Pollen Effects on Fruit Set, Seed Weight, and Shriveling of '73-S-20' Litchi- with Special Reference to Artificial Induction of Parthenocarpy

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Abstract. In this study, we documented whether pollen parents are required for fruiting and their effects on fruit set and seed characteristics in field-grown '73-S-20' litchi (Litchi chinensis Sonn.). The effects of preventing female (F) flower pollination to induce parthenocarpy, selling, and outcrossing with 'Haak Yip' and open-pollinated (OP) fruits derived from F flowers and the second wave of the male (M2) flowers of 'Haak Yip' blooming in synchrony were assessed. The correlation between the percentage of fruit set and shriveled seed/weight was found, indicating that seed abortion during development was not the key factor leading to low yield. We concluded that tiny parthenocarpic fruit could be obtained but that pollination is essential for the good yield of '73-S-20'. Outcrossing markedly increased fruit set and seed weight and decreased the percentage of shriveled seed. Factors other than the pollen parents may be involved in the seed development of '73-S-20'.

Besides normal seed, litchi (Litchi chinensis Sonn.) fruit may contain shriveled (chicken-tongue) seeds with aborted embryos (Stern and Gazit, 2003; Yen et al., 1984). Fruit with shriveled seeds is a character favored by consumers for its large edible part (Menzel and Simpson, 1990; Stern and Gazit, 2003). The shriveled seeds are caused by stenospermocarpy resulting from embryo abortion before fruit formation (Huang, 2001, 2005; Huang and Qiu, 1987).

'S-7-S-20' litchi, discovered in central Taiwan by Yen et al. (1984), is considered to have the best palatability among all other litchi commercial cultivars and has a high shriveled seed ratio (greater than 90%) (Menzel and Simpson, 1990; Yen et al., 1984). '73-S-20' has become one of the main cultivars in Taiwan both for local and export markets since the 1990s (Chang, 2004; Chang et al., 2009, 2012). However, its yield is irregular, large variation in shriveled seed ratio (26.6% to 87.1% over 3 years) occurs, and small, seedless fruits have occasionally occurred in recent years (Fig. 1) (Chang, 2004, 2008), limiting the development of the litchi industry in Taiwan (Chang et al., 2009, 2012). On the other hand, normal- and shriveled seeded and small seedless fruit may result from double fertilization, stenospermocarpy, and parthenocarpy simultaneously within different fruits of a cluster, but the causative factors for the various seed in this cultivar are still poorly understood (Chang, 2004; Chang et al., 2009).

Menzel et al. (1986) and Huang (2005) reported that the shriveled seed ratio in litchi may change with year, cultivar, locality, and weather. The effects of pollen source on seed development have been studied in litchi but with inconsistent results (Stern and Gazit, 2003). Litchi has been regarded as a cross-pollinated crop with self-compatibility (Galán Saúco and Menini, 1989). Three distinct flowering waves can usually be found in each litchi inflorescence with each wave comprising flowers of the same type. The first wave consists of male (M1), the second of female (hermaphrodite function as female, F), and the third of male (hermaphrodite function as male, M2) flowers, which bloom sequentially with partially overlapping bloom periods (Galán Saúco and Menini, 1989; Liu, 1954; Menzel, 1984; Mustard et al., 1953; Stern and Gazit, 2003). The outcrossed fruits of 'Floridian' with pollen from 'Mauritius' showed higher yield, heavier fruit, and greater seed weight than selfed fruits, reflecting xenia and metaxenia (Degani et al., 1995; Stern et al., 1993). However, the responses of the fruit characteristics of 'Bengal' and the shriveled seed ratio of 'Nuo Mi Chi' and 'Gui Wei' from the outcrossed fruit were variable, indicating that pollen parent is important in fruiting and seed development but that exotic pollen is not always more efficient than self-pollen (McConchie et al., 1991; Stern and Gazit, 2003; Xiang et al., 2001); pollen parent effects may greatly differ among litchi cultivars.

In Taiwan, middle-maturing 'Haak Yip' and late-maturing '73-S-20' are the main cultivars cultured in the central region and are usually grown in the same orchards. Because of fluctuating climate, their bloom periods have been closer or even simultaneous in recent years (Chang, 2008; Chang et al., 2009). 'Haak Yip' has a high yield whose normal seed weighs 2.6 g (Yen et al., 1984). We accordingly hypothesized that the peak bloom of F flowers in '73-S-20', with synchrony and crossing with the M2 flowers of 'Haak Yip', is one of the causes of erratic fruit set and the shriveled seed ratio of '73-S-20' (Chang, 2004; Chang et al., 2009).

