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Original article

Assessment of two sex-determining procedures in 'BH-65' papaya from an economical and developmental point of view

I. Salinas[1,3], M. Salehi[2], J.J. Hueso[3] and J. Cuevas[1,a]

[1] Department of Agronomy, University of Almería, ceiA3, La Cañada de San Urbano s/n, 04120 Almería, Spain
[2] Department of Horticultural Science, Faculty of Agricultural Science, University of Guilan, Rasht, Iran
[3] Cajamar Experimental Station 'Las Palmerillas', Paraje Las Palmerillas, El Ejido, 04710 Almería, Spain

Summary

Introduction – **The trioecious nature of papaya makes mandatory sex determination and plantlet selection. Recent advances permit determining plant sex at seedling stage with molecular markers. For the first time, a study is accomplished to compare the success of molecular sex-determining procedure (MSP) versus conventional sex-determining procedure (CSP) based on plant growth and fruit production and on economic aspects.** *Materials and methods* – **'BH-65' plantlets selected using both methods were grown under the same conditions in a greenhouse of South-East Spain. Seedlings were sexed molecularly during early leaf emergence and the hermaphrodites transplanted individually, while in CSP four seedlings per hole were initially planted and then three of them removed at flowering. Plant growth was seasonally monitored, and fruit number and quality compared at harvesting.** *Results and discussion* – **At first flowering, MSP plants were larger than CSP plants, whereas three months later, they were similar in size. In both treatments, the distance of first fruit from ground was the same. Harvest started just one week earlier in CSP than in MSP plants. No significant differences were observed between MSP and CSP fruits in total soluble solids, titratable acidity, firmness, and skin and pulp color. MSP plants were more expensive but produced a higher yield with fruits that were also significantly larger. Commercial yield and total cost paid for establishing a papaya orchard were 49% and 39% higher with MSP, respectively.** *Conclusion* – **These results confirm the suitability of using MSP for the protected cultivation of the 'BH-65' papaya.**

Keywords
papaya, *Carica papaya*, orchard management, roguing, profitability, protected cultivation, sex determination

Résumé

Évaluation de deux méthodes de détermination du sexe chez le papayer 'BH-65' du point de vue économique et développemental.

Introduction – **La nature trioécique du papayer rend obligatoire la détermination du sexe et la sélection des plantules. Des avancées récentes permettent de déterminer le sexe des plantes au stade du semis par des marqueurs moléculaires. Pour la première fois, une étude a été réalisée pour comparer le succès de la méthode moléculaire de détermination du sexe (MSP) par rapport à la procédure conventionnelle (CSP) basée sur la croissance des plantes et la production fruitière, et sur des aspects économiques.** *Matériel et méthodes* – **Les plantules du cv. BH-65 sélectionnées selon les deux méthodes ont été cultivées dans les mêmes conditions en serre au Sud-Est de l'Espagne. Les plants de semis ont été sexés par marqueurs moléculaires au début de l'émergence des feuilles et les plantes hermaphrodites ont été individualisés, tandis que par CSP, quatre plantules ont été repiquée par pot et trois d'entre elles ont été éliminées à la floraison. La croissance des plantes a été enregistrée de façon saisonnière et le nombre et la qualité des fruits ont été comparés à la récolte.** *Résultats et discussion* – **A la première floraison, les plantes MSP étaient plus grandes que les plantes CSP, alors que trois mois plus tard, elles avaient une taille similaire. Dans les deux traitements, la distance entre le premier fruit et le sol était la même. La récolte a débuté une semaine plus tôt sur les plantes CSP que sur celles MSP. Aucune différence significative n'a été observée entre les fruits MSP et CSP, que ce soit en matière soluble totale, en acidité titrable, pour la fermeté ou la couleur de la peau et de la pulpe. Les plantes MSP, plus onéreuses, ont produit un rende-**

Significance of this study

What is already known on this subject?

- Different molecular markers have been developed to determine the sex type of papaya plantlets. Since farmers prefer hermaphrodite plants, they can choose between the conventional (CSP) or the molecular (MSP) sex determining procedure.

What are the new findings?

- The present experiment supports a preference for MSP for the protected cultivation of 'BH-65' papaya. The cost of a new papaya orchard is about € 2,000 more expensive for MSP than for CSP. MSP plants grew more rapidly and yielded +49% fruit of larger size, more than compensating higher costs.

