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Influence of different fruit loads on the starch accumulation in the pistils of Chinese Chestnut cv. 'Zaodali'

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Summary

The experimental samples were collected from Chinese chestnut trees (*Castanea mollissima* Blume) of two different fruit loads, i.e., "high fruit load" and "low fruit load". No starch accumulation in the needle-shaped stigmas of all female flowers was observed while rapid starch accumulation took place in the transmitting tissues of the style just before the formation of complete ovules. In the cells of the ovary wall and the ovule, starch accumulation was obvious and reached the peak as the embryo sac was fully mature. Starch grains of ovule were mainly stored in the inner and outer integuments. Fruit loads of chestnut trees greatly affected level of starch accumulation in each part of the pistil. The starch accumulation in the ovary wall and the ovule of trees of "high fruit load" apparently surpassed that of trees of "low fruit load". The starch content in the outer integument could be of 17.50% for trees of "high fruit load" compared to a low percentage of 2.92% for trees of "low fruit load" in same time.

Introduction

Chinese chestnut (*Castanea mollissima* Blume) is an important crop in Yunnan Province, Southwestern China, but low yields and alternate-bearing affect the production development. The main reasons for low yield are the high empty cupule rate and fewer than 3 nuts per cupula at harvest. The rate of empty cupules in many chestnut orchards is usually 10 to 20%, but sometimes it can be as high as 90% (Bai, 1988; Xia et al., 1989; Shi, 2003; Shi and Stösser, 2005). Starch is the most important carbohydrate reserve in woody plants species (Chapin et al., 1990). According to previous studies, the accelerated growth of pollen tube when it entered into the transmitting tissues through the stigma was related to the change from autotrophy to heterotrophy, which means that the growth of pollen tube relied on the carbohydrate reserve in pistil for vegetative nutrition (Herrero and Dickinso, 1980; Mulcahy and Mulcahy, 1983). It was proved that starch accumulation existed in both the stigma and the transmitting tissues in apple pistil (Braun et al., 1986). As for peach trees, starch accumulation occurred in embryo sacs, integuments and the top of nucellus from anthesis to fertilization until the maturity of ovule (Arbeloa and Herrero, 1991). During fertilization, many large starch grains were observed in the integuments (Mogensen and Suthar, 1979). Accumulation and consumption of starch in the reproductive organs of fruit trees was of great significance for the pollen tube growth and the fertilization process as well as the stability of fruiting (Stösser, 2002).

The above cited data (Mogensen and Suthar, 1979; Mogensen and Suthar, 1979; Stösser, 2002) indicate that starch accumulation in the flower is critical for the fruiting process of fruit trees. The purpose of this paper is to determine if the levels of starch accumulation in pistils of the Chinese chestnut during the time of anthesis to fertilization could be correlated with the fruit set ability and thereby it would substantiate the delusive female flower quality concept.

Materials and methods

Material

Trees of the cultivars 'Zaodali' were selected for the study between 2005 and 2006 at the Yiliang County Experimental Station in Yunnan Province, Southwest China. Three "high fruit load" (HFL) and three "low fruit load" (LFL) trees planted in 1998 were selected. The "HFL" trees in 2004 and 2005 yielded less than 5% empty cupules, and the "LFL" trees yield 30% to 40% empty cupules during the same period. From each tree, three female catkins (cupules) per tree were sampled when the stigmas appeared in late April. Thereafter, sampling was done every 7 or 8 days until the end of May. The samples were immediately fixed in a solution FPA70 (containing 40% formaldehyde : propionic acid : 70% ethanol = 5: 5: 90 [v/v/v]).

