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STUDY ON THE CHEMICAL COMPONENTS OF SHOOTS AND LEAVES OF *CAMELLIA OLEIFERA* FROM DIFFERENT ELEVATIONS

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Abstract

The correlation between chemical components of shoot and leaf of *Camellia oleifera* and elevations were discussed, to provide theoretical basis for taking effective cultivation measures to promote the cold resistance of *C. oleifera* in the future "Northward Movement of *C. oleifera*". The ratio of bound water to free water of the leaf, as well as the contents of holocellulose, cellulose, lignin and benzene-ethanol extracts were determined. The results showed that: In different elevations, the ratio of bound water to free water of the leaf varied from 0.11-0.22, the contents of holocellulose, cellulose, lignin and benzene-ethanol extracts varied from 25.08%-62.72%, 7.72%-36.61%, 11.80%-30.29% and 7.66%-8.57% respectively. In the same elevation, the content of benzene-ethanol extracts of leaf was higher than that of shoot, and the contents of other materials were quite the contrary. The elevation was positively correlated with the ratio of bound water to free water and the contents of holocellulose, cellulose, lignin and benzene-ethanol extracs. The chemical components of shoot and leaf represented extremely significant difference ($p<0.01$) among different elevations. In the future northward movement of *C. oleifera*, for the climate characteristics of cooler regions and higher elevation, a serious of cultivation measures, improving the contents of holocellulose, cellulose, lignin, benzene-ethanol extracts and the ratio of water to free water, were taken to improve the cold resistance of *C. oleifera*.

Key words: *Camellia oleifera*; Elevation; Chemical composition; Cold resistance.

Introduction

Camellia oleifera Abel belongs to the family *Theaceae*, which is endemic woody oil tree species in southern China and equally famous like oil palm , olive and coconut, one of the top four oil source trees over the world, with an over 2,300-year-long history in cultivation and exploitation. Currently, it has been of great significance to the security of the grain and oil as *C. oleifera* are mostly grown in mountainous regions, because planting *C. oleifera* can not only reduce the high demand for edible oil supply, but also being able to replace the arable land for growing grain. In China, there are more than 55 million acres of *C. oleifera* forest mainly distributed in Hunan, Jiangxi, Guangxi, Anhui and other 18 provinces or regions, among which Anhui Province is the northern most boundary in China (Shu, 2013). Nowadays, *C. oleifera* has been tried to be planted at higher elevation areas as the response to the strategy of "northward and south-extended movement". However, it may be suffered from low temperature or any other climatic risks during the northward movement. Therefore, comprehending the theories of cold resistance and the ways of avoiding freeze injury must be concerned.

A significant corresponding relationship exists between elevation and temperature, (Saqib *et al*., 2011) *i.e.,* some climatic factors, such as temperature, air humidity, wind and illumination intensity changes according to the elevation, especially the temperature (Shaheen *et al*., 2012). For example, the temperature falls by 0.6 degrees with the increase of 100 m altitude. Lower temperatures affect the structures and some chemical components of the plant. Most researches show that there are significant differences between chemical components of plants and cold resistance as well as other functions (Fan & Wang, 2011; Zhao *et al*., 2013; Hultine & Marshall, 2000; Shinwari *et al*., 1998). Liu *et al*. (2011) analyzed the chemical components and fiber morphology of *Phyllostachys heterocycla* cv. *pubescens* plantations from different elevations, and believed that the contents of hot-water extractive, benzene-ethanol extractive, Klason lignin and ashy substance showed a tendency to increase, while 1% NaOH extractive, cellulose content and pH values showed a downward trend as elevation increased. Li *et al.* (2010) studied the physiological and biochemical index of *Pinus taiwanensis* Hayata from different elevations. Their results showed that when the plants were treated in low temperature, the relative conductivity of extract, the activity of Superoxide Dismutase (SOD) and molality of Methane Dicarboxylic Aldehyde (MDA) declined with increasing elevation. In the aspect of *C. oleifera*, a study on the chemical components of *C. oleifera* from different latitudes by Hu *et al.* (2012) found that annual average temperature and rainfall in ecotope had no correlation with the holocellulose content of shoot and leaf of *C. oleifera* but negative correlation with cellulose, lignin and benzene-ethanol extractive. But so far, the chemical components of *C. oleifera* in different elevations has not been reported. In this paper, *C. oleifera* forests (elevation ranging from 100m to 800m) from Shucheng county in Anhui province are selected, the contents of holocellulose, cellulose, lignin, benzene-ethanol extracive and the ratio of water to free water are determined. The relations between elevation and chemical components of *C. oleifera* are also analyzed, so that the advanced theoretical basis and more effective cultivation measures will be provided for the future cultivations of Northern *C. oleifera*.

