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# METABOLOMIC ANALYSIS OF FEMALE AND MALE PLANTS OF *PISTACIA CHINENSIS* BUNGE

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**Abstract**

*Pistacia chinensis* Bunge is dioecious. The female and male plants have different economic values. In addition to the large differences in floral organ shape on the outside, the male and female plants also have remarkable differences in ecological adaptability and physiological and biochemical characteristics. Thus, studying whether these differences in biological characteristics between male and female plants are caused by metabolic differences is necessary. Our results showed 56 kinds of different metabolites ($p \leq 0.05$) among the 235 metabolites detected between the female and male *Pistacia* plants, in which the contents of 20 kinds of metabolites in female plants were more than those in male plants, whereas the contents of 36 kinds of metabolites in male plants were more than those in female plants. Principal component analysis showed that the differences in peptide and hormone metabolites between the male and female plants of *P. chinensis* Bunge were the most significant. These differences may be the most important reason for the different biological characteristics between the male and female plants of *P. chinensis* Bunge.

**Key words:** *Pistacia chinensis* Bunge; Female and male plants; Metabolomics; Analysis.

## Introduction

*Pistacia chinensis* Bunge is a dioecious and deciduous tree of the *Anaeardiaceae* family and the *Pistacia* genus. *Pistacia* is often applied to urban greening because of its ecological value, including resistance to drought, low soil requirements, and strong resistance to sulfur dioxide, hydrogen chloride, and soot (Smith *et al*., 2008). Its roots, stems, leaves, and bark also have high medical value (Lu *et al*., 2003). In China, *Pistacia* has become a research hot spot of woody energy plants because the seed oil content of female plants is as high as 35%–50% (Wang *et al*., 2011). *P. chinensis* Bunge is dioecious, wherein female and male plants have different economic values. Large differences exist in their biological features, such as in physiological, biochemical, and ecological adaptability features, aside from the large difference in the floral organ morphology between the *Pistacia* male and female plants. Therefore, whether these differences in biological characteristics are related to the metabolic difference should be studied because the difference in metabolome is the basis of its biological characteristics differences. However, metabolomics research on the differences between *Pistacia* male and female plants has not been reported to date.

Metabonomics technology is no longer limited to the traditional targeted metabolite chemical composition analysis but carries on the determination of metabolites quantitatively and qualitatively from the angle of systems biology; hence, it has become a technology for detecting changes in complex organisms (Khakimov *et al*., 2013). As a new detection method, metabonomics causes minimal damage to the organism and can obtain a large amount of information. Compared with genomics and proteomics, metabonomics can be carried out more easily, and it has been widely used in plant metabolism variance analysis (Putri *et al*., 2013). This study analyzed the content differences in amino acids, sugars, and lipids between male and female *Pistacia* plants, mainly from the perspective of metabonomics, to provide a reference for deeply understanding the physiological differences between male and female *Pistacia* plants.

## Materials and Methods

**Plant material:** In July 2015, we collected *Pistacia* leaves of 30–40-year-old male and female plants as research material in a *Pistacia* natural forest farm located in the southeast of the Taihang Mountains (latitude: 113°23′–114°05′, longitude: 35°17′-36°12′). We selected the pinnately compound leaves from the central southern slope of the tree (Fig. 1). Six male or female plants were sampled, wherein leaflets from the lower part of the leafstalk in the upper direction to the number of third leaflets were obtained (Fig. 2). Male and female leaflets were mixed as test materials. The samples were physically selected, immediately frozen in liquid nitrogen, and stored in a −80°C ultra-low temperature freezer until further analysis.

**Method and Data analysis:** The samples were frozen by dry ice, carried to Shanghai Novel Bioinformatics Company, and underwent LC/MS and GC/MS for metabonomics analysis. After metabolic group extraction, derivatization, and gas chromatography–mass spectrometry detection, fingerprints of the male and female *Pistacia* plant leaf tissue metabolism group were obtained, and the obtained test data were analyzed using Welch Two Sample t-test. For other statistical designs we performed various ANOVA procedures.