The goals of this study were to document whether pollen parents are required for fruiting and their effects on fruit set and seed development per cluster in field-grown '73-S-20' trees. To identify the induction of parthenocarpic fruit, only F flowers were retained and bagged to prevent pollination before anthesis. To evaluate the pollen effects, selling and outcrossing with 'Haak Yip' by hand pollination was conducted. To compare results obtained from crossing trials by hand, OP fruits derived from F flowers at peak bloom of '73-S-20' synchronized with the second
wave of M_2 flowers in an adjacent ‘Haak Yip’ orchard were examined. To determine whether fruit set was influenced by shriveled seed/seed weight from outcrossing or selfing, the correlation between the percentage of fruit and shriveled seed/seed weight was calculated. The results may be useful for clarifying the fruiting mechanism and for developing a strategy for the optimal arrangement of different cultivars in ‘73-S-20’ orchards for fruit production.

**Materials and Methods**

**Plant materials.** Experiments were performed in an 8-year-old ‘73-S-20’ litchi orchard in Fenyuan Township, Changhua County, central Taiwan, in 2008. There was an adjacent ‘Haak Yip’ orchard at 10-m distance from the experimental orchard. All selected trees were uniform and spaced at 5 x 5 m.

**Non-pollination to induce parthenocarpic fruit.** Twenty-two inflorescences from five trees were randomly selected (Table 1). Each inflorescence was treated as one replicate and the variance among trees was ignored. Opened flowers and flowers of uncertain sex were removed from each selected inflorescence in advance, leaving only 15 to 20 open F flowers, which were bagged with paper to prevent pollination from 7 to 14 Apr. in 2008. The bags were removed at stigma browning, and the fruit set percentage was investigated at seed maturation (49 d after bloom) and harvest. Fruit characteristics were recorded at harvest.

**Hand and open pollination.** Six trees were randomly selected for each treatment, and five to six inflorescences/tree were pollinated (Table 1). Each inflorescence was treated as one replicate and the variance among trees was ignored. In a hand-pollination trial, opened flowers and flowers of uncertain sex were removed from each selected inflorescence, leaving only 15 to 20 unopened F flowers, which were bagged with paper before pollination. Considering the greatest numbers of M_2 flowers within the inflorescence (Galán Saáco and Menini, 1989; Stern and Gazit, 1998), only the fresh pollens of M_2 flowers in either ‘73-S-20’ or ‘Haak Yip’ were collected in advance. Female flowers were hand-pollinated with the M_2 pollens of ‘73-S-20’ and ‘Haak Yip’ (selfing: female ‘73-S-20’ × male ‘73-S-20’; outcrossing: female ‘73-S-20’ × male ‘Haak Yip’). In the mornings of 12 to 19 Apr. in 2008, when the bifurcated stigma opened into a V shape with a well-developed embryosac (Shih and Chen, 2000). After pollination, the bag was returned until stigma browning. The percentages of fruit set were recorded at seed maturation (49 d after bloom) and harvest. Fruit characteristics, including individual fruit weight, pericarp weight, aril weight, seed weight, transversal diameter, longitudinal diameter, and percentage of aril weight [aril weight/individual fruit weight] × 100% were recorded at harvest. For recording shriveled seed ratio according to embryo development, three seed patterns were defined: 1) normal seed containing a living embryo; 2) shriveled seed with a swollen seedcoat and containing an aborted embryo; and 3) seedless with no ovule development (Fig. 2).

**Result**

**Non-pollination to induce parthenocarpic fruit.** Parthenocarpic fruit could be induced without pollination. In total, 27.3% of inflorescences had parthenocarpic fruit at harvest (Table 1). The percentage of fruit set/clusters at 49 d after bloom and harvest was 21.3% and 9.2%, respectively (Table 2). Parthenocarpic fruit was tiny, containing a diminutive ovule without seed and weighing only 4.2 g on average. The ovule did not develop and only a tiny trace of it was observed (Fig. 2). In non-pollinated ‘73-S-20’ litchi, the percentage of aril and total soluble solids (TSS) content was 67.3% and 17.0 Brix, respectively (Table 3). Hand and open pollination. Fruit set per cluster derived from inflorescences after hand pollination with ‘73-S-20’ and ‘Haak Yip’ pollens was 27.6% and 56.7%, respectively (Table 1). Fruit set was 10.3% and 15.8%, respectively, after pollination with ‘73-S-20’ and ‘Haak Yip’ pollens 49 d after blooming and 3.1% and 10.4% at harvest, respectively (Table 2). Outcrossing had more fruit sets than that of selfing by 3-fold or even more (Table 2).

