Concentration of phenolic compounds of *Populus euphratica* and soil water contents in Ejina oasis, Inner Mongolia, China

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**Abstract:** With colorimetric methods, the concentrations of total phenolics, flavonoids and condensed tannins were analyzed in 9 categories of organs in *Populus euphratica*, including lanceolate leaves, ovate leaves, green branches, branches (*D* < 5 mm in diameter), branches (5–10 mm in diameter), barks, roots (*D* < 2 mm in diameter), roots (2–5 mm in diameter) and roots (5–10 mm in diameter). The results showed that phenolic compounds were present throughout the collected organs with the higher total phenolics concentrations in barks (27.93 mg/g), and the mean total phenolic concentrations in two categories of leaves, three classes of roots and three classes of branches were 17.64, 16.72 and 12.19 mg/g, respectively. The higher flavonoids were present in barks (51.30 mg/g), and the mean flavonoid contents in two categories of leaves, three classes of roots and three classes of branches were 28.45, 39.99 and 23.67 mg/g, respectively. The higher condensed tannin contents were found in roots (mean = 22.10 mg/g for three categories of roots), and the average condensed tannin contents in barks, leaves in two categories and branches in three classes were 8.41, 4.03 and 4.47 mg/g, respectively. There was no significant difference between the phenolic compounds of lanceolate leaves and ovate leaves (*P* > 0.05). Phenolic compounds in branches decreased with the branches maturing, resulting in the following orders: green branches > branches (*D* < 5 mm) > branches (5–10 mm). Condensed tannins in roots increased with the root diameter decreasing, and the highest condensed tannin contents were found in small roots (*D* < 2 mm) (25.95 mg/g). By analyzing correlation between phenolic compounds in all collected organs and soil water contents, it was indicated that the phenolic compounds in ovate leaves had negative relation with soil water contents (*P* < 0.05), and the *r* values for total phenolics, flavonoids and condensed tannins were −0.949, −0.923 and −0.944, respectively. Data reported here revealed the variation of phenolic compounds in different organs of *P. euphratica*, and their relationships with the environmental factors in extremely arid areas were discussed.

**Key Words:** *Populus euphratica*; phenolic compounds; total phenolics; flavonoids; condensed tannins; water contents

Phenolic compounds are the most important secondary metabolites in plants and defensive materials existing at molecular level exclusively[1]. Phenolics (including simple phenolics, hydrolysable tannins and condensed tannins) and flavonoids are two common phenolic compounds. The ecological roles of phenolic compounds in protecting plants from unfavorable internal or external environment have attracted great attention in ecology[2]. Recent research has shown that phenolic compounds are the main defensive materials resistant to herbivore or pathogen attack[3,4], extreme temperature, UV-B radiation[5,6] and antioxidation[7], and they also have influence on soil nutrient cycling[8–10]. Factors that determine the allocation of phenolic compounds in plants can be classified into biological and abiotic factors. Biological factors include plant species, genotypes, different parts or development stages of the same plant[11,12], while the abiotic factors in the environment include soil nutrient status, light illumination, temperature, atmospheric CO2 or O3 levels, water condition, soil pH value and so on[13]. The distribution of phenolic compounds in plants and their relationship with surrounding environment are important research fields in chemical ecology[14], which would provide basic data to further explain the role which the secondary me-
tabolites play in the interrelationship of plants-animals, plants-microorganism and plants-environments.

Resisting to saline alkali soil, meadow bog and sand storm, *Populus euphratica* is an exclusive riparian forest species in desert areas. It is a phreatophyte sensitive to soil moisture [15,16]. In recent years, *P. euphratica* forest in Ejina oasis declined gradually as the water table went down with the water supply declining from the upper reaches of Heihe River. Previous research mainly concentrated on the impact of water stress on plant growth, plant reproduction, seed germination and seed dispersal as well as species diversity in *P. euphratica* communities [17–22]. However, research on the variation of secondary metabolites in plants with different water stresses have not been reported yet. Furthermore, how water conditions influence the synthesis and allocation of secondary metabolites in plants is an important problem to reveal their initiative adaptation process to environment. In this study, *P. euphratica* in Ejina oasis was selected to analyze phenolic compounds in order to answer the following two questions: how phenolic compounds were distributed in different organs of *P. euphratica*, and how environment factors influenced the allocation of phenolic compounds in *P. euphratica*. In this paper, water condition was chosen as the primary environment factor to research its influence on the synthesis, transportation and storage of phenolic compounds in *P. euphratica*.

