CONTENTS

Biology and Breeding of Tea (*Camellia sinsensis* (L.) O. Kuntze) in Kenya

Samson M. Kamunya[1*], Richard M. Chalo[1], Tony Maritim[1], Robert Korir[1], Simon Ochanda[1], Kanja Thuku[2], Charles Mucheke[3], Julius King'oo[4], Brian Ngwiri[5]

1. KALRO-Tea Research Institute, Kericho 20200, Kenya; 2. Kenya Tea Development Agency, Nairobi, Kenya; 3. Nyayo Tea Zone Development Corporation, Nairobi, Kenya; 4. Tea Board of Kenya, Nairobi, Kenya; 5. East African Tea Traders Association, Mombasa, Kenya

Tea, *Camellia sinensis* (L.) O. Kuntze was introduced and cultivated commercially in Kenya during the first quarter of the 20th century using seeds from India. Its origin is south-east Asia in the region encompassing the Yunnan and Guangxi provinces of south western China home to *C. sinensis* var. *sinensis*, and the Assam-Burma region in north-east India, the origin of *C. sinensis* var. *assamica* and *C. sinensis* var. *assamica* spp. *lasiocalyx.* Thus, the Indian hybrid seed provenances from which pioneer seedling tea populations were established in Kenya were mixed random collections from popular landraces. These Jat selections were open-pollinated and hence seeds were polyclonal mixtures. The need to fast-track tea improvement led to adoption of rationalised breeding programme, initially capitalising on intra-specific hybridization using elite disparate parents, and later inter-specific hybrization, using tea germplasm introduced from China and Japan. The rationalised tea breeding in Kenya essentially consists of four phases; generation of genetic variability, selection of useful genotypes, comparative tests to demonstrate the superiority of the selected clones and exposing promising improved clones to multiple sites (genotype by environment interaction) for stability and adaptability. Since tea is grown in areas which differ widely in elevation, climatic and edaphic factors with profound effect on growth, productivity and quality of tea, the last phase of breeding is normally conducted in tea growers' fields through participatory clonal adaptability trials. Considering this, a collaborative study between the Tea Research Institute and the major tea growing stakeholders was initiated in 2011 to evaluate the performance and genetic stability of improved tea cultivars across nine tea growing sites in Kenya. Analysis of genetic stability with respect to yield data revealed that 19 improved cultivars performed better than average in good environments and worse in marginal environments (bi>1). Seven (7) cultivars exhibited broad stability as indicated by their responsiveness to environmental changes and took advantage of improved environment (bi=1), while 18 cultivars showed poor performaance in better environments but better in marginal environments. The generated biochemical data used to infer the level of quality of the processed tea (black or green) also showed that some cultivars had outstanding quality attributes suitable for diverse tea products. Other cultivars were only suitable for one product with environmental parameters significantly impacting on tea quality status adherence to uniform agronomic practices. notwithstanding. The analysis of genetic stability for yield and polyphenolic data, which was further butressed by drought damage and fermentability data helped in selecting climate resilient cultivars that are highly amenable to diversification of novel tea products diversification. Consequently, four elite cultivars viz; KTRI 616/1, KTRI 632/1, KTRI 713/1 and KTRI 895/22 that exhibited remarkable stability across the test sites and possessed traits for processing high value specialty tea products for niche markets were recommended for release and commercializatio in 2022.

Acknowledgement

This project was supported by the Kenya Agricultural & Livestock Research Organizaion (KALRO), National Research Fund (NRF) and the tea industry stakeholders.

***Correspondence**: samson.kamunya@kalro.org; samsonkamunya@gmail.com

Comparison of Leaf Ultrastructure, and Contents of Amino Acids and Catechins in Tea Plants with Yellow and Green Leaves at the First Harvest Time

Doo-Gyung Moon[1*], Yun-Suk Kwon[1], Yong Hee Kwon[1], Harim Hong[1], So Jin Lee[1], Eun Young Song[1], CHEN Liang[2]

1. Research Institute of Climate Change and Agriculture, NIHHS, RDA, Jeju 63240, Korea;
2. Tea Research Institute of Chinese Academy of Agricultural Sciences, Hangzhou 310008, China

Introduction

Tea [*Camellia sinensis* (L.) O. Kuntze] is an economically important lefty crop and an evergreen leafy plant cultivated worldwide. It contains more of the major amino acids and catechins than other plants. Tea germplasm has green, yellow, purple and white leaf colors. Among them, the tea plants with yellow and white leaves are called as albino tea plants. The albino leaves contain high concentration of amino acids, especially L-theanine which contributes to the umami taste and high quality of green tea. Recently, we discovered *Camellia sinensis* with yellow leaf (YL) which grows wild in Korea. Thus, we analyzed chloroplast ultrastructure, amino acids and catechin contents in YL at the first harvest time and then compared them with *Camellia sinensis* cv. *'Sangmok'* with green leaf (SM) of stable genetic traits.

Materials and Methods

An interesting characteristic of YL is that its young shoots are yellow at the first harvest times of spring and changes to green at the other seasons. The chlorophyll contents was estimated using the simple measuring instrument (SPAD-52, Konica Minolta, Japan) with one bud three leaves in both YL and SM. The experiment was performed in five biological replicates and the results were presented as mean ± SD. TEM observation was carried out according to the method described as the tender shoots from leaves in both YL and SM were fixed by immersion overnight using Karnovsky's at the first time. Theanine and each amino acid in extract samples were identified by High performance liquid chromatography (HPLC, Agilent 1200 series, USA). The catechin contents were identified by Ultra-performance liquid chromatography (UPLC, Waters Acquity, USA).

Results and Discussion

Leaf length, width and thickness of the YL were (6.60±0.72) cm, (3.00±0.46) mm and (0.41±0.06) mm, whereas those of SM were (8.20±0.48) cm, (3.40±0.31) mm and (0.47±0.06) mm, respectively. Moreover, YL exhibited decreased chlorophyll contents and chloroplast number. The chloroplast structure of YL was abnormal type compared to SM at the first harvest. Contents of total amino acid, L-theanine, arginine, glutamic acid and asparatic acid in YL were (93.2±2.1) mg/g, (61.7±1.1) mg/g, (12.06±0.95) mg/g, (7.52±0.15) mg/g and (4.34±0.10) mg/g, respectively. In SM, with the same order, (49.9±0.1) mg/g, (38.98±0.70) mg/g, (3.30±0.72) mg/g, (3.64±0.04) mg/g and (1.68±0.02) mg/g, respectively. The total amino concentration was 1.6~3.7 times higher in YL as compared with SM. The contents of EGCG ((-)-epigallocatechin gallate), EGC ((-)-epigallocatechin), ECG ((-)-epicatechin gallate) and EC

((-)-epicatechin) of YL were (22.6±3.0) mg/g, (8.8±0.8) mg/g, (11.4±1.2) mg/g and (4.8±0.4) mg/g, respectively. However, SM contained EGCG (34.8±1.4) mg/g, EGC (25.9±1.0) mg/g, ECG (12.5±0.5) mg/g and EC (8.5±0.3) mg/g. SM was 1.1~2.9 times higher than YL. YL would be more valuable tea plant resource for high quality green tea due to the higher amino acid contents, especially L-theanine and arginine.

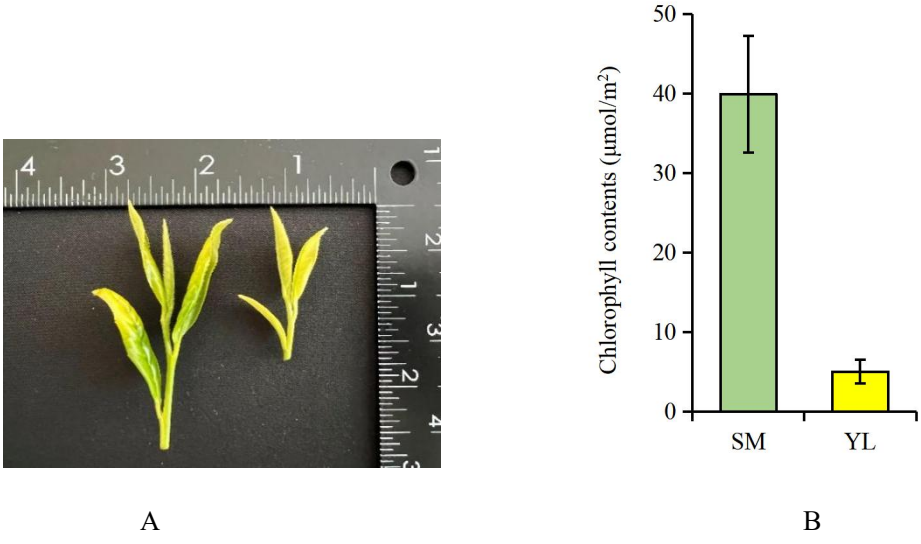


Figure 1 Phenotypic characteristics of yellow leaf (YL) and green leaf (SM) with the one bud and three leaves at the first harvest time (A). Chlorophyll contents of YL and SM with the one bud and three leaves at the first harvest time (B)

Left photo is *Camellia sinensis* cv. '*Sangmok*' (SM) and right photo is *Camellia sinensis* with yellow leaf (YL) in A.

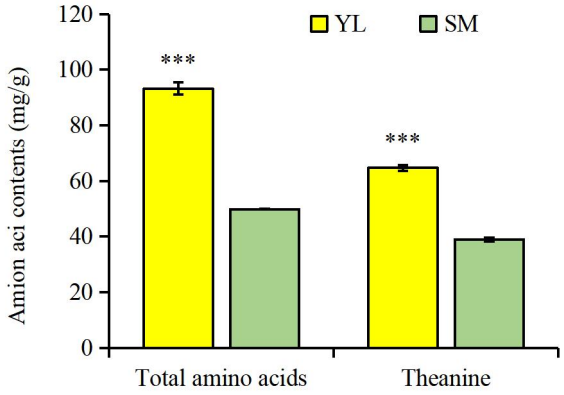


Figure 2 Total amino acid and theanine contents of yellow leaf (YL) and green leaf (SM) with the one bud and three leaves at the first harvest time

SM: *Camellia sinensis* cv. '*Sangmok*', YL: *Camellia sinensis* with yellow leaf (YL). Significant difference between YL and SM was measured by Student's-test (***P<0.001). Value mean±SD.

This work was carried out with the support of "Cooperative Research Program for Agriculture Science and Technology Development (Project No. PJ017194022023)" Rural Development Administration, Republic of Korea.

Indonesia Sinensis Tea Clones (*Camellia sinensis* var. *sinensis*) Screening for Good Quality and Nutrient Efficient for Climate Change Mitigation

Heri Syahrian, M. Khais Prayoga, Vitria P. Rahadi, Hilman Maulana, Shabri

Research Institute for Tea and Cinchona, Bandung 40972, Indonesia

Introduction

Tea production and quality rely on suitable temperatures, rainfall, and available nutrient uptake. The sustainable attempt to reduce the climate change impact on the productivity and quality of tea is by screening the Sinensis tea clones to have good quality, and nutrient uptake efficiency. In Indonesia, the clones of sinensis are diverse according to caffeine content, appearance, and aroma properties (Prayoga et al., 2022), and have high polyphenol and flavonoid through the panning method (Prawira-Atmaja et al., 2022). Research Institute for Tea and Cinchona (RITC) have 35 sinensis clones which untested to their taste performance and efficiency level of nutrient uptake. The research aimed to select the sinensis tea types' clones with good quality and nutrient use efficiency.

Materlal and Methods

The research was conducted at the Experimental Field and Product Processing Laboratory, Research Institute for Tea and Cinchona (RITC), Gambung, West Java, from July 2022 to January 2023. The materials used were 35 clones of *Sinensis* tea of RITC germplasm. The clones have been planted since 1994 in experimental field of RITC using Randomized Block Design with three replications. Each replication consists of 20 plants of tea and each clone in this study came from propagating cuttings. Cuttings from stems, grown in a nursery in polybags 12 cm × 25 cm with a thickness of 0.04 mm. After one year in the nursery, the plants are transferred to the field with a spacing of 90 cm between plants inside the row and 120 cm between rows (Figure 1).

Figure 1 Performance of 35 clones on test plot

Results

The results showed that Yabukita clone had significant differences in dry appearance, liquor color, and include into the premium taste. Comparatively, clone I.1.93 showed significant differences in weight before and after fertilization and NUE parameters of 237.18 kg/hm^2 and 64.10%, respectively.

Nitrogen and potassium increased in the leaves by 0.116% and 0.068%, respectively. Otherwise, the phosphorus content tended to decreased by 0.006%. Increasing nitrogen content in tea leave occurred in 22 clones. The phosphorus content decreased in 24 clones, while the potassium improved in 28 clones. The scatterplot of PCA showed the thirty-five tested clones were generally characterized with average weight per hectare (Figure 2). The clones are expected to absorb additional fertilizer effectively and maintain quality.

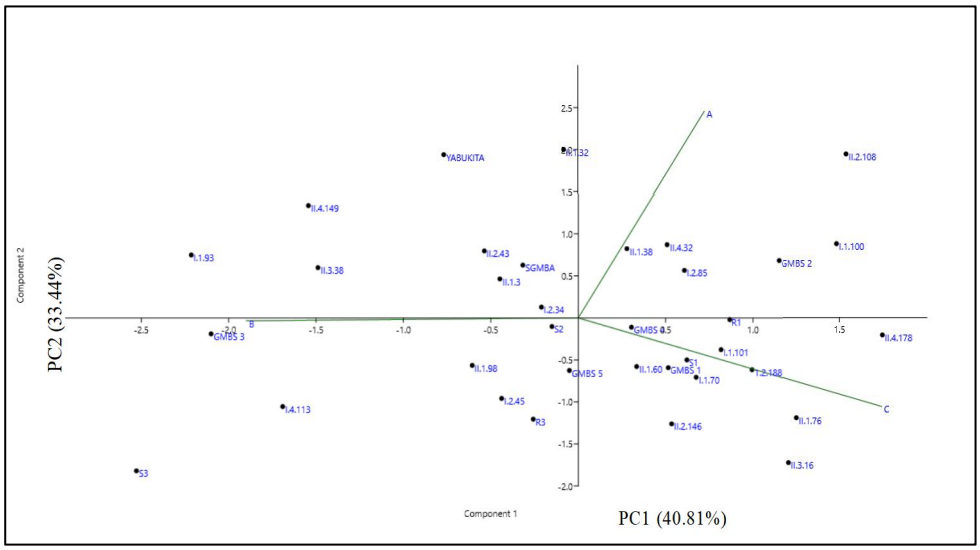


Figure 2 Principal component analysis of 35 *Sinensis* tea clones

The scatterplot was generated based on quality parameters (A), nutrient uptake efficiency (B), and average weight per hectare (C). Size: 9.5 cm (H) × 14.76 cm (W).

***Correspondence:** sekretariat.pptk@gmail.com

Tea Breeding Programs from 2018 to 2023 in Vietnam

Luu Ngoc Quyen[1*], Nguyen Van Toan[2], Nguyen Thi Minh Phuong[2],
Nguyen Van Thiep[2], Nguyen Ngoc Binh[3], Nguyen Thi Hong Lam[3],
Phung Le Quyen[3], Trinh Thi Kim My[4], Nguyen Hong Chien[4]

1. Director General of Northern Mountainous Agriculture and Forestry Science Institute, PhuTho, Vietnam; 2. Vietnamese Tea Science- Technology Association, Vietnam; 3. Tea Research and Development Centre, Northern Mountainous Agriculture and Forestry Science Institute , PhuTho, Vietnam; 4. Biotechnology and Crop Protection Department, Northern Mountainous Agriculture and Forestry Science Institute, PhuTho, Vietnam

By 2023, the tea growing area in Vietnam is more than 125 thousand hectares, with an average yield of 9.75 t/hm^2. In 2022, Vietnam produces about 200.0 thousand tons of dry tea, and exports about 146.0 thousand tons with a turnover of about 237 million USD, making it the country with the 6th largest tea output and the 5th largest export in the world. Large tea growing areas are concentrated mainly in the Northern Midlands and mountainous areas, followed by the Central Highlands, Northern Delta, and North Central region. Some provinces in Vietnam with large tea growing areas are Thai Nguyen (22.3 thousand hectares), Ha Giang (21.5 thousand hectares), Phu Tho (16.1 thousand hectares), Lam Dong (10.8 thousand hectares). Preserving tea genetic resources is important in tea breeding programs. Currently, the Northern Mountainous Agriculture and Forestry Science Institute (Nomafsi) is storing, researching and developing a collection of 406 tea cultivars collected from tea growing countries around the world, tea regions of Vietnam and cultivars created by different methods. Of these, 100 cultivars belong to the small-leaf China type, 63 cultivars belong to the large-leaf China type, 39 cultivars belong to the Assam type and 204 ones belong to the Shan type. From this collection, in the period from 2018 to 2023, Nomafsi has released 13 new tea cultivars, including: PH12, PH14, LCT1, PH22, PH276, LP18, LCT1, VN15, Huong Bac Son, TRI5.0, PH21, CNS141 and CNS831.

Research on tea is always carried out along the value chain from selecting tea cultivars to farming techniques for new tea cultivars, technology for processing products from new tea cultivars and finally the consumer market, thereby promoting the strengths of new tea cultivars when put into production. Among the new tea lines/varieties created in the period 2018—2023, the most outstanding varieties are Huong Bac Son, VN15, LCT1, CNS141, CNS831 and TRI5.0 created by hybridization and mutation method. These new tea varieties are an important source in building the tea variety structure by region, contributing to improving the value and sustainable development of Vietnam's tea industry.

Tea Breeding Programs

Hybridization is one of the main methods to obtain genetic variations and is an important method of breeding new varieties. Distant hybridization is a powerful method for expanding the genetic base of new varieties. At Nomafsi (Vietnam), a number of different breeding methods have been applied to create new tea varieties such as: sexual hybridization combined with embryo rescue technology, traditional hybridization and mutation treatment methods.

Traditional Hybridization and Mutation Treatment

Selecting and creating new tea varieties by hybridization method is carried out regularly and continuously. The advantage of this method is that it is simple, easy to implement, and the number of

individuals created is large; orienting parents in hybrid combinations; hybrid offspring with good characteristics of one parent; tea varieties with improved quality traits. Many hybrid combinations have been carried out with the aim of creating good quality tea lines/varieties. Applying this method, new tea varieties such as Huong Bac Son, VN15 and LCT1 were created. These are varieties with very good yield and quality, suitable for processing high quality green tea and Olong tea products (Table 1).

In addition to hybridization, mutation method has also been applied to create new tea varieties. The TRI5.0 tea variety was created by treating 5.0kr dose of gamma irradiation from Co60 source on the seeds of the TRI777 tea variety. TRI5.0 tea variety has a high yield and quite good quality, so it is suitable for processing both green tea and black tea (Table 1).

Table 1 Yield and some quality indicators of new tea varieties

Name	Breeding method	Yield (5-year-old plant)/(t/hm^2)	Tanin/%	Amino acids/%	Catechine/(mg/g)	Sensory evaluation score*		Product orientation
						Green tea	Black tea	
Huong Bac Son	HP	6.62	24.17	3.27	128.40	17.80		high quality green tea, Olong tea
VN15	HP	6.98	27.53	2.94	137.57	18.10		high quality green tea
LCT1	HP	8.95	29.73	2.84	152.20	18.04	16.7	high quality green tea
TRI5.0	Mutation	9.75	32.10	2.20	225.00	16.80	17.0	green tea and black tea
CNS141	HP	10.86	28.10	2.45	155.80	16.43	17.5	green tea and black tea
CNS831	HP	9.97	24.47	2.56	153.70	17.94	18.6	green tea and black tea

HP: Clones selected from hand-pollinated progenies.
*According to TCVN3218-2013, the maximum sensory evaluation score is 20.

Hybridization Combined with Embryo Rescue Techniques

The hybridization method combined with embryo rescue techniques has also been applied in selecting and creating new tea varieties. Tens of hybrid combinations were conducted in the direction of selecting tea varieties with high yield and good quality. Some hybrid combinations have very low fruiting rates, so embryo rescue techniques have been applied to increase the success rate of these hybrid combinations. The embryo rescue process includes the following main steps: collecting hybrid seeds, embryo genesis, inducing *in vitro* shoots and regenerating plants. The F1 hybrid plants are then transferred to green house, monitored their growth, and planted on fields to evaluate productivity and quality indicators. The advantage of this method is that it increases the success rate in out-crossing and thus can increase the number of hybrid individuals with different genetic base.

Hybrid tea plants from 2 out of 49 hybrid combinations were evaluated for productivity and quality indicators over a 5-year period. Results of evaluating yield indicators of 12 tea lines belonging to the THn hybrid combination and 8 lines belonging to the TKt hybrid combination show that two varieties CNS141 (hybrid plant of THn combination) and CNS831 (hybrid plant of TKt combination) have superior growth and productivity compared to their parents. Biochemical analysis results show that these two tea varieties have good quality, suitable for processing both green tea and black tea (Table 1).

Results of Transfer and Production Expansion

Every year, Nomafsi transfers from 5 to 7 million new tea seedlings and tens of millions of tea cuttings for new planting and replacement planting in tea areas nationwide. We have built and transferred technical packages along the value chain from varieties-farming techniques-processing-market. Technological processes for processing new tea products such as: Olong tea, high quality green tea and black tea, pressed tea, herbal tea, and flower-infused tea have also been transferred to many domestic businesses.

The area planted with these new tea varieties is gradually increasing over the years. At the end of 2022, the planted area of these 6 new tea varieties reached about 500 hectares. Currently, the demand for growing these new tea varieties is growing, especially in Thai Nguyen and the Northern Midlands and Mountainous provinces (Phu Tho, Yen Bai, Tuyen Quang, Son La, Lai Chau...).

In summary, tea breeding research in Vietnam mainly uses traditional methods combined with the application of modern technology. Although there are not so many new tea varieties released in the period of 5 years, they have made certain contributions to the Vietnamese tea industry and enriched the world's tea varieties. Tea breeding programs at Nomafsi are still ongoing with the main goal of getting new tea varieties with high yield, good quality, resistance to biotic and abiotic stresses, and some special characteristics (eg high catechin content...).

***Correspondence**: quyengret@yahoo.com

Tea Cultivation and Storage Study for K-Matcha Exportation

Jong Cheol Kim[1], Doobo Shim[1], Jung Gyu Hwang[1], Kyung Hwan Cho[1], Yong-Hwi Son[1], Gwang Hui Jeong[1], Jihun Shin[1], Changhyun Sung[1], Juwan Park[1], Ho Jin Heo[2]

1. Institute of Hadong Green Tea, Korea; 2. Gyeongsang National University

Introduction

Matcha is a fine powder made from tea (*Camellia sinensis*) leaves and generally called green tea powder or powdered green tea, however the making process is different from green tea process. The major different processing of matcha has no rolling process. Precisely the matcha should be cultivated under shading condition for some time. The quality of matcha depend on this shading cultivation, processing and grinding etc. The K-matcha is a Korean matcha brand in order to export to overseas including USA, Canada, EU, Mexico, and Asian countries etc. The K-matcha is exporting one hundred tons every year and especially 80% of these are exporting to USA StarbucksTM company. This presentation introduces a double shading cultivation to increase match quality and matcha storage study according to temperature changes.

Shading Cultivation Study for Increasing Matcha Quality

Morphologic and physicochemical changes of shading cultivated tea (*Camellia sinensis*) tree leaves were investigated during 26 days with 95% shading treatment. Shading cultivation showed higher leaf area (10%) and moisture content (12%) than non-shading cultivation. The shading cultivation increased the total free amino acid (3.4-fold), theanine (3.7-fold), arginine (10.8-fold) and caffeine levels (2.0-fold) but decreased the total catechin contents. The SPAD value and total chlorophyll contents of the shading cultivation were increased by 1.1- and 2.1-fold, respectively. The results of a color analysis showed that lightness (L^*) of non-shaded was higher than that of shading cultivated matcha. However, the greenness (a^*) and yellowness (b^*) of shading cultivated matcha were higher than those of non-shading cultivation. The surface color of shading cultivated matcha had higher chroma- and G-values than non-shading cultivated matcha, which represents real green color. Thus, the shading cultivation is suitable to prepare a high-quality matcha product

In addition, the sequential double shading method was applied to get high quality matcha and the growth and physiological changes of tea leaves were investigated.

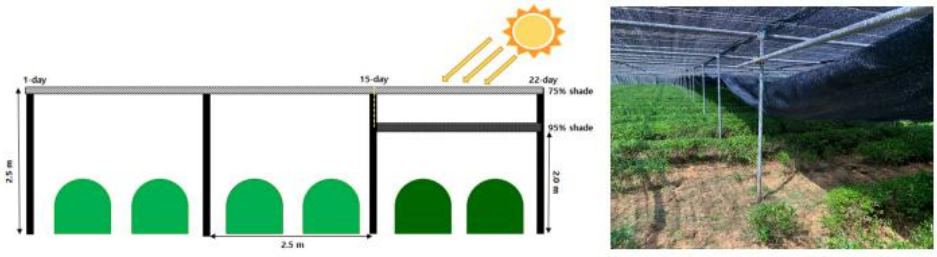


Diagram 1. Structure of sequential dobule-shading and actual installed image.

