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Relationship between petiole distribution and flower type in Maradol-type papaya populations

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Abstract

Determining the sex of papaya plants is complex, as flowers can be male, female, or hermaphroditic, which affects fruit production and quality. Identifying the sex of seedlings before flowering is key to optimizing resource use in the field. However, sex determination using molecular methods such as PCR (Polymerase Chain Reaction), while accurate, is costly and requires specialized infrastructure. Furthermore, the use of traditional methods based on morphology is confusing. On the other hand, the direction of petiole twist associated with flowering has not been studied. In some plants, the petiole twists clockwise or counterclockwise, which could be related to the plant's sex. The aim of this study was to determine the relationship between the direction of petiole twist and plant sex in selections of 'Maradol' papaya genotypes. Plants were observed during the flowering/fruiting stage, and the direction of petiole twist and flower type were recorded. Inferential statistics were used with the four possible combinations of petiole twist and plant sex: right (R)/female(F); right(R)/hermaphrodite(H); left(L)/female(F); left(L)/hermaphrodite (H). The analysis showed that leftward twisting was most strongly associated with hermaphroditic plants. Specifically, this twisting pattern has a 72 % probability of occurring in hermaphroditic flowers, whereas rightward twisting is more likely to be found in female flowers. This finding makes it possible to predict the flower type before blooming based on the twisting of the petiole, thereby facilitating the identification of hermaphroditic plants.

Keywords: *Carica papaya*, floral biology, female flower, hermaphroditic flower.

Introduction

As a fast-growing fruit species, *Carica papaya* L., is widespread across the world's vast tropical and subtropical regions (Antunes & Renner, 2012) and stands out among fruit trees for its remarkable productive potential. In 2023, Mexico allocated 20,334 ha to papaya cultivation, ranking fourth place among 73 producing countries worldwide (FAOSTAT, 2025). With a population exceeding 100 million Mexicans (National Institute of Statistics and Geography [INEGI, by its Spanish acronym], 2020), estimated *per cápita* consumption was 7.3 kg (Agri-food and Fisheries Information Service [SIAP, by its Spanish acronym], 2024). According to government statistics, in 2024, out of 32 states, 19 produced 1,148,546 tons of papaya, valued at $380.25 million U.S. dollars (USD), of which 34 % was exported (SIAP, 2025).

Given the importance of this crop, there are key management factors that directly influence yield, including, in addition to phytosanitary management (Alarcón et al., 2022), soil preparation, fruit-oriented nutrition (Vos & Arancon, 2019), and irrigation management (Ferreira et al., 2022). Additionally, a distinctive feature of this species is the complexity of its floral biology, where climatic variations destabilize its physiological development (Okereke et al., 2022). Conse-

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quently, the sexual forms of this species are altered (Dos Santos et al., 2022). In particular, *Carica papaya* L. generally produces three types of flowers: male, female, and hermaphroditic; and within the hermaphroditic flowers, four variants have been identified (Ramos et al., 2011). Of these, among plants that produce hermaphroditic flowers, the elongata variant is the most desirable from a commercial standpoint, as it produces elongated fruits (Ávila-Hernández et al., 2023).

However, in compact papaya genotypes, sex determination is only possible about two months after transplanting. Before this time, the plants' morphological characteristics are largely identical, and it is not until the plants produce flower buds that they can be distinguished by sex (Barrantes-Santamaría et al., 2019).

In a plant population, the typical sex ratio is 1 % male-flowering plants, 33 % female-flowering plants, and 66 % hermaphroditic plants (Barrantes-Santamaría & Sánchez-Barrantes, 2022). This ratio occurs when pollination is strictly controlled (Chen et al., 2019). Given this precedent, to ensure an adequate proportion of hermaphroditic plants under field conditions, up to four plants are commonly planted per planting site, so that plants with undesirable flower types can be subsequently removed. This leads to additional costs for labor, inputs, and time (Foram et al., 2019), which ultimately becomes a problem in the papaya production system.

