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Measurement of fruit color-heterogeneity index and their relation to *Jatropha curcas* L., oil in Colombia

Determinación del índice de color- heterogeneidad de frutos y su relación con el aceite de *Jatropha curcas* L., en Colombia

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Abstract

Jatropha curcas L. is a wild species in the domestication process with an oil and biofuel potential use. The evolutionary-adaptive processes have conferred a reproductive mechanism based on protogyny-geitonogamy, achieving a floral asynchrony addressed to ensure the outcrossing and survival in extreme climate and soil conditions. In the wild, this plant performs a fruit heterogeneity, an unattractive attribute for harvest timing. Therefore, this research aimed to determine the number of harvest, yield and proportion, and physiological states of fruit maturity with the maximum oil quantity and quality. A randomized complete block factorial arrangement of treatments design was performed. The varieties and stages of fruit ripeness, were the factors. A relation of 19: 31: 36: 14 with fruits pericarp colors: green: yellow: yellow-brown: brown, was obtained. The best harvest timing was during the yellow-brown state, which represented the highest oil content associated with high oleic acid and linoleic contents. Nevertheless, for a fruit harvest timing, the use of the epicarp color index is recommended. These results suggest the floral asynchrony breeding traits, requires the ensuring of the fruit homogeneity for mechanical or manual harvesting.

Keywords: Floral asynchrony, epicarp color index, oleic acid, linoleic acid.

Resumen

Jatropha curcas L., es una especie silvestre en proceso de domesticación con potencial para usar su aceite como biocombustible. Los procesos evolutivos-adaptativos le han conferido un mecanismo reproductivo basado en la protoginia-geitonogamia que garantiza la asincronía floral como una forma de asegurar la alogamia y supervivencia en condiciones extremas de clima y suelo. En condición silvestre esta planta presenta heterogeneidad de los frutos, atributo poco atractivo para la cosecha. Por esta razón, se realizó este estudio, para determinar el número de cosechas, rendimiento y la proporción y estados de madurez fisiológica del fruto con la máxima cantidad y calidad del aceite. Se utilizó un diseño de bloques completos al azar con arreglo factorial de tratamientos; los factores fueron variedades y estados de madurez del fruto. Se obtuvo una relación 19:31:36:14 de frutos con el color del pericarpio verde: amarillo: amarillo-café: café, respectivamente. La mejor época de cosecha fue el estado amarillo-café que representó el mayor contenido de aceite asociado con alto contenido de ácido oleico y linoelico. Para la cosecha oportuna del fruto se recomienda utilizar el índice de color del epicarpio. Estos resultados sugieren que el carácter asincronía floral requiere de mejora genética que asegure la homogeneidad de frutos para la cosecha manual o mecánica.

Palabras clave: Asincronía floral, índice de color-epicarpio, ácido oleico, ácido linoleico.

Introduction

The physiological fruit ripening is a process in which physical, biochemical and physiological changes take place and define the characteristics of a final product for either direct consumption or agro-industrial processes. The appropriate harvest timing is a key factor to guarantee the quality attributes and minimize the harvest and postharvest losses. In order to prevent this, harvest indexes are useful tool as a decision making. There are indexes of physical, biochemical and physiological type.

In annual-cycle crops, the identification of the fruit ripening degree is easy, with only a few indexes compared to the continual-cycle crops, which requires a combination of several indexes. In the first case, wheat, barley, soybean, bean and sunflower crops, the grain color and humidity (around 15%) and the harvest timing in terms of days, are useful to decide the appropriate moment for collection (Rondanini *et al.*, 2007; Vallejos, Rondanini, & Wassner, 2011).

In the continual-cycle crops or continuous harvest, it is recommended to combine the physical, biochemical and physiological indexes. In the case of apple (*Malus domestica* Borkh) and pear (*Pyrus communis* L.), the combination of several indicators is useful to describe the phenology (days from blooming to harvesting), fruit size and firmness, sugar content and ethylene concentration. In the case of feijoa (*Acca sellowiana* [Berg] Burret), the plant phenology (120 and 150 days after the flower buds) are correlated with the natural fruit absorption and the fruit shape and size (Quintero, 2012).

