Cluster analysis of the biochemical composition in 53 Sichuan EGCG3"Me tea resources

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Abstract. The EGCG3"Me contents in the young tea leaves of 102 tea resources in sichuan were analyzed accurately using HPLC-DAD. The results revealed that there was a wide variation in EGCG3"Me levels among different tea resources. The EGCG3"Me content in different tea resources was in a range from 0 to 11.04 mg/g, mean was 2.33 mg/g.53 tea resources contained EGCG3"Me,accounting for 51.96% of the total number of resources survey. Shucha5, Jinguanyin, Chengxi11, Fenghuang-dancong, Chongpi 71-1 were found to contain higher EGCG3"Me content (>10mg/g).Cluster analysis showed that: 53 Sichuan EGCG3"Me tea resources were divided into six groups and the difference was obvious between their biochemical composition; tea resources rich in EGCG3"Me were mainly distributed in Sichuan, Chongqing and Fujian Province, mostly were shrub and mid-leaf, mainly existed in tea resources which were suitable to make green tea, oolong tea. The morphological and biochemical distribution provided a good theoretical basis for selecting and utilizing higher EGCG3"Me resources.

1. Introduction
The catechins in tea are flavanols, accounting for over 80% of the polyphenols, which are mainly composed of (-) - epicatechin (EC), (-) - epigallocatechin (EGC), (-) - epigallocatechin gallate (EGCG), and (-) - epicatechin gallate (ECG). Catechins are beneficial in curing a wide variety of diseases [1, 5]. EGCG3"Me (3"-methyl-epigallocatechin gallate) is a methylated catechin of natural existence in tea [20, 21]. It has been proved stronger than EGCG in the aspect of anti-allergic [17, 18] inhibit inducible nitric oxide synthase (iNOS) expression [4],anti-hypertensive [11] and anti-obesity [10, 19] etc.

Sichuan is the birthplace of the world tea culture and the territory of tea resources is very rich, which mainly contain three categories: local resources, introduced resources and self-fertile resources. However, there are few reports about the catechins which contain many health functions. In some of the specific tea resources breeding, they mainly focus on the excavation of conventional components, such as, tea polyphenols, caffeine and amino acids [2, 3, 8]. There aren't studies on systematic screening of EGCG3"Me in Sichuan Tea Resources. At present, EGCG3"Me researches mainly focus on the separation, purification and preparation of tea leaves [9, 14, 24]. But, the reports on the screening of tea resources rich in EGCG3"Me (content> 10 mg / g) are fewer [13, 15]. There are more HPLC methods for simultaneous determination of 4 main catechins, CAF and GA in tea [6, 7, 12, 23]. The
Simultaneous detection of 4 main catechins and methylated catechins is less [22]. The simultaneous detection of 4 main catechins, methylated catechins, CAF and GA in tea is least [11, 25]. Therefore, in this study, the EGCG3"Me contents in the young tea leaves of 102 tea resources in Sichuan were analyzed firstly and accurately using a HPLC-DAD method [16]. The method has been proved to be a simple and sensitive in determining 1 methylated catechins (EGCG3"Me), 5 common catechin (EGC, C, EC, EGCG, ECG), caffeine (CAF) and gallic acid (GA) in tea. The tea resources rich in EGCG3"Me (content> 10 mg/g) were screened out. The 8 biochemical components of tea resources containing EGCG3"Me were analyzed by cluster analysis, in order to ascertaining the morphological and physicochemical characteristics of tea resources rich in EGCG3"Me. It provides a basis for the exploration of Sichuan tea resources rich in EGCG3"Me and the natural anti-allergy drugs in tea. Meantime, it is beneficial to the development of new functional tea. In addition, it is of great significance for the sustainable development of tea industry.

2. Materials

2.1 Materials

102 kinds of tea resources were respectively planted in Mingshan seed tea plantation in Sichuan Province, Sichuan Agricultural University tea plantation and Jiajiang Tianfu tea varieties garden in Sichuan.

