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ANALYSIS OF THE GROWTH IN WILD GENOTYPES OF *Carica papaya* L. GROWN *Ex Situ* AND cv. ´MARADOL ROJA ´

Análisis del crecimiento de un genotipo silvestre de *Carica papaya* L. cultivado *ex situ* y cv. `Maradol Roja´

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ABSTRACT. *Carica papaya* L. specie is native from the American tropic and one the most important fruit trees grown at tropical and subtropical regions of the world. In Cuba, many problems affect production of this fruit tree where the most important is reduction of number of exploded commercial varieties and low genetic diversity in germoplasma's bench. However, wild genotypes constitute an important source of genes for improvement programs and local explotation, which have not been characterized. Studies of growth analysis permit understanding his behavior in different planting periods and *ex situ* conditions and to identify initial growth characters which provoke more yield increase and favor improvement research works of the most productive genotypes. Due to before indicated, the aim of the present investigation was realized studies of the growth of the genotype wild papaya of the basin Almendares-Vento. The seeds for the experiments were obtained of fruits collected in situ. The direct measures in the plant were an area to foliate and dry weight. For the determination of the surface to foliate used a digital chamber and software. The analysis computarizado of the images of leaves obtained with the digital chamber allowed to estimate the area to foliate of simple, rapid and economic form. The major production of area to foliate of the wild genotype and similar behavior in the dry mass and rest of the evaluated indicators, indicates the availability of resources during the whole phase of growth, which associates with his performance.

Key words: physiology of the development, Indexes of the growth, biomass

RESUMEN. La especie *Carica papaya* L., es nativa del trópico americano y uno de los frutales más cultivados a nivel mundial. En Cuba existen problemas que afectan la producción de este frutal, considerados los más importantes el reducido número de cultivares explotados comercialmente y la baja diversidad genética presente en el banco de germoplasma. Sin embargo, existen genotipos silvestres que constituyen una fuente importante de genes para los programas de mejoramiento y explotación local, los cuales aún no se han caracterizado. Estudios de análisis del crecimiento permiten comprender su comportamiento en diferentes periodos de siembra en condiciones *ex situ* e identificar caracteres de crecimiento inicial que posibiliten el aumento del rendimiento y favorezcan los trabajos de mejoramiento en busca de genotipos más productivos. Debido a lo antes señalado, el objetivo de la presente investigación fue realizar estudios del crecimiento del genotipo papaya silvestre de la cuenca Almendares-Vento. Las semillas para los experimentos se obtuvieron de frutos colectados *in situ.* Las medidas directas en la planta fueron área foliar y peso seco. Para la determinación de la superficie foliar se empleó una cámara digital y un software. El análisis computarizado de las imágenes de hojas obtenidas con la cámara digital permitió estimar el área foliar de forma simple, rápida y económica. La mayor producción de área foliar del genotipo silvestre y similar comportamiento en la masa seca y resto de los indicadores evaluados, indica la disponibilidad de recursos durante toda la fase de crecimiento, lo cual se asocia con su rendimiento.

Palabras clave: fisiología del desarrollo, Índices de crecimiento, biomasa

INTRODUCTION

The specie *Carica papaya* L., from the Caricaceae family is native from the American tropic (1, 2) and one of the most grown cultivars in tropical and subtropical

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regions of the world for fresh consumption and for its different industrial uses[A] (3).

In Cuba, some 7 920 ha of papaya were planted in 2011 with a production of 135 700 t (4); however, crop management has influenced on the low yield and high production costs[B] (7). Another problem affecting papaya production is the reduced number of commercially exploited cultivars and the low current genetic diversity of the germplasm collection (5).

The Cuban archipelago has wild papaya genotypes that produce abundant fruits from small to medium sizes that are used by the population in their native regions (6). They are an important source of genes for breeding programs and local exploitation (7, 8). However, the information available on these genotypes is scarce so it is necessary to perform studies on their growth. Such studies are based on quantitative methods that describe the whole plant system with growth under natural, seminatural and controlled conditions (9), that permit to understand their behavior under *ex situ* conditions at different planting times (10).

Growth analysis is used as an alternative to explain growth differences of genetic origin or due to environmental modifications which is an efficient tool to identify promising materials. It allows the identification of the initial growth that favors the yield of the adult plant and facilitates breeding programs in search of more productive genotypes (11, 12, 13). Therefore, it is recommended to identify the existing plant material in relation to its assimilating system at consecutive intervals (14).

