Effect of Pollenizer Distance and Selective Fruitlet Abscission on Outcrossing Rate and Yield in 'Tommy Atkins' Mango

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ABSTRACT. Postzygotic self-incompatibility has been reported in several Indian mango (*Mangifera indica* L.) commercial cultivars. Floridian cultivars, on the other hand, have been planted in solid blocks and seem to be self-fertile. Isozyme analysis enabled us to determine outcrossing rates at the fruitlet and fruit stages in the Floridian 'Tommy Atkins' ('Tommy'). Two commercial mango orchards consisting of adjacent solid blocks of 'Maya' and 'Tommy' were studied. This combination offered a unique opportunity to identify each individual fruitlet or fruit as selfed or outcrossed by TPI isozyme analysis. A consistent and significant increase in outcrossing rate during fruit development was found: the average outcrossing rate increased from 10% and 13% in fruitlets to 66% and 73% in mature fruit in the two 'Tommy' blocks surveyed. This 6-fold increase is the result of selective abscission of selfed progeny. A significant inverse correlation was found between the distance of 'Tommy' trees from the 'Maya' block and the outcrossing rate in mature fruit. No significant correlation between distance from 'Maya', or outcrossing rate, and yield was observed, suggesting that the practice of planting 'Tommy' in solid blocks is sound.

Postzygotic self-incompatibility was reported in several commercial Indian cultivars ('Bombay Green', 'Chausa', 'Langra', and 'Dasher') in the early 1970s (Sharma and Singh, 1970; Singh and Sharma, 1972), and more recently confirmed (Rao et al., 1984). In those hand-pollination studies, initial fruit set did occur after self-pollination. However, the endosperm of selfed fruitlets showed clear signs of degeneration and ≈ 1 month after set they abscised. A similar phenomenon was found in the polyembryonic 'Turpentine' in Israel (Roizman, 1984).

Floridian mango cultivars ['Haden', 'Tommy Atkins' ('Tommy'), 'Kent', 'Keitt', and 'Van Dyke'] (Campbell, 1992) predominate in commercial orchards outside southeast Asia. Little evidence is available regarding the need for cross-pollination in these cultivars. 'Zill' pollen germinated faster on stigmas of detached 'Haden' flowers, relative to self pollen (de Wet et al., 1989). Conflicting information exists on the effect of adjacent pollenizers on 'Haden' yield in Florida. One report (Ruehle and Lynch, 1939) concluded that there is a clear beneficial effect, whereas another did not (Ledin, 1958). Young (1942) did not find any significant difference between the final set of selfed or crossed hand-pollinated 'Haden' flowers. In Israel it was found that 'Haden', 'Maya', 'Zillate', and 'Irwin' trees caged with a beehive produced good yields with or without the presence of pollenizer trees (Degani et al., 1992; Roizman, 1984; Yutko, 1995).

The fact that the Floridian cultivars investigated to date have been found to be self-fertile does not exclude the possibility that other Floridian cultivars may be self-sterile, or that cross-pollination may increase fruit set and yield in self-fertile mango cultivars, as suggested by Popenoe (1917). 'Tommy' is of specific interest, because it is one of the most widely grown commercial cultivars in

Florida (Campbell, 1992) and Israel, yet its productivity tends to be lower than that of the other leading commercial cultivar 'Keitt'. 'Tommy' is often planted in solid blocks and is considered self-fertile. However, there is no information on the effect of cross- vs. self-pollination on its yield.

The development of isozymes as genetic markers in mango (Degani et al., 1990) has enabled unequivocal determinations of the pollen parent of fruit in several specific combinations of two cultivars. In the first such study (Degani et al., 1997a), the outcrossing rate in mature fruit was determined in adjacent blocks of 'Maya' and 'Tommy', at different distances from the pollenizer blocks; however, yield was not recorded.

Hermaphroditic plants often produce far more flowers than mature fruit (Bawa and Webb, 1984; Lloyd, 1980; Stephenson,

Fig. 1. Schematic map of the mango blocks in the Merom Golan orchard (width and length of each block 120 and 132 m, respectively).

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Fig. 2. Schematic map of the mango blocks in the Neve Ur orchard (width and length of each block 90 and 125 m, respectively).

