Analysis of Growth and Physiological Variations Among Cyclocarya Species of Different Genotypes in a Clonal Seed Orchard

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Background: *Cyclocarya paliurus* (*C. paliurus*) is a woody species that has many medical benefits to human health. Geographical species has been formed among different natural forests of *C. paliurus*, but gradually decrease or extinct by human's excessively exploitation in recent years. So, it is worthy to conserve this native and valuable species in China. Environmental factors affected plant growth, and seed is the only way for breeding offspring among Cyclocarya species, herein, we assess plant growth of six genotypes (FJ, JX, TG, WF, JH, AJ) from 2017 to 2019 year and physiological variations from April to October in 2018 during the construction of a clonal seed orchard.

**Abstract**

**Background:** *Cyclocarya paliurus* (*C. paliurus*) is a woody species that has many medical benefits to human health. Geographical species has been formed among different natural forests of *C. paliurus*, but gradually decrease or extinct by human's excessively exploitation in recent years. So, it is worthy to conserve this native and valuable species in China. Environmental factors affected plant growth, and seed is the only way for breeding offspring among Cyclocarya species, herein, we assess plant growth of six genotypes (FJ, JX, TG, WF, JH, AJ) from 2017 to 2019 year and physiological variations from April to October in 2018 during the construction of a clonal seed orchard.

**Results:** The survival rate of plant reached 100% in a *C. paliurus* seed orchard. Plant height and basal diameter varied in different genotypes. Plant of six genotypes had different changes in water content and total soluble sugar content from April to October, Ca content reached maximum among the four detected mineral content, four kinds of antioxidant enzyme activity was in order of SOD > POD > CAT, and the highest content of phytochemical was total flavonoid. Plant growth and physiological changes during growth period was significantly correlated with environment factors by correlation analysis.

**Conclusions:** Plants of *ex situ* conservation adapted changeable environmental factors by physiological changes and showed the differentiation of plant growth in a clonal seed orchard. This would provide a foundation for *ex situ* conservation management in Cyclocarya species and selection of suitable cultivation provenance.

**Methods**

**plant materials and site description**

Seeds of *C. paliurus* from six genotypes were collected from natural forests in late October 2015 according to the method described by Deng et al. (2014) and information about six genotypes was seen in Fig.1. The collected seed were first subjected to removing wings, followed by exogenous gibberellin A3 (GA3) treatment, and then stratification treatment using a method described by Fang et al. (2006). After 5 months, the germinated seedlings were transplanted in cultivate bags (10.8 cm in diameter and 14.5 cm in height) and then kept in the nursery (Yongchun, Fujian, China). Each bag had a single plant.

1-year-old plants were selected with even growth and then transplanted to CSO in Xiayang, Fujian on March 2017 with a spacing of 2×2m. 50 plants of every genotype were conserved in each block. During the experimental period, environmental factors were collected from a local weather bureau and every index was seen in Tab.1.

After 1-year *ex situ* conservation, leaf samples were collected from the middle part of the current branch on April, June, August and October in 2018. Fresh leaves were divided into two parts, one was used for determining water content (WC), total soluble sugar content (TSS) and antioxidant enzyme activity...
(superoxide dismutase [SOD], peroxidase [POD], catalase [CAT], polyphenol oxidase [PPO]), another was dried to constant weight and then used for investigating mineral content (potassium [K], calcium [Ca], magnesium [Mg], sodium [Na]) and phytochemical content (total flavonoid, total triterpenoid and polysaccharides). Each experiment was repeated three times.

**Plant growth index determination**

In April, 2017, all plants were observed and the initial height (named \( H_i \)) and initial basal diameter (named \( BD_i \)) were measured, then all surviving plants were observed and plant height (named \( H_a \)) and basal diameter (named \( BD_a \)) were measured again in December 2017. The conduction of measurement method in 2018 and in 2019 was the same as that in 2017. The growth index was calculated as:

Net height of each surviving plant = \( H_a - H_i \)

Net BD of each surviving plant = \( BD_a - BD_i \)

Average net height = the sum of net height among the surviving plants / The number of surviving plants

Average net BD = the sum of net BD among the surviving plants / The number of surviving plants

**Physiological index determination**

**Determination of water content**

According to the method described by Stein et al. (1975), WC of leaves was calculated as WC (%) = \( \frac{FW - DW}{FW} \times 100 \), where FW mean fresh weight (FW) of leaf, DW mean constant weight of dried leaf (DW).