As a result, the chlorophyll content before harvesting of tea leaves was higher in order of sequential double shading (52.7) > single shading (49.9) > non-shading (40.2), and chlorophyll fluorescence (Fv/Fm) was measured in the order of sequential double shielding (0.73) > single shielding (0.67) > non-shielding (0.53). The order of the normalized difference vegetation index (NDVI) was sequential double shading (0.72) > single shading (0.70) > non-shading (0.65), and the simple ration index (SR) was in the order of sequential double shading (3.04) > single shading (2.82) > non-shading (2.30). The leaf length, leaf width and leaf area were higher in order of single shading > sequential double shading > non-shading, and the internode length was in the order of non-shading > sequential double shading > single shading. The G-value of the leaf surface was in the order of sequential double shading (62.9) > single shading (56.4) > non-shading (51.9). The overall physiological and growth status shows that the sequential double shading increases the color of the tea leaves better than does single shading, and sequential double shading is effective in reducing the shading stress of tea plants.

Effect of Storage Temperature on Matcha Stability

This study was conducted to evaluate the storage conditions of matcha (*Camellia sinensis*) according to temperature during 2 months. The moisture content of matcha tend to decrease with increasing temperature. To evaluate the brightness and green value of matcha, changes in L^* and G^* values were examined. These values decreased with increasing temperature and time. Total phenolic content and total flavonoid content also decreased with increasing temperature and time. ABTS and DPPH radical scavenging activities decreased with the increase in storage temperature and time. The content of catechins such as epicatechin, epigallocatechin, epicatechin gallate and epigallocatechin gallate showed a tendency to decrease gradually according to the storage temperature and time. Also, caffeine and rutin content in matcha significantly decreased according to storage temperature and time. This study could be used as basic data to determine optimal storage conditions by measuring physiological changes according to the temperature conditions of matcha.

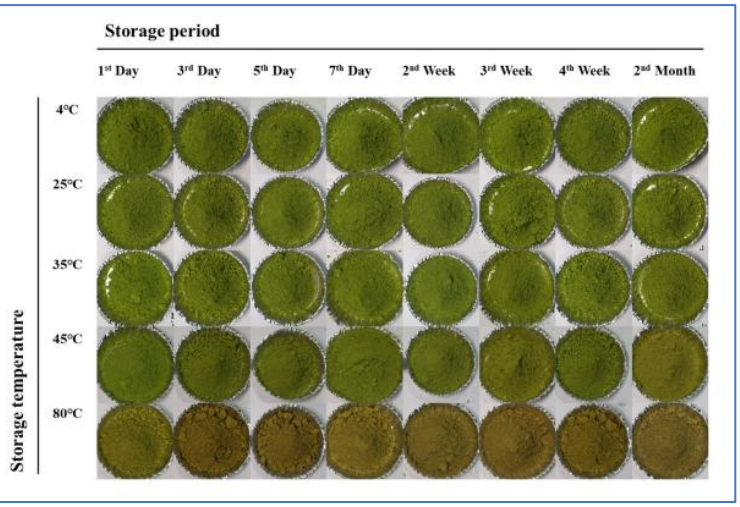


Fig. 1 Changes of the color of matcha in storage temperature and storage time during 2 months

This work was supported by Korea Institute of Planning and Evaluation for Technology in Food, Agriculture and Forestry (IPET) through Agri-Food Export Enhancement Technology Development Program, funded by Ministry of Agriculture, Food and Rural Affairs (MAFRA) (RS-2023-00233418)

Water-based Extraction of Bioactive Principles from Herbal Tea (Hawthorn, Blackcurrant Leaves and *Chrysanthellum americanum*): from Experimental Laboratory Research to Homemade Preparations

Phu Cao Ngoc[1,5], Laurent Leclercq[1*], Jean-Christophe Rossi[1], Isabelle Desvignes[1], Jasmine Hertzog[2,3], Anne-Sylvie Tixier[4], Farid Chemat[4], Philippe Schmitt-Kopplin[2,3], Hervé Cottet[1*]

1. IBMM, University of Montpellier, CNRS, ENSCM, Montpellier, France; 2. Analytical BioGeoChemistry, Helmholtz ZentrumMuenchen, Neuherberg, Germany; 3. Analytical Food Chemistry, TechnischeUniversitätMuenchen, Freising, Germany; 4. University of Avignon, INRA, UMR408, GREEN Extraction Team, Avignon, France; 5. Post-Harvest Technology Department, Northern Mountainous Agriculture and Forestry Science Institute, Phu Tho Province, Vietnam

This work deals with the question of standardization, repeatability and optimization of medicinal plant extraction in water. Three plants were selected, for which the complementary pharmacological activities such as hyaluronidase activity (against skin aging), anti-hypertensive activity (angiotensin-converting enzyme inhibition), anti-oxidant properties, anti-obesity (the pancreatic lipase inhibition) and anti-necrotic, anti-inflammatory, anti-oxidative effects based on different flavonoids were investigated, two of which are well documented (hawthorn flowering tops and blackcurrant leaves) with well-known properties, and the third one has been little studied (*Chrysanthellum americanum*). We established a general extraction protocol in water for these three plants that can be easily used by each of us, based on infusion that can afford a reproducible daily uptake of bioactive components (phenols, flavonoids, proanthocyanidin oligomers) at drinkable temperature. Granulometry was the most important factor to get the best extraction yields (about 22% for hawthorn, 26% for *Chrysanthellum americanum* and 28.5% for blackcurrant). Chemical composition of these plants was investigated by colorimetric methods, and also using performed and complementary analytical instrumentations (UHPLC-ESI-MS and FT-ICR MS). Blackcurrant extracts contained much more phenolic compounds (the main UV-detected components detected in UHPLC being flavonols) than the two other plants. Hawthorn extracts contained much more proanthocyanidin oligomers (the main UV-detected components in UHPLC being flavanols, flavonols and flavones) than the two other plants[1,2]. *Chrysanthellum americanum* and blackcurrant extracts contained similar amounts of flavonoids, the former one containing essentially hydrocinnamic acid derivatives, flavones, flavanones and aurones as UV-detected components. About 2 500 hints were obtained by FT-ICR MS for each plant, among which about 1 100 are common to all 3 plants and about 700 are specific to each plant. Quercetin and kaempferol derivatives were identified in blackcurrant leaves extracts, while vitexin-2-O-rhamnoside, hyperoside and isoquercetin were identified in hawthorn flowering tops extracts and flavanomarein and martitimein derivatives, and Oleanolic or Ursolic acid were identified in *Chrysanthellum americanum* extracts. A significant inhibition of hyaluronidase (≥ 90%) was reported for hawthorn extracts, much higher than that of the other two plant extracts. As for the anti-hypertensive activity, the *Chrysanthellum americanum* extracts demonstrated higher angiotensin-converting enzyme inhibition than the other two plant extracts. Regarding antioxidant activity, blackcurrant leaf extracts showed the highest antioxidant capacity. The pancreatic lipase (PL) inhibition activity by aqueous extracts of these three plants showed that the highest

PL inhibition was obtained by fresh hawthorn leaves extracts (37%±3%). Extract of *Chrysanthellum americanum* only revealed slight PL inhibition (14%±0.1%). The activation of PL of hawthorn flowers extracts as well as blackcurrant leaves extracts was (26%±1%) and (37%±0.1%), respectively (Figure 1).

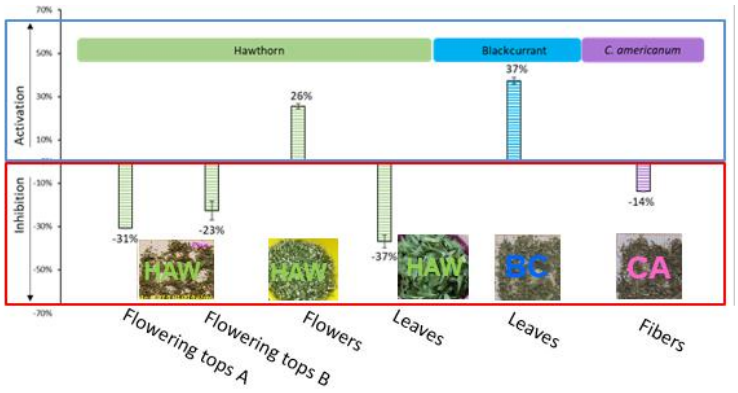


Figure 1 Inhibition and activation of pancreatic lipase activity by aqueous extracts of hawthorn (dry flowering tops, dry flowers, fresh flowers, fresh leaves), dry blackcurrant leaves and dry *Chrysanthellum americanum* at 1 mg/mL

The percentage of the inhibition and activation is represented relative to a control reactions conducted in the absence of the extracts. Error bars indicate the standard deviation on PL activity for n=3 experiments.

Finally, the experiments of anti-necrotic, anti-inflammatory and anti-oxidative effects of hawthorn extracts against colitis in rats were conducted, Colitis induced by trinitrobenzene sulfonic acid, hawthorn extracts tea (daily uptake: 100 kg/1 kg rat) was given via gavage for 21 days (2 weeks preventive uptake, 1 week curative uptake after colitis) and mesalamine drug (daily uptake: 100 mg/1 kg rat) was administrated during the period of disease onset. The results indicated that both hawthorn extracts and mesalamine reduced the disease parameters including: length and proportion of necrotic regions; pro-inflamatory cytokine and myeloperoxidase), it means that hawthorn tea given at 100mg/kg can be effective against colitis (Figure 2).

Figure 2 Apearance of necrotic lesions on the colonic mucosa of rats with colitis

***Correspondence**: herve.cottet@umontpellier.fr (H.C.) and laurent.leclercq@umontpellier.fr (L.L.)

A Novel CsRNF-CsHSF24-*CsF3'5'H1* Module Regulates Trihydroxy Catechins Biosynthesis in *Camellia sinensis*

LI Tong[1], YAO Xinzhuan[1], AN Haili[1], CHEN Hufang[1], LIU Shujun[2], ZHANG Baohui[1], TANG Hu[1], JIANG Yihe[1], JIAO Yujie[1], HE Yumei[1], ZHAO Qi[2], LU Litang[1,2*]

1. College of Tea Science, Guizhou University, Guiyang 550025, China; 2. College of Life Science, The Key Laboratory of Plant Resources Conservation and Germplasm Innovation in the Mountainous Region (Ministry of Education), Guizhou University, Guiyang 550025, China

Tea is rich in tea polyphenols, vitamins, amino acids, and other components that have many benefits to health. Catechins are the main functional components in tea, and it is also the main phenolic compound of tea polyphenols, accounting for about 70% of its total, and 12 to 24% of the dry quality of the tea. Catechins can be divided into dihydroxy catechins (C, EC, ECG) and trihydroxy catechins (GC, EGC, EGCG) based on the hydroxylation pattern of their B-ring. To date, catechins biosynthesis genes have been widely studied, and some transcription factors have been reported to regulate catechins biosynthesis. However, the regulatory mechanism underlying catechins biosynthesis control is not fully understood, especially the role of post-transcriptional modifications of transcription factors in catechin biosynthesis of tea plants remain still largely unknown. In this study, we identified tea plant heat shock transcription factor (CsHSF24) as an important positive regulator of trihydroxy catechins biosynthesis. Specifically, we found that CsHSF24 binds to the promoter of *CsF3'5'H1*, which is a key controller of trihydroxy catechins biosynthesis in tea plants, as its transcriptional activator. In addition, CsRNF, functions as a RING finger E3 ubiquitin ligase to ubiquitinate CsHSF24, leading to its degradation through the 26S proteasome pathway. The degradation of CsHSF24 reduced the expression of *CsF3'5'H1*, thereby inhibiting the biosynthesis of trihydroxy catechins.

Breeding Report of A New Tea Plant Cultivar with Leaf Color Heteromorphosis 'Jinfeng 2' (*Camellia sinensis* L.)

LIU Dongna[1], LI Lanying[1], XU Yaqiong[1], YAO Yu[1],
GONG Xuejiao[1], LUO Sheng[2], LUO Fan[1*]

1. Tea Research Institute, Sichuan Academy of Agricultural Sciences, Chengdu 610066, China；
2. Sichuan Academy of Agricultural Sciences, Chengdu 610066, China

In recent years, the emergence of well-known brands such as "Anji white tea" and "Guangyuan yellow tea", which are made of special yellow varieties of tea plants, has met the diversified needs of the market consumers, therefore, Leaf color-specific tea germplasm resources have become the focus of attention (Wang Xinchao et al., 2022).'Jinfeng 2', a new cultivar of *Camellia sinensis*, was selected and bred from the bud mutation of small and medium-leaf tea plant species in Zonggang Mountain, Mingshan County, Ya'an City, Sichuan Province by individual selection and systematic breeding. 'Jinfeng 2' obtained the registration certificate of non-major crop varieties issued by the Ministry of Agriculture and rural areas in August 2022. In this study, the characteristics of 'Jinfeng 2' were introduced, it includes plant morphological characteristics, field survival rate, tea seedling growth potential, phenological period, germination density, fresh leaf yield, main biochemical components, tea quality and resistance.

Materials and Methods

The tested tea variety is bred from a plant of wild population in Zonggang Mountain of Ya'an, Sichuan. The control variety are Fuding dabai tea (CK1) and Zhonghuang 1(CK2), two national varieties (*Camellia sinensis* L.).

Randomized plot design was adopted. The plant morphological characteristics, planting survival rate, new shoot growth, shooting density, yield, biochemical composition, sensory quality, adaptability and resistance of both the tested and control tea varieties were observed and identified.

Among the main biochemical components, the contents of water extract, tea polyphenols, free amino acids and caffeine were determined by the national standard methods; the content of soluble sugar was determined by anthrone colorimetry; and the major components of catechins were detected by high performance liquid chromatography (Phenomenex RP-MAX 4 μm, 250 mm × 4.6 mm, C12 reverse phase column).

Results and Analysis

Plant Morphological Characteristics

'Jinfeng 2' is shrubs with no distinguishable trunk, low branching position, half-opened plant type and medium branching. the survival rates of 'Jinfeng 2' were both higher than 93%, indicating that it had a strong adaptability and suitable to be cultivated in Sichuan Province and other areas with similar climate.

The leaves of 'Jinfeng 2' are narrow oval with length of 9.3 cm, width of 3.7 cm, belonging to middle leaf type. The color of new shoots is yellow green, the color of mature leaf is yellow green interlaced. 'Jinfeng 2'sprouts extremely early and neatly, which sprouting period is generally middle February, the peak period of one bud and two leaves is generally early March (Table 1). A bud and three leaves is 8.1 cm long, and weighs 41.74 grams per hundred shoots. The flowering period is generally middle of October,

and flowers are characterized by small corolla, white petal color, ovary fuzz, high style split position, and higher pistil than stamens (Figure 1).

Table 1 Phenology period of new shoots of the tested tea varieties

Survey time	Variety	Sprouting	One-leaf	Two-leaf	Three-leaf	Ending date of shooting
2020 year	Jinfeng 2	February 15	March 10	March 19	March 28	October 18
	Fuding dabai tea	February 27	March 19	March 24	April 7	October 25
	Zhonghuang	February 27	March 19	March 21	April 4	October 25
2021 year	Jinfeng 2	February 9	February 28	March 11	March 22	October 20
	Fuding dabai tea	February 25	March 17	March 20	April 2	October 28
	Zhonghuang	February 20	March 11	March 17	March 28	October 28

Figure 1 A new *camellia sinensis* cultivar 'Jinfeng 2'

Fresh Leaf Yield and Tea Quality

The fresh leaf yield of 'Jinfeng 2' was 302.24-310.93 kg, which was more than 11.50%-14.61% higher compared with that of Zhonghuang 1 (263.72-278.85 kg) , and lower than 23.91%-24.72% compared with that of Fuding dabai tea (401.50-408.63 kg), indicating that 'Jinfeng 2' has high yielding potential of fresh leaves.

'Jinfeng 2' is suitable for manufacturing green tea， black tea and yellow tea. The green tea has high aroma， flower fragrance， the taste is fresh and mellow. The total scores of aroma and taste quality of 'Jinfeng 2' were equivalent with 'Zhonghuang 1', and higher than those of Fuding dabai tea by 5.0 and 4.0 points.

Analysis on Main Biochemical Components

The water extract content of 'Jinfeng 2' was equivalent, and the tea polyphenols, free amino acids, caffeine, soluble sugar and water extract contents were 20.30%, 4.40%, 4.30%, 2.70% and 51.00%, respectively. The predominant catechin content of 'Jinfeng 2' were GC (0.04%) , EGC (2.72%), C (2.58%), EGCG (7.86%) , EC (1.06%), ECG (1.651%), respectively. This indicated that 'Jinfeng 2' has high intrinsic biochemical quality.

Table 2 Biochemical analysis of fresh leaves of the tested varieties

%

Variety	Tea polyphenols	Amino acids	Caffeine	Water extract	Soluble sugar
Jinfeng 2	15.92	4.94	5.11	51.92	3.43
Fuding dabai tea (CK1)	21.76	3.65	3.04	51.49	3.34
Zhonghuang 1(CK2)	18.50	4.50	4.72	50.50	3.60

Table 3 Detection results of catechins and components in the tested varieties

%

Variety	GC	EGC	C	EGCG	EC	ECG	Catechins
Jinfeng 2	0.04	2.72	2.58	7.86	1.06	1.65	15.86
Fuding dabai tea (CK1)	0.05	0.97	2.61	7.13	0.76	1.89	13.36
Zhonghuang 1 (CK2)	0.06	1.05	2.34	5.26	1.47	3.72	13.82

Characterization of Nitrate use Efficiency in Tea Plant based on Leaf Chlorate Sensitivity

ZHANG Wenjing, LONG Lizhi , NI Kang, MA Lifeng, RUAN Jianyun

Key Laboratory of Tea Plant Biology and Resources Utilization, Ministry of Agriculture,
Tea Research Institute, Chinese Academy of Agricultural Sciences, Hangzhou 310008, China

Introduction

Nitrate functions as a crucial form of inorganic nitrogen in soils, stimulating plant growth and metabolism. Nitrate (NO_3^-) is the primary nitrogen form that's lost through leaching in tea gardens. Therefore, enhancing its utilization efficiency is a critical step towards achieving sustainable tea production. Chlorate (ClO_3^-), as an analogue to nitrate, can be transported and reduced via the nitrate-pathway within plants. In this study, we employed a leaf chlorate sensitivity test to evaluate nitrate utilization efficiency across different tea varieties. Moreover, we examined the absorption and utilization of nitrate, along with its molecular regulatory mechanisms, among these varieties. The findings provide a theoretical foundation for identifying nitrate-use-efficiency phenotypes in tea plants and a precise breeding methodology.

Materials and Methods

Fresh branches of ten tea cultivars were hydroponically cultivated. The nutrient solution consisted of P 0.05 mmol/L, K 1.05 mmol/L, Mg 0.2 mmol/L, Ca 0.4 mmol/L, S 0.2 mmol/L, Fe 3.15 μmol/L, B 5 μmol/L, Mn 0.75 μmol/L, Zn 0.5 μmol/L, Cu 0.1 μmol/L, Mo 0.25 μmol/L. In addition, KNO_3 and $KClO_3$ were used with the concentration of 1 mmol/L in the control group and the treatment group, respectively. The culture period was five days, and daily measurements of SPAD and Fv/Fm were taken at a fixed foliar position. Differential expressed genes were screened based on transcriptional sequencing of leaves from two varieties ZN117 and TGY, which showed the most significant differences in chlorate sensitivity.

Results

Using Chlorate Sensitivity Test to Screen NO3- Utilization Efficiency

Tea branch of ten cultivars were cultivated hydroponically. The high-sensitivity variety, ZN117, and the low-sensitivity variety, TGY, were selected based on the time-course response of F_v/F_m. After five days of treatment, ZN117 showed signs of yellowing and wilting leaves, while TGY's leaves showed no obvious change (Figure 1A, 1B, 1C). The transpiration rate of the treatment group was lower than the control group, but there was no significant difference between ZN117 and TGY (Figure 1D). The SPAD and F_v/F_m values for ZN117 significantly decreased after three days of chlorate treatment, compared to values at day zero (Figure 1E, 1F). On the other hand, TGY showed no significant changes in SPAD values after five days of nitrate and chlorate treatment. The $^{15}NO_3^-$ isotope labelling experiment showed no significant difference in Ndff% between TGY and ZN117 (Figure 1G). However, the ^{15}N concentration in new buds of ZN117 was significantly higher than TGY (Figure 1H). Consequently, the proportion of ^{15}N in new buds of ZN117 was significantly higher than TGY, whereas the stem ^{15}N proportion in ZN117 was significantly lower than TGY (Figure 1I). This indicates that the nitrate utilization efficiency of ZN117 was higher than TGY. In conclusion,

genotypic differences in nitrate utilization efficiency between TGY and ZN117 were identified based on chlorate sensitivity testing. The critical stage of difference occurred after one day of chlorate treatment.

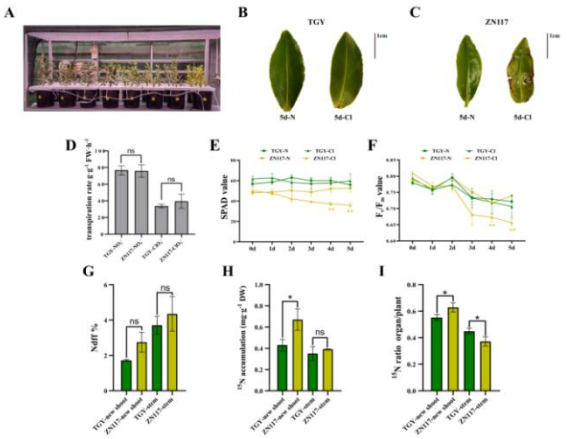


Figure 1 Growth and physiological responses of TGY and ZN117 after NO_3^- and ClO_3^- treatments

Differential Nitrate Transport and Utilization in Two Contrasting Tea Varieties: A Transcriptome Analysis

Transcriptome analysis was performed at five time points (0 h, 6 h, 12 h, 24 h, 72 h) under each treatment in two contrasting tea varieties. In ZN117, the expression of nitrate transporters *NRT2.4, NRT3.1, NPF1.10, NPF1.11, NPF4.6,* and *NPF6.1* significantly increased after nitrate treatment, while no change was observed in TGY. The upregulation of *NR* in ZN117 was more pronounced than in TGY. After 6 h nitrate treatment, *Fd-NiR* expression in ZN117 increased 4.48-fold and further increased to 5.64-fold after three days. This indicates that ZN117 has a more robust capacity for NO_3^- reduction than TGY. Similar changes were observed under chlorate treatment. Exogenous NO_3^- triggers the upregulation of the *bHLH* transcription factor which then regulates *NRT2.4, NRT3.1, NPF6.1, NPF4.6* to mediate NO_3^- transport to mature leaves, and *NPF1.10, NPF1.11* for transporting NO_3^- to new shoots for reduction and assimilation into ammonia. Concurrently, genes involved in chlorophyll synthesis (*HemA, CHLE, PAO, CAO*) were upregulated, and the chlorophyll degradation gene *CLH* was downregulated, thereby promoting chlorophyll synthesis for photosynthesis and supplying energy for plant growth. Based on the results, a regulatory model for NO_3^- transport and utilization in tea plants was proposed (Figure 2).

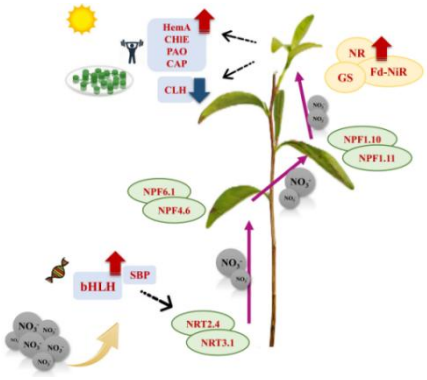


Figure 2 The model of NO_3^- transport and utilization in tea plants

Combined Multi-omics Approach to Analyze the Flavor Characteristics and Formation Mechanism of Gabaron Green Tea

HUANG Dongzhu[1], ZHENG Dongqiao[1], SUN Chenyi[1], FU Maoyin[1], WU Yuhan[1], WANG Hong[2], YU Jieyao[1], YANG Yunqiu[1], LI Yeyun[1], WAN Xiaochun[1], CHEN Qi[1,2*]

1. State Key Laboratory of Tea Plant Biology and Utilization, School of Tea and Food Science & Technology, Anhui Agricultural University, Hefei 230036, China; 2. Key Laboratory of Food Nutrition and Safety, Anhui Engineering Laboratory for Agro-products Processing, School of Tea and Food Science & Technology, Anhui Agricultural University, Hefei 230036, China

Introduction

As a unique tea with a high content of γ-aminobutyric acid (GABA) through specific processing, gabaron tea has gained much attention for its distinctive flavor and health benefits. The methods of industrialized production of gabaron tea have been developed quite maturely, among which the method of enriching the GABA in tea by anaerobic treatment is the most widely used. At present, most of the studies on gabaron tea have focused on its potential health-care functions or dynamic changes in the metabolic pathways of the tea plant due to its special processing, and there are few reports on its flavor. Therefore, in this study, combined multi-omics approach was applied to reveal the flavor characteristics and formation mechanisms of gabaron green tea (GAGT) under different degrees of N_2-filling anaerobic treatment.

Results and Discussion

In this study, the aroma extracts of six dried GAGTs which processing under different degrees of N_2-filling anaerobic treatment were extracted by SE-SPE and analyzed by GC-MS accompanied with two chromatographic columns with different polarities. A total of 103 aroma compounds were identified. Thirty-one differential volatile metabolites in GAGT were identified through nontargeted metabolomics, while thirteen odor-active compounds were regarded as the key contributor to the overall aroma characteristics of GAGT by applying of molecular sensory science. Benzeneacetaldehyde, nonanal, geraniol, linalool and linalool oxide III were further screened out as target metabolites by integrating the anaerobic stress response and aroma contribution. Differential and correlation analyses were performed on the transcriptome data, and some special CsERFs were screened out for which might be involved in mediating ethylene synthesis response to anaerobic stress and thus regulating the GABA accumulation and the targeted volatiles metabolism in GAGT.