2.2 Instruments and reagents

The DIONEX UltiMate 3000 UHPLC including: DGP-3600SD pump, WPS-3000 AutoSampler, TCC-3000 RS Column Compartment and Diode Array Detector, AcclaimTM 120 (5 μm, 120 Å, 4.6 mm×250 mm) C18 chromatographic column. It controls and processes some data with the help of the Chromelone 7 workstation (Dionex UltiMate). Velocity 18R centrifuge (Shimadzu, Japan), ALC-110.4 electronic balance (Germany Sartorius), DZKW-4 electrothermal thermostat water bath pot (Beijing Zhongxing Albert), Arium Comfort purified water machine (Germany Sartorius). Gallic acid, caffeine, C, EC, EGC, EGCG, ECG reference standards (Purchased from Sigma); EGCG3"Me reference standards and Japanese benifuji green tea (By the Key Laboratory of the Tea science, Ministry of Education, Hunan Agricultural University); KH2PO4, Phosphoric acid, Glacial acetic acid, ascorbic acid etc. are guarantee reagent. Acetonitrile and methanol are chromatographic grade, commercially available.

3. Method

3.1 Sample Preparation

Type According to the random sampling and stratified sampling method, 102 copies of tea resources, a bud and two leaves of autumn shoots, respectively, were picked up from mature disease-free shoots. Those shoots were collected with liquid nitrogen to save the back to the laboratory, steam cured, 90°C drying, -20°C refrigerator preservation.

3.2 Sample extraction

When Sample extraction was carried out according to the extraction method of literature [16].

3.3 EGCG3"Me content determination

Use The EGCG3"Me content was determined by referring to the chromatographic conditions of literature [16].
3.4 Data Analysis

Locate All the results were expressed as mean±SD (n=3), and the biochemical composition analysis and cluster analysis of EGCG3"Me tea resources were carried out with Excel 2013 and SPSS 17.0 statistical software.

4. Results and Analysis

4.1 Determination of EGCG3"Me in Sichuan Tea Resources

The EGCG3"Me contents in 102 tea resources in sichuan were analyzed accurately using HPLC-DAD. The results revealed that there were 53 tea resources containing EGCG3"Me. Also, there was a wide variation in EGCG3"Me levels among different tea resources. The EGCG3"Me content in different tea resources was in a range from 0 to 11.04 mg/g, and mean was 2.33 mg/g. The EGCG3"Me content was significantly lower than the main 4 catechins EGCG, EGC, ECG and EC (p <0.01) in most tea resources. The changes of catechins content were EGCG> EGC> ECG> EC> C. 53 tea resources contained EGCG3"Me, accounting for 51.96% of the total number of resources survey. Shucha5, Jinguanyin, Chengxi11, Fenghuangdancong, Chongpi71-1 were found to contain higher EGCG3"Me content (>10mg/g), respectively, 11.04 mg/g, 10.15 mg/g, 10.87 mg/g, 10.76 mg/g, 11.01 mg/g, accounting for 4.90% of the total number of resources survey. Origin of Sichuan accounted for 3 tea resources. It would be conducive to the promotion of owning good varieties (strains) in the west, and provide the basis for the exploration of Sichuan special tea resources. There were only 7 tea resources between 8 and 10 mg/g, accounting for 6.86% of the total survey resources. There were 2 tea resources with the content of 6~8 mg/g, accounting for 1.96% of the total survey resources, 8 tea resources with the content of 4~6 mg/g, accounting for 7.84% of the total survey resources; 31 tea resources in the range of 0~4 mg/g, accounting for 30.39% of the total survey resources. The part of the tea tree chromatogram was shown in Figure 1.

![Figure 1: Elution profiles of tea cultivar Qingxin-wulong; Note: 1.GA; 2.EGC; 3.CAF; 4.C; 5.EC; 6. EGCG; 7. EGCG3"Me; 8. ECG.](image-url)
4.2 Cluster analysis of biochemical components of 53 EGCG3"Me tea resources in Sichuan

4.2.1 Analysis of biochemical components of tea resources with EGCG3"Me. Never The statistical analysis of the 8 chemical components in 53 tea resources containing EGCG3"Me was carried out. The results were shown in table 1. As can be seen from table 1, there were obvious differences in the 8 biochemical components of tea resources. The variation range was large and the genetic diversity was abundant. The content of GA was low and was not detected in about 50% of tea resources, which caused the coefficient of variation greater than 1, so no further cluster analysis was done. The average variation of the other 7 biochemical traits was 45.75%, EGC (35.14%), CAF (37.59%), EC (25.30%), EGCG (41.28%), ECG (50.49%), ester-catechin (19.1%). The coefficient of variation of the 8 indexes ranged from 25% to 50%, and the variation range was larger. EGCG3"Me had the largest coefficient of variation (67.75%), followed by C (62.72%). The coefficient of variation of EGCG3"Me shown that there were abundant variation of tea resources with EGCG3"Me, which laid the foundation for the selection of fine varieties in the future.