What is the expected impact on horticulture?

- Papaya farmers are expected to rapidly adopt this sex determining procedure, especially if the cost for vigorous hybrids of papaya is reduced.

[a] Corresponding author: jcuevas@ual.es.

ment plus élevé avec des fruits également significativement plus gros. Le rendement commercial et le coût total investi pour l'établissement d'un verger de papayers étaient de 49% et 39% plus élevés avec MSP, respectivement. *Conclusion* **– Ces résultats confirment la pertinence de l'utilisation de MSP pour la culture sous abri du papayer 'BH-65'.**

Mots-clés
papayer, *Carica papaya*, culture sous abri, détermination du sexe, épuration variétale, gestion du verger, rentabilité

Introduction

Papaya (*Carica papaya* L.) is an edible tropical fruit crop indigenous to Central America and Mexico (Vázquez *et al.*, 2014), with significant health benefits (Ming *et al.*, 2008). Papaya fruit is the most nutritious of the 35 most commonly consumed fruits, and is recommended for the daily allowance of vitamins A and C, potassium, folate, riboflavin, niacin, thiamin, calcium, iron and fiber. Papaya is also cultivated for its milky latex that contains the proteolytic enzyme papain, applied for hydrolyzing beer peptides (chillproofing), tenderizing meats and other uses (Lintas, 1992; Chandrika *et al.*, 2003). Despite its economic importance and large world acreage, papaya is one of the very few fruit crops still propagated by seed. The greenhouse cultivation of papaya is under development in Turkey, Spain and other Mediterranean countries. In this cropping system, vigorous cultivars are not fully appropriate given the relatively low greenhouses (4–5 m height to roof) commonly utilized. In contrast to most papaya cultivars, the variety used in this experiment, 'BH-65', is dwarf (Gunes and Gübbük, 2012), making it more suitable for protected cultivation.

On the other hand, papaya is a trioecious species; that is, adult papaya plants may have three possible sexual forms: male, female, and hermaphroditic, being papaya sex expression controlled by a single gene with three alleles of pleiotropic effects (Storey, 1953). Sex determination in papaya is complex and has been a subject of much research (Parasnis *et al.*, 2000; Ming *et al.*, 2007). There are several reasons why the sex type of the seedlings should be known for papaya growers. First, hermaphrodite papaya plants are commercially preferred over females not only for their higher productivity, but also for the elongated shape of their fruits, more popular for marketing. Female plants produce, on the contrary, round fruits that require greater container volume for shipping than the pyriform shaped fruits of hermaphrodite plants. They also contain an unattractive large central cavity with few, if any, seeds (Singh and Sudhakarrao, 2011). Second, in some areas, the use of female plants for fruit production involves the loss of 6–10% of field space that must be allotted to growing male plants to pollinate the females (Fitch, 2005), although in most locations female plants set fruits parthenocarpically without pollination and no need for male plants exists.

Hermaphrodite flowers of papaya are mainly autogamous. Seeds from selfed hermaphrodite plants segregate into a 2:1 ratio of hermaphrodite to female. Seeds from female plants segregate at a ratio of 1:1 (hermaphrodite to female) when they are fertilized by pollen from a hermaphrodite plant, and at ratio 1:1 (male to female) when they are fertilized with pollen from male plants (Storey, 1953). Therefore, farmers, when establishing a new papaya orchard, have to plant three or four seedlings per hole, depending on the cross and on the parental pedigree, in order to almost guarantee the presence of at least one hermaphrodite plant per hole.

At the turning of the XXI century, sex-linked molecular markers, including RAPDs, ISSRs and AFLPs, were developed for sex identification of papaya, an advance that allows also the use of these markers for the differentiation of male, female and hermaphrodite plants at early seedling stages (Deputy *et al.*, 2002; Kim *et al.*, 2002; Aspeitia-Echegaray *et al.*, 2014; Aryal and Ming, 2014; Chaturvedi *et al.*, 2014). Despite this discovery was announced more than 15 years ago, no reports of using MSP plants in field are known in the scientific literature.