## Results

**Metabolites of LC/MS and GC/MS determination:** The experiment detected 235 kinds of biochemical substances, including 54 kinds of amino acids; 49 kinds of carbohydrates; 47 kinds of lipids; 18 kinds of cofactors, prosthetic groups, and electron carriers; 14 kinds of nucleotide; 1 kind of peptide; 1 kind of hormone metabolism; and 51 kinds of secondary metabolism. A total of 56 different kinds of metabolites ($p \leq 0.05$) were observed between male and female plants, in which 20 kinds of metabolites were higher in females, whereas 36 kinds were higher in males. Analysis of the metabolic pathways of these metabolites, mainly related to amino acid metabolism, fat metabolism, and carbohydrate metabolism, was conducted (Table 1).


Fig. 1. Leaf morphology of *Pistacia chinensis* Bunge (pinnately compound leaves, weight 0.3-0.5 g, width 5-10 cm, length 12-15 cm).

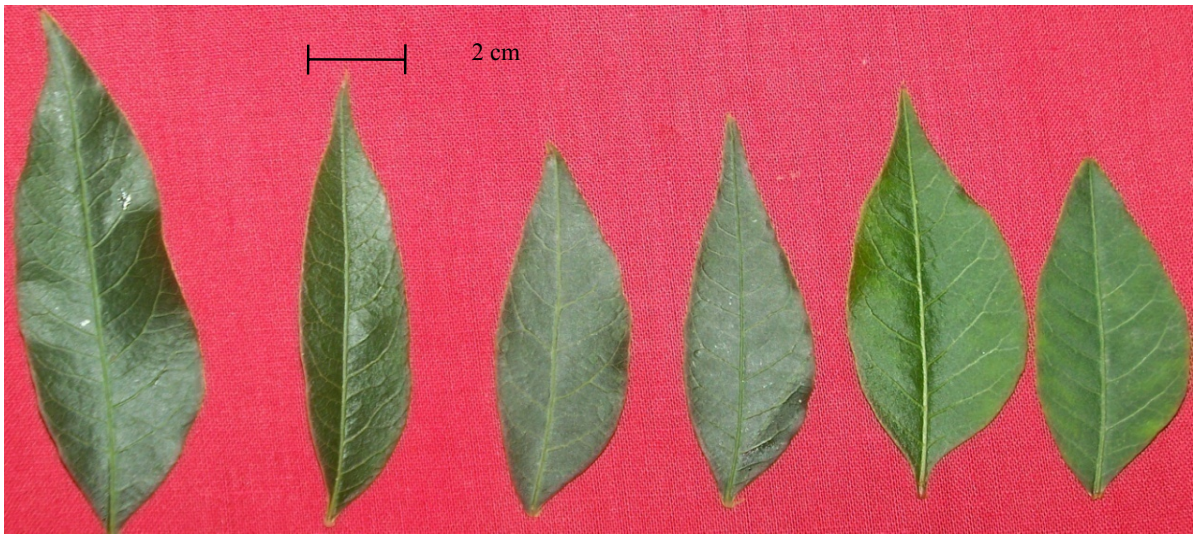


Fig. 2. Experimental leaflets (leaflets from the lower part of the leafstalk in the upper direction to the number of third leaflets, as shown in Fig. 1).

**Principal component analysis (PCA):** PCA was used to separate all the impact factors and obtain the contribution of the variation quantity of the different impact factors by analysis. Fig. 3 shows the PCA score difference concentration diagram for *Pistacia* male/female plant leaf extracts. The differences in the enrichment degree of the polypeptide and hormonal metabolites of *Pistacia* male/female plant leaf extracts were the highest at 4.123. The differences in the enrichment degree of the cofactors, prosthetic groups, and electron carriers were the lowest at 0.458, arranged in the following order: Peptides and Hormonal (4.123) > Nucleotide (1.767) > Secondary metabolite (1.132) > Lipid (1.053) > Amino acids (0.916) > Saccharides (0.757) > Cofactors, Prosthetic Groups, Electron Carriers (0.458). PCA analysis showed that the differences in peptides and hormonal metabolites between male and female *Pistacia* plants were the largest.

