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Analysis on Pigment and Photosynthetic Characteristics of Leaves of New Strain of *Rehmannia glutinosa*

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Abstract Through the analysis on the leaf color and photosynthetic characteristics of new strains and main cultivars of *Rehmannia glutinosa*, it is expected to provide theoretical basis for breeding of new varieties. Chlorophyll, anthocyanin, and net photosynthetic rate (Pn), stomatal conductance (Cond), transpiration rate (Tr), and intercellular CO_2 concentration (Ci) in 8 varieties of *Rehmannia glutinosa* were measured by spectrophotometer and LI-6400XT Portable Photosynthesis System. The results showed that the chlorophyll content of Huaidijin 8 (2.84 mg/g), Huaidi 81 (2.71 mg/g), Huaidi 85 - 5 (2.69 mg/g), Jinjiu (2.66 mg/g) and Huaidi 83 (2.63 mg/g) was higher; the anthocyanin content of Jinjiu (0.169) and Huaidijin 8 (0.165) was higher, while the anthocyanin content Huaidi 83(0.060) was the lowest; Pn of Huaidi 81[2.41 μmol/(m^2 · s)], Huaidi 83[2.37 μmol/(m^2 · s)]and Huaidijin 8[2.25 μmol/(m^2 · s)]was higher, and the anthocyanin content was positively correlated with Pn, while the anthocyanin content was negatively correlated with Pn; Huaidijin 8 and Huaidi 83 showed dominant advantages in single plant fresh weight, indicator component, and resistance over the main cultivars. This indicates that the new variety Huaidijin 8 and Huaidi 83 have excellent comprehensive traits and can be properly popularized.

Key words *Rehmannia glutinosa*, New strains, Chlorophyll, Anthocyanin, Net photosynthetic rate (Pn)

1 Introduction

Rehmannia glutinosa is *Scrophulariaceae* medicinal herb, is a common traditional Chinese medicine used for tonifying and replenishing, one of the four famous traditional Chinese medicines, and sells well both at home and abroad[1]. With the traditional Chinese medicine going to the world, extensive researches have been made on *Rehmannia glutinosa*. In recent years, researches focus on chemical composition, pharmacological effects, and processing methods[2-3]. However, there are few researches about the leaf color and photosynthetic characteristics of *Rehmannia glutinosa*, while the intensity of photosynthesis plays an important role in its high yield. The genuine medicinal material green production technology team of Henan Province hybridized Shengjin (female parent) and Huaidi 85 - 5 (male parent) in 2011, and obtained the new strain Huaidijin 8 through directed screening and reproduction; hybridized Huaidi 85 - 5 (female parent) and Huaidi 3 (female parent) in 2012, and obtained new strain Huaidi 83 through directed screening and reproduction. We measured leaf color and photosynthetic characteristics of Huaidijin 8 and Huaidi 83, and compared correlation between chlorophyll, anthocyanin, and net photosynthetic rate (Pn), stomatal conductance (Cond), transpiration rate (Tr), and intercellular CO_2 concentration (Ci), to provide theoretical support for breeding of new varieties of *Rehmannia glutinosa*.

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2 Materials and methods

2.1 Sample materials The model field was planted on April 20, 2015 and managed in accordance with the field management method. Samples were collected from Wen County Zhangsi *Rehmannia glutinosa* model field on July 29, 2015. Sample materials included Huaidijin 8, Huaidi 81, Huaidi 85 - 5, Huaidi 83, Huaifeng, Qinhuai, Beijing 3, and Jinjiu.

2.2 Instruments and reagents Instruments: UV-1800 Ultraviolet-Visible Spectrophotometer (Beijing Beifen-Ruili Analytical Instrument Group Co., Ltd.); FA2204B electronic balance (Shanghai Jinghai Instruments Co., Ltd.); LI-6400XT Portable Photosynthesis System (American LI-COR).

Reagents: ethanol, acetone, anhydrous ethanol, 0.1 mol/L HCl, distilled water.

2.3 Measurement indicators and methods

2.3.1 Determination of chlorophyll content. Four fresh leaves of different varieties of *Rehmannia glutinosa* were collected, cleaned, dried, main veins were removed, leaves were beaten into small round pieces using 0.2 cm^2 circular sampler, and evenly mixed. Precisely weighed 0.2 g samples separately, put into 25 mL brown volumetric flask, added 25 mL acetone-ethanol mixed extract (the volume ratio of acetone to anhydrous ethanol was 1∶1). Each variety (strain) repeated 4 times. The chlorophyll extract was shaken and poured into the cuvette. Taking the extract reagent as the control group, the absorbance of the chlorophyll extract was measured at 645 nm and 663 nm; the chlorophyll concentration was calculated as per Arnon formula: mass concentration of chlorophyll a (C_a) = 12.7A663 - 2.69 A645; mass concentration of chlorophyll b (C_b) = $22.9A_{645} - 4.68A_{663}$; total

mass concentration of chlorophyll (C_{a+b}) = C_a + C_b, and it can be converted into the chlorophyll content per gram of fresh leaves (mg/g)[4].