Based on multi-omics research and analysis methods, this study revealed the physiological mechanism of GABA accumulation and aroma characteristics change of gabaron green tea, and proposed for the first time that ethylene may be involved in tea aroma formation and GABA metabolism in response to anaerobic stress.

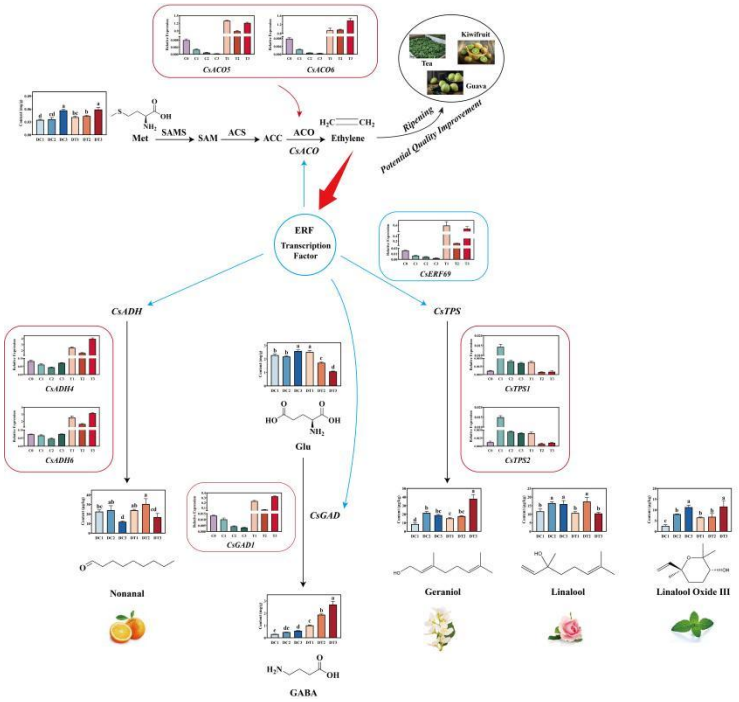


Figure 1 Model of regulation of gamma-aminobutyric acid accumulation and volatiles metabolism by ethylene in response to anaerobic stress in GAGT

Differences in Flavonoid Metabolism between *Camellia sinensis* and Lithocarpus Polystachyus

YANG Yusi, LU Jianliang*

Tea Research Institute, Zhejiang University, Hangzhou 310058, China

Flavonoids are a group of polyphenolic compounds ubiquitous in the plant kingdom, contributing to the health benefits of fruits, vegetables, and medicinal plants mainly by their antioxidant activities. They are generally classified into seven subclasses: flavonols, flavones, isoflavones, anthocyanidins, flavanones, flavanols, and chalcones. In addition to the aforementioned flavonoids, dihydrochalcones (DHCs), first identified from the bark of Malus domestica, are a kind of non-classic flavonoid widely distributed in the *Malus* species.

Polyphenol profiles are one of the characteristic features of different plant species and the mechanism of the diversities in the pattern of flavonoid accumulation in different plants has received great attention. Tea (*Camellia sinensis*) and sweet tea (*Lithocarpus polystachyus*) are two beverage plants with distinct tastes and flavors due to their different polyphenol composition, and the former tastes astringent, bitter, and umami, while the latter has a sweet taste. Catechins, caffeine, and L-theanine collectively contribute to the astringency, bitterness, and umami taste of tea infusion. Tea polyphenols, accounting for 18%-36% of the dry weight, are dominated by catechins, with (−)-epigallocatechin gallate (EGCG) taking up half of the total. In addition to catechins, flavonols, anthocyanins, and phenolic acids account for 3%, 0.01%, and 5% of the dry weight, respectively. In the current view, epicatechins, and their gallates are the principal bitter and astringent compounds of tea. *L. polystachyus*, also known as sweet tea due to its evident sweet taste, is consumed mainly restricted to the southwest of China and is less known by the public than *Camellia tea*. The polyphenol profiles of sweet tea are dominated by phloridzin and trilobatin, glycosides of phloritin, accounting for 21%-32% of the dry weight and the sweetness of trilobatin is 300 times that of sucrose.

In the old viewpoint, DHCs were exclusively biosynthesized in the *Malus* species. However, the polyphenol profiles of apples are dominated by flavonols, corresponding to 71%-90% of the polyphenolic compounds, whereas dihydrochalcones are merely minor components, accounting for 0.5-6% of total polyphenols. DHCs have been identified in more and more species nowadays. In species like *Fragaria x ananassa*, *Glycine max*, *Eleutherococcus senticosus*, *Prunus persica* and *Lactuca sativa*, phlorizin has been detected in trace amounts. In addition to the above species, DHCs have been found in high contents in medicinal plants used to make tea in the folk that has a sweet flavor, like *Docynia dcne*, and *Aspalathus linearis*. The detection of DHCs in many species indicates a common existence of the DHC biosynthetic pathway in the plant kingdom and efforts have been made to characterize key genes that direct the metabolic flux to DHC biosynthesis in DHC-rich plants. As shown in Fig 1, in the current view, phloretin, one of the DHCs, can be produced through a chalcone synthase (CHS) catalyzed reaction in which three molecules of malonyl-CoA are cyclized with one molecule of p-dihydrocoumaroyl-CoA that is previously generated from p-coumaroyl-CoA by catalysis of double bond reductases (DBRs). However, conflicting results have been achieved in the published work concerning the formation of p-dihydrocoumaroyl-CoA from p-coumaroyl-CoA, a process that happens whether directly or through some intermediates and the ability of DBRs to directly catalyze p-coumaroyl-CoA has been questioned.

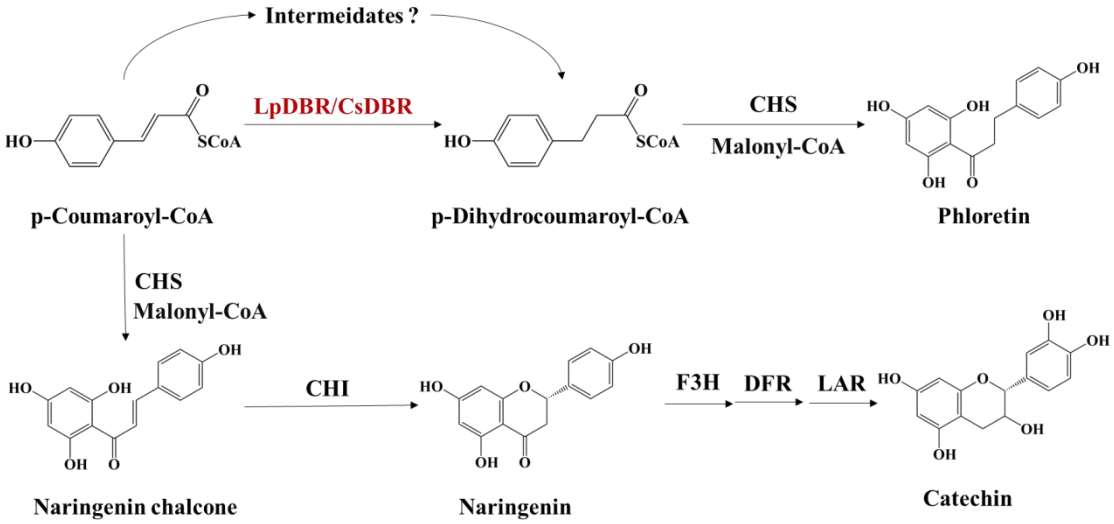


Figure 1 Biosynthetic pathway of major flavonoids from sweet tea and tea plant

In summary, both *C. sinensis* and *L. polystachyus* are medicinal plants with health-promoting functions and show distinct tastes due to different polyphenol profiles.

Acknowledgements

This work was financially supported by the National Natural Science Foundation of China (No. 31770728), the Zhejiang Science and Technology Major Program on Agricultural New Variety Breeding-Tea Plant (No. 2021C02067-6).

***Correspondence**: jllu@zju.edu.cn

Differentiation of Stress Resistance and Metabolism between *Camellia sinensis* cv. *Longjing 43* and Light-sensitive *Zhonghuang 1* in Response to High-light exposure

GE Shibei, ZHANG Zheng, YAN Peng, LI Zhengzhen, ZHANG Lan, HAN Wenyan, SHI Yuanzhi*, LI Xin*

Key Laboratory of Tea Quality and Safety Control, Ministry of Agriculture and Rural Affairs, Tea Research Institute, Chinese Academy of Agricultural Sciences, Hangzhou 310008, China

Abstract

Light is an important environmental factor for plants growth. However, tea plants are shade-requiring plants and the resistance to high light exposure varies with different tea varieties. 'Zhonghuang 1' is a novel chlorotic tea cultivar with bright yellow leaves, excellent quality, and special sensitivity to light. However, there is no in-depth study on the differences between green-leaf tea cultivars and chlorotic tea cultivars in terms of stress resistance and metabolism. Here, 'Longjing 43' (LJ) and 'Zhonghuang 1' (ZH) plants were exposed to normal light (NL) and high light (HL) for five hours. The results showed that LJ was more resistant to high-light exposure than ZH. The quality of tender leaves in ZH decreased sharply after high-light exposure, but LJ was almost unaffected. In addition, metabonomics analysis showed that there were obvious differences in amino acids and flavonoids metabolism between NL and HL treatments in LJ and ZH. This study revealed the stress response and metabolic changes of LJ and ZH tea plants under high-light exposure, which provided a basis for exploring the resistance of chlorotic tea varieties and the photoprotection mechanism of tea plants.

Results

LJ was More Resistant to High-light Exposure than ZH

After 5 hours of high light exposure, there were significant differences between LJ and ZH. Compared with LJ, ZH accumulated more lignin in mature leaves under high-light exposure (Figure 1b). There were withered leaves in tender leaves of ZH, while only a few black spots in LJ (Figure 1c). In addition, the MDA content in tender leaves of ZH was increased more than that in tender leaves of LJ (Figure 1d).

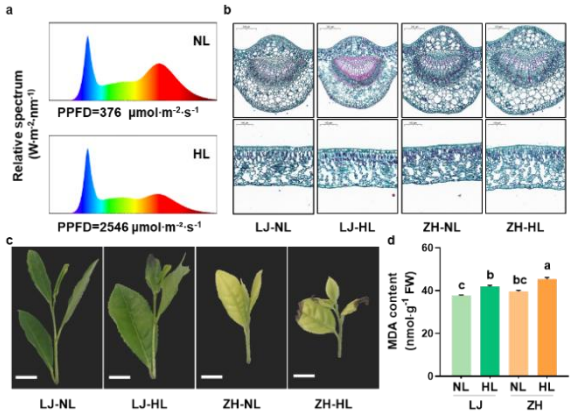


Figure 1 Plant phenotype and stress response of 'Longjing 43' (LJ) and 'Zhonghuang 1' (ZH) under normal light (NL) and high light (HL) conditions

(a) Relative spectrum of NL and HL. (b) Cross-section microstructure of mature leaves. (c) Phenotypes of tender leaves. (d) MDA content of tender leaves.

The Photoprotection Mechanism was Rapidly Started in LJ than that in ZH under High-light Stress

NPQ is an important photoprotection mechanism in plants. After 1.5 h of light treatment, the NPQ of LJ in HL was increased compared with NL. In contrast, the NPQ of ZH decreased in HL (Figure 2a). *PGR5* and *VDE* are positively related to NPQ, while ZEP plays negative functions in NPQ. After high light exposure, the expression of PGR5 and VDE was increased in LJ but decreased in ZH (Figure 2b-c). In contrast, the expression of ZEP was decreased in LJ but increased in ZH (Figure 2d).

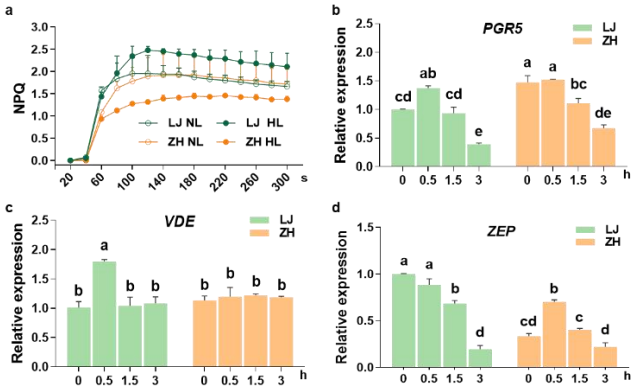


Figure 2 Photoprotection mechanism in tender leaves of 'Longjing 43' (LJ) and 'Zhonghuang 1' (ZH) tea plants under normal light (NL) and high light (HL)

(a) Non-photochemical quenching (NPQ) in tender (d) leaves. (b-d) relative expression of *PGR5, ZEP,* and *VDE* in tender leaves.

The Difference in Metabolic Response between LJ and ZH under High Light Exposure

Tea polyphenols and amino acids are the most important metabolic products in tea, and the ratio of tea polyphenols and amino acids could indicate the quality of tea. After high-light exposure, the quality of tender leaves in ZH significantly decreased while there was almost no difference in LJ (Figure 3a-c).

According to the metabonomics, metabolites such as amino acids and peptides, flavonoids and monosaccharides, were enrichment both in LJ and in ZH (Figure 3d-e).

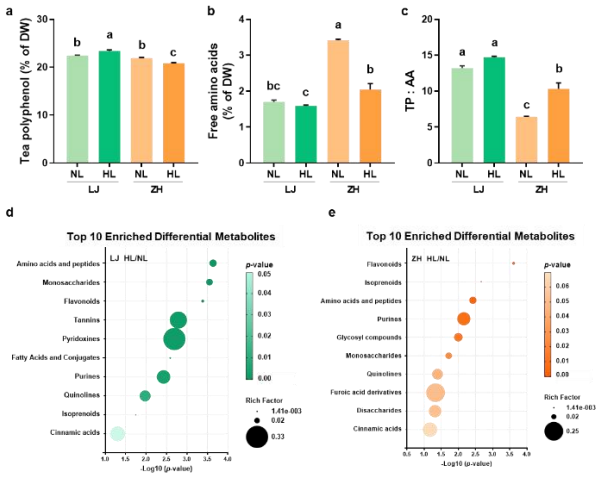


Figure 3 Quality components and enrichment metabolites in tender leaves of Longjing 43 (LJ) and Zhonghuang 1 (ZH) tea plants under normal light (NL) and high light (HL)

(a-b) The contents of tea polyphenol and free amino acids in tender leaves. (c) The ratio of tea polyphenol and free amino acids (TP: AA) in tender leaves. (d) Top 10 enriched differential metabolites in tender leaves of LJ. (e) Top 10 enriched differential metabolites in tender leaves of ZH.

***Correspondence**: shiyuanzhi@tricaas.com, lixin@tricaas.com

Excavation of Genes Related to Root Development and Theanine Metabolism of Tea Seedlings

FU Maoyin[1], GAO Yang[1], ZHENG Dongqiao[1], HUANG Dongzhu[1],
WAN Xiaochun[1], CHEN Qi[1,2*]

1. State Key Laboratory of Tea Plant Biology and Utilization, School of Tea and Food Science & Technology, Anhui Agricultural University, Hefei 230036, China; 2. College of Life Science and Health, University of Science and Technology, Wuhan 430081, China

Tea seed seedlings have developed taproot, strong vitality and resistance, can adapt to diverse environments, and can produce heritable variation, which is conducive to biological evolution. However, the genes related to root development during the growth and development of tea seed seedlings are still unknown. In this paper, Weighted Gene Coexpression Network Analysis was conducted on the transcriptome data of tea seed seedlings at the stage of development and on rhiza, stems, leaves and other organs, and four modules related to high root development were found, and the co-expression network of tea seed seedlings was constructed. Through trend analysis, 8 transcription factors that may be related to root development and consistent with theanine synthetase expression trend were selected. Through the above analysis, we hope to provide reference for the root growth and development of tea seed seedlings and theanine metabolism.

Materials and Methods

The transcriptome data of tea seed seedling during the development period were analyzed by using R packet WGCNA et al. Trend analysis using the Mfuzz package.

Results and analysis

Construction of Weighted Gene Coexpression Network Analysis in Rhizome and Stem Leaves of Tea Seedlings during their Development

In this paper, WGCNA analysis was performed on the transcriptome data (unpublished) and root (CsR) stem (CsST) leaf (CsL) tissues of tea seed seedlings during their development, and dynamic shear tree algorithm was used to identify gene modules, which were divided into 44 modules, as shown in Figure 1. Blue, yellow, purple, turquoise, four modules and the roots are related, and the correlation is greater than 0.6, salmon, module showed high negative correlation with root, correlation was 0.6. Among them, salmon and red modules were highly positively correlated with stem development, and the correlation was 0.68 and 0.69, respectively, while yelow modules were negatively correlated with stem development, and the correlation was 0.63. The module with positive correlation with leaf part (greenyellow) has the highest correlation and is only 0.57, while the blue module has a high negative correlation with leaf part (0.68). Through clustering analysis of modules and tissue parts, it was found that the roots were closest to blue and purple modules, the leaves were closest to darkturquiose,black and greenyellow, and the stems were closest to red and salmon clusters. As shown in Figure 1B, in order to explore the relationship between root height of tea seed seedlings and gene transcription, we constructed a co-expression network for the above modules related to root height of tea seed seedlings.

Analysis of the Expression Trend of Genes Highly Related to Root Development in the Module

In order to explore which genes are involved in the regulation of root growth and development, we conducted trend analysis on the genes in these four modules that are positively correlated with root height (Figure 2A), divided into 8 clusters, each representing an expression pattern. As is well known, theanine is mainly synthesized in the roots. In these modules, we found some genes related to theanine synthesis, such as *CsTSI*. By analyzing the expression patterns of TFs in these clusters (Figure 2B), a total of 8 TFs were found to be highly consistent with *CsTSI* expression patterns and have relatively high expression levels.

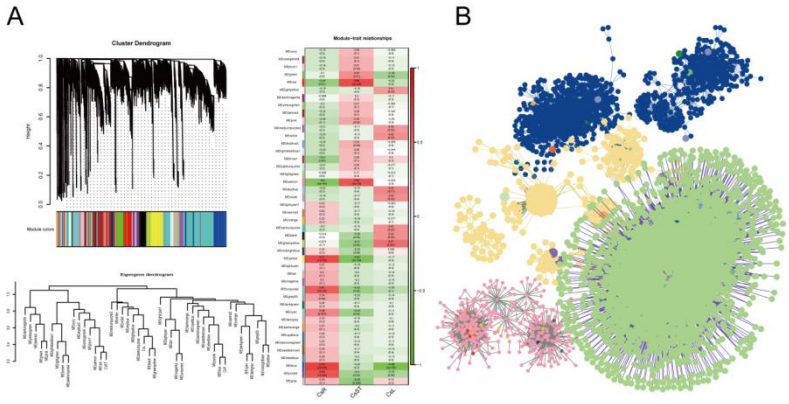


Figure 1 Construction of gene co-expression network

A: WGCNA analysis of all genes and roots, stems, and leaves during the development stage of tea seedlings, as well as cluster analysis of modules and roots, stems, and leaves; B: The co expression network of four modules, blue, yellow, purple, and turquoise, which are highly correlated with the root. The colors of blue, yellow, purple, and turquoise are consistent with the module name color, while other colors represent TFs.

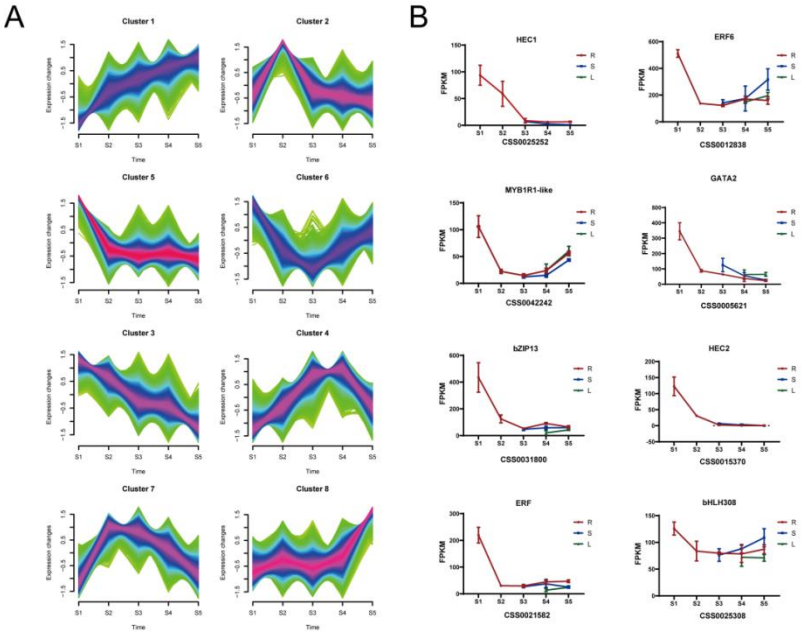


Figure 2 Expression trend analysis

A: Trend analysis of four module genes based on fuzzy clustering algorithm; B: TFs that are consistent with *CsTSI* trends in trend analysis.

Exogenous Activation of the Ethylene Signaling Pathway Enhances the Freezing Tolerance of Young Tea Shoots by Regulating the Plant's Antioxidant System

CHEN Yao[1,2], TANG Junwei[1], REN Hengze[1], LI Yuteng[1], LI Congcong[1], WANG Haoqian[1], WANG Lu[1], YANG Yajun[1], HAO Xinyuan[1*], WANG Xinchao[1]

1. National Center for Tea Plant Improvement, Tea Research Institute, Chinese Academy of Agricultural Sciences/Key Laboratory of Biology, Hangzhou 310008, China;
2. College of Food Science, Southwest University, Chongqing 400715, China

Tea plants (*Camellia sinensis* (L.) O. Kuntze) frequently suffer severe damage caused by cold spells in early spring, which severely affect tea quality and production in China. Emerging evidence has demonstrated that the ethylene signalling pathway plays important roles in tea cold responses. Here, we investigated the effects of ethylene signalling on freezing tolerance of tea sprouting shoots by promoting and inhibiting ethylene synthesis and perception. We observed that freezing resistance of tea plants correlated with high ethephon levels. Moreover, the ethephon-induced increase in freezing tolerance was associated with the activation of antioxidant enzymes. These results help us to further clarify the mechanism at play in ethylene-induced freezing tolerance of tea plants and provide a way to anticipate the threat to tea production and quality posed by spring cold spells.

Materials and Methods

First, we recorded the changes in climatic conditions during a typical cold spell and the damage symptoms caused by the cold spell in different tea cultivars and breeding lines. By simulating the low temperature of a cold spell under controlled conditions, comparative transcriptome and metabolic analyses were performed with sprouting shoots. Second, we verified that measurement of relative electrical conductivity in young shoots after one hour at -5 ℃ is a rapid way to evaluate cold tolerance in the laboratory. Furthermore, we investigated the effects of ethylene signalling on cold tolerance of tea sprouting shoots by promoting and inhibiting ethylene synthesis and perception.

Results

Exogenous ethylene application played a positive role in response to a cold spell in sprouting shoots of tea plants. Cold tolerance of tea shoots can be enhanced by alleviating cell injury, increasing antioxidant activities, and altering ethylene signalling pathway-related gene expression. Therefore, our study provides a sound theoretical basis for the development of a practical measure that tea farmers may use to effectively protect their tea plantations from cold injury by enabling tea plants to respond rapidly to an early spring cold-spell.

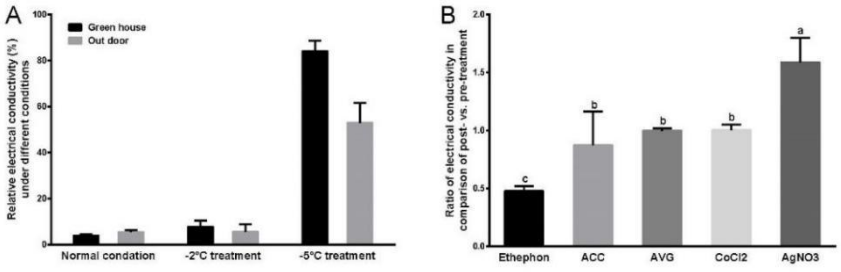


Figure 1 Relative electrical conductivity (REC) detection

(A) REC of tea shoots under different treatments. (B) Effects of an ethylene enhancer (ethephon, ACC) or inhibitor (AVG, $CoCl_2$ or $AgNO_3$) treatment on REC of tea shoots.

