Sexual Reproduction in the Chinese chestnut (*Castanea mollissima* Blume)

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Chinese chestnut (*Castanea mollissima* Blume) has noteworthy ecological, economic, and cultural importance in the Northern Hemisphere, especially in China. However, it is particularly prone to erratic fruit set showing very low and have little work on the reproduction biology. In order to clarify the sexual reproduction in Chinese chestnut, a cultivar named 'Yanshanzaofeng' was employed for microscopy analysis. The results showed that the catkins with floral primordia were formed in the buds of one-year olds shoots in later April. The sex differentiation was completed in early May. Later, the anther wall layers developed completely by middle May and consisted of epidermis, endothecium, middle layers and glandular tapetum, and the anther wall was of the basic type. Microspore mother cells underwent meiosis through simultaneous cytokinesis in later May and gave rise to tetrads of microspores, which were tetrahedrally arranged. Mature pollens contained two cells with three germ pores. Anthers were dehiscent and pollen grains shed on approximately on June 15th. While the female flowers were formed in early May, and could be divided into female flower cluster primordium differentiation phase, alabastrum primordium differentiation, stigma primordium differentiation, stigma elongation, ovary formation and blooming phase. The ovule primordium was formed in middle June. The ovary were eight or nine locules, and each locule contained two anatropous ovules which were bitegmic and crassinucellate. The embryo sac was mature between later June and early July, and the development of embryo sac conformed to the Polygonum type. When the anthers were dehiscent, the pollen tube passed through the basal style about two weeks. The pollen tube came into the embryo sac between later June and early July, and the syngamy was of premitotic type. The development of the endosperm was nuclear endosperm. The zygote developed from the proembryo to the clavate, globular, torpedo-shaped and cotyledon stage embryoid, and the development of embryo was of Onagrad type. The mature embryo formed in early August and the endosperm disappeared between later August and early September. A large number of abortive ovules were observed in the development of female gametophytes in middle July which showed the cavity of embryo and degeneration of nucellus tissue. The abortive ovules were identified as one of the major factors causing the low seed set in 'Yanshanzaofeng'.