**Extraction and determination of total soluble sugar**

Total soluble sugar (TSS) was extracted and determined by Li et al. (2000), then calculated as following: Leaf TSS content (%) = \( \frac{(C \times 25)}{(W \times 0.5 \times 10^6)} \times 100 \), where \( C \) was obtained from standard curve and sugar was used as standard curve; \( W \) mean the weight of fresh sample.

**Extraction and determination of mineral content**

Samples were digested by electric-heating digestion method described by Feng et al. (2020). Mineral content (K, Na, Ca, Mg) was calculated by \( \frac{(C \times 0.025)}{DW} \), where \( C \) mean its concentration measured by ICP-OES (optima 7000DV, American), DW mean dried weight of sample.

**Extraction and analysis of antioxidant enzyme activity**

Antioxidant enzyme was extracted and four kinds of antioxidant enzyme (CAT, SOD, PPO and POD) activity were analyzed in accordance with the method of Feng et al. (2020).

**Extraction and analysis of phytochemical content**

Extraction was obtained using an ultrasonic-assisted method with a slight modification (Liu et al. 2018c). Each sample (about 1.0g) was added to 20mL 75% ethanol, then centrifuged at 25°C, 11000g for 15min after heating at 70°C for 60min with ultrasonic cleaner (KQ-800DE, China), and finally extraction was obtained.

Total flavonoid content was determined by method described by Liu et al. (2018c), then calculated by the standard rutin curve and expressed as milligrams rutin equivalent per gram of dry mass (mg/g).

Total triterpenoid content was assessed by using the Folin-Ciocalteu colorimetric method and then expressed as milligrams gallic acid equivalent per gram of dry mass (mg/g).

Polysaccharides content was conducted by the method described by Liu et al. (2018a), then calculated using the standard glucose curve and expressed as milligrams glucose equivalent per gram of dry mass (mg/g).

**Results**

**Analysis of plant growth**

Plant height and basal diameter grew faster after been cultivated one year later. Plant growth varied with different genotypes, FJ had the highest plant height but WF had the highest basal diameter up to 2019 (Tab.2).

**Analysis of water content**

WC in leaf was different with growth periods and genotypes. WC change was similar from June to October among FJ, AJ, TG, JX and WF. WC of JH gradually decreased and had the highest content on June (Fig.2).
Analysis of total soluble sugar content

Plants of different genotypes varied in TSS content during growth period. TSS content of FJ and JH had the highest value on August, of which change was opposite with that of AJ. TSS content of JX and TG gradually increased and reached maximum on October, which was contrary with that of WF (Fig.3).

Analysis of mineral content

K, Ca, Na and Mg were contained in leaves among 6 genotypes, the highest content was Ca (≥6.0mg/g), and then was K(≥3.0mg/g), finally was Mg and Na (≤2.0mg/g) (Fig.4).

Analysis of antioxidant enzyme activity

Four kinds of antioxidant enzyme activity was in order of SOD>PPO>POD>CAT. However, every antioxidant enzyme of different genotypes had different activity in growth period (Fig.5).

All of genotypes had the highest SOD activity on April and its activity was in order with AJ>FJ>JX>WF>JH>TG. Among six genotypes, SOD activity of FJ gradually decreased from April to October and similar change exit among FJ, JX and TG. SOD activity among WF, AJ and JH firstly decreased, then increased and finally decreased.

PPO activity of FJ was 30.04U/(g·min) on June and reached significant difference with others, of which change was contrary with that of AJ. Both of JX and JH had similar change of PPO activity and reached maximum on August. PPO activity of TG and of WF reached maximum on October, on August, respectively.

The different peak of POD activity was observed among six genotypes, both of JX and FJ had more than 3.0U/(g·min) on June, TG and JH had the highest activity on April and on October, respectively. There was similar change among FJ, JX, TG and JH, but AJ had an opposite change with WF.

CAT activity of JX and FJ was lower than 0.5U/(g·min) from April to October. Similar change was found between TG and JH, but its activity of TG was higher than that of JH. AJ and WF reached maximum on August and on June, respectively, and the peak was more than 2.0 U/(g·min).

Analysis of phytochemical accumulation

High content of total flavonoid and polysaccharides (≥40mg/g) was detected, but total triterpenoid content was found in lower amount (≤3.0mg/g) in ex situ conserved plants. The change of their content varied with growth period and genotypes (Fig.6).

Total flavonoid content of FJ firstly decreased and then increased from April to October, which was similar with that among JX, WF and AJ. However, there was no significant difference from June to October in JH and in TG.

