Low Female Gametophyte Fertility Contributes to the Low Seed Formation of the Diploid Loquat [Eriobotrya Japonica (Thunb.) Lindl.] Line H30-6

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Loquat is a widely grown subtropic fruit because of its unique ripening season, nutrient content, and smooth texture of its fruits. However, loquat is not well-received because the fruits contain many large seeds. Therefore, the development of seedless or few-seed loquat varieties is the main objective of loquat breeding. Polyploidization is an effective approach for few-seed loquat breeding, but the resource is rare. The few-seed loquat line H30-6 was derived from a seedy variety. Additionally, H30-6 was systematically studied for its fruit characteristics, gamete fertility, pollen mother cell (PMC) meiosis, stigma receptivity, in situ pollen germination, fruit set, and karyotype. The results were as follows. (1) H30-6 produced only 1.54 seeds per fruit and the fruit edible rate was 70.77%. The fruit setting rate was 14.44% under open pollination, and the other qualities were equivalent to those of two other seedy varieties. (2) The in vitro pollen germination rate was only 4.04 and 77.46% of the H30-6 embryo sacs were abnormal. Stigma receptivity and self-compatibility in H30-6 were verified by in situ pollen germination and artificial pollination. Furthermore, the seed numbers in the fruits of H30-6 did not significantly differ among any of the pollination treatments (from 1.59 ±0.14 to 2 ±0.17). (3) The chromosome configuration at meiotic diakinesis of H30-6 was 6.87I + 9.99II + 1.07III + 0.69IV + 0.24V (H30-6), and a total of 89.55% of H30-6 PMCs presented univalent chromosomes. Furthermore, chromosome lagging was the main abnormal phenomenon. Karyotype analysis showed that chromosomes of H30-6 had no recognizable karyotype abnormalities leading to unusual synapsis on the large scale above. (4) The abnormal embryo sacs of H30-6 could be divided into three main types: those remaining in the tetrad stage (13.38%), those remaining in the binucleate embryo sac stage (1.41%), and those without embryo sacs (52.82%). Therefore, we conclude that the loquat line H30-6 is a potential few-seed loquat resource. The diploid loquat...
line H30-6 was with low gametophyte fertility, which may be driven by abnormal meiotic synapses. The low female gamete fertility was the main reason for the few seeds. This diploid loquat line provides a new possibility for breeding a few-seed loquat at the diploid level.

**Keywords:** seedless fruit, male sterility, female sterility, gametophyte differentiation and development, meiosis

## INTRODUCTION

Loquat (Eriobotrya japonica), which originated in China, is a globally grown subtropical fruit and its fruits have high nutrition and a delicious taste, consumed directly or made into juices, jams, wines, and syrups (Lin et al., 1999; Lin, 2008). There are large seeds in loquat fruits, which is inconvenient for eating and processing. Therefore, the development of seedless and few-seed varieties has been the main goal of loquat breeders.

For a long period of time, polyploidization has been an effective approach to breeding seedless and few-seed loquat varieties. Early in 1984, Huang et al. (1984) reported that a tetraploid line (‘Min No. 3’) was obtained via colchicine induction, and the majority of fruits (91.3%) had only one seed. In the 1990s, triploids were employed to produce seedless loquats by Liang, and seedless varieties including “Wuhe Guoyu” (Guo et al., 2016), “Huaini Wuhe No. 1” (Dang et al., 2019a), and “Huayu Wuhe No. 1” (Dang et al., 2019b) have since been released. However, hormone treatment is necessary to increase the fruit setting rate of triploid loquat varieties (Maria et al., 2013). Some triploid lines were found to produce few-seed fruits when pollinated by diploid lines without hormone treatment (Kikuchi et al., 2014; Yang, 2021). In recent years, some tetraploids have been applied to breeding triploids on a large scale (Liang et al., 2018; Wang et al., 2021).

At the diploid level, seedless fruits could be obtained via hormone regulation, but the fruit qualities have been proven to be significantly lower than those of the seeded fruits (Gu and Zhang, 1990; Zhang et al., 1999; Chen et al., 2006; Deng, 2009b; Maria et al., 2013). “Tai Cheng No. 4” was reported to be a loquat variety with 1.3 seeds per fruit, and fruits with one seed were observed for up to 72.6% (Huang and Xu, 1980). However, there is no further information about this variety. An investigation reported by Jiang et al. (2009) indicated that 15 out of 128 lines bear fruits with 1–2 seeds, and there are two of the lowest lines (Guangmian and Hongxing), with 1.2 seeds per fruit. Zhang et al. (2014) reported that there were only 1.12 seeds per fruit of “Chuannong No.1”; however, the fruit setting rate of this variety was very low. Therefore, it can be concluded based on the above information that few-seed loquat resources are scarce.

It is logical that the abnormal meiosis of polyploids contributes to low fertility gametes, thus leading to seedless or few-seed fruit (Maria et al., 2013; Xu et al., 2016). But the few-seed mechanism of diploid loquat may be a more complex issue. While there have only been a few reports concerning this issue. Zhang et al. (2014, 2015) reported that gametophytic self-incompatibility resulted in the few-seed of “Chuannong No. 1”, and pollen inactivation caused by high temperatures contributed to a low fruit setting rate and few-seed fruit of “Chuanzao”.

Mutant shoot breeding, chance seedling selection, and cross-breeding play an important role in the seedless variety breeding (Ledbetter and Ramming, 1989; Deng et al., 1996; Fan et al., 2004; Xu et al., 2006; Ye et al., 2006; Dong et al., 2013; Li and Wang, 2019; Li et al., 2019). So, it is important to discover more few-seed germplasm and identify the mechanism underlying the production of low numbers of seeds for breeding few-seed loquats.

An open-pollinated progeny of a white-fleshed variety “Huabai No.1”, named H30-6, was shown to bear fruits with few seeds. This line thus is an ideal material to breed new few-seed varieties and to research the mechanism underlying low seed formation. In the current study, we observed the stability of the H30-6 fruit set and quality traits over successive years. Moreover, the flower structure, gamete fertility, pollen mother cell (PMC) meiosis, stigma receptivity, in situ pollen germination, fruit setting, karyotypes, and so forth were studied to determine the factors underlying the low seed formation of loquat line H30-6. Taken together, the results can be referred to in few-seed loquat variety breeding at the diploid level.