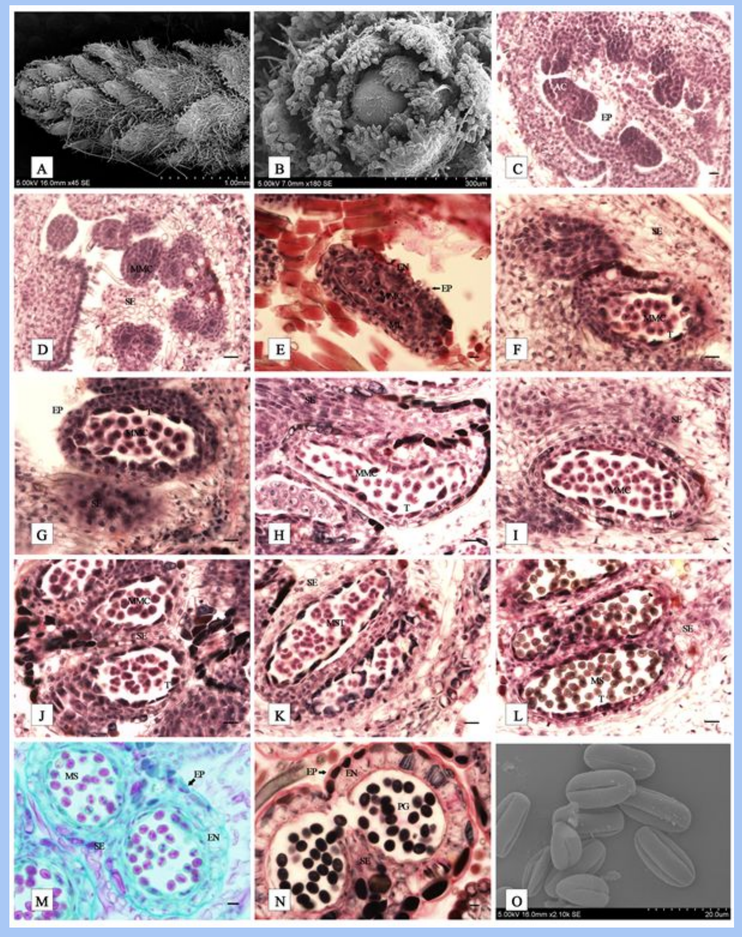

Plate 1 Development of the male gametophyte in chestnut
A, the catikin; B, floral primordia; C, the archesporial cell; D-E, the microspore mother cell; F, meiosis I prophase; G, meiosis I metaphase; H, meiosis I telophase; I, meiosis Ⅱmetaphase; J, meiosis Ⅱtelophase; K, the microspore tetrads; L, the uninucleate stage of microspores; M, the binucleate microspore stage; N-O, the mature pollen. (AC=archesporial cell; EN=endothecium; EP= epidermis; ML=middle layers; MMC=microspore mother cell; MS=microspore; MST=microspore tetrad; PG=pollen grain; SE=septum; T=tapetum.)

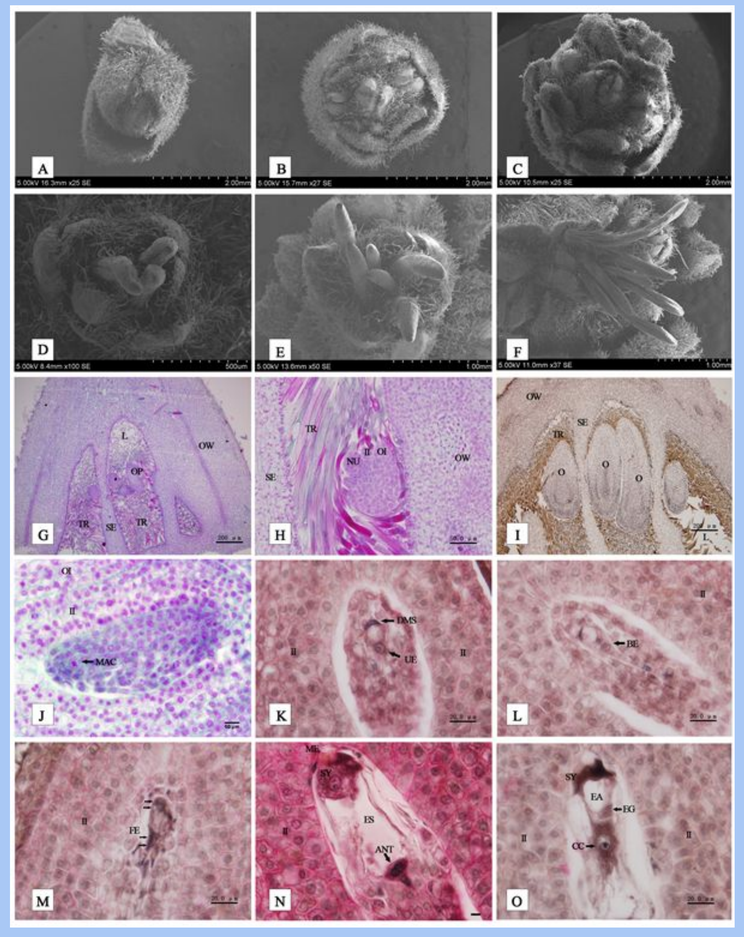

Plate 2 Development of the female gametophyte in chestnut
A, female flower cluster primordia differentiation stage; B, alabastrum primordium differentiation stage; C, stigma primordium differentiation stage; D, stigma elongation stage; E, ovary formation stage; F, blooming stage; G, the ovule primordia; H, the outer and inner of integument primordia; I, the anatropous ovule; J, the megaspore mother cell; K, one-nucleate embryo sac; L, two-nucleate embryo sac; M, four-nucleate embryo sac; N, the mature embryo sac, three antipodal cells and one synergid cell. O, the mature embryo sac, an egg apparatus with egg cell and a central cell. (ANT=antipodal cells; BE=binuclear embryo sac; CC=central cell; DMS=degenerate megaspores; EA=egg apparatus; EG=egg cell; ES=embryo sac; FE=4-nucleate embryo sac; Il=inner integument; L= locule; MAC=megaspore mother cell; ME=micropylar end; NU=nucellus; O=ovule; Ol=outer integument; OP=ovule primordial; OW=ovarian wall; SE=septum; TR=trichome; TT=transmitting tissue; SY=synergid; UE=uninuclear embryo sac.).

