*Journal of Tropical Forest Science* 34(3): 316–325(2022)
https://doi.org/10.26525/jtfs2022.34.3.316


# UNDERSTANDING FLOWER DEVELOPMENT, MEGASPOROGENESIS AND MICROSPOROGENESIS IN *EUCALYPTUS PELLITA* AS AN AID TO TRIPLOID INDUCTION

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*Submitted July 2021; accepted November 2021*

Induction of triploids through sexual polyploidisation requires in-depth understanding of plant reproductive biology. However, the method to identify the developmental stages of the gametes in *Eucalyptus* is still unclear. In this study, microsporogenesis and megasporogenesis of *Eucalyptus pellita* were revealed by staining with acetocarmine and paraffin sectioning. Results showed two types of stamens existed in a single flower, and the microsporogenesis in anthers of long stamens were significantly ahead of those in short ones. A temporal relationship between megasporogenesis and microsporogenesis could be found in a single flower where megasporogenesis was consistent with microsporogenesis in the long stamens. Although the flower bud development and meiosis process of a single flower bud at different positions on the branch were asynchronous, those positioned at the upper end of the branch were relatively consistent. From the study report, a simple and accurate method to identify the meiotic period of megasporocytes was proposed based on temporal relationships between megasporogenesis and microsporogenesis. The study not only deepened the understanding of *E. pellita* reproductive biology, but also provided important theoretical guidance for the genetic improvement of this tree species, especially the triploid breeding by sexual polyploidisation.

Keywords: Cytological characteristics, meiotic stages, gametes, polyploidy breeding, reproductive biology

## INTRODUCTION

*Eucalyptus* from the family Myrtaceae is one of the most important genera worldwide, which provides many species for the establishment of productive hardwood forest plantations (Ladiges et al. 2003, Wu et al. 2019). One species, the *Eucalyptus pellita* is fast growing and has good timber properties, which is an important plantation tree species in many countries and regions. Due to the advantages of wood color and disease resistance, it is also one of the most important parents plant for the *Eucalyptus* interspecific hybrid breeding (Hii et al. 2017, Dhakad et al. 2018). Due to the high utilisation value, *Eucalyptus* has been widely planted in more than 200 countries and regions (Forrester 2013, Bayle 2019).

Triploid breeding could effectively increase wood yield and improve wood quality, which has been proven in the genetic improvement of several tree species (Lu et al. 2013, Li et al. 2016). In polyploids induction, it is very important to obtain unreduced 2n gametes. Studies have shown that the key to artificially inducing unreduced gametes was to select a specific period of meiosis to induce mutation (Wang et al. 2010, Yao et al. 2020). The process requires a deep understanding of the timing and prediction of gametes development in a tree species, which is still unclear in *Eucalyptus*. In the past half century, there were some basic reports on the reproductive biology of *Eucalyptus* (Beardsell et al. 1993, Barbour et al. 2008, Potts et al. 2008). However, the complete process of microsporogenesis and megasporogenesis in *Eucalyptus* has not been studied (Davis 1968, Davis 1969, Yang & Kang 2015, Yang et al. 2015). In order to address the above-mentioned research gaps and deficiencies, *E. pellita* was chosen to study the flower bud development and cytological characteristics of microsporogenesis and megasporogenesis. The relationship between

microsporogenesis and megasporogenesis were analysed in order to identify the meiotic stages of megasporocytes over time. Results of this study would increase the understanding of *E. pellita* reproductive biology and provide important theoretical guidance for the genetic improvement of this tree species, especially on the triploid breeding by sexual polyploidisation.

## MATERIALS AND METHODS

### Plant materials

The branches and flower buds used in this study were selected from an *E. pellita* clone composed of 20 individual ramets. The clone was named P3, which is fast growing with straight trunk was planted in a seed orchard at the Guangxi Dongmen Forest Farm (Guangxi Zhuang Autonomous Region, China) in 2015. After several years of observation, growth and flowering period within this clone were confirmed to be relatively consistent.