Through the growth analysis, the ideal planting time and transplanting can be defined. Moreover, relations between the source and demand are also studed. On the operations referred to, direct measurements like dry mass (*W*), total foliar area (AF), time (*t*) and derived indexes like the relative growth rate (TRC), foliar area index (IAF), net assimilation rate (TAN), crop growth rate (TCC), among others, that should be reached by functional analysis calculations(15). The foliar area index is one of the most useful parameters to characterize vegetation[C].

The foliar area is associated with many agronomical and physiological processes influencing growth, photosynthesis, transpiration, and photons between perception and energy balance[D]. For that reason, measurements in increased foliar area and dry mass are significant parameters for plant growth evaluation. Its adequate determination during the crop cycle makes possible its growth and development, photosynthetic efficiency and consequently the total production of the plant (16).

Nevertheless, in spite of the abundant literature on the different methods used to calculate foliar area, its determination is not easy without the necessary equipment to do it simply (17, 18). However, with the development of computers, new possibilities opened to measure the foliar area (19). Some researchers use a flatbed scanner and software to process images as an effective, fast, and economic method (20). In spite of this, crops like *Carica papaya* L. with leaves of more than 80 cm of diameter and 50 cm long (21), they cannot be scanned so these methods need to be modified to achieve the desired purpose.

For all the above, the objective of this research has been the determination of the foliar area using a digital camera and a free software for image processing (ImageJ) that permits the growth analysis of wild papaya genotypes of Mayabeque province, and compare them with the cultivar `Maradol Roja´.

MATERIALS AND METHODS

Experiments were conducted at the Physiology and Biochemistry Department of the National Institute of Agricultural Sciences (INCA), located at km 3 ½ of San José to Tapaste road, San José de las Lajas municipality, Mayabeque province, and at 23°00’ of North latitude and 82°12’ of West longitude and 138 m a. s. l.

For *ex situ* experiments, seeds from fruits of wild papaya trees collected in the source heights of Almendares-Vento and Northeast Havana basin were used. This area is within the geographical coordinates: 23° 00’ 00” N and 23° 03’ 27” S, 82° 01’ 27” E y 82°

[A] Evan, E.A. y Balle, F.H. *Una mirada a la producción, el comercio y el consumo de papaya a nivel mundial* [en línea], [FE917], l Departamento Food and Resource Economics, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida, Gainesville, F, Florida, USA, 2013, [Consultado: 21 de agosto de 2015], Disponible en: <https://edis.ifas.ufl.edu/fe917>.

[B] Statistics Division. *FAOSTAT* [en línea], 2012, [Consultado: 21 de agosto de 2015], Disponible en: <http://faostat.fao.org/site/526/default.aspx>.

[C] Cifuentes Sánchez, V.J. y Navarro Cerrillo, R.M. *Determinación del índice de superficie foliar (leaf area index) en masas forestales usando imágenes landsat-tm. Conclusiones de un primer estudio en la sierra norte de Córdoba. Mapping, 58*, 1999.

[D] Hernández, J.; Lomelí, N.; Santiago, M.A. y Arenas, E. *Estimación del área foliar en maíz*, 2012, p. 9.

08’ 20” W. As control, the cultivar ´Maradol Roja´ from certified seeds was used for being of Cuban origin and the one of major economic importance in Cuba and in countries of the area[E].

In order to locate wild papaya plants *in situ*, the literature available was consulted as well as the criteria of local settlers on these papaya populations. For prospection, expeditions and tours were organized from February to May 2008. Participants included experts from the Institute of Ecology and Systematics (IES), from INCA, from the Flora and Fauna Station “Escaleras de Jaruco” (EFFEJ) and farmers or people living in the prospection area. The methodology used consisted in locating on a map the region of interest and select plots or quadrants and plan the route to follow according to the characteristics of the area and the information provided by local population[F]. The size of the quadrants ranged from 100 and 1000 m^2 depending on the topography (22).

Once the plants were located, a follow-up was practiced to those that had fruits (11 plants) till harvest, after the emergence of three or four yellow streaks on the fruits (5). When the fruits totally changed color, seeds were extracted and placed on shadow and in the open. Once dried, they were placed on flask with cap and conserved at room temperature till planting.

