Pak. J. Bot., 47(6): 2301-2308, 2015.

REPRODUCTIVE CHARACTERISTICS OF THREE BAMBOO SPECIES

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Abstract

Most bamboo species seldom flower. We gathered the pseudospikelets of *Dendrocalamus giganteus*, *Neosinocalamus affinis* and *Bambusa sinospinosa*, to compare the differences in their structures and developmental processes. Self-pollination was observed in some florets of *N. affinis*. Five types of abortions were summarized in the three flowering bamboo species. *D.giganteus* was the typical male sterility due to the degeneration of all microspores in all anthers. The developmental characteristics of reproductive organs of the three bamboo species were the same. Besides, not all endothecium of both *D. giganteus* and *B. sinospinosa* became fibrous. There are more antipodal cells in mature embryo sac of *D. giganteus* than in that of *B. sinospinosa*.

Key words: Anther wall development, Male gametogenesis, Abortion, Difference.

Introduction

Bamboo often flowered and died after a long time interval (Janzen, 1976). In bamboo species of *Phyllostachys edulis*, *P. glauca, P. viridis, P. nigra and P. dulcis* the vegetative growth intervals was ever reported as 50-60 years and in *Arundinaria faberi* it was also reported as 50 years (Lin *et al*., 2010). *Chimonobambusa quadrangularis* and *P. fimbriligula* were also reported that have about 60 years of vegetative growth intervals (Zhang *et al*., 1994; Cheng & Ren, 1995). Just due to so long time of interval of vegetative growth, few researches were focused on the anatomy of bamboo flowers and embryo development, and even both taxonomy and genetic relationship of different bamboo species are not easy to be carried out and identified. At the same time most bamboo species have relatively low seed production (Zhou, 1998), and even in *Dendrocalamus giganteus* there are no reports that the seeds have ever been gathered. However, only a few researches have been reported and had some discussions involved in the mechanism of low seed production until now (Pang *et al*., 1994; Hu *et al*., 1994; Huang *et al*., 1999; Wang *et al*., 2006; Lin *et al*., 2009; Lin & Ding, 2012 and 2013; Guo *et al.*, 2015 and Lin *et al.*, 2015).

In bamboo classification systems, the classification definition between *Bambusa* and *Dendrocalamus* is not clear and confusing, although they belong to Bambuseae and Dendrocalameae separately. It is just because one group of bamboo species, such as *Neosinocalamus affinis*, of which culm sheaths are more similar to that of *B. intermedia*, however their culm characteristics are more similar to *D. minor*, and meanwhile the spikelets of *N. affinis* has also the similar characteristics of both *Bambusa* and *Dendrocalmus* (Li, 1994). Yi *et al*. (2008) still merged the species of *Neosinocalamus affinis* and other several varietas into the genus of *Neosinocalamus*. However, Wu *et al*. (2006) merged the *Neosinocalamus* into *Bamusa* and the bamboo species of *Neosinocalamus affinis* was renamed "*Bambusa emeiensis*".

In this paper spikelets from three bamboo species i.e. *Dendrocalamus giganteus*, *Neosinocalamus affinis*, *Bambusa sinospinosa*, were used as materials to compare the differences in their spikelets structure, megasporogenesis, microsporogensis, male and female gametogenesis, so as to explore the causes of low seed yield and analyze the differences between these bamboo species during their developmental process of reproductive organs.

Material and Method

The spikelets of *D. giganteus* and *N. affinis* were both gathered from the bamboo garden of Southwest Forestry University in April, 2011 and the spikelets of *B. sinospinosa* were gathered from the bamboo gardens of Expo Park of Kunming, China, in June, 2011.

All spikelets from various developmental stages were soaked in FAA fixative (45% alcohol, 0.25% acetic acid and 1.85% formaldehyde) and then were dehydrated in a graded series of alcohol (begins at 50%) and technology of paraffin section was used. Transverse sections (7μm) were cut using a rotary microtome and double stained with 1% alcoholic Safranin O (Sigma S-2255) (in 50% ethanol), distilled water, and 1% Fast green (Fluka 05500) and dehydrated in a graded series of ethanol. The sections were permanently mounted in Canada balsam.

The sections were observed and captured via a video camera linked to a converted fluorescence microscope (Zeiss Axiovert 200M) and a Lenovo computer. The structure of each floret was observed with an Olympus anatomical lens and images were taken by using of the Carl Zeiss Imaging systems.

Results

Inflorescence and morphology: The spikelets of the three bamboo species were all pseudospikelets. The pseudospikelets of *N. affinis* were purple and only about 1.0-1.3cm long (Fig. 1A). Stigmas came out of florets earlier than anthers, showing the dichogamy and protogyny. It could also be seen that both stigmas and anthers did not always grow out of florets and lots of pollen grains stuck on stigmas due to the rupture of anther walls in

the floret of some spikelets, implied the self-pollination (Fig.1B). The ovary began to swell after self-pollination (Fig.1C). Florets of *N. affinis* often had 6 stamens and 3 lodicules (Fig.1D and E). Pistils usually had 1-3 plumous stigmas. Due to the elongation of filaments, most mature anthers usually hanged outside of florets. Some stamens could also mature in florets because of the un-elongation of filaments, to finish their self-pollination.