### Flower bud collection

Primary branches in *E. pellita* developed several orders of sub-branching, with flowering occurring on the lowest sub order branches. There were 120 flower branches selected for sampling with the same height from the ground, facing the same direction (south), and a relatively consistent number of flower buds. From the flower buds occurring on the branch, the development of flower buds and microsporogenesis were continuously monitored. When the leptotene stage of microsporocytes was observed, the first sampling (time at 0 h) was performed. Subsequently, flowering buds were sampled every 24 hours. Arabic numerals were used to number the sampled flower branches according to the different positions of the flower branches from the bottom of the tree. Furthermore, the umbels from the bottom to the top on each flower branch were numbered serially using letters (A, B, C...) according to their location on the branch. As an example, 2-D is the sample from the fourth umbel on the second flower branch from the bottom of the tree (Figure 1). The single flower bud collected from the umbel was measured three times by using digital Vernier caliper. The measurements with up to 2 decimals places were calculated for the mean diameter. Subsequently, flower bud was immediately fixed in a centrifuge tube with cold Carnoy's fixative solution (acetic acid/ethanol; 1/3) at 4 °C for 24 hours. The buds were finally placed in 70% ethanol for long-term storage at 4 °C.

### Cytological observation of microsporogenesis

In order to observe microsporogenesis in *E. pellita*, flower buds collected at different times were taken out from the storage condition. Anthers containing cells during meiotic periods were extracted from flower buds, transferred to slides, squashed and stained in 2% acetocarmine for 5 min. The slides containing microsporocytes were observed using a microscope and microphotographs were taken using a camera system.

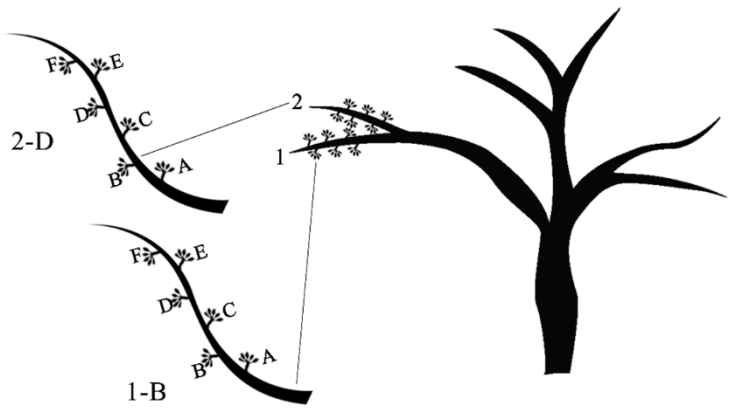


**Figure 1** Location of umbels on branches of the tree in *Eucalyptus pellita*
Each umbel was identified by a letter sequentially from the bottom to the top of the flower branch; flower branches were numbered using Arabic numerals in sequence from bottom to top according to their growth order on the tree

### Cytological observation of megasporogenesis

*E. pellita* is a hermaphroditic plant. While investigating microsporogenesis, the remaining parts of the flower buds were used for cytological observation of megasporogenesis. The epidermis and ovary wall were carefully cut and peeled off using a scalpel. Placentas attached with ovules were dehydrated with ethanol solution (from 70% to 100%). Subsequently, they were treated with dimethylbenzene and embedded in paraffin. The embedded placentas were sectioned at 8 µm thickness using a slicer and stained with 0.5% hematoxylin. Finally, the slides containing the sections were observed and photographed under a microscopic camera system.

### Statistical analysis

The SPSS version 24.0 statistical package was used in the statistical analysis. The ratios of the number of flower buds during meiosis to the total number of flower buds showed the percentages of flowers buds in each meiotic stage. The mean diameters of flower buds were analysed by GLM-Univariate to reveal the differences between different locations on the branch (Yang et al. 2015). The relationships between megasporogenesis and microsporogenesis in the same flower bud were examined using Pearson's correlation coefficients.

