Breeding of a Novel Cultivar 'Chengming No. 1' of *Chuanminshen violaceum*

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Abstract [**Objectives**] To obtain a novel cultivar of *Chuanminshen violaceum* with robust growth, high yield, and stable genetic traits. [**Methods**] A systematic selection method was employed to conduct a multi-point testing and regional production trial utilizing *C. violaceum* strain CMS1, which was sourced from a semi-wild population in Langzhong, Sichuan Province, as the experimental material. In contrast, *C. violaceum* CMS2, derived from a cultivated population in Langzhong, Sichuan Province, along with a mixed population of *C. violaceum* cultispecies from the same region, served as the control material. Through a comparative analysis of phenological periods, agronomic traits, yield, and quality, a novel cultivar, 'Chengming No. 1', was ultimately selected and developed based on its superior comprehensive evaluation. [**Results**] In the phenological period survey conducted as part of a two-year comparative study, the CMS1 strain exhibited a shorter growth cycle compared to others. Furthermore, the agronomic characteristics of the CMS1 strain were superior to those of both CMS2 and CK. The average yields of CMS1, CMS2, and CK in the 2019 cultivar comparison test and yield trial were 468.88, 448.52, and 422.15 kg/667 m^2, respectively. This resulted in an average yield increase of 11.07% for CMS1 compared to CK and 6.25% for CMS2 compared to CK. The average yields of CMS1, CMS2, and CK in the 2020 cultivar comparison test and yield trial were 482.69, 467.54, and 436.82 kg/667 m^2, respectively. CMS1 exhibited an average yield increase of 10.50% compared to CK, while CMS2 demonstrated an average yield increase of 7.03% relative to CK. Furthermore, the average yield of CMS1 per 667 m^2 achieved a statistically significant level compared to CK in both years of the study. In multiple-point comparison and yield trials conducted in 2019 and 2020, the CMS1 strain exhibited a total ash content of 15.30%, an acid-insoluble ash content of 1.30%, a moisture content of 10.80%, and water-soluble extract amounting to 11.40%. All of the indicators conformed to the criteria established by the *Sichuan Standards for Chinese Medicinal Materials* (*2010 Edition*). [**Conclusions**] The CMS1 strain successfully passed the field technical appraisal for the novel cultivar of *C. violaceum* in 2021. This cultivar is characterized by high yield, excellent quality, and stable traits. In 2022, it received validation from the Sichuan Provincial Committee for the Certification of Non-Staple Crop Varieties and was officially named 'Chengming No. 1' (CRY 2022002). This cultivar demonstrates significant potential for widespread cultivation.

Key words *Chuanminshen violaceum* M. L. Sheh & R. H. Shan, Systematic breeding, New cultivar, 'Chengming No. 1'

1 Introduction

Chuanminshen violaceum M. L. Sheh & R. H. Shan is a species belonging to the genus *Chuanminshen* in the family Umbelliferae. This plant is predominantly found in various locations in Sichuan, including Langzhong, Qingbaijiang, and Jintang[1]. *C. violaceum* contains many medicinal components, including polysaccharides, coumarins[2], imperatorin[3], *etc.* The dried root of this plant can be utilized for medicinal purposes, exhibiting pharmacological effects that include cough relief, phlegm elimination[4], immunomodulation[5–6], and antioxidant activity[7–8]. *C. violaceum* has been utilized for centuries as both a medicinal herb and a food source, contributing to the preparation of various culinary and medicinal products. Its consumption is associated with significant health benefits[9].

Currently, significant issues exist concerning the degradation of *C. violaceum* germplasm in various regions of Sichuan[10–11]. Despite the recent standardization of the cultivation and processing of *C. violaceum*[12–13], many farmers continue to utilize their own retained seeds without implementing a systematic seed selection process. This practice has resulted in increased variability in the cultivation of *C. violaceum*, which is evident in the form of reduced yields and inferior quality of the herbs. Consequently, this situation has led to diminished economic benefits for the farmers, adversely impacting their motivation to engage in cultivation. Currently, research aimed at enhancing the quality of *C. violaceum* primarily focuses on environmental factors, fertilization, and farming practices[14–16]. However, there is a notable absence of studies addressing the selection and breeding of new varieties of *C. violaceum*. Consequently, it is imperative to undertake the selection and breeding of new varieties that exhibit high yield, superior quality, and stable genetic traits.

