



Article Spatial Proximity of 'Ataulfo' to 'Haden' Cultivar Increases Mango Yield and Decreases Incidence of Nubbins

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Abstract: Mexico is the leading exporter of mangos worldwide, and 'Ataulfo' is one of the most popular cultivars. However, their production has dramatically dropped in recent years due to the high incidence of nubbins. One of the possible causes is the presence of a delayed self-incompatibility found in this cultivar; thus, proximity to compatible cultivars may help to reduce this incidence. Nevertheless, there is a lack of studies that have rigorously tested this hypothesis in this cultivar. For two consecutive years, the present study evaluated the incidence of nubbins, as well as the quality and quantity of commercial fruits of 'Ataulfo' trees located at 10, 30, and 50 m away from 'Haden' cultivar. Additionally, the yield and economic income of different planting designs were estimated. During both sampling periods, our results clearly indicated that at 10 m away from 'Haden' individuals, 'Ataulfo' trees presented a lower incidence of nubbins and higher production of commercial fruits, and higher yield and total income per hectare than at 30 or 50 m away from them. These results indicate that planting designs of 'Ataulfo' trees located 10 m away from 'Haden' will help to satisfy the increasing demand for mangos of this cultivar in the international market.

Keywords: crop breeding; mango niño; parthenocarpy; plantation design; pollen donor; self-incomp-atibility; stenospermocarpy

1. Introduction

Mango is one of the most cultivated and commercialized tropical fruits worldwide [1,2], and Mexico is the main exporter [3]. Among the different mango cultivars cultivated in Mexico, 'Ataulfo' shows greater acceptance at the international market and supports the highest volume and the highest commercial value at a national level [4]. However, crop yield and productivity of this cultivar have been significantly diminished in several localities [5–7], mainly due to the high incidence of parthenocarpic or stenospermocarpic fruits of smaller size and weight that have little or no commercial value, known as nubbins or in Spanish as "mango niño" [8,9].

One of the leading proposed causes of nubbins in 'Ataulfo' is the presence of a late selfincompatibility mechanism [10], also found in other mango cultivars [1,11–13]. Given that mango cultivars are propagated by grafting [14,15], self-incompatible pollen deposition may increase in orchards cultivated with only one cultivar [16]. One of the strategies used by some mango producers is planting trees of a compatible cultivar ("pollen donor",



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Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). from now on) with the cultivar of commercial interest on the same orchard to minimize the harmful effects of inbreeding depression since it has been observed that this practice increases the production of fruits in other crops such as pears [17], apples [18,19], and also in other mango cultivars [20,21].

Under this scenario, pollen donor's spatial arrangement within the orchard is crucial in order to increase the transfer of compatible pollen [17,22], fruit set, and fruit quality [23–27]. Quinet and Jacquemart [17] reported that pollination efficiency in pear cultivars increased when the distance with another cultivar decreased. Buccheri and Di Viao [18] found in apple orchards, more seeds per fruit, a higher percentage of commercial fruits, and a lower percentage of deformed fruits in trees located at a shorter distance from pollen donors. Likewise, the quantity of developing and developed fruits in mango trees from the cultivar 'Tommy Atkins' was greater when the distance to pollen donor diminished [20,21].

Perez-Barraza et al. [6], without any statistical inference, found a higher incidence of nubbins in 'Ataulfo' orchards located more than 200 m away from 'Tommy Atkins' orchards compared to orchards located next to this cultivar. Even when the proximity to compatible cultivars is expected to reduce the incidence of nubbins in 'Ataulfo' cultivar, there are no studies that evaluate the effect of the distance among 'Ataulfo' trees and pollen donors on the incidence of nubbins and on the quality and yield of commercial fruits rigorously. Considering that pollen transfer occurs mainly among neighboring trees [28,29] due to the behavior of pollinating insects [17,30-32], it is expected that the incidence of nubbins will be lower whereas the production and quality of commercial fruits will be higher in 'Ataulfo' trees located near to pollen donor. Moreover, given that the selected pollen donor in this study (i.e., 'Haden' cultivar) is among the most profitable cultivars in Mexico [33], it is expected that orchards with an arrangement of proximity among 'Ataulfo' and 'Haden' trees will present higher yield and economic income than orchards with greater separation among cultivars. This study aimed to compare the incidence of nubbins and the quality and quantity of commercial fruits of 'Ataulfo' in trees located at different distances from 'Haden' as pollen donor, as well as to compare the yield and economic income considering different distance scenarios of 'Ataulfo' from 'Haden'.