Polysaccharides content of FJ reached 100.16mg/g on April, compared with that, its content decreased by 50.52%, 57.29% and 20.39% from June to October, respectively. Other five genotypes had the highest content of polysaccharides on October , there was similar change between WF and AJ, between JH and TG.

The highest content of total triterpenoid reached on August among FJ, JX, JH and TG; and their changes were similar, but were contrary with that of WF and AJ. AJ had no significant difference in triterpenoid content from June to October, similar results were observed in WF and in JX.

Correlation analysis among parameters

Plant height was significantly related with basal diameter, both of them were also related with AT, AP and AS (Tab.3).

Both of T and P seemed predominantly impact on various physiological index, (Fig.7). In conformity with CCA, both of T and P was significantly correlated with K, Ca, polysaccharides and total flavonoid. S had correlation with POD and WC (Tab.4).

There was significant correlation between total triterpenoid and Mg, between Na and Ca, between K and SOD activity, between K and Na. WC had significant correlated with K, Ca and TT. Total flavonoid was also related with Na, Ca and TP (Tab.4). The similarities are presented in cluster analysis (Fig.8).

Discussions

Plant growth is influenced by genotype and environment (Enßlin et al. 2011). From our findings, plant height and basal diameter among six genotypes were significantly correlated with environmental factors (annual sunshine hours (AS), annual average temperature (AT) and annual precipitation (AP) (Tab.3), which was disagreement with the finding of Deng et al. (2015) whose findings indicated environmental factors were not correlated with height growth of C. paliurus. This could be caused by difference in cultivation sites and genotypes.

As an essential macroelement for plants growth, mineral elements (K, Ca, Mg and Na) have an important role in physiological functions, like osmotic adjustments, water balance, water use efficiency improvement and stomata control (Coskun et al. 2013, Gattward et al. 2012, Schachtman et al. 1997). Environmental factors affected mineral uptake and distribution from soil to leaf in plants (Baghour et al. 2002, 2003), this was in agreement with our findings that mineral content varied with growth period and had significant negative relation with temperature and precipitation (Tab.4, Fig.7).
Plants have developed antioxidant defense systems to adapt changeable environmental factors during growth period. In the enzymatic defense mechanism, SOD, in combination with CAT, PPO and POD, eliminated excessive H$_2$O$_2$ and O$_2$ in tissue of plants (Burduea et al. 2019, Gill and Tuteja 2010). Results in our study also showed that SOD activity was the highest among the detected four enzymes and varied with genotypes and growth period (Fig.5). These suggested that SOD might be the main enzyme to interfere and metabolize excess H$_2$O$_2$ and O$_2$ at a high rate (Burduea et al. 2019, Gill and Tuteja 2010).

Variation of phytochemical content was also influenced by genotype and environmental conditions during growth period (Zhou et al. 2019, Liu et al. 2018a, 2018b, Tohidi et al. 2017, Itidel et al. 2013, Satì et al. 2013). Djerrad et al. (2015) reported geographic variation and environmental conditions had important effect on essential oils in Pinus halepensis. Deng et al. (2015) indicated that genotype and growth environment significantly affected flavonoid accumulation of C. paliurus, with environmental effects being more predominant. Under the same environment, genotypes had a significant role in flavonoid accumulation of C. paliurus (Fang et al. 2011). In accordance with previous researches (Djerrad et al. 2015, Cui et al. 2013, Fang et al. 2011, Sosa et al. 2005, Hare 2002), our findings also showed that variations in TR, TF and TT content were found from different genotypes and phytochemical accumulation was related with environmental factors during growth period(Fig.6, Fig.8).

According to our results, leaves of C. paliurus were rich in flavonoid and polysaccharides, which was consistent with previous works (Shang et al. 2018, Liu et al. 2018a, 2018b, Yang et al. 2017, Xie et al. 2012, Fang et al. 2011). But total flavonoid content in the C. paliurus was far beyond that of previous studies (Liu et al. 2018a, 2018b, Zhou et al. 2019), however both of polysaccharids content and total triterpenoid content was lower than previous results (Zhou et al. 2019, Deng et al. 2017). This may be caused by difference in the extraction method.