## RESULTS AND DISCUSSION

### Microsporogenesis in *E. pellita*

The results from the microsporogenesis process study showed that microsporocytes of *E. pellita* had obvious nuclei with thick cytoplasms before meiosis began. During the leptotene stage of microsporogenesis (Figure 2a), these cells had thick cytoplasm and the chromatin condensed into chromosomes that intertwined into filament forms. In the pachytene stage (Figure 2b), the chromosomes shrank, thickened, overlapping and entwining with each other. In the diplotene

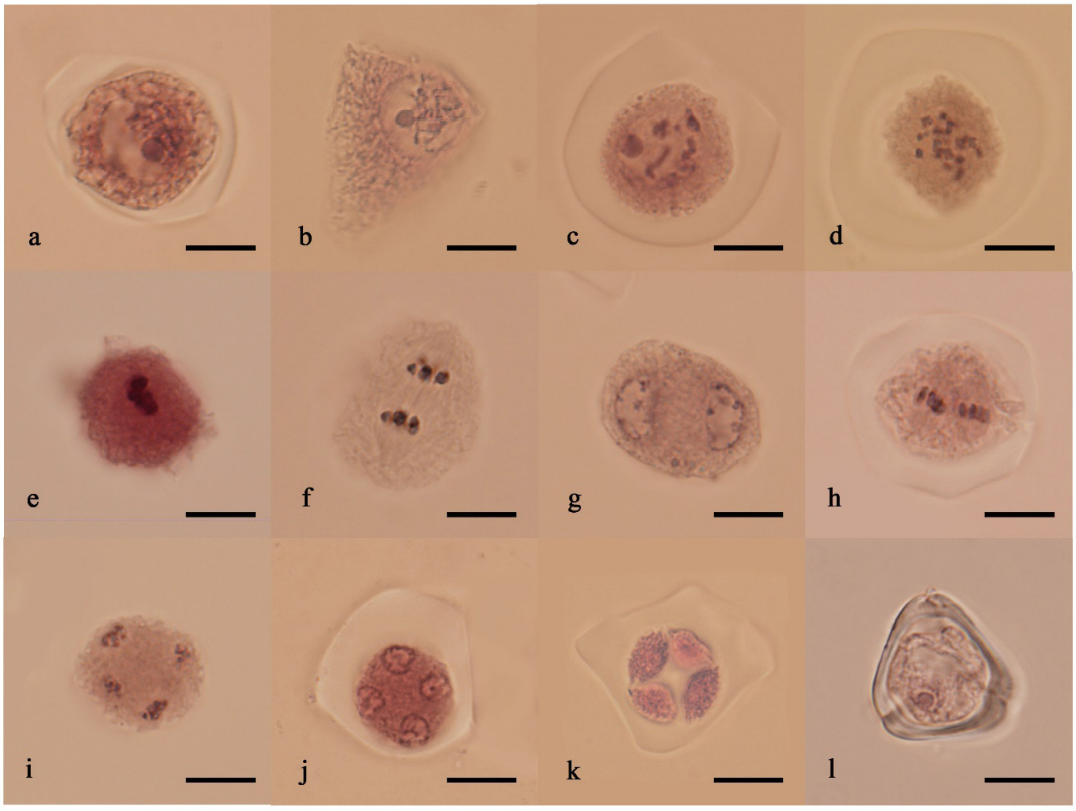


**Figure 2** Microsporogenesis in *Eucalyptus pellita*
Scale bar =10 µm; (a) Leptotene, (b) Pachytene, (c) Diplotene, (d) Diakinesis, (e) Metaphase I, (f) Anaphase I, (g) Telophase I, (h) Metaphase II, (i) Anaphase II, (j) Telophase II, (k) Tetrad, (l) Microspore

stage (Figure 2c), homologous chromosomes began to separate from each other, and a cross knot or circular cross structure could also be observed. The nucleolus and membrane disappeared completely in the diakinesis stage (Figure 2d). The chromosomes condensed into dots or strips that were scattered in the cell and easy to count. Subsequently the microsporocytes underwent two meiosis to form tetrad, and the tetrad cytokinesis was simultaneous (Figure 2e–k). This process was consistent with the results of at least three other taxa of *Eucalyptus* (Davis 1968, Davis 1969, Yang & Kang 2015, Yang et al. 2015). Next, the four microspores had separated from each other with the projections seen under the microscope as triangles (Figure 2l). The clear structure of germinating pores could be seen at the top of triangles.