2. Materials and Methods

2.1. Study Site

The experiment was carried out in two sampling periods: December 2018–March 2019 and December 2019–March 2020, in several orchards of mango 'Ataulfo', located in the municipalities of Atoyac de Álvarez (10 orchards), Tecpan de Galeana (9 orchards), and Benito Juárez (2 orchards), Guerrero, Mexico (Figure 1, Table S1). These municipalities are located in one of the regions (i.e., Costa Grande) with the highest mango production in Mexico [33]. In this region, the dominant native vegetation type is a tropical dry forest with a warm sub-humid climate (Aw), average annual precipitation of 1100 mm with a rainy period from June to November (total precipitation \approx 950 mm), and a dry period from December to May (total precipitation <70 mm). The average annual temperature is 26.6 °C, with a maximum of 32 °C in April-May and a minimum of 18 °C in December-January [34].

Orchard selection criteria included similar management (e.g., use of pesticides and synthetic fertilizers, trees around 14 years old), surface of at least one-hectare, and no beehives introduced for pollination. Different orchards were selected in each sampling period to cover greater spatial variation. Mango trees on the orchards were distributed under a square planting system at a distance of 10 m between rows and trees.

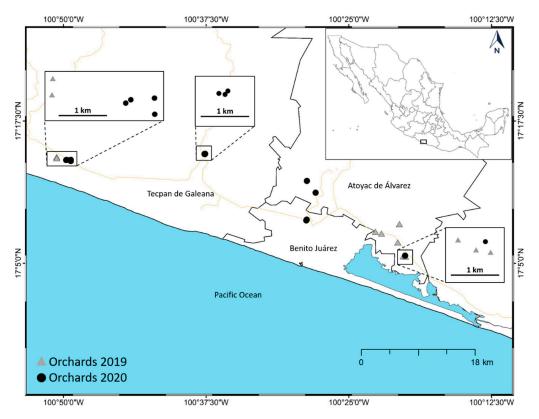


Figure 1. Location of orchards in Costa Grande Guerrero, Mexico. Orchards sampled in 2019 (gray triangles) and 2020 (black circles) are shown.

2.2. Study Species

Mango (*Mangifera indica* L.) is an andromonoecious tree (i.e., male and hermaphrodite flowers on the same plant) with panicle-shaped inflorescences, whose flowers depend on animals to set fruit [35]. The aperture of the flowers is diurnal, and its main floral visitors are flies, bees, ants, and beetles [36].

Trees from the cultivar 'Ataulfo' were used as the focal cultivar on which the response variables were estimated, whereas trees from cultivar 'Haden' were selected as pollen donor, as it blooms at the same time as 'Ataulfo' and since our data show that manual crosses among these cultivars produce commercial fruits [37].

2.3. Incidence of Nubbins and Production of Fruits

Three distances, 10, 30, and 50 m among 'Ataulfo' and 'Haden' trees were selected to evaluate the effect of spatial proximity from pollen donors on the incidence of nubbins and the production of commercial fruits. On each orchard, two 'Ataulfo' trees were randomly selected from each distance. In both sampling periods for each tree, twenty panicles were selected with similar characteristics (e.g., located at the same height from the ground, similar size and development stage) and were monitored until fruits reached physiological maturity (~60 days). At this stage, the number of nubbins, the number of commercial fruits, and fruit set per panicle were registered. The incidence of nubbins was estimated as the number of nubbins divided by the total fruits per panicle. In the sampling period of 2020, the incidence of nubbins, the total number of nubbins, and commercial fruits present in all the inflorescences of each tree were estimated in the same individuals.

2.4. Quality of Commercial Fruits

Five fruits at physiological maturity from each tree at each distance were selected randomly to compare the quality of commercial fruits in both sampling periods. Three traits were estimated on each mature fruit: fresh weight with a digital scale (Ohaus Corporation,

Model Scout Pro SP401, Parsippany, NJ, USA, precision of 0.1 g), and polar and equatorial diameter with a digital caliper (Mitutoyo Corp., Model CD-8"ASXL, Kanagawa, Japan, precision of 0.01 mm).

2.5. Yield and Economic Income

Yield and economic income per hectare were calculated, considering the tree yield per distance calculated above. For this, the average weight per fruit for each distance was multiplied by the number of fruits per tree [38] and then multiplied by the density of 'Ataulfo' trees per hectare considering each scenario: 84 trees for 10 m, 96 trees for 30 m, and 99 trees for 50 m.