## MATERIALS AND METHODS

### Plant Materials

From 2016 to 2021, we obtained materials from vigorously growing plants in the orchard of fruit germplasm resources belonging to the Key Fruit Lab, of the College of Horticulture and Landscape Architecture, Southwest University. H30-6 is an open-pollinated progeny of a white-fleshed variety “Huabai No.1”, which is red-fleshed. In the orchard, the H30-6 line was represented by one 10-year-old individual and five 3-year-old grafted trees. So, in this study, we observed the stability of the H30-6 fruit set and quality traits over successive years. Moreover, the flower structure, gamete fertility, pollen mother cell (PMC) meiosis, stigma receptivity, in situ pollen germination, fruit setting, karyotypes, and so forth were studied to determine the factors underlying the low seed formation of loquat line H30-6. Taken together, the results can be referred to in few-seed loquat variety breeding at the diploid level.

### METHODS

#### The Yield and Fruit Quality

The yield of the 10-year-old tree of H30-6 was measured from 2019 to 2021, and the yield of the 3-year-old grafted trees was measured in 2021. In 2019, 70 naturally set H30-6 fruits were used to calculate the seed number per loquat fruit, which was repeated three times. From 2019 to 2021, fruits of the three varieties were used each year to assess fruit quality, including average fruit weight, seed number per fruit, fruit edible rate, total sugar (measured using Fehling’s reagent titration), reducing
sugar (measured using Fehling's reagent titration), titratable acid (measured using sodium hydroxide titration), sugar-acid ratio (total sugar/titratable acid), and vitamin C (measured using 2,6-dichlorophenol sodium indophenol). The fruit edible rate, fruit shape index, and sugar-acid ratio were calculated using the following formulas:

\[
\text{Fruit edible rate} \ (\%) = \frac{\text{Flesh weight}}{\text{Fruit weight}} \times 100\%
\]

\[
\text{Fruit shape index} = \frac{\text{Longitudinal diameter per fruit}}{\text{Transverse diameter per fruit}}
\]

\[
\text{Sugar acid ratio} \ (\%) = \frac{\text{Total sugar}}{\text{Titratable acid}} \times 100\%
\]

**Flower Organ Observations and Quantification**

The flower buds were dissected 1 day before opening (DBO) to count the flower organs and measure the stigma length. The evenness of stigma length was calculated as the ratio of the number of flowers with stigmas of the same length to the number of all flowers observed. There were 30 flowers of H30-6, H411, and B336 which were observed separately. Many of the anthers of the flowers dissected with 1 DBO were collected, dried naturally at 25°C, and stored at 4°C for subsequent experiments. We collected images of blooming flowers to show the anther dehiscence state of the three varieties in the field. Additionally, the dried anthers were observed and imaged under a Carl Zeiss Jena Axio Zoom version 16 microscope (Jena, Germany).

**Pollen Morphology, Pollen Viability, and Pollen Quantity**

To study pollen morphology, dried pollen grains were adhered onto the sample stage with conductive adhesive, coated with gold in a KYKY SBC-12 vacuum evaporation instrument, and observed under a Phenom ProX scanning electron microscope (Thermo Fisher Scientific, Waltham, MA, USA). For H30-6, H411, and B336, three pollen group photos were taken separately by scanning electron microscope to count the pollen with normal or abnormal shapes. The total numbers of pollen counted were 255, 159, and 297 for H30-6, H411, and B336, respectively.

There were 10 dried anthers which were placed into a 1.5 ml centrifuge tube and then crushed in 200 µl BK medium with tweezers to count the pollen in a blood counting chamber. The test was processed three times for H30-6, H411, and B336 separately.

We slightly modified Hu Shiyi’s pollen viability method (Hu, 1993) to test pollen viability. The dry anthers were placed into a 1.5 ml centrifuge tube with 200 µl BK medium, and the tube was shaken to allow pollen dispersion until the pollen density was the same among H30-6, H411, and B336. Then, the anthers were removed with tweezers. Later at 45 min, 50 µl pollen suspension was transferred into a new centrifuge tube and then placed into the dark constant temperature incubator upside down at 25°C for 4 h. After culture and thorough shaking, 15 µl suspension was dropped onto a glass slide, covered with a coverslip, and observed and imaged using an Olympus (BX35) fluorescence microscope (Tokyo, Japan). At least 6 times were repeated in each material. Each repeat took at least three pictures of different visions. The germinated pollen and ungerminated pollen were counted in each repeat. The total numbers of the pollen count in the viability test were 2,743, 1,724, and 2,568 for H30-6, H411, and B336, respectively.

**Anthers Development**

Buds with anthers with microspore mother cell before meiosis or at meiosis stage and buds 1 DBO were collected, fixed in a 38% formalin-acetic acid-70% alcohol solution (FAA, volume ratio = 1:1:18) for 24 h, and then preserved in 70% alcohol solution at 4°C. Anthers were dissected from fixed buds for serial paraffin sections following the protocol described by Li (1987), including safranine staining, dehydration, transparency, wax immersion, and embedding. Thin serial sections of 8–10 µm were cut with a microtome (RM2235, Leica, Wetzlar, Germany). This was followed by safranine staining, dehydration, Fast Green counterstaining, and rehydration prior to examination under a light microscope (BX53, Olympus, Tokyo, Japan).

**PMCs Meiosis**

Anthers were dissected from buds, fixed in methyl alcohol: acetic acid solution (3:1) for 24 h, rinsed with deionized water, and then treated with 3% (w/v) cellulose, 1% (w/v) macerozyme pectinase, and 1% (w/v) snailase for 4 h. Slides were prepared using the chromosomal smear technique (Liang and Li, 1991) with Na2HPO4 solution prestaining for 30–40 min and 5% Giemsa staining for 10–15 min and then observed under a light microscope (BX53, Olympus, Tokyo, Japan).

**Stigmas Receptivity**

The benzidine staining method (Dafni et al., 2005) was used to assay stigma receptivity. The staining solution contained 1% benzidine, 3% H2O2, and H2O (volumetric ratio = 4:11:22), and 3% H2O2 was prepared when needed. Five flowers at 1 DBO of H30-6, H411, and B336 were chosen separately, and the stigmas were revealed by removing the sepals, petals, and anthers. The stained stigmas were observed and imaged under a Carl Zeiss Jena Axio Zoom version 16 microscope (Jena, Germany).