## Microsporogenesis asynchrony between long stamens and short stamens

After dissection, two types of stamens were found in the individual flower buds of *E. pellita*; the long stamens and the short stamens. Before the operculum falls off, the filament was in a curly state where the long stamens were close to the style and the short stamens were far from the style. Cytological observation showed that the developmental stages during microsporogenesis in anthers of long stamens were ahead of those in anthers of short stamens (Table 1). When almost all of the microsporocytes in the long stamens were in the microspore stage, most of the microsporocytes in the short stamens were in the tetrad stage. This result was consistent with the findings in *E. urophylla* × *E. grandis* and *E. urophylla* × *E. tereticornis* (Yang & Kang 2015, Yang et al. 2015). The phenomenon of microsporogenesis asynchrony was reported in many tree species (Carrizo-Garcia et al. 2017). As an example, microsporogenesis in different anthers in one flower of *Eucommia ulmoides* and *Hevea brasiliensis* had demonstrated asynchrony (Gao et al. 2006, Yao et al. 2017). This was considered to be the result of plants adapting to the environment (Wyatt 1982). In order to ensure the well-timed implementation of breeding work, the microsporogenesis of long stamens was chosen which was ahead of that on short stamens, as the standard for analysing meiotic period in the flower bud.

## Megasporogenesis in *E. pellita*

Megasporocytes were observed by continuous paraffin sectioning. The results showed that the megasporogenesis processes were mostly the same as that of microsporogenesis. The megasporocytes underwent two meioses and the homologous chromosomes in the same cell separated from each other and moved to the two poles under the pull of the spindle during the anaphase II to form a tetrad (Figure 3a–h). There was only one cytokinesis and this occurred during megasporogenesis. The observation was different from the pattern that Davis (1968, 1969) observed in *E. stellulata* and *E. melliodora* where the megasporocytes formed a dyad after the first meiosis. The tetrad consisted of four megaspores arranged in a straight line and three megaspores at the distal end of the chalazal degenerated and

**Table 1** Asynchronous meiosis among stamens of different lengths in *Eucalyptus pellita*

| Flower buds | Percentage of flowers buds in microsporocyte meiotic stages (%) | | | | | | | | Total no. of flowers examined |
|---|---|---|---|---|---|---|---|---|---|
| | Stamen types | Microsporocyte | Leptotene-Pachytene | Diplotene-Diakinesis | Metaphase I-Telophase I | Prophase II-Telophase II | Tetrad | Microspore | |
| Group 1 | Short | 71.43 | 19.05 | 9.52 | | | | | 84 |
| | Long | | 42.86 | 38.10 | 19.05 | | | | 84 |
| Group 2 | Short | | 3.57 | 7.14 | 46.43 | 42.86 | | | 81 |
| | Long | | | | 3.70 | 11.11 | 44.44 | 40.74 | 81 |
| Group 3 | Short | | | | 12.50 | 25.00 | 62.50 | | 79 |
| | Long | | | | | | 5.89 | 94.12 | 79 |

Flower buds were divided into three groups according to sampling time. Group 1, Group 2 and Group 3 represented 48–96, 144–192 and 240 hours of microsporogenesis in *Eucalyptus pellita*