2.3.2 Determination of relative content of anthocyanin. Precisely weighed 1 g material, leaves were beaten into small round pieces using 0.2 cm^2 circular sampler, put into the beaker, added 10 mL 0.1 mol / L HCl to the beaker, covered the beaker opening tightly, placed at 32 ℃ incubator, and soaked for more than 4 h, filtered with qualitative filter paper, and the filtrate was the anthocyanin extract. Taking the extraction reagent as the control group, the absorbance value was measured for the extract of anthocyanin at 530 nm by the spectrophotometer. Suppose the anthocyanin concentration with the absorbance value of 0.1 as 1 unit (namely, using the measured absorbance multiplied by 10 as the relative concentration of anthocyanin) to compare the relative content of anthocyanin[5].

2.3.3 Determination of photosynthetic parameter. The photosynthetic parameter was measured at 9: 00 – 11: 00 on July 29, 2015. Randomly selected ripe leaves of *Rehmannia glutinosa* (the second leaves from the top), measured the net photosynthetic rate (Pn), stomatal conductance (Cond) and transpiration rate (Tr) by LI-6400XT Portable Photosynthesis System, and selected 3 leaves for each treatment, and recorded 3 – 5 relatively constant values for each leaf.

3 Results and analyses

3.1 Comparison of chlorophyll and anthocyanin in different varieties of *Rehmannia glutinosa* From Table 1, it can be known that the chlorophyll content of the new strains and main cultivars of *Rehmannia glutinosa* was 2.51 – 2.84 mg/g; the chlorophyll content was Huaidijin 8 > Huaidi 81 > Huaidi 85 – 5 > Jinniu > Huaidi 83 > Huaifeng > Qinhuai > Beijing 3; the relative content of anthocyanin was Jinniu > Huaidijin 8 > Beijing 3 > Huaidi 81 > Huaifeng > Qinhuai > Huaidi 83.

There were extremely significant differences in the chlorophyll content between Huaidijin 8 and other varieties (strains); the difference in chlorophyll content was extremely significant between Huaidi 83, Qinhuai, Beijing 3, Huaidijin 8, Huaidi 81, and Huaidi 85 – 5, and not significant between Jinjiu. The difference in relative content of anthocyanin was not significant between Huaidijin 8 and Jinjiu, and not significant between all other varieties (strains); the relative content of anthocyanin was the lowest and showed extremely significant difference with all varieties (strains).

Through SPSS calculation and analysis, the coefficient of correlation between 8 varieties (strains) of *Rehmannia glutinosa* Pn and the chlorophyll content was 0.678, showing the positive correlation, failing to reach the significance level; the coefficient of correlation between 8 varieties (strains) of *Rehmannia glutinosa* Pn and relative content of anthocyanin was – 0.077, and there was negative correlation between relative content of anthocyanin and Pn.

Table 1 Comparison in the chlorophyll content and relative content of anthocyanin between new varieties and main cultivars of *Rehmannia glutinosa*

No.	Variety (Strain)	Chlorophyll content // mg/g	Relative content of anthocyanin // mg/g
1	Huaidijin 8	2.84 ± 0.034 aA	0.165 ± 0.0019 aA
2	Huaidi 81	2.71 ± 0.011 bB	0.105 ± 0.0058 cC
3	Huaidi 85 – 5	2.69 ± 0.038 bB	0.087 ± 0.0079 eD
4	Huaidi 83	2.63 ± 0.025 cCD	0.060 ± 0.0019 fE
5	Huaifeng	2.56 ± 0.030 dDE	0.103 ± 0.0034 cdC
6	Qinhuai	2.52 ± 0.028 deE	0.097 ± 0.0058 dCD
7	Beijing 3	2.51 ± 0.025 eE	0.136 ± 0.0026 bB
8	Jinjiu	2.66 ± 0.021 bcBC	0.169 ± 0.0038 aA

Note: capital and small letters separately denote significant difference at 0.01 and 0.05, the same below.