1 Materials and methods

1.1 Study sites

Ejina oasis is located in the northwest of Inner Mongolia, where the second largest desert riparian *P. euphratica* forests are distributed along the lower reaches of Heihe River. The weather and vegetation conditions there have been reported [8]. The study site (42°00′N,101°14′E) was the core area of the National Nature Reserve of *P. euphratica* forests, fenced in 1947.

1.2 Plant materials

All samples were collected in July, 2006. In each plot, 5 healthy *P. euphratica* trees (named No.1–No.5) with approximately 16 cm in diameter at breast height (DBH) were selected to collect leaves, branches, barks and roots. Barks with sere parts were peeled from trunks at the height of about 1.3 m. Leaves exposed to the sun were sampled and classified into lanceolate leaves and ovate leaves. Branches were classified into green and lignified branches based on their maturity, and then the latter were further divided into branches (*D < 5 mm*) and branches (*5 mm < D < 10 mm*). Roots at depth of 20 cm to 80 cm were demarcated into roots (*D < 2 mm*), roots (*2 mm < D < 5 mm*) and roots (*5 mm < D < 10 mm*). All samples (45 in total) were dried under shade in the laboratory at room temperature (about 25°C), then cut into pieces, mixed well, grounded to pass a 0.5 mm screen and stored at 1°C for the chemical extraction.

The mean soil water contents at depth of 30 cm and 60 cm (3 repeat in each layer) were measured to estimate the rhizosphere soil moisture of each sampled tree. Soil samples were weighed by a portable electronic scale in fields, then dried in oven at 105°C for 12 hours and weighed again. Soil water contents (*θm*) were calculated by the following formula: \(\theta_m = (M_w - M_r) / M_r \times 100\%\), where *Mw* represents wet weight of soil samples, and *Mr* is dry weight of soil samples.

1.3 Extraction and analysis

2.00 g powered and air-dried samples were weighed, extracted 3 times with 40 ml, 30 ml and 20 ml 40% acetone (acetone volume:distilled water volume = 4:6) in 45°C water bath for 1 h each time and then filtered. Orthogonal experiment has proved that this procedure can extract maximum phenolics from samples. Although a part of phenolics couldn’t be extracted completely, the procedure was considered to be adequate for the relative comparisons [23]. After being evaporated under reduced pressure to remove acetone, the combined extract was fixed with distilled water for analysis.

Total phenolics were assayed colorimetrically by the Folin-Dennis procedure [24]. Gallic acid (Ouhi Fine Chemistry Corp., China) was used as the standard in the experiment. Flavonoids were assayed with the method of Al(NO3)3-NaNO2 [25] and Routine as the standard (Sigma Corp., USA). Condensed tannins were assayed by the vanillin-HCl method [26] and Catechin as the standard (Sigma Corp., USA). The detailed assay process was slightly modified in this study according to references. The contents of total phenolics, flavonoids and condensed tannins were expressed by relative contents of Gallic acid, Routine and Catechin, respectively.

1.4 Statistics analysis

The classical statistical method was used to calculate the mean soil water contents of roots for each tree. Tukey’s least significant difference (LSD) was used to locate difference of average phenolics contents in different organs when ANOVA results were significant. Pearson correlation analysis was used to evaluate the relationship between soil moisture and phenolics contents in leaves, branches and barks.

2 Results

2.1 Content and allocation of phenolic compounds in *P. euphratica*

The DBH of the sampled trees (No.1–No.5) were 16.9, 16.2, 16.9, 17.2 and 17.5 cm, respectively. Pearson correlation analysis showed no significant correlation between phenolic compound contents and DBH (*P > 0.05*). Phenolic compounds were present in all collected organs with higher total phenolics concentrations in barks (27.93 mg/g). The mean total phenolics contents in two categories of leaves, three classes of roots and three classes of branches were 17.64, 16.72 and 12.19 mg/g, respectively. The higher flavonoids were present in barks (51.30 mg/g), and the mean flavonoid contents in two
categories of leaves, three classes of roots and three classes of branches were 28.45, 39.99 and 23.67 mg/g, respectively. The higher condensed tannin contents were found in roots (mean = 22.10 mg/g in three categories of roots), and the average condensed tannin contents in two categories of barks, two categories of leaves and three classes of branches were 8.41, 4.03 and 4.47 mg/g, respectively. Fig. 1 showed the phenolic compounds in different organs with the post-hoc comparison results. One-way ANOVA analysis showed that there was significant difference among phenolic compounds in 4 kinds of organs \( (P < 0.0001) \).