The economic income per hectare was estimated by multiplying the yield (kg/ha) by the market price of 'Ataulfo' and adding the economic income of 'Haden' trees considering the yield (i.e., 87 kg/tree) reported by Avilan et al. [39] and the density of 'Haden' trees per hectare in each scenario: sixteen trees for 10 m, four trees for 30 m, and one tree for 50 m). The market price used in these analyses for each cultivar was estimated as the average value per kilogram reported for March 2020 at a national level (i.e., 'Ataulfo': \$0.93 and 'Haden': \$0.74 US dollars) [40].

2.6. Orchards of 'Ataulfo' without 'Haden'

Three 'Ataulfo' orchards without any 'Haden' tree were selected to estimate the incidence of nubbins, fruit production, quality of commercial fruits, and yield and economic income per hectare as above. Given the small sample size of these data, we used them only as a reference value without any statistical purpose.

2.7. Statistical Analyses

Generalized linear mixed models (GLMM [41]) were used to compare the influence of the distance from 'Haden' cultivar on 'Ataulfo' mango production, the incidence of nubbins, and the quality of commercial fruits. A Poisson error distribution and logarithmic link function (due to counts data) were used to the number of the commercial and total number of fruits per panicle. Conversely, a negative binomial distribution (due to overdispersion) was used for the number of nubbins per panicle and the number of nubbins, commercial, and total fruits per tree. Finally, binomial distribution and a logit link function (due to proportional data) were used to the incidence of nubbins, while a gamma distribution and an identity link function were used for polar diameter, equatorial diameter, and the weight of commercial fruits.

A GLMM with a gamma distribution and an identity link function (due to continuous data) were used to compare the yield (per tree and hectare) and economic income in each plantation scenario. The distance (i.e., 10, 30, and 50 m) and the orchard were used, respectively, as fixed and random factors. Analyses were carried out separately for the 2019 and 2020 sampling periods.

For each analysis, the chi-square values were obtained from comparisons between the model that included the explanatory variable (complete model) and a null model (that is, with the explanatory variable, discarded). The lowest value of the Akaike Information Criterion (AIC) was used to classify the most parsimonious model, and Tukey's test was used for post hoc comparisons. All analyses were performed with R software [42], using GLMM's with the glmer function in the "lme4" package; whereas multiple comparisons of means were calculated with the "emmeans" package and the ggemmeans function from the "ggeffects" package to obtain means and standard error of the models [43].

3. Results

3.1. Incidence of Nubbins and Production of Fruits

In both sampling periods, all response variables, with exception of total fruit production per tree, were influenced by the distance of 'Ataulfo' from 'Haden' individuals (Figure 2, Figure 3). Specifically, the number of commercial fruits per panicle (2019: $\chi^2 = 199.85$,

p < 0.0001; 2020: $\chi^2 = 121.87$, p < 0.0001; Figure 2A) and per tree ($\chi^2 = 11.62$, p = 0.002; Figure 3), and the number of total fruits per panicle (2019: $\chi^2 = 23.26$, p = 0.01; 2020: $\chi^2 = 15.54$, p = 0.0004; Figure 2B) on 'Ataulfo' trees decreased as the distance to individuals of 'Haden' trees increased. Trees at 10 m away from 'Haden' in 2019 and 2020 produce, respectively, 126% and 108% more commercial fruits per panicle than orchards without 'Haden' (1.04 ± 0.001 fruits; Figure 2A) and, in 2019, 20% more total fruits per panicle (2.40 ± 0.002 fruits; Figure 2B).

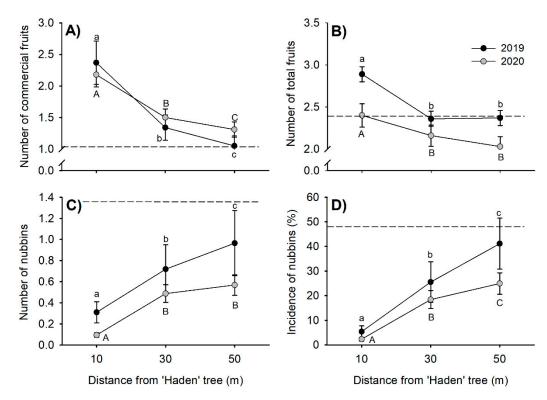


Figure 2. Mango production per panicle of 'Ataulfo' trees (mean \pm standard error) located at different distances from trees of 'Haden' in 21 orchards (9 in 2019, *n* = 360 panicles per distance; 12 in 2020, *n* = 420) from the Costa Grande, Guerrero, Mexico. (**A**) Number of commercial fruits, (**B**) Number of total fruits, (**C**) Number of nubbins; and (**D**) Incidence of nubbins. Dotted lines indicate the average values of 'Ataulfo' trees in orchards without 'Haden' individuals. Different letters indicate statistical differences among distances for each sampling period according to GLMM and Tukey test (*p* < 0.05).