Higher content of K and Ca in leaf of C. paliurus (Fig.8) were clustered into one group and had significant negative with temperature and precipitation (Tab.4), suggesting that relatively large amount of Ca and K could act on similar biological metabolize (Coskun et al. 2013, Gattward et al. 2012). These were also observed in the other results by Peiter (2011), Ahmad and Maathuis (2014). Mg directly or indirectly participates in biological processes in plants, like the synthesis of chlorophyll (Masuda 2008), Ribulose-1,5-bisphosphat-carboxylase/oxygenase (Rubisco) activity (Portis 2003). Mg deficiency reduced the absorption and utilization of light energy, which induced the production of reactive oxygen species (ROS) (Guo et al. 2016). Antioxidant enzyme developed in plants is expected to cope with the harmful effects of ROS, like SOD and CAT(Tewari et al. 2004; Tang et al. 2012). This could be a reason that Mg had positive relation with SOD and CAT, especially one group was formed between Mg and CAT (Tab.4, Fig.8).

Between the four tested mineral elements and three kinds of phytochemical, Ca and Mg was significant positively correlated with polysaccharides and total flavonoid without fertilization condition, respectively, which was disagreement with Deng et al. (2019b) who reported Ca and Mg had a significant negatively correlation with total flavonoid accumulation under five nitrogen fertilization levels (Deng et al. 2019b). This could be caused by nitrogen availability, which influence nutrient balance and further affected phytochemical accumulation in C. paliurus (Deng et al. 2019b).

**Conclusions**

Plants of C. paliurus of six genotypes were conserved in a clonal seed orchards at Quanzhou, Fujian province. Variations in plant growth and physiological changes in leaf were analyzed and results showed that (1) plant growth among 6 genotypes was different in plant height and basal diameter. (2) Difference in WC and TSS content among 6 genotypes varied from April to October. Four kinds of antioxidant enzyme and mineral element content was in order with SOD, CAT, Ca, Mg, Na, respectively. Total avonoid accumulation in leaf was the highest, then was polysaccharides, and finally was total triterpenoid. (3) Both of plant growth and physiological index had relation with environmental factors (mean temperature, precipitation, sunshine hours) and various physiological index also related with each other by relation analysis. These results suggested that plants of 6 genotypes had a better adaptation in CSO by physiological changes during growth period and different genotypes varied in plant growth and nutrient accumulation. Therefore, results from this study provide a foundation for ex situ conservation management of Cyclocarya species and selection for breeding programs.

**Declarations**

**Authors’ contributions**

YF designed the work plan and preparation of manuscript. JPH carried out the construction of a clonal seed orchard of C. paliurus and carried out mineral nutrient content determination, GXL measured water content and total soluble sugar content in the laboratory, ChQZh determined antioxidant enzyme activity, XYY performed phytochemical content measurement, XFL performed the statistical analysis.

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**Availability of data and materials**

The data that support the findings of this study are available from the authors (School of Resource and Environmental Science, Quanzhou Normal University China) upon reasonable requests and with permission from Quanzhou Normal University (China).

**Ethics approval and consent to participate**
Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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## Tables

### Tab.1 Environmental parameters in the experimental site

| Indexes | Year | 2017 | 2018 | 2019 | Indexes | Month | April | June | August | October |
|---------|------|------|------|------|---------|-------|-------|------|--------|---------|
| AT (℃) |      | 20.6 | 20.4 | 18.6 | T (℃)   |       | 18.4  | 23.5 | 24.7   | 18.1    |
| AP (mm) |      | 1296.4 | 1794.7 | 1570.7 | P (mm) |       | 64.3  | 227.7 | 353.7  | 53      |
| AS (h)  |      | 1874.6 | 1825.6 | 1600.6 | S (h)  |       | 152   | 123.8 | 177    | 159.2   |

* Climatic parameters were recorded in 2018. AT mean annual mean temperature; AP mean annual precipitation; AS mean annual sunshine hours. T mean average temperature, P mean precipitation, S mean sunshine hours.

### Tab.2 Variation in plant growth index among six genotypes of *Cyclocarya paliurus*

| Index | Year | FJ     | JX     | WF     | TG     | JH     | AJ     |
|-------|------|--------|--------|--------|--------|--------|--------|
| H     | 2017 | 10.67±3.34b | 15.70±1.51c | 7.08±0.79c | 18.00±4.47c | 18.98±1.53b | 10.71±0.29c |
|       | 2018 | 48.15±16.46b | 88.75±6.51b | 100.06±7.05 b | 59.42±2.98b | 72.32±12.19b | 72.57±15.50b |
|       | 2019 | 300.17±29.33a | 148.33±18.00a | 269.16±28.13a | 168.29±14.48a | 236.82±14.19a | 133.58±27.42a |
| BD    | 2017 | 3.39±1.27b | 2.56±0.79c | 2.62±0.76c | 1.54±0.45c | 1.80±0.22c | 1.11±0.14c |
|       | 2018 | 7.92±4.24b | 9.07±1.76 b | 10.36±1.88b | 8.13±1.20b | 9.74±1.61b | 9.03±1.81b |
|       | 2019 | 27.12±3.73a | 21.84±1.49a | 20.49±4.52a | 19.78±1.63a | 53.19±5.45a | 25.27±1.09a |

H denoted the net height of every plants, BD denoted the net basal diameter of every plants. FJ denoted Yongchun, Fujian provenance; JX denoted Jinggangshan, Jiangxi provenance; AJ denoted Anji, Zhejiang provenance; TG denoted Tonggu, Jiangxi provenance; JH denoted Jianhe, Guizhou provenance; WF denoted Wufeng, Hubei provenance.