There was no significant difference in fruit weight, transversal diameter, longitudinal diameter, aril weight, or TSS content among the two hand pollination methods and OP (Table 3). The percentage of shriveled seed was 66.7%, 18.8%, and 77.0%, and seed weights were 1.1, 1.9, and 0.7 g from hand pollination with ‘73-S-20’ and ‘Haak Yip’ pollens and OP, respectively (Table 3). Fruits

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**Table 1.** Pollen sources, treated trees, clusters, flowers, and fruit numbers for ‘73-S-20’ litchi used in this study.

| Pollen source | Treated tree no. | Treated inflorescence no. | Cluster with fruits | Cluster with fruits (%) | Treated female flowers no. | Fruit no. |
|---------------|-----------------|---------------------------|--------------------|------------------------|---------------------------|-----------|
| 73-S-20       | 6               | 29                        | 8                  | 27.6                   | 354                       | 20        |
| Haak Yip      | 6               | 30                        | 17                 | 56.7                   | 469                       | 48        |
| Open pollination | 9           | 28                        | 28                 | 100.0                  | 449                       | 202       |
| Non-pollination | 5             | 22                        | 6                  | 27.3                   | 449                       | 43        |

*Fruits derived from non-pollination were considered as parthenocarpic.*
from outcrossing with ‘Haak Yip’ pollen showed significantly higher seed weight and lower shriveled seed ratio than selfed or OP fruits (Table 3).

Although fruit set and seed weight after outcrossing with ‘Haak Yip’ pollen were higher than those from pollination with ‘73-S-20’, there was no significantly positive correlation between the percentage of fruit set and shriveled seed/seed weight, irrespective of pollen parents (Table 4).

**Discussion**

According to the seed development, the seed types of litchi could be classified as normal seeds with living embryo, shriveled seed with aborted embryo covered with a shriveled seedcoat, and seedless with these three types resulting from double fertilization, stenospermocarpy, and parthenocarpy, respectively (Huang, 2001, 2005; Huang and Qiu, 1987).

Parthenocarpy has been found in a highly natural seedless litchi cultivar, Hexiachuan, irrespective of the occasional occurrence of normal seed and shriveled seed fruit (Huang, 2005; Wang et al., 1996; Zhang et al., 1990). However, it is quite rare and cannot be induced in most cultivars. For example, in ‘Wuheli’ and ‘Dazao’, all fruitlets dropped within 4 to 5 d after bloom in the absence of pollination (Liang et al., 2012). In the present study, parthenocarpic fruit were induced without pollination for the first time in ‘73-S-20’ litchi, indicating that pollen is not always required for fruiting in this cultivar. The incidence of seedless fruit in ‘73-S-20’ is more apparent when blooming occurs during light rainy weather (Chang, 2008). A reduction in pollinator activity, pollen viability, or pistil function in rainy weather may lead to incomplete pollination, facilitating the formation of seedless fruit. Treatments used to increase fruit size deserve further study.

Fruit set in most litchi cultivars ranges from less than 4.1% to 19.5%, depending on the cultivar, environment, and tree (Menzel and Simpson, 1990). However, natural fruit set was only 0.8% to 2.4% in ‘73-S-20’ with two to three waves of fruit drop, particularly the first wave (Chang, 2004). In our study, fruit set in ‘73-S-20’ was more than 3-fold greater after outcrossing with ‘Haak Yip’ than after selfing (Table 2), indicating that outcrossing can markedly increase yield and offers the potential for the optimal arrangement of different cultivars in a single ‘73-S-20’ orchard without considering the incidence of shriveled seed fruit. Chang (2004) revealed a negative correlation between shriveled seed ratio and retained fruit number per cluster in ‘73-S-20’ and indicated that efficient pollination and fertilization is essential for reducing the initial mass fruit drop. Although both fruit set ratio and seed weight were found to have increased by outcrossing with ‘Haak Yip’, there was no positive correlation between the percentage of fruit set and shriveled seed/seed weight (Table 4), confirming that seed abortion during development was not the key factor leading to low yield reported by Chang (2004), which was unlikely the cultivar ‘Floridiana’ that showed a significant positive correlation between the percentage of fruit set and shriveled seed ratio than selfed or OP fruits (Table 3).