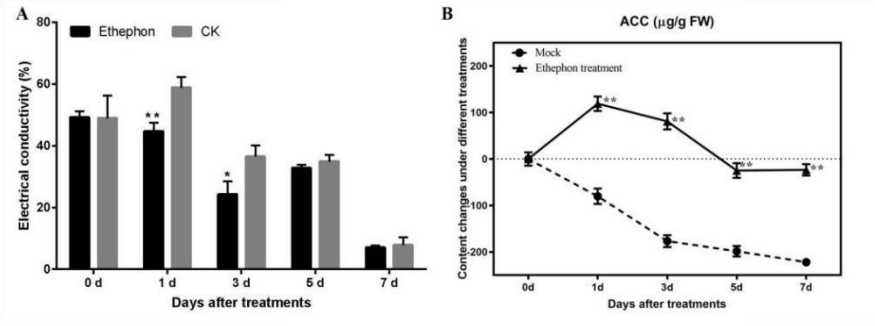


Figure 2 Relative electrical conductivity detection and ACC content measurement of tea shoots under ethephon treatment

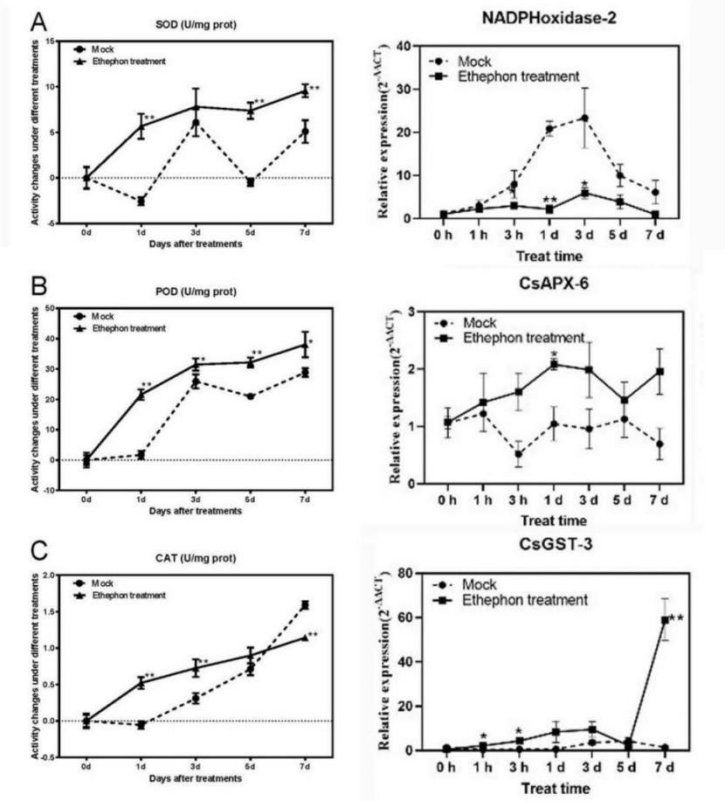


Figure 3 Antioxidant enzyme activity and genes expression in tea plants at the indicated times after ethephon treatment for different time

Exploring the Functional Components and Health Effects of Hawk Tea

ZENG Liang[1,2,3]

1. College of Food Science, Southwest University, Chongqing 400715, China; 2. Chongqing Key Laboratory of Speciality Food Co-Built by Sichuan and Chongqing, Southwest University, Chongqing 400715, China; 3. Integrative Science Center of Germplasm Creation in Western China (Chongqing) Science City/College of Food Science, Southwest University, Chongqing 400715, China

Hawk tea, also known as Laoyin tea, belongs to the evergreen tree of the camphor family. Its scientific name is Litsea coreana var. *lanuginose*, which is mainly distributed in Sichuan, Chongqing, Guizhou, Fujian, Yunnan and other regions. It has been recorded by the Compendium of Materia Medica as having the effect of lowering blood glucose and lipids for hundreds of years. At the same time, modern pharmacological studies have shown that eagle tea has the properties of lowering blood lipids, lowering blood sugar and anti-inflammatory. From this point of view, the material basis of Hawk tea's exercise of health effects and the mining of other health effects have aroused our great interest.

First, we investigated the chemical composition, sensory quality, antioxidant activity and potential liver protective effects of decocted (PFHT), sun-dried (SDHT) and fermented (FHT) Hawk tea (improving acute alcoholic liver injury model in mice). The results showed that Hawk tea mainly contained flavonoids and phenols, followed by polysaccharides. It not only has high antioxidant activity, but also can increase the tolerance of mice to acute alcohol exposure and reduce the liver damage caused by acute alcohol intake. Based on this, 191 flavonoid molecules of eagle tea were extracted and identified, among which Reynoutrin, Avululin, Guaijaverin, Cynaroside and kaempferol-7-o-Glucoside had the highest content. Considering the bioavailability and sorting, the highest contribution of guaiac flavonoids was 0.016 mg/ tree. Secondly, gavage experiments in mice showed that Hawk tea flavonoids reduced liver lipid deposition (inhibition of TG, TC and LDL-C) by reducing oxidative stress-mediated inflammation in the liver (up-regulation of NRF2/HO-1 and down-regulation of TLR4/MyD88/NF-kB pathway) and remodeling intestinal microbiota (increase of Lactobacillus, bifidobacterium and Bacillus). Subsequently, we will separate and purify the polysaccharide from Hawk tea, and systematically study its physicochemical properties, antioxidant activity, structural analysis, *in vitro* simulated digestion and glycolysis characteristics, anti-alcohol and brain effects and liver protection activities.

Acknowledgement

This project is supported by the Eagle Tea Processing and Deep Processing Technology Integrated Innovation and Demonstration (2021-2), Eagle Tea Tea Garden Quality Improvement Technology Promotion (2022-3) project fund.

Gene-pair Acted in JA Signaling Pathway from Tea Plant (*C. sinensis*): 12-oxophytodienoate Reductase (OPR)/ lncRNA (OPRL)

JIANG Ting[1], JIAO Tianming[2], LI Tongtong[1], LIU Cheng[1], LIU Yajun[1], JIANG Xiaolan[1], XIA Tao[2,3*], GAO Liping[1,3*]

1. School of Life Science, Anhui Agricultural University, Hefei 230036, China; 2. State Key Laboratory of Tea Plant Biology and Utilization / Key Laboratory of Tea Biology and Tea Processing of Ministry of Agriculture; 3. Anhui Provincial Laboratory of Tea Plant Biology and Utilization, Anhui Agricultural University, Hefei 230036, China

Long non-coding RNAs (lncRNAs) have attracted substantial attention owing to their important role in plant growth and development as well as biotic and abiotic stress resistance. It is difficult to manipulate gene expression in *Camellia sinensis* (*Cs*) (tea plant) through modern molecular biology methods, and the functions of lncRNAs in this plant species remain elusive. In this study, we designed a strategy to screen for trans-functionally conserved lncRNAs (trans-FC lncRNAs) in plants using highly consistent base sequences in the exon overlapping region between trans-lncRNAs and their target genes. trans-FC lncRNAs were found to be involved in the growth, development, and disease resistance of plants. Several pairs of 12-oxophytodienoate reductase (*OPR*) and its lncRNAs (*OPRL*s) were identified from *Cs*, *Arabidopsis*, alfalfa, potato, and rice. From the perspective of evolution, the *OPRL* gene cluster of each species should originate from a replication event of the *OPR* gene cluster. Gene manipulation and gene expression analysis revealed that *CsOPRL* affected disease resistance by participating in the positive feedback function of regulating jasmonic acid biosynthesis induced by MeJA treatment in tea plants. Knockout of *OPRL1* in *Solanum tuberosum* resulted in abnormal growth characteristics and strong resistance to fungal infection. Altogether, this study suggests a strategy for the screening and functional verification of trans-FC lncRNAs.

Materials and Methods

Camellia sinensis var. *sinensis* (*CSS)* cultivar "Zhongcha 108" (ZC108) was used in this study. It was cultivated in the tea experimental field of Anhui Agricultural University (East longitude, 117.27; North longitude, 31.86). Using a Camellia Co Camelliae pathogenic isolate TYDY-2 infects tea trees. Methyl jasmonate (MeJA), and diethyldithiocarbamic acid (DIECA, an inhibitor of JA synthesis) were purchased from Hefei Yili Biotechnology Co., Ltd.

Results and Analysis

According to the phylogenetic tree, the OPR family in algae, gymnosperms, and angiosperms was divided into seven subgroups (Figure 1A). The seven OPR genes found in tea plants were distributed in the first and second subgroups. The results of chromosome mapping of OPRs and OPRLs found in tea plants, rice, *Arabidopsis*, alfalfa, and potato are shown in Figure 1B. The seven OPRs of tea plants were found to be distributed on three chromosomes, with four of them (*CsOPR9-1*, *CsOPR9-2*, *CsOPR9-3* and *CsOPR9-4*) being distributed on chromosome 9 in tandem. Similarly, 1–3 tandem OPR gene clusters were found in other plants. The target OPRs of OPRLs were distributed in the first (dicotyledonous plants) or the third (monocotyledonous plants) subgroup and existed in the form of a gene cluster.

Based on the abovementioned results, we speculate that the *OPRL* cluster originates from a pseudogene generated during the replication of the *OPR* cluster. Given the similarity of the overlapping regions between *OPRs* and *OPRLs* in different plants and their similar chromosomal locations, the *OPRs–OPRLs* pairs may be conserved in angiosperms.

In this study, we found through fluorescence quantitative analysis that exogenous MeJA treatment promoted the expression of CsOPR9-1/2/3/4, CsOPR3-1, and CsOPR6-1, inhibited the expression of CsOPRL, while DIECA had the opposite effect. These results indicate that the expression of CsOPRL in tea plants is regulated at the JA level. AsODN mediated genes significantly reduced the expression of CsOPRL in tea leaves, with only CsOPR9-1/2/3/4 expression significantly upregulated, and significantly enhanced the resistance of tea leaves to anthracnose. The schematic diagram of the role of CsOPRL in the positive feedback control system for jasmonic acid biosynthesis is shown in Figure 2D.

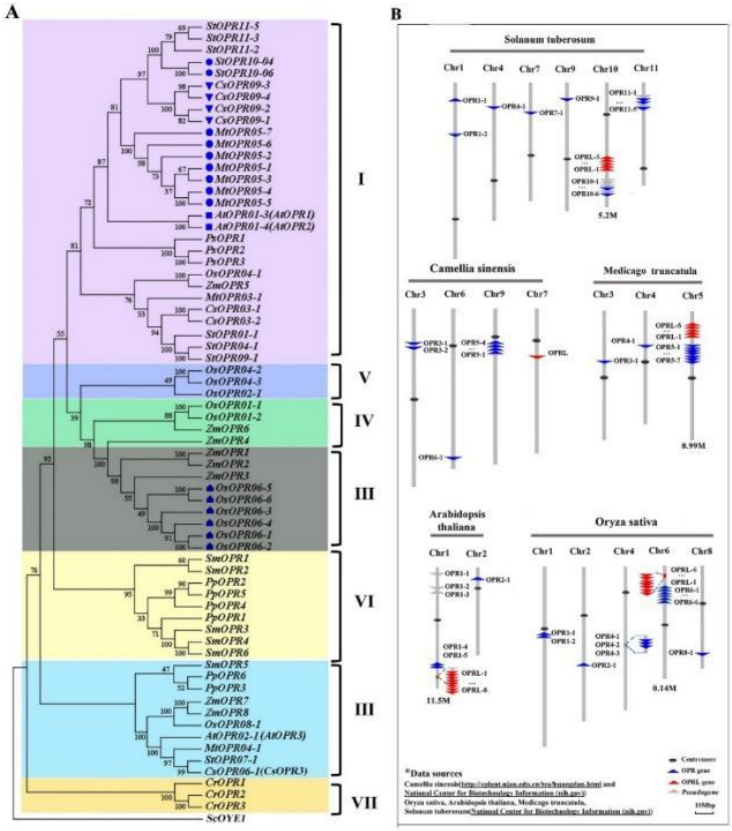


Figure 1 Phylogenetic analysis of OPR genes in 10 representative plants and chromosome mapping of 5 pairs of 12-oxophytodienoate reductase (OPR) and OPRL

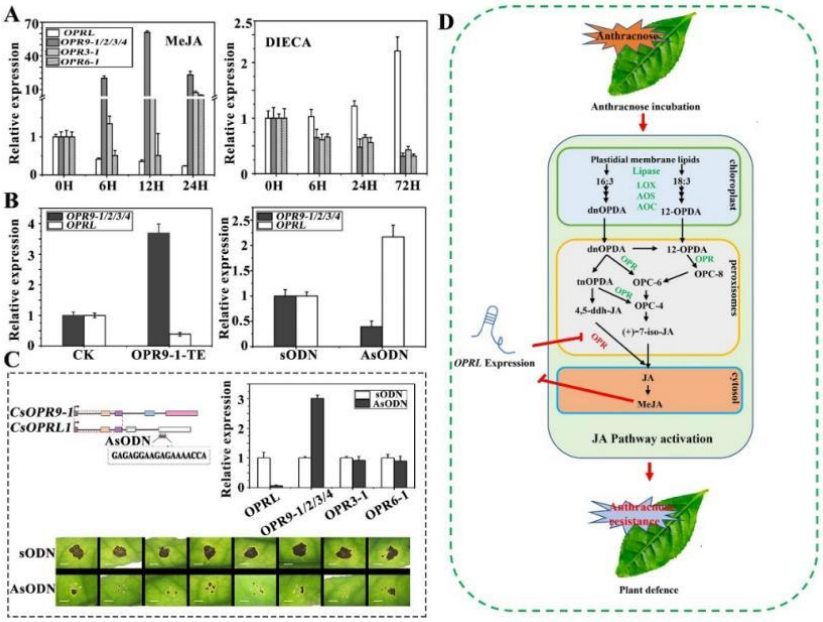


Figure 2 Role of CsOPRL in the positive feedback control system of jasmonic acid biosynthesis

Genome-wide Analysis and Expression Profiling of YUCCA Gene Family in Developmental and Environmental Stress Conditions in Tea Plant (*Camellia sinensis*)

ZHANG Liping[1], JIN Shan[2], BAI Peixian[1], GE Shibei[1], YAN Peng[1], LI Zhengzhen[1], ZHANG Lan[1], HAN Wenyan[1], ZENG Jianming[1*], LI Xin[1*]

1. Key Laboratory of Tea Quality and Safety Control, Ministry of Agriculture and Rural Affairs, Tea Research Institute, Chinese Academy of Agricultural Sciences, Hangzhou 310008, China; 2. Key Laboratory of Tea Science in Universities of Fujian Province, College of Horticulture, Fujian Agriculture and Forestry University, Fuzhou 350002, China

YUCCA (YUC) flavin monooxygenases are the rate-limiting enzymes of the most important indole-3-acetic acid (IAA) biosynthetic pathway in plants. A total of 17 *CsYUC* members were identified and phylogenetically classified into three subfamilies. The expression profiles of *CsYUCs* were analyzed. Combined with previous studies, it can be concluded that *YUC10* may play key roles in seed development. Low temperature markedly induced the expression of *CsYUC2.2*, *-11.8*, and *-11.9*. Furthermore, *CsYUC* genes that might play key roles in the specific development stages and involve enhancing the resistance to drought and NaCl stress were screened, respectively. This study could provide a research basis for deeply studying the gene functions of the *CsYUC* family in the tea plant.

Results

1 Identification and Bioinformatics Analysis of the CsYUC Gene Family in Tea Plants

A total of 17 unique *CsYUC* members were identified from the genome database of the tea cultivar 'Longjing 43' (LJ43) (Figure 1A). From Figure 1B, we can see that all of the *CsYUCs* contain the conserved flavin-binding monooxygenase (FMO) domain.

2 Expression Profiles of the CsYUC Family Numbers in Tea Plants

2.1 *CsYUC* Gene Expression Analysis during the Development Processes of Tea Leaf and Flower

CsYUC1, *-2.1*, *-7*, *-8*, *-11.5*, and *-11.9* play key roles in the early stage of tea leaf development, while *CsYUC2.2*, *-2.3*, *-11.3*, and *-11.4* play key roles in the process of tea leaf maturation and senescence (Figure 2A,B). *CsYUC2.1*, *-2.2*, *-6*, and *-11.9* played roles in the early stages, and *CsYUC1* and *-11.3* played roles in the later stages, whereas *CsYUC2.3*, *-8*, and *-11.8* played key roles in the three stages of flower development (Figure 2C).

2.2 The Regulatory Roles of *CsYUC* Genes in Response to Low Temperature

Low temperatures, including moderate low temperature (ML) and severe low temperature (SL), significantly induced the transcription of *CsYUC2.2*, *-11.8*, and *-11.9*. (Figure 3A). The results of qRT-PCR showed that, after low-temperature (LT) treatment, the transcript levels of *CsYUC2.2*, *-11.8*, and *-11.9* significantly increased (Figure 3B-F).

2.3 The Roles of *CsYUC* Genes in Response to Drought and NaCl Stress

The transcript of nine *CsYUC* genes, including *CsYUC6*, *-7*, *-8*, *-11.3*, *-11.4*, *-11.6*, *-11.7*, *-11.8*, and *-11.9*, may be involved in the positive regulation of the drought stress resistance of tea plants. There are eight *CsYUC* genes, including *CsYUC1*, *-7*, *-11.3*, *-11.4*, *-11.6*, *-11.7*, *-11.8*, and *-11.9*, which may be involved in the positive regulation of NaCl stress resistance of tea plants (Figure 4).

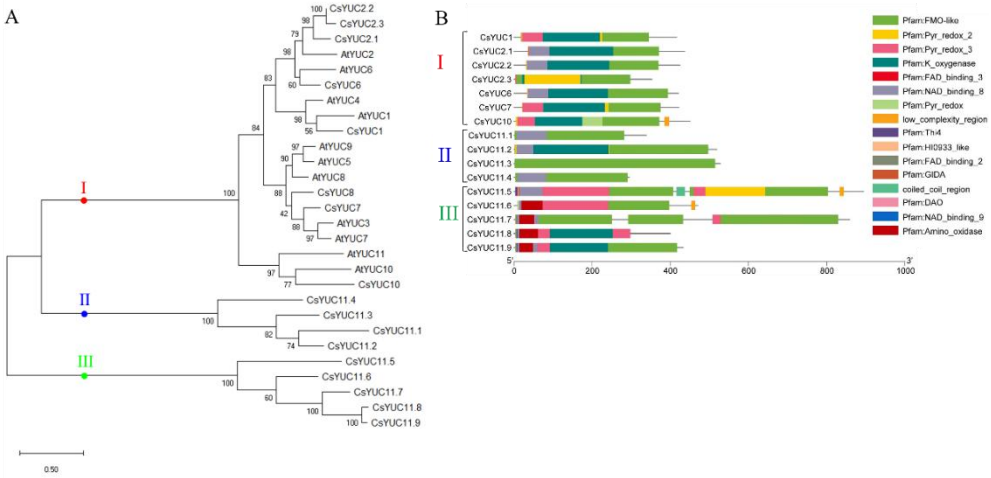


Figure 1 Phylogenetic relationships of *CsYUCs*, together with their *Arabidopsis* counterparts, respectively (A). Conserved domain analysis of *CsYUCs* (B)

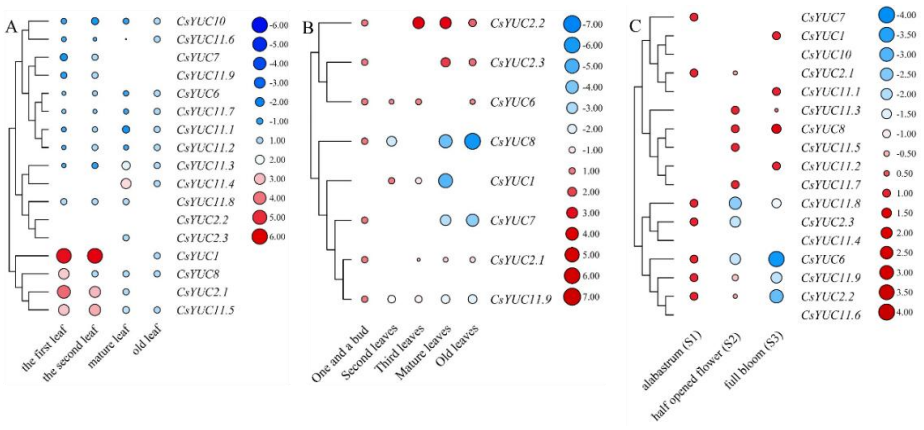


Figure 2 Expression profiles of *CsYUCs* during the process of leaf development and senescence (A,B). Expression profiles of *CsYUCs* during the process of flower development (C)

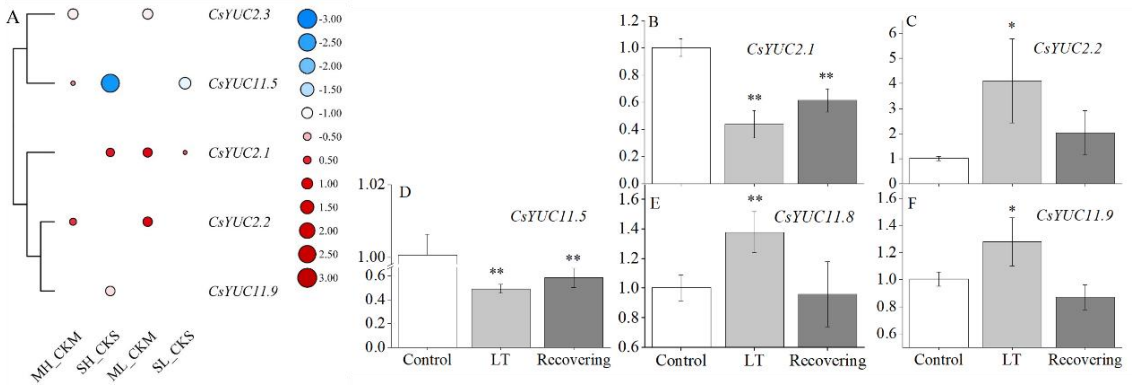


Figure 3 Transcriptional levels of *CsYUC* genes under temperature stresses (A). Expression profiles of *CsYUCs* in response to low temperature (LT) detected by qRT-PCR (B)

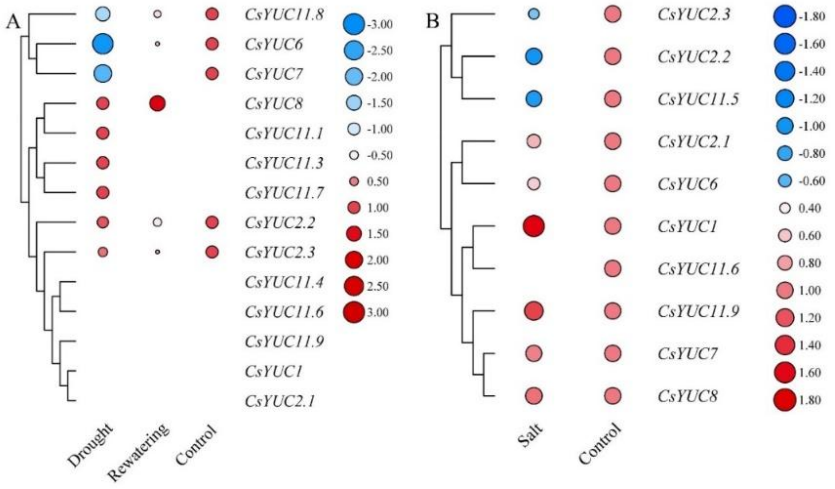


Figure 4 Expression profiles of *CsYUC* genes in response to drought and salt stresses

***Correspondence:** zengjm@tricaas.com (J.Z.); lixin@tricaas.com (X.L.)

Genome-wide Identification of Abscisic Acid Receptor PYL Gene in Tea Plant and Its Expression Pattern under Various Abiotic Stresses

ZHENG Dongqiao[1], SUN Chenyi[1], FU Maoyin[1], HUANG Dongzhu[1], WAN Xiaochun[1], CHEN Qi[1,2*]

1. State Key Laboratory of Tea Plant Biology and Utilization, School of Tea and Food Science & Technology, Anhui Agricultural University, Hefei 230036, China;
2. College of Life Science and Health, University of Science and Technology, Wuhan 430081, China

Abscisic acid receptor PYL protein family is an important participant in plant hormone abscisic acid signal transduction, which can sense abscisic acid signal and mediate downstream effects. In view of the important role of PYL protein in plant growth and development and environmental stress response, the study of its molecular structure and functional mechanism is of great significance for further understanding of abscisic acid signal transduction pathway. In this paper, we screened the PYL gene of tea plant in the whole genome, and identified 15 PYL genes, named *CsPYL1-15*, of which 3 genes with large fragment deletion (*CsPYL11-13*) may not have function. Phylogenetic tree analysis showed that members of the *CsPYLs* family were divided into three subfamilies. The analysis of genetic structure and tissue-specific expression showed that there were differences among members of *CsPYLs* gene family. Tea plant *CsPYLs* gene showed different response strategies to different abiotic stress, in which the negative response of *CsPYL14* gene to drought treatment was the strongest, and with the increase of drought time, the response value was lower, while the positive response of *CsPYL15* to drought treatment was the highest. This study provides theoretical support for the functional study of abscisic acid receptor PYL gene in tea plant.

Materials and Methods

Identification and Biological Analysis of CsPYLs

Using Arabidopsis thaliana gene annotation group database TAIR (http://www.arabidopsis.org/) , rice genome annotation database RCAP (http://rice.plantbiology.msu.edu/) and verified PYLs gene sequences from Arabidopsis thaliana, rice and other species obtained from NCBI (https://www.ncbi.nlm.nih.gov/) , Blast homologous sequences were searched in tea genome interpretation library TPIA (http://tpdb.shengxin.ren/) , and the genomic sequences, CDS sequences and amino acid sequences of tea *CsPYLs* family were obtained.

The genomic sequence of tea plant *CsPYLs* gene and the corresponding CDS sequence were submitted to the online gene structure analysis website Gene Structure Display Server 2.0 (http://gsds.cbi.pku.edu.cn/index.php) for gene structure analysis. In order to analyze the phylogeny and evolution of *CsPYLs* gene family in tea plant, a phylogenetic tree was constructed by using neighbor-joining pattern in MEGA 7.0 software and AtPYL1-13 sequence in Arabidopsis thaliana.

Transcriptome Data Analysis

Transcriptome data of members of *CsPYLs* gene family under drought, saline-alkali and low temperature treatments were obtained from NCBI. After the data are normalized, the Multiple Array Viewer software is used for heat map analysis.

Results and Analysis

Screening and Identification of *CsPYLs* Gene

According to the reported PYLs gene query in Arabidopsis thaliana and rice, 15 *CsPYLs* genes were found in the tea plant genome number note release library TPIA (http://tpia.teaplant.org/), which was named *CsPYL1-15*, of which 3 genes with large fragment deletion (*CsPYL11-13*) may not be functional.

