Cross-pollination improves 'Orri' mandarin fruit yield

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ABSTRACT

'Orri', a selection of 'Orha' mandarin [Temple (Citrus temple hort. ex Y. Tanaka) × Dancy (Citrus tangerina hort. ex Tanaka)], is a new high-quality Israeli mandarin which, in the last decade, has become one of the leading varieties in Israel. 'Orri' has an excellent taste, the rind is deep orange in color and easily removed, and it contains few or no seeds. However, 'Orri' grown in Israel suffers from inadequate yield and no published studies have yet addressed this problem. In the present study we determined that 'Orri' productivity depended on conditions being favorable to cross-pollination. Under cross-pollination conditions a positive correlation ($R^2 = 0.97$) was found between yield per tree and number of fruits per tree, and more than 90% of the fruits exceeded 60 mm: the most profitable size range. These data suggest that the number of fruits per tree, and not fruit size, is the limiting factor for yield improvement in 'Orri' orchards. Studying seed set showed that 'Michal' mandarin (Citrus reticulata Blanco) is a compatible pollenizer for 'Orri' flowers: the number of seeds per 'Orri' fruit increased as the distance from 'Michal' trees decreased. The present study demonstrated that cross-pollination of 'Orri' resulted in yield improvement, yet at the price of increased seed set.

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1. Introduction

There is increasing consumer interest in seedless citrus fruit, including mandarin (Campbell et al., 2004). Seed set reduction is encouraged when parthenocarpy, (i.e., fruit formation without fertilization or embryo abortion) is coupled with self-incompatibility or male sterility, under self-pollination conditions (Vardi et al., 2008). Since many mandarin varieties exhibit some degree of parthenocarpy, the cultivation of mandarins in isolated blocks is common, although cross-pollination usually improves fruit-set and yield (Krezdorn, 1967; Hearn et al., 1968; Garcia-Papi and Garcia-Martinez, 1984; Wallace and Lee, 1999; Wallace, 2004; Chao, 2005).

It was reported that for 'Imperial' mandarin (Citrus reticulata Blanco) (Wallace et al., 2002) and for 'Orlando' tangelo (Citrus reticulata Blanco × Citrus paradisi Macf.) (Krezdorn and Robinson, 1958) the number of seeds per fruit decreased with increasing distance from the pollenizer. Seed set was correlated with fruit weight in 'Imperial' and with the number of fruits per tree in 'Orlando'. Thus, solid-block orchards with low-seeded fruits may suffer from low yields. Seed set of 'Imperial' mandarins from row 1, adjacent to the pollenizer, was significantly higher than fruits from row 3. Fruits from rows 5 to 15 did not differ significantly among

themselves. However, isozyme analysis suggested that all seeds in the block originated from the pollenizer, indicating pollen dispersal to distances of more than 75 m, i.e., about 15 rows (Wallace et al., 2002). Later Chao et al. (2005) found that pollen could be dispersed in the citrus orchard to distances as great as 1000 m. Nevertheless, the number of successful crosses (as indicated by seed number per fruit) decreased significantly beyond the third row from the pollenizer (Wallace et al., 2002). A possible explanation is that the number of viable pollen grains from the pollenizer that were deposited on each stigma decreased with increasing distance from the pollenizer row.

'Orri' is a new Israeli mandarin variety, selected for seedlessness and low pollen grain viability from irradiated bud wood of the cultivar 'Orah' mandarin [Temple (Citrus temple hort. ex Y. Tanaka) × Dancy (Citrus tangerina hort. ex Tanaka)] (Vardi et al., 2008). In the north of Israel the 'Orri' fruits mature in February and March; they have a delicious taste, the peel is deep orange in color and easy to remove, and the fruit has few or no seeds, i.e., 0–4 seeds per fruit. The first commercial 'Orri' plantation was established in 1996, and with passing time it became one of the main mandarin plantations in Israel. However, the Israeli 'Orri' suffers from inadequate yield, and so far no published studies have addressed this problem.