In conventional sex-determining procedure (CSP), growers distinguish their plants' sex when the first flowers appear; that is, approximately 6 months after germination depending on the variety and growing conditions (Ming *et al.*, 2007), or 3–4 months after transplanting if plantlets proceed from a nursery as seedlings. After first flowering, roguing performed by qualified workers must be practiced selecting only hermaphrodite plants. This system is regarded as wasteful of time, plants, labor, water, and nutrients (Paterson *et al.*, 2008). This misusage is especially negative for the protected cultivation of papaya, a crop system recently adopted in South-East Spain. On the other hand, molecular sex-determining procedure (MSP) involves sampling and high laboratory costs making more expensive these plantlets. Nevertheless, when selecting a procedure, it is necessary to take into account not only the basic costs, but also the performance of the plants selected according to each sex-determining procedure. To the best of our knowledge, no data have been reported on the field performance of MSP, nor a comparison of growth parameters and yielding of MSP and CSP papaya plants has been done so far. The present experiment ultimately aims to reduce the costs incurred during the establishment of papaya orchards by introducing the most efficient sex-determining method, through the screening of the detailed costs for each procedure (MSP and CSP) along with considering some plant growth attributes and fruit quality of 'BH-65' papaya grown under a plastic greenhouse.

Material and methods

Plant material and growth conditions

This study was carried out in a papaya crop cultivated in a plastic greenhouse located at the Cajamar Experimental Station 'Las Palmerillas', sited in El Ejido (Almería, Spain) (2°43'W, 36°48'N, 151 m above sea level). The greenhouse used was a multi-tunnel type provided with eight chapels 7.5 m wide each one and E-W orientated and covered with low density polyethylene. The greenhouse had 3.4 m height to the eaves and 5.4 m to the ridge. Natural ventilation through one zenithal window per chapel and two laterals panels improved climate conditions inside the greenhouse.

Papaya cultivar 'BH-65' was selected as a plant material since it has relatively shorter height and do not reach rapidly the ceiling, making it more appropriate for the most common greenhouse type in use in the Mediterranean countries. Treatments included two different sex-determining procedures for the above-mentioned cultivar. They were assayed as follows; for the first treatment, known as molecular sex-determining procedure (MSP), a piece of leaf from each papaya seedling was taken soon after germination and leaf appearance (5 weeks after sowing) and transferred to the laboratory for determining the sex-type via molecular markers base on SNPs (Single Nucleotide Polymorphism). This determination was carried out by a private Company, SP Laboratorios, sited in Almería. Once the hermaphrodite plants were identified in the lab, only one plant per hole was transplanted to the field at April 6th, 2016. For the second treatment, conventional sex-determining procedure (CSP), papaya seeds were taken to a private nursery where they were sowed and grown until seedlings reached a height of 10 cm. Next, four unselected seedlings were transplanted in each planting hole at the same date as we did with MSP seedlings. Visual determination of sex plant in CSP was carried out on them when the first flowers appeared, at mid-September, 165 days approximately after transplanting. Then, the first hermaphrodite plant reaching bloom was selected, while the other three plants were removed and taken out of the orchard. Only one plant per hole remained. Final plant spacing was 2.5 × 1.5 m in both treatments. The plantation was terminated before winter cold at December 20th, 2017, completing a 20-month cycle as usual in papaya.

All plants were irrigated regularly through drip pipes containing the same nutrient solution throughout the experiment. The same amount of irrigation water and fertilizers were used despite the number of plants was initially four times higher in CSP. Random disposition of the treatments in the irrigation lines imposed us this approach. Irrigation and fertilization was not a limiting factor for plant growth. Temperature and relative humidity inside the greenhouse was monitored during the whole season.

The two-different sex-determining procedures, molecular and conventional, were scrutinized and compared based on both, economic aspects and plant performance. To do so, effectiveness and reliability, cost, plant growth, yield and fruit quality and economic results were determined for each sex-determining procedure.

Effectiveness and costs of sex-determining procedures

Despite planting four seedlings per hole in CSP, there are still some chances for all four seedlings resulting female. To compare the effectiveness and reliability of CSP and MSP in obtaining only hermaphrodite plants at the final plantation design, we compare the percentages of hermaphrodite plants under both procedures inspecting all definitive plantlets at full bloom.

Taking the results on reliability into account, the related expenses were calculated for each sex-determining procedure, considering the costs and success in obtaining hermaphrodite plants. For CSP, farmers have to pay to the nursery for the seeds and the raising of the seedlings (up to 10 cm height). For MSP, farmers pay to the nursery only for selected hermaphrodite seedlings. In this cost, the nursery charge to the farmers for seeds, plant care and MSP process itself. MSP includes the labor cost of leaf sampling as well as of the molecular assessing in the lab. In addition, in CSP farmers incur in the cost of labor for both, visual sex-determining process in the field and the removal of the discarded plants.