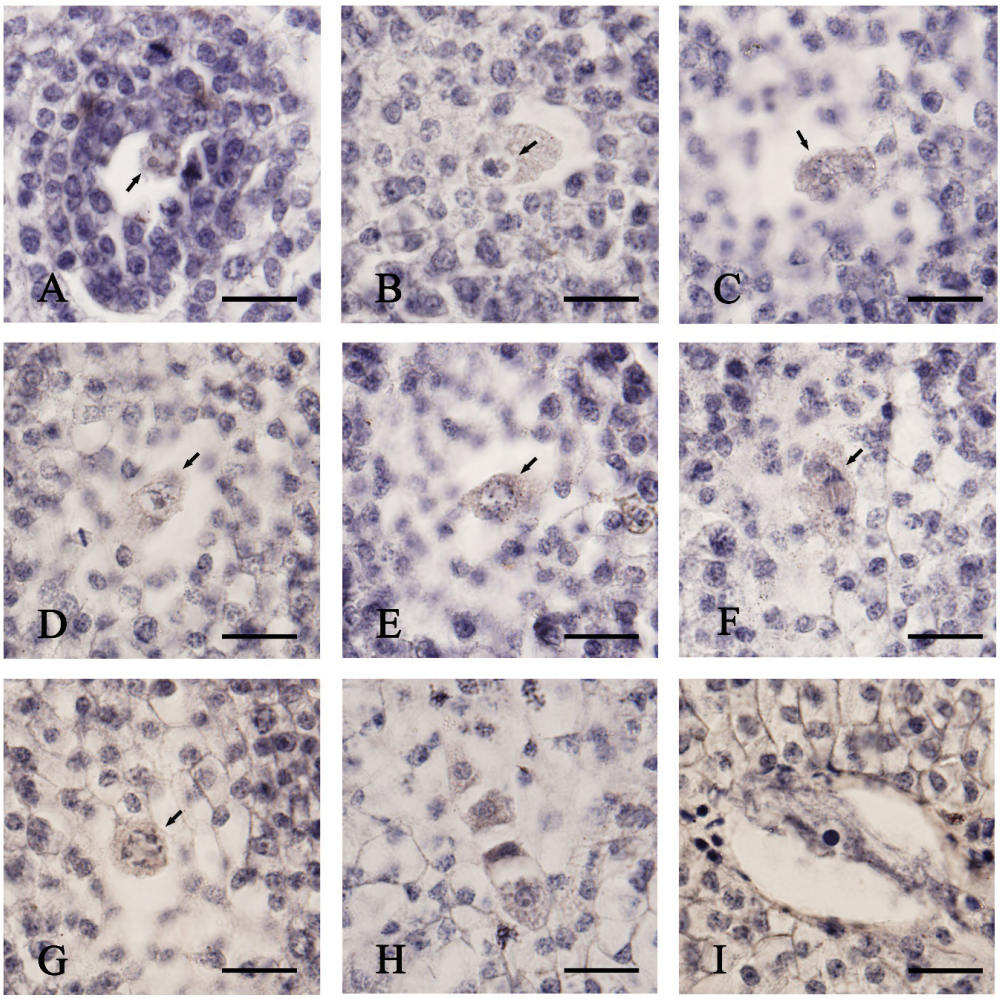


**Figure 3** Megasporogenesis in *Eucalyptus pellita*
Scale bar = 10 μm; (A) Early leptotene, (B) Late leptotene, (C) Pachytene, (D) Diplotene, (E) Diplotene, (F) Anaphase I, (G) Anaphase II, (H) Tetrad, (I) Megaspore; the arrows indicate the megasporocyte in the meiosis stage

disappeared, while the remaining megaspore closest to the chalazal end continued to develop into functional megaspore (Figure 3i). Most of these processes of megasporogenesis in *E. pellita* are similar to *E. stellulata* and *E. melliodora* (Davis 1968, Davis 1969). The difference of cytokinesis pattern between *E. pellita* and other *Eucalyptus* could be a taxonomic feature to distinguish the different species.

## Temporal relationship between megasporogenesis and microsporogenesis

*E. pellita* is hermaphroditic plant, although megasporocytes are usually buried in the ovules and are difficult to observe, microsporogenesis which is easily observed could be used as a reference to identify the process of meiosis of the megasporocytes in the same flower (Li et al. 2008). Therefore, it is very important to make known the relationship between megasporogenesis and microsporogenesis. The meiotic stages of most megasporocytes in the flower bud were regarded as the meiotic stage of the megasporocytes in one flower bud. It was found that megasporogenesis and microsporogenesis in long stamens within the same flower bud had a clear temporal correlation (Table 2). Among the flower buds in which the microsporocyte meiotic stage in long stamens was not observed, 78.38% of them also did not exhibit meiosis in megasporocytes. When the microsporocytes in the long stamens were in the stage from leptotene to diakinesis (Figure 2a–e) in the corresponding flower buds, the megasporocyte meiotic stage concurred with the microsporocyte meiotic stage in long stamens in