| Component | Mean | Min | Max | S   | CV (%) |
|-----------|------|-----|-----|-----|--------|
| GA        | 0.53 | 0.02| 2.71| 0.63| 119.67 |
| EGC       | 41.72| 19.95| 71.82| 14.66| 35.14 |
| CAF       | 33.02| 12.83| 59.52| 12.41| 37.59 |
| C         | 1.40 | 0.04| 4.08| 0.88| 62.72 |
| EC        | 15.52| 4.71| 26.91| 3.93| 25.30 |
| EGCG      | 70.11| 22.06| 137.04| 28.94| 41.28 |
| EGCG3"Me | 4.54 | 0.46| 11.04| 3.07| 67.75 |
| ECG       | 16.55| 2.77| 38.76| 8.35| 50.49 |
4.2.2 Cluster Analysis of tea resources Containing EGCG3"Me. All the 7 biochemical components of 53 tea resources were clustered by mean Euclidean distance (figure 2). The biochemical components of each group were compared and analyzed according to the cluster results (table 2). In the genetic distance coefficient of 0.985, 53 tea resources were divided into 6 groups. Group I consisted of 18 resources and divided into two subgroups. One subgroup were Fuxuan4, Yucha2, Fujianshuixian, Longjingchanye, Baxiancha, Orange Osmanthus, Cuiyu, Meizhan, from top number 43 to bottom number 22. Another subgroup were Zhenong117, Mengshan29, Xicha5, Qingfeng, Huangyezao, Xiapu-yuantea, Huangguanyin, Mengshan16, Mingshan213, Chongqingpipacha, from top number 41 to bottom number 38. Group II included 14 resources, also divided into two subgroups. The first subgroup were Shuyong906, Shuyong401, Mabianlv1, Yinghong1, Jinxuan, Tianfu24, Sijichun, from top number 62 to bottom number 13. The second subgroup were Anxihsuixian, Huangdan, Lianandayecha, Shuyong2, Chengxi13, Tianfu28, Beichuan10, from top number 12 to bottom number 55. The III group included 6 resources, respectively, Yuenandaye, Yinghong2, Huangyeshuixian, Fuxuan9, Zhongcha102, Zhongcha302, from top number 95 to bottom number 23. The IV group included 3 resources, respectively, Tieguanyin, Maoxie, Shuyong808, from top number 77 to bottom number 21. The V group included 5 germplasm, respectively, Shucha5, Jinguanyin, Chengxi11, Fenghuang-dancong, Chongpi71-1, from top number 16 to bottom number 17. The VI group included 7 resources, respectively, Rougui, Chuannong2, Mengshan11, Fujian1, Qingxinwulong, Mingmeng8, Chengxi8, from top number 20 to bottom number 71.

Figure 2: Biochemical composition Dendrogram of 53 tea resources which contain EGCG3"Me

As can be seen from Table 2, there were significant differences in biochemical composition among groups. Group I and Group II had moderate levels of component contents. The highest average content of CAF and ECG in group III was 43.34 mg/g and 24.84 mg/g. EGCG3"Me had the lowest average content of 0.66 mg/g in group III. The average content of EGC in group IV was the highest, which was 57.19 mg/g. EC had the lowest average content of 11.51 mg/g in group IV. The highest content of EGCG3"Me in group V was 10.77 mg/g, which was the group of rich in EGCG3"Me. In addition, the average content of EC was the highest, 17.52 mg/g. The difference between the average content of EC in group V and group VI was not significant. In the group V, the average content of EGCG3"Me was relatively high, which was 8.62 mg/g, and the average CAF content was the lowest, which was 27.34 mg/g.
Table 2: Comparison of biochemical compositions among six clusters

| Component | Cluster I | Cluster II | Cluster III | Cluster IV | Cluster V | Cluster VI |
|-----------|-----------|-----------|-------------|------------|-----------|------------|
| EGC       | 38.50±3.31b | 37.92±3.76b | 38.49±5.74b | 57.19±8.12a | 52.63±6.29ab | 45.98±5.31ab |
| CAF       | 35.87±2.82ab | 28.60±3.19ab | 34.34±4.88a | 32.95±6.89ab | 30.79±5.34ab | 27.34±4.51b |
| C         | 1.50±0.20a | 1.00±0.23a | 1.48±0.35a | 2.11±0.50a | 1.72±0.39a | 1.33±0.33a |
| EC        | 16.24±0.91ab | 14.21±1.03ab | 16.53±1.57ab | 11.51±2.21b | 17.52±1.72a | 15.76±1.45a |
| EGCG      | 73.32±6.71a | 60.97±7.60a | 94.69±11.61a | 59.09±6.43a | 67.21±12.72a | 65.89±10.75a |
| EGCG3'Me | 3.92±0.10d | 2.36±0.11e | 0.66±0.17f | 6.25±0.25c | 10.77±0.19a | 8.62±0.16b |
| ECG       | 18.82±1.80ab | 11.66±2.04b | 24.84±3.12a | 13.33±4.41b | 16.54±3.41ab | 14.76±2.89b |

Note: Small letters represent the significantly different at 5% level (comparison between groups).

