{13}C$ /%
CK_1	2.13±0.12	−30.15	2.14±0.05	−28.70	1.75±0.16	−27.66	1.65±0.07	−27.29
CK_2	2.24±0.09	−30.81	1.91±0.07	−28.32	1.95±0.07	−27.38	1.67±0.03	−27.24
J_1	1.93±0.23	−30.58	1.91±0.07	−29.62	1.82±0.11	−28.91	1.70±0.26	−26.30
J_2	2.51±0.09	−29.30	2.63±0.09	−27.92	2.54±0.08	−27.24	2.58±0.16	−26.43
J_3	2.18±0.12	−30.01	2.18±0.04	−28.29	1.76±0.11	−26.69	1.78±0.09	−26.35
J_4	2.25±0.11	−30.20	1.87±0.06	−28.33	2.00±0.03	−27.09	1.82±0.12	−26.44
J_5	1.89±0.06	−30.62	1.59±0.03	−28.39	1.63±0.11	−28.70	1.50±0.05	−26.79
J_6	2.17±0.20	−29.67	1.78±0.05	−29.06	2.38±0.21	−26.99	2.34±0.16	−25.92
J_7	2.46±0.10	−29.33	2.20±0.11	−27.90	2.24±0.08	−27.40	2.07±0.18	−26.98
J_8	2.14±0.09	−30.65	2.17±0.07	−28.86	2.18±0.04	−27.22	2.14±0.51	−26.54
J_9	2.33±0.08	−30.02	2.19±0.07	−27.60	2.32±0.07	−26.89	2.25±0.12	−26.99
J_{10}	1.63±0.06	−30.63	1.32±0.09	−28.65	1.44±0.07	−27.34	1.77±0.14	−27.09

2.3.2 Long-term water use efficiency

The value of WUE_L (mg/mL) was determined by the ratio of the total dry biomass accumulated during the water control period to the total amount of irrigation water.

2.3.3 Carbon isotope composition

For each clone in the four water treatments, three seedlings were selected at the end of the experiment. One leaf (the ninth leaf from the seedling top) was picked. Three leaves were mixed as one sample. The total number of mixed samples was 48. These samples were dried in the oven at 105°C for 0.5 h, and then dried up to a constant weight at 70°C for about 72 h. After grounding and sieving, the samples were burnt to release CO_2, then the $\delta^{13}C$ values of samples were measured by a MAT-251 mass spectrograph according to Eq. (1):

$$\delta^{13}C = \left(R_p - R_s\right)/R_s \quad (1)$$

where R_p and R_s are the $^{13}C/^{12}C$ values of plant tissue and Pee Dee Belemnite (PDB), respectively.

Table 2 Single-element (clone) variance analysis of WUE_L under severe water stress

Source of variance	*df*	SS	MS	*F* value
Clones	11	4.80	0.44	11.03*
Random errors	36	1.43	0.04	
Total	47	6.23		

$F_{0.01}(11,36) = 2.89$.
*Significant at the 0.01 level.

3 Results and analysis

3.1 Total dry biomass

As shown in Fig. 1, total dry biomass markedly decreased with the aggravation of water stress. In every water treatment, J_2 had the highest total dry biomass and proved to be a clone with good adaptability, followed by J_7, J_8, and J_9, whose total dry biomass values remarkably exceeded those of CK_1 and CK_2. The total dry biomass of J_6 was not good in well-watered and slight water stress treatments, ranking seventh and tenth, respectively. However, J_6, just second to J_2, had a good total dry biomass in moderate and severe water stress treatments. J_6 was preferred for planting in arid conditions.

Table 3 Multiple comparisons of WUE_L for different clones under severe water stress

Clones	WUE_L	Significance	
		0.05	0.01
J_2	2.58	a	A
J_6	2.34	ab	AB
J_9	2.25	abc	ABC
J_8	2.14	abcd	ABCD
J_7	2.07	bcde	ABCD
J_4	1.82	cdef	BCDE
J_3	1.78	cdef	BCDE
J_{10}	1.77	cdef	BCDE
J_1	1.7	def	CDE
CK_2	1.67	def	DE
CK_1	1.65	ef	DE
J_5	1.5	f	E

q test is used for multiple comparisons. The same letters mean that the variation between clones is not significant, while different letters indicate that the variation between clones is significant.
Significance levels: $D_{0.05}$ = 0.49 mg/ml and $D_{0.01}$ = 0.58 mg/ml.

Table 4 Two-factor (water treatment and clone) variance analysis of δ^{13}C

Source of variance	*df*	SS	MS	*F* value
Treatments	3	77.48	25.83	107.12**
Clones	11	6.08	0.55	2.43*
Random errors	33	7.96	0.24	
Total	47	91.51		

$F_{0.05}(3,33) = 2.89$, $F_{0.01}(3,33) = 4.43$, $F_{0.05}(11,33) = 2.10$, and $F_{0.05}(11,33) = 2.85$.
*Significant at the 0.05 level.
**Significant at the 0.01 level.