**Table 2** Temporal relationship between megasporogenesis and microsporogenesis

| Microsporogenesis | Percentage of flowers buds in megasporocyte meiotic stages (%) | | | | | | | | | Total no. of flowers examined |
|---|---|---|---|---|---|---|---|---|---|---|
| | Megasporocytes | Leptotene | Pachytene | Diplotene | Diakinesis | Metaphase I - Telophase I | Prophase II - Telophase II | Tetrad | Megaspore | |
| Microsporocyte | 78.38 | 12.16 | 9.46 | | | | | | | 370 |
| Leptotene | 13.33 | 24.59 | 23.33 | 16.67 | 13.33 | 8.75 | | | | 305 |
| Pachytene | 15.10 | 11.11 | 25.00 | 19.44 | 16.67 | 12.68 | | | | 355 |
| Diplotene | 10.34 | 6.90 | 17.24 | 24.14 | 20.69 | 13.79 | 6.90 | | | 290 |
| Diakinesis | 7.50 | 7.50 | 15.00 | 17.50 | 30.00 | 12.50 | 7.50 | 2.50 | | 400 |
| Metaphase I - Telophase I | | 6.98 | 13.95 | 18.60 | 20.93 | 23.26 | 11.36 | 4.92 | | 415 |
| Prophase II - Telophase II | | | 10.20 | 14.16 | 14.41 | 16.33 | 20.41 | 14.29 | 10.20 | 409 |
| Tetrad | | | 14.10 | 12.82 | 19.23 | 2.56 | 8.97 | 23.08 | 19.23 | 390 |
| Microspore | | | 4.59 | 5.56 | 4.59 | 2.75 | 6.42 | 21.10 | 54.99 | 270 |

most flower buds. Among the flower buds in the microspore stage in the long stamens, 54.13% of the buds showed that megasporocytes had developed into functional megaspores (Figure 3i). The result showed that the developmental stages of microsporogenesis in the long stamens were temporally consistent with the megasporogenesis stages in the same flower bud. Pearson's correlation analysis was used to examine the relationship between the meiotic stages of megasporocytes and microsporocytes within the same flower bud. The results showed that the meiotic stage of the megasporocytes and the meiotic stage of the microsporocytes in the long stamens in the same flower bud had significant correlation ($r = 0.89$, $P < 0.01$).

The temporal relationship between megasporogenesis and microsporogenesis could be used as guidance for *E. pellita* polyploid breeding as an effective approach to induce 2n female gametes to produce polyploid. Therefore, it was of great significance to immediately determine the meiotic period of megasporocytes and treat female gametes in appropriate time to induce 2n female gametes (Li et al. 2008, Wang et al. 2011). The temporal correlation between megasporogenesis and microsporogenesis could be used as an indirect way to determine the megasporocyte stage in time (Li et al. 2008). In triploid induction in *E. urophylla*, Yang et al. (2018) obtained seven triploids, and the triploid induction rate was very low. The main reason for the low rate was the inaccurate prediction of the meiotic period of the megasporocytes. Results obtained in this current study could provide guidance for accurately identifying the process of megasporogenesis in polyploid breeding in *E. pellita*.

## Asynchrony of bud development and microsporogenesis at different positions

In order to explore the development patterns among different flower buds, the diameter of flower buds at each different position and sampling time were measured for further analysis (Table 3). In addition, the flower diameter and meiosis developed at the same time where the development patterns among different flower buds could be used as a reference for megasporogenesis and microsporogenesis. Results showed that the diameter of flower buds collected at the same time was different among the different positions. Variance analysis was conducted on the diameters of flower buds (Table 3) and the results showed that except at 0 h, the flower bud diameters at different positions were significantly different at each sampling period in *E. pellita*. The average diameter of the flower buds at an upper location of the branch was larger than that at a lower location on the same flower branch. At 240 h of microsporogenesis, the average diameter of flower buds at the F position was 0.89 mm larger than that at the A position. Furthermore, least significant difference multiple comparisons showed that there was no significant difference in the diameter between the buds at positions C to F, but there was a significant difference in the diameter between the buds at positions A and B, indicating development of flower bud diameters from positions C to F was more advanced and relatively consistent.