For *ex situ* evaluations, two planting periods were used. It was taken into account that despite papaya planting with assured irrigation can be done throughout the year, the recommended months are those with low populations of vectors or where virus development is slow, which coincides with low-temperature and less rainy months (21). For planting without irrigation, growers take advantage of the phenological stages of growth and development that coincide with the rainiest period.

For all the above, the first planting time was named “winter”, because the treatment for pre-germination of the seeds was in December and planting was in January. This period coincided with the months of lower temperatures and rains, from November till April. The other planting period was named “summer” because the planting was done in May, when temperatures start to rise, so transplanting, growth and development stages coincided with the months of maximum rainfall and temperatures, from June to September.

For the study of crop growth and development, four growth indexes were included: relative growth rate (RGR), net assimilation rate (NAR), foliar area index (FAI) and crop growth rate (CGR) at transplanting, flowering and fruiting, in the winter and summer of 2010, in order to contribute with the best knowledge on these wild genotypes.

Direct measurements were foliar area and dry weight of the plants. In order to determine the foliar surface at transplanting, flowering and fruiting were evaluated in five representative plants by genotype with a use of digital camera and a free software for image processing (ImageJ) shown in Figure 1.

Growth evaluation in dry mass of plant organs (grams of leaves and stems per $plant^{-1}$) was made through destructive sampling. Organs were weighed and then heat-dried at 80 °C till reaching a constant dry mass. For the calculation of the total dry mass, dry masses of the different organs were added up.

From direct measurements, proposed formulas (23), were used to calculate growth indexes by the following derivatives:

$$RGR = (LnW2 – LnW1) / (T2 – T1), (g\ g^{-1}\ d^{-1})$$

$$NAR = (W2 – W1) / (T2 – T1) * (Ln\ FA2 – Ln\ FA1) / (FA2 – FA1), (g\ m^{-2}\ d^{-1})$$

$$CGR: (1/SA) * (W2 – W1) / (T2 – T1), (g\ m^{-2}\ d^{-1})$$

$$FAI = FA / SA, (Adimensional)$$

where: FAI: Foliar area index; FA: foliar area; SA: soil area; RGR: relative growth rate; W: dry weight; T: time; TCC: crop growth rate; TAN: net assimilation rate

The statistical analysis consisted in Confidence Intervals with a signification of P<0,05. Data processing was done with the software package SPSS v. 21 (24), to check differences between sampling, under a totally randomized experimental design.