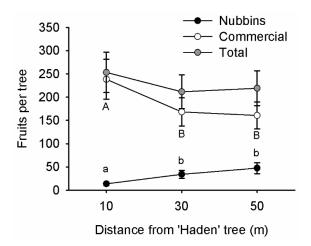


Figure 3. Mango production per tree (mean \pm SE; n = 24 trees per distance) of 'Ataulfo' trees located at different distances from 'Haden' trees in 12 orchards of the Costa Grande, Guerrero, Mexico in 2020. Different letters indicate statistical differences among distances according to GLMM and Tukey test (p < 0.05).

On the other hand, the number of nubbins per panicle (2019: $\chi^2 = 127.28$, p < 0.0001; 2020: $\chi^2 = 42.46$, p < 0.0001; Figure 2C) and per tree ($\chi^2 = 36.54$, p < 0.0001; Figure 3), as well as the incidence of nubbins per panicle (2019: $\chi^2 = 130.54$, p < 0.0001; 2020: $\chi^2 = 137.74$, p < 0.0001; Figure 2D), increased as the distance from 'Ataulfo' trees to 'Haden' trees increased. Trees at 10 m away from 'Haden' in 2019 and 2020 produce, respectively, 77% and 93% less number of nubbins per panicle than orchards without 'Haden' (1.36 ± 0.002 fruits; Figure 2C) and 43% and 46% lower incidence of nubbins in 2019 and 2020, respectively ($48.18\% \pm 0.0006$; Figure 2D).

3.2. Quality of Commercial Fruits

For both sampling periods, no influence of the distance of 'Ataulfo' to 'Haden' trees was found in most of the quality traits of commercial fruits considered in this study. During 2019, only the fresh weight of fruits was greater in 'Ataulfo' trees near from 'Haden' individuals than from trees further away ($\chi^2 = 14.15$, p = 0.0008; Figure 4C). In addition, the weight of fruits in trees 10 m away from 'Haden' increased 27% in 2019 compared with trees in orchards without 'Haden' (245.1 ± 2.50 g; Figure 4C). Conversely, during 2020, no differences were found in polar diameter (2019: $\chi^2 = 1.08$, p = 0.58; 2020: $\chi^2 = 0.35$, p = 0.83; Figure 4A), nor in the equatorial diameter (2019: $\chi^2 = 0.96$, p = 0.58; 2020: $\chi^2 = 1.19$, p = 0.54; Figure 4B) or in the fresh weight of fruits ($\chi^2 = 1.67$, p = 0.43; Figure 4C).

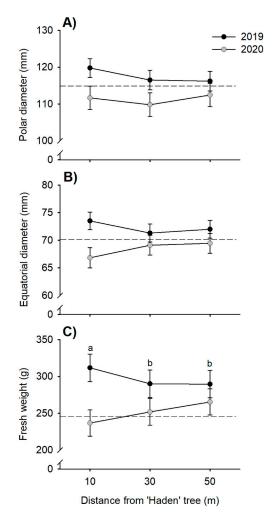


Figure 4. Quality of commercial fruits (mean \pm SE) produced in trees of 'Ataulfo' located at different distances from trees of 'Haden' in 14 orchards (8 in 2019, *n* = 80 fruits per distance; 6 in 2020; *n* = 60) of the Costa Grande, Guerrero, Mexico. (A) Polar diameter, (B) Equatorial diameter, and (C) Fresh fruit weight. Dotted lines indicate the average values of 'Ataulfo' trees in orchards without 'Haden' individuals. Different letters indicate statistical differences according to GLMM and Tukey test (*p* < 0.05).