In addition, there were obvious differences in the meiotic periods of flower buds that were collected at the same time (Table 4). Meiotic periods of flower buds containing stamens and

**Table 3** Average diameter of flower buds at different positions and sampling times

| Position | Average diameter of flower buds at different times (mm) | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 48 | 96 | 144 | 192 | 240 |
| A | 6.25 ± 0.34 $^{b}$ | 6.31 ± 0.38 $^{b}$ | 6.35 ± 0.53 $^{b}$ | 6.44 ± 0.43 $^{b}$ | 6.52 ± 0.39 $^{b}$ | 6.53 ± 0.57 $^{b}$ |
| B | 6.36 ± 0.41 $^{b}$ | 6.50 ± 0.23 $^{b}$ | 6.57 ± 0.27 $^{b}$ | 6.69 ± 0.30 $^{b}$ | 6.73 ± 0.39 $^{b}$ | 6.76 ± 0.27 $^{b}$ |
| C | 6.77 ± 0.56 $^{a}$ | 6.84 ± 0.19 $^{ab}$ | 6.96 ± 0.33 $^{ab}$ | 6.93 ± 0.47 $^{ab}$ | 7.07 ± 0.26 $^{ab}$ | 7.12 ± 0.35 $^{ab}$ |
| D | 6.86 ± 0.38 $^{a}$ | 7.07 ± 0.22 $^{a}$ | 7.13 0.33 $^{a}$ | 7.21 ± 0.37 $^{a}$ | 7.28 ± 0.47 $^{a}$ | 7.34 ± 0.27 $^{a}$ |
| E | 6.89 ± 0.22 $^{a}$ | 7.11 ± 0.10 $^{a}$ | 7.16 ± 0.17 $^{a}$ | 7.20 ± 0.24 $^{a}$ | 7.33 ± 0.28 $^{a}$ | 7.38 ± 0.15 $^{a}$ |
| F | 6.95 ± 0.24 $^{a}$ | 7.08 ± 0.17 $^{a}$ | 7.18 ± 0.25 $^{a}$ | 7.23 ± 0.25 $^{a}$ | 7.35 ± 0.27 $^{a}$ | 7.42 ± 0.28 $^{a}$ |
| Significance | NS | ** | ** | * | * | ** |

See Figure 1 for locations. Different lowercase letters within the same line represent significant differences at the 5% level as determined by LSD multiple comparison test, the same letter represents no significant difference at the 5% level;
*** for $P$ 0.001, ** for 0.001 < $P$ 0.01, * for 0.01 < $P$ 0.05 and NS = not significant for $P > 0.05$

**Table 4** Asynchrony of microsporogenesis in flower buds at different positions

| Time (h) | Positions | Number of flower buds in stages of microsporogenesis | | | | | | | | | Total no. of flowers examined |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Microsporocyte | Leptotene | Pachytene | Diplotene | Diakinesis | MetaphaseI-Telophase I | Prophase II-Telophase II | Tetrad | Microspore | |
| 0 | A | 47 | | | | | | | | | 47 |
| | B | 40 | 3 | | | | | | | | 43 |
| | C | 31 | 13 | | | | | | | | 44 |
| | D | 27 | 17 | | | | | | | | 44 |
| | E | 26 | 16 | | | | | | | | 42 |
| | F | 24 | 18 | | | | | | | | 42 |
| 48 | A | 41 | 5 | 2 | | | | | | | 48 |
| | B | 30 | 10 | 9 | | | | | | | 49 |
| | C | 17 | 14 | 15 | 5 | | | | | | 51 |
| | D | 5 | 18 | 18 | 10 | | | | | | 51 |
| | E | 3 | 19 | 20 | 12 | | | | | | 54 |
| | F | 4 | 17 | 23 | 13 | | | | | | 57 |
| 96 | A | 23 | 8 | 3 | 1 | | | | | | 35 |
| | B | 15 | 12 | 7 | 7 | | | | | | 41 |
| | C | 2 | 5 | 20 | 12 | 6 | | | | | 45 |
| | D | | 7 | 9 | 19 | 15 | 5 | | | | 55 |
| | E | | 7 | 10 | 20 | 16 | 7 | | | | 60 |
| | F | | 6 | 9 | 18 | 16 | 8 | | | | 57 |
| 144 | A | | | 8 | 13 | 7 | 6 | | | | 34 |
| | B | | | 4 | 7 | 17 | 3 | 2 | | | 33 |
| | C | | | | 3 | 11 | 18 | 10 | | | 42 |
| | D | | | | | 2 | 11 | 21 | 12 | 3 | 49 |
| | E | | | | | 1 | 11 | 18 | 13 | 2 | 45 |
| | F | | | | | 1 | 10 | 19 | 15 | 4 | 49 |
| 192 | A | | | | | 5 | 22 | 17 | 9 | 2 | 55 |
| | B | | | | | 3 | 13 | 21 | 15 | 7 | 59 |
| | C | | | | | | 5 | 14 | 19 | 13 | 51 |
| | D | | | | | | | 12 | 23 | 16 | 51 |
| | E | | | | | | | 10 | 22 | 18 | 50 |
| | F | | | | | | | 9 | 20 | 19 | 48 |
| 240 | A | | | | | | | | 38 | 19 | 57 |
| | B | | | | | | | | 26 | 24 | 50 |
| | C | | | | | | | | 6 | 30 | 36 |
| | D | | | | | | | | | 41 | 41 |
| | E | | | | | | | | | 45 | 45 |
| | F | | | | | | | | | 43 | 43 |