Materials and Methods

Location and species: In this experiment, in order to better demonstrate the influences to the growth and development of *C. oleifera* in high elevation, latitude and low temperature, *C. oleifera* were selected at the highest elevation in northern China where *C. oleifera* is distributed in Shucheng County in Anhui province, located in the northeast slope of Dabie Mountain area (North 31°01'—31°05' by East 116°31'), belonging to northern subtropical humid monsoon climate, frost-free period of 200ds, with average 13.6°C and 1574 mm rainfall annually. The highest elevation of *C. oleifera* planting field can be as high as at least 810 m. The species is ″Cold Dew Seed″ type in normal *C. oleifera*, around 40-year-old.

Sample collection: Collecting was scheduled for cold winter (late in December 2011). Using MAGELLAN explorist 500 type GPS to measure altitude. Sampling was located in the same slope direction, sunny slope, at the elevation of 100 m, 200 m, 300 m, 400 m, 500 m, 600 m, 700 m and 800 m respectively. 40 samples were selected in total with 5 samples for each elevation. Annual shoots in the middle and upper part of crowns were collected and preserved in sealed bags.

Methods: (1) Bound water/free water: 0.5g fresh leaves were cut into pieces and soaked into 65% sucrose solution for 4h. The changes of sucrose concentration were determined by Abbe Refractometer. The contents of free water and bound water and the ratio of bound water to free water were calculated (Van Wagner, 1967).

(2) Content of chemical components: The samples (shoots and leaves) were pre-dried to a constant weight under 85°C (namely, the mass was determined once per hour, if the differences of the two adjacent results are within 2 mg, the latter one is the constant mass) and weighted. Samples were smashed and completely mixed. The determinations of all the chemical components were carried out in accordance with the national standards (Qu, 1992), of these, the holocellulose was determined by sodium chlorite (GB/T 2677.10-1995); Cellulose was determined by nitric acid-ethanol method; Lignin was determined by GB/T 2677.8-1994 with volume fraction of 72% in sulfuric acid; Benzene-ethanol extractive was determined by GB/T 2677.6-94. The contents of all chemical components refer to mass fraction.

Statistical analysis methods: All the datum were statistically analyzed and graphically processed using Microsoft Excel 2003 and spss17.0 software, among which the data in a chart were the average values for three times, and all the variances of statistics and average values were indicated by standard deviation. Moreover, one-way Analysis of Variance (ANOVA) combined with SSR (Duncan) were applied to analyze the differences among elevations.

Results

The effects of elevations on bound water/free water ratio: There is a very close relationship between the ratio of free water to bound water and cold resistance in plant growth. More exactly, if free water is more abundant, the metabolism of plant tissue or organs tend to be active while the ability for resisting cold is expected to be weak. On the contrary, if the content of bound water is higher, the growth will be slow, whereas the cold resistance is likely to be dominant. Therefore, we generally use the ratio of bound water to free water as cold resistance index. The differences of bound water/free water of *C. oleifera* from different elevations are shown in Fig. 1.

An increasing tendency of bound water/free water ratio is illustrated in Fig. 1, which ranged from 0.11 to 0.22, with the increasing elevation. To be precise, initially, the ratio of bound water to free water sharply rose by 0.05 from 100m to 300 m, from this point backwards, the ratio went up slightly by only 0.06 from 300m to 800m. Besides, ANOVA showed that the differences of bound water/free water of leaf had achieved extremely significant level ($p<0.01$) at different elevations.

The effects of elevations on holocellulose content of shoot and leaf: It can be seen from the Fig. 2, the holocellulose content of shoot rose consistently to 62.72% from 45.18% between 100m and 800m, and the holocellulose content of leaf increased from 25.08% to 37.13%. Furthermore, ANOVA and multiple comparison analysis showed that the holocellulose content of shoots and leaves achieved extremely significant level ($p<0.01$) at different elevations, particularly, the content of holocellulose reached extremely significantly different level in shoots between 100 m and other elevations.

