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Identification of Phenological Growth Stages of Four *Morus* Species Based on the Extended BBCH-Scale and Its Application in Fruit Development with Morphological Profiles and Color Characteristics

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Abstract: Mulberry (*Morus* L.; Moraceae; Rosales) is an economically important tree with a long history in China and valued for its rich nutrient, flavor content, medicinal value and useful ecological functions. *Morus* species are widely distributed in Asia, Europe, North and South America and Africa and exhibit obvious differences in morphological characteristics and phenological stages due to being distributed in diverse climate zones. The morphological description of the phenological stages of black mulberry (*Morus nigra* L.) has been established in Mediterranean climates and this study extended the BBCH scale for application in different *Morus* species, especially for mulberry trees grown in subtropical monsoon climates. In this study, we used the BBCH (Biologische Bundesanstalt, Bundessortenamt and CHemische Industrie) scale to describe in detail the phenological growth stages for *Morus* species in Wuhan, China (a subtropical monsoon climate). Based on this general scale, eight principal stages, i.e., bud, leaf and shoot development stage, inflorescence emergence stage, flowering stage, fruit development stage, fruit maturation stage, senescence and beginning of dormancy stage, were described. We provide photographic images of some primary and secondary developmental stages to better define and standardize morphological characteristics and phenological descriptions of these mulberries (*Morus* spp.). In addition, because the color of mulberry fruit varies from white, purple, black, or pink to red at maturity, with mostly monosexual flowers, we have also presented detailed descriptions of morphological traits of flower sexuality and fruit color among these four species. In addition, two late phenological germplasms (late bud sprouting associated with late blooming and late fruit ripening) were screened out according to the BBCH scale. Overall, this study will contribute to advance the field of mulberry breeding and implementation of agronomic practices and facilitate convenient communication between mulberry cultivators and researchers in different areas.

Keywords: mulberry; BBCH scale; growth stage; phenology; sexuality; color

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1. Introduction

Mulberry, genus *Morus* (Rosales, Moraceae), is a fast-growing deciduous woody tree that grows widely in Asia, Europe, North and South America and Africa [1]. *Morus* trees show high genetic diversity in Asia, especially in China and Japan [2]. Mulberry are multipurpose and economically important plants, which can be used not only in sericulture, but also in wine, juice, jam and canned food, as well as in medicinal products [3].

The taxonomy of *Morus* has long been controversial due to its wide geographical distribution, morphological and sexual plasticity, interspecific hybridization, long history of domestication, and the introduction and naturalization of species [4–6]. Therefore, various studies have recognized a variable number of *Morus* species [7] and most of the classification and description of *Morus* was conducted using morphological characters of leaves and female reproductive traits [8–12]. Nonetheless, 10-16 species in *Morus* are widely cited and recognized [11,13], with 12 species recognized in China alone [11]. However, Zeng et al. [7] suggested that *Morus* should be classified into eight species using information from internal transcribed spacer (ITS) genetic sequences and found that some generally accepted *Morus* species (e.g., *M. multicaulis* and *M. wittiorum* in this work) are synonyms or subspecies/varieties of *M. alba*.

Mulberry is an out-breeding and wind-pollinated perennial tree with the fastest pollen release rate in the plant kingdom, at more than half the speed of sound [14]. Compared to seed dispersal, the proportion of gene flow via pollen dispersal is higher in wind-pollinated trees [15], which results in highly heterozygous mulberry. Although many researchers have classified *Morus* based on flower traits, especially the style length, minimal research has focused on sex expression. The sexuality of various mulberry cultivars is diverse, with monoecious, dioecious and bisexual individuals [16]. Jolly et al. [17] reported the sex composition of 124 *Morus* accessions from diverse geographical sources in India, of which 75 were dioecious (17 bearing male flowers and 58 bearing female flowers), 44 were monoecious and five were non-flowering, consistent with the findings of Minamizawa [18]. There are fewer and fewer male-flower-only (androecious) germplasms due to their elimination over the long history of mulberry cultivation. However, as an important material for mulberry breeding, it is necessary to protect androecious germplasms.

Color and size are essential parameters for fruit quality identification. The color of mulberry fruit is green at first, but varies at maturity from white, purple, black, or pink to red due to disparities in anthocyanin concentration. In addition, the length of mulberry fruit is one of the bases for *Morus* classification and varies greatly in different cultivars. There are three fruit lengths, long (>3.0 cm), medium (between 2.0 cm and 3.0 cm) and short (<2.0 cm). Mulberry fruit, known botanically as sorosis, is juicy and has a sweet taste with some sourness that is more prominent in less mature fruits [19]. However, mature sorosis are softer and have a tendency to bruise and decay during storage and distribution [20].

Mulberry can be exploited for successful cultivation in tropical and subtropical climates. It is hardy and can tolerate short periods of drought, as well as withstand heavy rainfall and floods. Mulberry survives up to 7 m of flooding in parts of drawdown zones and grows successfully in high-salt and low-temperature regions of northern China [21,22]. For successful mulberry cultivation, it is necessary to develop crop management schedules and production practices that include knowledge of periodic phenological events such as bud sprout, leaf flushing, flowering and fruit development under different climatic conditions. It is also vital to identify and describe the distinct phenological stages of a crop species to schedule agronomic practices and conduct plant breeding programs and to study the effect of climate change on crop production. The extended BBCH (Biologische Bundesanstalt, Bundessortenamt and CHemische Industrie) scale [23] provides uniform coding of phenologically similar growth stages of all mono- and dicotyledonous plant species, which is extremely useful for studying plant phenological behavior under different climatic and experimental conditions [24].