**In Situ Pollen Germination**

The aniline blue staining protocol was used to test in vivo pollen tube growth according to Hu (1982), and the tissues were observed under a light microscope (BX53, Olympus, Tokyo, Japan). Five pollination methods were used on H30-6 to test for in situ pollen germination. First, natural pollination with bags was applied: some branches with many flowers were bagged before the flowers opened, and then each flower was marked on the day it started opening. More than 20 flowers were collected and fixed 2, 3, and 4 days after opening (DAO). Second, open pollination was allowed to proceed: when the flowers naturally opened, they were marked. More than 20 flowers were collected and fixed 2, 3, and 4 DAO. Third, artificial selfing was performed: some branches with many flowers were bagged before the flowers opened, and the flowers 1 DBO were emasculated and pollinated with dry pollen of H30-6. More than 20 flowers were collected 2 days after pollination (DAP) and fixed. Fourth, cross-pollination with H411 was applied: some branches with many flowers were bagged before the flowers opened, and 1 DBO, the
flowers were emasculated and pollinated with dry pollen of H411. More than 20 flowers were collected 2 DAP and fixed. Fifth, cross-pollination with B336 was conducted: several branches with many flowers were bagged before the flowers opened, and flowers at 1 DBO were emasculated and pollinated with dry pollen of B336. More than 20 flowers were collected 2 DAP and subsequently fixed.

**Embryo Sac Observations**
The ovules were separated from flowers 1 DBO, fixed in FAA for 48 h, and then transferred to 70% ethanol for 24 h. We followed the protocol of Zilli et al. (2015) for dehydration and removal, with the following modifications: 10% H₂O₂ (30 min), 10% H₂O₂ (2 h), 70% ethanol (2 h), 85% ethanol (2 h), 95% ethanol (2 h), 100% ethanol (2 h), 100% ethanol (overnight), methyl salicylate/ethanol 50:50 v/v (3 h), methyl salicylate/ethanol 75:25 v/v (3 h), methyl salicylate/ethanol 85:15 v/v (3 h), methyl salicylate (3 h), and methyl salicylate until observations performed through a light microscope (BX53, Olympus, Tokyo, Japan). The numbers of ovules observed in this test were 142, 289, and 123 for H30-6, H411, and B336, respectively.

**Pollination Treatments**
From 2017 to 2021, reciprocal crosses were performed among H30-6, H411, and B336, and pollen was collected and saved in the same way as germination in the in situ experiment. In the meantime, the flowers of H30-6, H411, and B336 were processed by natural pollination with bags, open pollination, and artificial selfing separately. Each year, the fruits in each treatment were harvested, and the numbers of fruits and seeds were counted. The fruit setting rate, average seed number per fruit, and seed ovule ratio were calculated using the following formulas:

- **Fruit setting rate (%)**
  \[
  \text{Fruit setting rate} = \frac{\text{Total fruit number of one treatment}}{\text{Total flower number of the treatment}} \times 100\%
  \]

- **Seed ovule ratio (%)**
  \[
  \text{Seed ovule ratio} = \frac{\text{Total seed number of one treatment}}{\text{Flower number of the treatment} \times 10} \times 100\%
  \]

- **Seed number per fruit (%)**
  \[
  \text{Seed number per fruit} = \frac{\text{Total seed number of one treatment}}{\text{Total fruit number of the treatment}} \times 100\%
  \]

**Chromosome Karyotype Analysis**
Root tips and shoot tips were placed into 0.002 mol/L 8-hydroxyquinoline aqueous solution for 4 to 5 h in the dark, fixed in methyl alcohol: acetic acid solution (3:1, v/v) for 24 h, rinsed with deionized water, and then treated with 3% (w/v) cellulose and 1% (w/v) macerozyme for 3 h. Slides were prepared and observed as described for the meiosis slides. Chromosome karyotype analysis was according to Li and Chen (1985) standard. Chromosome types and relative lengths were determined according to Levan et al. (1964). While karyotype classification was performed according to the Stebbins (1971) standard, the asymmetrical karyotype coefficient was calculated as described by Arano (1963).

**Data and Image Processing**
Microsoft Excel (Redmond, Washington, USA) and SPSS 17 software (IBM, Armonk, NY, USA) was applied for statistical analysis. Adobe Photoshop CS5 (San Jose, USA) was applied for image processing. The parameters in the fruit quality test, the pollen quantity, normal shape pollen ratio, germination pollen ratio, fruit setting rate, seed ovule ratio, and No. of seeds per fruit were presented by mean ± standard error (SE). ANOVA for mean comparisons (LSD/Dunnett T3, P < 0.05) was performed. The percentage data were transformed using the arcsine square root transformation to normalization separately before the significant difference tests.

**RESULTS**

**The Yield of H30-6 and Fruit Quality of Naturally Seated Fruit**
The average yield of the 10-year-old tree of H30-6 was 14.43 kg (15.19, 18.36, and 9.75 kg, from 2019 to 2021 separately), and the average yield of the 3-year-old grafted tree was 7.3 kg. The results showed that the seed numbers of open-pollinated H30-6 fruits were between 0 and 3 (Figure 1), and the percentage of fruits with one seed was 56.67%, followed by two-seeded fruit (35.24%). Subsequently, similar observations were made in the last 2 years, when the average seed number per fruit was 1.54, which was significantly lower than that of the controls (H411, 4.63 ± 0.22; B336, 3.23 ± 0.12). All the results proved that loquat line H30-6 was a few-seed resource. Furthermore, the successive three-year fruit quality data revealed that H30-6 had the highest fruit edible rate (70.77 ± 0.61%), and the other detection indexes were equivalent to those of the two controls (Table 1). Therefore, loquat H30-6 can be used as a commercial variety.
Thus, the H30-6 line exhibited low male fertility. According to Deng (2009a) standard, the H30-6 strain was a male-sterile resource, and the controls were both fertile. We also found that the pollen quantity of H30-6 was significantly lower than that of the two controls (Figure 3K).

**Anter Anatomical Structure**

The anter paraffin sections revealed no obvious structural differences among H30-6, H411, and B336 during the developmental process (Figure 4). However, in male gametangia dissected from flower buds 1 DBO, the controls had plump pollen (Figures 4F,I), while the pollen of H30-6 was distorted and shrunk (Figure 4C). Therefore, we hypothesize that the reason for the lower pollen release involved the pollen mother cells, not the other tissues.