1981). Apparently excess flowers allow the plant to control the genetic quality of its offspring by aborting inferior progeny (Janzen, 1977; Lloyd, 1980; Stephenson, 1981). Selective abscission of selfed progeny was found in *Litchi chinensis* Sonn. (Degani et al., 1995), *Persea americana* Mill (Degani et al., 1986; 1989), *Banksia spinulosa* Smith (Vaughton and Carthew, 1993), and other crops (Stephenson, 1981; Stephenson and Winsor, 1986).

The aim of this study was to 1) determine the effect of self vs. cross-pollination on mango fruitlet survival and 2) determine the effect of pollenizer proximity on outcrossing rate and yield.

Materials and Methods

CAGED TREES. Two 'Tommy' trees were caged at the end of February 1994 before flowering, with a 15-mesh screen (openings of 1.5×1.5 mm) before flowering at the Faculty of Agriculture experimental mango orchard in Rehovot. A beehive was placed inside each cage on 20 Mar. Twenty fruitlets, weighing 2 to 6 g, were sampled from each tree to confirm that the isozyme pattern of selfed 'Tommy' fruitlet endosperm is the same as that of mature leaves.

ORCHARD STUDIES. We located two commercial mango orchards (Merom Golan and Neve Ur) consisting of adjacent solid blocks of 'Maya' and 'Tommy'. This arrangement allowed the identification of each

individual fruitlet or fruit as selfed or outcrossed by TPI (triosephosphate isomerase) isozyme analysis. For the locus *Tpi*, 'Maya' and 'Tommy' are homozygous *aa* and *bb*, respectively (Degani et al., 1990). Thus, hybrid fruit of 'Tommy' $\times$ 'Maya' is identified by its heterozygous (*ab*) *Tpi* genotype, and 'Tommy' selfed-fruit is identified by its *bb* genotype.

Young 'Tommy' fruitlets (2 to 6 g) and mature fruit were randomly sampled in 1994 in Neve Ur and in 1995 in Merom Golan. In 1994, only mature 'Tommy' fruit were randomly sampled in Merom Golan.

The Merom Golan orchard is located to the north of the Sea of Galilee, and was studied for 2 consecutive years. The 'Tommy' block had been planted in 1983 and consisted of 20 rows (6 m apart), each with 42 trees (5 m apart). At its northern side it bordered with a 'Maya' block (Fig. 1). In both years, mature fruit were sampled in July from distances of 10, 15, 25, 45, 85, and 165 m away from the 'Maya' block. In 1994, 60 fruit were sampled at each distance: four replications (individual trees) $\times$ 15 fruit per replication. In 1995, 120 fruitlets and fruit were sampled at each distance: Four replications (groups of three trees each) $\times$ 30

Fig. 3. Hybrid rate and yield in 'Tommy' fruit at six distances from a 'Maya' block; study conducted at Merom Golan, in 1994. The bars are SE.

Fig. 4. Hybrid rate and yield in 'Tommy' fruitlets and fruit at six distances from a 'Maya' block; study conducted at Merom Golan, in 1995. The bars are SE.

fruitlets/fruit per replication.

The Neve Ur orchard is located in the Jordan Valley. The 'Tommy' block had been planted in 1980 and consisted of 15 rows (6 m apart), 25 trees per row (5 m apart). The block had adjacent 'Tommy' blocks on three sides, and a 'Maya' block at its western side (Fig. 2). 'Tommy' fruitlets were sampled in May and mature fruit in July, from distances of 10, 15, 25, 45, and 85 m from the 'Maya' block. At each distance, 80 fruitlets and fruit were sampled: four replications (individual trees) $\times$ 20 fruitlets/fruit per replication.

ISOZYME ANALYSIS. Extracts for electrophoresis were prepared from endosperm of fruitlets or mature leaves of seedlings. Horizontal starch electrophoresis and staining for triosephosphate isomerase (TPI; EC 5.3.1.1) were carried out as described by Degani et al. (1990).