Plant growth

For comparing growth attributes of the plants of the two treatments (MSP versus CSP), the below parameters were measured every other three months beginning at September 20th, 2016, just after sex determination, and finishing at December 20th, 2017. The parameters were height of plant (HP) (in cm) using a graded bar, trunk perimeter (TP) (in cm too, at 15 cm above ground) and the distance of first commercial fruit from ground (DF) measured (in cm) from the fruit peduncle to the soil surface. Plant height and trunk perimeter data were subjected to regression analysis.

Yield and fruit quality components

Yield (amount and dates) and fruit quality were also compared. Fruits were harvested as soon as they reached ripening (when at least one third of the skin fruit turned to yellow) throughout the experiment. After harvesting, each single fruit was weighed. The sum of weight of the harvested fruits during all harvesting operations was considered the final yield. The number of commercial and non-commercial, misshapen fruits (carpelloid and pentandric), that reach harvest was annotated and their weight recorded.

Fruit size (length and perimeter considering the widest part) was measured using seamstress tape ruler for each harvested fruit in both treatments.

Fruit skin color was measured at the middle of papayas on two opposite sides of each fruit by a colorimeter (Model RC-300, Minolta Co. Ltd., Osaka, Japan). Papaya fruits were then divided into two halves and the pulp color was measured. Colors are reported as a combination of lightness (L*), green and red (a*) and blue and yellow (b*). Numerical values of a* and b* were converted into hue angles ($H° = \tan^{-1} b^*/a^*$) and into chroma values [$chroma = (a^{*2} + b^{*2})^{1/2}$]. Lightness represents the general illumination of the color, where 0 = black, 100 = white; a* indicates chromaticity on a* green (−) to red (+) axis, and b* indicates chromaticity on a b* blue (−) to yellow (+) axis. A change in hue indicates fruit ripening from green to yellow or red, where 0 = red, 90 = yellow, and 180 = green. The chroma indicates the purity of hue or color saturation regardless of the lightness or darkness of the skin or pulp. A highly chromatic color looks very luminous or concentrated, whereas a color with a low chroma looks dull, gray or dilute (Francis, 1980).

After color measurement, papaya skin was removed with a sharp knife, and the pulp next to the skin was used for firmness penetration. Firmness was measured in the middle on two opposite sides of each fruit using a texture analyzer (TA-XT2, Stable Micro Systems Ltd., UK) with a 5 mm diameter plunger with a constant moving rate of 20 mm min^{-1} for a depth of 5 mm. The mean values for the maximum force obtained are reported in Newtons (N). The fruit firmness values were measured for 6 randomly selected fruits per replicate.

Finally, once the fruits were cut longitudinally, the skin, placenta and seeds were removed. Then, the pulp of the papaya from each replicate was homogenized using a blender. A drop of the centrifuged homogenate was placed on an ATAGO digital refractometer (Tokyo, Japan) at 25 °C and the content of total soluble solids (TSS) expressed as °Brix. The centrifuged homogenate was used to measure organic acids by titration. 5 mL of homogenate containing two drops of 1% phenolphthalein were titrated with 0.1 N NaOH until the solution turned permanently pink. Titratable acidity (TA) was expressed as percentage of citric acid as indicated by Guleria (2000).

Statistical analysis

The experiment was designed with four replicates (four central rows of a group of plants, using three plants of each row as replicate) for each treatment. The results obtained were expressed as means ± SE (standard error). Analysis of variance was performed by GLM procedures (SAS 9.1 for

Windows). The separation of means was performed according to LSD tests. The P value < 0.05 was considered statistically significant.