In response to this challenge, various efforts have been explored, but the results are limited because they do not clearly distinguish hermaphroditic plants, although they do distinguish female and male plants, such as cytological studies (Datta, 1971), colorimetry (Jindal & Singh, 1976), trans-cinnamic acid chromatography, and isoenzyme analysis (Sriprasertsak et al., 1988).

Recently, DNA analysis using PCR (Aryal & Ming, 2014; Prakash et al., 2018), offered a solution to the problem of plant sex determination, as it is a highly effective method; however, like previous studies, it requires individual tissue analysis for each seedling. Thus, once processed, the results are obtained, and each seedling is identified to be selected or discarded, which becomes impractical when analyzing large populations. An example of this is the number of plants required to establish a one-hectare area, which amounts to around 2,000 seedlings. Given that the sex ratio is estimated at 66 % hermaphroditic plants, this means evaluating 3,000 seedlings to achieve the appropriate density, since female plants will be discarded. Despite its accuracy, the complexity of the procedure motivates the exploration of alternatives.

To date, no practical and accessible method has been developed to determine the sex of plants at early stages, which poses a significant challenge in papaya production. In the association of floral characteristics and certain morphological traits (Odu et al., 2006), the possible relationship between the helical arrangement of petioles–following a clockwise or counterclockwise orientation depending on the relative position of the nearest petiole on the stem–suggests an alternative of practical agricultural interest. Based on the above, the aim of this study was to determine the relationship between the direction of petiole orientation and plant sex in selections of 'Maradol' papaya genotypes.

Materials and methods

Healthy and vigorous populations were observed in the papaya-growing region of the valley of Apatzingán, Michoacán, Mexico, through field surveys of commercial plots of the 'Maradol' papaya variety, ranging in age from 2 to 14 months, whose phenological stage indicated floral differentiation at the time of data collection, across sets serial lines. In the study area, the predominant climate according to García (2004), is classified as Bs_1 = semi-arid steppe, the driest of the warm semi-arid climates, with species representative of the lowland deciduous forest (García & Linares, 2012), and, generally, the soils characteristic of the area are of the vertisol pelic type (INEGI, 2016). During field surveys, the climatic variation behaved as shown in Table 1.

Papaya populations (plots) were concentrated in four municipalities, and in each stand (of the 13 selected) five rows were randomly sampled, with a sample size of 100 plants. A total of 1,300 plants were observed. The order and identification of the populations were as follows: one in Apatzingán, five in Mújica, four in Parácuaro, and three in Tepalcatepec, Michoacán (Table 2).

Based on the matrix developed for the 13 populations, visual observations were conducted to identify and record the orientation of the petiole on the stem. The observation involved recording the direction of the petiole, starting from the base of the stem toward the apex; the sex of the flower was also recorded. The results were recorded based on the classification of the four possible combinations of petiole orientation and plant sex (Table 3).

Each population was sampled using a completely randomized experimental block design with four treatments (petiole orientation/flower sex). Subsequently, to ensure compliance with the assumptions, the data were processed and analyzed as percentages; they were subjected to tests for normality and homogeneity of variances. Additionally, an analysis of variance (ANOVA) was performed, following transformation using the arcsine fusion of the square root of the original value, and means were compared using Tukey's test (P = 0.05). The statistical software SAS 9.4 version (SAS, 2019) was used.

Results and discussion

Figure 1 shows an overview of the distribution of petiole orientation/flower sex across all 13 populations. The

Table 1. Climatic variation during the experiment, fall-winter cycle.

Climate Variables	Average values (September-February)
Maximum temperature (°C)	34.01
Minimum temperature (°C)	17.63
Average temperature (°C)	25.82
Precipitation (mm)	36.46
Evaporation (mm)	5.09

Source: Department of Hydrometry. CONAGUA Irrigation District 097, Mexico.