In the initial phase of this experiment, germplasm resources of *C. violaceum* were collected from 11 regions, including Jintang in Sichuan Province. A novel strain of *C. violaceum* was identified, characterized by broad basal leaves, dark green leaves, vigorous growth, an increased number of bolting, and a reduced incidence of pests and diseases. In this study, we conducted a multi-point comparison and production test of new strains of *C. violaceum* to evaluate the differences in phenological periods, agronomic traits, yield, and quality in comparison to traditional cultivars in

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the production area. Notably, the new cultivar of *C. violaceum*, 'Chengming No. 1', successfully passed cultivar certification in 2022.

2 Materials and methods

2.1 Materials The test strain CMS1 is a superior line derived from the semi-wild population of *C. violaceum* in Langzhong, Sichuan Province, China, through a process of systematic selection and breeding. Similarly, CMS2 is a superior line selected from the cultivated population of *C. violaceum* in the same region, also through systematic selection and breeding. The control group, designated as CK, consists of a traditional cultispecies sourced from the production area, which encompasses a mixed population. The aforementioned seed sources were identified by Professor Zhang Li from the College of Science at Sichuan Agricultural University.

2.2 Methods

2.2.1 Design of cultivar comparison test. In 2019 and 2020, four sets of three-point trials were conducted in Group 6 and Group 8 of Kufeng Village, Qingquan Town, Chengdu City; Group 14 of Sanyuan Village, Renhe Township, Qingbaijiang District, Chengdu City; and Yunding Village, Huaikou Township, Jintang County, Chengdu City. Each trial employed a one-way randomization method and included three replications at each site. A rectangular plot with an area of 10 m^2 was established, bordered by protective rows approximately 60 cm in width. The production and planting conditions were consistent across all plots. Sandy loam soil was chosen as the test site, characterized by flat terrain, medium to high fertility, uniformity of the preceding crop, absence of fences or large trees that could shade the surrounding area, and a considerable distance from livestock.

2.2.2 Survey of phenological period. The experimental materials were sown after the End of Heat period, specifically on August 22, 2019, and August 22, 2020. The seedling emergence stage was defined as the interval from sowing until 50% of the seedling buds were visible. The full seedling stage was characterized by the time at which 90% of the seedling buds were evident. The budding stage was recorded as the date when flower buds were observed on 1% of the plants. The initial flowering stage was noted as the date when flowers opened on 1% of the plants, while the full blooming stage was defined as the period when flowers were open on 90% of the plants. The growth and overall condition of *C. violaceum* plants were systematically monitored throughout the phenological period.

2.2.3 Cultivation management. The materials were planted in early September. The fertilizer was uniformly distributed into the soil, followed by thorough excavation and the unclogging of drainage ditches. After trenching the field, the surface of the ridges was shaped to resemble tiles. On the day for seedling planting, horizontal ditches were excavated with a spacing of 25 cm between each line. Well-rotted farmyard manure was applied within the ditches, and the seed roots were positioned at intervals of 7 cm between individual plants, adhering to the specification that the diameter of the central portion of the root should range from 0.5 to 1.0 cm. The buds were oriented upwards, and the root systems were spread out and straightened before being covered securely with loose soil. This procedure was implemented to protect the seedlings from direct sunlight and to maintain adequate moisture levels. Subsequently, 70% of the coverings were removed once the seedlings emerged. Following the emergence of seedlings, two weeding activities were conducted in December and early February of the subsequent year. Subsequently, based on the growth of weeds, additional weeding was performed as necessary to maintain soil aeration. Fertilizer was applied twice after the seedlings emerged; the initial application occurred when the seedlings developed 3 – 4 true leaves, while the second application took place after the completion of weeding following the Spring Festival. In early to middle March, the removal of manual bolting was performed prior to the emergence of buds and flowers. Timely drainage should be implemented during the rainy season. Diseased plants must be promptly removed upon detection; the affected areas should be sterilized with lime powder, and the dead branches and leaves of the diseased plants should be collected and incinerated.