Results and discussion

Effectiveness and cost of sex-determining procedures

As previously explained, papaya is a polygamous species with three sex types: male, hermaphrodite and female. In most cases, hermaphrodite plants are preferred for production. So far, farmers must indispensably wait for flowering in order to distinguish and select the first hermaphrodite seedlings. Nonetheless, and despite the installment of four seedlings per hole, sometimes all four seedlings may result into female plants. In CSP, 96% of the plantation holes had at least one hermaphrodite plant, while in the remaining 4% of the planting sites, only female plants were obtained. Theoretically, the percentage of hermaphrodite plants is 98.8% when using four plants per hole for this cross and genotype. On the other hand, the reliability of MSP has not been checked yet in commercial fields. In our case and in this regard, MSP was a complete success since 100% of the selected seedlings in the lab were confirmed hermaphrodite in the field. Saalu *et al.* (2009) reached a success of 96% positives when identified sex type of 'Pococi' plants using PCR markers. A complete success was observed, on the contrary, on 'Maradol' variety by Aspeitia-Echegaray *et al.* (2014). Sex determination system in papaya is particularly intriguing as it shows frequent sex reversal caused by environmental factors (Damasceno *et al.*, 2008; Ramos *et al.*, 2011). In our study, no completely sex reversal was observed in any experimental plant of the two sex-determining procedures, and the ratio above expressed was maintained until the end of the trial.

Economic assessment is one of the most mandatory analyses for farmers to help them to select the most cost-effective cultivation procedures. All expenses related to conventional procedure for sex-determining are shown in Table 1. Total cost for one hectare of CSP papayas was around € 5,200, while it was circa € 7,300 for MSP (that is, 39% higher). The higher cost of MSP was due to the lab process involved. In this regard, the cost per planting hole (one plant at the final orchard design) was estimated on € 1.80 versus € 2.70 for CSP and MSP, respectively. It is important to note that the cost of MSP could be as high as € 3.80 per planting hole in the case of hybrid genotypes, due to 1:1 (female to hermaphrodite) segregation. On the other hand, CSP required more labor for transplanting, tagging the hermaphrodite plants once visually assessed, and removing the discarded plants. This labor totals € 350 more per hectare for CSP (Table 1).

All expenses related to drip irrigation were equally devoted to MSP and CSP plants. However, two irrigation lines and more emitters are needed in CSP while in MSP transplanting from the beginning only one plant per hole allows installing only one irrigation line per plant row. In the present study, we bought the molecularly-confirmed hermaphrodite seedlings from a private company, and then just a single seedling was cultivated in each hole. Finally, it is worthwhile to mention that total costs have to be paid at the same time in MSP, when buying the hermaphrodite seedlings, while the expenses of farmers for CSP are not simultaneously incurred, since some expenses are paid at the beginning of the plantation when buying the seeds/seedlings but others as plant selection and removal occurs 4–6 months later. The disposal of vegetable residues in the greenhouses of Almería and its transport and elimination in a certified processing plant have a cost of circa € 1,000 ha^{-1} (€ 990 in fact).

Plant growth

The first measurement of plant growth was done at September 20th, 2016, immediately after sex determination. The MSP plants were about 8% taller than CSP plants at September 2016, first date when only one plant remained in each planting hole (Figure 1). However, three months later, at December 2016, both MSP and CSP plants had nearly the same height. In this regard, MSP plants had increased a 21% in vertical growth, while this growth was a 30% in CSP plants during this 3-month period, September to December, once flowering started in plants of both treatments. Although both MSP and CSP plants had very limited growth during winter due to the prevalent low temperatures, they grew as rapid as a 22% more in spring, from late March to late June. Both kinds of plants had virtually the same height in the last measurements (Figure 1).

The transverse growth of MSP plant trunk was also significantly ($P < 0.05$) larger than that of CSP at the time of first flower appearance (Figure 2), which could be attributed to a better initial situation for obtaining light and nutrients under non-competitive conditions, since in MSP only one plant per hole was planted. Contrarily to MSP, four CSP seedlings were cultivated simultaneously in a hole and they grew initially together in a close proximity until the first flowers appeared, and the removal of the three discarded plants per hole was performed. As observed for plant height, the initial differences favoring MSP diminished with time and the last measurements showed slightly higher trunk diameter for MSP plants. Unlike plant height, the transversal growth of the trunk did not stop during winter neither in MSP nor CSP, as they both had a 10% increase in trunk perimeter during this season. From these results, we may assume that papaya cultivar 'BH-65' seedlings grew initially more both vertically and transversely when it was planted separately as occurred for MSP.

Plant height and trunk diameter were linearly related ($P < 0.001$), showing coefficients of determination (r^2) values of 0.74 and 0.81 for MSP and CSP, respectively. The regression analyses of plant height and trunk diameter increases along the studied period significantly fitted several logis-

Table 1. The costs related to conventional sex-determining procedure (CSP) and molecular sex-determining procedure (MSP) in papaya orchard.