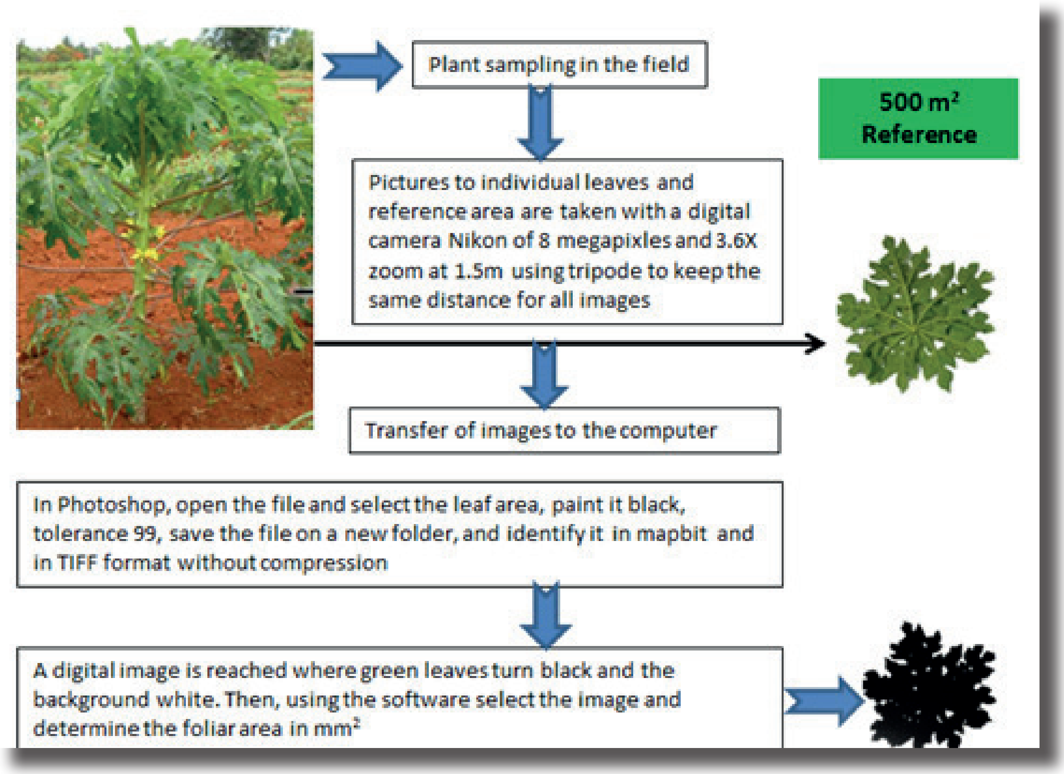
RESULTS AND DISCUSSION

Variables and indicators of growth and development

The accumulation of biomass at the areal part of the plant (leaves and stems) and foliar area in the early growth, transplanting, flowering and fruiting stages showed a similar tendency with the highest values at the reproductive stage (Table).

[E] Trujillo, I. y Cubillas, D. *Crecimiento de plántulas de papayo (Carica papaya L.) en contenedores cuadrados y cilíndricos puestos sobre un lecho de sustrato y plástico [T.E.R.]*, Universidad Veracruzana, Facultad de Ciencias Agrícolas, 2011, p. 44.

[F] Matos, J. *Manual de Manejo de flora Silvestre, para especialistas y técnicos de áreas protegidas*, Empresa Nacional para la Protección de la Flora y la Fauna, 2006, p. 242.



Fugure 1. Methodology to estimate the foliar area in *Carica papaya* L. by morphometric methods

Table. Variables used to determine growth and development indicators in wild papaya and cv `Maradol Roja´

Trials	Time	Number of leaves		Organs	Dry mass (g)		Foliar area (cm^2)	
		Wild papaya	`Maradol Roja´		Wild papaya	`Maradol Roja´	Wild papaya	`Maradol Roja´
				Leaves	0,35 ± 0,10	0,41 ± 0,08		
	Transplanting	11,3±0,8	10,6±0,6	Stem	0,18 ± 0,04	0,16 ± 0,03	107,2 ± 30,4	115,2±18,8
				Total	0,53 ± 0,14	0,58 ± 0,09		
				Leaves	111,8±14,7	97,1±17,5		
Winter 2010	Flowering	35,7±2,4	33,2±1,1	Stem	94,6±13,7	74,7 ± 15,5	12 444,0±860,2	10 404,3±1 099,6
				Total	206,4 ± 28,2	171,7 ±32,9		
				Leaves	139,9 ± 19,1	130,9±14,5		
	Fruiting	39,9±2,3	38,2±1,6	Stem	103,0 ± 21,8	101,7±16,4	14 929,8±902,8	13 175,1±839,1
				Total	243,0 ± 38,8	232,6±30,1		
				Leaves	0,36 ± 0,07	0,48 ± 0,12		
	Transplanting	11,1±,0,5	10,8±0,4	Stem	0,23 ± 0,09	0,20 ± 0,10	116,3±34,8	128,8±20,9
				Total	0,59 ± 0,18	0,68 ± 0,22		
				Leaves	74,7 ±13,7	68,1 ± 13,6		
Summer 2010	Flowering	28,8±0,86	26,4±0,9	Stem	51,6 ±16,7	43,6 ± 11,8	9 732,9±715,2	8 281,7±721,1
				Total	126,3 ±30,4	111,6 ±14,7		
				Leaves	98,7 ±11,5	100,3 ±16,1		
	Fruiting	33,2±1,04	31,8±1,1	Stem	75,7 ± 9,9	69,86 ± 14,5	12 904,7±893,7	11 146,6±773,6
				Total	174,4±21,0	170,2 ±29,7		

Average values data. Signification represents a confidence interval of 95 %

It could be due to the fact that in vegetative stages of annual species, plants produce biomass efficiently and sustained in response to the photosynthetic capacity.