3.2 Long-term water use efficiency

According to Table 1, the WUE_L of clones with high total dry biomass, such as J_2, J_6, J_7, J_8, and J_9, changed little with the aggravation of water stress in the four treatments, while that of CK_1, CK_2, and other clones decreased markedly. Under slight water stress, there were significant differences in WUE_L, among which J_2 had the most significant, followed by J_7, J_9, J_3, J_8, and CK_1, and, the least significant, CK_2, J_1, J_4, and J_6.

Table 2 shows that the WUE_L difference among clones was remarkable under severe water stress. It indicates in Table 3 that the WUE_L of J_2, J_6, J_7, J_8, and J_9 markedly exceeded that of CK_1, CK_2, and others. The WUE_L difference among J_2, J_6, J_7, J_8, and J_9 was not obvious. It was found that J_2, J_6, J_7, J_8, and J_9 were clones with high WUE_L under severe water stress. The results in other water treatments were consistent (i.e., J_2, J_6, J_7, J_8, and J_9 were clones with high WUE_L under the same water treatment).

3.3 Carbon isotope composition

It was shown in Table 1 that foliar δ^{13}C increased along with the aggravation of water stress. In severe water stress treatment, δ^{13}C was 3.50‰ higher than that in the well-watered treatment. The δ^{13}C difference in the same water treatment was evident. The maximum differences of δ^{13}C in the four water treatments were 1.51‰, 2.02‰, 2.22‰, and 1.37‰, respectively, with an average of 1.78‰.

Two-factor (water treatment and clone) variance analysis of δ^{13}C (Table 4) revealed that the δ^{13}C difference among water treatments was extremely significant and that the δ^{13}C difference among clones was significant. By comparing MS (25.83 and 0.55) and *F* values (107.12 and 2.43), it could be concluded that the difference induced by water treatments was larger than that among clones. Thus, water treatment was the root cause of δ^{13}C differences under water stress.

3.4 Correlation between δ^{13}C and WUE_L

The above analysis showed that δ^{13}C and WUE_L differences among clones were all significant. The correlation between δ^{13}C and WUE_L was studied. As shown in Fig. 2, there was a positive relationship between δ^{13}C and WUE_L in the four water treatments. The correlation coefficients were 0.749,1, 0.701,9, 0.732,7, and 0.775,6, respectively.

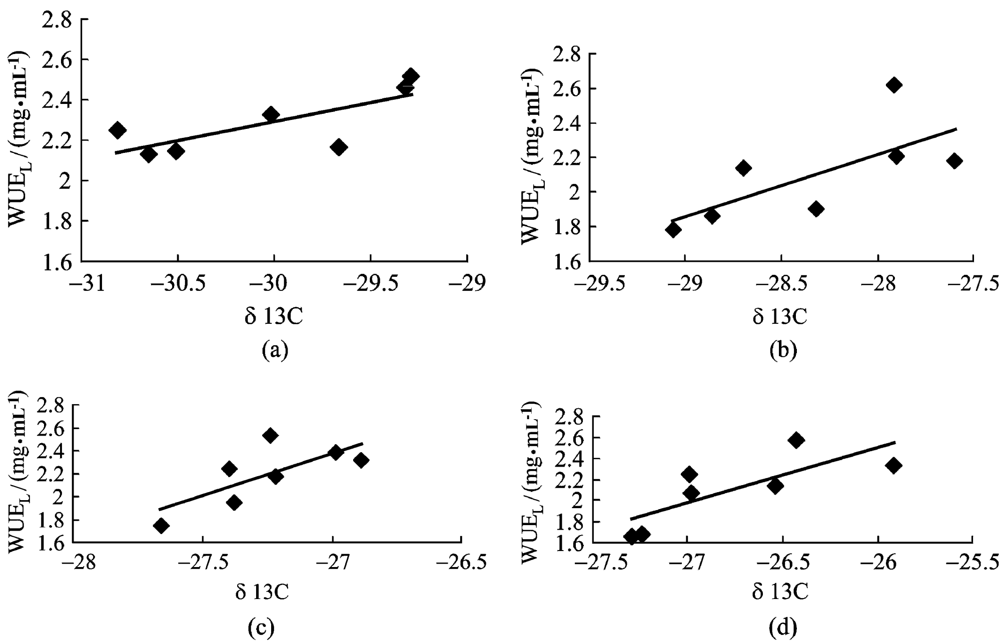

Fig. 2 The relationship between δ^{13}C and WUE_L. **a** Well-watered ($y = 3.065{,}2x + 129.78$, $r = 0.749{,}1$). **b** Slight water stress ($y = 4.209{,}8x + 143.47$, $r = 0.701{,}9$). **c** Moderate water stress ($y = 6.192{,}2x + 186.77$, $r = 0.732{,}7$). **d** Severe water stress ($y = 3.578{,}1x + 0.601{,}6$, $r = 0.775{,}6$)

4 Discussion and conclusion

4.1 WUE_L difference among *P. deltoids* clones

The WUE_L difference among *P. deltoids* clones (J_2, J_6, J_7, J_8, and J_9 elite clones, whose WUE_L markedly exceeded those of CK_1, CK_2, and other clones) was remarkable in the same water treatment.

