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Scientia Horticulturae

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A comparison of the costs of flowering in 'Feizixiao' and 'Baitangying' litchi

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ARTICLE INFO

Article history: Received 9 August 2011 Received in revised form 24 September 2012 Accepted 28 September 2012

Keywords: Litchi Panicle size Fruit set Pollen viability Carbohydrates Amino acids Minerals

ABSTRACT

In this study, we investigated panicle size, blooming pattern, pollen viability and fruit set as well as changes in starch and soluble sugars in shoots and leaves, and N, P, K and free amino acids in panicles and flowers during panicle growth and blooming in litchi (Litchi chinensis Sonn. cvs. 'Feizixiao' ('FZX') and 'Baitangying' ('BTY'). This was done in order to understand the nutritional costs for flowering and its relation to fruit set, 'FZX' had significantly larger panicles than 'BTY' but similar final set (2.8 fruit vs 2.9 fruit per panicle, averaged over three seasons). The averaged fruit retention rate to harvest over three seasons was 18.5% in 'FZX' and 28.2% in 'BTY'. 'FZX' also had lower pollen viability. During panicle growth and especially blooming, the concentrations of carbohydrates in the shoots and leaves declined in both cultivars, with a greater decline in 'FZX'. As a result, 'FZX' had a fewer carbohydrate reserves available for fruit development than 'BTY'. 'FZX' panicle had a higher concentration of N, which provided nutrition for a larger panicle bearing a larger quantity of flowers. Abscising male flowers continued to accumulate N, P and K, which were not remobilized or reused but lost with flower shed. Before blooming, concentrations of total free amino acids increased in both cultivars. The concentration of Arg was higher than the other amino acids, suggesting flowering had a high demand for this amino acid. 'FZX' panicles had higher concentrations of amino acids than 'BTY'. These results suggest that the nutritional cost of flowering is higher in 'FZX' than in 'BTY'. The lower fruit retention rate in 'FZX' is related to the excessive consumption of carbohydrate reserve by flowering, leaving little for fruit set. Therefore, both pollen limitation and resource limitation contributed to the poor fruit set in 'FZX'.

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1. Introduction

Litchi (*Litchi chinensis* Sonn.) is one of the most important subtropical fruits in the family of Sapindaceae. The crop produces far more female flowers than fruit that the tree can bear, with a final fruit set rate of less than 5% (Stern and Gazit, 2003). This rate is even lower in 'Feizixiao' ('FZX'), the most widely cultivated cultivar in China and becoming more and more popular across the world (Chen, 2008).

Flowering consumes a large amount of tree resources. Urban et al. (2004) found that in mango leaf nitrogen concentration decreased during flowering and was lower in leaves on shoots with panicles than in leaves of vegetative shoots. Yuan et al. (2008, 2009a) showed that total sugar, starch, protein, phosphorus and

potassium concentrations in leaves of bearing shoots decreased during flowering. These results indicate that panicle draws nutrients from the leaves.

The production of large panicles with large numbers flowers increases the chances of successful pollination, fertilization, and fruit set. However, this can deplete tree's resources, which may have a negative impact on subsequent fruit growth. Therefore, competition for resources between flowering and fruit set exists within a panicle. Removing some male flowers enhances fruit set (Huang, 2005). In 'FZX', a panicle can produce up to 3000 flowers but generally has poor fruit set (Wu et al., 2000), and panicle pruning improves fruit set (Wu et al., 2000; Chang and Lin, 2003). This response indicates resources limit fruit set in this cultivar (resource limitation). However, fruit set is also limited by poor pollination (pollen limitation) (Berjano et al., 2011). In the present study, we compared panicle size, blooming pattern, flower biomass, pollen viability, fruit set and changes in nutrition reserves in 'FZX' with large panicles and in 'Baitangying' ('BTY') with small panicles. The main objective of the study was understand the nutritional costs of flowering in litchi and the causes of the poor fruit set in 'FZX'.

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2. Materials and methods

2.1. Materials

The experiments was carried out in Huaxiang Orchard, Yangjiang County in 2009 and in the experimental orchard of South China Agricultural University, Guangzhou in 2010 and 2011, with three 8- to 10-year-old trees each of 'FZX' and 'BTY' planted close-by with synchronous flowering.

2.2. Field investigation and sampling

In each season, 10 panicles from each tree at different positions of the canopy were chosen for observations of panicle growth and fruit set. The length of each panicle was measured before anthesis. The numbers of freshly opened male and female flowers on five of the panicles were counted every other day. Initial fruit set on each panicle was recorded on April 20, 16 or 18, and final fruit set on June 7, 3 or 5 in 2009, 2010 and 2011, respectively. Initial fruit set rate and final fruit set rate were calculated as the percentages of initial and final numbers of fruit set against the number of female flowers, respectively, whereas the fruit retention rate as the percentage of the final fruit set against the initial fruit set.

In 2009, three panicle-bearing shoots were sampled biweekly from each tree from January 17 to April 20. The panicles and ten randomly selected mature leaves from the latest flushes on the shoots were collected and the shoots cut into 10 cm segments. They were then dried in an oven at 65 $^{\circ}\text{C}$ for two days, weighed, ground and stored in a drier for analyses for carbohydrates, minerals and amino acids.