In *J. curcas* L., there is a lack of research records about the indexes of the appropriated physiological ripening of the fruit harvest timing. The yellow-brown color of the epicarp (Silva, Santos-Días, Milagres, & Santos-Días; Santos *et al.*, 2012); yellow with brown patches (Dransky *et al.*, 2010); yellow (Garay *et al.*, 2012), and fruit clusters with less than 10% of yellow color (Silip, Tambunan, Hambali & Surahman, 2011), have proven to be useful in order to obtain the highest performance of seed dry matter, oil contents and quality. In other crops, *Ricinus communis* L. (Silva, Martins, Machado, & Nakagawa, 2009), *Peltophorum dubium* (Spreng) (Aquino *et al.*, 2006) and *Caesalpinia echinata* L. (Aguiar, Pinto, Tavares, & Kanashiro, 2007), these same index—color fruit—are useful to determine the harvest timing maturity and the physiological quality.

In the case of new species such as *J. curcas* L., with a potential use in biofuels. The first studies were twenty years ago in Africa and India, and more recent, in America (Henning, 2004). Due to its wild condition, this plant is associated to poor

soils and low precipitation, where the protandry mechanism (69%) and geitonogamy (81%) (Neto *et al.*, 2010), ensure the xenogamy-allogamy (89%), as an adaptive and co-evolutionary strategy to guarantee its survival in extreme conditions. Nevertheless, in order to achieve a production on a commercial and rentable scale, the domestication throughout a plant breeding and the technological mastering of good harvest practices, is already necessary.

Actually, the low performance and fruit heterogeneity is the most limiting aspect of the *J. curcas*. This results in a mechanical harvesting unviable and it is caused by the floral asynchrony which determines the repeated cycles with different states of ripening in a harvest (Henning, 2004).

There is a lack of basic studies that could indicate an accurate harvest interval, performance and appropriate state of the fruit harvest timing in Colombia. The aim of this research was to determine the harvest number by crop cycle and intervals, the fruit performance, the state of fruit ripening and the epicarp color identification to establish the physiological period of maturity on harvest timing, which provides the highest quantity and quality of the *Jatropha* oil.

Material and methods

Location

The research was carried out in the Nataima – Corpoica Research Center, located in the town of Espinal, Tolima department, Colombia at 323 m. a. s. l., 29°C average temperature, 1380 mm annual precipitation, 74% average relative humidity, with a location of 04° 09' north latitude and 74° 53' west longitude.

Type of experiments

Two experiments were carried out in field conditions. The first was established to determine the harvest number and intervals among harvests correlated with crop cycle, the fruit and seed performance and the predominant fruit proportion according to their physiological maturity; the second experiment was addressed to evaluate the usefulness of the epicarp color index in order to recognize the appropriate physiological maturity of the fruit for harvest timing. In both experiments, two varieties of *J. curcas* identified as CJ3 and CJ6 were evaluated.

Experiment 1: Determination of number of harvests, interval among harvests, performance and proportion of fruits according to the maturity state at harvest timing

A field experiment was performed as a base in a randomized complete block design (RCBD) to control the fertility gradient with two varieties (CJ3=high performance and CJ6=low performance) and three replicates. The useful experimental unit was settle out of 20 plants with a sowing distance of 3x2 m and a growing density of 16600 plants.ha⁻¹, the harvest timing of 20 *Jatropha* trees of each useful experimental unit are associated to the CJ3 and CJ6 varieties and the consequently repetition was carried out.

Experiment 2: Fruit heterogeneity

A randomized complete block design (RCBD) was performed in order to control the soil fertility gradient with a factorial treatment arrangement of two factors and three repetitions. The first factor: varieties, which consisted of two varieties from the *Jatropha* Colombian collection (CJ3 and CJ6), and the second factor, the epicarp color associated to the four states of ripening of the predominant fruit founded in the experiment 1. The levels of this factor were: EMF1= 100% color green fruits; EMF2= 100% color yellow fruits; EMF3= 100% color in transition yellow-brown; and EMF4= 100% color brown fruits with fruit dehiscence. The ripening degree of the *J. curcas* L. fruit, was defined by the scale proposed by Dranski *et al.*, (2010), and adjusted by Campuzano, (2015) (Table 1).

Table 1. Color scale of the epicarp fruit for the states of ripening in *J. curcas* L., (Dranski *et al.*, 2010). Adjusted by (Campuzano, 2015).

Fruit state	Epicarp color	Color description	Munsell Scale
EMF1		Green	7, 5 GY 6/6
EMF 2		Yellow	5, 0 Y 8/10
EMF 3		Yellow-Brown	5, 0 Y 8/8
EMF 4		Brown-Dehiscence	5, 0 YR 3/2

A fruit proportion of 19:31:36:14 (EMF1:EMF2:EMF3:EMF4), was performed as a control (EMF5). The experimental unit was 20 plants with a sowing distance of 3x2 m. and a growing density of 16600 plants.ha⁻¹.