2.2.4 Survey of agronomic traits. The materials were harvested in late March of the subsequent year following planting, and 10 whole-plant samples were randomly collected from each trial site to investigate agronomic traits. Stems and leaves were selected from healthy, upright portions of the plant, ensuring they were free from disease, damage, and drooping. The longest roots from multiple branches were chosen for analysis. Leaf coloration was recorded, and the number of stems was quantified using a counting method. Additionally, the following measurements were taken using vernier calipers and a straightedge: the central portion diameter of the stems, the terminal lobe width, the root length (defined as the distance from the threaded section to the tip of the longest root), and the root thickness (measured as the diameter of the central portion of the root). The fresh weights of the stems, leaves, and roots were measured using a balance. Subsequently, the roots were dried in a constant temperature oven at 50 ℃ and subsequently weighed to determine the moisture content. The data from 10 samples were averaged and analyzed.

2.2.5 Yield measurement. Following the harvesting process, a regional assessment was performed to evaluate the yield. The yields from the four experimental sites were documented during the two-year cultivar comparison test. Subsequently, Excel was utilized to organize the data, facilitating the calculation of the annual equivalent yield per 667 m^2, the average equivalent yield per 667 m^2 over the two-year period, and the percentage increase in yield relative to CK. The analysis was conducted using SPSS software, specifically employing the one-way ANOVA calibration to compare the average of the dependent variables, which included equivalent yield per 667 m^2 and percentage yield increase over

CK. The cultivar name was designated as the factor. Post hoc comparisons were performed using the Least Significant Difference (*LSD*) and Duncan's multiple range test, with the relevant descriptions selected. The processing and analysis of the results were carried out by assessing the significance of multiple comparisons to evaluate the feasibility of the test. Additionally, the analysis incorporated the homogeneous subset, as well as calculations of the average and standard deviation, to implement the Letter labeling method for the significant analysis of differences.

2.2.6 Measurement of quality indicators. The total ash content, moisture content, and water-soluble extract of the herbs were measured in accordance with the method outlined in the *Sichuan Standards for Chinese Medicinal Materials (2010 Edition)*.

3 Results and analysis

3.1 Survey of phenological period The results of the survey into the phenological periods of 2019 and 2020 (Table 1) indicate that there were notable differences among various strains of *C. violaceum* during each phenological period. The seedling emergence stage and the full seedling stage of CMS1 occurred approximately 1 – 4 d earlier than those of CMS2 and CK. The differences in the duration required for the three strains to progress from the initial flowering stage to the full blooming stage were minimal, with all strains requiring approximately 3 – 4 d. CMS1 exhibited minimal differences from CMS2 during both the budding and initial flowering stages, with a variation of approximately 1 – 3 d. In 2020, CMS1 reached the budding stage concurrently with CMS2; however, there was a temporal gap of approximately 4 – 5 d when compared to CK. The growth of CK was characterized as average and mixed, whereas the other two strains exhibited robust and orderly growth. In summary, all three strains of *C. violaceum* exhibited a fertility period of approximately 210 d. CMS1 exhibited excellent performance during the seedling emergence stage, budding stage, and initial flowering stage. In contrast, CMS2 demonstrated a slightly extended duration for seedling emergence, while the overall performance of CK was deemed ordinary.

Table 1 Phenological periods of different *Chuanminshen violaceum* strains

Phenological period	2019			2020		
	CMS1	CMS2	CK	CMS1	CMS2	CK
Planting date//(year/month/day)	2019/08/22	2019/08/22	2019/08/22	2020/08/22	2020/08/22	2020/08/22
Seedling emergence stage//(year/month/day)	2019/09/20	2019/09/24	2019/09/21	2020/09/19	2020/09/22	2020/09/21
Full seedling stage//(year/month/day)	2019/09/26	2019/09/29	2019/09/30	2020/09/25	2020/09/26	2020/09/30
Budding stage//(year/month/day)	2020/01/22	2020/01/25	2020/01/27	2021/01/25	2021/01/25	2021/01/29
Initial flowering stage//(year/month/day)	2020/02/10	2020/02/12	2020/02/15	2021/02/09	2021/02/10	2021/02/13
Full blooming stage//(year/month/day)	2020/02/14	2020/02/15	2020/02/19	2021/02/13	2021/02/14	2021/02/16
Harvest date//(year/month/day)	2020/03/20	2020/03/20	2020/03/20	2021/03/20	2021/03/20	2021/03/20
Growth	Robust	Robust	Ordinary	Robust	Robust	Ordinary
Evenness	Neat	Neat	Mixed	Neat	Neat	Mixed

NOTE The seedling emergence stage is characterized by 50% of seedlings having emerged from the soil; the full seedling stage is defined as the point at which 90% of seedlings have emerged; the budding stage is identified when 1% of plants exhibit flower buds; the initial flowering stage is marked by 1% of plants having opened flowers; the full blooming stage is defined as the occurrence of flowers in 90% of the plants.