**PMCs Meiosis**

The PMCs of B336 behaved quite normally throughout all the meiosis stages. Additionally, the normal behavior rate of H411 PMCs ranged from 78.31 to 95.64%, whereas those of H30-6 ranged from 0 to 41.03% (Table 3, Figure 5, Supplementary Figure 2). The high-frequency abnormalities of H30-6 included incorrect chromosome pairing, chromosome lag, abnormal spindle formation, unequal division, and micronucleus formation. To analyze homologous chromosome pairing, chromosome configurations at diakinesis were observed and quantified. The results showed that the average chromosome configurations were $6.87I + 9.99II + 1.07III + 0.69IV + 0.24V$ (H30-6), $0.70I + 16.50II + 0.02III + 0.06IV$ (H411), and $0.11 + 16.99II$ (B336) (Supplementary Table 1). From these configurations, we found that the chromosome configuration of H30-6, H411, and B336 was mainly bivalent, however, H30-6 also showed a high frequency of univalents, and 89.55% of the H30-6 PMCs presented as univalent (Supplementary Table 1). Due to the randomness of the bipolar spindle fibers, univalent travel to one pole will be delayed. In the later meiosis process, chromosome lag was the main phenomenon, which accounted for 65.31, 95.24, 48, 55.13, 95, and 46.32% of the observations at metaphase I, anaphase I, telophase I, metaphase II, anaphase II, and telophase II, respectively (Table 3, Figure 5). Usually, lagging chromosomes are distributed randomly, as well as losing or forming micronuclei. Some of the immature microspores grew to the mature pollen stage. This was proven by the pollen images obtained by scanning electron microscopy, which showed many small pollen grains. Therefore, we believe that the high frequency of univalent chromosomes is the reason for the chromosome lag, which may cause a decrease or an increase in the inherited material, leading to an influence on the fertility of the pollen. In addition, we found that the percentages of PMCs with doubled chromosomes were as high as 16.33 and 41.03% at metaphase I and metaphase II, respectively.

**Stigma Receptivity and in situ Pollen Germination**

We observed that loquat stigmas were heart-shaped or triangular. The stigmas of H30-6, H411 and B336 were stained dark brown (Figures 6A–C), meaning that all the stigmas were receptive. However, the stained areas showed some differences, especially

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### Table 1: Fruit qualities during three consecutive years (2019 to 2021).

| Lines/varieties | H30-6 | H411 | B336 |
|-----------------|-------|------|------|
| Average fruit weight (g) | $32.59 \pm 1.47$ | $31.58 \pm 0.86$ | $36.89 \pm 0.76$ |
| Seed number per fruit | $1.54 \pm 0.09$ | $4.63 \pm 0.22$ | $3.23 \pm 0.12$ |
| Edible rate (%) | $70.77 \pm 0.61$ | $65.3 \pm 1.03$ | $65.76 \pm 0.69$ |
| Fruit shape index | $1.30 \pm 0.02$ | $0.90 \pm 0.01$ | $1.11 \pm 0.02$ |
| Soluble solids | $9.94 \pm 0.35$ | $12.43 \pm 0.56$ | $9.28 \pm 0.36$ |
| Total sugar (g/100 ml) | $7.61 \pm 0.13$ | $10.72 \pm 0.59$ | $7.66 \pm 0.50$ |
| Reducing sugar (g/100 ml) | $6.75 \pm 0.20$ | $6.93 \pm 0.16$ | $5.80 \pm 0.17$ |
| Titratable acid (g/100 ml) | $0.24 \pm 0.01$ | $0.37 \pm 0.03$ | $0.28 \pm 0.01$ |
| Sugar acid ratio | $31.41 \pm 0.60$ | $30.69 \pm 1.29$ | $27.39 \pm 1.16$ |
| Vc (mg/100 ml) | $4.65 \pm 0.43$ | $3.82 \pm 0.26$ | $3.98 \pm 0.45$ |

ANOVA for mean comparisons (LSD, $P < 0.05$) was performed among H30-6, H411, and B336 (the average fruit weight, fruit shape index, soluble solids, total sugar, and titratable acid were analyzed by Dunnett T3 test among different treatments). Lowercase letters indicate differences at the level of significance of 5%; values are presented as mean ± SE.

### Table 2: Flower organ numbers and evenness degree of stigma length of H30-6, H411, and B336.

| Lines/varieties | Average petal number | Average sepal number | Average anther number | Average ovule numbers | Average style number | the evenness degree of stigma length (%) |
|-----------------|----------------------|----------------------|-----------------------|-----------------------|----------------------|----------------------------------------|
| H30-6           | 5                    | 5                    | 20                    | 10                    | 5                    | 80                                     |
| H411            | 5                    | 5                    | 21.87                 | 10                    | 5                    | 40                                     |
| B336            | 5                    | 5                    | 10                    | 10                    | 5                    | 80                                     |

Flower Organs

Among the flower organs, we observed the numbers of petals, sepals, anthers, and ovules, and the stigma length evenness (5, 5, 20, 10, 80%) was equal to that of B336, but H411 had more anthers (21.87) and lower stigma length evenness (40%) (Table 2, Figures 2A–F). Therefore, the number of flower organs is not the reason for the low seed formation of H30-6. Field observations of anther dehiscence showed that H411 and B336 released large amounts of pollen while almost no pollen was released from H30-6 (Figures 2G–I). Similarly, the dried anthers released little pollen. These results revealed that there are abnormalities in the anthers or pollen of H30-6 (Figures 2J–L).

Pollen Morphology, Viability, and Quantity

The scanning electron microscopy results (Figures 3A–F) showed that the loquat pollen grains were medium in size, prolate, and tricolporate. We also found that the pollen sexine ornamentation of the three varieties was striated, with sparse holes between the ridges. However, there were significant differences in normal pollen ratios among H30-6, H411, and B336. The H30-6 line presented the lowest normal pollen rate (34.98%), while those of H411 and B336 were 76.91 and 92.29%, respectively (Figures 3D–F). The viability test presented similar results, as the germination rates of the H30-6, H411, and B336 lines were 4.04, 61, and 72.83%, respectively (Figures 3G–J). Thus, the H30-6 line exhibited low male fertility. According
those on the stigmas of H30-6, where only the small areas of two opposite angles were stained. The aniline blue staining results showed that the pollen germinated and that the pollen tubes passed through the styles, which also indicated that the stigma receptivity was normal. We calculated the percentages of stigmas with pollen, styles with germinated pollen, and styles penetrated by pollen tubes for each of the five pollination methods. For the first method (natural pollination with bag), there was no pollen on the stigmas and no pollen germination on the styles, regardless of whether the observation was made 2, 3, or 4 DAO (Figures 6D–F, Supplementary Table 2). For the second method (open pollination), from 2 DAO to 4 DAO, the percentages of stigmas with pollen, styles with germinated pollen, and styles with pollen tubes that passed through increased (21.7, 41.18, and 46.67%; 21.71, 39.5, and 45.56%; 0, 4.2, and 16.67%; respectively) (Figures 6G–I, Supplementary Table 2). The staining results of styles pollinated with H30-6 pollen (2 DAP) showed that the percentages of stigmas with pollen, styles with germinated pollen, and styles with pollen tubes that passed through were much lower than the percentages observed under pollination with H411 and B336 pollen (Figures 6J–L, Supplementary Table 2). These results suggest that H30-6 pollen can germinate on H30-6 styles but that its low pollen viability may cause a lower in situ germination rate and a lower percentage of style pollen tube
passage, also proving that the natural fruits of H30-6 are likely hybrid fruits.