M. multicaulis, *M. alba*, *M. laevigata* and *M. wittiorum*, but particularly *M. alba*, are the main cultivated mulberry species in China and are thus widely distributed. They are the most representative species of mulberry in commercial cultivation in China, vary in color from white, purple and black to red at maturity and exhibit different flower sexualities. In this work, we recorded the phenological growth stages and defined the chronological progression of principal growth stages of these four species to provide a useful tool for scientific research and agronomic management.

2. Materials and Methods

Data were collected from healthy, adult trees (4–22 years old) of six cultivars derived from four species (*M. multicaulis* Perr., *M. alba* L., *M. laevigata* Wall. and *M. wittiorum* var. *mawa* Koidz.). Table 1 shows the mulberry cultivars, their species, origin, pedigree and some agronomic traits. All test materials were planted at a spacing of 0.5 × 2.0 m in the Mulberry Repository of Hubei Province (latitude: 30°48′7″, longitude: 114°33′4″ and altitude: 28 m), Wuhan, China.

Table 1. Names, species, abbreviations, origin, pedigree and some agronomic traits of the mulberry cultivars examined in this study.

Cultivar	Species	Abbreviation	Accession Number	Origin	Pedigree	Sexuality	Style Length	Sorosis Length	Sorosis Color
Zijing	*M. multicaulis* Perr.	ZJ	hbgs0001	Wuhan, China	'Zhushan 3' × 'Yueyou 78'	Female	Absent	Medium	Black
Zhenzhubai	*M. alba* L.	ZZB	hbgs0011	Liqing, China	Local cultivar	Female	Very short	Medium	White
Xinjiang Baisang	*M. alba* L.	XJ-BS	hbzy0003	Aksu, China	Local cultivar	Female	Very short	Medium	Purple
Taiwan Changguosang	*M. laevigata* Wall.	TW-CGS	hbzy0002	Taiwan, China	Local cultivar	Female	Very short	Long	Red
Xianfeng Changsuisang	*M. wittiorum* var. *mawa* Koidz.	XF-CSS	hbzy0005	Enshi, China	Local cultivar	Female	Short	Long	Red
Changsui Xiongzhu	*M. laevigata* Wall.	CS-XZ	hbzy0009	Unknown, China	Local cultivar	Male			

Wuhan is located in the middle of the Yangtze River Delta, which has a typical subtropical monsoon climate, with weather featuring distinct seasons and abundant rainfall. The climate data of the last 20 years (from 1999 to 2018) show a mean annual temperature for this location of 17.4 °C, average maximum temperatures of 29.6 °C in July, average minimum temperatures of 4.1 °C in January, with annual rainfall of 1269 mm (data extracted from the statistical yearbook of Wuhan). Another notable feature of this region is that a quarter of the urban area is covered by water bodies, which brings a humid and sweltering summer in Wuhan that lasts for nearly half a year each year (135 days) [25,26].

In this work, codification and description of different growth stages were performed using the extended BBCH scale [23]. Data on vegetative and reproductive phases of different developmental stages were collected during two annual growing seasons (2020–2022), additionally described in other years. Phenological observations and measurements were carried out on five individual trees from each cultivar, which were randomly selected and tagged. For two years, measurements were taken 2–3 times per week (between January and May) or once per week (from May onwards) for representative principal and secondary growth stages.

Subsequently, for fruit size estimation, the weight, size and diameter of 30 fruits from each selected tree were recorded weekly or biweekly, depending on the development stage, using an electronic balance (ME203E, Mettler Toledo Technology Co., LTD, Shanghai, China) and a digital vernier caliper (3V Battery Digital Caliper, Guilin Guanglu Measuring Instrument Co., LTD, Guilin, China). Moreover, in order to estimate fruit ripeness, the anthocyanin contents of mulberry fruits were measured using a pH differential method [27] and the contents of chlorophyll and carotenoid in mulberry fruit were determined with a 95% ethanol extraction method.

The calculation of the chilling requirements (expressed in chilling units, CUs) was obtained based on the Utah method [28]. In this model, the calculation of CUs is initiated in the fall after the accumulation of the largest number of negative CUs occurred and the starting point of low temperature accumulation is determined as 20 November 2020 and 21 November 2021 in Wuhan.

3. Results

The extended BBCH scale is divided into ten principal growth stages (numbered from 0 to 9), starting with the development of buds (stage 0) and ending when the fruit approaches its final size and is ready for harvest (stage 9). Within the primary growth stage, the secondary stages are also numbered from 0 to 9 and 42 secondary growth stages are described in this work (Figures 1–6). The different phenological stages are defined below:

Figure 1. Stages of bud development for mulberry according to the BBCH scale. Buds are densely covered with hairs in CS-XZ.