**Table 2. Effects of pollen parents and pollination methods on the fruit set of ‘73-S-20’ litchi at seed maturation and harvest stages.**

| Pollen source | Treated cluster no. | Treated inflorescence no. | Cluster with fruits (%) | Treated female flowers no. | Fruit no. | Fruit set (%)<sup>6</sup> |
|---------------|---------------------|---------------------------|-------------------------|---------------------------|-----------|---------------------------|
| 73-S-20       | 29                  | 22                        | 75.9                    | 526                       | 55        | 10.3 ± 1.6 ns             |
| Haak Yip      | 30                  | 26                        | 86.7                    | 469                       | 74        | 15.8 ± 2.4 ns             |
| Non-pollination<sup>5</sup> | 22                  | 15                        | 68.2                    | 449                       | 95        | 21.3 ± 5.4 ns             |
| 73-S-20       | 29                  | 8                         | 27.6                    | 554                       | 20        | 3.1 ± 1.2 b               |
| Haak Yip      | 30                  | 17                        | 56.7                    | 469                       | 48        | 10.4 ± 2.4 a<sup>7</sup>   |
| Non-pollination | 22                  | 6                         | 27.3                    | 449                       | 43        | 9.2 ± 3.9<sup>7</sup> ab  |

<sup>5</sup>Fruit set (%) = fruit number/pollinated flower number per cluster.
<sup>6</sup>Means followed by different letters within the same column are significantly different by least significant difference test at P = 0.05.
<sup>7</sup>Percentage data were transformed into angle by Bliss table before analysis.
<sup>8</sup>Fruits derived from non-pollination were considered as parthenocarpic.

**Table 3. Effects of pollen parents and pollination methods on fruit characteristics and percentage of fruits with shriveled seeds in ‘73-S-20’ litchi.**

| Pollen source | Fruit wt (g) | Transversal diam (mm) | Longitudinal diam (mm) | Pericarp wt (g) | Aril wt (g) | Seed wt (g) | Aril % (wt) | Total soluble solid (°Brix) | Fruit with shriveled seed (%) |
|---------------|-------------|-----------------------|------------------------|-----------------|-------------|-------------|-------------|---------------------------|-----------------------------|
| 73-S-20       | 15.9 ± 0.6 a| 31.7 ± 0.3 a          | 31.4 ± 0.5 a           | 3.0 ± 0.2 ab    | 11.8 ± 0.6 a| 1.1 ± 0.3 b | 74.5 ± 2.6 b| 19.0 ± 0.4 a               | 66.7 ± 17.8 a                |
| Haak Yip      | 17.4 ± 0.5 a| 32.6 ± 0.3 a          | 32.3 ± 0.3 a           | 3.2 ± 0.1 a     | 12.3 ± 0.3 a| 1.9 ± 0.2 a | 70.8 ± 1.0 b| 18.1 ± 0.4 a               | 18.8 ± 0.7 b                 |
| Open pollination | 16.2 ± 0.4<sup>a</sup> | 31.8 ± 0.3 a | 31.4 ± 0.3 a | 2.8 ± 0.1 b<sup>a</sup> | 12.7 ± 0.3 a | 0.7 ± 0.1 b | 78.5 ± 0.8 a | 18.8 ± 0.2 a | 77.0 ± 5.7 a |
| Non-pollination | 4.2 ± 0.3   | 19.7 ± 1.0            | 22.0 ± 0.6             | 1.3 ± 0.1       | 2.9 ± 0.3   | 0.00        | 67.3 ± 2.0  | 17.0 ± 0.2                | All seedless                 |

<sup>a</sup>Each value represents mean ± SE.
<sup>b</sup>Means followed by different letters within the same column except non-pollination are significantly different by least significant difference test at P = 0.05.
<sup>c</sup>Fruits derived from non-pollination were considered as parthenocarpic.