The pseudospiklets of *B. sinospinosa* were about 2.5-3.5cm long, green and flat, and their rachillae often disarticulated (Fig.1F). Each floret often had 6 stamens, 2 ciliate lodicules and 1 pistil with 3 plumous stigmas (Fig.1G and H). However, the pseudospiklets of *D. giganteus* was purple yellow and about 1.3 cm long, which usually had 6 stamens, 1pistil with only 1 plumous stigma and no lodicules (Fig.1I, J and K).

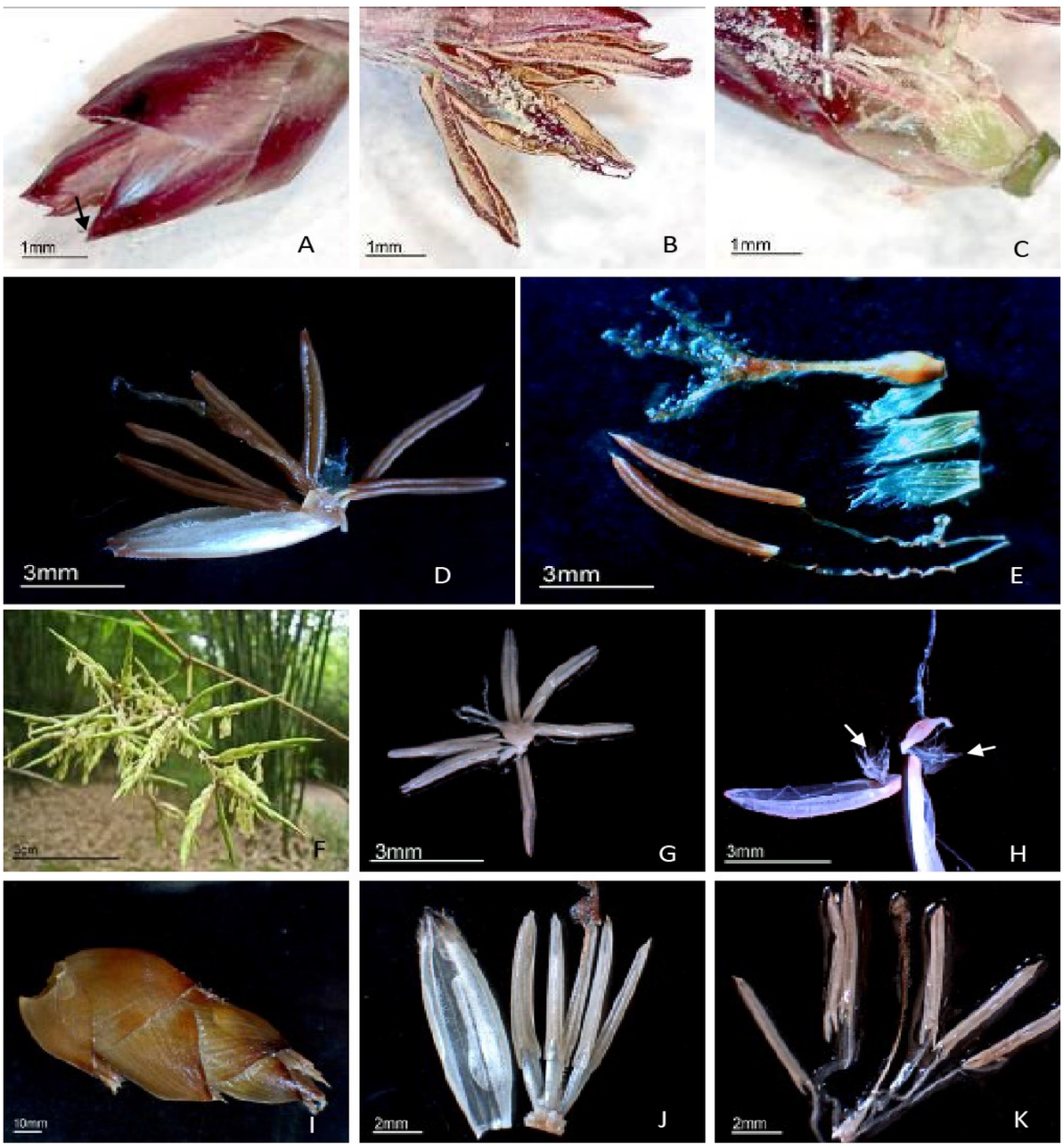

Fig. 1. Morphological characteristics of bamboo flowers. A-E: The flowers of *Neosinocalamus affinis*. F-H: The flowers of *Bambusa sinospinosa*. I-K: The flowers of *Dendrocalamus giganteus*. A: The spikelet of *N. affinis*. Stigmas (arrow) stretched out of the flower earlier than stamens. B: Anther walls dehisced and pollen grains scattered on the feathery stigma inside the floret. C: The inflated ovary after fertilization. D: The matured stamens with short filaments. E: The matured stamens with long filaments and three lodicules in one floret. F: The spikelets of *B. sinospinosa*. G: 6 stamens and 1 pistil with 3 stigmas in one floret. H: Lodicules with ciliate margins (arrow). I: The spikelet of *D. giganteus*. J: 6 stamens and 1 pistil with only 1 stigma in one young floret. K: 6 stamens with elongated filaments, 1 pistil and no lodicule in one matured floret.