As shown in Table 1, the WUE_L of J_2, J_6, J_7, J_8, and J_9 changed little with aggravation of water stress, while the WUE_L of CK_1, CK_2, and other clones decreased sharply. The WUE_L of J_2, J_6, J_7, J_8, and J_9 was more than 2 mg/mL. It was considered that the WUE_L of plants would increase under water stress [18]. Dickmann et al. [19] had pointed out that *P. deltoids* would produce 3.5–4.4 mg of dry biomass with 1 mL of water. However, the results of WUE_L in Table 1 were relatively lower, with the maximum being 2.63 mg/mL. The main reason would be that WUE_L was calculated by the total amount of irrigation water and that the amount of water consumed by soil evaporation had not been eliminated. The proportion of water consumed by soil evaporation under water stress was much higher than that under the well-watered treatment. If WUE_L were calculated by the amount of water consumed by plant transpiration, WUE_L values would be higher than those in Table 1. Thus, the WUE_L of each clone would increase within the extent of water stress. In that way, the results would be consistent with those of Farquhar et al. [2] and most researchers [17,20–22] who had studied WUE. The reasons why the same amount of irrigation water was adopted for all seedlings in the same water treatment lay in three aspects. First, this method almost simulated natural soil-drying process. Second, this method was more suitable for intensive management of artificial planting. Third, total dry biomass differences among clones could be compared easily with the same amount of irrigation water.

4.2 Correlation between $\delta^{13}C$ and WUE_L

There existed a positive correlation between $\delta^{13}C$ and WUE_L under the same water condition. $\delta^{13}C$ was a reliable indirect index used to measure the WUE_L difference among *P. deltoids* clones.

There are two carbon isotopes in nature. ^{12}C accounts for 98.89%, and ^{13}C accounts for only 1.108%. $^{13}C/^{12}C$ values of plant tissue samples are compared with those of PDB, and the deviating values are expressed as $\delta^{13}C$. PDB is a kind of shell fossil in the ocean found in South Carolina, USA, whose ^{13}C content is 1.124% [9]. The $\delta^{13}C$ difference among different plants is marked, and the $\delta^{13}C$ of the same plant individual changes greatly in different environment conditions. Farquhar [2] named the plant discrimination against ^{13}C as $\Delta^{13}C$, which has a negative relationship with $\delta^{13}C$. $\Delta^{13}C$ is caused by stomatal diffusion discrimination and carboxyl enzyme discrimination against ^{13}C. C_3 plants only produce Rubisco enzyme in photosynthetic carbon fixation; thus, the stoma is the main factor affecting changes in $\Delta^{13}C$ in different environments.

The stoma is the main pathway of gas exchange for green plants. Its behaviors are determined by genes and are sensitive to environmental factors, among which water is the primary factor. In C_3 plants, stomatal behavior differences, such as stomatal frequency and stomatal size, lead to differences in stomatal conductance (G_S) under the same water treatment. The clones with lower G_S would have lower transpiration (T_r) and lower intercellular CO_2 concentration C_i/C_a. The impacts of G_S on net photosynthetic rate (P_n) would be lower than on T_r; thus, even though there was a C_i/C_a difference among clones, the P_n difference among clones remained unmarked. In this case, the clones with lower C_i/C_a would have higher WUE. At the same time, because more ^{13}C would be used in photosynthetic carbon fixation, clones with C_i/C_a would have high $\delta^{13}C$. This is the theoretical basis of the finding that a high $\delta^{13}C$ is a reliable indirect index used to estimate WUE among clones.

The C_i/C_a difference among genotypes is determined by inherent genetic factors under the same water treatment, which is the basis of the WUE difference. Stress factors, especially water stress, would lead G_S to decrease and would subsequently cause C_i/C_a to decrease. The decreasing extent of C_i/C_a caused by water stress would far exceed the C_i/C_a difference among genotypes. As shown in Table 1, the $\delta^{13}C$ difference of 3.50‰ caused by water stress was far greater than the $\delta^{13}C$ difference of 1.78‰ among clones in the same water treatment. The two-factor (water treatment and clone) variance analysis of $\delta^{13}C$ (Table 4) also revealed this. Thus, the $\delta^{13}C$ difference among clones could only be compared in the same water treatment. Comparisons of $\delta^{13}C$ differences among clones in different water treatments would be meaningless. Many studies showed that there was negative or no correlation between $\delta^{13}C$ and WUE [9], which might be due to the results having been obtained in different water treatments. In addition, if seedling growth were restrained by water stress, the C_i/C_a would decline and then the $\delta^{13}C$ would increase. As shown in Table 1, $\delta^{13}C$ values of J_2 and J_3 with low WUE_L were −27.24‰ and −26.69‰ under moderate water stress, and those of J_2 and J_1 with low WUE_L were −26.43‰ and −25.8‰ under severe water stress. The two factors analyzed above weakened the correlation between $\delta^{13}C$ and WUE_L; this might be the explanation for the correlation coefficients between $\delta^{13}C$ and WUE_L having been only near 0.7 in this study.

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