3.2 Survey of agronomic traits The agronomic traits of *C. violaceum* were categorized into aboveground and underground parts, with the results of the measurements conducted in 2019 and 2020 presented in Table 2. The results pertaining to the aboveground part of the plants indicate that the stem diameters of CMS1 over a two-year period were measured at 0.49 and 0.48 cm, respectively. These measurements were found to be 0.05 to 0.06 cm greater than those of the other two strains. While all three strains exhibited green stem coloration, CMS1 demonstrated a greater abundance of stem strips and leaves, with a darker green leaf coloration. In contrast, CMS2 and CK exhibited fewer stem strips, which contributed to a notable difference in the fresh weight of the stems and leaves compared to CMS1, as well as a lighter coloration of the stems and leaves. The terminal lobe width of CMS1 ranged from 1.0 to 2.0 cm, exhibiting a difference of approximately 0.4 cm when compared to the other two strains. Additionally, the root length of CMS1 was found to be 1.53 – 1.77 cm greater than that of CMS2 and CK in both years, specifically in the underground part. The variation in root thickness among the strains was minimal, measuring around 0.1 cm. The root weight of CMS1 was significantly greater, with individual root weights in 2019 and 2020 measuring 2.82 and 2.40 g, respectively, above the average weight recorded in the experiment. In contrast, the root fresh weights of the other two strains were below the average. The root moisture content among the three strains exhibited minimal variation, with all measurements being ≤13% or ≤14%. In summary, the stem of CMS1 is characterized by increased thickness, while the terminal lobe width ranges from 1.0 to 2.0 cm. The leaf exhibits a dark green coloration, with clearly defined veins. The top lobe is three-lobed, and the root is both thick and cylindrical. The root possesses a sweet taste, a hard and brittle texture that facilitates easy breakage, and a cross-section that is light yellow, semi-transparent, and exhibits a waxy luster (Fig. 1). Additionally, the fresh root weight of a single plant exceeds 40 g, and the moisture content of the root is ≤13%.

Table 2 Agronomic traits of different *Chuanminshen violaceum* strains

Part	Measurement indicator	2019				2020			
		CMS1	CMS2	CK	Average	CMS1	CMS2	CK	Average
Above-ground	Central portion diameter of the stem//cm	0.49	0.44	0.43	0.45	0.48	0.44	0.40	0.44
	Number of stems//strip	5-6	2-3	2-5	3-5	5-6	2-3	3-6	3-5
	Fresh weight of stems and leaves//g	64.21	53.71	53.58	57.17	60.18	53.64	54.75	56.19
	Leaf color	Dark green	Light green	Mixture of dark green and green		Dark green	Light green	Mixture of dark green and green	
	Terminal lobe width//cm	1.0-2.0	0.6-1.8	0.6-2.0	0.7-1.9	1.0-2.0	0.6-1.8	0.6-2.0	0.7-1.9
Under-ground	Root length//cm	27.93	26.57	26.40	26.97	28.11	26.61	26.34	27.02
	Root thickness//cm	1.21	1.11	0.98	1.10	1.20	1.09	0.97	1.09
	Root fresh weight//g	40.44	36.53	35.88	37.62	40.30	37.73	35.67	37.90
	Root moisture content//%	≤13	≤13	≤14	≤13.33	≤13	≤13	≤14	≤13.33

Fig.1 Comparison of whole plant (left) and root (right) of *Chuanminshen violaceum* strains CMS1, CMS2, and CK