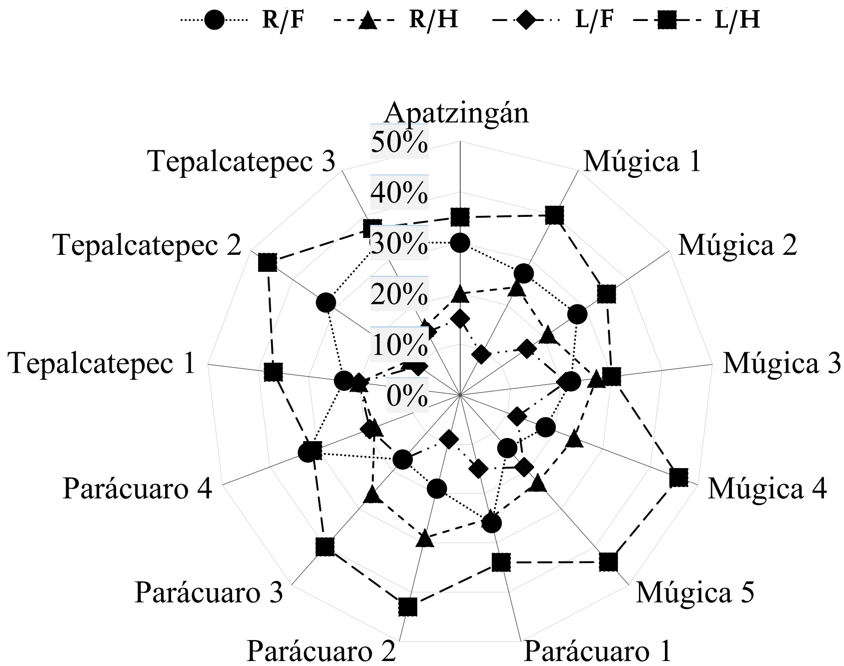
Table 2. Characterization of the surveyed commercial papaya orchards.

Identifier	Municipality	Locality	Coordinates North/West	Area (ha)	Altitude (m)
Apatzingán	Apatzingán	Puerta de Alambre	19°03´33´´/ 102°24´51´´	6	314
Mújica 1	Mújica	Nueva Italia	18°55´32´´/ 102°09´48´´	8	421
Mújica 2	Mújica	Nueva Italia “Aguacate”	18°58´53´´/ 102°10´04´´	8	378
Mújica 3	Mújica	Ceñidor “Canal”	18°58´44´´/ 102°10´52´´	8	359
Mújica 4	Mújica	Ceñidor “Carretera”	18°59´34´´/ 102°11´35´´	10	327
Mújica 5	Mújica	Ceñidor “Carretera”	18°57´27´´/ 102°09´33´´	6	364
Parácuaro 1	Parácuaro	Ciudad Morelos	19°00´15´´/ 102°17´20´´	6	328
Parácuaro 2	Parácuaro	Antúnez	18°57´40´´/ 102°13´14´´	8	324
Parácuaro 3	Parácuaro	Antúnez “La Perla”	18°56´09´´/ 102°13´35´´	4	319
Parácuaro 4	Parácuaro	Las Yeguas	19°00´42´´/ 102°14´52´´	9	312
Tepalcatepec 1	Tepalcatepec	Cholula	19°07´54´´/ 102°50´34´´	7	370
Tepalcatepec 2	Tepalcatepec	Atascadero	19°08´13´´/ 102°51´58´´	10	342
Tepalcatepec 3	Tepalcatepec	Calderitas	19°10´22´´/ 102°50´25´´	9	376

Table 3. Basic elements of the matrix left/right petiole orientation, female/hermaphrodite flower.

Petiole orientation	Flower sex	Result
Petiole orientation to the right (R)	Female (F)	R/F
	Hermaphrodite (H)	R/H
Petiole orientation to the left (L)	Female (F)	L/F
	Hermaphrodite (H)	L/H

Figure 1. Performance of the petiole orientation/flower sex combination in 'Maradol' papaya populations.



R=Right, L=Left, F=Female, H=Hermaphrodite.