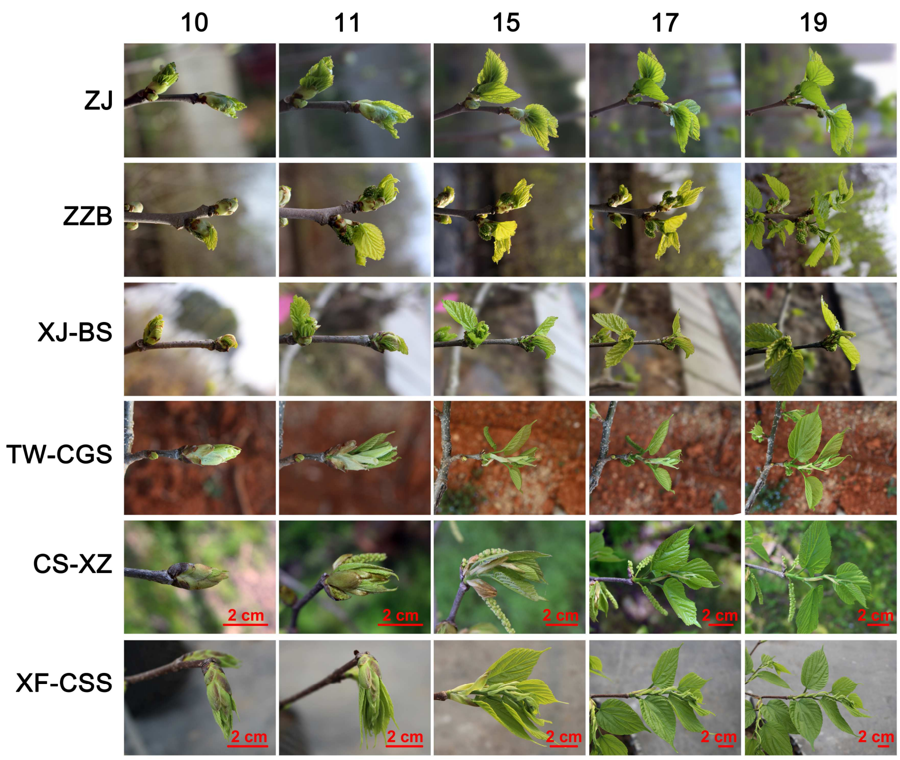


Figure 2. Stages of leaf development for mulberry according to the BBCH scale. Buds are densely covered with hairs in CS-XZ.

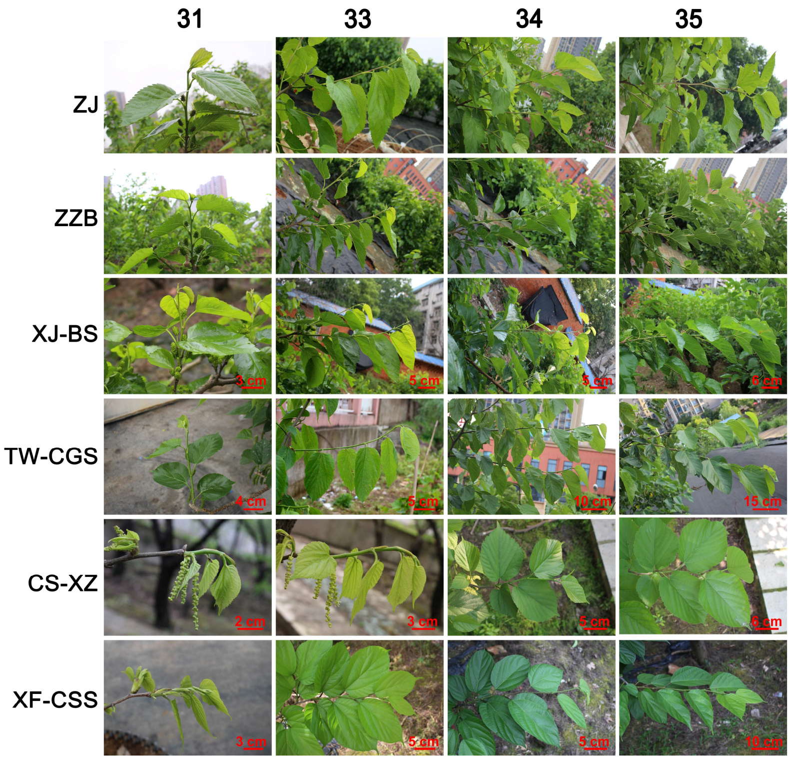


Figure 3. Stages of shoot development for mulberry according to the BBCH scale.