3.3 Cultivar comparison test of *C. violaceum* strains across different years In 2019, the average dry yield of the CMS1 strain was 468.88 kg/667 m^2 across four experimental test sites, representing an increase of 11.07% compared to CK (Table 3). This yield was significantly higher than that of the other two strains, positioning CMS1 as the highest yielding strain among the three evaluated. In contrast, the average yield of CK was 422.15 kg/667 m^2 across the same four experimental test sites, which was significantly lower than the yields of the other two strains. In 2020, the average yield of CMS1 across four experimental test sites was 482.69 kg/667 m^2, representing a 10.50% increase compared to CK. This yield was the highest among the three strains evaluated. Furthermore, the difference in yield between CMS1 and CMS2 was not statistically significant. In contrast, the average yield of CK was 436.82 kg /667 m^2 across the same four experimental sites, which was significantly lower than that of the other strains. In summary, the CMS1 strain exhibited the highest average yield in two consecutive years of cultivar comparison tests, ranking first among all evaluated strains. A comparison with CK indicated that the CMS1 strain achieved the most significant yield increase in both 2019 and 2020, demonstrating commendable stability.

Table 3 Cultivar comparison test of different *Chuanminshen violaceum* strains

Indicator	Strain	2019	2020	Two-year average
Yield//kg/667 m^2	CMS1	468.88 ± 5.45^a	482.69 ± 14.63^a	475.79 ± 9.68^a
	CMS2	448.52 ± 8.04^b	467.54 ± 13.67^a	458.03 ± 13.35^{ab}
	CK	422.15 ± 6.42^c	436.82 ± 4.22^b	429.52 ± 10.42^b
Yield increase compared to CK//%	CMS1	11.07 ± 1.69^a	10.50 ± 2.30^a	10.77 ± 0.43^a
	CMS2	6.25 ± 0.79^b	7.03 ± 2.48^b	6.64 ± 0.54^b
	CK	0^c	0^c	0^c

NOTE Different lowercase letters for the same indicator within the same year signify statistically significant differences ($P < 0.05$).

3.4 Measurement of quality indicators of the CMS1 strain The assessment was conducted in accordance with the *Sichuan Standards for Chinese Medicinal Materials (2010 Edition)*. The specifications stipulate that the total ash content must not exceed 18.0%, the acid-insoluble ash must not exceed 1.5%, the moisture content must not exceed 12.0%, and the water-soluble extract must be no less than 10.0%. During the analysis, the water-soluble extract of CMS1 was found to be 11.40%, the total ash content was 15.30%, the acid-insoluble ash content was 1.30%, and the moisture content was measured at 10.80%. All indicators were found to be in compliance with the established regulations.

Based on the aforementioned studies, the CMS1 strain, which possesses a well-defined origin, stable genetic characteristics, and reliable field performance, fulfills the criteria for a new cultivar of *C. violaceum*. This strain was officially recognized by the Sichuan Provincial Committee for the Certification of Non-Staple Crop Varieties in 2022 and was officially named ‘Chengming No. 1’ (CRY 2022002).

4 Discussion

In 2008, our research group identified a promising single plant, designated as CMS1, within a semi-wild population of *C. violaceum* located in Langzhong City. The self-pollinated seeds from this promising single plant CMS1 were collected in 2009 and were subsequently propagated in August of the same year. Between 2010 and 2015, the CMS1 strain was developed through a process of successive plant selection and self-pollination aimed at seed preservation. Subsequently, from 2016 to 2018, the CMS1 strain underwent three consecutive years of propagation, which was characterized by vigorous growth, high yield, and stable genetic traits. In 2019 and 2020, multi-point cultivar comparison tests were conducted, revealing that CMS1 exhibited robust growth, high yield, and stable traits in comparison to other strains. In 2021, CMS1 successfully underwent a field technical appraisal conducted by an expert group. Subsequently, in 2022, it received validation from the Sichuan Provincial Committee for the Certification of Non-Staple Crop Varieties, and was officially named ‘Chengming No. 1’ (CRY 2022002).

C. violaceum has a longstanding history of cultivation, achieving yields of 250 – 260 kg/hm^2 in certain production areas of Sichuan Province, including Qingbaijiang and Yunding. In contrast, the cultivar ‘Chengming No. 1’ demonstrated favorable performance across both years of yield assessment in the cultivar comparison test. The prolonged field mixing of common *C. violaceum* plants has resulted in the degradation and mutation of their desirable traits. Consequently, the rapid dissemination and application of the new *C. violaceum* cultivar ‘Chengming No. 1’ is of considerable importance.

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