3.2 Comparison in photosynthetic parameter between different varieties (strains) of *Rehmannia glutinosa* From Table 2, it can be seen that Pn of the new cultivar and the main cultivars of *Rehmannia glutinosa* was 1.83 – 2.41 μmol/(m^2 · s), Pn of Huaidi 81, Huaidi 83, and Huaidijin 8 was higher, and Pn of Qinhuai was the lowest. Pn of Huaidijin 8 was 2.25 μmol/(m^2 · s), the difference between Huaidi 81, Huaidi 83, and Jinjiu was not significant, and the difference between all other varieties was extremely significant; Pn of Huaidi 83 was 2.37 μmol/(m^2 · s), the difference between Huaidi 81 and Huaidijin 8 was not significant, and the difference between all other varieties (strains) was extremely significant. Cond of Huaidijin 8 was 0.292 μmol/(m^2 · s), the difference between Huaidi 81 was not significant, and the difference between all other varieties (strains) was extremely significant; Cond of Huaidi 83 was 0.051 μmol/(m^2 · s), the difference between Huaifeng and Qinhuai was not significant, and the difference between all other varieties (strains) was extremely significant. Tr of Huaidijin 8 was 4.48 mmol/(m^2 · s), the difference between Beijing 3 and Huaidi 81 was not significant, but significant with Jinjiu, and the difference between all other varieties (strains) was extremely extremely; Tr of Huaidi 8 was 2.12 mmol/(m^2 · s), the difference between Qinhuai, Huaifeng, Huaidi 85 – 5, and Jinjiu, and the difference between Huaidi 81, Huaidijin 8, and Beijing 3 was extremely significant. Ci of Huaidijin 8 was 340.89 μL/L, the difference between Huaidi 81 and Huaidi 83 was significant and the difference between other varieties (strains) was extremely significant; Ci of Huaidi 83 was 336.2 μL/L, the difference between Huaidi 81 was not significant, the difference between Huaidijin 8 was significant, and the difference between all other varieties (strains) was extremely significant.

Through correlation analysis on Cond, Tr, and Ci by SPSS software, the results indicate that the coefficient of correlation between Pn and Cond was 0.235, showing positive correlation; the coefficient of correlation between Pn and Tr was 0.204, showing positive correlation, both failed to reach the significance level; the coefficient of correlation between Pn and Ci was −0.795, showing negative correlation, failing to reach the significance level.

Table 2 Comparison in photosynthetic parameter between new varieties and main cultivars of *Rehmannia glutinosa*

No.	Variety (Strain)	Pn//μmol/(m^2 · s)	Cond//μmol/(m^2 · s)	Tr//mmol/(m^2 · s)	Ci//μL/L
1	Huaidijin 8	2.25 ± 0.161ab AB	0.292 ± 0.020 bB	4.48 ± 0.28 aAB	340.89 ± 1.58 eD
2	Huaidi 81	2.41 ± 0.048 aA	0.290 ± 0.011 bB	4.31 ± 0.67 aAB	334.48 ± 2.51 fD
3	Huaidi 85 −5	2.02 ± 0.047 cdB	0.151 ± 0.014 cC	2.70 ± 0.62 bC	358.72 ± 0.97 cB
4	Huaidi 83	2.37 ± 0.045 aA	0.051 ± 0.020 dD	2.12 ± 0.45 bC	336.20 ± 3.98 fD
5	Huaifeng	1.85 ± 0.070 dC	0.061 ± 0.013 dD	2.36 ± 0.80 bC	362.88 ± 1.09 bAB
6	Qinhuai	1.83 ± 0.051 dC	0.048 ± 0.008 dD	1.99 ± 0.72 bC	362.72 ± 1.96 bAB
7	Beijing 3	1.91 ± 0.113 cdC	0.372 ± 0.074 aA	5.38 ± 0.89 aA	346.91 ± 2.50 dC
8	Jinjiu	2.08 ± 0.081 bcB	0.197 ± 0.010 cC	2.79 ± 0.24 bBC	367.96 ± 1.60 aA

4 Conclusions and discussions

In this experiment, different varieties (strains) of *Rehmannia glutinosa* were simultaneously planted in the same model field for unified management in accordance with the same standard, to avoid the impact of objective factors on the experimental results, to make the results more objectively and realistically reflect the difference between varieties (strains).