Fruitlets were kept up to 7 d at 4 °C, for endosperm extraction. The minuscule amount of endosperm extracted did not allow repetition of the isozyme analysis. The number of fruitlets successfully analyzed was 252 in Neve Ur, 1994 and 546 in Merom Golan, 1995. Seeds were extracted from mature fruit, weighed and sown;

mature leaves from the seedlings were used for the TPI analysis (Degani et al., 1990). Germination rates were 80%, 70%, and 38% for Neve Ur 1994, Merom Golan 1994 and 1995, respectively. The low rate in 1995 apparently occurred because germination media from 1994 was reused without sterilization. The factor responsible for low germination rate in 1995 did not discriminate against selfed seeds; outcrossing percentage was slightly lower in 1995 compared to 1994 (Figs. 3 and 4). The number of seedlings analyzed was 319, 253 and 273, for Neve Ur 1994, Merom Golan 1994 and 1995, respectively.

Results

TPI-GENOTYPE IN THE ENDOSPERM OF FRUITLETS FROM CAGED 'TOMMY' TREES. All 40 fruitlets sampled were found to have endosperm with the same *Tpi* genotype (*bb*) as in leaves from the parent 'Tommy' trees (Degani et al., 1990). This result confirmed that fruitlet endosperm could be used for unequivocal paternity analysis.

OUTCROSSING RATE IN FRUITLETS AND FRUIT AT INCREASING DISTANCES FROM THE POLLENIZER BLOCK. Outcrossing (hybrid) rates in 'Tommy' fruitlets and fruit at increasing distances from the 'Maya' block (Figs. 1 and 2) are presented for Merom Golan in Figs. 3 (mature fruit only) and 4, and for Neve Ur in Fig. 5. The percent of outcrossed progeny for mature fruit was $\approx$ 6-fold higher than percent outcrossed for young fruitlets (Figs. 4 and 5). The percent of outcrossed fruitlets were 10 and 13, and for mature fruit, 66 and 73, for Merom Golan and Neve Ur orchards, respectively.

In the two orchards, the increase was consistent and significant ($P < 0.01$) at all distances from the 'Maya' block.

There was a significant decrease in the proportion of mature outcrossed fruit with increasing distance from the 'Maya' pollenizer block (Figs. 3–5). This inverse correlation was represented by linear regression curves: $OC = 0.87 - 0.02r$ for Neve Ur 1994 ($R^2 = 0.91$); $OC = 0.91 - 0.01r$ ($R^2 = 0.90$) and $OC = 0.79 - 0.01r$ ($R^2 = 0.94$) for Merom Golan in 1994 and 1995, respectively (OC = Outcrossed fruit, r = row from 'Maya' block). In both orchards, the percent of outcrossed fruitlets was also much lower at great distance from the pollenizer block than at close proximity (Figs. 4 and 5), but, no consistent trends were found. Both sites were characterized by mid-distance outcrossing rates higher than expected, at 45 m in Neve Ur and at 25 to 45 m in Merom Golan. We rechecked all trees in the studied 'Tommy' blocks. In Neve Ur we found, 45 m from the 'Maya' block, one 'Tommy' tree with a big 'Maya' branch, and in Merom Golan we found a 'Palmer' tree (*Tpi* genotype *ab*) 50 m from the 'Maya' block.

THE RELATIONSHIP BETWEEN FREQUENCY OF OUTCROSSED FRUIT, DISTANCE, AND YIELD. The number of mature fruit carried by the sampled trees was determined at harvest (Figs. 3–5). Neither distance nor frequency of outcrossed mature fruit was correlated with yield.

Fig. 5. Hybrid rate and yield in 'Tommy' fruitlets and fruit at five distances from a 'Maya' block; study conducted at Neve Ur, in 1994. The bars are SE.

POLLEN PARENT EFFECT ON FRUIT AND SEED MASS. A comparison between pericarp and seed mass of self versus cross-pollinated 'Tommy' mature fruit is presented in Table 1. Although the difference in seed mass was significant, with outcrossed seeds being 15% heavier than selfed seeds, there was no significant difference in pericarp mass resulting from the two pollen parents.