Harvest and sample preparation

The fruit from the evaluated varieties assessed corresponds in both experiments— to the sixth harvest (514 days — 17.1 months) after the field conditions establishment. On each experimental unit and repetition 250 fruits from 20 plants were taken; the seed was obtained from the manual discarding of the pulp fruit.

Determined variables

The determined variables are described as following: 1) number of harvests and intervals among harvests expressed in days; 2) fruit and dry seed performance expressed in kg.ha⁻¹, respectively; 3) seed dry matter: determined in 100 seeds throughout the stove method at 36°C, and expressed in grams.100 seeds⁻¹; 4) oil contents, through the chemical extraction using the Soxhlet method (AOAC International, 2007), and expressed in percentage; 5) oleic and linoleic acids determined through Gas Chromatography with a Flame Ionization Detector (GC-FID) and is expressed in percentage.

Statistical analysis

The compliances of the assumptions taken from the variance analysis, the procedures and statistical analysis were ran with SAS, version 9.3™, in order to establish the differences among medians and the Tukey test (P=0, 05) was performed.

Results and discussion

Harvest number and fruit performance

In 17.1 months of harvest, the two varieties of *J. curcas* evaluated in soil and weather conditions of the Espinal-Tolima, Colombia, presented six cycles of fruit harvest. The first fruit harvest timing was at 174 days, this date was correlated as cohort zero; the second was at 255 days and 81 days cohort; the third at 326 days and 71 days cohort; the fourth at 368 days and 42 days cohort; the fifth at 480 days and 112 days cohort; and the sixth at 514 days and 34 days cohort (Table 2).

Table 2. Number of harvests, interval among harvests, fruit and dry seed performance of two varieties (CJ3, CJ6) of *J. curcas* L., in harvest conditions in Espinal, Tolima, Colombia.

Harvest	Harvest days	Interval among harvest days	Performance kg.ha ⁻¹ (CJ3)		Performance kg.ha ⁻¹ (CJ6)	
			Fruit	Seed	Fruit	Seed
1	174	0	154	40	43	12
2	255	81	1653	430	554	144
3	326	71	1759	457	541	141
4	368	42	1513	393	524	136
5	480	112	1244	323	507	132
6	514	34	503	13	63	17
1+6	514	4	6826	1656	2232	582

CJ3=high performance variety; CJ6=low performance variety

The heterogeneity of the days among each harvest interval was: 0, 81, 71, 42, 112 and 34 days, respectively, as a result of the *J. curcas* floral

asynchrony which results in a heterogeneity of the fruit maturity.

The accumulated performance of the CJ3 variety was 6826 kg.ha⁻¹ of fruit, equivalent to 1656 kg.ha⁻¹ of dry seed. Whereas the CJ6 variety, performed 2232 kg.ha⁻¹ of fruit and 582 kg.ha⁻¹ of dry seed. CJ6 variety presented a fruit and dry seed performance superior to CJ3 in 4594 kg and 1074 kg, respectively. Both varieties, performed an ascendant and descendant production behavior; lowest in the first harvest, with a significantly gradual increase in the second and third harvest timing and a gradual reduction from the fourth and fifth harvest.

The highest accumulated performance at 17.1 months obtained in the CJ3 variety was 1656 kg.ha⁻¹ of dry seed, equivalent to 96.8 kg.ha⁻¹ to month, is not enough to represent the balance point in order to obtain economic benefits. In that regard, Rucoba & Munguia (2013), describes a balance point for *J. curcas*, in monoculture in Mexico: 282.6 kg.ha⁻¹.month of dry seed, with a production cost similar to the obtained in Colombia:

\$3000.ha⁻¹ accumulated upon the second year of harvest.





The worldwide information about the real performance of *J. curcas* in commercial condition is speculated; the common denominator is a low performance in India with 0.5 to 1.4 mg.ha⁻¹.year⁻¹, South Africa with a 0.35 mg.ha⁻¹.year⁻¹ and Tanzania with a 2.0 mg.ha⁻¹.year⁻¹ (Gopinathan & Sudhakaran, 2009).

These results allowed to corroborate the performance of *J. curcas* in non-domesticated plant genetic resources and not corroborates the economic balance point to originate recommendations on a commercial level. This low performance is associated to the floral asynchrony phenomenon and the low relation of male: female flowers (30:1). Typical attributes of species still are correlated to a lowest degree of domestication and adaptive development of evolutionary strategies in soil and weather restrictions.