Treatments	Seedlings cost (€) per			Labor cost (€ ha^{-1})			Total cost (€ ha^{-1})
	Unit	Hole*	Ha**	Transplanting	Tagging plants	Roguing plants	
CSP	0.45	1.80	4,800	90	108	216	5,214
MSP	2.70	2.70	7,200	72	-	-	7,272

* Four seedlings per hole.

** Calculated based on a plant spacing of 2.5 × 1.5 m.

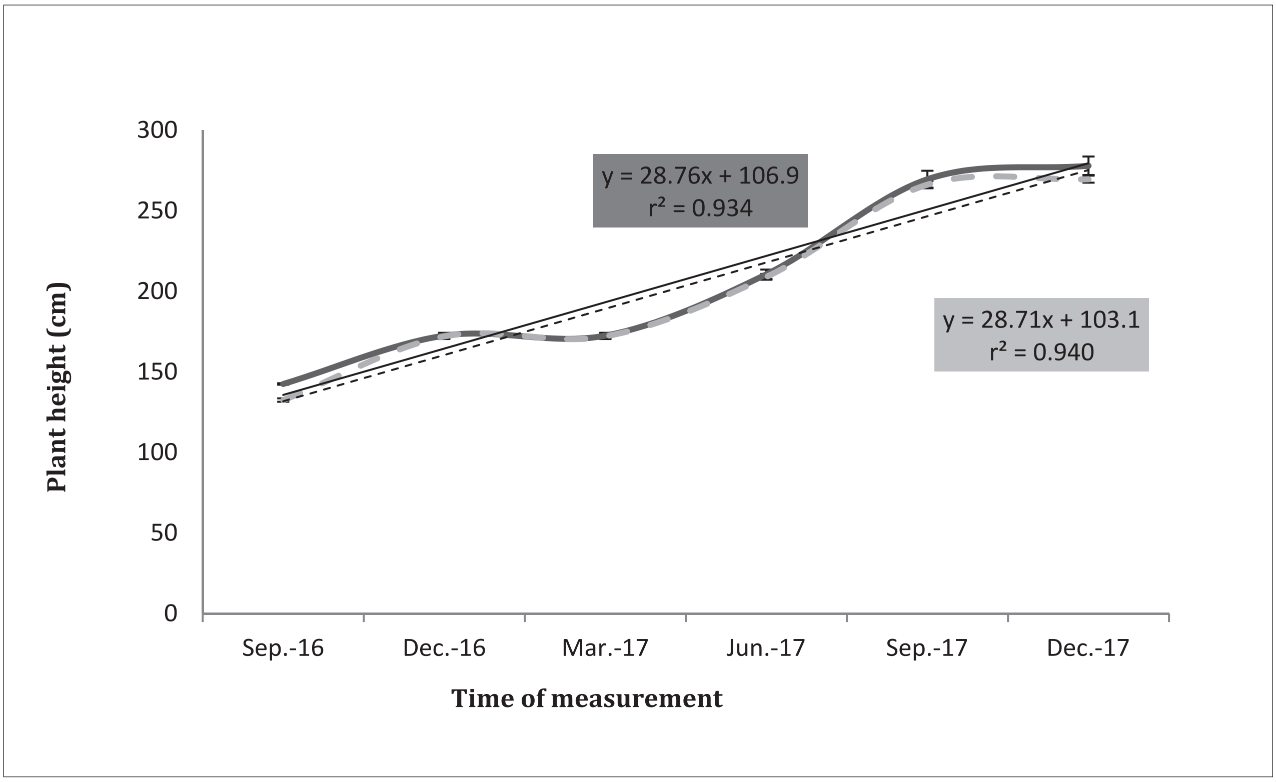

Figure 1. The vertical growth rate of plants of papaya cv. BH-65, selected based on two different methods, molecular sex-determining procedure (MSP; solid line) and conventional sex-determining procedure (CSP; dashed line) during a complete cultivation cycle (September 2016 to December 2017). The first measurement was performed just after sex determination (September 20, 2016).