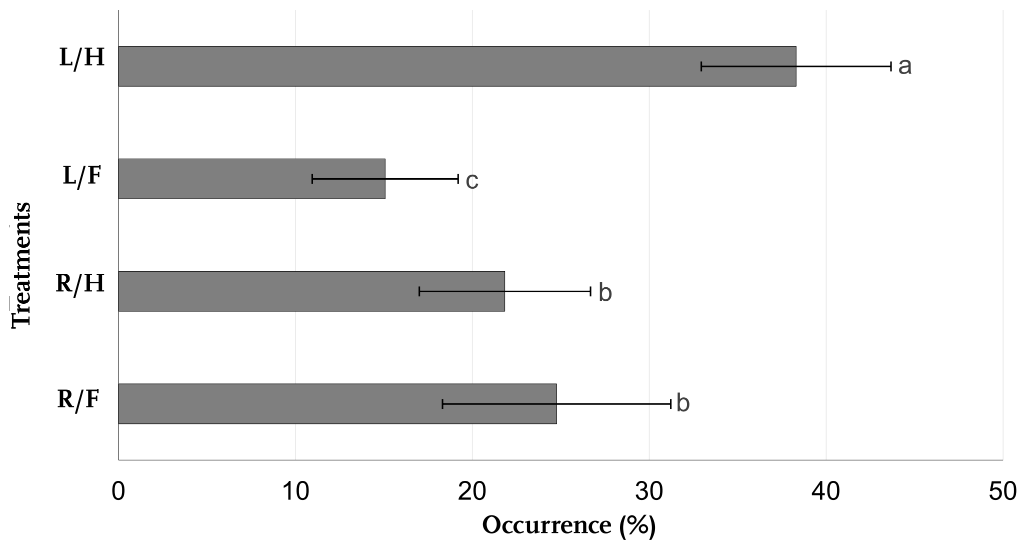
observed patterns in each population, particularly for the petiole orientation/flower sex L/H combination, ranged from 31 % to 46 % in frequency, making it the most common combination and the one that stood out across all populations. In contrast, the petiole orientation/flower sex L/F showed the lowest percentages in most populations, ranging from 9 % to 21 % occurrence. The other combinations, petiole orientation/flower sex, both R/F and R/H, showed intermediate occurrence in most populations.

During its various stages of development, the papaya faces conditions that can negatively affect its productivity and fruit quality due to its sensitivity to environmental factors. Among the main challenges is its sexual instability, typical of an open-pollinated species, which complicates the selection of suitable plants for commercial cultivation. In this context, it is essential to prioritize the selection of hermaphroditic plants, since, in addition to being fertile, they produce fruit with characteristics that are more highly valued by consumers. The 'Maradol' genotype, one of the most widely used for its fruit's good size, color, and texture, often exhibits problems with sterility and fruit deformities, especially during seasons with extreme temperatures (Santamaría et al., 2016). These problems are related to instability in the sexual expression of flowers, influenced by both genetic and environmental factors (Ramos et al., 2011). This helps explain the patterns observed in petiole orientation/flower sex combinations, where variable sexual expression in hermaphroditic plants appears to be determined by these interactions.

Furthermore, the analysis of the conducted variance–based on the petiole orientation/flower sex combination factor and its four possible combinations (treatments)–found that significant differences exist. The petiole orientation/flower sex combination L/H was superior, as it had the highest occurrence rate (38.31 %), followed by the petiole orientation/flower sex combinations R/F and R/H, with both treatments being equal. Meanwhile, the petiole orientation/flower sex combination L/F was the treatment with the lowest occurrence rate, reaching 15.08 %, and was grouped at the bottom of the range (Figure 2).

Early sex determination in papaya remains a major challenge in crop management due to the absence of distinctive morphological characteristics in early stages and the complexity associated with the presence of heteromorphic sex chromosomes. This limitation prevents the effective selection of individuals during the seedling stage, forcing researchers to wait until flowering for accurate classification (Deputy et al., 2002; Gangopadhyay et al., 2007). Although certain morphological indicators, such as seed coat color or root shape, have been proposed, the results have been inconsistent and lack solid scientific validation. Some studies, such as those by Kumar (1951) and Choudhary et al. (1957), suggest physiological and biochemical differences between sexes, but their practical application remains limited. On the other hand, Soni et al. (2017) identified a possible association between seed color and sexual expression, particularly in dioecious and gynodioecious genotypes. However, these approaches do not yet

Figure 2. Probability of occurrence of petiole orientation and flower sex in 'Maradol' papaya populations.