**Fig. 2. Three types of fruits in ‘73-S-20’ litchi. (A) Normal fruits with normal seeds, (B) normal fruits with shriveled seed, and (C) parthenocarpic fruits with no seeds.**
relationship between fruit set and seed weight (Stern et al., 1993). ‘Nuo Mi Chi’, ‘Gui Wei’, and ‘Salathiel’ usually have almost exclusively shriveled seed (Menzel and Simpson, 1990; Stern and Gazit, 2003). Other cultivars such as No Mai Chee standard, Tai So, Kwai May Pink, and Feizixiao have varying percentages of shriveled seeds (Galán Saúco and Menini, 1989; Menzel and Simpson, 1990), depending on the weather at flowering and pollination (Huang, 2001). Stern and Gazit (2003) revealed some studies investigating the influence of pollen parent on yield, seed and fruit characteristics, and incidence of shriveled seed using hand pollination (McConchie et al., 1991; Xiang et al., 2001) or isozyme analysis of the seed embryo from OP fruits (Degani et al., 1995; Stern et al., 1993). Although isozyme can be used to analyze the progeny in normal seed samples, it cannot be used for the shriveled seed samples, which have no embryos. For this reason, isozyme analysis was not used in this study. The percentage of shriveled seed decreased and the seed weight increased by ‘Haak Yip’ pollen comparing with selfed and OP, suggesting that outcross is one of the causes for variable shriveled seed ratio in ‘73-S-20’. ‘Haak Yip’ pollens also clearly affected seed development in ‘73-S-20’ with 81.2% seeds containing a living embryo (Table 3). This result is similar to that achieved in fruits of ‘Floridian’ outcrossed with pollen from ‘Mauritius’, a normal-seed cultivar, resulting in higher yield and higher fruit and seed weight than selfed fruits (Degani et al., 1995; Stern et al., 1993). It has been reported that pollen parents may exert a strong xenia effect on the seed characteristics of nut crops (Sedgley and Griffin, 1989). The influence of ‘Haak Yip’ pollen on seed development of ‘73-S-20’ may also represent a xenia effect. Alternatively, embryo abortion, leading to shriveled seed, may be a result of inbreeding depression (Stern and Gazit, 1993) and may occur near the global stage of embryo development (Xiang et al., 2001), but the actual mechanism remains unclear (Chang, 2008; Huang, 2001, 2005; Stern et al., 1993). Variable responses in fruit characteristics resulted from the outcross of ‘Bengal’ with four pollenizers (McConchie et al., 1991) and only one pollen parent increased fruit weight. A range of shriveled seed ratios was also observed in ‘Nuo Mi Chi’ and ‘Gui Wei’ pollinated with seven pollenizers: 7.5% to 92.3% and 4.7% to 75%, respectively (Xiang et al., 2001). ‘Nuo Mi Chi’ showed a more stable shriveled seed ratio after pollination with six pollenizers than ‘Gui Wei’, and only ‘Dazao’ pollen decreased the shriveled seed ratio to 7.5%, indicating that pollen was an important factor in fruit characteristics and seed development but that the influence was not absolute, further indicating the existence of other unknown factors. Similar results were obtained in the present study (Tables 2 and 3). Although outcrossed fruit have greater seed weight and a lower shriveled seed ratio, it is noteworthy that 33.3% seeds had well-developed embryo after self-pollination (Table 3). Moreover, 23.0% seeds contained embryo from OP fruit, which bloom the closed M2 flowering of ‘Haak Yip’ (Table 3), possibly resulting from high outcrossing rate as a result of a short distance from a pollinator (Stern et al., 1993) and showing that factors other than outcross/pollen parents may affect seed development in ‘73-S-20’.

Galán Saúco and Menini (1989) indicated that litchi was regarded as a cross-pollination crop but was generally self-compatible and that a partial overlap in the blooming of the F and male flower usually occurred in different trees of a single orchard, so that a monocultivar orchard was feasible for commercial production. However, because M2, F, and M3 bloom consecutively in each litchi inflorescence (Liu, 1954; Mustard et al., 1953), there is no or little flowering behavior among these waves in the same inflorescence. Such overlap may facilitate cross-pollination, even inside a single tree. Therefore, planting two cultivars with F flower blooms that coincide with one type of the male flower blooms (especially the potent M2 as a result of its greatest numbers of flowers in inflorescence and higher pollen viability) of the other cultivar has the potential to improve pollination by ensuring pollen availability throughout the F flower blooms (Stern and Gazit, 1998; Stern and Gazit, 2003). Thus, cross-pollination in litchi orchards may have beneficial effects on production (Fromeman et al., 2012; Galán Saúco and Menini, 1989), but outcrossing may decrease the shriveled seed ratio in shriveled seed cultivars as ‘73-S-20’. To the best of our knowledge, this is the first report to demonstrate that double fertilization, stenopomaropocy, and parthenocarpy occur naturally and all together on a ‘73-S-20’ tree. To increase the percentage of shriveled-seeded fruits, monocultivar cultivation has been suggested for ‘73-S-20’ litchi production, but outcrossing may increase cluster yield and seed weight and decrease shriveled seed ratio. However, the presence of factors other than pollen parent that affect seed development cannot be excluded. Further studies for increasing both fruit set and shriveled seed may be required. Besides efficient pollination and fertilization to reduce the initial mass fruit drop and the sufficient flushing of bearing shoots to reduce the second wave of fruit drop owing to an inadequate supply of carbohydrates (Chang and Lin, 2008), perhaps spraying synthetic auxins or gibberellic acids may potentially raise yield by reducing the initial dropping of embryo-aborted fruits (Stern and Gazit, 2003).
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