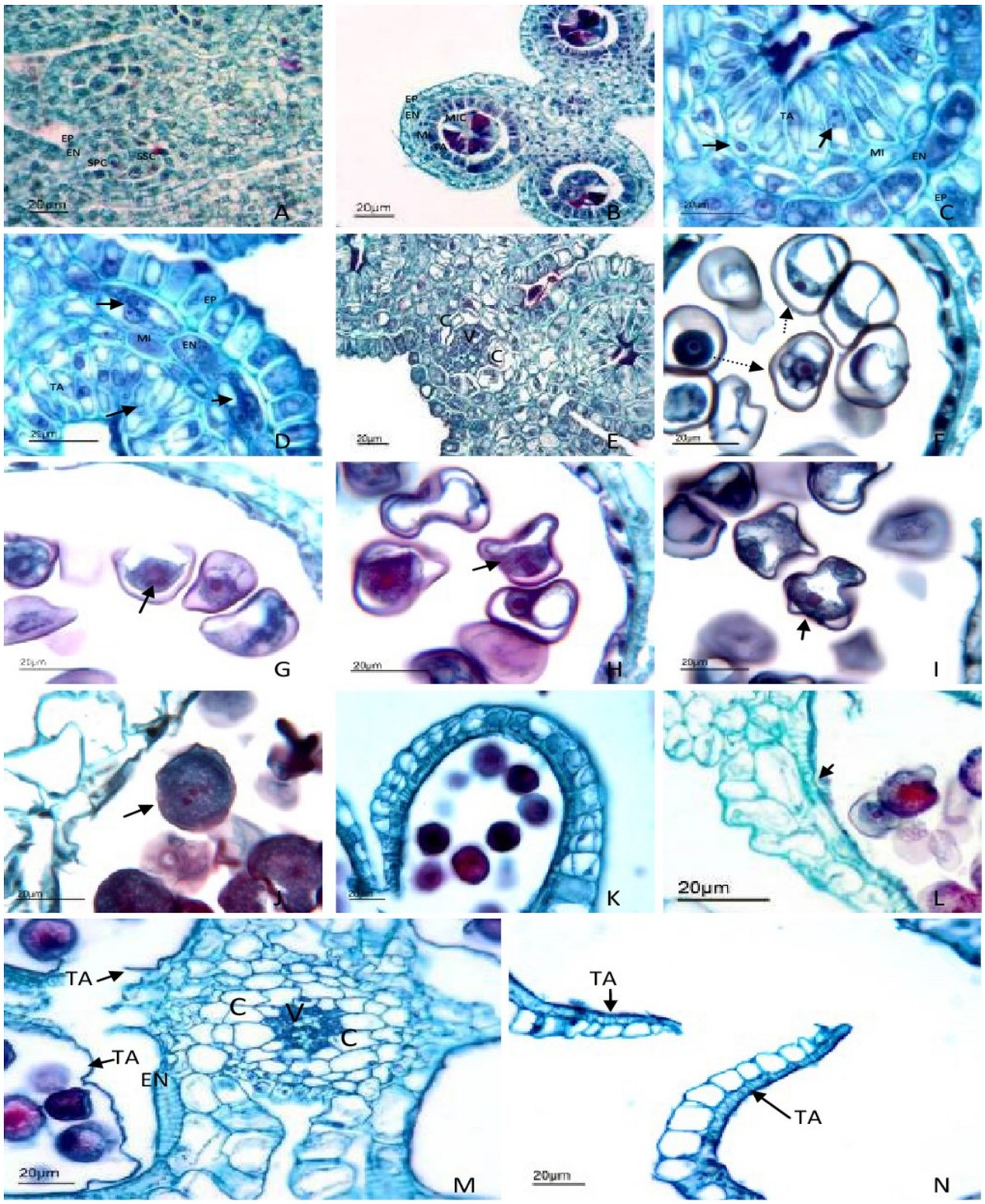

Fig. 2 Anther wall development, microsporogenesis and microgametogenesis of *Neosinocalamus affinis*. A: Only a few sporogenous cells in young anthers. Anther walls have three layers. B: The increase of number of sporogenous cells and wall layers. C: A abortive anther. The tapetal cells elongate dramatically and some cells have two nuclei (arrow). Some middle layer cells also divide into two cells (arrow). D: The division of endothecium and tapetum cells (arrow) in the abortive anther. E: The vascular bundles and connective tissue with more dense protoplasm in the abortive anther. F: The uninucleate microspore. The nucleus is pressed to be close to the cell wall with the formation of the large vacuole, indicated as the arrows. G: The metaphase of mitosis (arrow). H: The anaphase of mitosis (arrow). I: A binucleate pollen grain (arrow) and degenerating tapetum. J: A trinucleate pollen (arrow) and the anther wall without formation of fiber layer. K: An anther with mature pollen grains. L: The residual nucleoli after the degeneration in matured anther. M: The matured anther with the residual tapetum layer. N: The anther wall close to stomium after the release of pollen grains. There is no protoplasm in most epidemic cells. C: connective tissue; EN: endothecium; EP: epidermis; MI: middle layer; SPC: secondary parietal cell; SSC: secondary sporogenous cell; MIC: microsporocyte; TA: tapetum; V: vascular bundle.