In the present study we determined the influence of the pollenizer on 'Orri' seediness and fertility, i.e., number of seeds per fruit, total yield, number of fruits per tree and fruit size.
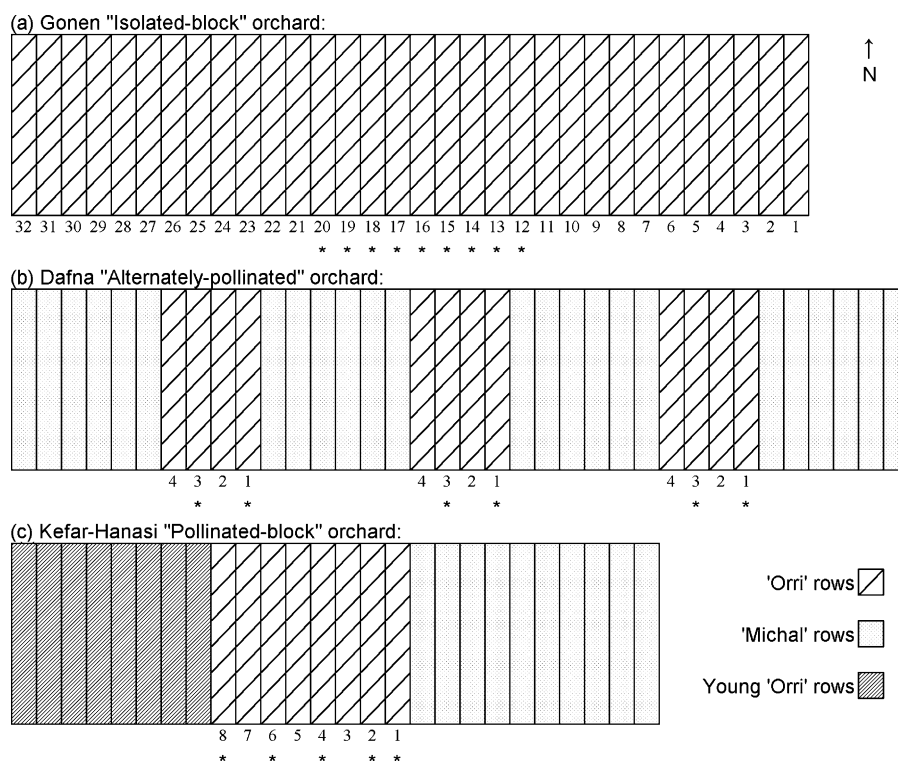
Fig. 1. Schematic diagram of the three types of 'Orri' orchards tested. The rows, from which the data were collected, are marked with an asterisk.

2. Material and methods

2.1. Experimental sites

The experiments were conducted in three different commercial 'Orri' mandarin orchards in Upper Galilee in northern Israel, located within 55 km of each other (32.6–33.1°N; 35.4°E; alt. 100–250 m) (Fig. 1): Gonen – an "isolated-block" orchard; Dafna – an "alternately-pollinated"; and Kefar-Hanasi – a "pollinated-block" orchard.

The "isolated-block" orchard in Gonen consisted of a 2 ha (150 m × 130 m) solid block of 'Orri', about 2 km from the nearest pollenizer – an orchard containing a selection of mandarin cultivars. The trees were planted in 1997 at a spacing of 3 m × 5 m (667 trees/ha). The orchard was subsequently lightened by graded displacement of trees: after the harvest of 2005 every fourth tree was removed from each row; after the harvest of 2006 the inner trees from triplets in the odd rows were removed; and after the harvest of 2007 the inner trees from triplets in the even rows were removed. The spacing of the trees after the displacement was 6 m × 5 m (333 trees/ha).

The "alternately-pollinated" orchard in Dafna comprised three blocks of four 'Orri' rows followed by six 'Michal' pollenizer rows on each side. The trees were planted in 1999 at a spacing of 2 m × 5 m (1000 trees/ha), and were subsequently displaced by removing every fourth tree after the harvest of 2004, and the inner tree from each triplet after the 2006 harvest. The spacing between the trees after the displacement was 4 m × 5 m (500 trees/ha).

The "pollinated-block" orchard in Kefar-Hanasi, which was planted in 1996, consisted of eight 'Orri' rows adjacent to 10 'Michal' pollenizer rows on the east side and eight young 'Orri' rows, which had been grafted in 2005, on the west side. Tree initial spacing and displacement were the same as in Gonen orchard.

All the 'Orri' trees were grafted on Troyer Citrange rootstock and were uniform in size. Row direction was north–south. 'Orri' and

'Michal' flowered with full overlap and the bee activity was constant through flowering season.