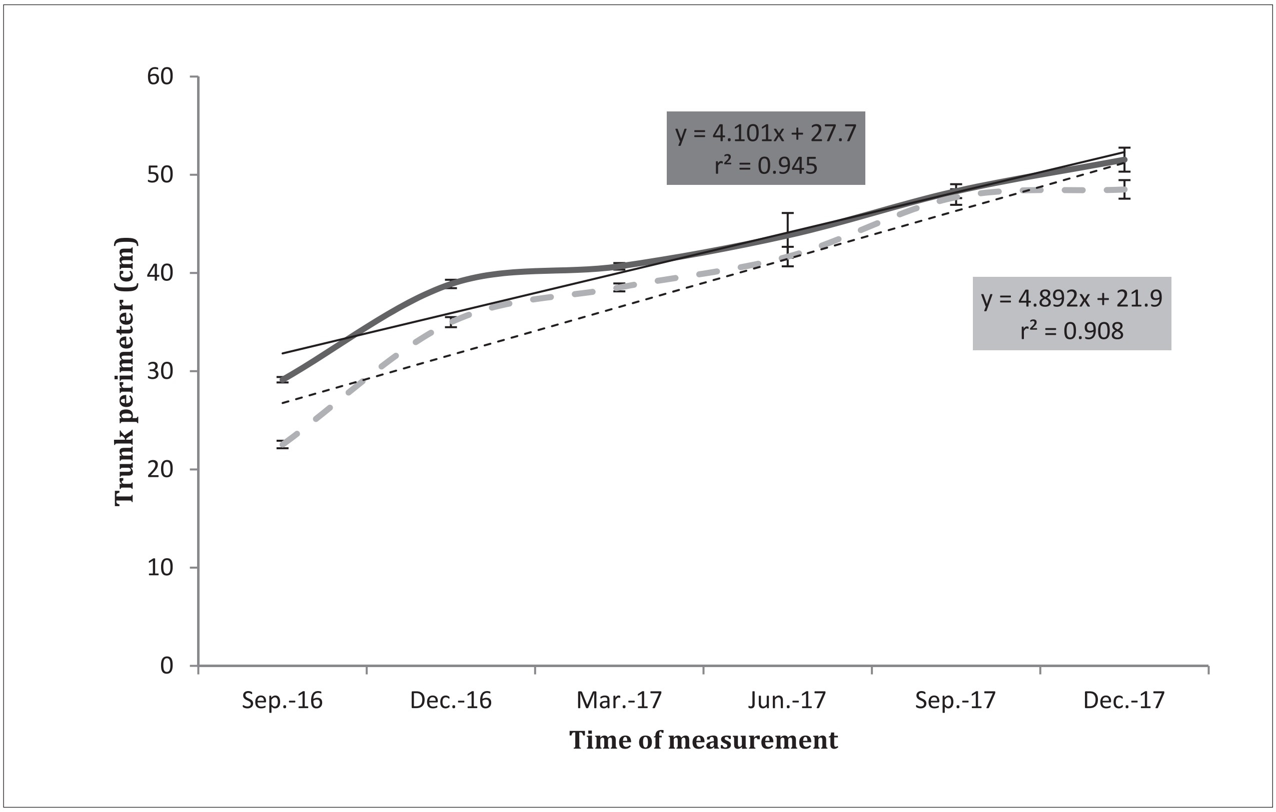

Figure 2. The transverse growth rate of trunk in plants of papaya cv. BH-65, selected based on two different methods, molecular sex-determining procedure (MSP; solid line) and conventional sex-determining procedure (CSP; dashed line) during a complete cultivation cycle (September 2016 to December 2017). The first measurement was performed just after sex determination (September 20, 2016).

Table 2. L*, a*, b*, hue angles (H°) and chroma values in fruit skin and pulp of papaya cv. BH-65, as cultivated based on two different sex-determining procedures, CSP and MSP.

Treatments	L*	a*	b*	H°	Chroma
			Skin color		
CSP	49.3	-6.8	40.5	100.6	41.6
MSP	49.9	-11.5	38.0	107.1	39.9
			Pulp color		
CSP	55.7	26.1	42.6	58.6	50.1
MSP	57.1	25.3	42.2	59.0	49.2

Table 3. Fruit quality parameters measured at harvest time for CSP and MSP, harvested when approximately one third of the skin fruit color turned to yellow, and commercial and non-commercial yield.