Leaves were the aerial organs that tended to accumulate more biomass which is related to the fact that these increase their compexity and functionality along the crop cycle (25). Moreover, leaves are the main organs of the plant in this stage and most of the resulting photoassimilates of plant metabolism are devoted to their formation and growth (26).

There were no differences between genotypes at transplanting in any of the variables. The foliar area ranged from 107,2 cm^2 in wild papaya to 128,8 cm^2 in cultivar `Maradol Roja´. These values are slightly lower than those previously reached in plants 40 days after seed germination for this cultivar[E], where average values for the foliar area between 144,3 and 154,2 cm^2 (27) were found.

After transplanting, there was an accelerated increase of growth variables in both genotypes till flowering and it continued till fruiting. It happened because in the first stages of the plant, its vegetative growth is notably favored and consequently the increased foliar area is directly proportional to the increased mass in this organ. Experts recognize the intensity of foliar area, plant efficiency in this expansion stage in terms of sun radiation usage (27). Moreover, the time between transplanting and the phenological flowering stage is longer than the rest of the evaluated stages which favored that sun radiation had influenced on the speed of the growth and temperature rates as main conditions for development.

When the total dry mass was compared at flowering and fruiting times, there were no differences among genotypes, but among planting times did. In wild papaya the accumulated value was 206,4 g in winter and 126,3 g in summer, while in ´Maradol Roja´ it was 171,7 g and 111,6 g for equal planting times. It confirmed that the genetic potential of plants and environmental conditions affect plant growth speed. This result coincided with other authors who pointed out that temperature is one of the physical factors of major importance that directly influence on plant growth and length during its vegetative cycle (28).

Total dry mass production is the result of the foliar efficiency in grasping and using sun radiation available during the growth cycle (29). However, this efficiency can be influenced by the quantity of sun radiation, leaf ability for the photosynthesis, the foliar area index, plants arquitecture, respiration, among others, which is summarized in internal growth factors related to the genotype and external factors related to the environment and management practices used during the cycle (30, 31).

In contrast to the dry mass, during flowering and fruiting, the foliar area, in addition to the difference between planting times, also showed differences between genotypes in both periods. It is indicative of a higher sun radiation activity in wild genotypes. Increased foliar area also meant increased dry mass, but to a certain degree where the optimum foliar area index is found (32).

These results revealed that leaves from wild genotypes and those of the cultivar `Maradol Roja´ could be of different forms. It is also possible that the limbo of the commercial cultivar is thicker than wild papaya’s so it influenced more on the dry mass than on the foliar area. It could be due to the adaptation of the commercial cultivar to abundant irrigation, while the wild genotypes tend to accumulate less water in the leaves because of the lack of irrigation in their natural areas. It could be the reason of a thinner limbo. The differences as to foliar areas between winter and summer, both in flowering and fruiting, were due to the fact that in winter plants accumulated more leaves than in summer because they needed more time to flower, since flowering in summer is more precocious compared to winter[G].

Relative growth rate (RGR)

The RGR index is shown in Figure 2. There was a similar behavior between genotypes in the two periods and different evaluation times. RGR tendency was ascendant during the nursery stage, with values from 0,2030 $gg^{-1}d^{-1}$ to 0,2306 $gg^{-1}d^{-1}$ at transplanting in `Maradol Roja´ and wild papaya, respectively. This result was due to an increased foliar area in this period since RGR depends on the dry mass in time, equivalent to the activity on demand (33). Lower values were reached in plants evaluated during the nursery stage of the cultivars `Maradol Roja´ and `Tainung-1´ (34).

After transplanting there was a delay or reduction in the speed of sharp growth till flowering. It continued but less accelerated during anthesis, with the lowest values during fruiting, where 0,0896 $gg^{-1}d^{-1}$ and 0,0950 $gg^{-1}d^{-1}$ were found in winter-10, while in summer-10 the

[G] Rancel, J.; Lobo, J. y Rodríguez, P. *Estudio sobre la fenología y pos¬cosecha de la papaya (Carica papaya, L.) en los cultivares «Sunset», «Baixinho de Santa Amalia» y «BH-65», bajo invernadero de malla en la ver¬tiente sur de Tenerife* [Trabajo (Fin de Carrera)], Escuela Técnica Superior de Ingeniería Agraria, Universidad de La Laguna, Tenerife, 2006, p. 377.

recorded value were 0,0871 $gg^{-1}d^{-1}$ and 0,0990 $gg^{-1}d^{-1}$ in wild papaya and `Maradol Roja´, respectively. It happened because when the dried mass increased, maintenance respiration (Rm) was higher than in the previous stage and therefore TCR decreased.