2.2. 'Orri' and 'Michal' pollen viability and pollen germination in 'Orri' stigma

Pollen grains from one fresh flower of 'Orri' or 'Michal' were suspended in 0.5 ml of germination medium, containing 10% sucrose, 2×10^{-3} M H_3BO_3 and 3×10^{-6} M $Ca(NO_3)_2$ in distilled water. The vitality of the pollen was deduced from the proportion of pollen grain germinating after 24 h of incubation at 25 °C. The procedure was repeated one to four times in all orchards during the 2007 and 2008 blooms, each time with 5–10 different flowers of each cultivar.

For pollen germination in the stigma, 'Orri' flowers were caged at the balloon stage with 15-mesh, 30%-shade screen nets, to prevent honeybees and other insects from reaching them. Ten flowers were hand pollinated to excess with fresh 'Orri' or 'Michal' pollen. After 24 h the styles were collected into Histochoice solution (Amersco, Solon, OH) and stained with 1% aniline blue (Martin, 1959). Pollen grain germination in 'Orri' stigmas was determined by counting the number of germinating pollen grains with the aid of a light microscope.

2.3. Effect of 'Michal' pollenizer on 'Orri' production

The yield, number of fruits per tree, average fruit size and fruit-size distribution were determined in three 'Orri' blocks of the Dafna orchard during four consecutive years, 2004–2007. Data were collected from the 'Orri' trees in rows number 1 and 3 (Fig. 1b) i.e., an adjacent row and a distant row, respectively. Rows 2 and 3 are identical for the aspects of this experiment (the experiment was conducted on rows 3 and not on row 2 because of technical limitations in the orchard). In 2004 and 2005 10 'Orri' trees were examined in each row. In 2006 and 2007 the data were
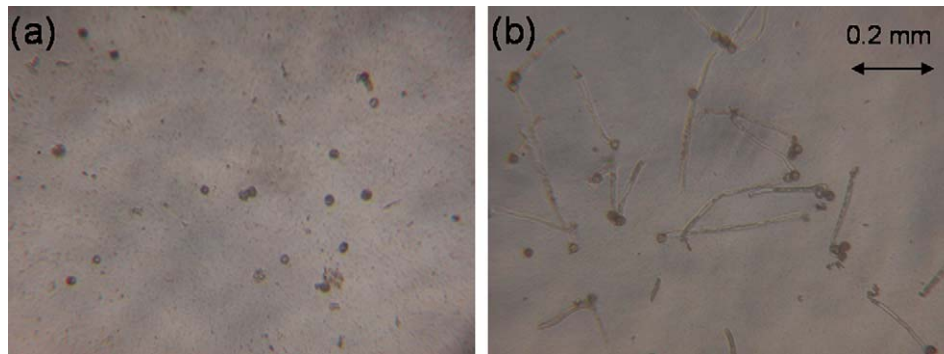
Fig. 2. 'Orri' (a) and 'Michal' (b) pollen germination after 24 h incubation at 25 °C.

collected from six and four trees per block, respectively. The number of trees was reduced due to tree displacement, as described above. Fruit characteristics (soluble solids content (SSC) and titratable acids (TA)) were determined annually in 10 fruits per row in each block. SSC was determined with a digital refractometer (PR-100 Palette; Atago, Tokyo, Japan) and 2 ml juice was titrated with 0.1 M NaOH to pH 8.2 for TA determination.

2.4. Effect of 'Michal' pollenizer on 'Orri' seediness

In the Gonen orchard the number of seeds per fruit was examined in 50 fruits, picked at random from the center of the plot (Fig. 1a). In the Dafna orchard the number of seeds per fruit was determined in 25–30 'Orri' fruits, picked at random from the middle of rows number 1 and 3 (Fig. 1b). Fruits from row 1 were examined from both the facing and non-facing sides to the adjacent row of 'Michal'. Fruits from row 3 were examined only from the side facing row 2. In the Kefar-Hanasi orchard the number of seeds per fruit was examined in 40–50 'Orri' fruits, randomly picked from both facing and non-facing sides to the 'Michal' block in row number 1, and in rows 2, 4, 6 and 8 only from the facing side (Fig. 1c).

2.5. Statistical analysis

Percentage data were subjected to arcsin transformation, to provide a normal distribution before analysis. Data were analyzed for statistical significance by the general linear model (GLM) procedure of SAS (SAS, 1990). Duncan's new multiple range test was applied to compare treatments when ANOVA showed significant differences among means.