State of fruit ripening

The fruit proportion obtained during the sixth harvest (514 days after the sowing) and classified by epicarp color index in the two varieties, performed the following fruit relation: 18% to 19% green; 30% to 31% yellow; 35% to 36% yellow-brown; and 13% to 14% brown (Table 3).

Table 3. Fruit proportion (%) and standard deviation for the ripening states EMF1=green, EMF2=yellow, EMF3=yellow-brown and EMF4=brown of two varieties of *J. curcas* in harvest conditions in Espinal-Tolima, Colombia.

Variety	EMF1	EMF2	EMF3	EMF4
				
CJ3 (n=300)	18,0±1,2	31,7±1,6	35,7±2,1	14,6±1,3
CJ6 (n=300)	19,6±0,8	30,5±1,2	36,5±2,6	13,4±0,9

This heterogeneous relation of fruit proportion (different states of physiological maturity) and the variation in the number of cohorts among harvests, are associated to the genetic constitution and the condition of wildness of the evaluated varieties. This phenomenon is attributed to the reproductive mechanism of protogyny. These results agreed with (Silip, Tambunan, Hambali, Sutrisno, & Surahman, 2010), who reported the non-uniformity in the blooming and development of fruits in *J. curcas*.

State of fruit ripening and its relation to oil quantity and quality

The variance analysis performed a high significantly differences ($P=0.01$) for oleic acid in the variety factor and for all the variables in the fruit state of ripening factor. In the linoleic acid variable, a statistic differences were detected ($P=0.01$) for the variety interaction by fruit state of ripening. This indicates that the fruit state of ripening factor performed a higher influence in all the evaluated variables with the exception of linoleic acid, which corroborates a differential interaction variety by fruit state of ripening (Table 4).

Table 4. Mean squares for dry matter, oil contents, oleic and linoleic acids of two varieties of *J. curcas* L., by effect of five fruit ripening state in harvest conditions in Espinal-Tolima, Colombia.

Mean square (M.S)					
Variation source	gl	Dry matter	Oil contents	Oleic acid	Linoleic acid
Repetition	2	4.14	0.21	0.21	0.12
Variety (V)	1	0.73 ns	0.056 ns	38.08 **	0.23 ns
Fruit ripening state (FRS)	4	1094.25 **	375.67 **	15.49 **	10.69 **
V x FRS	4	6.57 ns	1.49 ns	0.92 ns	0.42 **
Error	18	1.72	0.40	0.35	0.13
Cv (%)		1.64	2.40	1.42	1.09

ns: non-significant differences; ** Significant difference at 0.01%.

The seed dry matter performed a gradual increase from 59.20 g in EMF1 (green) state to 85.77 g in EMF4 (brown) state with a mass

increase of 26.57 g, attributable to the loss of water during the physiological process of fruit ripening. The highest seed dry matter content was observed in EMF3=92.98 g. The oil content in the *J. curcas* seed by effects of the fruit ripening state, performed an ascendant behavior from the EMF1 state to the EMF3 state, and descendant in the EMF4. The fruit ripening state based on the epicarp color index showed the highest oil content EMF3 with a 33.62% superior and statistically different ($p=0.01$) correlated to EMF1=21.32%, EMF2=26.67 and EMF4=28.97% maturity states. The control EMF5, performed the lowest oil content statistically different ($p=0.01$) to all the studied ripening states (22.18%). The fruit harvest in the states of maturity before, after and close to the EMF3, showed an oil content reductions of 12.30%, 6.95% and 4.65% for the EMF1, EMF2 and EMF4, respectively, and of 11.44% with the control EMF5 (Table 5). In relation to the oleic acid, the highest contents were obtained in three states of fruit ripening EMF2=42.72 %, EMF3=42.38% and EMF4= 42.40%. These values did not show any statistical differences among them ($p=0.01$). The oleic acid contents of EMF5=control, was the lowest record with a 38.92% statistically different from the other evaluated treatments and performed the highest reduction value (3.46%) in relation to EMF3. The fruit harvest in ripening states EMF2 and EMF4 control the reduction in oleic acid contents in relation to EMF3, which not surpass the 3% (Table 5).

Table 5. Average values of oil and oleic acid contents of two varieties of *J. curcas* by effect of five fruit physiological maturity states and their increase or decrease in relation to EMF3 in harvest conditions in Espinal-Tolima, Colombia.