Treatments	TSS (°Brix)	TA (%)	Firmness (N)	Fruit perimeter (cm)	Fruit length (cm)	Distance of first commercial fruit to ground (cm)	Misshapen fruits* (kg ha^{-1})	Commercial yield (kg ha^{-1})
CSP	10.0	0.54	62.8	27.3 a	15.8 a	108.1	215	9,500
MSP	10.1	0.58	78.4	29.6 b	17.7 b	108.8	380	14,200

Mean values having different letters in the columns are significantly different ($P < 0.05$).
* Carpelloid and pentandric fruits harvested.

tic models, especially well to Richards and Gompertz. The slopes of the linear equations for plant height growth were very similar for both sex-determining procedures (Figure 1), The increase in time of the trunk diameter was slightly lower in MSP, and hence, despite the larger trunks observed initially in MSP, final trunk size was about the same in both procedures (Figure 2). These results highlight that the higher competition as that derived of planting four seedlings per hole in CSP did not result in a permanent narrow trunk and/or plants of larger height as feared in plants thriving in poor light conditions. The situation could be different in the case of hybrids of much higher vigor.

Fruit attributes

According to the statistical analyses, no significant differences were observed between MSP and CSP papaya plants regarding fruit quality parameters, including TSS, TA, firmness, and both skin and pulp color. On the contrary, some significant differences were found in fruit size. In this regard, the fruits of MSP were significantly ($P < 0.05$) larger and wider than those of CSP (Tables 2–3). At June 16th, 2017, 340 days after transplanting, first fruits of CSP plants started to ripen. One week later, at June 23rd, the same occurred for MSP plants. Although CSP fruits could be harvested just a week sooner than MSP fruits, they were not as large and wide as those from MSP. As MSP plants have been planted solely in a hole, it may be assumed that they had a suitable opportunity to save sufficient nutrients during the vegetative stages under non-competitive condition, and used these stored reserves for the enhancement of productivity and for producing larger fruits.

Based on our study, there was not a significant relation between the sex-determining procedure during orchard establishment and qualitative properties of the fruits such as color and taste. Although the quality of papayas is strongly affected by growing conditions and practices adopted during its cultivation (Nunes *et al.*, 2010), the genetic feature is predominant in some physiological characteristics of papaya fruits (Oliveira *et al.*, 2005). In our study, we have used the same genetic resource, 'BH-65', but under two different growing conditions during vegetative phase. In both MSP and CSP plants, the distance of first commercial fruit to the ground (DF) was the same (Table 3). Our results indicate that if farmers use each of the sex-determining procedure, there would not be a difference neither in harvesting dates nor in total soluble solids (TSS), titratable acidity (TA), firmness and color of the fruits (Table 3).

In our experiment, MSP plants had a higher proportion of misshapen fruits, resulting from the formation of carpelloid and pentandric flowers, than CSP did (Table 3). These misshapen fruits are not commercially valid. These variations of the hermaphrodite flower, classified as floral abnormalities, reduce the commercial yield and increase the seasonality as they appear in a very different magnitude depending on the season (Salinas *et al.*, in press). This seasonal effect leads to supply oscillations and consequently to price variations of papaya in the market. Although, we have not observed an entire plant to have sex reversal, some flowers present in each treatment, especially in MSP plants were exposed to sex disorders, resulting in carpelloid and pentandric fruits.

Finally, according to our results, the commercial yield of MSP plants was about 49% higher than that of CSP (Table 3). This improvement derives in higher income coming from MSP plants that may compensate the higher orchard establishment cost, considering that current prices paid to farmers in Spain for papaya fruit are in the edge of € 1 kg^{-1}. These results confirm the suitability of using MSP for the protected cultivation of 'BH-65'. However, the results do not support a preference for MSP in papaya hybrids, since the plant cost charge for the lab in this case is as high as € 3.80 plant^{-1}. Additional studies on taller cultivars of papaya where vigorous growth could be more troublesome for greenhouse cultivation are needed before the most suitable procedure for sexing papaya is recommended worldwide.

Conclusion

The establishment of a 'BH-65' papaya orchard using MSP was about 39% more expensive than planting 'BH-65' seedlings sexed visually by CSP. Nonetheless, MSP plants produced a commercial yield 49% higher with fruits of larger size (in both length and perimeter) than CSP plants did. These results confirm the suitability of MSP for the protected cultivation of 'BH-65'. Although MSP plants grew initially more, and that faster growth derived in higher yield, the differences in height and perimeter were attenuated with time. No significant differences in fruit quality (TSS, TA, firmness, color) between MSP and CSP plants were found.

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