pistils were both asynchronous among different positions. Microsporogenesis in the long stamens was temporally consistent with megasporogenesis in the same bud, it was more appropriate to use microsporogenesis in long stamens as the calibration standard to show asynchronous flower development. Results showed that developmental stages of microsporogenesis in the flower buds at the upper positions were ahead of those at the lower positions on the same flower branch. And the developmental stages of microsporogenesis at the D, E, and F positions were relatively consistent with each other. Among the multiple meiotic stages of flower buds at positions D, E and F with the largest proportion of flower buds at each position were usually the same or two adjacent stages in the development process. But the stages of A, B and C were quite different. As an example, at 96 h of microsporogenesis, the flower buds at the D, E and F positions were mainly in the diplotene to diakinesis stages (Table 4). However, the main meiosis stages at positions A, B and C were the microsporocyte, leptotene and pachytene stages, respectively; indicating the developmental stages of microsporogenesis were relatively more advanced and synchronous in the upper positions of flower branches than in the lower positions.

Asynchronous flower development has been found in many tree species (Kang et al. 2000, Gao et al. 2004, Yao et al. 2017). In *Eucalyptus*, the flower bud diameter of *E. urophylla* × *E. tereticornis* was very small and about half of that in *E. pellita* when both were in meiosis. The main structures of flower buds of these two *Eucalyptus* species were similar while the flower bud development was also asynchronous in *E. urophylla* × *E. tereticornis* (Yang & Kang 2015). It was worth noting that the average diameter of flower buds at the lower position was greater than that at the upper position of the branch and the developmental stages of microsporogenesis were more advanced at the lower position in *E. urophylla* × *E. tereticornis* (Yang & Kang 2015). However, the pattern was opposite in *E. urophylla* × *E. grandis*, where the flower bud diameter and microsporogenesis at the lower positions was slower than that at the upper positions of the branch (Yang et al. 2015). In the current study, *E. pellita* was consistent with *E. urophylla* × *E. grandis*, although there are some differences in bud development at different positions on the flower branches where the buds near the top of the flower branches are relatively consistent in diameter development and stages of megasporogenesis and microsporogenesis. These results could be used in triploid breeding of *E. pellita* and possibly also on some other species of the genus *Eucalyptus*. The efficiency of triploid induction could be effectively improved by using the upper flower buds of the branches to reduce development asynchrony and to determine the meiotic stage of megasporocytes in a timely as well as accurate manner based on the temporal correspondence of megasporogenesis and microsporogenesis.

## CONCLUSION

Detailed features of flower bud development and the meiosis of microsporocytes and megasporocytes were revealed in the reproductive biology study of *E. pellita*. The meiosis process and diameter development of flower buds near the top of the branch were relatively synchronous. In addition, the megasporogenesis and microsporogenesis had an obvious corresponding temporal relationship within the same flower bud. Based on this temporal relationship, a simple and accurate method to identify the meiotic period of megasporocytes was developed, which provided important theoretical guidance for the genetic improvement of this tree species, especially the triploid breeding by sexual polyploidisation.

## ACKNOWLEDGEMENTS

Our sincere appreciations to Mr Tao Xiong from Guangxi Dongmen Forest Farm for collecting materials and additional assistance. The study was supported the National Key R&D Program of China during the 14th Five-Year Plan Period (2021YFD2200105) and the Fundamental Research Funds for the Central Universities (2021ZY62).

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