**Embryo Sac Structure**

As we observed ovules at 1 DBO, the embryo sacs should have been mature with 8 nuclei. H30-6, H411, and B336 all had normal embryo sacs, but the normal embryo sac rate of H30-6 was only 22.54%, which was obviously lower than that of the controls (H411 71.79%, B336 95.93%) (Figure 7, Supplementary Table 3). Each ovary had 10 ovules, so in H30-6, the normal ovule number per ovary was 2.25. This number was close to the average seed number of H30-6 natural fruits (1.54), so we speculate that embryo sac abnormality may be the most restrictive factor for lower seed formation. The abnormal embryo sacs could be divided into three main kinds: those remaining in the tetrad stage (with one functional macrospore or with four macrospores, 17.27%), those remaining in the binucleate embryo sac stage (1.82%), and those without an embryo sac (68.18%) (Figure 7, Supplementary Table 3). We hypothesize that majority of functional macrospores were disabled, which caused the differentiation shutoff or degradation of female gametes.

**Fruit and Seeds Obtained Through Various Pollination Treatments**

The pollination treatments performed over consecutive years showed that when artificial selfing was applied to H30-6, the average fruit setting rate, average seed number per fruit and average seed ovule ratio were 9.93 ± 6.08%, 1.82 ± 0.28%, and 2.11 ± 1.44%, respectively (Table 4). Therefore, we believe that H30-6 is a self-compatible line. H30-6 pollination with H411 or B336 pollen dramatically improved the fruit setting rate (25.2 ± 6.23% and 26.68 ± 6.37%, respectively) compared with those under artificial selfing (9.93 ± 11.64% and 0.47 ± 2.94%) and natural pollination with bagging (2.13 ± 5.46% and 0.09 ± 1.24%) (Table 4). When H30-6 was open-pollinated, the fruit setting rate was significantly increased than those under natural pollination with bagging. These results showed the low fertility of H30-6 pollen was a limiting factor for the fruit setting. However, all pollination treatments to H30-6 showed no significant difference in seed number per fruit (from 1.59 ± 0.14 to 2 ± 0.17), which proved that the male gametes were not the main reason for the low seeds’ formation in the natural fruits of H30-6 and may indicate that at least 1 to 2 seeds were usually needed for the loquat fruit set of H30-6.
The average seed number per fruit was also close to the former results of the number of ovules with normal embryo sac (2.25) in the ovary of H30-6. All these results proved that embryo sac abnormality was the most important restrictive factor for seed formation of H30-6. The seed ovule ratios were dramatically increased when H30-6 was pollinated with H411 or B336 pollen than that natural pollinated with the bag. But there was no significant difference in seed ovule ratio among the four pollination treatments, pollination with pollen of H411, pollination with pollen of B336, open pollination, and artificial selfing when H30-6 was the female parent (Table 4). Meanwhile, the fruit setting rate and seed ovule ratio were without significant difference among pollination treatments (pollinated with pollen of B336 or H30-6, and artificial selfing) to H411 (Table 4). There was no significant difference in fruit setting rate and seed ovule ratio among pollination treatments (pollinated with pollen of H30-6 or H411, and artificial selfing) to B336 (Table 4). The results above showed that H30-6 can be used as a male parent in loquat breeding.

### Somatic Cell Karyotypes

The small amount and low vitality of pollen and the differentiation shutoff or degradation of the female gametes showed that H30-6 had low gametophyte fertility, which caused the low seed formation of the loquat fruit. We believe that there must be some common reasons for the low gametophyte fertility. As the abnormal meiosis of PMCs caused the male gametes to be sterile and the disabled functional macrogametes came from the MMCs, we observed the chromosomes of H30-6 to try to determine a common underlying factor.

The chromosome number shoot and stem-tip cells showed that H30-6 was diploid (2n = 2x = 34), which was the same as that of the controls; thus, the univalent was observed during PMC meiosis was not caused by an unbalanced chromosome number. Then, we analyzed the chromosome karyotype. The relative length (%) (long arm + short arm = full length), arm ratio (long/short) and chromosome type were assessed (Supplementary Table 4). The karyotype formulas were as follows: 2n = 2x = 26m + 8sm (H30-6), 2n =
### TABLE 3 | Counting of pollen mother cell (PMC) meiosis phenomena.