The results showed that the chlorophyll content was the highest in new variety Huaidijin 8, extremely significantly different from all other varieties (strains); the chlorophyll content of Huaidi 83 was higher than main cultivars Huaifeng, Qinhuai, and Beijing 3; the difference between Huaifeng was significant, and the difference between Qinhuai and Beijing 3 was extremely significant. Wei Shuluan *et al.* [6] stated that the level of chlorophyll content can directly reflect the intensity of photosynthesis. Researches of the relationship between chlorophyll content and photosynthetic in many crops have shown that chlorophyll has a positive correlation with photosynthetic rate within a certain range[7]. The results showed that the correlation coefficient between chlorophyll content and Pn was 0.678, which was consistent with the above results.

The relative content of anthocyanin in Huaidijin 8 was lower than that of all the other cultivars (strains), which was not significantly different from that of all other cultivars (strains). The relative content of anthocyanin was the lowest in Huaidi 83, showing extremely significant difference from all other varieties (strains). Anthocyanin is not photosynthetic pigment, but anthocyanins and plant photosynthesis had a close relationship[8], Pietrini *et al.* [9] hold that the anthocyanin in maize leaves could absorb larger portion of light with wavelength of 400 −700 nm, and calculated and compared the leaves containing and not containing anthocyanin, the it needed 60% quantum more for assimilating the equal amount of CO_2. This experiment showed that the relative content of anthocyanin was negatively correlated with Pn, which is consistent with the above results.

Pn of Huaidijin was lower than Huaidi 81 and Huaidi 83, and higher than all other varieties, the difference between Huaidi 81, Huaidi 83, and Jinjiu was not significant, the difference between Huaidi 85 −5 was significant, and the difference between all other varieties was extremely significant; Pn of new strain Huaidi 83 was lower than Huaidi 81, higher than all other varieties (strains), and the difference between Huaidi 81 and Huaidi 83 was not significant, but the difference between all other varieties (strains) was extremely significant. Net photosynthetic rate is a comprehensive reflection of photosynthetic physiological characteristics of plant, and also a direct indicator of the photosynthetic efficiency[10], indicating that the new strains Huaidijin 8 and Huai 83 have high photosynthetic efficiency.

The correlation results showed that chlorophyll content was positively correlated with Pn, and anthocyanin was negatively correlated with Pn, Cond and Tr were positively correlated with Pn, and Ci was negatively correlated with Pn.

In sum, the new strains Huaidijin 8 and Huai 8 have higher content of chlorophyll and higher Pn, and Huaidijin 8 has higher content of anthocyanin, while Huaidi 8 has the lowest content of anthocyanin. Previous researches showed that the Huaidijin 8 and Huaidi 83 have dominant advantages in single plant fresh weight, indicator component, and resistance over the main cultivars, dominant advantages in acteoside content over main cultivars, and relative advantage in Catalpol, and the indicator component is much higher than provisions in *Chinese Pharmacopoeia*, and they have higher resistance against leaf diseases[11]. This indicates that the new strains Huaidijin 8 and Huaidi 83 have excellent comprehensive traits and can be properly popularized.

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establish a professional team with high-level and high-quality media and ideological and political knowledge through rigorous selection procedures and diversified selection methods. In addition, it is recommended to strengthen the contact and communication with media professionals, and regularly invite prestigious professionals to participate in the media work of Tibetan agricultural colleges and universities, to enrich the ability and level of information dissemination. Tibet agricultural colleges and universities should regularly train this professional and full-time team, make the team have new ideas, master new knowledge, new skills, new methods, always be able to adapt to Tibetan economic and social requirements and demands of Tibetan agriculture, ensure this team always have higher ideological and political level, and have media theories and skills, so as to improve the ideological and political work of the media of Tibetan agricultural colleges and universities.

6.4 Stressing the effective integration of students' ideological and political work and the media work in Tibetan agricultural colleges and universities to ensure the formation of a tremendous joint effort Characteristics of the media of Tibetan agricultural colleges and universities determine that it has inborn affinity and integration with the students' ideological and political work. Tibet agricultural colleges and universities should combine the factors reflecting modern media in the teaching of ideological and political courses, to improve the quality and effectiveness of teaching. Especially, Tibetan government and the agricultural colleges and universities should fully improve the understanding of the students' ideological and political work, deepen the understanding level, and make effort to issue related rules and regulations as soon as possible, to ensure effective integration of them. Firstly, it is recommended to effectively promote their integration at the policy level. Secondly, it is recommended to implement their organic integration by various measures and means according to actual work situations. Besides, it is necessary to further improve the treatment and welfare of students' ideological and political workers in Tibetan agricultural colleges and universities, improve their sense of superiority and presence, to ensure the formation of a strong joint effort, so as to realize coordinated development and progress, and work together to create a bright future for Tibetan agricultural higher education.

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