Development of male and female gametophyte of *N. affinis*: During the early developmental stage of secondary sporogenous cells, there were only a few secondary sporogenous cells identified in loculus. The young anther walls usually consisted of three layers: from the outer to the inner layers, epidermis, endothecium and secondary parietal cell layer (Fig. 2A). The number of sporogenous cells increased further by mitosis and the number of anther wall layers also increased from three layers to four layers that consisted of epidermis, endothecium, middle layer and conspicuous tapetum (Fig. 2B). However, in some anthers the sporogenous cells degenerated and the tapetal cells enlarged and enlongated radially. As a result the tapetal cells occupied the whole space of microsporocyte and meanwhile the enlarged taptal cells often had more nuclei in nucleus than normal tapetal cells (Fig. 2C). In these sterile anthers, tapetal and endothecium cells increased the number of wall layers by mitosis (Fig. 2D). There were more dense cytoplasm in both connective cells of filament and anther wall layers of these sterile anthers (Fig. 2E) than in those cells in fertile anthers (Fig. 2M).

The nucleus of the early uninucleate microspore was located at the center of microspore. Many small vacuoles were generated in cytoplasm and at last one large vacuole was formed, which pushed the nucleus to one end of microspore (Fig. 2F). At this period, all tapetal cells had also degenerated obviously. After two mitoses, the binucleate and trinucleate pollen grains were formed in succession (Fig. 2G-J). In the mature period of pollen grain there was still conspicuous cytoplasm in epidermis cells,which indicated that the epidermis cells play an important role in the nutrition transport for maturing pollen (Fig. 2K). The endothecium also became fibrous . Some residual nucleoli of tapetal cells were still observed after the degeneration in matured anthers (Fig. 2L). When the anther walls dehisced from the stomium region, the residual tapetum could still be observed and it could be observed even after all pollen grains were released (Fig. 2M and N). The cytoplasm of all cells in both epidermis and connective tissue had degenerated completely by this time.

The ovary was unilocular with one anatropous and dual-integument ovule. There was one large and prominent megasporocyte beneath two layers of nucellus cells in young pistil (Fig. 3A). The megasporocyte developed into a normal and mature embryo sac after the stage of tetranucleate embyo sac (Fig. 3B and C). Most pistils could be fertilized successfully, developed into normal embryos and went to seed (Fig. 3D).

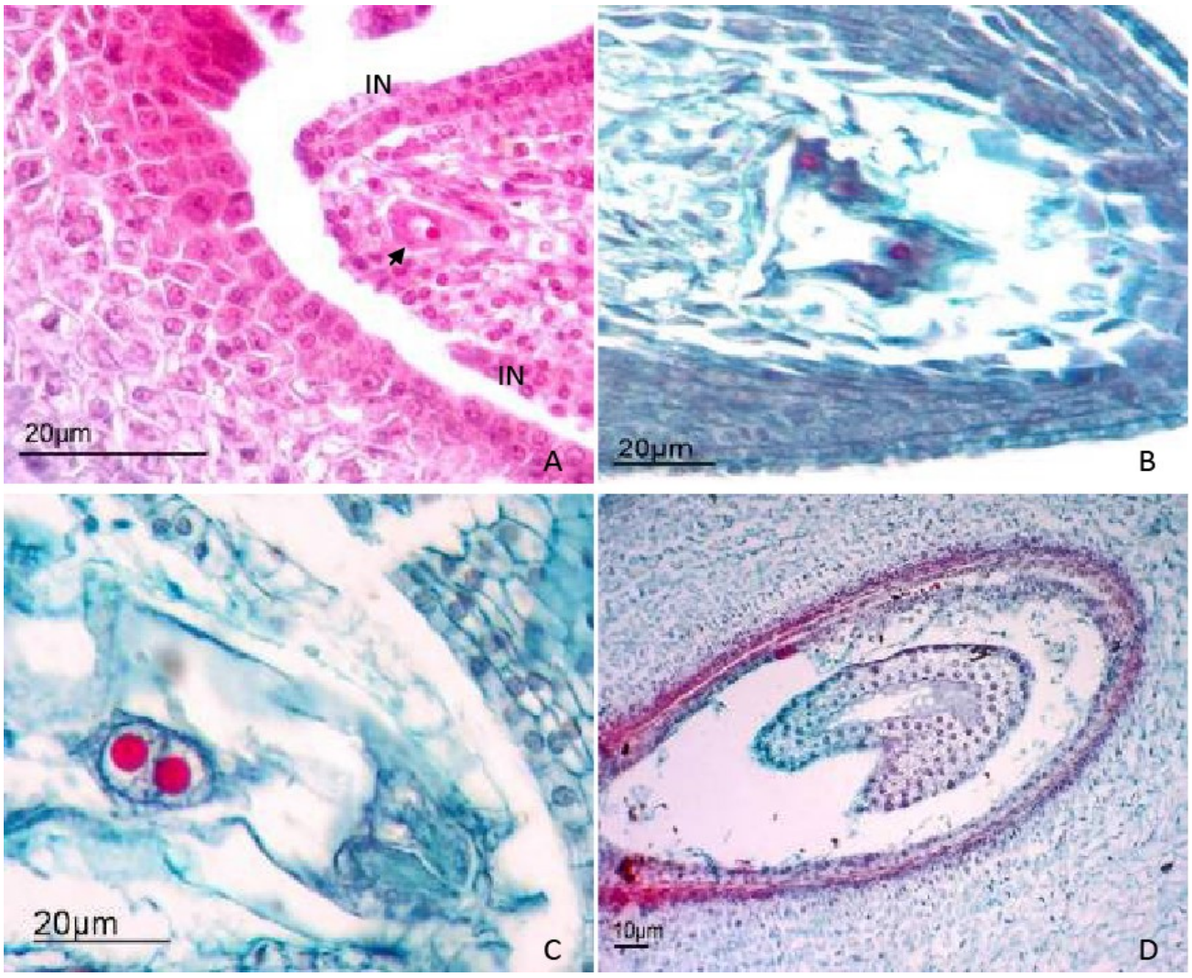

Fig. 3. Development of female gametophyte of *Neosinocalamus affinis*. A: Megasporocyte (arrow). B: Tetranucleate embryo sac. C: Mature embryo sac, showing the prominent central cell and egg apparatus. D: The developing embryo. IN: inner integument.