Methods

The sampled tissues were dehydrated with an ethanol series (70%, 80%, 90%, 96%), at least 4 hours in each step, and the dehydrated samples embedded in glycolmethacrylate-methyl methacrylate (Rudell, 1967). After dehydration, the tissues were transferred to 50:50 (v/v) mixture of methacrylic-acid-hydroxyethyl ester and methyl methacrylate and stored for 24 hours at 4 °C. They were then transferred to a mixture containing 60ml methacrylic acid-hydroxyethyl ester, 20ml methyl methacrylate, 16ml ethylene glycol monobutyl ether, 2ml polyethylene glycol 400 (Serva), and 270mg benzoyl peroxide (solution A), and stored for 24 hours at 4 °C. Polymerization was achieved by transferring the tissue to the same amount of solution A and by adding 100µl N,N-dimethylaniline (solution B). All reagents were obtained from Merck, Darmstadt, Germany. Sections (5µm thick) were obtained using a Reichert-Jung 2050 microtome (Leica, Bensheim/Germany), and sections were stained with the Lugol's solution and tested for starch. Starch content of the ovary and ovule cells was determined by percentage starch (grains) coverage over the total cell area, and three measurements were done in each typical area. The image analyzing system consisted of a microscope (Axioskop, Fa. Zeiss) and a digital camera (Axioskop, Fa. Zeiss) linked to a computer. The analyzing software of the Firma SIS (Soft Imaging System) was used (Lai-Dihn and Stösser, 2004). The paraffin embedding method (Shi, 2003) was used to observe the development of female gametophyte.

Statistical significance of starch content (%) for the pistil between the fruit loads was analyzed by using the software program SAS, version 6.12 (Cary, USA), with $P<0.05$.

Results

Starch accumulation in the stigma and style

The female flowers of chestnut have a needle-shaped stigma and an ovary with 6 to 9 locules (Shi, 2003). No starch accumulated in the stigmas from the emergence of the cupula to the formation of the embryo (Fig. 1a). However, once the integument primordium appeared, starch grains appeared mainly in the transmitting tissues

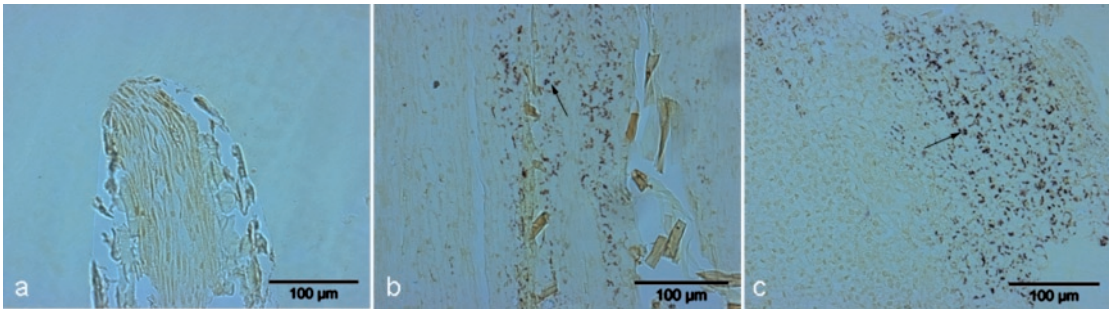

Fig. 1: Starch accumulation in the ovary: a) the needle-shaped stigma of HFL trees (May 8th, 2006), b) cells in the style of LFL trees (May 1st, 2006), c) cells at the ovary bottom of LFL trees (May 1st, 2006). An arrow (→) is indicating a starch grain.