**Amino acid metabolites:** The 54 kinds of detected amino acid metabolites included 6 kinds of serine family (phosphoglycerate-derived), 9 kinds of aromatic amino acid metabolism (PEP-derived), 8 kinds of aspartate family (OAA-derived), 14 kinds of glutamate family (alpha-ketoglutarate-derived), 1 kind of branched chain amino acid (OAA-derived) (isoleucine), 11 kinds of branched chain amino acids (pyruvate-derived), 2 kinds of amines and polyamines, and 3 kinds of glutathione metabolism.

The contents of 12 kinds of amino acids showed significant differences between male and female *Pistacia* plants ($p \leq 0.05$), in which the contents of nine kinds of amino acids (i.e., O-acetylserine, phenethylamine, tyramine, 2-aminoadipate, arginine, N-acetylglutamate, pyroglutamine, methylsuccinate, and 2-isopropylmalate) in male plants were higher than those in females. By contrast, the contents of three kinds of amino acids, namely, putrescine, oxidized glutathione (GSSG), and reduced glutathione (GSH), in female plants were higher than those in male plants.

**Table 1. Metabolomic analysis results between the male and female plants of *Pistacia chinensis* Bunge [shows only the metabolites with significant differences ($p \leq 0.05$)].**

| Super pathway | Sub pathway | Biochemical name | Platform | Comp ID | Female/ Male |
|---|---|---|---|---|---|
| Amino acid | Serine family (phosphoglycerate derived) | O-acetylserine | GC/MS | 15947 | 0.19 |
| | Aromatic amino acid metabolism (PEP derived) | Phenethylamine | GC/MS | 12142 | 0.3 |
| | | Tyramine | LC/MS pos | 1603 | 0.38 |
| | Aspartate family (OAA derived) | 2-Aminoadipate | GC/MS | 6146 | 0.47 |
| | Glutamate family (alpha-ketoglutarate derived) | Arginine | LC/MS pos | 1638 | 0.27 |
| | | N-Acetylglutamate | LC/MS pos | 15720 | 0.42 |
| | | Pyroglutamine* | LC/MS pos | 32672 | 0.24 |
| | Branched chain amino acids (pyruvate derived) | Methylsuccinate | GC/MS | 15745 | 0.38 |
| | | 2-Isopropylmalate | LC/MS neg | 15667 | 0.44 |
| | Amines and polyamines | Putrescine | GC/MS | 1408 | 3.36 |
| | Glutathione metabolism | Glutathione, oxidized (GSSG) | LC/MS pos | 27727 | 1.93 |
| | | Glutathione, reduced (GSH) | LC/MS pos | 2127 | 4.96 |
| Carbohydrate | Glycolysis | Glucose | GC/MS | 20488 | 1.8 |
| | | Pyruvate | GC/MS | 599 | 0.48 |
| | TCA cycle | Fumarate | GC/MS | 1643 | 0.41 |
| | Calvin cycle and pentose phosphate | Sedoheptulose-7-phosphate | GC/MS | 35649 | 0.54 |
| | Amino sugar and nucleotide sugar | Erythritol | GC/MS | 20699 | 0.28 |
| | | Lyxose | GC/MS | 15787 | 0.33 |
| | Sucrose, glucose, fructose metabolism | Fructose | GC/MS | 31266 | 2.05 |
| | | Raffinose | LC/MS neg | 586 | 2.2 |
| | C5 branched dibasic acid metabolism | Citramalate | GC/MS | 22158 | 0.52 |
| Lipids | Free fatty acid | 2-Hydroxypalmitate | LC/MS neg | 35675 | 2.04 |
| | Oxylipins | 13(S)-HpOTrE | LC/MS pos | 39621 | 0.45 |
| | Glycerolipids | 1,2-Dipalmitoylglycerol | GC/MS | 35727 | 2.53 |
| | | 1-Linoleoylglycerol (1-monolinolein) | LC/MS neg | 27447 | 2.49 |
| | | 2-linoleoylglycerol (2-monolinolein) | LC/MS neg | 32506 | 4.4 |
| | Phospholipids | 1-Linoleoylglycerophosphocholine (18:2n6) | LC/MS pos | 34419 | 0.1 |
| | | 1-Oleoylglycerophosphocholine (18:1) | LC/MS pos | 33960 | 0.5 |
| | | 1-Palmitoylglycerophosphocholine (16:0) | LC/MS pos | 33955 | 0.09 |
| | | 2-Linoleoylglycerophosphocholine | LC/MS pos | 35257 | 0.16 |
| | | Glycerophosphorylcholine (GPC) | LC/MS pos | 15990 | 2.74 |
| | Choline metabolism | Choline phosphate | LC/MS pos | 34396 | 0.26 |
| | Sphingolipid | Phytosphingosine | LC/MS pos | 1510 | 0.2 |
| Cofactors, Prosthetic Groups, Electron Carriers | Ascorbate metabolism | Threonate | GC/MS | 27738 | 0.43 |
| | Chlorophyll and heme metabolism | Pheophorbide A | LC/MS pos | 35879 | 0.07 |
| Nucleotide | Purine metabolism | Adenine | LC/MS pos | 554 | 0.46 |
| | | Allantoin | GC/MS | 1107 | 0.48 |
| | | Urate | GC/MS | 1604 | 0.3 |
| | Pyrimidine metabolism | Orotate | GC/MS | 1505 | 0.52 |
| | | Uridine | LC/MS neg | 606 | 0.55 |
| | | Beta-alanine | GC/MS | 55 | 3.03 |
| Peptide | Dipeptide Derivative | Cyclo (ala-val) | LC/MS pos | 43577 | 1.96 |
| Hormone metabolism | Abscisic acid metabolism | Abscisate | LC/MS neg | 21156 | 2.31 |
| Secondary metabolism | Benzenoids | 4-Hydroxy catechol | GC/MS | 37430 | 0.31 |
| | Flavonoids | Catechin | LC/MS pos | 17668 | 1.87 |
| | | Cyanidingalactoside | LC/MS pos | 34122 | 0.34 |
| | | Kaempferol | LC/MS pos | 17785 | 0.36 |
| | | Quercetin | LC/MS neg | 17780 | 3.78 |
| | | Quercetin-3-o-glucoside | LC/MS neg | 37131 | 2.02 |
| | | Quercitrin | LC/MS neg | 21186 | 0.44 |
| | | Rutin | LC/MS neg | 18382 | 0.31 |
| | | Kaempferol-3-rhamnoside | LC/MS neg | 40041 | 0.33 |
| | | Chrysin | LC/MS neg | 40431 | 2.47 |
| | Phenylpropanoids | Isoferulate | LC/MS neg | 37450 | 1.89 |
| | | 4-Methoxycinnamic acid | GC/MS | 42992 | 0.4 |
| | Phenolic lipids | (15:2)-Anacardic acid | LC/MS neg | 41397 | 5.41 |