Development of male and female gametophyte of *B. sinospinosa*: The anther development of *B. sinospinosa* was similar to that of *N. affinis*, due to the fact that the number of second sporogenous cells increased by meitosis (Fig. 4A and B). The anther wall also consisted of epidermis, endothecium, middle layer and tapetum. At this stage, the tapetal cells close to vascular bundle degenerated firstly. Like the microsporocyte degeneration of *N. affinis*, the tapetal cells also expanded abnormally and occupied the space of microsporocyte (Fig. 4C). Microsporocytes either in neighbouring sporangia or within the same sporangium were not synchronized (Fig. 4D). It was observed that the microsporocytes in sporangium I began to degenerate and the tapetal cells began to enlarge radially and invaded the space of microsporocyte. However there were normal microsporocytes and tapetum in sporangium II. The tapetum degenerated remarkably in sporangium III and IV and even there were no microsporocytes in sporangium III. The nucleus of uninucleate microspore was pushed against the microspore by the large vacuole in a similar way to that of *N. affinis* (Fig. 4E). Tapetum degenerated to be only a layer of remnant at the same time. Meanwhile, it could be observed that most uninucleate microspores shrank and degenerated completely, to support the growth of the left microspores in some anthers (Fig. 4F). It could also be observed that microspores in most anthers developed abnormally (Fig. 4G-K). Even if the trinucleate pollen grains were formed, their pollen walls were shrink and aberrant (Fig. 4L). Hence, almost no normal pollen grains could be produced in anthers of *B. sinospinosa*. Besides, other types of abortions were also observed. In some other anthers all microspores lost their nucleus (Fig. 4G), or shrunk completely (Fig. 4H-J), or only a little residual protoplasm was left in shrunken anther (Fig. 4K). Endothecium cells of some anthers observed fibrous thickness (Fig. 4J), this was not the case in that of other anthers.