Discussion

The mango flower is entomophilic; it has a tiny stigma and only one stamen with a very small amount of pollen. Pollination is carried out mainly by flies and bees (Anderson et al., 1982; Dag and Gazit, 1996; Davenport and Nunez-Elisea, 1997; Singh, 1988). The flowers open early in the morning. At anthesis, the stigma is already receptive, and a few hours later pollen is shed (Davenport and Nunez-Elisea, 1997; Singh, 1960). The flower does not have any morphological or temporal mechanism to prevent self- or close-pollination or encourage cross-pollination. Thus, self pollination within the flower or close pollination between flowers of the same trees and adjacent trees

should predominate. Nevertheless, Degani et al. (1997a) found a very high outcrossing rate in mature fruit of 'Tommy' and 'Maya' mangos, even 108 m away from the pollinizer block. Dag et al. (1997) found the same phenomenon in 'Lily' at 60 m from 'Tommy'. Our results corroborate these findings for 'Tommy' (Figs. 3–5); even at 165 m away from the 'Maya' block, about half of the mature fruit originated from outcrossing. Mango breeders using open pollination progeny should be aware of these findings.

The 6-fold increase in the frequency of outcrossed progeny from the 2 to 6 g fruitlet stage (≈ 1 month old) to mature fruit indicates that the high rate of outcrossed mature fruit in mango (Figs. 3–5) (Degani et al., 1997a) does not reflect the actual rate of cross-pollination; it is mainly a result of selective fruitlet abscission. The actual increase in outcrossing rate from fruit set to fruit maturity is apparently much higher. A mature mango tree produces several millions of flowers. Only $\leq 0.1\%$ of its perfect flowers develop into mature fruit (Singh, 1960). Massive fruitlet abscission was specifically reported for 'Tommy' (Nunez-Elisea and Davenport, 1983), with most of the fruitlet drop occurring during the first month. Thus, it seems reasonable that the increase in outcrossing rate was much higher than 6-fold. As a result, a minuscule rate of cross-pollination could be transformed by selective abscission to a substantial percentage of hybrids at harvest. The same phenomenon was found in other fruit crops (Degani et al., 1986, 1989, 1995, 1997b), suggesting that selective abscission is a widespread phenomenon for plants that have profuse bloom and low rate of final fruit set.

In unisexual or self-incompatible cultivars, effective cross-pollination can be determined readily and accurately by monitoring fruit set. In several such plants, pollinated by insects, fruit set rate was found to decrease sharply with increasing distance from the foreign pollen source. In male sterile cotton, the decrease found was 10-fold or more for the first 20 m (Mungomery and Glassop, 1969). In self-incompatible plum, fruit set decreased from 7% in trees adjacent to the pollinizer to $\leq 1\%$, four rows or more away (Free, 1962). In contrast, when the plant is self-fertile, there is no easy way to differentiate between cross- and self-pollination initial set. With 'Tommy' we had to wait until the amount of embryo and endosperm was large enough to enable separation and isozyme analysis.

Table 1. Pericarp and seed average mass of selfed vs. outcrossed 'Tommy' progeny, Merom Golan, 1994.

Progeny	Fruit examined (no.)	Pericarp mass (g)	Seed mass (g)
Outcrossed	202	279	19.5
Selfed	51	273	17.0
Significance		NS	*

NS,*Nonsignificant or significant at $P < 0.05$.

The fact that crossed 'Tommy' fruit had significantly heavier seeds than selfed ones (Table 1) indicates greater vigor of the former and probably reflects greater ability to compete for nutrients. However, competition and inbreeding depression may be involved with the massive fruitlet abscission in self fertile mango. As a result of inbreeding degeneration (Singh and Sharma, 1972), self 'Tommy' fruitlets may abscise even when there is no competition (Charlesworth and Charlesworth, 1987; Sedgley and Griffin, 1989). The fact that no correlation was found between the distance from the pollinizer block or the outcrossing rate and yield (Figs. 3–5) indicates that both selfed or crossed fruit may survive to realize the tree capacity. Apparently, a sufficient number of selfed 'Tommy' fruitlets had the ability to develop into mature fruit. Similar phenomenon was reported for 'Mauritius', a self-fertile lychee cultivar (Degani et al., 1995; Stern et al., 1993).

Our results (Figs. 3–5) show that 'Tommy' productivity was not influenced by its vicinity to a large 'Maya' block. Hence, there is no reason to change the practice of planting 'Tommy' in solid blocks. The absence of an adjacent pollinizer may even be an advantage, as selfed 'Tommy' fruit have a more desirable fruit to seed ratio (Table 1).

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