3.3. Yield and Economic Income

Fruit yield per tree was significantly greater in 'Ataulfo' trees located at 10 m (69.6 ± 16.14 kg) than those at 50 m (50.3 ± 15.13 kg) away from 'Haden' trees (χ^2 = 7.22, *p* = 0.027; Figure 5A). Trees at 10, 30, and 50 m away from 'Haden' produce, respectively, 42%, 4%, and 3% more yield per tree than orchards without 'Haden' (48.8 ± 0.55 kg). Similarly, the plantation design significantly influenced the yield per hectare (χ^2 = 18.83, *p* < 0.0001; Figure 5B), being higher in orchards with 'Ataulfo' trees located at 10 m away from 'Haden' (8.0 ± 0.19 t/ha) than in orchards with trees at 30 m (5.6 ± 0.23 t/ha) and 50 m (5.3 ± 0.24 t/ha) away from 'Haden'. Moreover, plantation designs at 10 m away from 'Haden' showed 64% more yield than orchards without 'Haden' (4.8 ± 0.05 t/ha).

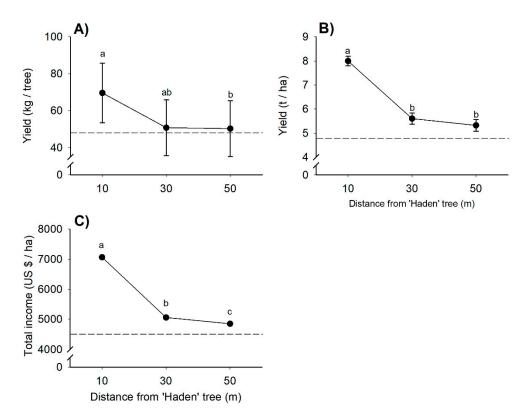


Figure 5. Estimated yield and economic income (mean \pm SE) of trees and orchards of 'Ataulfo' in three plantation designs considering different distances from 'Haden' trees. (**A**) 'Ataulfo' yield per tree, (**B**) Total yield per hectare, and (**C**) Total economic income per hectare. In B and C, yield and economic income, respectively, of both 'Ataulfo' and 'Haden' were considered. Dotted lines indicate the average values of 'Ataulfo' orchards without 'Haden' individuals. Different letters indicate statistical differences among plantation scenarios, according to GLMM and Tukey's test (*p* < 0.05).

Despite no differences were found in economic income per hectare among different plantation scenarios considering only 'Ataulfo' production ($\chi^2 = 3.68$, p = 0.15), there were significant differences when the economic income per hectare of both 'Ataulfo' and 'Haden' trees was considered ($\chi^2 = 15.54$, p = 0.0004; Figure 5C), being higher in orchards with 'Ataulfo' trees located at 10 m from 'Haden' individuals (\$7063 ± 11.93 US dollars), than at 30 m (\$5059 ± 16.17 US dollars) or 50 m (\$4852 ± 15.48 US dollars; Figure 5C). Furthermore, there was 56% more economic income in plantation designs with 'Ataulfo' trees located at 10 m from 'Haden' to orchards with 'Ataulfo' trees located at 10 m from 'Baden' individuals compared to orchards with 'Ataulfo' trees located at 10 m from 'Haden' individuals compared to orchards without 'Haden' (\$4516 ± 51.53 US dollars).

4. Discussion

The enormous incidence of nubbins found in several orchards of 'Ataulfo' in Mexico [5,6,44,45] is one of the main causes of lower yields and economic losses for several mango producers in recent years. Gehrke-Vélez et al. [10], found that 'Ataulfo' flowers pollinated with 'Ataulfo' pollen increase the production of nubbins, suggesting selfincompatibility in this cultivar. Thus, pollination with another cultivar compatible with 'Ataulfo' should reduce the incidence of nubbins. Accordingly, our results clearly showed for two consecutive sampling periods that at a shorter distance from 'Haden' individuals, 'Ataulfo' trees presented a lower incidence of nubbins, possibly due to the limitation of high-quality pollen (i.e., compatible pollen).

The appearance of nubbins in several mango cultivars has been associated with low temperatures (<10–15 °C), during mango flowering [5,46,47] or pollination [48,49]. However, the minimum temperature recorded in recent years in the study area has not decreased below 18 °C [34,50], so other factors rather than temperature should influence the high incidence of nubbins in this tropical region. In addition, if the temperature had influenced the incidence of nubbins, it would not be expected differences to be found in nubbins production at different distances from another cultivar. Therefore, our findings support the hypothesis that the incidence of nubbins must be mainly due to the presence of a delayed self-incompatibility mechanism in 'Ataulfo'.