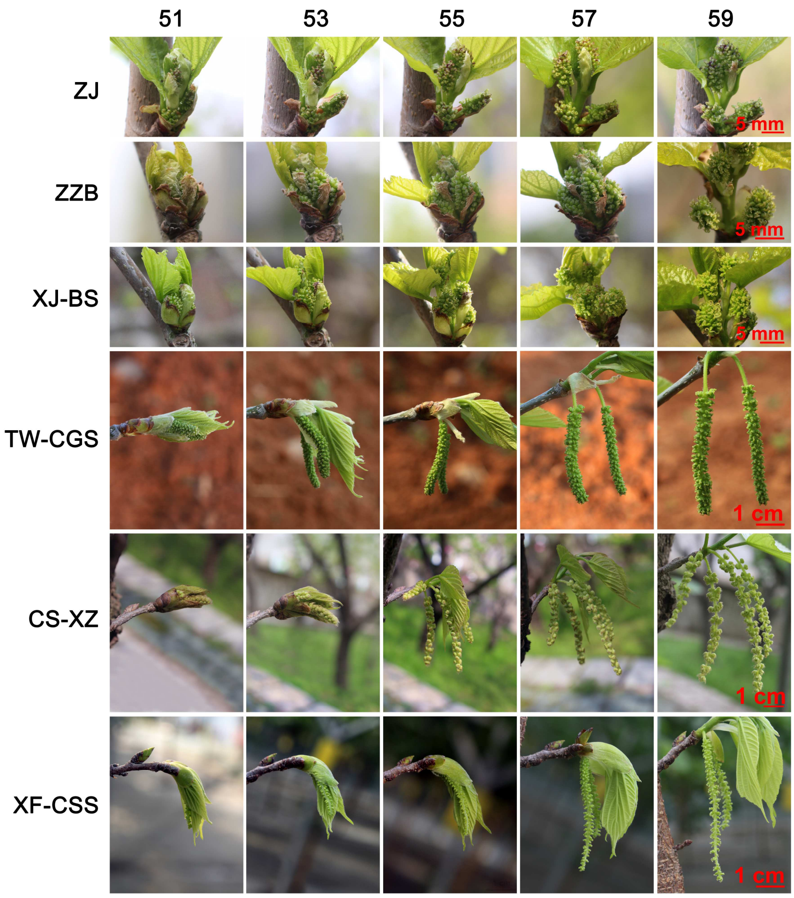


Figure 4. Stages of inflorescence emergence for mulberry according to the BBCH scale. Buds are densely covered with hairs in CS-XZ.

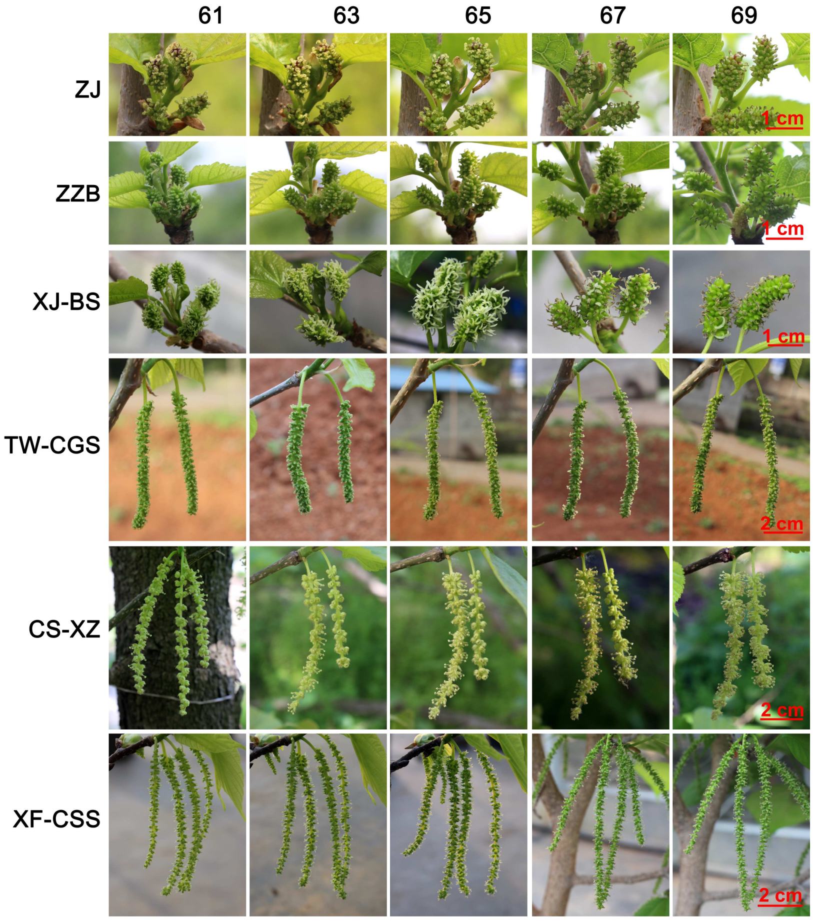


Figure 5. Flowering stages for mulberry according to the BBCH scale.

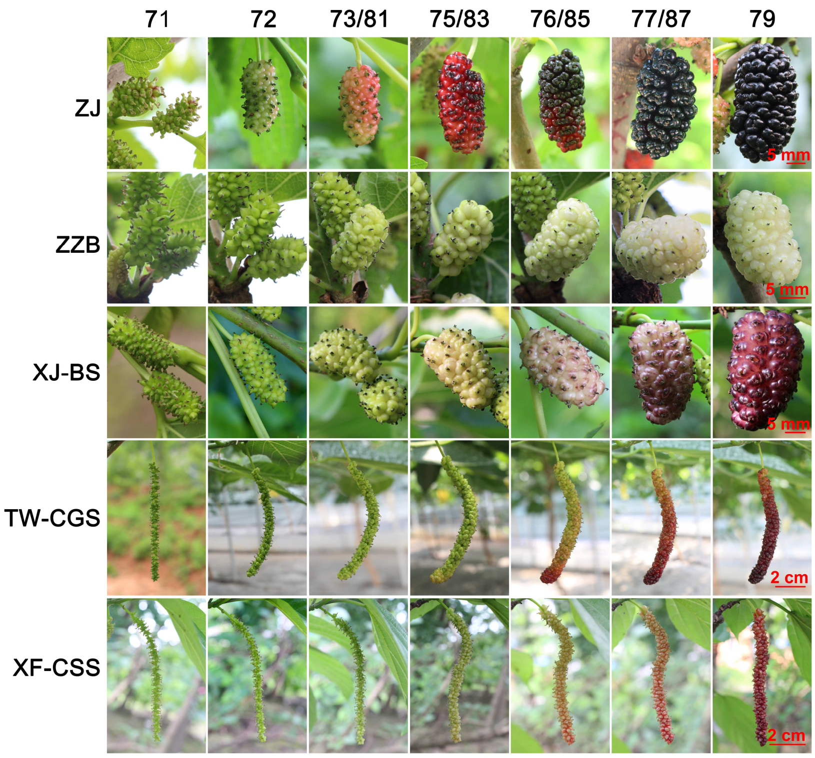


Figure 6. Stages of fruit development and maturity for mulberry according to the BBCH scale.