Note: Metabolite ratio of ≥1.71 or ≤0.55 indicates a significant difference ($p \leq 0.05$) between the male and female plants

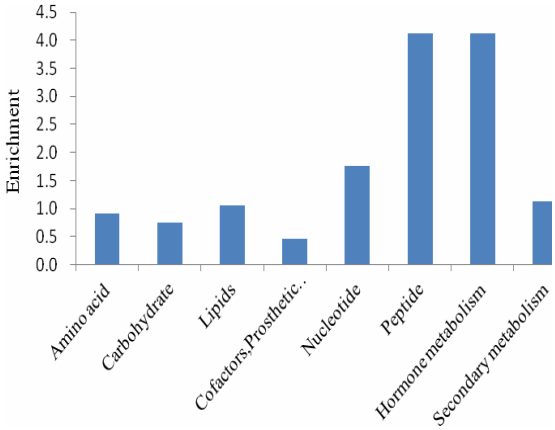


Fig. 3. Metabolomic principal component analysis results of *Pistacia chinensis* Bunge between male and female plants.

**Lipid metabolites:** In 47 kinds of detected lipid compounds, 10 kinds were free fatty acids, 3 were oxylipins, 8 were glycerolipids, 19 were phospholipids, 2 were choline metabolism, 1 was sphingolipid (phytosphingosine), 3 were sterols, and 1 was utin and cuticular (acetoacetate). The contents of 12 kinds of lipid metabolites showed significant differences ($p \leq 0.05$) in the leaves between male and female *Pistacia* plants. Seven kinds of lipid compounds (i.e., 13(S)-HpOTrE, 1-linoleoylglycerophosphocholine, 1-oleoylglycero-phosphocholine (18: 1), 1-palmitoylgly-cerophosphocholine (16: 0), 2-linoleoylglycerophosphocholine, choline phosphate, and phytosphingosine) were higher in male plants than in female plants. Five kinds of lipid compounds, namely, 2-hydroxypalmitate, 1, 2-dipalmitoylglycerol, 1-monolinolein, 2-linoleoylglycerol, and glycerophosphorylcholine, were higher in female plants than in male plants.