The results shown in figure 2 are attributed, first of all, to the evaluation times that were not done with similar frequency, so there was a higher difference days wise, between the evaluation made from transplanting till flowering, a stage that coincided with the recovery of the plants after the stress suffered at transplanting and during the dry period.

The RGR decreasing tendency coincided with that of studies made in *Physalis peruviana*, which concluded that RGR is a very sensitive growth parameter to the climatic conditions where the crop developed (35). An important aspect to take into account is that in the phenological development stage of the plant, reproductive organs are more active and demand more biomass which unfavorably influenced on the growth rate of new leaves and stems.

It confirms what was pointed out relative to the correlation between the growth and development process of the different parts of the plant (25). As more active growth is in one part of the plant, the higher will be the demand of available materials, and higher will be growth reduction in other parts.

The total dry mass increased as a function of time in each analyzed interval, was according to TRC in both genotypes which revealed their efficiency as producer of new materials depending on the total photosynthesis and respiration.

Net assimilation rate (NAR)

The net assimilation rate (NAR) shown in Figure 3, had a similar behavior to RGR analysis. There were no differences between wild papaya and cv `Maradol Roja´ at different evaluation times which indicated that photosynthetic efficiency was similar in both genotypes.

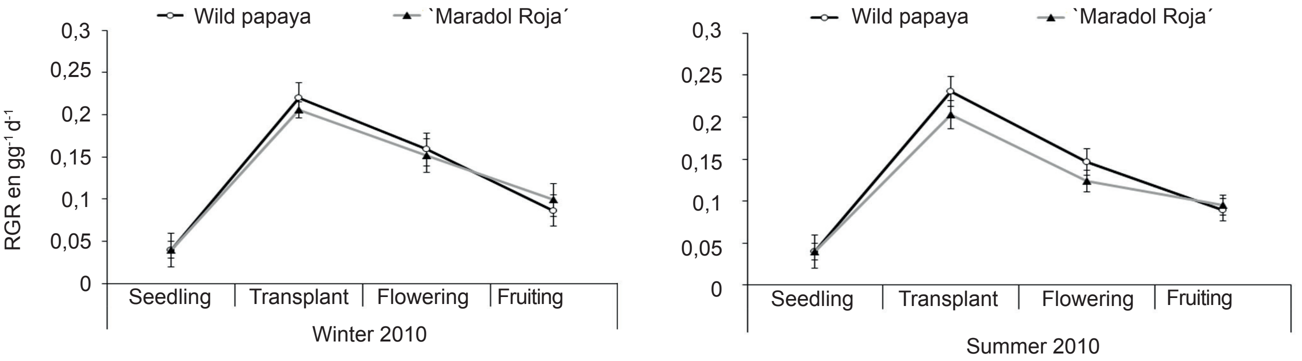


Figure 2. Evaluation of growth and development. Relative growth rate (RGR)

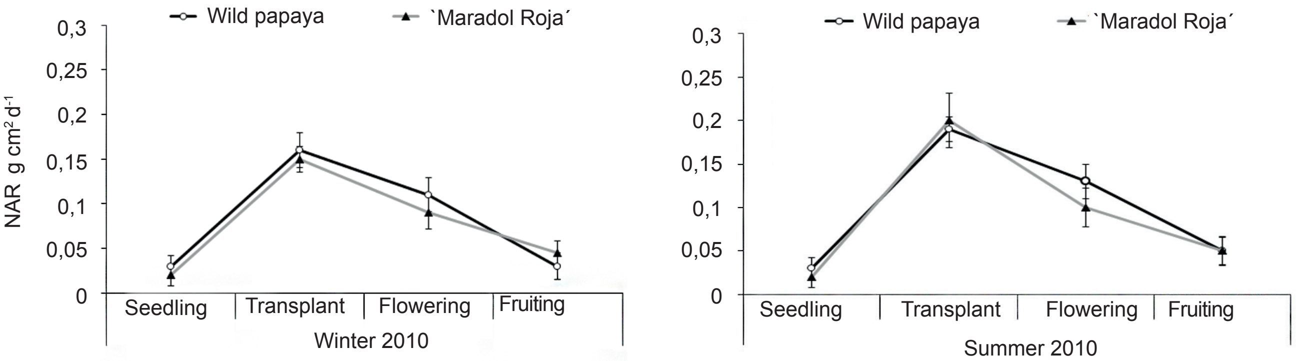


Figure 3. Net assimilation rate of the foliar area of wild papaya genotype and culktivar 'Maradol Roja', evaluated in two sowing periods