3.1. Principal Growth Stage 0: Bud Development (Figure 1)

00: Dormancy. Leaf and flower buds, closed and covered by brown scales.

01: Beginning of bud swelling. Buds visibly swollen.

02: Buds elongate further and burst. In addition to the brown scales, a few green scales can be seen.

03: End of bud swelling: brown scales slightly separated, more green color can be seen (and buds are densely covered with hairs in CS-XZ).

07: Beginning of bud burst: first green leaf tips just visible.

09: Green leaf tips visible: green leaf tip about 5–10 mm above bud scales.

3.2. Principal Growth Stage 1: Leaf Development (Figure 2)

10: Separating first leaves: green leaf tips around 10 mm above the bud scales; first leaves separating.

11: The first leaves unfolded.

15: More leaves unfolded.

17: The first leaves at about 70% of final expanding.

19: The first leaves completely unfolded and expanded.

3.3. Principal Growth Stage 3: Shoot Development (Figure 3)

31: Beginning of shoot growth: axes of developing shoots visible.

33: Shoots about 30% of final length.

34: Shoots about 40% of final length.

35: Shoots about 50% of final length.

3.4. Principal Growth Stage 5: Inflorescence Emergence (Figure 4)

51: Inflorescence buds swell: buds are closed and covered with light green scales.

53: Inflorescence buds swell further and burst (with catkin densely covered by hairs in CS-XZ).

55: Inflorescences extend to about 50% of final length.

57: Inflorescences extend to about 70% of final length.

59: End of inflorescence extension, but style of female flowers still folded, male flower still closed.

3.5. Principal Growth Stage 6: Flowering (Figure 5)

61: Beginning of flowering: about 10% of flowers are open.

63: Early flowering: about 30% of flowers are open.

65: Full flowering: 50% of flowers are open.

67: Flower fading: most flowers wilting and dry.

69: End of flowering: all flowers wilting and dry, fruits have already set.

3.6. Principal Growth Stage 7: Fruit Development (Figure 6)

72: Fruits reach 20% of final size.

73: Fruits reach 30% of final size.

74: Fruits reach 40% of final size.

75: Fruits at about 50% of final size.

77: Fruits at about 70% of final size.

79: Fruits at about 90% of final size.

3.7. Principal Growth Stage 8: Maturity of Fruit (Figure 6)

81: Initiation of skin color change (color-break): the skin and pulp of black cultivars appear red or reddish and the fruit color of other cultivars changes to light green. This growth stage proceeds in parallel with stage 73.

83: Advanced ripening: black cultivars have darkened fruit with cultivar-specific color. Cultivars of other colors have further lightened fruit color and have not yet shown cultivar-specific color. This stage proceeds in parallel with stage 75.

85: Advanced ripening: significant changes in fruit color with increasing intensity of cultivar-specific color. This stage proceeds in parallel with stage 76.

87: Fruit ripe for picking: the fruit skins of black cultivars have reached the characteristic color of the cultivar, other color cultivars increased in intensity of cultivar-specific color. This stage proceeds in parallel with stage 77.

89: Fruit color fully developed: the fruits become softer, have typical taste; beginning of senescence.

3.8. Principal Growth Stage 9: Senescence and Beginning of the Rest Period

91: Shoot growth completed; foliage still fully green.

92: The leaves begin to fade in color, some remain green/other leaves develop a yellow coloration.

93: Beginning of leaf fall and leaf discoloration increases.

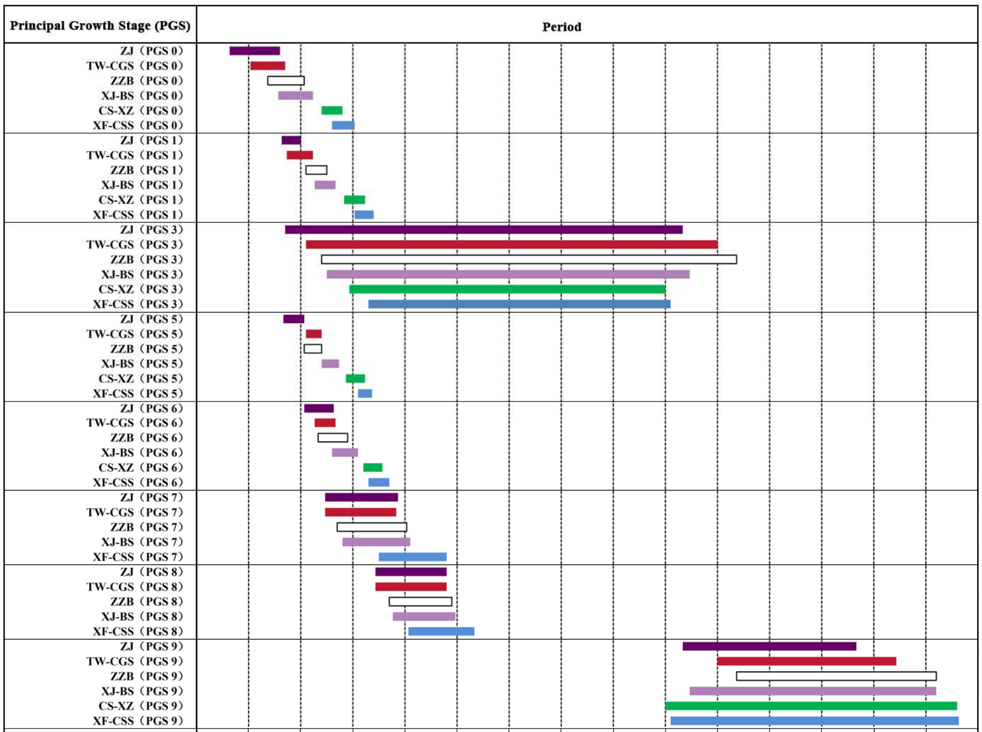
95: 50% of leaves fallen.