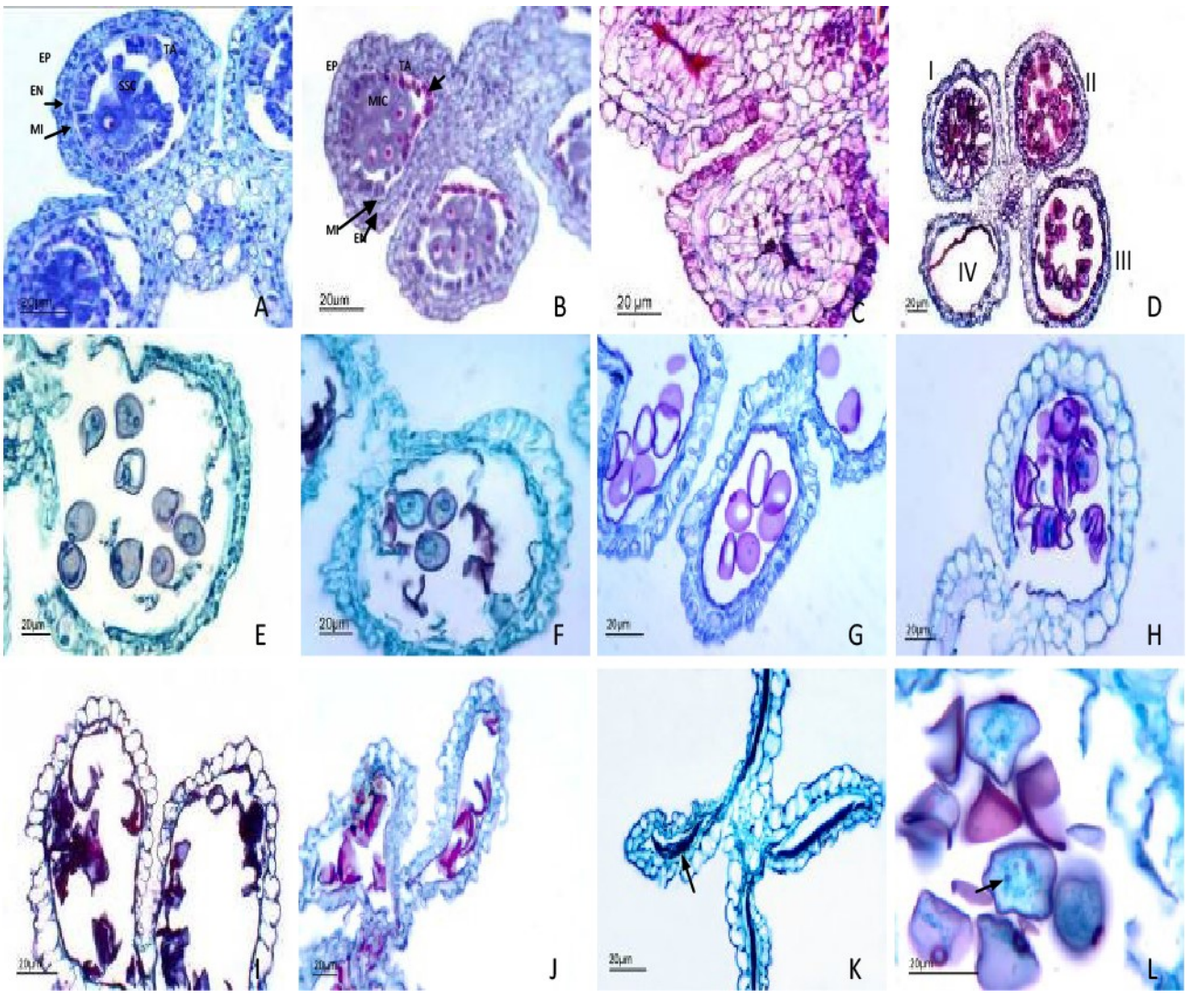

Fig. 4. Anther wall development, microsporogenesis and microgametogenesis of *Bambusa sinospinosa*. A: Four layers of anther wall around sporogenous cells. B: The occurrence of degeneration of tapetum (arrow). C: The elongation of tapetum. D: Tetrasporangiate anther showing two abortive sporangia (sporangium I and IV). E: Uninucleate microspore. F: Abortive microspores and several normal uninucleate microspores in the same loculus. G: Abortive microspores without nuclei and anther wall without fibrous thickness. H: Abortive microspores with irregular shape. I: Abortive microspores with extremely shrunken shape. J: Abortive anther with the fibrously thickening endothecium. K: Deformed anther with remnants of microspores (arrow). L: Trinucleate pollen (arrow). EN: endothecium; EP: epidermis; MI: middle layer; SSC: secondary sporogenous cell; MIC: microsporocyte; TA: tapetum.

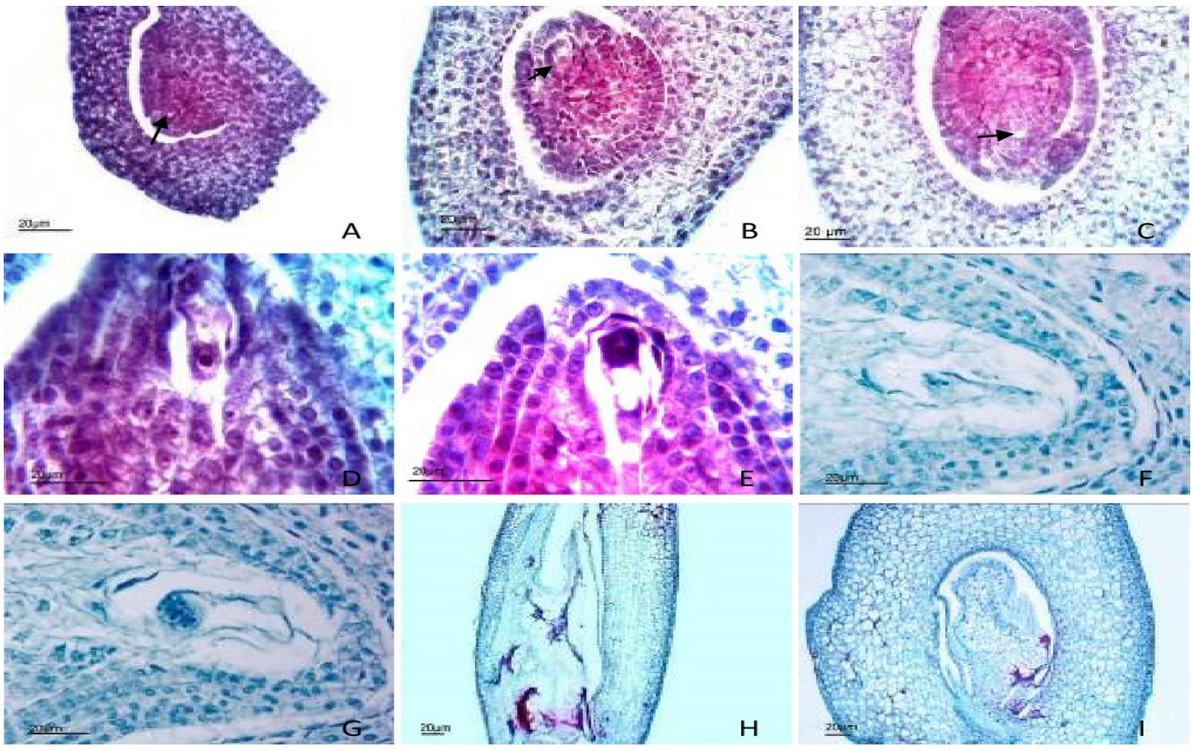

Fig. 5. Development of female gametophyte of *Bambusa sinospinosa*. A: Archesporial cell. B : Megasporocyte. C: Tetrad stage. D and E: Binucleate embryo sac. F: Central cell. G: Antipodal cell. H: Aberrant ovule. I: Aberrant cell.