| Meiosis stage | Phenomenon                  | H30-6 | Percentage (%) | H411 | Percentage (%) | B336 | Percentage (%) |
|---------------|-----------------------------|-------|----------------|------|----------------|------|----------------|
|               | Number of PMCs              |       | Percentage     |      | Percentage     |      | Percentage     |
| Metaphase I   | Normal                      | 10    | 20.41          | 490  | 81.53          | 135  | 97.82         |
|               | With chromosomes lag        | 32    | 65.31          | 104  | 17.3           | 3    | 2.17          |
|               | Chromosomes doubled         | 8     | 16.33          | 7    | 1.16           |      |               |
|               | Total number of PMCs        | 49    |                | 601  |                | 138  |               |
| Anaphase I    | Normal                      | 0     | 0              | 277  | 90.52          | 112  | 100           |
|               | With chromosomes lag        | 20    | 95.24          | 24   | 7.84           |      |               |
|               | With micronucleus           | 8     | 38.1           |      |                |      |               |
|               | With chromosomes bridge     | 5     | 1.63           |      |                |      |               |
|               | Total number of PMCs        | 21    |                | 306  |                | 112  |               |
| Telophase I   | Normal                      | 7     | 9.33           | 329  | 95.64          | 58   | 100           |
|               | With chromosomes lag        | 36    | 0.48           | 15   | 4.36           |      |               |
|               | With chromosomes bridge     | 1     | 1.33           |      |                |      |               |
|               | With micronucleus           | 32    | 42.67          |      |                |      |               |
|               | Total number of PMCs        | 75    |                | 344  |                | 58   |               |
| Prophase II   | Normal                      | 15    | 31.91          | 255  | 93.41          | 219  | 100           |
|               | With dissociative chromosomes | 17   | 36.17          |      |                |      |               |
|               | With micronucleus           | 12    | 25.53          | 18   | 6.59           |      |               |
|               | Unequal separation of chromosomes | 2    | 4.26          |      |                |      |               |
|               | Chromosomes doubled         | 1     | 2.13           |      |                |      |               |
|               | Total number of PMCs        | 47    |                | 273  |                | 219  |               |
| Metaphase II  | Normal                      | 5     | 3.21           | 261  | 84.74          | 148  | 98.01         |
|               | With chromosomes lag        | 86    | 55.13          | 43   | 13.96          | 3    | 1.99          |
|               | Chromosomes doubled         | 64    | 41.03          |      |                |      |               |
|               | With micronucleus           | 46    | 29.49          | 4    | 1.3            |      |               |
|               | With perpendicular spindle  | 6     | 3.85           |      |                |      |               |
|               | With splayed spindle        | 9     | 5.77           |      |                |      |               |
|               | Total number of PMCs        | 156   |                | 308  |                | 151  |               |
| Anaphase II   | Normal                      | 0     | 0              | 195  | 78.31          | 86   | 98.85         |
|               | With chromosomes lag        | 57    | 91.94          | 15   | 6.02           | 1    | 1.15          |
|               | With micronucleus           | 21    | 33.87          | 9    | 3.61           |      |               |
|               | With chromosomes bridge     | 21    | 12.05          | 30   |                |      |               |
|               | With splayed spindle        | 8     | 12.9           |      |                |      |               |
|               | With perpendicular spindle  | 2     | 3.23           |      |                |      |               |
|               | With cross spindle          | 1     | 1.61           |      |                |      |               |
|               | Total number of PMCs        | 62    |                | 249  |                | 87   |               |
| Telophase II  | Normal                      | 17    | 7.36           | 299  | 81.25          | 580  | 96.99         |
|               | With chromosomes lag        | 107   | 46.32          |      |                | 13   | 2.17          |
|               | Unequal separation of chromosomes | 50    | 21.65         | 3    | 0.82           |      |               |
|               | With micronucleus           | 97    | 41.99          | 38   | 10.33          | 2    | 0.33          |
|               | Dyad                        | 2     | 0.54           |      |                |      |               |
|               | Triad                       | 11    | 4.76           | 14   | 3.8            | 3    | 0.5           |
|               | Polyad                      | 5     | 2.16           | 12   | 3.26           |      |               |
|               | Total number of PMCs        | 231   |                | 368  |                | 598  |               |

2x = 18 m + 16 sm (H411), and 2n = 2x = 30 m + 4 sm (B336). The chromosome lengths varied from 1.54 ± 0.08-2.63 ± 0.09 µm (H30-6), 2.02 ± 0.16–3.59 ± 0.16 µm (H411), and 1.57 ± 0.05–2.5 ± 0.1 µm (B336). According to the chromosome field theory, the chromosomes belonged to the small chromosome. The ratios of the longest chromosome to the shortest were 1.71 (H30-6), 1.78 (H411), and 1.59 (B336), and chromosome arm ratios above 2 accounted for 0.03 (H30-6),...
0.13 (H411), and 0.06 (B336) of the total ratios. Therefore, according to Stebbins’s (1971) classification, the karyotypes were all 2A types. The asymmetrical karyotype coefficients were 59.03% (H30-6), 61.95% (H411), and 59.23% (B336). Figure 8 shows the chromosome images, karyograms, and idiograms of H30-6, H411, and B336, respectively. The chromosome observation results revealed no obvious chromosomal numerical or structural abnormalities.

DISCUSSION
Low Female Gametophyte Fertility Is Mainly Responsible for the Low Seed Formation of H30-6
Few-seed usually results from low gametophyte fertility (Xiao et al., 2007; Rédei, 2008; Qiu et al., 2012; Li et al., 2016; Royo et al., 2016), self-incompatibility (Zhang et al., 2012; Gambetta et al., 2013; Garmentia et al., 2019) and embryo abortion (Pearson, 1932; Tang, 2001). There was no self-incompatibility found for H30-6, which can be demonstrated proved by the result of artificial selfing and in situ pollen germination. As a result, low fertility should be responsible for the few-seed nature of H30-6. There were no abnormalities found in flower morphology, but only limited pollen existed on the dehiscent anther indicating a low male gamete fertility character. Additionally, this was verified according to the vertical sections of mature anther and pollen morphology observation. Further, the pollen viability test showed that only 4.04% of pollen could germinate in vitro. So H30-6 was a male sterile material depending on Deng (2009a) standard. However, the pollination treatment results showed that there was no significant difference in the seed number (1.59-2) per fruit when the pollen of H30-6, H411, and B336 was used or open-pollinated. Meanwhile, the former embryo sac observation results showed that there were only 2.25 normal ovules in each ovary of H30-6. It was reasonable to assume that abnormal embryo sacs should be responsible for low seed formation.

May Abnormal Meiosis Lead to Low Female Gametophyte Fertility of H30-6?
The formation of both male and female gametophytes is a precisely coordinated process, including the formation of gametophyte mother cells, meiosis, and gametophyte development. Therefore, there are three likely mechanisms leading to low gametophyte fertility: pre meiosis abnormality, such as a lack of sporogenous cells (Schiethaler et al., 1999; Yang et al., 1999) and defects in meiotic entry (Hong et al., 2012), meiosis abnormalities, such as arrests at one meiosis period (Siddiqi et al., 2000) and aberrant chromosome distribution (Hoyt and Geiser, 1996; d’Erfurth et al., 2008; De Storme and Geelen, 2011), and postmeiotic abnormality, especially reproductive companion cell abnormalities, which will cause gametophytes to be sterile (Chaubal et al., 2000; Guo et al., 2001; Suzuki et al., 2001; Smith et al., 2002; Kawanabe et al., 2006; Li et al., 2006; Zhang et al., 2006). In this study, we found no premeiotic or post-meiotic abnormalities but a multitude of meiosis abnormal phenomena of PMCs. Therefore, low male fertility is caused by abnormal meiosis. Although we did not observe the meiosis of megasporocytes, the differentiation shutoff or degradation of female gametes showed that most of the functional macrospores were disabled, which we speculated could be due to that there were meiotic abnormalities in megasporocytes.