Phylogeny, Gene structure and tissue specific expression of *CsPYLs*

In order to identify candidate PYL proteins in tea plants, Arabidopsis AtPYL was collected and phylogenetic trees were constructed by MEGA7.0 (Figure 1A). Phylogenetic tree analysis showed that members of the *CsPYLs* family were divided into three subfamilies. Among them, *CsPYL13,14* belongs to the same subfamily, *CsPYL4-10,15* belongs to the same subfamily, and *CsPYL11-13* belongs to the same subfamily.

In order to further understand the *CsPYLs* gene family of tea plant, the genomic sequences of 15 *CsPYLs* genes and their corresponding CDS sequences were analyzed. As shown in figure B. In the *CsPYLs* gene family, most of the family members are conservative and have no introns or few introns. *CsPYL4/5/6/11/12/14/15* has neither intron nor untranslated region. *CsPYL1/3* has one intron, *CsPYL7/8/9/13* has two introns and *CsPYL2/10* has five introns. Only three family members have untranslated regions, *CsPYL8* has untranslated regions downstream, and *CsPYL7/9* has untranslated regions at both ends.

In order to study the expression of *CsPYLs* gene family in different tissues and organs of tea plant, the expression data of *CsPYLs* in 8 tissues and organs (bud, tender leaf, mature leaf, old leaf, stem, root, flower and seed) were obtained from tea plant transcriptome database (TPIA). The result is shown in Figure C. Except for 3 genes with large fragment deletion (*CsPYL11-13*) in the *CsPYLs* family, which may not be functional, the other 11 genes are expressed in all tissues and organs. The expression of *CsPYL1* was higher in shoot and root, the expression of *CsPYL2/9* was similar in old leaves and flowers, the expression of *CsPYL3/4* was similar in mature leaves and roots, the expression of *CsPYL3* was the lowest in mature leaves and roots, the expression of *CsPYL7/8/10* in fruit was slightly higher, while that of *CsPYL5/6* was higher in mature leaves, the expression of *CsPYL14* in roots and flowers was higher, and that of *CsPYL15* in roots and seeds was higher.

Effect of Abiotic Stress on *CsPYLs* Gene Expression Pattern

ABA signal transduction family is an important part of plant stress resistance. In order to study the expression characteristics of *CsPYLs* gene family under different abiotic stress treatments, we obtained the transcriptome data of *CsPYLs* under drought, saline-alkali and low temperature chilling treatment from NCBI. As shown in Figure B, An is the expression result of *CsPYLs* family under different abiotic stress treatments. The expression of *CsPYLs* family was different under different abiotic stress treatments. Under drought treatment, the expression of *CsPYLs* gene family showed different expression patterns, except for *CsPYL3* without transcriptome data, *CsPYL24/6/14* showed down-regulated expression, in which *CsPYL14* gene had the strongest negative response to drought treatment, and with the increase of drought time, the response value was lower. The expression of *CsPYL1/8/9/10/15* gene was up-regulated, and *CsPYL15* had the highest positive response to drought treatment. Under saline-alkali stress, the expression of

CsPYL37/8/9/10/14 was down-regulated, and the negative response of *CsPYL3* gene to saline-alkali treatment was the strongest, and the stronger the gene response was with the extension of salt treatment time. The expression of *CsPYL14/5/6/15* gene was up-regulated, and the degree of response was similar. *CsPYL2* changes from positive response to negative response with the increase of processing time. Under low temperature treatment, except for *CsPYL2/15* non-transcriptional group data, most of the *CsPYLs* gene family showed down-regulated expression, of which *CsPYL4/5* had the strongest negative response, only *CsPYL1/14* was up-regulated, and *CsPYL1* had a strong positive response.

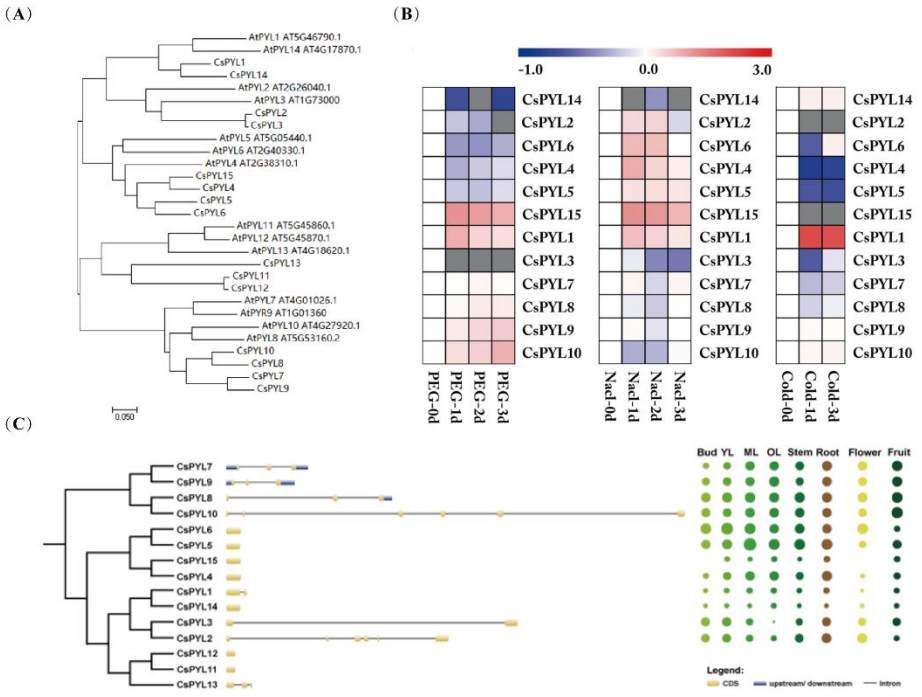


Figure 1 Biological analysis of *CsPYLs* gene family

(A) Construction of phylogenetic Tree of *CsPYLs* Family; (B) Effect of abiotic stress on *CsPYLs* gene expression pattern; (C) Gene structure and tissue specific expression of *CsPYLs*.

Glutamate Dehydrogenase Isogenes CsGDHs Cooperate with Glutamine Synthetase Isogenes CsGSs to Assimilate Ammonium in Tea Plant (*Camellia sinensis* L.)

JIAO Zixin[1,2,3], TANG Dandan[1,2,3], ZHANG Qunfeng[1,2*], LIU Meiya[1,2*], RUAN Jianyun[1,2]

1. Tea Research Institute, Chinese Academy of Agricultural Sciences, Hangzhou 310008, China; 2. Key Laboratory of Tea Plant Biology and Resources Utilization (Ministry of Agriculture and Rural Affairs), Hangzhou 310008, China; 3. Graduate School, Chinese Academy of Agricultural Sciences, Beijing 100081, China

Glutamate dehydrogenase (GDH) is a central enzyme in nitrogen metabolism, assimilating ammonia into glutamine or deaminating glutamate into α-oxoglutarate. Tea plants (*Camellia sinensis* L.) assimilate ammonium efficiently, but the role of *CsGDH* in ammonium assimilation remains unclear. We confirmed that tea has three GDH isogenes: *CsGDH1-3*. Bioinformatic analysis showed that *CsGDH1* encodes the β-GDH subunit, *CsGDH2/3* encodes the α-GDH subunit (Figure 1), and their proteins all feature an NADH-specific motif. *CsGDH1* is mainly expressed in mature leaves and roots, *CsGDH3* is mainly expressed in new shoots and roots, and *CsGDH2* has the highest expression level in flowers compared to the other five tissues. Expression patterns of *CsGDHs* and glutamine synthetase isogenes (*CsGSs*) under different ammonium concentrations suggested that *CsGDHs* cooperate with *CsGSs* to assimilate ammonium, especially under high ammonium conditions. Inhibition of GS and its isogenes resulted in significant induction of CsGDH3 in roots and *CsGDH2* in leaves, indicating their potential roles in ammonium assimilation. Moreover, *CsGDHs* transcripts were highly abundant in chlorotic tea leaves, in contrast to those of *CsGSs*, suggesting that *CsGDHs* play a vital role in ammonium assimilation in chlorotic tea mutants (Figure 2). Altogether, our circumstantial evidence that *CsGDHs* cooperate with *CsGSs* in ammonium assimilation provides a basis for unveiling their functions in tea plants.

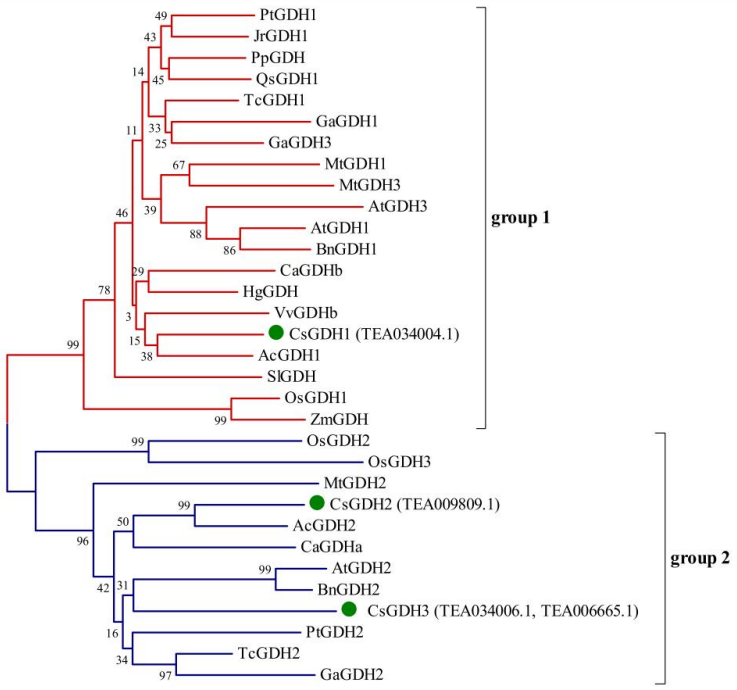


Figure 1 Phylogenetic analysis of GDHs

Amino acid sequences were aligned using Clustal X (using default parameter values), and the phylogenetic tree was reconstructed using MEGA 5.0 with the neighbor-joining method (bootstrap=1 000). *CsGDHs* are highlighted with green circles, and classified into different groups.

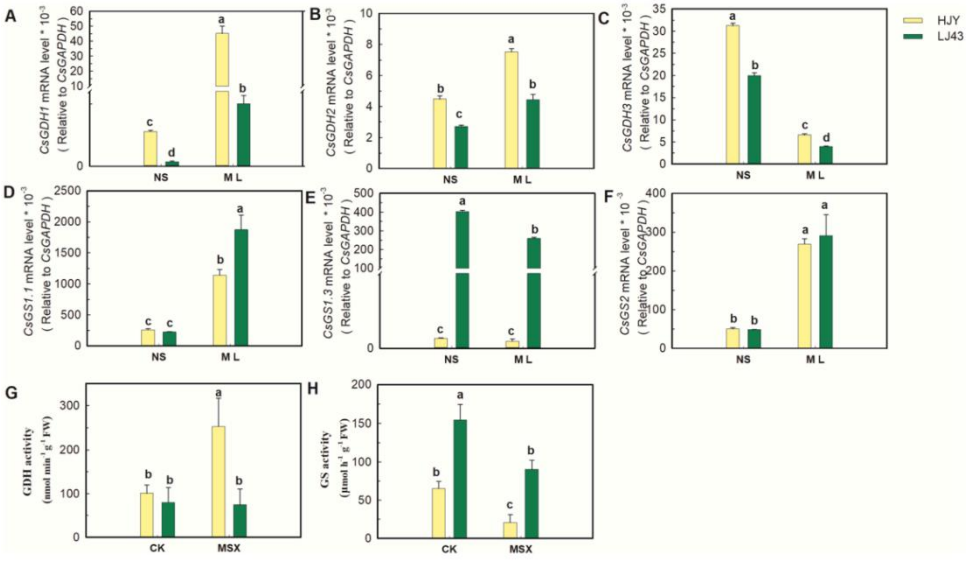


Figure 2 Expression profiles of *CsGDHs* and *CsGSs* in new shoots (NS) and mature leaves (ML) of *C. sinensis* cultivars 'Huangjinya' (HJY) and 'Longjing43' (LJ43). And GS activity (G) and GDH activity (H) in mature leaves of HJY and LJ43 in control (CK) and methionine sulfoximine (MSX)-treated tea leaves

Expression levels were normalized to that of the *CsGAPDH* gene. Expression levels were normalized to that of the *CsGAPDH* gene.

High-density Genetic Map Construction and QTL Mapping of a Zigzag-shaped Stem Trait in a Tea Plant BC1 Population

LIU Dingding, YE Yuanyuan, TANG Rongjin, GONG Yang, ZHANG Chenyu, MEI Piao, CHEN Jiedan, CHEN Liang*, MA Chunlei*

Key Laboratory of Biology, Genetics and Breeding of Special Economic Animals and Plants, Ministry of Agriculture and Rural Affairs, Tea Research Institute of the Chinese Academy of Agricultural Sciences, Hangzhou 310008, China

Stem zigzag-shaped phenotype is a very unique trait in tea plants and has great ornamental value. These tea resources with the trait are very rare. The creation of a BC1 artificial hybrid populations lays a foundation for elucidating the genetic machanism of stem zigzag-shaped in tea plant from the perspective of forword genetics. Genetic analysis showed that the the zigzag-shaped trait was a qualitative trait (Figure 1A and 1B). Then, we constructed a high-density genetic map via a whole-genome resequencing strategy based on the population (Figure 1C). For the consensus linkage map, a total of 5,250 markers spanning 3,328.51 cM were mapped to 15 linkage groups, with an average marker interval distance of 0.68 cM. the 15 linkage groups of genetic map anchored 15 chromosomes of *Camellia sinensis* 'Shuchazao' genome. Based on this map, a QTL of zigzag-shaped trait were mapped on chromosome 4 from 61.2 to 97.2 Mb. And the percentage of phenotypic variance explained (PVE) of the QTL to the zigzag-shaped phenotype was 13.62% (Figure 1D). Furthermore, A total of 6 candidate genes were identified within the target QTL. To further identify the candidate genes regulating zigzag-shaped stem in tea plant. Gene expression analysis were performed among different tissues of individual with straight stem and in nodes and internode of tea plants with the straight and zigzag-shaped stem, respectively (Figure 1E and 1F). Combined with the content of two auxins (2-oxindole-3-acetic acid and Indole-3-acetyl-L-aspartic acid) in nodes and internode of tea plants with zigzag-shaped stem (Figure 1G), the result showed that *CsXTH* (*CSS0035625*) and *CsCIPK14* (*CSS0044366*) may be involved in the formation of zigzag-shaped stem. These results lay the groundwork for functional genetic mapping, map-based cloning, and marker-assisted selection in tea plant.

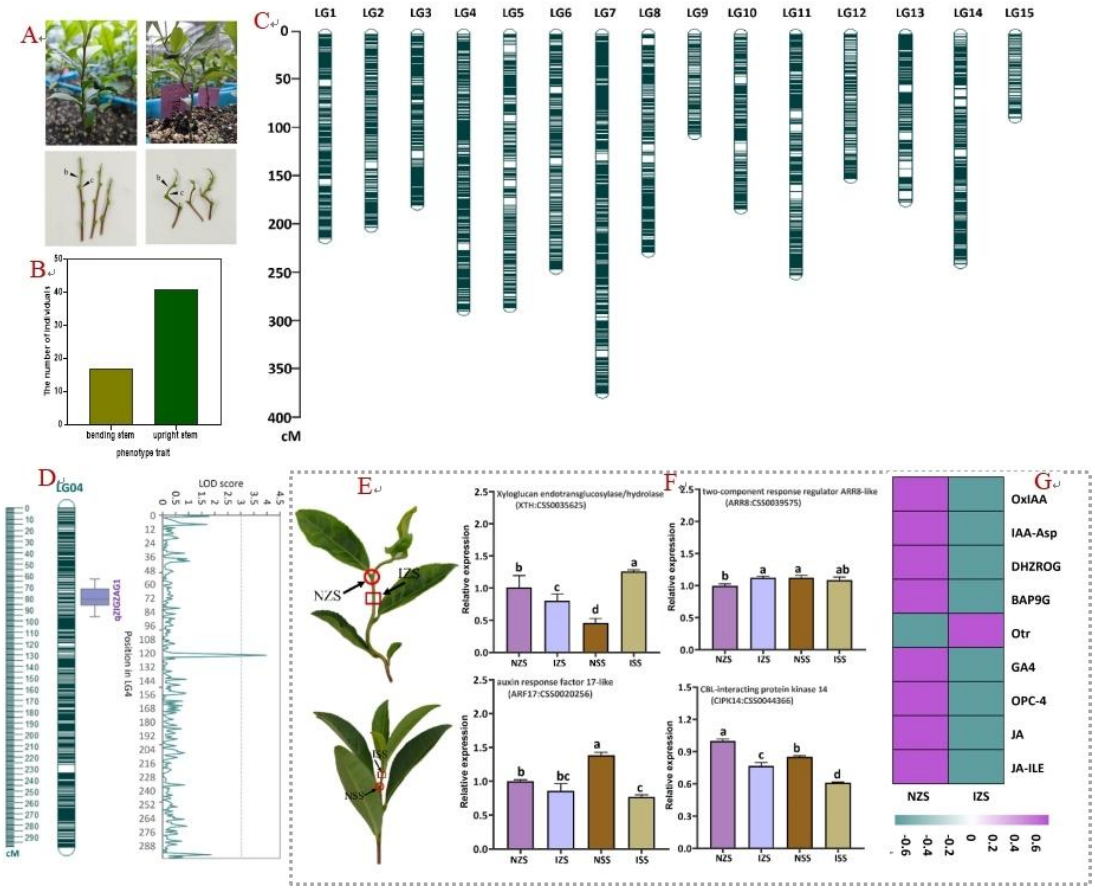


Figure 1 (A) the phenotype of individual of upright and bending stem in tea plant; (B) the number of individuals of upright and bending stem in tea plant; (C) Distribution of SNP markers on the 15 linkage group; (D) The position of QTL associated with zigzag-shaped trait in the BC1 population; (E) The branch morphology of tea plants with the straight and zigzag-shaped stem; (F) Differential expression analysis of candidate genes in node and internode of tea plants with the straight and zigzag-shaped stem. NZS: node of zigzag-shaped stem; NSS: node of straight stem; IZS: internode of zigzag-shaped stem; ISS: internode of straight stem; (G) heatmap of hormone content in axillary bud growth point and internode sites of tea plants with the zigzag-shaped stem. OxIAA: 2-oxindole-3-acetic acid; IAA-Asp: Indole-3-acetyl-L-aspartic acid; DHZROG: Dihydrozeatin-O-glucoside riboside; BAP9G: N6-Benzyladenine -9-glucoside; oTR: ortho-Topolin riboside; GA4: Gibberellin A4; OPC-4: 3-oxo-2-(2-(Z)-Pentenyl) cyclopentane-1-butyric acid; JA: Jasmonic Acid; JA-ILE: Jasmonoyl-L-isoleucine

Hydroxycinnamoylputrescines Catalyzed by a BAHD-family Acyltransferase Contributes to both Constitutive and Inducible Resistance to Herbivore in Tea Plant

YU Yongchen[1,2#], QIAN Xiaona[1,2#], LI Xiwang[1,2#], LIN Songbo[1,2], ZHANG Xin[1,2], CHAI Zhichao[1,2], ZHANG Jin[1,2*], SUN Xiaoling[1,2*]

1. Tea Research Institute, Chinese Academy of Agricultural Sciences, Hangzhou 310008, China;
2. Key Laboratory of Tea Biology and Resources Utilization, Ministry of Agriculture, Hangzhou 310008, China

Hydroxycinnamoylputrescines (HPs) are a group of important defensive compounds against herbivores in herbaceous plants. Yet, whether HPs also play an important role in regulating the herbivore resistance in woody perennials remains largely unknown.

Tea plant is famous as a leaf-usage beverage woody perennial cash crop, and its distinctive properties are based on its extreme diversity of second metabolites in leaves. Most studies have mainly focused on studying healthy functions and flavor contributions of the tea second metabolites. Casually, three catechin compounds have been demonstrated to act as the inducible defense agents against *Ectropis grisescens* (Eg) directly, while volatiles of (*Z*)-3-hexenol, α-farnesene, linalool and indole have been verified to induce and/or prime the tea plant resistance. Therefore, the potentially novel metabolites that function against tea pests are needed to be excavated deeply. Fascinatingly, we find the basal level of cinnamoylputrescine (Cin-Put) is abundant in tea plant, but only a trace amount of Cou-Put and Fer-Put are detected in healthy tea plants. While, *p*-coumaroylputrescine (Cou-Put) and Feruloylputrescine (Fer-Put) are markedly elicited upon Eg feeding. Our objective of this study is to reveal that the Fer-Put, Cou-Put and Cin-Put are direct defense agents in tea plants and CsAT1 acts as an authentic synthase of them.

Our results show that the content of Cin-Put is really high in tea plant comparing to the other two congeners and its accumulation is not elicited by chewing herbivore Eg feeding, JA- and SA- treatment; importantly, an artificial diet supplemented with Cin-Put significantly delay the larval growth, and the effect is dependent on the concentration and feeding time. From this, we conclude that Cin-Put behaves as a constitutive resistance factor in tea plant. Eg feeding strongly elicit the accumulation of Cou-Put and Fer-Put in tender leaves. The expression level of *CsERF53*, *CsERF55*, *CsERF60*, *CsMYB73*, *CsbHLH14* and *CsWRKY71* are significantly elicited upon herbivore feeding, along with the upregulated expression level of a subset of genes (*CsPAL*, *CsC4H*, *Cs4CL*, *CsCCoAOMT*) involved in phenylpropanoid pathway, genes (*CsADC*, *CsODC*, *CsArginase*) involving in the formation of putrescine skeleton, and genes encoding acyltransferases (*CsAT1*) catalyzing the final steps of HPc in higher plants. Crucially, an artificial diet complemented separately with Cou-Put or Fer-Put delays the growth of Eg, and the larval mass gain is dependent on the concentration of the above HPs and feeding time. The holistic investigation strongly recommends that Cou-Put and Fer-Put are effective inducible direct function agents against Eg in tea plants.

Most of acyltransferase enzymes belong to the characterized BAHD protein superfamily. Previously, several polyamine *N*-hydroxycinnamoyltransferases have been identified in several herbaceous plants. The tea acyltransferase CsAT1 belongs to the BAHD-acyltransferase superfamily with two conserved motifs: HXXXD and DFGWG, and the CsAT1 protein being structurally very similar to the OsPHT4, OsPHT3 and

NaAT1 proteins. The recombinant proteins CsAT1 exhibited putrescine hydroxycinnamoyl acyltransferases properties, which catalyzed putrescine (acetyl acceptor) with acetyl donors *p*-coumaroyl-CoA, cinnamoyl-CoA and feruloyl-CoA to form Cin-Put, Cou-Put and Fer-Put, respectively.

In conclusion, we identify and characterize *in vitro* the first gene encoding putrescine hydroxycinnamoyl acyltransferase of tea plant, whose protein product mediates *p*-coumaroyl-CoA, cinnamoyl-CoA and feruloyl-CoA conjugation to putrescine, and verify that the downstream products mediated tea plant constitutive or inducible resistance to herbivore in depth.