We can find the distribution of Morphology and adaptability in the 6 groups tea resources from table 3. Group I and Group II resources were dominated by shrubs, mid-leaf and suitable green tea resources. The group III resources were mainly in small tree, mid-leaf and suitable green tea resource. Group IV resources were dominated by shrubs and mid-leaf. The group V rich in EGCG3'Me and the group VI of higher content mostly were shrubs and mid-leaf, mainly existed in tea resources which were suitable to make green tea, oolong tea.

Table 3: Morphological characteristics and processing suitability of tea resources among 6 clusters

| Morphological characteristics and processing suitability | Cluster I | Cluster II | Cluster III | Cluster IV | Cluster V | Cluster VI |
|---------------------------------------------------------|-----------|-----------|-------------|------------|-----------|------------|
| Tree-type                                              |           |           |             |            |           |            |
| Shrub                                                  | 11        | 6         | 2           | 2          | 3         | 5          |
| Small tree                                             | 6         | 5         | 3           | 1          | 2         | 2          |
| Half tree                                              | -         | 1         | -           | -          | -         | -          |
| Tree                                                   | 1         | 2         | 1           | -          | -         | -          |
| Small leaf                                             | -         | -         | -           | -          | -         | 1          |
| Leaf-type                                              |           |           |             |            |           |            |
| mid-leaf                                               | 15        | 10        | 4           | 2          | 5         | 5          |
| Big leaf                                               | 3         | 4         | 2           | 1          | -         | 1          |
| Green tea                                              | 8         | 5         | 3           | -          | 2         | 4          |
| Oolong tea                                             | 2         | 3         | -           | -          | 1         | 1          |
| Processing suitability                                  |           |           |             |            |           |            |
| Oolong & Green tea                                     | -         | -         | -           | 1          | -         | 1          |
| Oolong, Black, Green tea                               | 4         | 1         | 1           | 1          | 1         | 1          |
| Black & Green tea                                      | 2         | 3         | -           | 1          | 1         | -          |
| Black tea                                              | -         | 2         | 2           | -          | -         | -          |

We can find the source and distribution of tea resources in the six groups from table 4. The group I resources were mostly dispersed in Fujian, Sichuan, Zhejiang. The group II resources were mainly in Sichuan. The group III resources were mainly dispersed in Zhejiang and Guangdong. The group IV resources came from Chongqing and Fujian. The group V rich in EGCG3'Me came from Sichuan, Chongqing and Fujian. The group VI of higher EGCG3'Me came from Sichuan and Fujian. Thus, the tea resources rich in EGCG3 were mainly distributed in Sichuan, Chongqing and Fujian, and most of them were shrub and mid-leaf. They were mainly suitable to make green tea, oolong tea or its concurrent varieties.

Table 4: Origins and distribution of tea resources among 6 clusters

| Province | Cluster I | Cluster II | Cluster III | Cluster IV | Cluster V | Cluster VI |
|----------|-----------|-----------|-------------|------------|-----------|------------|
| Taiwan   | 1         | 2         | -           | -          | -         | -          |
| Fujian   | 6         | 2         | -           | 2          | 2         | 3          |
| Sichuan  | 4         | 7         | -           | 2          | 2         | 4          |
5. Conclusion
The study found that the tea resources rich in EGCG3"Me, such as Shucha5, Jinguanyin, Fenghuang dancong and Chongpi 71-1, were suitable for Green Tea and Oolong Tea. In general, there are significant differences in biochemical composition among the six groups. The highest content of EGCG3"Me groups was group V, which is rich in EGCG3"Me, with an average content of 10.77 mg/g. In addition, the group has the highest average content of EC. The tea resources rich in EGCG3"Me are mainly found in shrubs, mid-leaf and green tea, oolong tea and its concurrent varieties. Those tea resources are distributed mainly in Sichuan, Chongqing and Fujian. These species distribution and physicochemical characteristics provide a good theoretical basis for the selection and development of rich in EGCG3"Me resources.

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