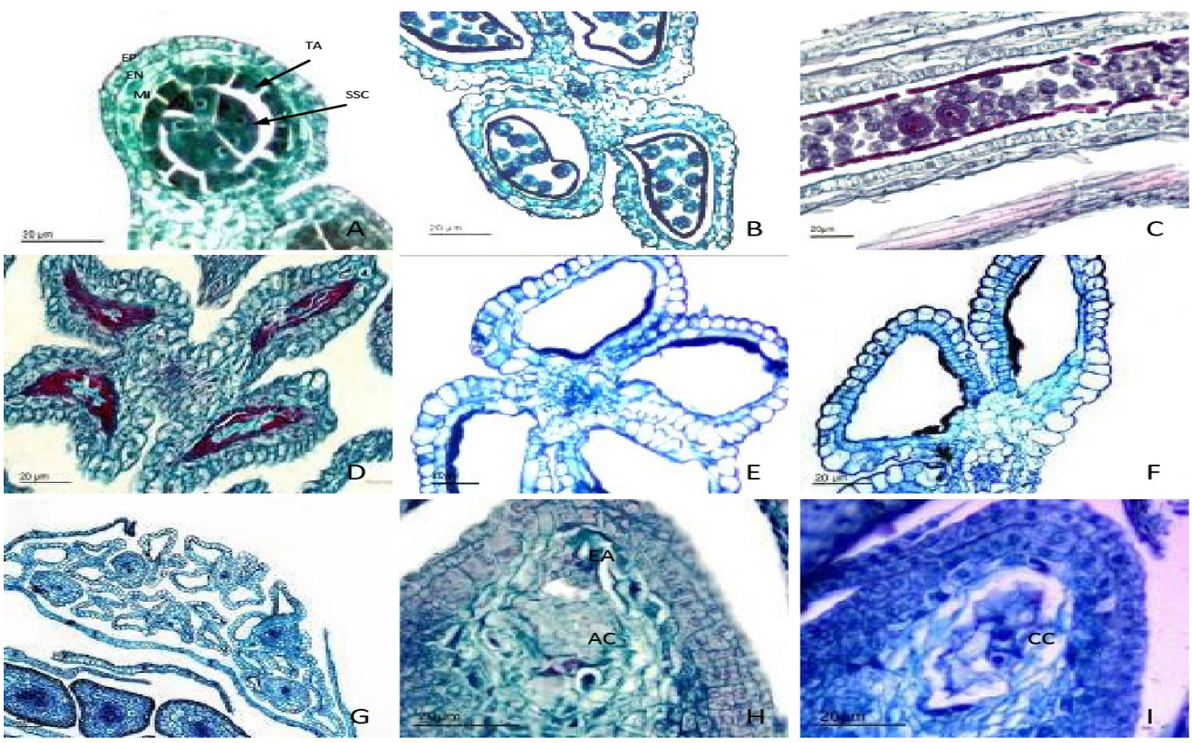

Fig. 6. Anther development and normal embryo sac of *Dendrocalamus giganteus*. A: Four layers of anther wall around sporogenous cells. B: Transverse section of uninucleate microspore. C: Longitudinal section of uninucleate microspore. D: Deformed anther with degenerated microspores. E: Remnants of microspores and endothecium without fibrous thickening. F: Abortive anther with fibrous thickening. G: Transverse section of a floret showing most anthers without microspores. H: Antipodal cell and egg apparatus. I: Central cell. AC: antipodal cell; CC: central cell; EA: egg apparatus; EN: endothecium; EP: epidermis; MI: middle layer; SSC: secondary sporogenous cell; TA: tapetum.

The ovary of *B. sinospinosa* was also unilocular with one anatropous, dual-integument ovule, which was similar to that of *N. affinis*. Archesporial cell was beneath the single layer of nucellar epidermis and differentiated into the megasporocyte (Fig. 5A and B). The ovule was crassinucellate. Although only central cell and antipodal cells were observed and the egg apparatus was not observed in embryo sac (Fig. 5F and G), it could still be inferred that the megasporocyte could develop into normal and mature embryo sac successfully after the stage of tetrad megaspores and binucleate embryo sac. Meanwhile, lots of aberrant ovules were observed in mature pistils (Fig. 5C, D and E).

Development of male and female gametophyte of *D. giganteus*: At the stage of secondary sporogenous cells, the anther walls of *D. giganteus* also consisted of four layers of walls, i.e. epidermis, endothecium, middle layer and tapetum (Fig. 6A). Secondary sporogenous cells could developed into uninucleate microspores successfully (Fig. 6B and C), but almost all of them would degenerate, leaving only the residual protoplasm and the shrunken anthers (Fig. 6D). At this time, the wall of these abortive anthers usually consisted of epidermis and endothecium. During the following developmental stage, the residual protoplasm continued to degenerate and only the remnant of protoplasm was left in most anthers (Fig. 6E, F and G). The endothecium of some anthers developed fibrous thickenings (Fig. 6F) and some others did not become fibrous (Fig. 6G).

Although all anthers of *D. giganteus* were sterile, their ovary could still develop into mature embryo sac, consisting of egg apparatus, central cell and antipodal cells (Fig. 6H and I). The embryo sac of *D. giganteus* had more antipodal cells than that of *B. Sinospinosa*.

Discussion

Morphological characteristics: According to our observations on the three bamboo species, no seeds could be produced from *B. sinospinosa* and *D. giganteus*, but a lot of seeds could be gathered from *N. affinis*. Du *et al*. (2000) pointed out the same conclusion and they also considered that most cultivated species including *B. sinospinosa*, *D. giganteus*, *N. affinis* and other bamboo species usually flowered fragmentarily. Meanwhile, cross-pollination was ensured by the dichogamy that had been reported in many bamboo species, such as *D. sinicus* (Wang *et al*., 2006), *Menstruocalamus sichuanensis* (Lin *et al*., 2009) and *Shibataea chinensis* (Lin & Ding, 2012). Just because of flowering fragmentarily and dichogamy, the low seed setting rate was caused.