Means ± standard deviation, previously transformed to the arcsine of the squared root of the ratio. $DMSH_{0.05} = 6.42$. Different letters indicate significant differences, Tukey ($P \leq 0.0001$). R=Right, L=Left, F=Female, H=Hermaphrodite.

Table 3. Probability of occurrence and assertiveness based on petiole orientation and flower sex in the 'Maradol' papaya variety.

Sex	By petiole orientation direction (occurrence %)		Twist	By flower sex (occurrence %)	
	L	R		F	H
Female	28	53	L	38	64
Hermaphrodite	72	47	R	62	36

n = 1,300 plants. R=Right, L=Left, F=Female, H=Hermaphrodite.

replace reliable identification methods. In this context, the results obtained from the analysis of variance support the need for reliable tools to predict sexual expression, since the differences observed in the occurrence of specific combinations (such as L/H) could be leveraged to improve early selection, thereby optimizing the crop's productive and genetic efficiency.

Based on the overall combined values from the sample (n = 1,300 plants), which are determined by the probability of a specific flower or petiole twist, Table 3 presents the degree of prediction for the occurrence of both petiole orientation and flower type. Regarding petiole twist, if only leftward twisting is considered, approximately 72 % corresponded to plants with hermaphroditic flowers. In this regard, the remaining 28 % may occur in plants with female flowers. As for rightward twisting, the occurrence of plants bearing female flowers was correctly predicted in 53 % of cases. In the remaining 47 %, the flowers may be hermaphroditic. Based on the above, hermaphroditism in papaya can be increased by 6 % from the baseline proportion observed in compact genotypes. Similarly, using the overall combined values, the probability that petiole orientation will occur to the right may be 62 % if the plants have female flowers. Meanwhile, if plants with hermaphroditic flowers are observed, approximately 64 % may coincide with leftward petiolar twist; this provides an estimate based on the information obtained from the 13 'Maradol' papaya populations studied.

The complexity of the papaya's reproductive system, characterized by the coexistence of male, female, and hermaphroditic flowers, poses a significant agronomic challenge, as it directly impacts fruit quality, shape, and yield (Ávila-Hernández et al., 2023). The ability to identify the sex before flowering would allow for the optimization of resource use, avoiding the subsequent removal of unwanted individuals, mainly those that develop male flowers. Adding to this complexity is the presence of sexual reversal, observed in male and hermaphroditic plants, whose behavior can vary depending on environmental conditions (Shukoor et al., 2023).

In contrast, female plants are more stable in terms of their sexual expression and productive capacity, both in tropical and subtropical climates (Prakash et al., 2018). Although molecular methods such as PCR exist for early sex determination, they require specialized infrastructure and are costly to implement on a larger scale. Therefore, the development of prediction strategies based on simple morphological characteristics observable in the field is important. The results obtained in this study suggest a positive and significant correlation between petiole twist and sex type, which represents a useful tool for early prediction of flower sex. Specifically, the petiole orientation towards the left showed a higher coincidence with the hermaphroditic expression, while the inverse observation, based on flower type, reinforces this association. Although other combinations did not present consistent patterns, the established relationship between the petiole twist and flower sex allows for the consideration of a prediction model of easy application, which could complement early selection systems in breeding programs and commercial production.

Conclusions

The analysis conducted on 13 populations of the 'Maradol' papaya variety shows a clear correlation between petiole orientation and flower sex combinations, which could be used to predict the sex of plants prior to flowering. In particular, a leftward petiole twist was primarily associated with hermaphroditic plants, while a rightward twist corresponded mostly to female flowers. This association held true when observing both petiole twist and flower type, suggesting that both factors can serve as predictors of the plant's sexual expression. This morphological relationship could become a key tool for early plant selection, optimizing resources and improving production efficiency.

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