**Cofactors, prosthetic groups, and electron carriers:** Eighteen kinds of detected cofactors, prosthetic groups, and electron carriers comprised 1 kind of CoA metabolism (pantothenate), 6 kinds of nicotinate and nicotinamide metabolism, 2 kinds of oxidative phosphorylation, 1 kind of quinone metabolism [phytonadione (Vitamin K1)], 4 kinds of ascorbate metabolism, 1 kind of tocopherol metabolism (alpha-tocopherol), 1 kind of vitamin B metabolism (B6 or B12) (pyridoxate), and 2 kinds of chlorophyll and heme metabolism. Only two kinds of metabolite content differences between male and female plants were significant ($p \leq 0.05$), and the contents of these two kinds of metabolites (i.e., threonate and pheophorbide A) in male plants were higher than those in female plants.

**Nucleotide metabolites:** In the 14 kinds of detected nucleic acids metabolites, 8 kinds were purine metabolism and 6 kinds were pyrimidine metabolism. The contents of three kinds of purine metabolism and three kinds of pyrimidine metabolism showed significant differences between male and female plants. Five kinds of nucleotide contents in male plants were higher than those in female plants, including adenine, allantoin, and urate of purine metabolism, as well as orotate and uridine of pyrimidine metabolism. The contents of three kinds of purine metabolites in male plants were all higher than those in female plants ($p \leq 0.05$). Only one kind of nucleotide showed higher content in female plants than in male plants, namely, beta-alanine of pyrimidine metabolism.

**Peptide metabolites:** Dipeptide derivative [cyclo(ala-val)] was the only kind of polypeptide metabolites detected, and its content in female plants was 1.96 times than that in male plants. The present study suggested that dipeptide derivative was an active ingredient of several medicinal plants, and it played an important physiological role in the body. PCA analysis indicated that the content difference of polypeptide metabolites was the largest between male and female plants. This trial detected only one kind of polypeptide in male and female *Pistacia* plants, which does not explain many problems but is worthy of further study.

**Hormone metabolites:** Abscisate of ABA metabolism was the only kind of hormone metabolite detected. ABA plays an important role in the processes of seed development, seed dormancy, seed germination, plant vegetative growth, and environmental stress response (Hao *et al*., 2009). The ABA content in female *Pistacia* plants was 2.31 times than that in male plants ($p \leq 0.05$), which may be closely related to the role of female *Pistacia* plants. PCA analysis indicated that the differences in contents of hormone metabolites were the largest between male and female plants.

**Secondary metabolites:** Secondary metabolites, which are produced from secondary metabolism, are not necessary small molecule organic compounds for cell life activities or normal operations of plant growth and development. The production and distribution of secondary metabolites usually involve specific species, organs, tissues, and growth and development period. In the 51 kinds of secondary metabolites detected, 8 were benzenoid metabolites, 1 was a fatty acid and sugar derivative [galactarate (mucic acid)], 1 was a fatty acid derivative [(15:0)-anacardic acid], 22 kinds were flavonoids, 16 were phenylpropanoids, 1 was a tannin (catechin gallate), 1 was a terpenoid (alpha-amyrin), and 1 was a phenolic lipid [(15:2)-anacardic acid]. Among these metabolites, 13 secondary metabolites showed significant differences between male and female plants ($p \leq 0.05$). The contents of six kinds of secondary metabolites (i.e., catechin, quercetin, quercetin-3-o-glucoside, chrysin, isoferulate, and (15:2)-anacardic acid) in female plants were higher than those in male plants. The contents of seven kinds of secondary metabolites (i.e., 4-hydroxy catechol, cyanidingalactoside, kaempferol, quercitrin, rutin, kaempferol-3-rhamnoside, and 4-methoxycinnamic acid) in male plants were higher than those in female plants.