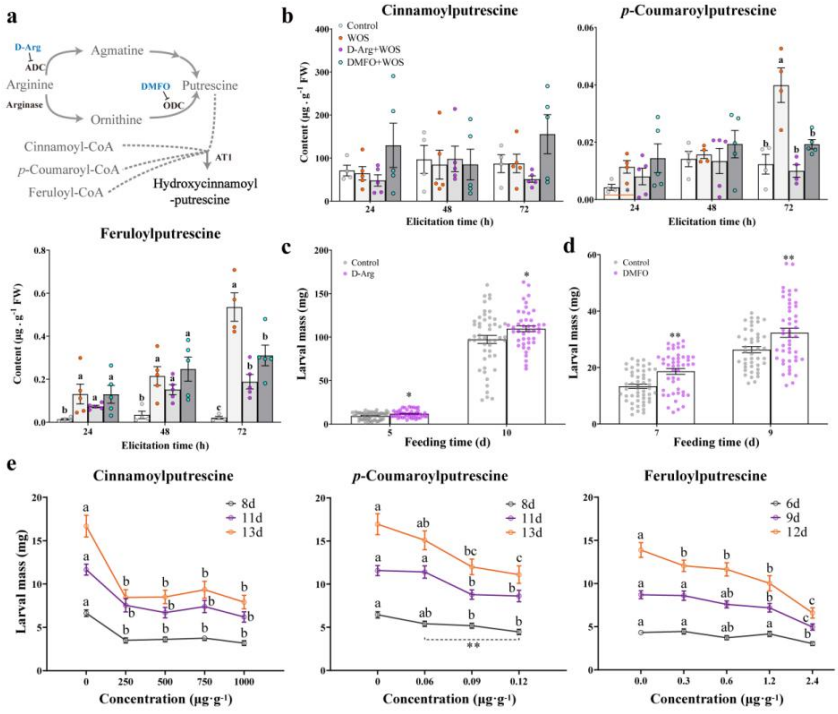


Figure 1 Cinnamoylputrescine, p-coumaroylputrescine and feruloylputrescine retard *Ectropis grisescens* larval growth

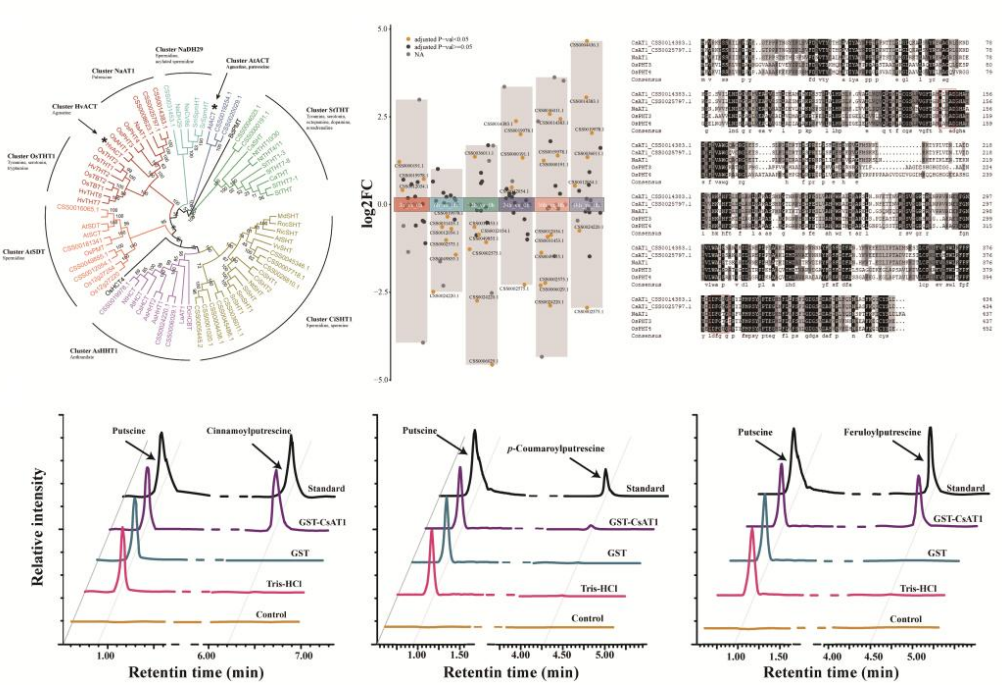


Figure 2 Identification CsAT1 as a putrescine hydroxycinnamoyl acyltransferase

Identification and Expression Analysis of Expansin Gene Family in *Camellia sinensis*

XU Yaqiong, LI Lanying, HUANG Fan, LUO Fan*

Tea Research Insitute of Sichuan Academy of Agricultural Sciences, Chengdu 610066, China

Expansin (EXP) is an important component of plant cell wall, which is involved in plant growth and stress process. In order to reveal the composition, characteristics and functions of the EXP gene family in tea plant, 38 EXP genes were identified from the whole genome of Camellia sinensis through bioinformatics, and their biological characteristics were comprehensively analyzed, which include protein physicochemical properties, chromosome localization, gene structure, and promoter acting elements. By analyzing the basic information of the EXP gene family in C. sinensis, this study provides a basis for the comprehensive analysis of the function of the EXP.

Materials and Methods

The genome of the tea plant (*Camellia sinensis* var. *sinensis* cv. '*Shuchazao*', SCZ), which was used in this study, were downloaded from the TPIA database (http://tpia.teaplant.org/). To identify the number of EXPs family in tea plants, Hidden Markov Model (HMM) was employed to identify the EXP gene family protein sequence; Homologous alignment was made between the EXP gene family protein sequence of Arabidopsis thaliana and the whole genome protein sequence of tea plant by MAGE7; Motif analysis of the protein sequences were conducted on the MEME website (http://meme-suite.org/). Cis-acting regulatory elements were annotated using PlantCARE online tool (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/). The prediction results of motif and Cis-acting regulatory elements were visualized by TBtools software.

Results

Genome-wide Identification of the EXP Gene Family

A total of 38 EXP genes were identified in the 'Shuchazao' genome based on HMM model. Except *CsEXPA22~CsEXPA26* and *CsEXLA2* located in contig 98, contig 227, contig 971, contig 1 040, and contig 1 054, all other EXP genes are unevenly distributed on 13 chromosomes (Figure 1).

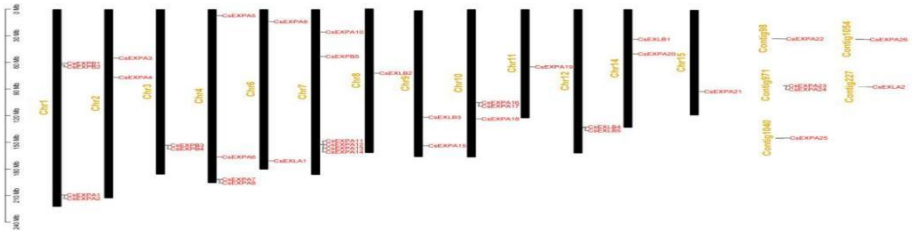


Figure 1 Distribution of expansin genes on 13 chromosomes in *C. sinensis*

Phylogenetic Analysis of the EXP Gene Family

To investigate the evolutionary relationship of EXP genes, a total of 38 EXP amino acid sequences from SCZ constructed a phylogenetic tree together with Arabidopsis (Figure 2). All sequences are divided into four subfamilies (CsEXPA, CsEXPB, CsEXLA and CsEXLB) based on their sequence similarities. Among them, class CsEXPA contains 26 EXP genes, while class CsEXPB contains 2 EXP genes, class CsEXPB and class CsEXLB both included 5 EXP genes respectively.

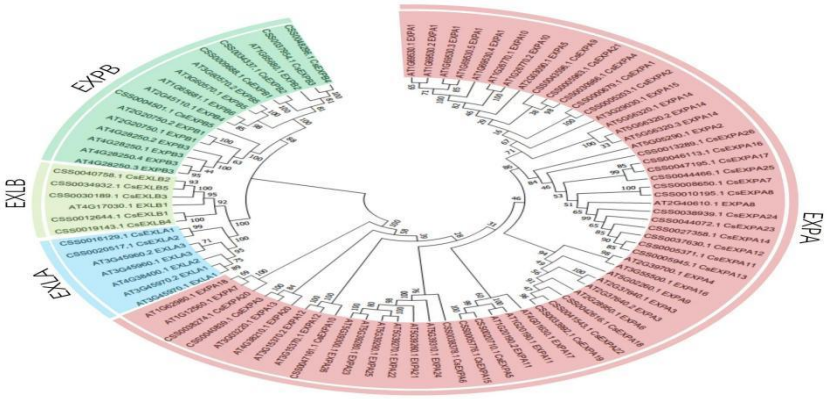


Figure 2 Phylogenetic tree analysis of expansins in *C. sinensis* and *A. thaliana*

Motif, Domain and Gene Structure Analysis of the EXP Gene Family

To reveal the internal relationship of the EXP gene family, conserved motifs of EXP gene were predicted by MEME. The results show that the EXP gene all contain Motif 3 and Motif 6.

Gene structure analysis showed that the number of exons in EXP genes family of tea plants were 1, 2,3, and 4, respectively. Among them, the exon with the least number was 1, such as *CsEXPA3*.

The maximum exon number of *CsEXLA2*, *CsEXLA1*, *CsEXLB4* and *CsEXLB1* genes is 4 (Figure 3).

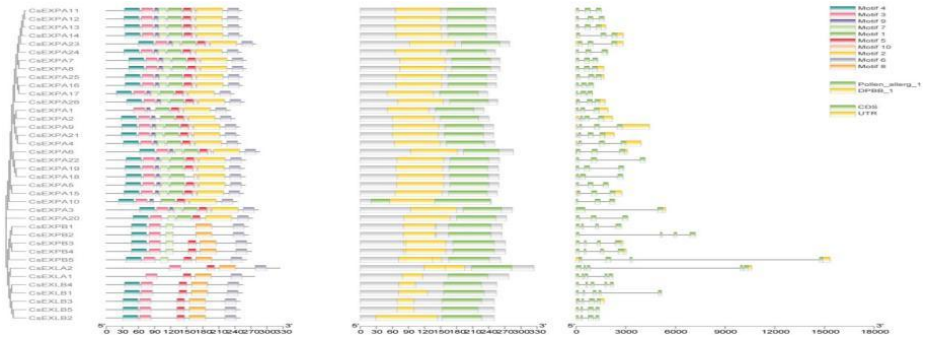


Figure 3 Phylogenetic (A), conserved motifs (B), conserved domain (C), gene structure (D) of expansin gene family of *C. sinensis*

Cis-acting Regulatory Elements Analysis of the EXP Gene Family

To better understand the regulatory network of EXP genes, we analyzed the promoter regions of EXP genes. Two thousand upstream sequences of EXP genes were collected as their promoter, and cis-acting elements were identified by PlantCARE online website. We found stress, light, hormone, regulatory and protein binding cis-elements in the promoter region of EXP genes. In addition, we also identified some growth and development-related action elements in the promoter region of EXP gene. These results suggesting that the EXP genes play an important role in the abiotic stress resistance.

Figure 4 The cis-elements of expansin gene family in *C. sinensis*

Identification of Qintang Maojian Green Tea of Different Cultivars using Non-targeted Metabolomics and Multi-elemental Analysis with Chemometrics

LIU Huahong[1], WU Yuxin[1], ZHAO Ziwei[1], LIU Zhi[2], LIU Renjun[1], PANG Yuelan[3], YANG Chun[3], ZHANG Yun[1], NIE Jinfang[1]

1. College of Chemistry and Bioengineering, Guilin University of Technology, Guilin 541004, China; 2. College of Agriculture and Biotechnology, Hunan University of Humanities, Science and Technology, Loudi 417000, China; 3. Guangxi Research Institute of Tea Science, Guilin 541004, China

Qintang Maojian (QTMJ) tea as one of the top ten most popular Chinese green teas, its authentic products are often deliberately counterfeited with inferior tea cultivars for illegal profits. In this paper, the combination of non-targeted metabolomics and multi-element analysis were used to investigate the impact of five different cultivars on the sensory quality of QTMJ tea and identify candidate markers for varietal authenticity assessment. With chemometric analysis, a total of 54 differential metabolites were screened with the abundances significantly varied in the tea cultivars. By contrast, the QTMJ tea from Yaoshan Xiulv (XL) monovariety presents much better sensory quality as result of the relatively more abundant anthocyanin glycosides and the lower levels of 2'-*O*-methyladenosine, denudatine, kynurenic acid and L-pipecolic acid. In addition, multi-elemental analysis found 14 significantly differential elements among the cultivars (VIP>1 and P<0.05). The differences and correlations of metabolites and elemental signatures of QTMJ tea between five cultivars were discussed by Pearson correlation analysis. Element characteristics can be used as the best discriminant index for different cultivars of QTMJT with the predictive accuracy of 100%. Therefore, this strategy is promising to be a feasible method for verifying the cultivars of QTMJ tea, ensuring the development of the tea industry.

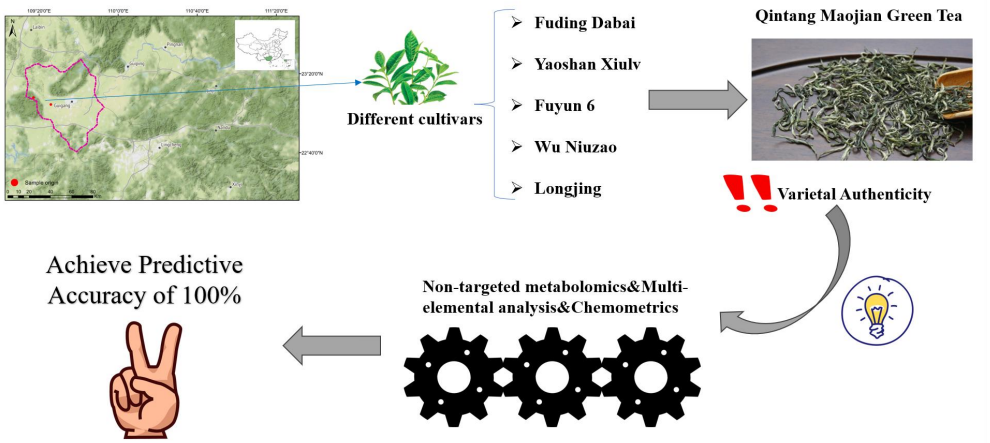


Figure 1 Graphical abstract

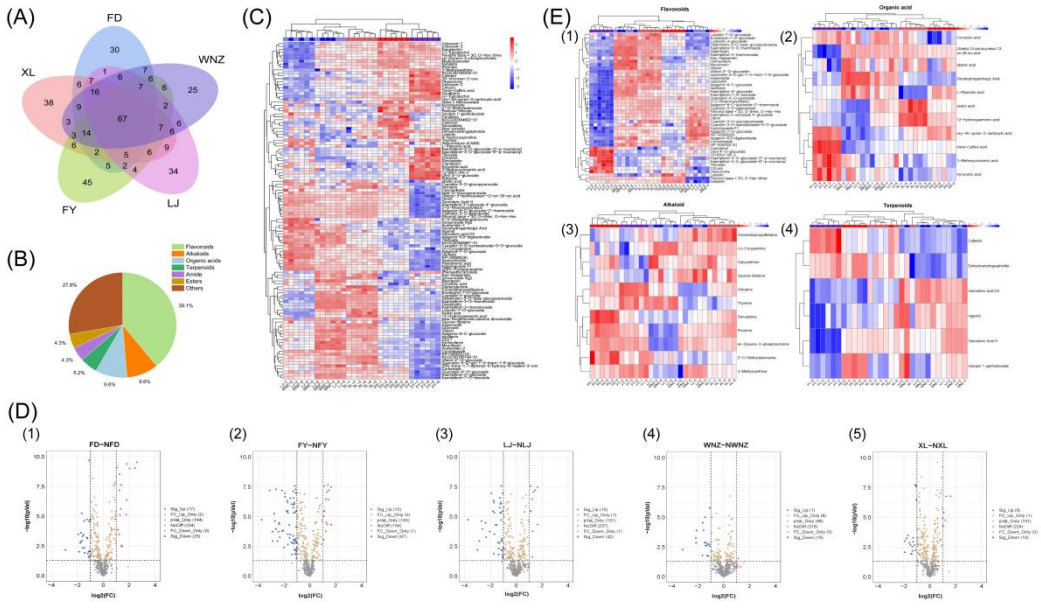


Figure 2 Metabolic profiles of Qintang Maojian green tea between different cultivars

(A) Wayne diagram; (B) Pie chart of differential metabolite species; (C) Heat map analysis of differential metabolites; (D) Volcanoes of me-tabolites between different species; (E) Heat map analysis of major species differential metabolites (The more red the color, the higher the content, and the more blue the color, the lower the content).

Integrative Transcriptome and Whole-genome Bisulfite Sequencing Analyses of a Temperature-sensitive Albino Tea Plant Cultivar

ZHANG Kexin[1], FENG Xia[1], LIU Ying[1], YANG Yajun[1], HAO Xinyuan[1,2], LI Dongliang[2], WANG Xinchao[1,2*], WANG Lu[1,2*]

1. Key Laboratory of Tea Biology and Resources Utilization, Ministry of Agriculture and Rural Affairs of the People's Republic of China/National Center for Tea Improvement/Tea Research Institute, Chinese Academy of Agricultural Sciences, Hangzhou 310008, China; 2. Sanya Institute, Hainan Academy of Agricultural Sciences, Hainan 572025, China

Green tea made from albino buds and leaves has a strong umami taste and aroma. The cultivar 'Zhonghuang 2' (ZH2, *Camellia sinensis*) is a natural mutant with young shoots that are yellow in spring and green or yellow-green in summer. However, the mechanism of leaf color change remains unclear. Here, we obtain the albino phenotype under low temperature (LT) treatment compared to the high temperature (HT), and we conformed that ZH2 is a temperature-sensitive cultivar. And this albinism is caused by the poorly stacked grana in the chloroplasts of young shoots. RNA-seq results showed 1279 genes differentially expressed in the young shoots grown at LT compared to HT, including genes related to cytochrome synthesis, chloroplast development, photosynthesis, and DNA methylation. A whole-genome bisulfite sequencing assay revealed that the dynamics of DNA methylation levels in the CG, CHG, and CHH contexts decreased under LT. Furthermore, most of genes had significant changes in both expression and DNA methylation levels, and they were related to cytochrome synthesis, chloroplast development, photosynthesis, transcription factors, and signaling pathways. These results demonstrate that DNA methylation is involved in the LT-regulated albino processes of ZH2. Changes in DNA methylation levels were associated with changes in gene expression levels, affecting the structure and function of chloroplasts, which may have a phenotypic impact on shoot and leaf color.

LT Caused Albinism of Young Shoots in ZH2

The young shoots of ZH2 were albinistic under the LT treatment, whereas they were green under HT (Figure 1a). And the chlorophyll contents of the first ZH2 leaves at LT were significantly lower than those at HT (Figure 1b). The transmission electron microscopy results showed that the leaves exhibited a typical chloroplast ultrastructure consisting of grana and thylakoids under HT conditions, and he grana stacks disappeared, and only a few thylakoids remained under LT conditions (Figures 1c–f). These observations suggested that ZH2 was a temperature-sensitive albino tea plant cultivar and that the albinism of young shoots caused by LT was due to abnormal chloroplasts.

Identification of DEGs between LT and HT Conditions

RNA-seq results identified 1 279 DEGs between the LT and HT groups, of which 483 were upregulated and 796 were downregulated. GO enrichment analysis of all the DEGs showed that the most significantly enriched items were related to photosynthesis, chloroplast and thylakoid development, and so on. KEGG pathway enrichment analyses suggested that the DEGs were mostly involved in photosynthesis, oxidative phosphorylation, and nitrogen metabolism pathways. These results indicated that the transcription

levels of genes related to photosynthesis and chloroplast development were significantly changed during the young shoot albino process. Notably, we also identified 31 DEGs related to DNA methylation, which accounted for a large proportion of the DEGs in other pathways. Therefore, we performed the WGBS assay to assess changes in DNA methylation.

LT reduced the Genome DNA Methylation Levels

To explore the changes in genome-wide DNA methylation levels during the albino process of young shoots caused by LT, we examined the whole-genome methylation patterns of young shoots grown under HT and LT conditions. We determined the DNA methylation levels in the promoter, exon, and intron regions and the upstream 2 kb, gene body, and downstream 2 kb regions in the CG, CHG, and CHH contexts. Genome-wide DNA methylation levels were reduced in each context under LT conditions compared with those under HT. The average DNA methylation levels decreased in all three contexts under LT, and the CHH context showed the most significant change. The proportions of these three methylated contexts to all the methylcytosine contexts also changed. The proportion of mCHH in all methylcytosine contexts decreased from 30.96% to 27.19%, whereas the proportion in the other two contexts increased. These results indicated that DNA methylation levels could change during young shoot albinism caused by LT.

Comparison of DMRs in Three Contexts between LT and HT Conditions

The identification of DMRs could comprehensively highlight epigenetic differences in the different colors of young shoots of ZH2. To explore the dynamics of DNA methylation and evaluate how the methylation levels of DMRs were altered during the albino process in young shoots, we determined the distribution of DMRs in the three contexts. The total number of hypomethylated DMRs was much higher than that of hypermethylated DMRs. To determine the distribution of DMRs in gene and non-gene regions, we classified the genomic DNA into six groups: promoter (2 kb upstream of TSS), TSS, exon, intron, TES, and other regions. In addition to other regions, the number of DMRs in the intron region was the largest in all contexts, followed by the promoter region; the number of DMRs in the TES region was the smallest. To clarify the function of DMRs in the albino process that occurs under LT treatment, GO and KEGG enrichment analyses of DMRs were performed to identify pathways that were enriched in response to temperature change. GO terms involved in iron ion, heme, and tetrapyrrole binding had the highest number of gene entries in the three contexts. Chlorophyll metabolism, carotenoid biosynthesis pathways, and porphyrin were also identified by KEGG pathway analysis, indicating that the chlorophyll and carotenoid contents changed in the albinism of ZH2. The results indicated that DNA methylation changes in genes related to these pathways above might affect the expression levels and physiological characteristics of ZH2 in response to different temperatures.

Integrated Analysis of DEGs and DMGs in Albino Young Shoots of ZH2

Results from the integrated analysis comparing DEGs and DMGs helped understand the potential linkages between DNA methylation and gene expression. DNA methylation affects gene expression by blocking or suppressing transcription, and suppressed genes show hypermethylated DNA levels in specific functional regions. Conversely, hypomethylated genes usually show increased expression levels. We analyzed the relationship between DEGs and DMGs and found that the number of hypomethylated upregulated genes was higher than that of hypermethylated downregulated genes in all contexts under the LT treatment than under the HT treatment. Hypermethylated downregulated genes were the least abundant in the CHH context. A total of 11 genes related to chloroplast function and the cytochrome biosynthesis pathway, 12 genes related to transcription factors, 19 genes related to signaling pathways, and 8

transporters were identified as DEGs and DMGs. Therefore, we summarized a pattern diagram for the albino process of ZH2 under LT based on all the results (Figure 2).

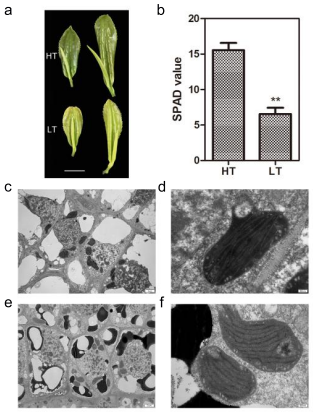


Figure 1. Young shoot phenotype and chloroplast ultrastructure of ZH2 grown under LT and HT conditions. (a) Young shoot phenotype of ZH2 grown under HT and LT (bar = 1 cm). (b) SPAD values of the first leaves. Nine first leaves of ZH2 plants were measured in each treatment. (c, d) Chloroplast ultrastructure of the first leaf under HT. (e, f) Chloroplast ultrastructure of the first leaf under LT. (c, e) Bar = 2 μm. (d, f) Bar = 200 nm.

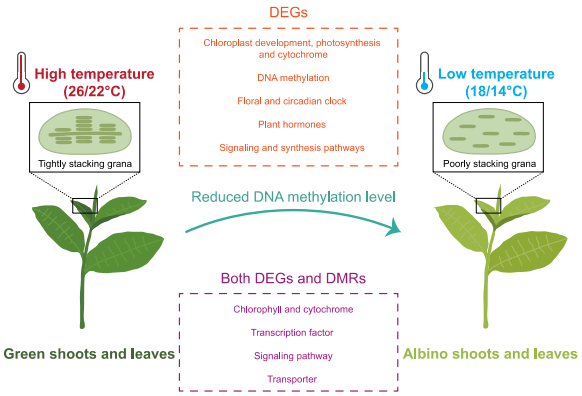


Figure 2. A model of a proposed mechanism for albinism in shoots of ZH2. Under the HT conditions (26/22°C), the young shoots and leaves of ZH2 turned green, and the grana stacking of chloroplast in the leaves is normal. Under LT (18/14°C) conditions, the young shoots and leaves of ZH2 showed an albino phenotype, with abnormal stacking of grana in the leaves. Compared with the green leaves, there were significant changes in the transcription levels of genes related to chloroplast development, photosynthesis, and cytochrome in albino leaves. The DEGs were also present in plant floral, circadian clock, plant hormones, signaling pathways, and synthesis pathways. In addition, the DEGs related to DNA methylation also exhibited significant transcriptional changes (orange boxes and text). The DNA methylation levels in albino leaves decreased under LT compared with those in green leaves under HT (blue arrow and text). The genes related to chlorophyll and cytochrome, transcription factor, signaling pathway, and transporter had been discovered in both DEGs and DMRs. The significant changes in the transcriptional abundance of these genes were likely due to changes in their DNA methylation levels (purple boxes and text).

Isolation and Functional Analysis of the *F3'H* Promoter from *Camellia sinensis*

LIU Yufei[1,3,4], PANG Dandan[1,3,4], LI Youyong[1,3,4], CHEN Chunlin[1,3,4], TIAN Yiping[1,3,4], CHEN Linbo[1,3,4*], YANG Tianyuan[2*]

1. Tea Research Institute, Yunnan Academy of Agricultural Sciences, Menghai 666201, China; 2. State Key Laboratory of Tea Plant Biology and Utilization, Anhui Agricultural University, Hefei 230036, China; 3. Yunnan Provincial Key Laboratory for Tea Science, Menghai 666201, China; 4. Yunnan Technology Engineering Research Center of Tea Germplasm Innovation and Supporting Cultivation, Menghai 666201, China

Tea plant *F3'H* is a key enzyme in the catechin synthesis pathway. To investigate the regulatory role of the *F3'H* gene promoter in gene expression in tea plants, the full-length promoter sequence of the F3'H gene was cloned by RT-PCR from the tender leaves of tea cultivar 'Yunkang 10' (YK10). The cis-acting elements of different promoter fragments of the *F3'H* gene were analyzed by bioinformatics methods, and the role of cis-acting elements was preliminarily analyzed using transcriptome data. Through the double enzyme digestion method, plant expression vectors were constructed for the full length promoter sequence of the *F3'H* gene and the 5' fragment deletion promoter sequence, respectively. Finally, the activity of the *F3'H* gene promoter and key active regions were analyzed by GUS histochemical staining and enzyme activity measurement. In this study, a 1806 bp promoter sequence upstream of the *F3'H* gene start codon (ATG) was cloned, and four 5' fragment deletion sequences of the *F3'H* gene were obtained, *F3'HP2-F3'HP5*, with sequence lengths of 804 bp, 479 bp, 195 bp, and 154 bp, respectively. In addition to the basic elements of TATA-box and CAAT-box, the *F3'HP1* sequence also has response elements related to low temperature, light, and gibberellin. Transcriptome data showed that *F3'H* is responsive to low temperature. The results of GUS histochemical staining and enzyme activity measurement showed that *F3'HP2* had the strongest promoter activity, rather than *F3'HP1*. In addition, the region between *F3'HP2* and *F3'HP3* was a key region to ensure the high activity of the *F3'H* promoter, and the region between *F3'HP*3 and *F3'HP4* contained basic elements that satisfied the activity of the *F3'H* promoter. This study lays a foundation for exploring the relationship between factors such as light, hormones, and stress conditions and the promoter of the *F3'H* gene, as well as analyzing the regulatory mechanism of the *F3'H* gene.