3. Results

3.1. 'Orri' and 'Michal' pollen viability and pollen germination in 'Orri' stigma

'Orri' flowers contained very few pollen grains, in comparison with those of 'Michal' (Fig. 2). 'Orri' pollen grain germination in the medium was negligible, whereas 'Michal' pollen grain viability was 73% and 42% in 2007 and 2008, respectively (Table 1). Similarly, only 0–10 pollen grains germinated in 'Orri' stigmas after hand pollination with 'Orri' flowers, whereas more than 50 pollen grains germinated in all 'Orri' stigmas that were hand pollinated with 'Michal' flowers (Table 1).

3.2. Effect of 'Michal' pollenizer on 'Orri' production

The influence of the presence of 'Michal' on 'Orri' productivity was examined in an "alternately-pollinated" orchard during four consecutive years, 2004–2007. Table 2 summarizes the data from 'Orri' rows number 1 and 3. In 2004–2007 the average yield and average number of fruits per tree were higher in the adjacent row than in the distant row, at 62 kg or 484 fruits per tree and 47 kg or 327 fruits per tree, respectively. The average fruit weights did not differ significantly between the two rows, at 135 and 147 g per fruit, respectively. It should be noted that the higher yields in the adjacent row in 2006 did not impair the productivity in 2007.

A high positive correlation ($R^2 = 0.97$) between yield per tree and number of fruits per tree was found for both rows (Fig. 3A), although fruit weight decreased as the number of fruits per tree increased ($R^2 = 0.66$) (Fig. 3B). The maximum 'Orri' yield per tree and maximum number of fruits per tree were higher in the adjacent row than in the distant row (Fig. 3A), and this was found

Table 1
'Orri' and 'Michal' pollen grain viability and pollen grain germination in 'Orri' stigma.

Year	2007	2008
Pollen grain viability (%)		
Orri	0[a]	0[a]
Michal	73 ± 8	42 ± 3
Number of pollen grains germinated in 'Orri' stigma		
Orri	0–10	0–5
Michal	50–200	50–200

[a] An occasional germinating pollen grain was found.

Table 2
The effect of distance from 'Michal' pollenizer on yield, number of fruits per tree and average fruit weight in 'Orri' ("alternately-pollinated" orchard in Dafna).

Year	2004	2005	2006	2007	Average
Mean yield (kg trees^{-1})					
Adjacent row[a]	57 ± 1A	68 ± 6	93 ± 19	30 ± 4	62 ± 8a
Distant row	41 ± 3B	61 ± 6	57 ± 13	29 ± 4	47 ± 5b
Mean yield (Tons/ha)					
Adjacent row	57 ± 1A	51 ± 5	69 ± 14	15 ± 2	48 ± 7a
Distant row	40 ± 3B	46 ± 4	43 ± 10	14 ± 2	36 ± 4b
Fruits per tree					
Adjacent row	441 ± 25	524 ± 74	776 ± 192	198 ± 34	484 ± 77a
Distant row	278 ± 38	444 ± 52	397 ± 106	187 ± 28	327 ± 41b
Average fruit weight (g)					
Adjacent row	132 ± 7	132 ± 9	125 ± 9	153 ± 6	135 ± 5
Distant row	148 ± 12	138 ± 5	146 ± 6	155 ± 2	147 ± 4

Means ± standard errors (SE) are presented. Mean values of a given measure within a column that differ significantly ($P = 0.05$) are followed by different letters.
[a] Adjacent 'Orri' rows are rows number 1, distant 'Orri' rows are rows number 3 (Fig. 1b).
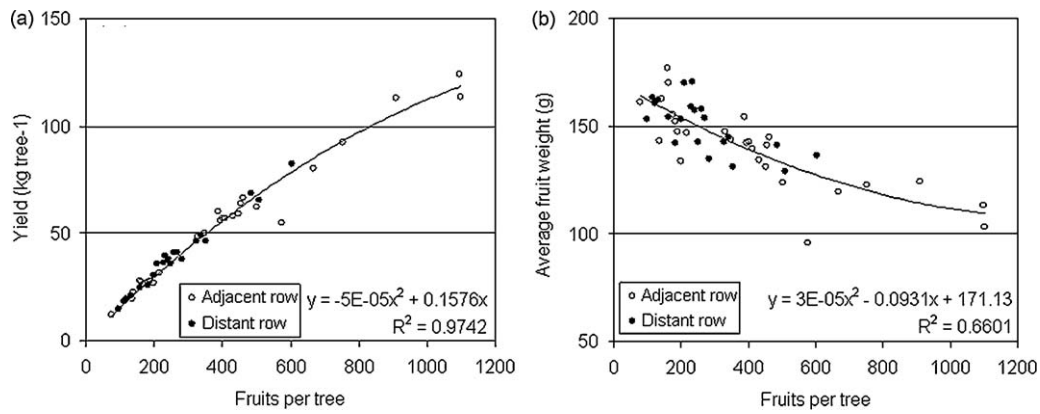
Fig. 3. The relationships between 'Orri' yield (a) or average fruit weight (b) and number of fruits per tree ("alternately-pollinated" orchard in Dafna, 2004–2007). Adjacent 'Orri' rows are rows number 1, distant 'Orri' rows are rows number 3 (Fig. 1b). Each point relates to a single tree in a different year.