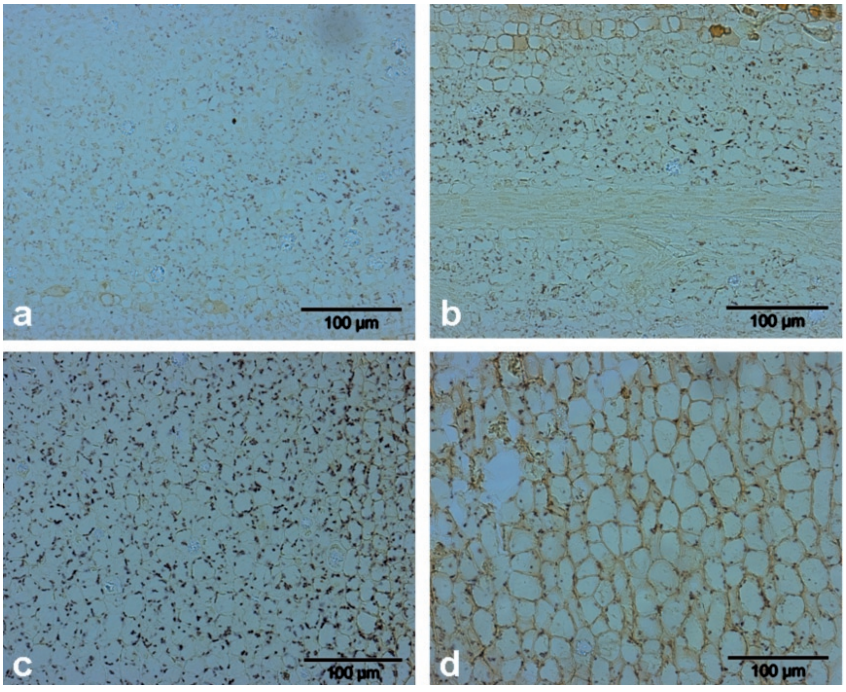

Fig. 2: Starch accumulation in the tissues of the ovary wall etc. (vertical section): a) ovary wall cells of trees of LFL (on May 1st, 2006), b) ovary wall cells of trees of LFL (on May 8th, 2006), c) ovary wall cells of trees of HFL (on May 16th, 2006), d) ovary wall cells of trees of HFL (on 24 May 24th, 2006).

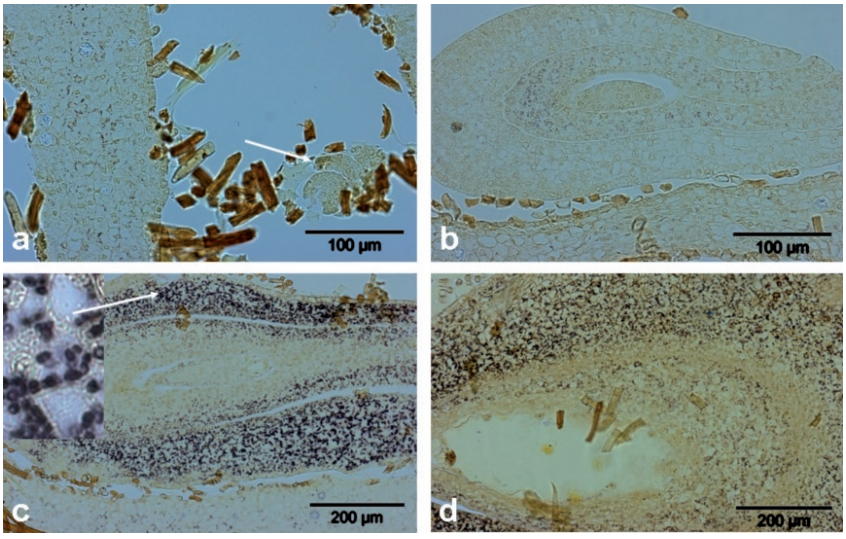

Fig. 3: Starch accumulation in the ovule (vertical section): a) ovule of tree of LFL (on May 1st, 2006), an arrow (→) is indicating a ovule, b) ovule of tree of LFL ((on May 8th, 2006), c) ovule of tree of HFL (sampled on May 16th, 2006), an arrow (→) is indicating a starch grain, d) ovule of tree of HFL (on May 24th, 2006).

of the style (Fig. 1b). These disappeared after the completion of ovule formation or during the formation of megaspore mother cell, with obvious starch grains only apparent in the ovary tissue cells connecting the cupula (Fig. 1c).

Starch accumulation in the ovary wall

Starch grains began to accumulate in a few cells of the ovary walls when the ovule was club-shaped in late April (Fig. 2a). These then grew when the integument primordium appeared. They were accumulated remained in the ovary wall (especially in the outer wall) as the ovule and the female gametophyte developed (Figs. 2b, c, d and Tab. 1). For "HFL" trees, starch content increased to a maximum value of 2.90% on May 16th when the embryo sac was fully matured, and then decreased to 2.30% on May 24th. In contrast, the corresponding starch contents for "LFL" were 0.60% on May 24th (Tab. 1).

Starch accumulation in the ovule

When the integument primordium emerged, a few starch grains were observed only at the chalaza (Fig. 1a). In the "LFL" trees this occurred on May 1st. When the ovule was fully developed, starch accumulated in both the inner and outer integuments (Fig. 3b and Tab. 2). When the embryo sac was mature, the starch content was a maximum value of 17.5% in the outer integument and 4.9% in the inner integument of the "HFL" trees (Fig. 3c and Tab. 2). No starch accumulated in the nucellus cells (Figs. 3b and 3c). The starch level in the ovule decreased after fertilization (Fig. 3d and Tab. 2).