However, self-pollination was observed in some florets of *N. affinis*. There were two types of stamens in mature florets with elongated and un-elongated filaments separately. The elongated filament usually caused the cross-pollination and the un-elongated filament caused the self-pollination. Self-pollination was also the first time to be observed in bamboos, and moreover, it could also increase the seed setting rate and avoid effectively risks from cross-pollination. Brys & Jacquemyn (2011) had ever reported that autonomous selfing may guarantee reproductive assurance. Therefore, *N. affinis* could bear more seeds than other other bamboo species when flowering framentarily.

As for the morphological characteristics of pseudospikelets, it could be seen that the pseudospikelet of *N. affinis* was more similar to that of *D. giganteus* than the pseudospikelet of *B. sinospinosa*. The pseudospikelets of *B. sinospinosa* were longer than that of *N. affinis* and *D. giganteus*. However, only the pseudospikelets of both *N. affinis* and *B. sinospinosa* had lodicules and their pistils usually had three feathery stigmas. There were no lodicules in the pseudospikelets of *D. giganteus* of which the pistils were single stigma.

Abortions in development of stamens and pistils: Bamboos seldom flowered and went to seed. Even if there were some sporadic clump of bamboos flowered, few or no seeds could be gathered. Du *et al*. (2000) had ever recorded sixty one bamboo species belonging to twenty three genera in Yunnan province, which had been in flowering and fruit bearing in the period of 15 years. They also considered that both *B. sinospinosa* and *D. giganteus* never went to seed.

The flowering bamboos of *N. affinis* could produce lots of seeds because almost no abortions occurred in the development of their stamens and pistils. Actually, there were still some anthers in which the abortion was observed. In these anthers, the enlarged tapetal cells fully occupied the whole spaces of sporogenous cells and the sporogenous cells degenerated. In other bamboo species, such as and *S. chinensis* (Lin & Ding, 2012), this phenomena was also observed. Meanwhile, it was also reported in other plants, such as *Excentrodendron hsienmu* (Tang *et al*., 2006).Therefore, it could be concluded that the aberrant tapetal behaviour was associated with male sterility (Meric *et al*., 2003; Shi *et al*., 2010; Li *et al*., 2010). With regard to the aberrant tapetum caused male sterility, Holford *et al*. (1991) summarized three types of abnormal tapetal behavior in a male sterile onion, such as the premature breakdown of the tapetum at the tetrad stage, the hypertrophy of the tapetum after the diad stage followed by its premature autolysis and the tapetum remaining in good condition but for an abnormal long period of time. Buyukkartal *et al*. (2005) considered that persistent tapetum blocked nutrient transportation and then leaded to low pollen fertility and affected the proportion of seed setting and grain formation. Beside the premature breakdown of tapetum was also reported in *B. multiplex* (Lin *et al.*, 2015). Therefore, the enlarged tapetal cells, which caused the abortion of anthers, were a common phenomenon in plants. *N. affinis* could produce lots of seeds, which benefited from the normal development of most stigmas and pistils and the self-pollination.

In flowering bamboos of *B. sinospinosa*, it could be observed that there were other types of abortions in anthers, except for the enlarged tapetum. At the vacuolated uninucleate microspore stage, some microspores shrank seriously, resulting in deformation and degeneration of these microspores, in order to save or supply nutrition to the surviving microspores. Meanwhile, in some anthers almost all microspores deformed. Similar phenomenon was also reported in other bamboos, such as *M. sichuanensis* (Lin *et al*., 2009) and *S. chinensis* (Lin & Ding, 2012).

Besides, Li *et al*. (2010) also reported that the cease of development leaded to the deformation of uninucleate pollen in one cultivars of sterile chrysanthemum.

In some anthers of *B. sinospinosa*, microspores lost their nuclei and only the empty microspores were left. This was also observed in *S. chinensis* (Lin & Ding, 2012). Meanwhile, it could also be seen that microspores and taptal cells degenerated together and the anthers shrunk, leaving only one layer of remnants. Even if some microspores could develop into pollens and these pollens were deformed. The reason that caused the low seed setting of *B. sinospinosa* was not only the aberrant development of microspores and tapetal cells, but also the aberrant development of ovule. Therefore, almost no florets of *B. sinospinosa* could go to seed.

With regard to *D. giganteus*, no uninucleate microspores could successfully develop into pollen grains. They usually deformed and degenerated into a lump of protoplasm in the shrunk anthers at uninucleate stage. At last all anthers were empty in mature pseduospikelets, and however their mature embryo sacs were normal. Therefore, *D. giganteus* belonged to the typical male sterility.

In summary, the developmental characteristics of pistils and stigmas of the three bamboo species was the same, including four layers of anther wall, fibrous thickening of endothecium, crassinucellate ovule, anatropous bi-integument ovules, and as so on. Besides, the endothecium did not become fibrous in some anthers of *B. sinospinosa* and *D. giganteus*. Five types of abortions in bamboos could be concluded as follow: tapetum enlarged aberrantly, nuclei of microspores were lost, deformed microspores, tapetal cells and microspores degenerated into a lump of protoplasm and ovule developed aberrantly.

Acknowledgements

The project was funded by the Science and technology projects of Yunnan Province (2015FB150), National "Twelfth Five–Year" Scientific and Technological Support Plan (2012BAD23B05) and the National Natural Science Fund of China (31560196).

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(Received for publication 15 December 2014)