Phenolic compound contents in two categories of leaves, three classes of branches and three classes of roots were presented in Figs. 2–4, respectively. Phenolic compound contents in lanceolate leaves were not significantly different from those in ovate leaves \( (P > 0.05) \). The order of phenolic compound contents in three kinds of branches was as follows: green branches > branches \( (D < 5 \text{ mm}) > \text{branches (5–10 mm)} \). Total phenolics and flavonoid contents in green branches were significantly different from those in branches \( (5–10 \text{ mm}) \) \( (P = 0.022 \text{ for total phenolics and } P = 0.023 \text{ for flavonoids}) \). The order of phenolic compound contents in three kinds of roots was as follows: roots \( (D < 2 \text{ mm}) > \text{roots (2–5 mm)} > \text{roots (5–10 mm)} \). Condensed tannin contents in roots \( (D < 2 \text{ mm}) \) was significantly different from those in roots \( (5–10 \text{ mm}) \) \( (P > 0.05) \).

2.2 Relationship between phenolic compounds and soil water contents

Soil water contents of No.1–No.5 were 1.81%, 10.90%, 10.86%, 7.76% and 9.18%, respectively. Pearson correlation analysis was used to show a negative correlation between phenolic compound contents in ovate leaves and soil water contents \( (P < 0.05 \text{, shown in Table 1}) \).

3 Discussion

Lanceolate leaves and ovate leaves are two basic phylliforms of \( \textit{P. euphratica} \). Ovate leaves are the main phylliform of mature \( \textit{P. Euphratica} \), while lanceolate leaves usually grow on immature \( \textit{P. euphratica} \) and the lower-crown branches of the mature. As the immature \( \textit{P. euphratica} \) grows up, lanceolate leaves are gradually substituted by ovate leaves, and even disappeared. Previous research has paid great attention to phylliform variation and the function discrepancy of \( \textit{P. euphratica} \). Su\textsuperscript{[27]} found that ovate leaves are more efficient than lanceolate leaves with respect to photosynthesis and water use. In this research, phenolic compound contents for both lanceolate leaves and ovate leaves were analyzed, and no significant difference was found \( (P > 0.05) \), which meant that phylliform was not correlative with phenolic compound contents. Higher total phenolics and flavonoids (27.93 and 51.30 mg/g, respectively) in barks represented that \( \textit{P. euphratica} \) intended to synthesize and accumulate more phenolic compounds in epi-

dermis. Wang\textsuperscript{[25]} studied the flavonoid contents in different organs of \( \textit{Larix gmelini} \) and concluded that flavonoid contents
were positively related with solar radiation intensity. Rozema\textsuperscript{6} also found that the contents of secondary metabolites in plants which could absorb UV-B radiation such as phenolic compounds increased with illumination intensity. Higher phenolic compounds in leaves and barks would be beneficial for \textit{P. euphratica} to resist strong UV-B radiation, high temperature and other extreme factors in the arid environment, and to maintain their normal physiological activities.

Phenolic compound contents in plants were influenced intensively by environmental factors. Usually in extremely acid and arid soils, much more phenolic compounds were presented in plants and litter\textsuperscript{28,29}. As water condition is the primary limiting factor in extremely arid areas, its relationship with phenolic compounds was studied and it was found that there was positive correlation between phenolic compounds in ovate leaves and soil water contents (Table 1). Phenolic compounds had strong hydrophilicity with phenolic hydroxyl and other hydrophilic groups in molecules in spite of hydrophobic parts such as aromatic rings\textsuperscript{30}, which meant that phenolic compounds could hold internal water of plants and reduce water loss in drought environment. So leaves with higher phenolic compounds would lessen transpiration. This research primarily proved that \textit{P. euphratica} could synthesize and accommodate phenolic compounds to adapt to the variation of soil water.