Influence of different fruit loads on starch accumulation

Before the integument primordium developed, there had been few differences in the accumulation of starch in pistil of trees with different fruit loads. However, later stages of growth were more responsive. The levels of starch in the ovary wall and the ovule of the "HFL" trees were apparently higher than those of the "LFL" trees (Tab. 1 and 2). The female gametophytes of the "LFL" trees developed later than those of the "HFL" trees. For instance, the proembryo of 3 to 4 cells was developed in the "HFL" trees on May 24th, while the "LFL" trees only had 4- to 8-nucleate embryo sacs (Fig. 3d and Tab. 2). Fruit loads of the chestnut 'Zaodali' affected starch accumulation in the style. On May 24th, the levels of starch in the outer integument of the "HFL" trees was a maximum value of 14.08% followed by that in the "LFL" trees only 2.94%. At the same stage of female flower development (mature embryo sacs), the level of starch of the "HFL" trees was 17.50% in the outer integument and 2.92% in the "LFL" trees (Tab. 2).

Discussion

Starch accumulated in the transmitting tissues of the style before the formation of megaspore mother cell. The starch grains then disappeared. This is similar to which Hu (1982) described for wheat when the pollen tube pierced the style and also for peach trees (Herrero and Arbeloa, 1989). In the present study, no starch accumulated in the needle-shaped stigmas of Chinese chestnut. The nutrients required by the pollen tube to penetrate stigma cells were mainly supplied from the secretions of the top of the stigma. There was no starch grain in the tissues of the nucellus. Instead

Tab. 1: Starch content (%) in the ovary wall of Chinese chestnut in 2006.

Date	Low fruit load		High fruit load	
	starch content[1]	stage of development[2]	starch content	stage of development
April 24th	Beginning	Club-shaped ovule	0.17	Club-shaped ovule
May 1st	0.28a	Inte. [3]primordium appeared	0.73b	Inte. parallels the nucellus
May 8th	0.39a	Inte. completely enclosed nucellus	0.89b	Tetrad stage
May 16th	0.51a	Ovule fully developed	2.90b	Mature embryo sac
May 24th	0.60a	Embryo sac of 4 to 8 nucleates	2.30b	2 to 4 cells proembryo

[1] starch content: percentage of area coverage by starch grains over the total area of ovary wall.
[2] develop.: development of female flower.
[3] Inte.: Integument.
*Statistical difference is indicated by different letters in same date.

Tab. 2: Starch content (%) in the ovule of Chinese chestnut in 2006.

Date	Low fruit load High fruit load starch Content[1]		stage of development[2]	starch content		stage of development
	Outer	Inner Inte.[3]		Outer	Inner Inte.	
May 1st	none	none	Inte. primordium appeared	none	none	Inte. parallels the nucellus
May 8th	Beginning	Beginning	Inte. completely enclosed nucellus	1.44	0.52	Tetrad stage
May 16th	0.03a	0.05a	Ovule fully developed	17.50c	4.87b	Mature embryo sac
May 24th	2.92b	1.35a	Embryo sac of 4 to 8 nucleates	14.08c	2.46b	2 to 4 cells proembryo

[1] starch content: percentage of area coverage by starch grains over the total area of ovary wall.
[2] develop.: development of female flower
[3] Inte.: Integument.
*Statistical difference is indicated by different letters in same date.

starch grains accumulated in the inner and mostly outer integument. Starch accumulation and depletion occurred the pistil and the female gametophyte development. Starch accumulation in the ovary and the ovule was a maximum when the embryo sac was mature. This was presumably to support the growth of the pollen tube in the ovary and fertilization. Levels of starch were low in the pistils of the "LFL" trees under supply of poor nutrition the development of embryos arrested and embryos aborted in Chinese chestnut (SHI, 2003). Therefore, this experiment should be repeated in the future to determine that there is a relation between the starch accumulation in the pistils and actual fruit load of the chestnut cv. 'Zaodali'.

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