Plants efficiency as assimilating system was observed during the nursery stage with ascending tendency till transplanting, which suggested a higher average photosynthetic efficiency when the gain in dry mass per assimilatory tissue unit and per time unit rose, as previously stated (25).

NAR decreased in time in both genotypes till fruiting because the radiation absorption tended to reduce as plant increased its foliar area and its size in general. Therefore, the foliage arquitecture and radiation absorption will be more important in the next plant growth, because as a plant grows up and produces more leaves, the selfshade among them is also increased. It creates a very heterogeneous luminous environment among foliage units that in turn generates a great heterogeneity in photosynthetic yield of the different individual leaves (36).

Consequently, the relationship between growth and foliar area will be less direct for bigger plants with more complex foliage, regarding the commonly observed in studies with small plants. In papaya cultivars it was shown that for the development of nursery seedlings, the light intensity effect, temperature, and foliar selfshade allowed to explain 40 % of the differences in the photosynthetic efficiency of the plants (37).

Foliar area index (FAI)

The results of the FAI are shown in figure 4. It was observed that in both genotypes fthe behavior of the two periods and times of evaluation were similar. `Maradol Roja´ reached extreme values between 2.11 in plants at transplanting in winter and 2,60 in the summer fruiting.

The pronounced ascendance of the foliar area index at transplanting was caused by the fast plant growth in a short time during the nursery stage. In addition, the soil area in the bags is very much reduced in relation to the foliar area that in papaya shows bigger leaves according to their height.

The accumulation of assimilates directly responds to the expansion and duration of the foliar area, so when it increases, maintenance respiration (Rm) also increases so a higher efficiency of the photosynthetic and root systems is needed to send photoassimilates and nutrients to the sinks (34).

However, after transplanting and with a wider spacing, foliar dry mass and foliar area increased which evidenced a higher correspondence between the foliar area and the soil area with influence on the leaf area index reduction during flowering as compared to transplanting with values from 0,19 in summer for `Maradol Roja´ and 0,41 in winter for wild papaya.

From flowering and during anthesis and fruiting the foliar area index tended to slightly increase. It was then confirmed what has been said on the expansion and duration of the foliar area in the first stages to then rise in anthesis and fruit development (38). Foliar development favors sun radiation, one of the key factors for plants for its implication in the photosynthetic process so the differences in the light absorpotion capacity can also lead to important differences in growth and reproduction (39).

Crop growth rate (CGR)

CGR (Figure 5) was ascendant till transplanting in both genotypes, due to the activated growth of the plants in this stage; however, no differences were observed between both planting periods. This result contrasts with those of evaluations made to papaya genotypes (40). These authors found very slow growth rates from mid-November till March, when temperatures were lower, to then go through an accelerated growth when temperatures were higher.