Phenolic compound contents were not significantly different between mature ovate leaves and immature lanceolate leaves. However, phenolic compounds in branches decreased with maturation. The contents of total phenolics, flavonoids and condensed tannins in branches are ranked as follows: green branches > branches (\(D < 5 \text{ mm}\)) > branches (5–10 mm). There was significant difference in total phenolics and flavonoid contents between green branches and branches (5–10 mm) (total phenolics: \(P = 0.022\); flavonoids: \(P = 0.023\)). The variation laws of different phenolic compounds may be caused by the microenvironments in which leaves and branches grew. Leaves were exposed to higher temperature and UV-B radiation, and underwent much more intense environmental stress than branches, and thus ovate leaves and lanceolate leaves would accumulate certain phenolic compounds to resist worsening environment conditions. There were probably two approaches through which the phenolic compounds gradually decreased in branches during maturation: firstly, phenolic compounds in lignified branches were transferred to maintain comparatively higher concentrations in less lignified or green branches; secondly, as the premise matter of lignin\textsuperscript{31}, some phenolic compounds changed into lignin during lignification. This research showed that transition of phenolic compounds in \textit{P. euphratica} was considerably active and intensively influenced by environment factors such as sun light, temperature and so on.

Root biomass is an important component in plants, especially for desert plants whose root biomass accounts for the most part. However, there has been no report on the secondary metabolites in the root systems of \textit{P. euphratica}. In this paper, \textit{P. euphratica} roots were found to contain relatively higher phenolic compounds; especially, the average content of condensed tannins was 22.10 mg/g, more than that in the above-ground parts. As the most important materials resisting to herbivore and pathogen attack\textsuperscript{31}, condensed tannins decreased with root diameters increasing, showing that thinner roots underwent more environmental stress. In this research, phenolic compounds were inferred to play an active role in maintaining thin roots with normal physiological function.

Since high phenolic compounds concentrated in roots and litter, a plenty of phenolic compounds entered into rhizosphere soil, and would play an important role in the nutrient cycling of \textit{P. euphratica} forest ecosystem. In general, the decomposition rate of litter would reduce with the increase of phenolic compounds in rhizosphere soil\textsuperscript{32-34}. Tannin-protein polymers were hard to be decomposed; it would slow down the \(N\) cycling rate, and then restrict the ecosystem nutrient cycling. Thus high phenolic compound contents in \textit{P. euphratica} forest soil might lead to the reduction of nutrient cycling rate. However, it is uncertain whether the reduced rate of nutrient cycling is advantageous or disadvantageous for \textit{P. euphratica} forest ecosystem.

\textbf{Table 1} Correlation between soil water contents and phenolic compound concentrations (mg/g DW) of ovate leaves

<table>
<thead>
<tr>
<th>Phenolic compound concentrations (mg/g DW)</th>
<th>Regression equation (y = )</th>
<th>(r)</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total phenolics</td>
<td>(-1.701x+30.424)</td>
<td>(-0.949)</td>
<td>0.014</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>(-1.524x+38.670)</td>
<td>(-0.923)</td>
<td>0.026</td>
</tr>
<tr>
<td>Condensed tannins</td>
<td>(-0.375x+8.042)</td>
<td>(-0.944)</td>
<td>0.016</td>
</tr>
</tbody>
</table>

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4 Conclusions

In this study, environmental stress led to the content difference of phenolic compounds in \emph{P. euphratica} organs, reflecting its intensive influence on the synthesis of phenolic compounds. With respect to the phenolic compound contents in different \emph{P. euphratica} organs, the order of total phenolics contents in different organs was: barks > leaves > roots > branches; the order of flavonoid contents was: barks > roots > leaves > branches; the order of condensed tannins was: roots > barks > branches > leaves. These results showed that \emph{P. euphratica} accumulated more phenolic compounds in epidemics. Phenolic compound contents in lanceolate leaves were not significantly different with those in ovate leaves ($P > 0.05$); phenolic compounds in branches decreased with the maturation, resulting in the order as follows: green branches > branches ($D < 5 \text{ mm}$) > branches (5–10 mm). Total phenolics and flavonoid contents in green branches were significantly different with those in branches (5–10 mm) ($P = 0.022$ for total phenolics and $P = 0.023$ for flavonoids). It was found that there were relatively higher phenolic compounds in \emph{P. euphratica} roots, especially condensed tannins, more than those in the above-ground parts. Condensed tannins decreased with root diameters increasing, showing that fine roots underwent more environmental stress. Phenolic compounds in ovate leaves had negative relation with soil water contents ($P < 0.05$), and the $r$ values for total phenolics, flavonoids and condensed tannins were $-0.949$, $-0.923$ and $-0.944$, respectively, which showed that the synthesis of phenolic compounds in ovate leaves was influenced intensively by water condition. This research showed that in extremely arid environment \emph{P. euphratica} could accommodate the synthesis, transfer and conversion of phenolic compounds to adapt to the environmental stress.

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