Table 3
Average numbers of seeds per fruit, seedless fruit percentages and maximum numbers of seeds per fruit in the three types of 'Orri' orchards.

'Orri' row number and side with respect to 'Michal'	Average seeds per fruit		Seedless fruit (%)		Maximum seeds per fruit	
	2006	2007	2006	2007	2006	2007
(a) "Isolated-block" orchard in Gonen						
	1.4 ± 0.2	1.5 ± 0.2	33%	36%	4	5
(b) "Alternately-pollinated" orchard in Dafna						
Adjacent[a]-F[b]	4.7 ± 0.4a	4.7 ± 0.4a	0%	0%	9	10
Adjacent-N	3.0 ± 0.4b	3.6 ± 0.4b	4%	0%	8	10
Distant-F	3.2 ± 0.3b	3.5 ± 0.3b	7%	4%	7	8
(c) "Pollinated-block" orchard in Kefar-Hanasi						
1-F	–	3.5 ± 0.2a	–	0%	–	8
1-N	–	2.8 ± 0.3b	–	4%	–	8
2-F	–	2.1 ± 0.2c	–	8%	–	5
4-F	–	1.7 ± 0.2cd	–	8%	–	4
6-F	–	1.0 ± 0.2e	–	38%	–	4
8-F	–	1.3 ± 0.2de	–	20%	–	4

Means ± standard errors (SE) are presented. Values within a column and referring to a given orchard, followed by different letters, are significantly different ($P < 0.05$).
  [a] Adjacent 'Orri' rows are rows number 1, distant 'Orri' rows are rows number 3 (Fig. 1b).
  [b] The side of 'Orri' row facing towards (F) or non-facing (N) the 'Michal' block.

every year (data not shown). The mean maximum values for 2004–2007 were 77 kg or 689 fruits per tree and 60 kg or 437 fruits per tree in the adjacent and distant rows, respectively. The distance from the pollenizer was insignificant for the fruit characteristics SSC and TA ($P > 0.3$).

3.3. Effect of 'Michal' pollenizer on 'Orri' seediness

The average seed number per fruit, the seedless fruit percentage and the maximum seeds per fruit found in the 'Orri' "isolated-block", "pollinated-block" and "alternately pollinated" orchards (Table 3). 'Orri' fruits from the middle of the "isolated-block" orchard (2 km from a potential pollenizer) contained about 1.5 seeds per fruit, 33–36% of the fruits were seedless and the maximum number of seeds per fruit was 4–5. In the "alternately-pollinated" orchard the number of seeds per 'Orri' fruit decreased significantly from 4.7 to 3.2–3.5 seeds per fruit in the facing side of row number 1 and row 3, respectively. In the "pollinated-block" orchard the average and maximum numbers of seeds per fruit decreased and the seedless fruit percentage increased as the distance from the 'Michal' block increased ($R^2 = 0.71$ for average seed number) (Table 3 and Fig. 4). In the 'Orri' row adjacent to the 'Michal' block the average number of seeds per fruit was 3.5, none of the fruits were seedless, and the maximum number of seeds per fruit was 8. However, in rows 6 and 8 from 'Michal' the average numbers of seeds per fruit were 1.0 and 1.3 respectively; 38% and

20%, respectively, of the fruits were seedless; and in both rows the maximum number of seeds per fruit was 4 (Table 3).