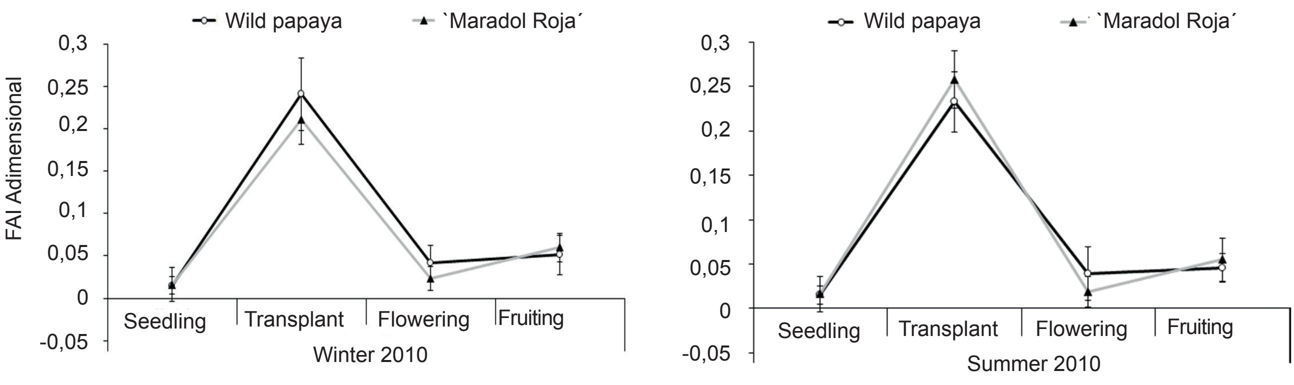


Figura 4. Índice del área foliar del genotipo papaya silvestre y cultivar `Maradol Roja´ evaluados en dos periodos de siembra.

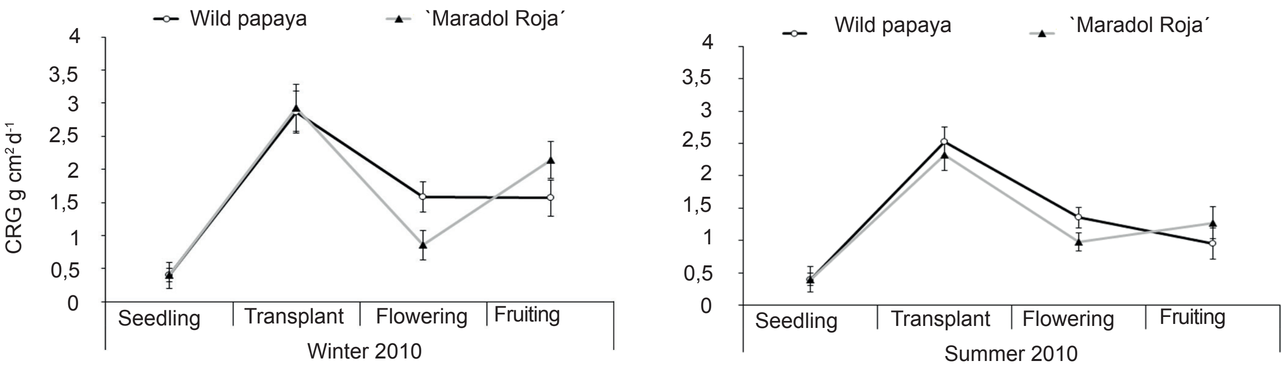


Figure 5. Crop growth rate of foliar area of wild papaya genotype and cultivar 'Maradolk Roja', evaluated in two sowing period

In spite of a greater dry mass and foliar area observed from transplanting to flowering (Table), CGR was lower in this period compared to the nursery stage. Nevertheless, in the flowering stage of both planting times, the wild papaya surpassed `Maradol Roja´. This confirmed that wild genotypes have a higher gain of plant biomass per surface area occupied by the plant in that stage. This is an important aspect, because carbohydrates are synthesized in leaves and they are distributed among the different organs (14) which allow a higher vegetative growth rate that influenced on the crop increased yield.

After flowering and till fruiting, growth speed in CGR was less pronounced in the wild papaya, while in `Maradol Roja´ it tended to increase; however, in the fruiting stage, there were no differences between genotypes for respective planting times.

Under natural conditions, wild papaya genotypes depend on climate so growth is highly depended on the radiation that foliage can grasp and on the conversion efficiency of such radiation into dry matter which in turn depends on the available resources and on the genotype. In these genotypes, the accelerated growth in the early stages allows grasping sun rays and projects a greater shadow area on the soil. Thus, water loss by evaporation is avoided and plants competing for the habitat are controlled.

CONCLUSIONS

- The computer analysis of leaf images taken with a digital camera allowed the simple, fast, and economic determination of the leaf area in the specie *Carica papaya* L.
- Differences found in the foliar area of both evaluated genotypes and TCC during flowering are due to their genetic expression. Although the phenotype was influenced by the planting time, the genetic component was determinant in the behavior of both, as shown by the results. The highest production of foliar area of the wild genotypes and similar behavior of the dry mass and the rest of the evaluated indicators reflects the availability of resources during growth, which are associated to yield. The adaptation of wild genotypes to *ex situ* conditions was evident as well as the possibility of its recommendation for breeding programs looking for new more productive cultivars.

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