## Discussion

The number of amino acids with high contents in male *Pistacia* plants (nine kinds) was higher than that (three kinds) in female *Pistacia* plants. This result was similar to previous findings of ginkgo, kiwifruit,

eucommia, and asparagus leaves. Zhao (1993) analyzed 17 kinds of amino acids of ginkgo leaf in different genders and showed the largest difference between tyrosine and cysteine. The tyrosine content of male seedlings was 40.94% higher than that of female seedlings. Consistent with his results, the tyramine content in male *Pistacia* plants was 2.63 times than that of female plants. Zhang *et al*. (2014) showed that the half essential amino acid (arginine and histidine) content of male *Trichosanthes kirilowii* plants (1.93%) was higher than that of female plants (1.74%). The arginine content of male *Pistacia* plants was 3.67 times that of female plants, which was consistent with the results of previous studies. High aspartic acid and N-acetyl glutamate contents in male *Pistacia* plants were observed in all plant proteins, thereby playing an important role in metabolism. These two amino acids can also eliminate toxic ammonia formed during metabolism by forming asparagine and glutamine, as well as prevent aspartic acid and N-acetyl glutamate oxidation. Glutamate also has a signaling molecule function, and it influences or regulates important physiological molecular processes in plants (Song *et al*., 2012).

Putrescine and glutathione in female plant leaves were higher than those in male plants. Putrescine can inhibit protease and RNA enzyme activity, delay leaf senescence and decomposition of chlorophyll, regulate growth and morphogenesis associated with phytochromes, and regulate the flowering process. Glutathione is an important plant chelate precursor that controls cell heavy metals (Grill *et al*., 1989), as well as an important antioxidant and reducing agent (Foyer *et al*., 1976). In addition, glutathione also has a protective gene effect (Meister, 1988) and controls the redox of cell division (Sanchez, 1997). Thus, female *Pistacia* plants can gently and obviously maintain nutritional vitality for a long period compared with male plants to meet the development of seeds; male plants become yellow and age earlier after flowering than female plants. We believe that the high amount of amine substance and glutathione in female *Pistacia* plants compared with male plants ensures seed maturation and late aging of female plants (Zhao *et al*., 1993). This finding was also consistent with our results that the browning of female plants was significantly lighter than that of male plants in the process of tissue culture. The content differences of amino acids between male and female plants have significance reference in medicinal applications.

The 49 kinds of detected carbohydrates included 8 kinds of glycolysis metabolites; 7 kinds of TCA cycle metabolites; 1 kind of Calvin cycle and pentose phosphate (sedoheptulose-7-phosphate); 15 kinds of amino sugar and nucleotide sugar; 2 kinds of inositol metabolism; 15 kinds of sucrose, glucose, and fructose metabolism; and 1 kind of C5 branched dibasic acid metabolism (citramalate). Nine kinds of carbohydrates showed significant differences between male and female plants ($p \leq 0.05$), in which six kinds (i.e., pyruvate, fumarate, sedoheptulose-7-phosphate, erythritol, lyxose, and citramalate) had higher contents in males than in females. The contents of three kinds, namely, glucose, fructose, and raffinose, were higher in females than in males.

In this study, the contents of glucose and fructose in female *Pistacia* plants were 1.8 and 2.05 times than that in male plants, respectively. Some evidence indicated that glucose, fructose, and sucrose of soluble carbohydrates and fructan of low degree polymerization may be the signaling substances (Gibson, 2000). The sugar signaling activity in female plants may be stronger than that in male plants. In recent years, the "sugar signal" in the process of plant growth and development has gained increasing attention. A study showed that glucose can promote the synthesis of abscisic acid (ABA) by hexokinase (Chen *et al*., 2005). This result was the same as that of the ABA content in female *Pistacia* plants, which was 2.31 times than that in male plants. The raffinose content in female plants was 2.2 times than that in male plants. Raffinose series oligosaccharides function as low-temperature protective agents, accumulate in mature seeds, and are closely related to seed vigor, resistance to storage, and dehydration property (Pennycooke *et al*., 2003).