On the other hand, since pollinators tend to forage among nearby plants [17,31,32], the production of developed fruits should increase with the proximity of the compatible cultivar. Accordingly, we demonstrated that the production of commercial fruits of 'Ataulfo' for the two evaluated periods increased with the proximity of cultivar 'Haden', which seems to serve as a compatible pollen donor. Similarly, some studies have also found that trees near pollen donors produce higher fruit set, including apple [19], chestnut [25], and olive [26].

Contrary to our expectations, the quality of commercial fruits (here evaluated as size and weight) did not increase in 'Ataulfo' trees located near pollen donor (with exception of fruit weight in 2019 sampling period). Some authors suggest that pollen's source directly affects the shape, size, flavor, color, maturity, and/or composition of fruits [51]. For example, in avocado, cross-pollination with other cultivars increased the size of the fruits compared to self-pollination [52]. In mango 'Tommy Atkins', the seed's weight was higher as a result of cross-pollination with another cultivar [20]. On the other hand, it has been documented that a greater load of fruits may cause a higher cost for the tree, causing a decrease in the quality of fruits [53]. Probably, the greater quality of pollen grains of other cultivar deposited on the stigma of flowers of 'Ataulfo' compensates the cost of the greater load of fruits. Therefore, our results suggest that pollen grains of 'Haden' were responsible for the maintenance or increase of weight of fruits and the increase of the number of commercial fruits of 'Ataulfo'. However, these benefits were reduced as the distance to pollen donors increased, due to a reduction in the transfer of compatible pollen by pollinators.

Traditionally, 'Ataulfo' mango orchards consist of monoculture plantations, derived from clones propagated by grafting on native cultivar patterns [14,15]. Here, we found that planting designs including a suitable pollen donor (e.g., 'Haden') increased the yield and economic input of these orchards. Specifically, the plantation designs that include 'Haden' at 10 m distance from the 'Ataulfo' trees showed the best results by increasing their yield considerably and the total economic income compared to plantation designs at 30 and 50 m away from 'Haden' and to orchards planted exclusively with 'Ataulfo' trees. This study presents a strategy to increase yields and maximize economic income for producers. Therefore, the inclusion of 'Haden' within the orchards in 'Ataulfo' planting designs should become a common practice among mango producers. These results coincide with other studies on gene flow, showing that donor pollen dispersed mostly within the first 10 to 15 m [17,28,29]. Thus, the density of pollen donors and location within the orchard are very important to improve efficiency in cross-pollination [17,54], especially in crops that depend on pollinators to set fruit.

It is important to note that the transfer of compatible pollen not only depends on the distance of pollen donor but also on the visitation rate of pollinators [55–57]. If pollen

donors were more attractive to pollinators than the cultivar of interest, higher yields might be due to both quantity (i.e., number of visits) and quality (i.e., transfer of compatible pollen) of visits. Further studies considering the composition and visitation rate of pollinators in trees located at different distances from pollen donors are necessary to better understand the causes of yield production in mango orchards and propose better management practices and plantation designs.

5. Conclusions

This study provides clear evidence that the proximity of a compatible cultivar decreased the incidence of malformed fruits and increased commercial 'Ataulfo' fruit production, without reducing their quality. Furthermore, with this information, we propose that with the occurrence of 'Haden' mango trees within 10 m distance from 'Ataulfo' trees, mango producers could find the best spatial arrangement to increase fruit yield and total economic income per hectare in their orchards. Many other cultivars that show malformed fruits or a high incidence of fruit abortion due to the harmful effects of inbreeding depression could be diminished through the plantation with compatible cultivar at specific distances.

Supplementary Materials: The following are available online at https://www.mdpi.com/2073-439 5/11/3/450/s1, Table S1: Geographical coordinates and characteristics of the selected orchards in the study area.

Author Contributions: Conceptualization, R.L.-G., V.R.-G. and L.A.-F.; methodology, R.L.-G., V.R.-G. and L.A.-F.; formal analysis R.L.-G. and V.R.-G.; investigation, R.L.-G. and V.R.-G.; resources, R.L.-G., V.R.-G. and L.A.-F.; writing—original draft preparation, R.L.-G. and V.R.-G.; writing—review and editing, all authors; visualization, R.L.-G. and V.R.-G.; supervision, V.R.-G., L.A.-F., R.C.A.-N., J.V.-G. and J.G.K.-D. All authors have read and agreed to the published version of the manuscript.

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