The effects of elevations on cellulose content of shoot and leaf: Cellulose is a kind of polysaccharide composed of glucose molecules, which is an important component of the primary cell wall of green plants (Li & Zhang, 1994; Cheng, 2008; Li, 2001; Wang, 2006). According to the Fig. 3, the content of cellulose in shoot showed a slight growth from 100m (19.05%) to 800m, reaching the highest point at 36.61%. Similarly, the content of cellulose in leaf also represented an upward trend from 100m (7.72%) to 800m (14.20%). However, the contents of cellulose in leaf were consistently lower than that of shoot among these eight elevations.

Additionally, one-way ANOVA and multiple comparison analysis showed that the cellulose content of leaf differed significantly ($p<0.05$) between 200m and 300m, and there was no significant difference between 300m and 400m, the rest elevations showed extremely significant difference ($p<0.01$) between each other.

The effects of elevations on lignin content of shoot and leaf: Lignin is a kind of complicated macromolecular compounds, which is one of the major components of green plant (Chen *et al*., 2005; Han *et al*., 2000; Yu *et al*., 2003; Chen, 1995). According to the Fig. 4, the content of lignin experienced a modest growth in shoot from 23.28% to 30.29% with the increase of elevation, and the upward trend of leaf was basically the same as shoot but more slight, from 11.8% to 17.38%. One-way ANOVA showed that the difference of the contents of lignin of shoot and leaf had achieved extremely significant level ($p<0.01$) at different elevations.

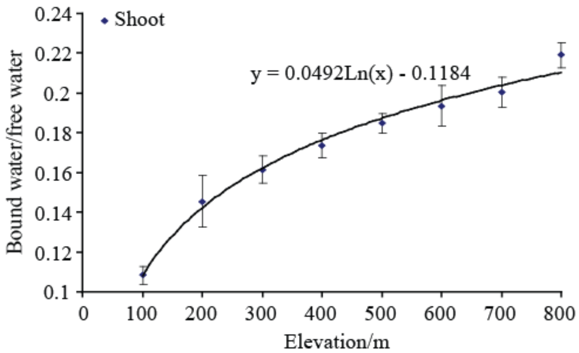

Fig. 1. Comparison of bound water/free water at different elevations.

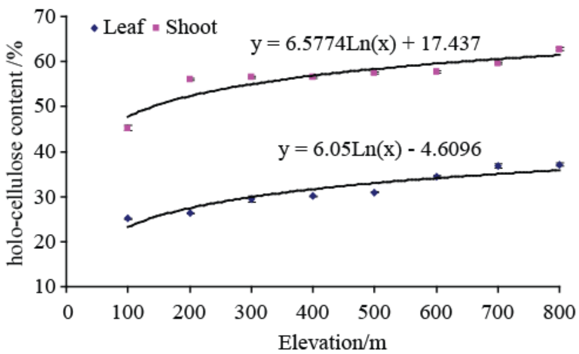

Fig. 2. Holocellulose content of shoots and leaves at different elevation.

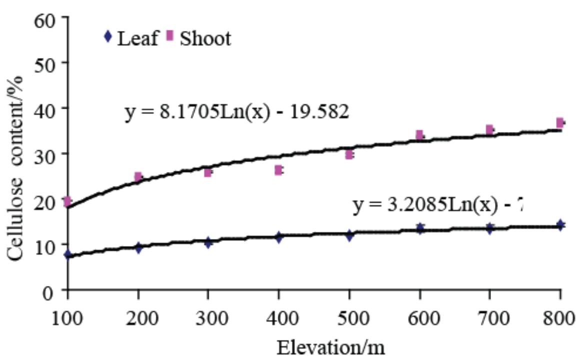

Fig. 3. Cellulose content of shoot and leaf at different elevations.

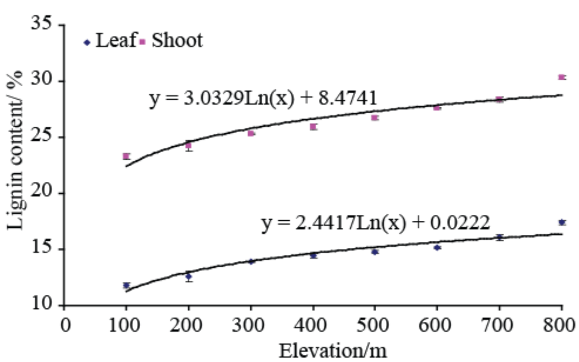

Fig. 4. Lignin content of shoot and leaf at different elevations.