Possible Mechanism of H30-6 Abnormal Meiosis
Chromosome number and structural changes can lead to abnormal meiosis (Grandont et al., 2014; Rocha et al., 2019). Low fertility is often found in polyploids, which have been used for loquat seedless and few-seed breeding (Maria et al., 2013). H30-6 was identified as being a diploid line. That was to say, the abnormal meiosis of H30-6 was not caused by polyploidization. A recent report showed that a 2.09 Mb chromosome fragment translocation causes abnormalities during meiosis and leads to pollen semisterility and fewer seeds in watermelon (Tian et al., 2021). Additionally, the fragment translocation results in one quadrivalent ring of four chromosomes at prometaphase I during meiosis. During the meiosis of H30-6, the earliest abnormalities were found during diakinesis, and a high frequency of univalent chromosomes (6.87) was the main abnormality. The most likely reason was that homolog pairing failure caused abnormal meiosis.

Homolog pairing is subdivided into three levels: preselection of homologous chromosomes, homologous connections by the synaptonemal complex (the process also known as synapsis), and the precise matching of DNA molecules in the process of genetic exchange and conversion (the process also known as gene recombination) (Moens, 1969; Loidl, 1990). Abnormalities occurring in all three of these levels may result in the univalent characteristics found in diakinesis.

When or how homologous chromosomes select each other is still unknown (Bozza and Pawlowski, 2008); However, chromosomal movements that are performed prior to and as a precondition to any homologous interaction have been widely supported (Loidl, 1990; Dawe et al., 1994; Bass, 1997), especially when considering telomere mediated movement (Lange, 1998; Nimmo et al., 1998; Trelles-Sticken and Scherthan, 2000; Seps and Schwarzacher, 2020). Varas et al. (2015) reported that two Sad1/UNC-84 (SUN)-domain proteins (AtSUN1 and AtSUN2) play an important role in telomeres movement during meiosis, and the double mutant exhibited severe meiotic defects, including a delay in the progression of meiosis, an absence of full synapsis, and a reduction in the mean cell chiasma frequency.

The synaptonemal complex stabilizes the presynaptic alignment of the axes of the homologous chromosomes and promotes the maturation of crossover recombination events, which is widely conserved in sexually reproducing eukaryotes with the lateral elements (LEs), central elements (CEs), and transverse element (transverse filaments, TFs) (Gillies and Moens, 1984; Christa, 1996; Dawe, 1998). Thus far, several proteins involved in LE, such as budding yeast Hop1 and Red1;
FIGURE 5 | Images of abnormal phenomena of PMCs meiosis: (A1–H4), images of PMCs meiosis of H30-6: (A1–A3), diakinesis, (A1) showing the univalent, (A2) showing the trivalent, (A3) showing the quadrivalent (arrowhead) and pentavalent (arrow); (B1–B3), metaphase I, (B1) showing a chromosomes lag, (B2,B3) showing doubled chromosomes; (C) showing chromosomes lag and micronucleus in anaphase I; (D1,D2), Telophase I, showing chromosomes lag, micronucleus (Continued)
FIGURE 5 | and the bridge of chromosomes, (E1–E4), prophase II, (E1) showing the dissociative chromosome, (E2) showing the unequal separating of chromosomes, (E4) showing the doubled chromosomes, (F1–F4), metaphase II, (F1) showing chromosomes lag and micronucleus, (F2) showing doubled chromosomes, (F3) showing perpendicular spindle, (F4) showing splayed spindle; (G1–G3) anaphase II, (G1) showing the splayed spindle chromosomes lag, (G2) showing perpendicular spindle and chromosomes lag, (G3) showing cross spindle and micronucleus; (H1–H4), telophase II, (H1) showing chromosomes lag and micronucleus, (H2) showing unequal separation of chromosomes, (H3) showing triad and micronucleus, (H4) showing polyad. (I–P5), images of PMCs meiosis of H411: (I) showing the univalent (arrowhead), trivalent (arrow), and quadrivalent (red arrow); (J1,J2), metaphase I, (J1) showing chromosomes lag, (J2) showing doubled chromosomes. (K1,K2), anaphase I, (K1) showing chromosome lag and chromosome bridge, (K2) showing chromosomes bridge; (L) showing chromosomes lag and micronucleus in telophase I; (M) showing micronucleus in prophase II; (N) showing chromosomes lag in metaphase II; (O1,O2), anaphase II, (O1) showing chromosomes lag and chromosomes bridges, (O2) showing chromosome bridge; (P1–P5) telophase II, (P1) showing unequal separating of chromosomes, (P2) showing dyad, (P3) showing triad and micronucleus, (P4,P5) showing polyad and micronucleus. (Q–R2), images of PMCs meiosis of B336: (Q) showing chromosome lag and chromosomes bridge in anaphase II; (R1) showing micronucleus, (R2) showing a triad. Scale: 10 µm.

FIGURE 6 | Images of the stigmas receptivity test and pollens in situ germination: (A–C) the stained area showing the stigmas receptivity of H30-6, H411, and B336 respectively. (D–F) showing pollen in situ germination at 2, 3, and 4 days after H30-6 bagging natural pollination receptivity. (G–I) showing pollen in situ germination at 2, 3, and 4 days after H30-6 open pollination receptivity. (J–L) showing pollen in situ germination at 2 days after H30-6 artificial selfing, pollination with pollens of H411, and pollination with pollens of B336.

mice SYCP2 and SYCP3; nematode HIM-3, HTP-1, HTP-2, and HTP-3; and plants proteins ASY1/PAIR2 and ASY3/PAIR3 have been reported (Hollingsworth et al., 1990; Smith and Roeder, 1997; Yang et al., 2006; Sanchez-Moran et al., 2008; Ferdous et al., 2012; Lui and Colaiacovo, 2013). Furthermore, yeast Zip1, mice SYCP1, Drosophila C (3) G, nematode SYP-1/2/3/4, Arabidopsis thaliana ZYP1, and rice ZEP1 have been reported to be components of TFs (Sym et al., 1993; Page and Hawley, 2001; De Vries et al., 2005; Higgins et al., 2005; Wang et al., 2010; Lui and Colaiacovo, 2013).
FIGURE 7 | Images of normal and abnormal embryo sacs: (A–C) normal embryo sacs of H30-6, H411, and B336, respectively. (D–F) abnormal embryo sacs of H30-6: (D) embryo sac at the tetrad stage, (E) embryo sac at the binucleate embryo sac stage, (F) without embryo sac. The embryo sac area was magnified in the small box on each image. Scale: 20 μm.