96: 60% of leaves fallen.

97: All leaves fallen. Winter dormancy period.

Cultivars differed considerably in their phenological growth stages, even intra-species. Therefore, we summarized a schematic representation showing the chronological progression of principal growth stages for the vegetative and reproductive phenology in the six mulberry cultivars (Figure 7). In stage 0, the bud development of 'ZJ' and 'TW-CGS' began at the third week of January and first week of February in winter, earlier than other mulberries, followed by 'ZZB' and 'XJ-BS'. In contrast, the buds of 'CS-XZ' and 'XF-CSS' developed much later than the above mulberries, starting in mid-March and continuing for 12–13 days. Despite 2–3 week inter-year shifts in dates of bud development onset, the order of bud development in different cultivars remained constant (Figure 7). The order of leaf and shoot development (stages 1 and 3) in different cultivars were consistent with bud development. In stage 3, shoot development began with shoot emergence and ended with abortion and abscission of apical meristems of the long shoots, which further provided evidence of the temperate origin of mulberry (Figure 7).

Usually, the reproductive growth phases of flower and fruit development (stages 5–8) proceed in parallel with vegetative growth phases. In stage 6, the flowering period lasted 11–17 days, depending on variability of rainfall and temperatures (Figure 7). Full blooming of 'ZJ' and 'TW-CGS' took place the earliest, while 'ZZB' and 'XJ-BS' were 8–10 days later than the above two cultivars. Inflorescences of 'CS-XZ' and 'XF-CSS' appeared and bloomed much later than other mulberries, with full blooming on 13th April and 15th April in 2021, respectively (Figure 7). Late flowering is essential for mulberry fruit to avoid infection by pathogens that cause sclerotiniosis, which usually infect flowers in the spring (late February to early April in Wuhan, China) when mulberry trees bloom and the climate is suitable [29]. Flowering in April is not easily affected by low temperatures of late spring, which are more conducive to natural or artificial pollination. The fruit development (stage 7) and maturation (stage 8) durations of five different cultivars investigated here were approximately 39–42 days and 36–41 days, respectively (Figure 7). Among them, the periods of fruit development and maturation were almost synchronous and earlier than other mulberries for 'ZJ' and 'TW-CGS', but the coloring time of 'ZJ' mulberry was earlier than that of 'TW-CGS'. 'XF-CSS' fruit development and maturation were the latest and took place from 14 April to 25 May and 3 May to 10 Jun in 2021, respectively. The order of fruit that was ripe for picking was as follows: 'TW-CGS', 'ZJ', 'ZZB', 'XJ-BS' and 'XF-CSS'.



Date	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar
Min. teamperature (°C)	1.17	6.14	8.11	13.42	18.27	23.26	26.09	24.57	21.61	13.42	5.87	0.12	0.52	−0.34	8.71
Max. teamperature (°C)	9.57	15.76	16.82	20.29	26.31	31.86	32.97	31.98	33.63	23.53	20.14	14.48	9.56	9.96	20.22
Mean teamperature (°C)	5.51	10.88	12.76	16.77	22.27	28.02	29.37	27.91	27.24	17.98	13.09	7.63	5.08	5.32	14.76

Figure 7. Schematic representation of the growth stages for six mulberries (data gathered from 2021 to 2022). The purple rectangle represents the 'ZJ'; the red rectangle represents the 'TW-CGS'; the white rectangle represents the 'ZZB'; the pink rectangle represents the 'XJ-BS'; the green rectangle represents the 'CS-XZ'; the blue rectangle represents the 'XF-CSS'.

3.9. Morphological Profiles and Color Characters of the Mulberry Fruits

In order to better understand the fruit morphological characteristics, we have measured the fruit length, diameter and weight at different developmental stages of five mulberry genotypes in detail, respectively (Figures 8 and S1–S3). Compared with short fruit type varieties, the fruit length and diameter of the long fruit type varieties 'TW-CGS' and 'XF-CSS' did not change significantly during the fruit development (Figures 8b,e and S1–S3b). In contrast, the fruit weight of all genotypes increased significantly with fruit development (Figures 8b,e and S1–S3b). The fruit color changes of five mulberry varieties, i.e., 'TW-CGS', 'ZJ', 'ZZB', 'XJ-BS' and 'XF-CSS', showed obvious differences in the development of fruits (Figures 8a,d and S1–S3a). The contents of chlorophyll and carotenoid in the fruit of five mulberry genotypes continuously decreased during the fruit development (Figures 8c,f and S1–S3c). However, the difference is that the 'TW-CGS', 'ZJ', 'XJ-BS' and 'XF-CSS' fruit continuously accumulated anthocyanins and became a purple-red color; the higher the anthocyanin contents, the darker the flesh color (Figures 8a,c,d,f, S2 and S3a,c), except for the ZZB cultivar fruit, which turned a jade-white color due the absence of the accumulation of anthocyanin and the continuous degradation of chlorophyll and carotenoid (Figure S1a,c).