The contents of sphingosine and choline phosphate in male *Pistacia* plants were 5 and 3.84 times than those in female plants, respectively. Plant sphingolipid is a necessary component of plant cell (Chen *et al*., 2006), and it plays an important role in membrane stability, cell signaling, stress responses, pathogen defense, and programmed cell death (Napier *et al*., 2002). Choline phosphate is an important biosynthesis precursor of two kinds of plant stress-resistant factors (phosphatidylcholine and betaine). The high or low content of phosphatidylcholine and betaine is especially important to improve the ability of plants to adapt to adverse environments. Phosphatidylcholine, the most abundant phospholipid in the plant biomembrane system, participates in the formation of biomembranes, maintains the normal biological function of biomembranes, and improves the ability of plants to survive in adverse environment (Nuccio *et al*., 2000). We speculated that male *Pistacia* plant resistance was better than female *Pistacia* plant resistance, and the ecological adaptability of male plants was stronger than that of female plants. This speculation was consistent with the result of our field investigation in the Taihang Mountains, where male *Pistacia* plants showed higher growth altitude and better drought adaptability than female plants.Three kinds of glycerides detected in female plants of this experiment were significantly greater than that in male plants ($p \leq 0.05$), which may be closely related to the high oil content in *Pistacia* seeds of female plants.

The threonate content in male *Pistacia* plants was 2.32 times than that in female plants; hence, vitamin C metabolism levels of males were higher than those of females. Xu *et al*. (2009) showed that the *Populus cathayana* Rehd chlorophyll content of male plants was higher than that in female plants growing in the same environment. The annual seedlings showed greater differences, consistent with the annual average chlorophyll content in ramie male plants, which was greater than that in female plants (Xing, 2011). These findings were also consistent with the results of this study. The pheophorbide A content of male *Pistacia* plants was 14.29 times than that of female plants, indicating that male *Pistacia* plants had higher photosynthesis levels than female plants.

Three kinds of detected purine metabolites showed higher contents in male plants than in female plants ($p \leq 0.05$), indicating that purine metabolic activity of male *Pistacia* plants was stronger than that of female plants. Among the three kinds detected pyrimidine metabolites, the contents of orotate and uridine contents in male plants were higher than those in female plants ($p \leq 0.05$). By contrast, the beta-alanine content in female plants was 3.03 times than that in male plants ($p \leq 0.05$), indicating that male *Pistacia* plants had vigorous pyrimidine compound metabolism than female plants. However, uracil metabolism showed the opposite trend, in which female plants demonstrated vigorous uracil metabolism than male plants. Thus, nucleic acid metabolism levels in male plants were stronger than those in female plants; however, uracil metabolism in female plants was stronger than that in male plants.

Taking the flavonoid secondary metabolites as examples, Tab. 1 shows that the contents of quercetin, quercetin-3-o-glucoside, chrysin, and isoferulate in female plants were 3.78, 2.02, 2.47, and 1.89 times than those in male plants, respectively, and reached significant difference levels ($p \leq 0.05$). This finding was similar to the result that the flavonoid content of *Hippophae rhamnoides sinensis* Rousi female leaves is greater than that in male leaves (Li *et al*., 2000), but different from the result that the flavonoid content of ginkgo male plants is higher than that in female plants (Wu *et al*., 2010). These results showed that flavonoid contents varied between male and female plants in different tree species. In practical applications, the differences in secondary metabolite content between male and female plants were significantly related to medicinal material selection.

## Conclusion

The experimental results in this study indicated that male and female *Pistacia* plants had various and obvious differences in the metabolome. In the 235 kinds of metabolites detected, 56 metabolites showed significant differences in male and female *Pistacia* plants ($p \leq 0.05$), with an overall difference rate of 23.83%. The contents of 20 kinds of these metabolites in female plants were higher than those in male plants, whereas the contents of 36 kinds of these metabolites in male *Pistacia* plants were higher than those in female plants. Analysis of the metabolic pathways of these metabolites indicated that these metabolites were related to amino acid metabolism, fat metabolism, and glucose metabolism. PCA analysis indicated that the contents of peptide and hormonal metabolites between males and females showed the largest difference, which should be the direction of focus of future research on physiological differences between male and female *Pistacia* plants. The differences in metabolic characteristics between male and female *Pistacia* plants may be the reason for the differences in their physiological metabolism and ecological adaptability. Whether these differences may be attributed to one or a number of sex-determining genes warrants further research.

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