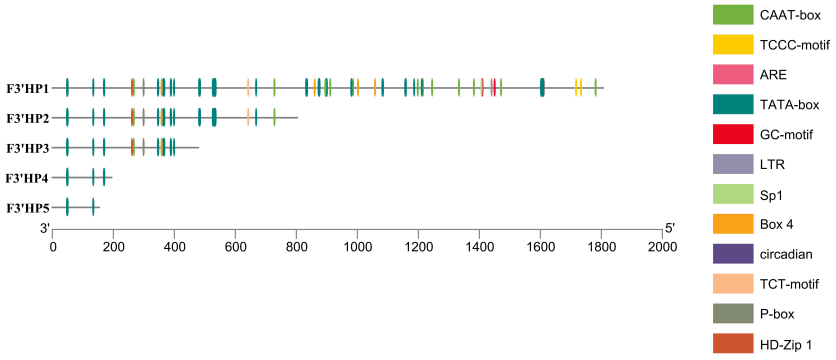


Figure 1 Analysis of the position of cis-acting elements in the *F3'H* promoter fragment

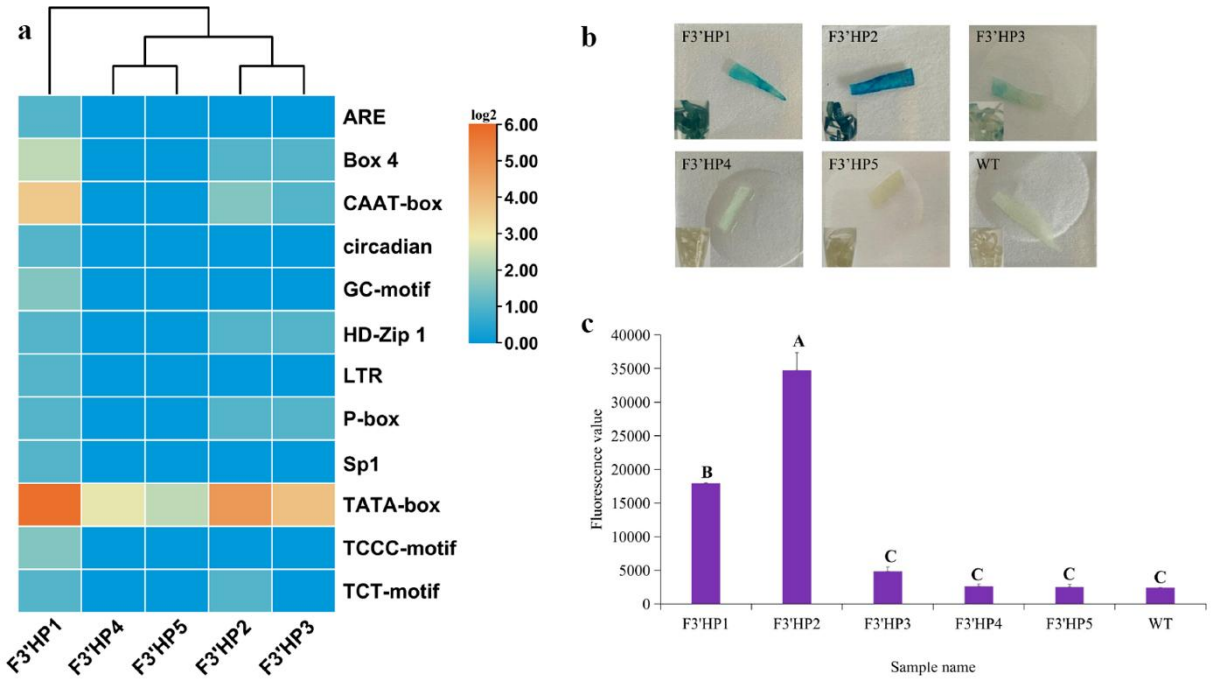


Figure 2 Analysis of the number of cis-acting elements in the *F3'H* promoter fragment and activity analysis of promoters with different lengths of *F3'H* gene

Metabolomics Reveals Relationship of Metabolite Variation and Sensory Attributes among Tea Cultivars

WANG Jing[1], LI Yong[3], LIU Huahong[1], HUANG Yingyi[1], PANG Yuelan[2], YANG Chun[2], ZHANG Yun[1], NIE Jinfang[1]

1. College of Chemical and Bioengineering, Guilin University of Technology, Guilin 541004, China;
2. Guangxi Research Institute of Tea Science, Guilin 541004, China;
3. Institute of Food Safety and Nutrition, Jiangsu Academy of Agricultural Sciences, Nanjing 210014, China

The beneficial effects of tea have long been attributed to its rich composition of natural bioactive compounds; however, the influence of variations in these compounds on taste and aroma remains poorly understood. In this study, we aimed to explore the relationship between metabolic profiles and tea quality characteristics by conducting a comparative analysis of four fresh tea varieties in Guangxi. The investigated varieties included Ying Hong No.9 (YH), Gui Hong No.3 (GH), Gui Cha No.2 (GC), and Meizhan (MZ). Through sensory evaluation, GH and MZ were identified as exhibiting the highest quality for green and black tea, respectively, but YH displayed the lowest quality for both tea types. Metabolomics analysis revealed significant differences in the metabolic profiles among the four tea varieties. MZ and YH demonstrated higher levels of flavonoids, whereas GC and GH showed higher levels of organic acids. Pathway analyses of GH and MZ revealed that the differentially enriched pathways mainly pertained to linoleic acid metabolism and flavonoid and flavanol production. Quantitative analysis showed that most of the catechins and flavonol glycosides were high in MZ. Correlation analyses indicated that the sensory quality of green tea was closely associated with fatty acids and organic acids, while the sensory quality of black tea showed a strong correlation with the content of flavonoids. This study contributes to a better understanding of the metabolic characteristics and processing adaptability of tea tree cultivars in the Guangxi region, shedding light on the intricate relationship between metabolite variation and sensory attributes among different tea varieties.

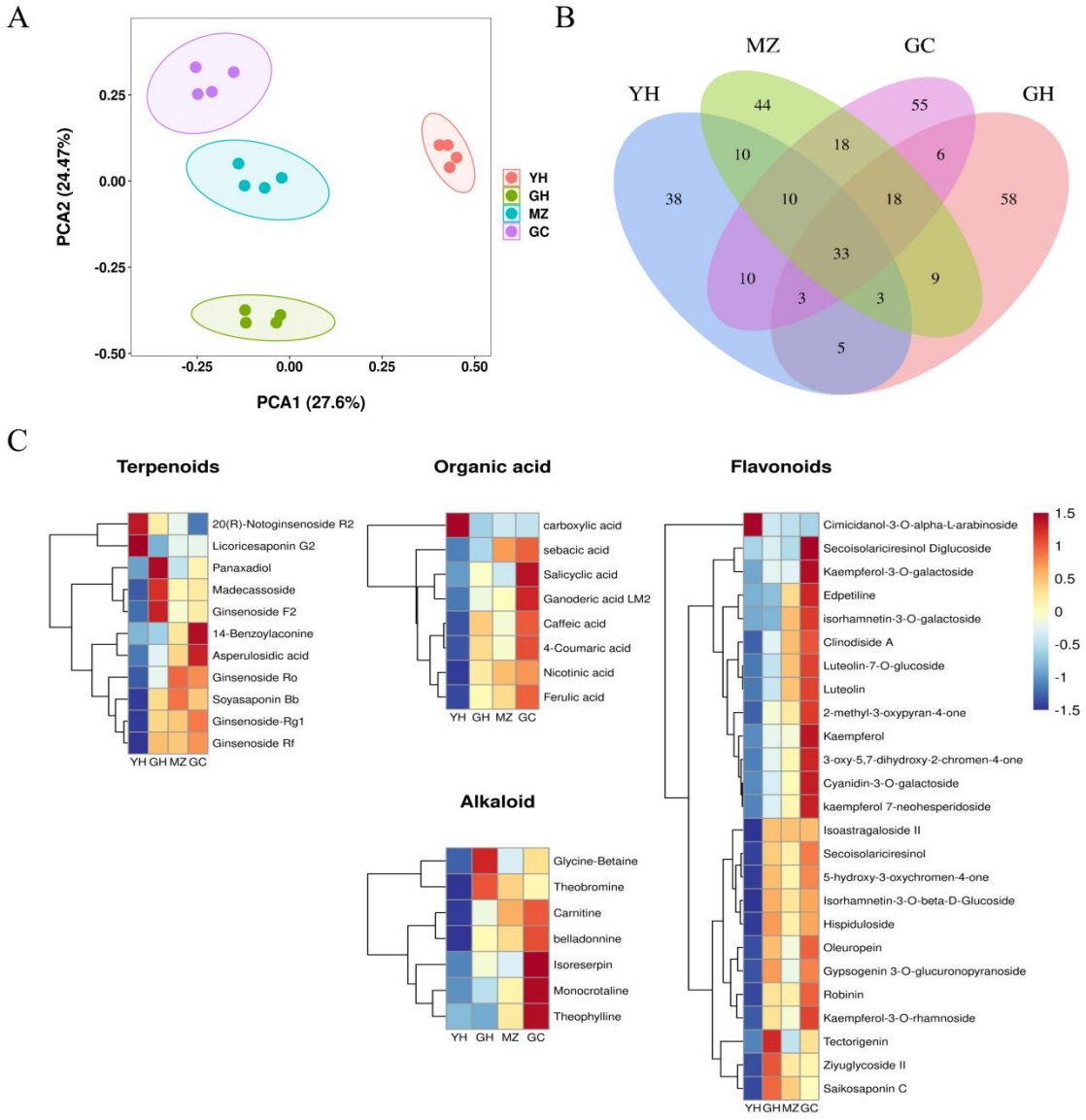


Figure 1 Metabolic profiles of tea between different cultivars
(A) PCA score plot; (B) Wayne diagram; (C) Heat map analysis of differential metabolites.

Residue Analysis of Cyflumetofen and Iits Metabolites in Tea and Tea Infusion by New Nano Adsorbents Purification and Ultra-performance Liquid Chromatography–tandem Mass Spectrometry Determination

NING Yating[1], HU Yue[1], LUO Fengjian[1], CHEN Liezhong[2], ZHANG Xinzhong[1*]

1. Tea Research Institute, Chinese Academy of Agricultural Sciences, Hangzhou 310008, China; 2. State Key Laboratory for Managing Biotic and Chemical Threats to the Quality and Safety of Agro-products, Institute of Agro-product Safety and Nutrition, Zhejiang Academy of Agricultural Sciences, Hangzhou 310021, China

Cyflumetofen is a potential new type of acaricide for tea gardens. A simultaneous residue analysis method of cyflumetofen and its three metabolites (B-1, B-3, AB-1) in fresh tea leaves, green tea and black tea, green tea infusion and black tea infusion was developed by ultra performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS). Cyflumetofen and its metabolites in different samples were extracted with acetonitrile containing 1% of formic acid, followed by a combination of multi-walled carbon nanotubes (MWCNT) and nano-ZrO2 absorbents in conjunction with low-temperature clean purification. There were exhibited wonderful linearities in the matrix-matched calibration concentration range of 0.005-2.0 mg/L (except B-1, which was 0.01-2.0 mg/L), with correlation coefficients (R2) greater than or equal to 0.9931. The average spiked recoveries of cyflumetofen and its metabolites in different tea matrixes were of 71.9%-116.9% with the relative standard deviations (RSDs) below 15.1%. The limits of quantification (LOQs) of cyflumetofen and its metabolites in fresh tea leaves, as well as black and green tea were at 0.005 mg/kg and B-1 were at 0.01 mg/kg in black and green tea, the LOQS were at 0.5 μg/L in black and green tea infusion and B-1 was at 1.0 μg/L. After application of 20% cyflumetofen SC, cyflumetofen and its three metabolites B-1, B-3 and AB-1 were detected in real fresh tea leaf samples obtained from the field experiment, and the half-life of cyflumetofen in fresh tea leaves was 1.18 d.

Screening of Discriminant Indexes for Suitable Varieties of Chongqing Tuo Tea

LUO Hongyu, ZHONG Yingfu, WANG Yi, CHANG Rui, CHEN Shanmin

Tea Research Institute of Chongqing Academy of Agricultural Sciences, Chongqing 402160, China

The differential of varieties will lead to different quality of Tuo tea. Conventional Chongqing Tuo tea was made of small leaf species baking, stir-frying and Yunnan big leaf species sun-dried tea, which resulted in the poor quality stability and non-significant native characteristics. In order to excavate the advantages of tea germplasm resources in Chongqing, the processing suitability of fresh tea leaves of main tea cultivars and local tea cultivars for Tuo tea production, and discriminant indexes were researched in this paper.

Materials and Methods

Tea Samples

Fresh tea leaves (one bud with two leaves of *Camellia sinensis* 'Sichuan small and medium leaf group species'， *Camellia sinensis* cv. Fuxuan 9', *Camellia sinensis* cv. 'Fuding-dabaicha', *Camellia sinensis* cv. 'Shuyong 2', *Camellia sinensis* 'Nanchuan-dashucha', and *Camellia sinensis* cv. 'Shuyong 1') were obtained from Chongqing, China, on May and June 2022, and were used to prepare Chongqing Tuo tea numbered as 1, 2, 3, 4, 5 and 6, respectively. All tea samples were stored at −20 ℃ for analysis. All experiments were repeated three times.

Chemicals

The main chemicals included methanol, sodium carbonate, folin phenol, ninhydrin hydrate, caffeine, gallic acid and 7 kinds of catechins.

Results

The Quality of Different Varieties of Fresh Tea Leaves

Taste components analysis showed that tea polyphenols (TPs), water extract and TPs to amino acids (TPs/AAs) ratio were lower in No. 1 than others, while γ-aminobutyric acid reached the highest content (0.012%). No. 2 had the highest content of AAs (5.26%) due to abundant umami and sweet AAs. No. 3 had the lowest content of caffeine. TPs, theanine and TPs/AAs were the highest level in No. 4. Soluble sugar content was high in No.5, but the content of ester-catechins, umami and sweet AAs were the lowest. Bitter AAs, caffeine and water extract were high in No. 6.

Volatile components analysis showed that these six varieties had a large number of alocohols (26.12% to 58.91%) and alkenes (22.97% to 37.40%), the content of aldehydes, ketones and esters were lower (2.83% to 14.52%). No.1 had abundant α-farnesene. Oleanols, cis-β-ocimene, γ-elemene, α-mullene, β-cadinene and β-sesquiphellandrene were the main volatile components in No.2. Dimethyl sulfide were rich in both No.1 and No.2. The content of nerolidol, 4,8-dimethyl-1,3,7-nonatriene, isovalerate, methyl dimethylvalerate together with indole were high in No.3. No.4 had high content of β-linalool, trans-3,7-linalool oxide II and olive alcohol. No.5 had high content of β-caryophyllene and

trans-3,7-linalool oxide I. No.6 had abundant olefins such as limonene, aldehydes such as nonanal, and ketones such as β-ionone.

The Quality of Different Varieties of Tuo Tea

According to the methodology of sensory evaluation of tea, the sensory quality of No.1 and No.2 were the best because of their deep green color of dry tea, deep yellow of tea soup, faint scent and sweet aroma and mellow taste, followed by No.3. No.4 presented acrimony aroma, No.5 presented sweet potato aroma. No.6 had the worst sensory quality due to its dull odour, bitter and astringency taste. These results were different from the electronic tongue analysis results, which might be due to the sensory quality was closely related to the interaction of taste substances.

Screening of Suitable Varieties and Discriminant Indexes

According to the sensory quality, taste and volatile components, and the correlation coefficient between them, combined with OPLS-DA and Fisher discrimination analysis, the optimum variety was No.1, followed by No.2 and No.3. It was preliminarily proposed that tea polyphenols, water extract and total ester catechins were used as indicators for judging the suitability of Chongqing Tuo tea.

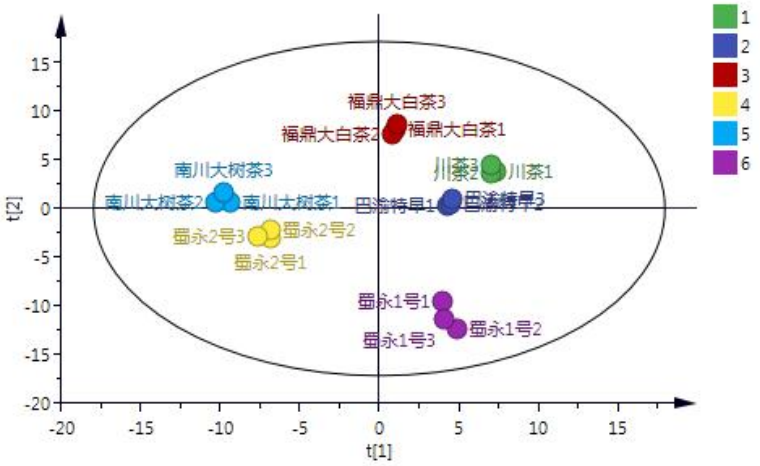


Figure 1 OPLS-DA score plot of taste and aroma components of fresh tea leaves

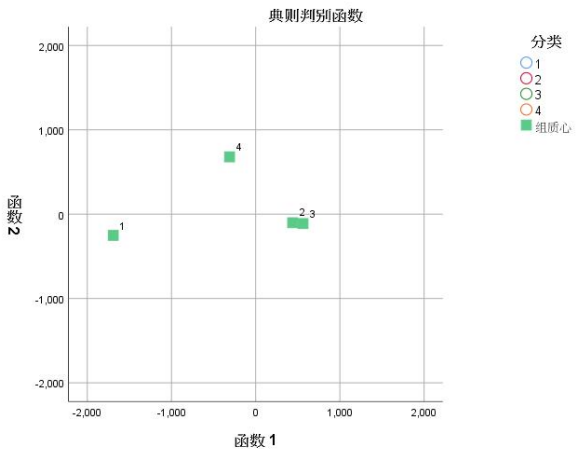


Figure 2 Linear discriminant score plot of fresh tea leaves

Sugar Signal Participates in the Biosynthesis of Flavonoids in Tea Plants

Lü Yiqing, LU Lu, ZHENG Xinqiang, LU Jianliang, YE Jianhui*

Tea Research Institute, Zhejiang University, Hangzhou 310058, China

Background

Flavonoids, as the important secondary metabolites in tea plants, contribute to the bitterness and astringency of tea as well as health benefits like antioxidant, anticarcinogenic, and cardiovascular protective effects. Sugars not only supply carbon skeletons as substrates for sink tissue growth, but also act as signal molecules or stimuli by influencing metabolic processes or regulating relevant gene expression. Studies have shown that sugar signal plays a role in regulating flavonoid biosynthesis in plants, which have been reported in many plants, e.g. grape berries, apple, and *Arabidopsis*. However, there are few studies on the involvement of sugar signal in tea flavonoid biosynthesis.

Results

1. Based on weighted gene coexpression network analysis (WGCNA, Figure 1), the key gene modules (Modules 2 and 3) related to the varying relationship between sugar metabolism and flavonoid biosynthesis as well as the corresponding hub genes were obtained. Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis showed that the transcription factors (TFs) in Modules 2 and 3 were mainly enriched in the pathway of plant hormone signal transduction.

2. An *in vitro* study showed that the transcriptional levels of *ERF1B-like* TF for hexokinase inhibitor and sucrose treatments were upregulated, being respectively 28.1- and 30.2-fold higher than in the control, suggesting that *ERF1B-like* TFs participate in the sugar-induced regulation of flavonoid biosynthesis.

3. The results of yeast one-hybrid (Figure 2) and dual-luciferase assays demonstrated that *CsF3'H*, encoding flavonoid 3'-hydroxylase, was the target flavonoid biosynthetic gene for CsERF1B-like TF.

Our study identified the potential key regulators participating in the metabolism of sugars and flavonoids, providing new insights into the crosstalk between sugar metabolism and flavonoid biosynthesis in tea plants.

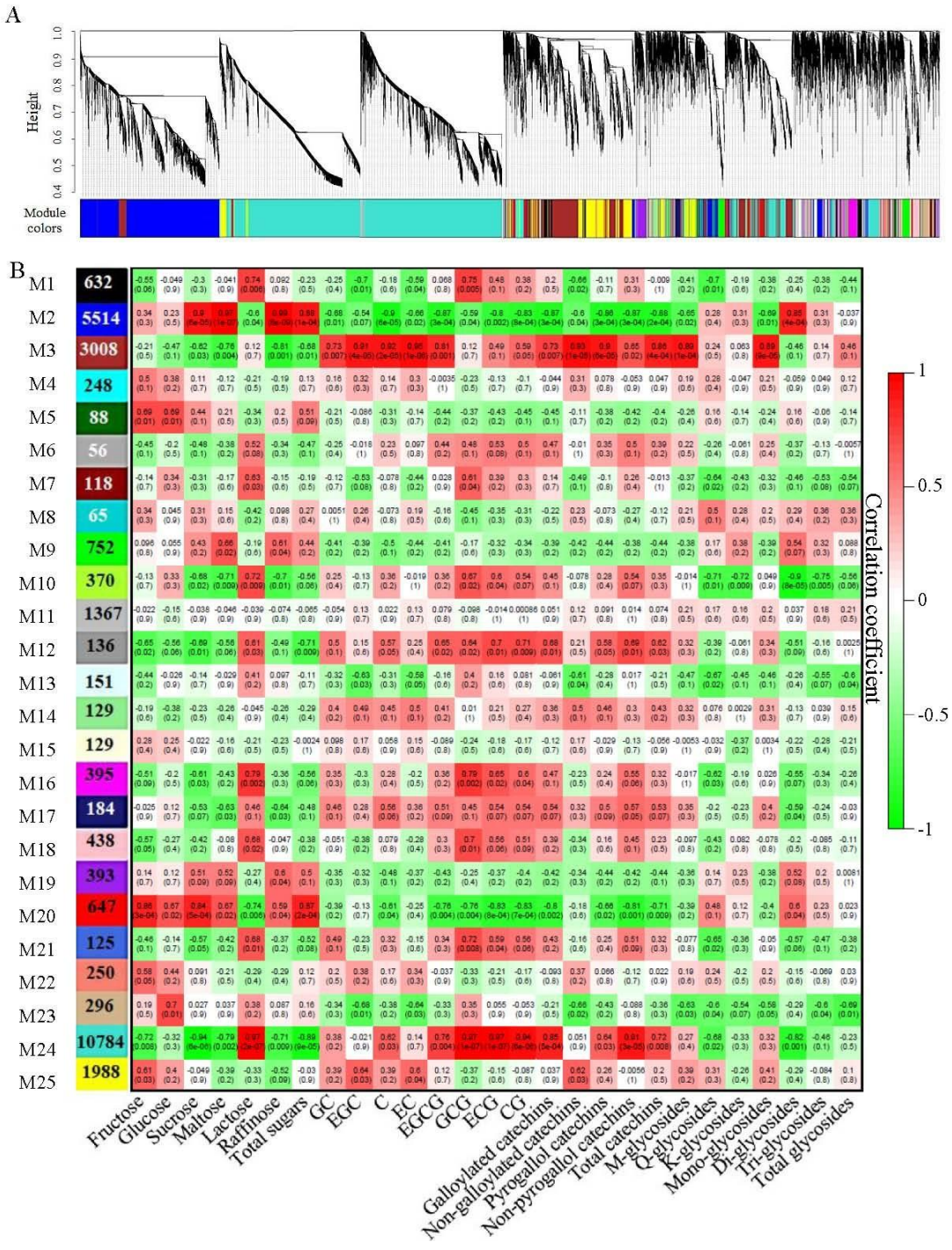


Figure 1 Signalling interactions between chemical compositions and global gene expressions at different growth stages

(A) Hierarchical clustering dendrogram of the average network adjacency for the identification of co-expression modules. The major tree branches constitute 25 modules labelled by different colors. (B) Module treatment association, with correlation coefficients (upper) and P-values (lower) annotated in each cell.

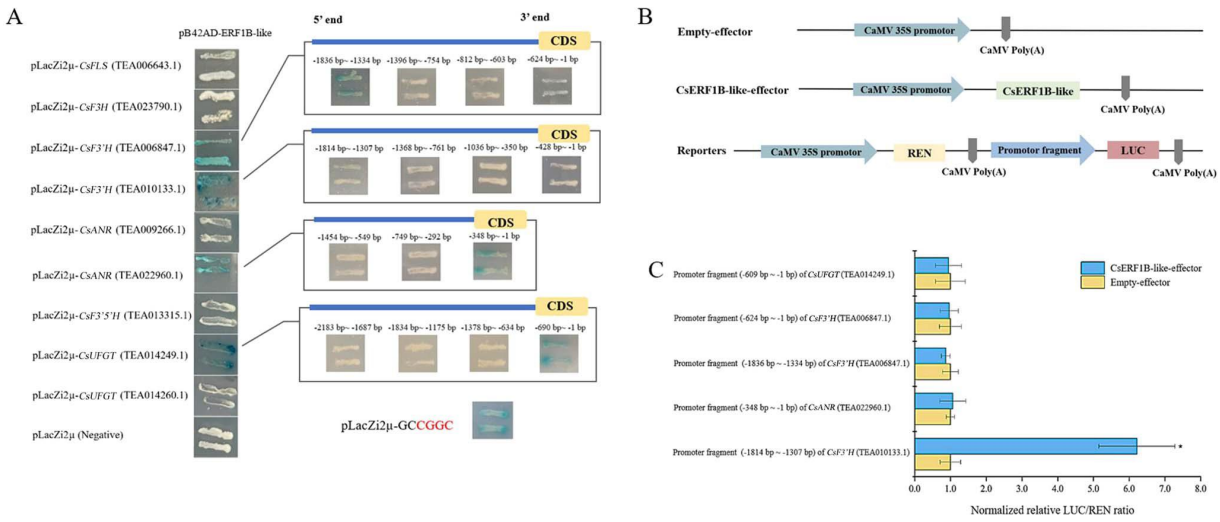


Figure 2 Characterization of CsERF1B-like interactions with the structural gene promoters of the flavonoid biosynthesis pathway in Y1H and dual-luciferase assays

A: Interactions of CsERF1B-like proteins and the promoter fragments of *CsFLS*, *CsF3H*, *CsF3'Hs*, *CsANRs*, *CsF3'5'H*, and *CsUFGTs* in yeast cells. pLacZi2μ was used as a negative control. SD/Gal/Raf/−Ura−Trp/+X-gal medium indicates yeast nitrogen base containing galactose, raffinose, and X-gal, without Ura and Trp. B: Construction diagrams of effector and reporter vectors for dual-luciferase assays. C: Normalized relative LUC/REN ratio.