### Tab.3 Correlation between plant growth and environmental factors

| Factors | H      | BD     | AT     | AP     | AS     |
|---------|--------|--------|--------|--------|--------|
| H       | 1.00   | 0.83** | -0.10  | -0.19  | -0.90**|
| BD      | 1.00   | -0.06  | -0.22  | -0.85**|
| AT      | 1.00   | -0.95**| -0.02  |
| AP      | 1.00   | 0.35   |
| AS      | 1.00   |

Note: H, BD, AT, AP, AS denotes plant height, plant basal diameter, annual mean temperature, annual precipitation, annual sunshine light, respectively. *means difference level at 0.05, ** means difference level at 0.01.

### Tab.4 Correlation between environmental factors and physiological index
| Factors | T     | P     | S     | WC    | TSS   | SOD   | CAT   | POD   | PPO   | K     | Na    | Ca    | Mg    | TP    | TF    | TT    |
|---------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| T       | 1     |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |
| P       | 0.97**| 1     |       |       |       |       |       |       |       |       |       |       |       |       |       |       |
| S       | -0.01 | 0.23  | 1     |       |       |       |       |       |       |       |       |       |       |       |       |       |
| WC      | -0.04 | -0.19 | -0.62**| 1     |       |       |       |       |       |       |       |       |       |       |       |       |
| TSS     | -0.11 | 0     | 0.44  | -0.60**| 1     |       |       |       |       |       |       |       |       |       |       |       |
| SOD     | 0.43  | 0.41  | -0.07 | 0.25  | -0.35 | 1     |       |       |       |       |       |       |       |       |       |       |
| CAT     | 0.21  | 0.18  | -0.09 | 0.15  | -0.1  | 0.02  | 1     |       |       |       |       |       |       |       |       |       |
| POD     | 0.19  | 0     | -0.73**| 0.37  | -0.34 | 0.13  | 0.12  | 1     |       |       |       |       |       |       |       |       |
| PPO     | -0.04 | -0.02 | 0.08  | -0.17 | 0.1   | 0.32  | -0.28 | -0.21 | 1     |       |       |       |       |       |       |       |
| K       | -0.47*| -0.57**| -0.42 | 0.48* | -0.05 | -0.50*| 0.13  | 0.4   | -0.35 | 1     |       |       |       |       |       |       |
| Na      | -0.17 | -0.26 | -0.41 | 0.49* | -0.27 | 0.32  | 0.34  | 0.17  | 0.04  | 0.21  | 1     |       |       |       |       |       |
| Ca      | -0.54*| -0.48*| 0.17  | -0.2  | 0.09  | -0.31 | -0.11 | -0.42 | 0.23  | -0.05 | 0.12  | 1     |       |       |       |       |
| Mg      | -0.34 | -0.4  | -0.27 | 0.43  | 0     | 0.01  | 0.19  | -0.06 | -0.17 | 0.37  | 0.58**| 0.13  | 1     |       |       |       |
| TP      | -0.80**| -0.72**| 0.19  | 0.03  | -0.01 | -0.03 | -0.31 | 0.08  | 0.18  | 0.31  | 0.56* | 0.29  | 1     |       |       |       |
| TF      | -0.48*| -0.53*| -0.27 | 0.19  | -0.15 | 0.21  | -0.25 | 0.04  | 0.04  | 0.09  | 0.59**| 0.42  | 0.45* | 0.56**| 1     |       |
| TT      | 0.02  | 0.14  | 0.49* | -0.60**| 0.4   | -0.14 | -0.11 | -0.25 | -0.12 | -0.09 | -0.15 | 0.1   | -0.28 | 0.12  | 0.18  | 1     |

T mean average temperature, P mean precipitation, S mean sunshine hours, WC mean water content, TSS mean total soluble sugar content, TT denoted total triterpenoid, TF denoted total flavonoid, TP denoted polysaccharide.