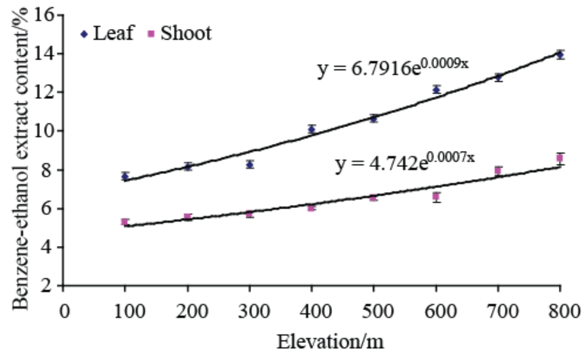

Fig. 5. Benzene-ethanol extracts content of shoot and leaf at different elevation.

The effects of elevations on benzyl-alcohol extractives content of shoot and leaf: The ingredients of benzyl-alcohol extracts are resin, wax, fat and tannin material, etc. (Huang & Fang, 2005). At the same elevation, content of benzyl-alcohol extracts of leaf were higher than those of shoot (Fig. 5). More specifically, the content of benzene-alcohol extracts of shoot and leaf all went up with elevation rising, from 5.28% to 8.57% in shoot and from 7.66% to 13.95% in leaf. In addition, one-way ANOVA and multiple comparison analysis showed that the content of benzene-alcohol extracts of shoot and leaf had extremely significant difference ($p<0.01$) among different elevations.

Discussion

The main chemical components of *C. oleifera* shoots and leaves in Shucheng county, Anhui province are determined and compared. The results show that the ratio of bound water to free water and the contents of holocellulose, cellulose, lignin and benzene-ethanol extractives are positively correlated with elevations. According to one-way ANOVA, the chemical components of *C. oleifera* shoots and leaves are extremely significantly different ($p<0.01$) among different elevations. This conclusion is the same to *Phyllostachys heterocycla* cv. *pubescens* plantations from different altitudes by Liu *et al*. (2011), except the variation trend of cellulose content, probably because of the differences between the two species.

The ratio of bound water to free water in tissue increased dramatically under low-temperature (Yu & Xu, 2003), i.e. if the bound water accounts for larger proportion, the growth and metabolism will be slow, whereas the cold resistance is likely to be dominant. This study shows that the ratio of bound water to free water of *C. oleifera* leaf will be increased with increasing elevation, and the growth would be slower whereas the cold resistance would be stronger.

Holocellulose, the mass of remaining hemicellulose and cellulose after removing lignin from the cellulosic materials in green plant, and along with hemicellulose and cellulose are the main components of the cell wall (Li & Zhang, 1994; Cheng, 2008), being closely associated with the lignifications of shoots and leaves (Li, 2001). Therefore, there is a certain relationship between holocellulose and plant lodging resistance and drought resistance (Wang, 2006). Moreover, lignin not only enables the cell wall to be more rigid, but also enhances the ability of cells and tissues against other adversities (Chen *et al*., 2005; Han *et al*., 2000; Yu *et al*., 2003; Chen, 1995; Kidokoro *et al*., 2009). The contents of holocellulose, cellulose and lignin content of shoots and leaves in *C. oleifera* increase with increasing elevation, this depicts that shoots are increasingly heavily lignified along with the elevation increasing, which will play a positive role in improving the capability of resisting cold, snow and wind.

In the previous report, the relationships between benzene-ethanol extracts and plant resistance might be due to lipids, wax and tannin in benzene-ethanol extracts (Zhang & Xiao, 1997). In the final analysis, we deem that benzene-ethanol extracts have a certain correlation with plant cold resistance from its upward trend as elevation increasing.

With the rise of elevation, many ecological factors, such as temperature, air humidity, moisture condition, sunlight and ultraviolet radiation changes at different degrees, and such environmental factors have been exerting a long-term effects on the same species and regarded as evolutionary pressure, maintaining the characteristic of the species in the long run (Fan & Wang, 2011; Shinwari *et al*., 2012). In this study, the growth resistance of *C. oleifera* rises with the elevation increasing under such evolutionary pressure. Nowadays, effective cultivation measures can be applied into "Northern Movement of *C. oleifera*", for example, selecting resistance varieties at relatively high elevation, establishing seedling base and using resistant culture. This is of great significance to the success of "Northern Movement of *C. oleifera*".

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