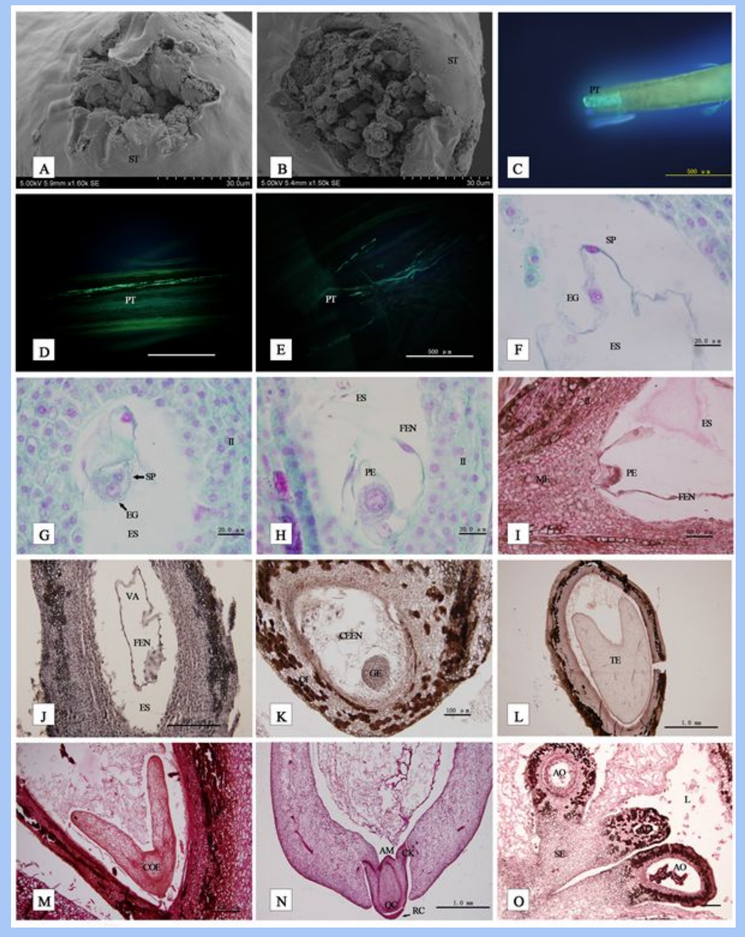

Plate 3 Pollination and fertilization, and embryo development in chestnut
A-B, the pollen germinate on the stigma under the scanning electron microscope; C, the pollen germinate on the stigma under the fluorescence light; D, the pollen tube grow in the style. E, the pollen tube pass through the base of style; F, the sperm cell enter into the embryo sac; G, the sperm and egg cell begin to fuse; H, the proembryo; I, the clavate proembryo; J, the free endosperm nuclear; K, the globular embryo; L, the torpedo-shape embryo; M, the cotyledon embryo; N, the mature embryo; O, the abortive ovule. (AO=abortive ovule; AM=apical meristem; CEEN=cellular endosperm; CK=cotyledonary knots; COE=cotyledon embryo; EG=egg cell; ES=embryo sac; GE=globular embryo; Il = inner integument; L = locule; ME = micropylar end; Ol = outer integument; OW = ovary wall; PEN = primary endosperm nucleus; PE = proembryo; PT = pollen tube; QC = quiescient center; RC = root cap; SE = septum; SP = sperm; ST = stigmatic; TE = torpedo embryo; VA = vacuole).