Moreover, in both the "alternately-pollinated" and "pollinated-block" orchards the average number of seeds per fruit was significantly higher on the side of the 'Orri' facing 'Michal' than the non-facing side (Table 3).
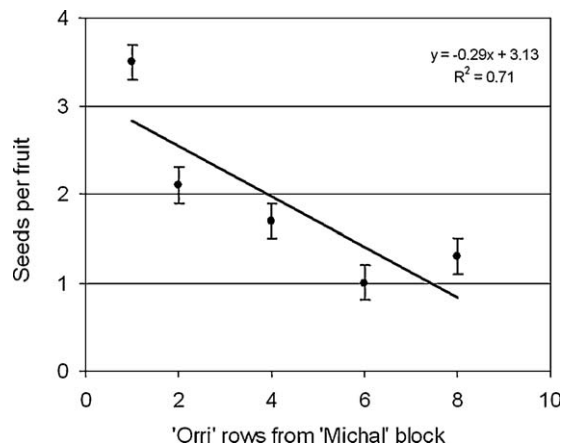
Fig. 4. The relationship between average number of seeds per 'Orri' fruit and row distance from 'Michal' block ("pollinated-block" orchard in Kefar-Hanasi, 2007).

4. Discussion

The results demonstrate that 'Orri' productivity was increased by cross-pollination with 'Michal'. The mean yield of the "alternately-pollinated" orchard (four 'Orri' rows planted alternately with six 'Michal' rows) during four consecutive years, 2004–2007, was 36–48 Tons/ha, whereas 'Orri' orchards in Israel are known to produce only 30 Tons/ha (Holtzman et al., 2005). In the 'Dafna' orchard the average yield and average number of fruits per tree were significantly higher in 'Orri' rows adjacent to 'Michal', i.e., row number 1, than in distant rows, i.e., row number 3. Nevertheless, more than 90% of the fruits from both rows exceeded 60 mm in diameter, which is the most profitable size range (data not shown). Moreover, the positive correlation ($R^2 = 0.97$) found between yield per tree and number of fruits per tree in both rows suggests that the number of fruits per tree and not fruit size is the limiting factor for yield improvement in 'Orri' orchards. The data show that the number of fruits per tree can be improved by optimizing cross-pollination conditions: in 'Orri' rows adjacent to 'Michal' the number of fruits per tree reached 1100, compared with a maximum of 600 fruits per tree in the distant rows.

Our study revealed that in the Israeli "isolated-block" orchard 'Orri' is not a seedless cultivar, although the number of seeds found was usually small. An isozyme analysis of the progeny is needed in order to determine whether these seeds resulted from self-fertilization by the few viable pollen grains in 'Orri' flowers or were a consequence of cross-fertilization with "foreign" pollen transported by insects such as honeybees, over a distance of more than 2 km.

Examination of the seed set in the "alternately-pollinated" and "pollinated-block" orchards showed that 'Michal' is a compatible pollenizer of 'Orri' flowers: the number of seeds per 'Orri' fruit increase and the seedless fruit percentage decreased as the distance from 'Michal' decreased. Krezdorn and Robinson (1958) examined 'Orlando' tangelo seediness and fertility in a "pollinated-block" orchard adjacent to a 'Temple' orange block on one side and a 'Valencia' orange block on the other side. For both pollenizers the numbers of seeds per fruit and of fruits per tree began to decline in the second row from the pollenizer. Brosh and Monselise (1977) reported that 'Topaz' mandarin fruits growing three rows away from the 'Mineola' pollenizer contained fewer seeds than those in the row adjacent to the pollenizer. Wallace et al. (2002) found that 'Ellendale' mandarin fertilized all 'Imperial' mandarin ovules at a distance of 15 rows, i.e., >50 m, although the number of successful crosses, as indicated by number of seeds per fruit, decreased significantly beyond three rows, i.e., ~15 m. They explained that the number of viable 'Ellendale' pollen grains deposited on each stigma decreased with increasing distance from 'Ellendale'. Our present study revealed a significant difference in seed numbers per fruit even between the two sides of 'Orri' row adjacent to the 'Michal' block, i.e., the facing and non-facing sides.

The present study demonstrated that cross-pollination of 'Orri' resulted in yield improvement. On the other hand, higher seed set, which downgraded the fruit, is the price of it. 'Orri' is an excellent quality mandarin and its fruits are highly demanded worldwide, with no conditions stipulated up to now, regarding seed set. Thus, cross-pollination could be used as a method to increase 'Orri' orchard yields.

Acknowledgements

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