In 2010, 100 each of unopened, freshly opened and abscised male flowers and freshly opened female flowers were randomly collected from each tree during full bloom. The samples were dried at 65 °C for two days. The samples were weighed before being ground into powder for analyses of N, P and K.

2.3. Pollen viability

Pollen grains were collected from newly opened male flowers during the second wave of male blooming and scattered on solid 1% (w/v) agar, supplemented with 3% sucrose (w/v) in petri dishes. The growth of the pollen tubes was observed under a light microscope after incubation at 30 °C for 3 h. Germination rate was calculated as the percentage of total counted pollen grains with tube against the total number of pollen grains (>1000) observed within each dish.

2.4. Determination of sugars and starch

Soluble sugars were extracted from 0.2 g dried shoot or leaves by grinding the powder in 5 ml of 80% (v/v) alcohol. The homogenate was centrifuged at 5000 x g for 10 min and the supernatant collected for soluble sugar analysis using the anthrone method (Zhang, 1990). Starch extraction from the sediment after centrifuge as well as quantitative analysis was conducted according to Xu et al. (1998). The sediment was re-suspended with 10 ml solution of 80% (w/v) calcium nitrate, bathed in boiling water for 20 min, and centrifuged at $8000 \times g$ for 10 min after cooling down. Then, 0.1 ml of 0.01 mol l⁻¹ iodine-potassium iodide (I-KI) solution was added to 2 ml of the supernatant, and OD 630 nm was read on a photospectrometer. A perfect linear curve of starch concentration versus OD_{630nm} (Y=461.8X, R^b = 0.999) was constructed with a series of standard solutions (10, 20, 40, 60, 80 and $100\,\mu g\,ml^{-1}$), and the concentrations of starch in the tissues were calculated. The above analyses were conducted with 3 replicates, using samples from the 3 trees of each cultivar.

2.5. Analyses for N, P, K and free amino acids

For mineral analyses, dried panicle or flower samples were digested in 10 ml concentrated sulfuric acid at 420 °C. Nitrogen concentration was determined with a KJELTEDC AUTO-2300 automatic nitrogen analyzer, phosphorous was analyzed with Mo–Sb–ascorbic acid colorimetric method, and K using flame photometry.

For free amino acid analysis, 0.2 g dried panicle powder was ground with 3 ml of 75% alcohol solution in a mortar. The homogenate was bathed at 85 °C for 20 min and then centrifuged at $6000 \times g$ for 10 min. The sediment was re-suspended with 3 ml of 75% alcohol solution and bathed at 85°C for 20 min before centrifuge at $6000 \times g$ for 10 min. The supernatants of the two centrifuges were mixed in a centrifuge tube and set in a water bath at 55 °C to remove the alcohol. The condensed extracts were diluted with 2 ml of deionized water, and then centrifuged at 15000 g for $20\,\mathrm{min}$. The supernatant was forced through a Millipore TM filter $(20 \, \mu m)$ and used for amino acid analysis on a Hitachi L-8800 amino acid autoanalyzer. The amino acids analyzed included glycine (Gly), glutamic acid (Glu), aspartic acid (Asp), alanine (Ala), leucine (Lue), proline (Pro), serine (Ser), lysine (Lys), valine (Val), threonine (Thr), isoluecine (Ile), arginine (Arg), phenyalanine (Phe), tyrosine (Tyr), histidine (His), methionine (Met) and cysteine (Cys). The analysis was conducted with 3 replicates using samples from the 3 trees of each cultivar.

2.6. Statistics

The above measurements or analyses were conducted with 3 replicates using samples from 3 trees for each cultivars. SPSS 10.0 was used to conduct *t*-tests to compare various parameters, including panicle size, panicle dry mass, flower numbers, fruit set, and concentrations of N, P, K, carbohydrates and free amino acids between the two cultivars. Standard errors of numbers of opening flowers and concentrations of carbohydrates in leaves and shoots were calculated with Excel 2003.

3. Results and analysis

3.1. Panicle size, blooming pattern and fruit set in 'FZX' and 'BTY'

Panicles of 'FZX' were significantly larger than those of 'BTY' in terms of axis length, dry mass and the number of flowers (Fig. 1 and Table 1). Blooming generally started from a small wave of male flowers, followed by a wave of female flowers and then by a second larger wave of male flowers (Fig. 2). The three waves were all higher in 'FZX' than in 'BTY'. Hence, the number of flowers was larger in 'FZX' (Table 1).

In 2009, initial fruit set was similar in the two cultivars, and final set at harvest slightly higher in 'BTY'. However, initial fruit set rate and fruit retention rate were all higher in 'BTY' than in 'FZX' (Table 1). In 2010, both initial fruit set and final fruit set were higher in 'FZX', initial fruit set rate was identical, while fruit retention rate was higher in 'BTY' (Table 1). In 2011, initial set was higher in 'FZX' but final set was similar, and the calculated fruit retention rate was significantly higher in 'BTY' (Table 3). Clearly, fruit set varied with season and cultivar. The lower fruit set in 2010 compared with the other seasons was related to cold weather around fruit set.

Pooling the data of final fruit set in the three seasons revealed that the overall final fruit set per panicle was similar in the two cultivars (2.9 in FZX vs 2.8 in BTY). However, the final fruit set rate and especially fruit retention rate were higher in 'BTY'. These results showed that 'FZX' had poorer setting.

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