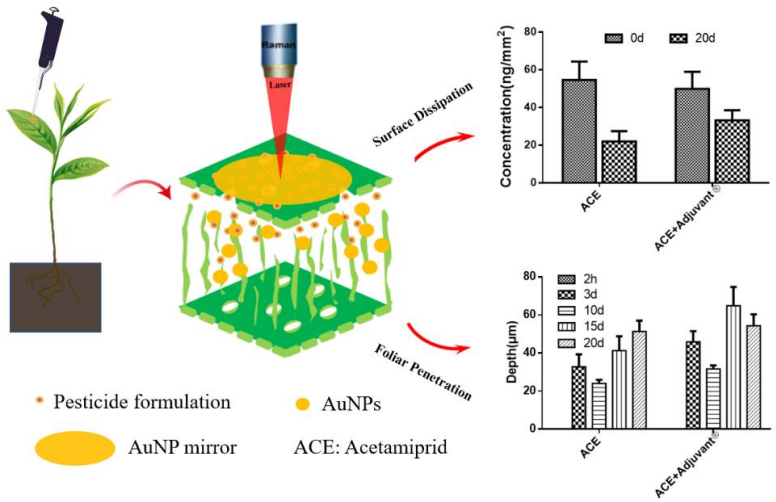
Surface Dissipation and Foliar Penetration of Acetamiprid on Tea Leaves in the Presence or Absence of Adjuvants

GAO Wanjun[1,2], HE Lili[2*], LUO Fan[1*]

1. Tea Research Institute, Tea Refining and Innovation Key Laboratory of Sichuan Province, Sichuan Academy of Agricultural Sciences, Chengdu 610066, China; 2. Department of Food Science, University of Massachusetts, Amherst, Massachusetts 01002, United States

The objective of this study is to investigate the surface dissipation and foliar penetration of a model insecticide, acetamiprid, following application to live tea plants in the presence and absence of two adjuvants (Alligare 90 and Peptoil). A surface-enhanced Raman scattering (SERS) mapping protocol with a self-assembled gold nanoparticle (AuNP) mirror as a substrate was utilized for in situ and real time pesticide analysis on live tea plants. The results showed that both adjuvants enhanced the spreadability of the pesticide. The SERS analysis revealed that the adjuvants had no significant effect on the characteristic peak of acetamiprid but reduced the SERS intensity significantly in the case of Peptoil due to the presence of a paraffin base petroleum oil used in its formulation. The surface dissipation analysis showed that acetamiprid with Alligare 90 had the lowest dissipation rate (34%) compared to acetamiprid alone (60%) or with Peptoil (44%), indicating the addition of Alligare 90 was more effective in reducing the surface dissipation of acetamiprid than Peptoil. The study also investigated the foliar penetration of acetamiprid into tea leaves in the presence and absence of adjuvants using SERS and liquid chromatography coupled with mass spectrometry. The results showed that the adjuvants improved the foliar penetration of acetamiprid. The study concludes that the two adjuvants tested can enhance the spreadability, surface stability, and foliar penetration of acetamiprid applied on tea leaves. The information from this study will facilitate the development and application of efficient and safe pesticide formulations.

TOC



The MADS-box Transcription Factor *CsAGL9* Plays Essential Roles in Seed Setting in *Camellia sinensis*

WEI Kang

Key Laboratory of Tea Biology and Resources Utilization, Ministry of Agriculture, National Center for Tea Improvement, Tea Research Institute Chinese Academy of Agricultural Sciences, Hangzhou 310008, China

The number of seed setting (NSS) is an important biological trait that affects tea propagation and yield. In this study, the NSS of an F_1 tea population (n=324) generated via a cross between 'Longjing 43' and 'Baihaozao' was investigated at two locations in two consecutive years. Quantitative trait locus (QTL) mapping of the NSS was performed, and 10 major QTLs were identified. In total, 318 genes were found in these 10 QTLs intervals, and 11 key candidate genes were preliminarily identified. Among them, the MADS-box transcription factor *AGAMOUS LIKE 9* (*CsAGL9*, CSS0037962) located in the most stable QTL (*qNSS2*) was identified as a key gene affecting the NSS. *CsAGL9* overexpression in *Arabidopsis* promoted early flowering and significantly decreased the length and number of pods and number of seeds per pod. Transcriptome analysis demonstrated that the auxin pathway, a key hormone pathway regulating plant reproduction, was highly affected in the transgenic lines. The auxin pathway was likewise the most prominent in the gene co-expression network study of *CsAGL9* in tea plants. In summary, we identified *CsAGL9* is essential for seed setting using QTL mapping integrated with RNA-seq, which shed a new light on the mechanism NSS of seed setting in tea plants.

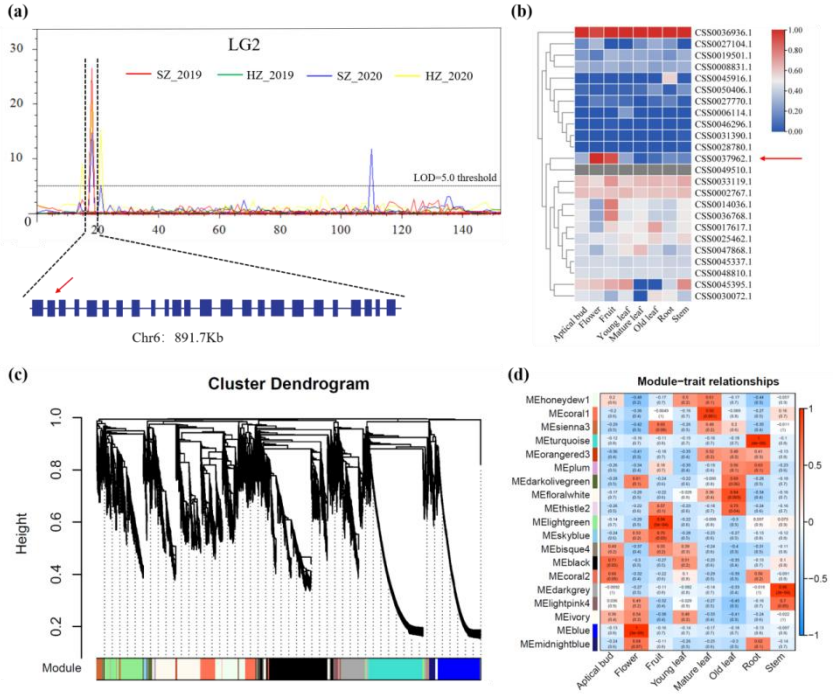


Figure 1 Fine mapping of the major QTL associated with the NUF trait and identification of flower-related genes

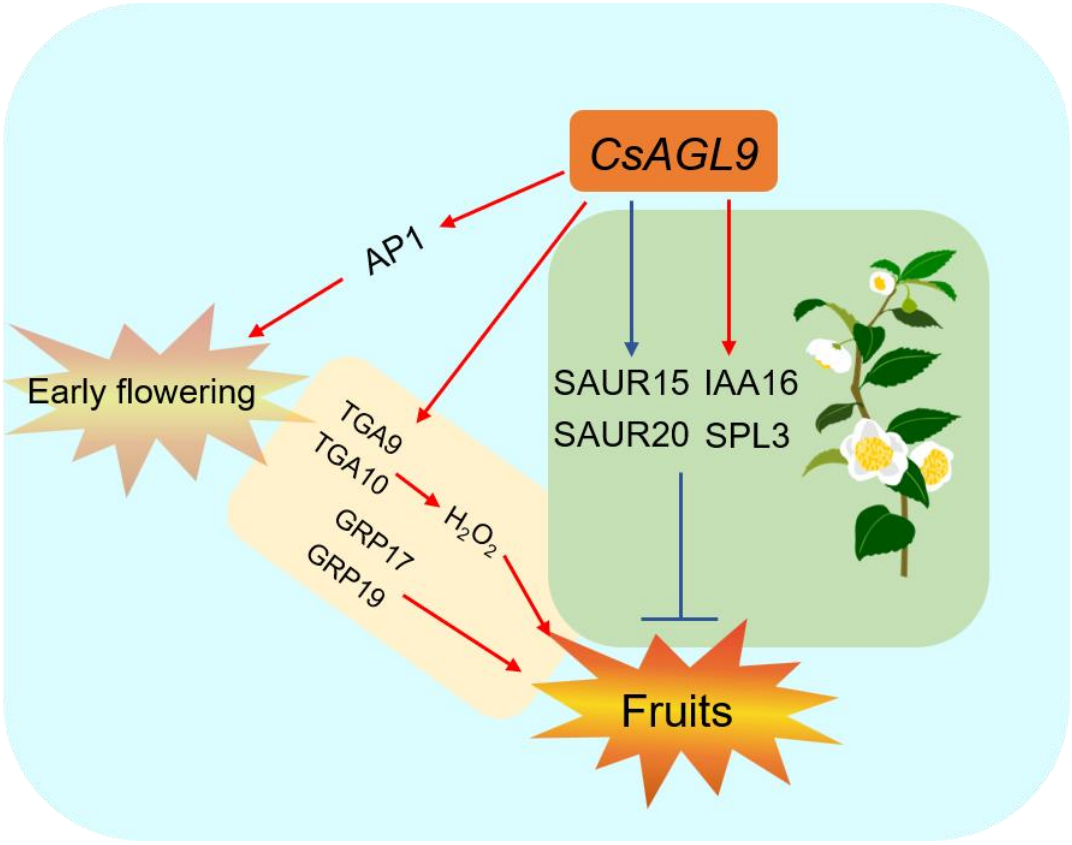


Figure 2 A speculated model for the molecular regulation mechanism of early flowering and fruit number of *CsAGL9* in tea plants

Transcriptome and Biochemical Analyses of a Chlorophyll-deficient Bud Mutant of Tea Plant (*Camellia sinensis*)

LI Yuanyuan*, ZHANG Chenyu*, MA Chunlei, CHEN Liang, YAO Mingzhe

Key Laboratory of Biology, Genetics and Breeding of Special Economic Animals and Plants, Ministry of Agriculture and Rural Affairs, Tea Research Institute of the Chinese Academy of Agricultural Sciences, Hangzhou 310008, China

Tea leaf-color mutants have attracted increasing attention due to their accumulation of quality-related biochemical components. However, there is limited understanding of the molecular mechanisms behind leaf-color bud mutation in tea plants. In this study, a chlorina tea shoot (HY) and a green tea shoot (LY) from the same tea plant were investigated using transcriptome and biochemical analyses. The results showed that the chlorophyll a, chlorophyll b, and total chlorophyll contents in the HY were significantly lower than the LY's, which might have been caused by the activation of several genes related to chlorophyll degradation, such as SGR and CLH. The down-regulation of the CHS, DFR, and ANS involved in flavonoid biosynthesis might result in the reduction in catechins, and the up-regulated GDHA and GS2 might bring about the accumulation of glutamate in HY. RT-qPCR assays of nine DEGs confirmed the RNA-seq results. Collectively, these findings provide insights into the molecular mechanism of the chlorophyll deficient-induced metabolic change in tea plants.

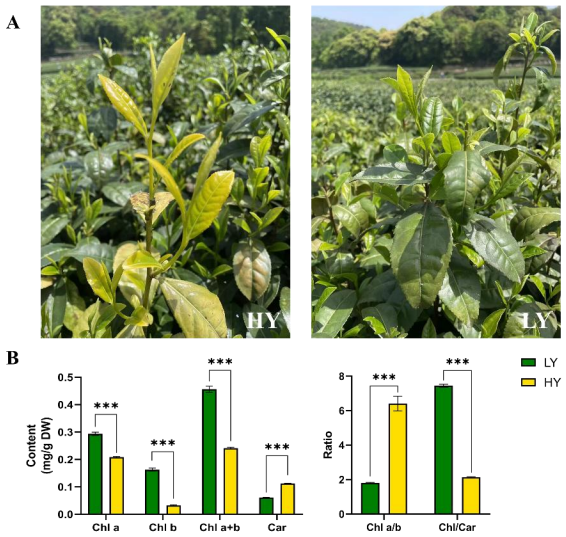


Figure 1 Plant phenotypes (A, captured on 16 April 2023) and comparisons in the contents of pigments (B) of the HY (the yellow bud mutant shoot) and the LY (the green tea shoot) tea shoots

The plots are presented as mean ± SEM (standard error of the mean, n = 3). The significant differences (*** $P < 0.001$) between LY and HY are determined by Student's t-test. Chl a, chlorophyll a; Chl b, chloro-phyll b; Chl a+b, the content of total chlorophyll a and chlorophyll b; Car, total carotenoids; Chl a/b, the ratio of Chl a to Chl b; and Chl/Car, the ratio of Chl a+b to Car.

*These authors contributed equally to this work.

Uncovering the Complex Regulatory Network of Spring Bud Sprouting in Tea Plants: Insights from Metabolic, Hormonal, and Oxidative Stress Pathways

TANG Junwei[1†], CHEN Yao[1†], HUANG Chao[1], LI Congcong[1], FENG Yue[2], WANG Haoqian[1], DING Changqing[1], LI Nana[1], WANG Lu[1], ZENG Jianming[1], YANG Yajun[1], HAO Xinyuan[1*], WANG Xinchao[1]

1. Key Laboratory of Biology, Genetics and Breeding of Special Economic Animals and Plants, Ministry of Agriculture and Rural Affairs/National Center for Tea Plant Improvement, Tea Research Institute, Chinese Academy of Agricultural Sciences, Hangzhou 310008, China; 2. Zhejiang Provincial Seed Management Station, Hangzhou 310020, China

Background

The sprouting process of tea buds is an essential determinant of tea quality and taste, thus profoundly impacting the tea industry. Buds spring sprouting is also a crucial biological process adapting to external environment for tea plants and regulated by complex transcriptional and metabolic networks. Tea cultivars that exhibit early and late bud sprouting can display a difference of over one month in their sprouting periods, even when exposed to the same environmental conditions.

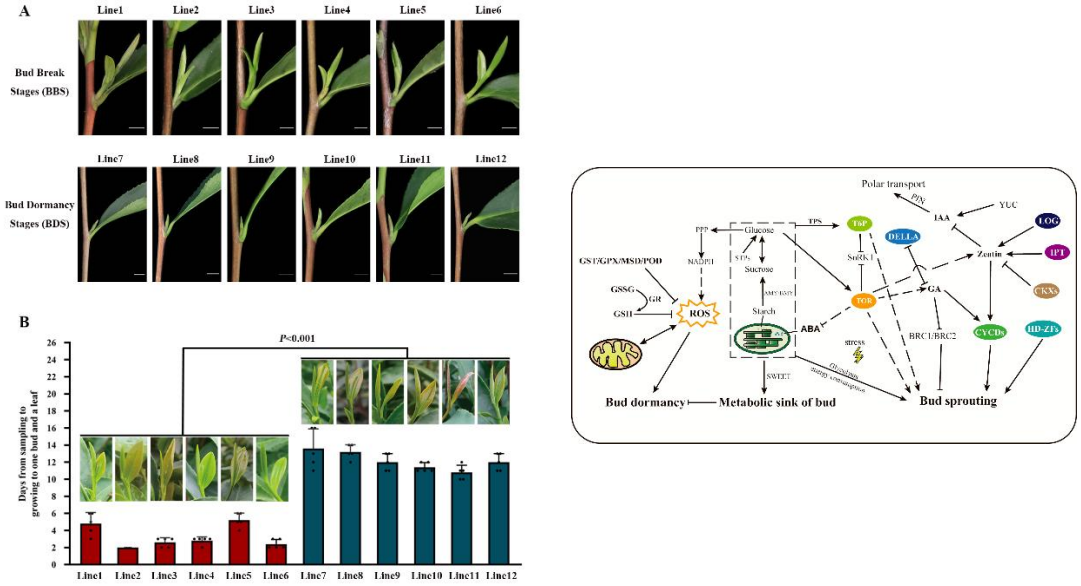
Materials and Methods

Experimental materials were planted in the germplasm resource garden located at the Tea Research Institute of the Chinese Academy of Agricultural Sciences (Hangzhou, Zhejiang Province, China (30°N18′, 120°E08′)). In the same plot, twelve lines were selected and sampled on April 13, 2022, in which six lines of ‘Yuqilin’, ‘Leibo 23’, ‘Guizhoudamaiya’, ‘Ankangzhong’, ‘Shubei’, ‘Wuyi 90’ were in bud break stage (respectively named BBS-1, BBS-2,BBS-3, BBS-4, BBS-5, BBS-6) and six lines of ‘Wuyi 43’, ‘Yunhun’, ‘Wuyi 83’, ‘Mandihong’, ‘Magucha’, ‘Ningzhou 6’ were in bud dormancy stage (respectively named BDS-1, BDS-2,BDS-3, BDS-4, BDS-5, BDS-6). The axillary buds of each line were collected for gene expression, transcriptional, and metabolism analyses. A portion of the bud samples were embedded in glutaraldehyde for histological analysis. Meanwhile, the sprouting time was recorded for each line until bud sprouting (one bud and a leaf).

Results

This study aimed to investigate the molecular basis of bud sprouting in tea plants firstly based on the comparisons of metabolic and transcriptional profiles of buds at different developmental stages. Results notably highlighted several essential processes involved in bud sprouting regulation, including the interaction of plant hormones, glucose metabolism, and reactive oxygen species scavenging. Particularly prior to bud sprouting, the accumulation of soluble sugar reserves and moderate oxidative stress may have served as crucial components facilitating the transition from dormancy to active growth in buds. Following the onset of sprouting, zeatin served as the central component in a multifaceted regulatory mechanism of plant hormones that activates a range of growth– related factors, ultimately leading to the promotion of bud growth. This process was accompanied by significant carbohydrate consumption. Moreover, related key

genes and metabolites were further verified during the entire overwintering bud development or sprouting processes. A schematic diagram involving the regulatory mechanism of bud sprouting was ultimately proposed, which provides fundamental insights into the complex interactions involved in tea buds.



Conclusions

In this study, tea buds in different sprouting states were selected to investigate the molecular basis of tea bud sprouting and identify key factors involved in the process. Transcriptional and metabolic analyses identified key genes and metabolites involved in bud sprouting, and a comprehensive pathway that governs bud sprouting in tea plants was suggested. This pathway is intricately linked to various factors such as carbohydrate content, energy metabolism, oxidative stress, and antioxidant capacity within the buds. Notably, the metabolism of soluble sugars, such as glucose and sucrose, as well as the effective clearance of reactive oxygen species, have emerged as pivotal elements preceding bud sprouting. Additionally, plant hormones, especially zeatin, was unveiled the central role in orchestrating bud growth. And regulatory proteins like CYCDs and TOR contribute to this complex regulatory process. Overall, this study provides insights into the complex mechanisms underlying tea plant bud sprouting.

Water-based Extraction for the Analysis of Nitenpyram and Pymetrozine in Tea

YU Huan[1], LIANG Yabo[1], PAN Canping[3], ZHOU Li[1,2*]

1. Tea Research Institute, Chinese Academy of Agricultural Sciences, Hangzhou 310008, China;
2. Key Laboratory of Tea Quality and Safety Control, Ministry of Agriculture and Rural Affairs, Hangzhou 310008, China;
3. Department of Applied Chemistry, College of Science, China Agricultural University, Beijing 100193, China

Nitenpyram and pymetrozine are widely used polar pesticides as well as in tea cultivation. More costly and toxic organic reagents were involved in the present detection methods. There is a continuing search for more eco-friendly methods. An eco-friendly approach was established and optimized to analyze the presence of two hydrophilic insecticides including nitenpyram and pymetrozine in tea. The method involved the use of boiling water instead of organic solvent, followed by PCX solid phase extraction (SPE) cleanup and determination with ultra-high-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS). On average 83.2%-98.8% of nitenpyram and pymetrozine were recovered from tea with relative standard deviations (RSDs) below 7%. The limits of quantification (LOQs) were 0.025 mg/kg. This organic solvent-saving approach offers a reliable and effective alternative for detecting nitenpyram and pymetrozine from tea samples, which can serve as a practical tool for tea safety and market supervision.

Yangta Dabai Tea

Breeding unit: Tea Research Institute of Yunnan Academy of Agricultural Sciences, Tea and Characteristic Biological Industry Development Center in Jinggu Dai and Yi Autonomous County.

Breeders: TIAN Yiping, LIAO Shengqiang, LIANG Mingzhi, LU Chuankun, CHEN Chunlin, SU Qilan, PANG Dandan, ZENG Yong, DENG Shaochun, FENG Mei, XU Pizhong, ZHANG Zhengyong, LI Dachang, ZHU Zhikun

Variety number: Non Major Crop Variety Registration No. GPD Tea Tree (2022) 530052

Variety source: The Tea Research Institute of Yunnan Academy of Agricultural Sciences and the Tea and Characteristic Biological Industry Development Center of Jinggu Dai and Yi Autonomous County has selected from the Jinggu 'Yangta Dabai Tea' group variety by single plant breeding. Selected as the leading agricultural variety in Yunnan Province in 2023.

Characteristics: Arbor type, large leaf type, medium bud species. The tree has an open posture, strong growth potential, high branching positions, and sparse branching density. The leaves grow upwards, the leaf shape is medium elliptical, with a length of 16.8 cm and a width of 6.7 cm, the leaf color is dark green, the blade tip has a sharp shape. The mining period is generally in early March, with one bud and two leaves blooming in late February. The germination density is medium, with much fuzz. The blooming period is in early October every year. One bud with three leaves is 12.1 cm long, and one bud with three leaves weighs 150.0 grams per hundred buds. The diameter of the corolla is 4.7 cm × 4.1 cm, with 6-8 petals, hairy ovary, and 3-lobed style. The biochemical samples of one bud and two leaves in spring contain 29.9% tea polyphenols, 3.8% amino acids, 5.2% caffeine, and 49.2% water extracts. This variety is suitable for making green tea, black tea, and white tea, black tea has a fresh, sweet and tender aroma, and a mellow and refreshing taste, green tea has a high, fresh, chestnut and floral aroma, a rich and sweet taste, the aroma of white tea is rich, sweet, and floral, with a sweet and refreshing taste. The first growth cycle produces 438 kilograms of fresh leaves per mu, an increase of 5% compared to the control 'Yunkang 10', the second growth cycle produces 453 kilograms of fresh leaves per mu, an increase of 4% compared to the control 'Yunkang 10'. Resistance to tea leaf blight, tea cake disease, and moderate resistance to tea green leafhopper, moderate drought resistance and weak cold resistance.

Yuncha Chunyun

Breeding unit: Tea Research Institute of Yunnan Academy of Agricultural Sciences

Breeders: BAO Yunxiu, HUANG Mei, YANG Xingrong, LIANG Mingzhi, LI Youyong, CHEN Linbo, TIAN Yiping, XU Pizhong, SUN Xuemei

Variety Number: Yunnan Province Non Major Crop Variety Registration No. Yunnan Registration Tea Tree No. 2012001

Variety source: The Tea Research Institute of Yunnan Academy of Agricultural Sciences has selected individual plants from the F1 generation of artificial pollination between Yunkang 14 (♀) and Fuding Dabai tea (♂). Selected as the leading agricultural variety in Yunnan Province in 2023.

Characteristic: Small arbor type, large leaf type, early bud species. The tree has an open posture, strong growth potential, high branching positions, and dense branching density. The leaves grow upwards, the leaf shape is medium elliptical, with a length of 12.1 cm and a width of 4.4 cm, the leaf color is green, the blade tip has a sharp shape. The mining period is generally in early March with one bud and two leaves blooming in mid to late February. The germination density is dense and there are much fuzz. The blooming period is in mid October every year. One bud with three leaves is 8.3 cm long, and one bud with three leaves weighs 70.2 grams per hundred buds. The diameter of the corolla is 4.3 cm × 3.9 cm, there are 7-8 petals, the ovary is hairy, and the style is 3-lobed. The biochemical samples of one bud and two leaves in spring contain 28.4% tea polyphenols, 2.7% amino acids, 3.7% caffeine, and 44.6% water extracts. This variety is suitable for making green tea, green tea has a green and moist appearance, showing buds, a tender green and clear color of the tea water, a long-lasting aroma, a fresh and mellow taste, and bright green foliage fundus. The first growth cycle produces 646 kilograms of fresh leaves per mu, an increase of 23% compared to the control 'Yunkang 10', the second growth cycle produces 728 kilograms of fresh leaves per mu, an 18% increase compared to the control 'Yunkang 10'. Resistant to tea green leafhopper, tea cake disease, strong cold and drought resistance.