TABLE 4 | The continuous years of pollination treatments results (2018 to 2021).

| Female parent (♀) | Pollination treatment | No. of flowers | No. of fruits | No. of seeds | Fruit setting rate (%) | No. of seeds pre fruit | Seed ovule ratio |
|-------------------|-----------------------|----------------|--------------|--------------|------------------------|-----------------------|-----------------|
| H30-6 × H411 (♂) | 903                   | 196            | 360          | 25.20 ± 6.23 | 1.90 ± 0.15            | 4.84 ± 1.04           |
| × B336 (♂)       | 904                   | 204            | 400          | 26.68 ± 6.37 | 2.00 ± 0.17            | 5.60 ± 1.73           |
| Artificial setting | 887                   | 96             | 200          | 9.93 ± 6.08  | 1.82 ± 0.28            | 2.11 ± 1.44           |
| Open pollination | 720                   | 159            | 261          | 14.20 ± 3.84 | 1.59 ± 0.14            | 2.28 ± 0.73           |
| Bagging natural pollination | 1,203              | 6              | 11           | 0.47 ± 0.35  | 1.88 ± 0.13            | 0.09 ± 0.06 |
| H411 × H30-6 (♂) | 762                   | 144            | 547          | 19.06 ± 8.94 | 3.22 ± 0.72            | 7.29 ± 4.70           |
| × B336 (♂)       | 780                   | 222            | 874          | 28.22 ± 10.63 | 3.63 ± 0.40           | 11.08 ± 5.39         |
| Artificial setting | 943                   | 287            | 1,187        | 31.56 ± 10.83 | 3.89 ± 0.60            | 12.93 ± 5.79         |
| Open pollination | 585                   | 98             | 346          | 13.15 ± 4.26 | 3.39 ± 0.49            | 4.90 ± 2.46           |
| Bagging natural pollination | 1,219              | 81             | 224          | 8.40 ± 3.73  | 2.62 ± 0.33            | 2.41 ± 1.15 |
| B336 × H30-6 (♂) | 731                   | 167            | 574          | 20.68 ± 10.53 | 2.66 ± 0.70            | 6.96 ± 4.84           |
| × H411 (♂)       | 706                   | 256            | 932          | 35.70 ± 7.45 | 3.62 ± 0.63            | 13.29 ± 4.03         |
| Artificial setting | 618                   | 260            | 953          | 53.20 ± 20.23 | 3.47 ± 0.41           | 18.71 ± 6.86         |
| Open pollination | 615                   | 104            | 302          | 12.33 ± 1.77 | 3.06 ± 0.52            | 3.55 ± 1.16           |
| Bagging natural pollination | 1,216              | 60             | 138          | 10.86 ± 7.95 | 2.22 ± 0.15            | 2.55 ± 1.95 |

ANOVA for mean comparisons (LSD, P < 0.05) was performed among different treatments of the same female parent (the seed ovule ratio was analyzed by Dunnett T3 test among different treatments when H30-6 was a female parent). Lowercase letters indicate differences at the level of significance of 0.05; values are presented as mean ± SE.

The gene recombination structure, which is also known as the visualized crossover (CO), links the homologs after diplotene in most organisms. Typically, every chromosome undergoes at least one obligate CO event to ensure proper segregation at metaphase I (Jones, 1984). The double-strand break repair model (DSBR) explained the gene recombination (Szostak et al., 1983; Mercier et al., 2015). Meiotic DNA DSBs are formed by the highly conserved SPO11 protein (Edlinger and Schlogelhofer, 2011; Bernard, 2013) and PRD1, PRD2, AtPRD3/OsPAIR1, DFO, and CRC1 proteins in plants (De Muyt et al., 2009; Miao et al.,...
In most species, homologous chromosomes do not pair properly in mutants that do not normally form meiotic DNA double-strand-breaks (DSBs) (Peoples-Holst and Burgess, 2005; Yu et al., 2010; Ji et al., 2016; Robert et al., 2016). The plant MRE11, RAD50, and COM1 genes are functionally conserved in the early steps of DSB processing, and their respective mutants all display strong chromosome fragmentation at anaphase I (Mercier et al., 2015). In Arabidopsis, DMC1-mediated inter-homologous (IH) DNA repair is the predominant pathway of DSB repair, and when DMC1 is missing, RAD51 works as a backup pathway leading to a complete absence of synopsis and bivalent formation (Couteau et al., 1999; Deng and Wang, 2007). Finally, the CO formation relies on two pathways: the ZMM pathway, which relies on a group of proteins called ZMMs, as well as proteins MLH1 and MLH3, and the non-ZMM CO pathway, in which protein MUS81 has been characterized in the non-ZMM CO pathway in plants (Mercier et al., 2015). The mutants of the ZMM pathway and MUS81 were reported to exhibit various degrees of decrease in COs (Mercier et al., 2015).

Therefore, to clarify the molecular mechanism of H30-6 abnormal meiosis, the above proteins involved in the homolog pairing constitute the main points of this study.

**CONCLUSIONS**

The diploid loquat line H30-6 is a potentially commercial few-seed (1.54 per fruit) loquat, with high fruit edible rate (70.77%). The diploid loquat line H30-6 is a male sterile material, and this sterility is caused by the abnormal meiotic synapses. The low fertility of H30-6 pollen was a limiting factor for the fruit setting. The main reason for the few seeds character of H30-6 was the female gamete abnormality.

**DATA AVAILABILITY STATEMENT**

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding authors.
**AUTHOR CONTRIBUTIONS**

GL, QG, and JD conceived and designed the research and revised the manuscript. QX performed the experiments and wrote and revised the manuscript. XW and SX helped with the data analysis and revised the manuscript. PW and SL managed the material collection. XL and QH helped with the material filed management. HS, DW, XX, SW, and DJ helped with the fund management, drug purchase, and instrument management. All the authors read and approved the final manuscript.

**FUNDING**

This research was funded by the National Key R&D Program of China (No. 2019YFD1000900), the National Nature Science Foundation of China (32171820), the Chongqing Science and Technology Commission (cstc2021jscx-gksbX0010 and cstc2021jcyj-sxmX1156), the Innovation Research Group Funds for Chongqing Universities (CXTQ19005), and the Chongqing Forestry Administration (YuLinKeYan2022-14).

**ACKNOWLEDGMENTS**

QX expresses her great and deep appreciation to all the laboratory members for so much help and so many great moments. We also thank the reviewers and the editor for the helpful comments and suggestions on this manuscript.

**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2022.882965/full#supplementary-material

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