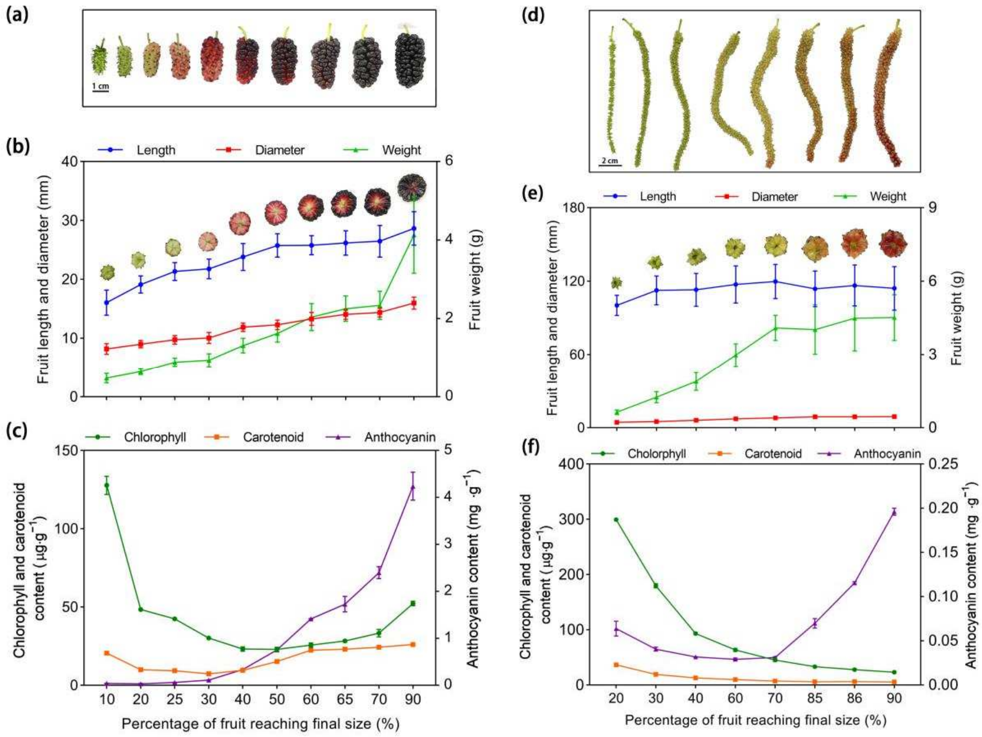


Figure 8. Fruit photographs, size and pigment content in 'ZJ' and 'XF-CSS' mulberries at different developmental stages (stages 7 and 8) (data gathered in 2022). (**a**–**c**) Fruit photographs, size and pigment content in 'ZJ'; (**d**–**f**) fruit photographs, size and pigment content in 'XF-CSS'.

4. Discussion

The BBCH codification scale has been widely used to describe plant growth stages in various fruit trees [30–39], more commonly in northern latitudes. Although the BBCH scale of black (*M. nigra*) mulberry was established in a Mediterranean climate by Sánchez-Salcedo et al. [33], the other main *Morus* species, especially the most popular *M. alba* species, were not described. In addition, we found that the previous description of fruit maturity (stage 8) is imprecise. Therefore, an updated phenological scale and codification is required to cover the whole life cycle of mulberry species with different flower sexualities and fruit colors.

In mulberry, the period of true dormancy almost parallels that of leaf senescence. Like other deciduous fruit trees, mulberries require a period of accumulative chilling to transition from dormancy to bud-burst [40–42], which is analogous to the chilling requirement of vernalization for flowering in winter annuals [43]. As an important prerequisite for mulberry dormancy release, the chilling requirement, which depends on the variety, serves to characterize the naturalization environment [40–42,44]. In this study, 'ZJ' (*M. multicaulis*) and 'TW-CGS' (*M. laevigata*) had low chilling requirements and released their dormancy before the other cultivars and their bud burst started on 26 January and 13 February in 2021, respectively (Table S1). In addition, the chilling requirements of 'ZJ' (1156 CUs) and 'TW-CGS' (1440 CUs) are lower than 'ZZB' and 'XJ-BS' (1645.25 CUs). These results coincided with findings by Chang et al. [44], who reported that the chilling requirements of *M. bombycis* and *M. alba* are higher than those of *M. atropurpurea* and *M. laevigata* and are stable within species.

Furthermore, our results suggest that the dormant buds of *M. wittiorum* ('XF-CSS' and 'CX-XZ') sprout on 24 March and 17 March in 2021, respectively, both of which represent later bud sprouting, flowering and fruit maturation. Sprouting and flowering are considered influential characters for mulberry production and breeding. Late blooming could also involve late ripening, meaning that the harvest time of mulberry fruits can be extended in production by planting certain varieties. With global climate change, accurate knowledge of the timing of bud burst and its control are critical for the productivity of fruit trees, as increasing or irregular seasonal temperatures impact optimal timing for important phenological traits [45].

Mulberry have "indirect flowering", where the differentiation of flower buds occurs before dormancy, while blooming and fruit development take place after dormancy release [46]. The timing of flowering and fruit ripening highlighted in our study were also reported by previous studies, showing that 'Elongated fruit No. 1' (*M. laevigata*) bloomed fully on 15th February and fruits were ready for harvest on 14 April in Miaoli (24.3° N; 120.5° E), Taiwan [47,48], 8–9 days earlier than Wuhan. The fruit maturation of *M. alba* trees occurs in July in the Indian trans-Himalaya region [19] and in June in the Coruh valley of Turkey [49], much later than that of *M. alba* trees in China and Japan. These findings highlight that phenological stages of the same species of mulberry vary wildly in different parts of the world. We investigated the data of two primary phenological growth stages (flowering stage and fruit maturity period) for six mulberry cultivars in different regions of China, i.e., Institute of Economic Crop Research of Shiyan (Shiyan city, Hubei province), Hubei Academy of Agricultural Sciences (Wuhan city, Hubei province), Sericulture Technology Extension Station of Guangxi Zhuang Autonomous Region (Nanning city, Guangxi province) and Sericultural Research Institute, Sichuan Academy of Agricultural Sciences (Nanchong city, Sichuan province) (Table S2). Among these regions, the phenological period of mulberries in Nanning was 15–20 days earlier than that of Wuhan and the phenological period of mulberries in Wuhan was 7–10 days earlier than that of Shiyan (Table S2).

Sexuality, fruit length and color are essential traits for mulberry cultivation, selection and artificial cross-breeding. The sex of mulberry flowers depends on the cultivar, with some that are monoecious and others dioecious or androgynous [16]. The staminate flowers (male), which are relatively common in mulberry trees, are essential germplasm for mulberry breeding, but are easily eliminated and ignored by cultivators. In comparison with the *M. nigra* BBCH scale previously reported [33], we investigated the phenological records of six mulberry cultivars from over two growing seasons in Wuhan. These six cultivars exhibit three important morphological traits: sexuality, fruit length and color. The 'CS-XZ' catkin is a long flower type and its time of full flowering is shared with 'XF-CSS', a late phenological cultivar. It has a great potential to be used as a pollen donor for mulberry breeding in late phenological germplasm. 'ZJ', 'ZZB' and 'XJ-BS' are important commercial cultivars with medium fruit lengths of 28.65 mm, 24.05 mm and 25.65 mm and average fruit weights of 4.13 g, 2.54 g and 3.03 g, respectively (Figure 8b and Figure S1–S2b). In contrast, 'TW-CGS' and 'XF-CSS' had longer fruits, with average fruit weight of 6.18 g and 4.52 g, respectively (Figure 8e and Figure S3b).

The color of fruit is also an important quality attribute for consumer acceptance [50]. In the fruit organs of horticultural plants, anthocyanins, carotenoids, betalains and chlorophyll are the four main pigments for coloration [51,52]. Like strawberry, the fruit coloration of mulberry is due to both chlorophyll and anthocyanins. Our results confirmed that the anthocyanins and chlorophyll are the main pigments for coloration in mulberry fruits (Figures 8 and S1–S3), which have various colors ranging from white to purplish red, purple and black (Figures 8 and S1–S3), similar to apples, grapes and pears. In our results, the fruit ripening process is accompanied by the degradation of chlorophyll and carotenoid and synthesis of anthocyanin, except in 'ZZB' (Figures 8c,f and S2–S3c). Moreover, we found that the secondary growth stages 0–9 cannot be represented by the degree of coloration (the percentage values of anthocyanin content) as previously reported [33]. For example, at stage 83 (advanced fruit ripening), the anthocyanin content of 'ZJ' mulberry was 0.33 mg/g, which was 7.1% of the final anthocyanin content instead of 30% as reported by Sánchez-Salcedo et al. [33] (Figure 8c). This result was also supported by the findings of Lee and Hwang [20]. When the fruit fully developed, the fruit colors of 'ZJ', 'XJ-BS', 'TW-CGS' and 'XF-CSS' were black, purple, purplish red and purplish red and the anthocyanin concentrations were 4.24 mg/g, 0.12 mg/g, 0.37 mg/g and 0.20 mg/g, respectively (Figures 8c,f and S2–S3c). Interestingly, the fruit coloration of 'XJ-BS' is mainly in the peel

(Figure S2b), while other cultivars are colored throughout the fruit (Figures 8b,e and S3b). In this study, the anthocyanins are abundant in purple or black mulberry fruits but not in white fruits, the differences in the levels and proportions of anthocyanins result the differently colored mulberry fruits (white, purplish red, purple and black) (Figures 8 and S1–S3), as reported in previous studies [53–56].

5. Conclusions

In conclusion, our study used the BBCH scale to accurately and uniformly describe the phenological growth of four *Morus* species other than *M. nigra* in subtropical-monsoon-climate conditions. These species comprise six commercial cultivars and exhibit different flower sexualities and fruit color and length. In addition, two late phenological germplasms (late bud sprouting associated with late blooming and late fruit ripening) were screened out according to the BBCH scale. The information of phenological phase will not only guide agronomic practices, but also facilitate convenient communication between researchers of mulberry in different areas.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae8121140/s1, Figure S1: Fruit photographs (a), size (b) and pigment content (c) in 'ZZB' mulberry at different developmental stages (stages 7 and 8). Figure S2: Fruit photographs (a), size (b) and pigment content (c) in 'XJ-BS' mulberry at different developmental stages (stages 7 and 8). Figure S3: Fruit photographs (a), size (b) and pigment content (c) in 'TW-CGS' mulberry at different developmental stages (stages 7 and 8). Table S1: Time of bud breaking in six mulberry cultivars and estimation of their bud chilling requirement by Utah model in Wuhan. Table S2: Primary phenological growth stages for six mulberry cultivars in different provinces. Table S3: The phenological data of stage 0–9 for six mulberry cultivars. Table S4: Field resistance evaluation of nine Morus species against mulberry fruit sclerotiniosis disease in 2022.

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