Fruit ripening state	Dry matter g	Oil contents %	Increase or decrease in relation to EMF3	Oleic acid %	Increase or decrease in relation to EMF3
EMF1=green	92.98 a	21.32 d	-12.30	40.71 b	-1.67
EMF2=yellow	85.77 b	26.67 c	-6.95	42.72 a	+0.34
EMF3=yellow-brown	73.62 c	33.62 a	-	42.38 a	-
EMF4=brown	59.20 d	28.97 b	-4.65	42.40 a	-0.02
EMF5 = Control	87.38 b	22.18 d	-11.44	38.92 c	-3.46

Medians with the same letter in a vertical direction are statistically the same (Tukey $P=0.01$).

The performed of the linoleic acid showed a differential response to varieties and fruit ripening state. In the two evaluated varieties (CJ3 and CJ6), the lowest content of linoleic acid was obtained in the ripening states EMF1 and EMF5 control. In the CJ3 variety, a linoleic acid content of 30.90% and 30.43% was obtained, and in the CJ6 variety: 30.73% and 30.30% for the ripening states EMF1 and EMF5 control, respectively. The differential response for this variable was

observed in the ripening state to reach the highest content of linoleic acid. The CJ3 variety reached it in EMF2 (33.03%) contrary to CJ6 variety which obtained it in EMF4 (33.63%). Nevertheless, in both varieties, the linoleic acid values obtained in EMF3 were statistically equal ($p=0.01$) to the maximum value obtained in EMF4 (33.63%) (CJ3=32.73% and CJ6=33.2%) (Table 6).

Table 6. Average and standard deviation of dry matter and linoleic acid contents by effect of the variety factor and physiological states of maturity of the fruit in *J. curcas* under harvest conditions in Espinal-Tolima, Colombia.

Variety	Fruit ripening state	Linoleic acid %
CJ3	EMF1=green	30.90 \pm 0.30 c
	EMF2=yellow	33.03 \pm 0.32 ab
	EMF3=yellow-brown	32.73 \pm 0.15 ab
	EMF4=brown	32.67 \pm 0.40 ab
	EMF5=Control	30.43 \pm 0.40 c
CJ6	EMF1=green	30.73 \pm 0.15 c
	EMF2=yellow	32.76 \pm 0.15 ab
	EMF3=yellow-brown	33.20 \pm 0.65 a
	EMF4=brown	33.63 \pm 0.38 a
	EMF5=Control	30.30 \pm 0.26 c

Medians with the same letter in a vertical direction are statistically the same (Tukey $P=0.01$).

This could indicates the variety interaction x fruit ripening state, which is considerably weak.

Previous studies of morpho-agronomic and lipid characterization of the Colombian *Jatropha* Collection, in which the varieties CJ3 and CJ6 can be found, performed a constitution of oils predominantly oleic-linoleic, with results correlated to the previously reports of *J. curcas* on a worldwide level. The predominant fat acid was the oleic acid (44.62%) in the monounsaturated, the linoleic acid (33.95%) in the polyunsaturated, and in a lowest level, the palmitic (12.41%) and stearic (7.43%) in the saturated acids (Campuzano *et al.*, 2015). For biofuel applications, the predominant lipid constitution was oleic-linoleic, the first fat acid confers the biodiesel of the *J. curcas* a better performance in cold flow properties if compared to the palm oil biodiesel which exhibited bad cold flow properties.

These results, considered the first obtained for this species in Colombia, agreed with the obtained by Santos *et al.* (2012), in *J. curcas* and Silva *et al.* (2009), in castor-oil plant (*Ricinus communis* L). The first authors mentioned the yellow-brown fruit ripening state in *J. curcas*, performed the highest performance in seed and oil dry matter. The second authors, in castor-oil plant, a species relative to *Jatropha* sp., performed a similar behavior in the definition of fruit ripening state for harvest timing. For the biodiesel industry, the most relevant factors at the time of buying raw material for processing is the quality and oil

contents determined by the lipid profile, mainly the oleic acid. Due to the above mentioned, the appropriate harvest timing of the *Jatropha* fruit based on the color index of the ripening states EMF3, represents the highest oil quantity, a high content of oleic acid, which is the main acid associated to the best characteristics of *J. curcas* oil for biodiesel.

Complementary studies are suggested in relation to storage time of the harvested fruit and its acidity, phosphorus contents and oil oxidative stability correlated with the effect of the fruit variety and ripening state factors.

Conclusion

The *J. curcas* harvest for the CJ3 and CJ6 varieties in their wild condition without domestication with floral asynchrony in Espinal-Tolima, carried out at 514 days (17.2 months) after the field conditions establishment, presented four states of fruit ripening identified by the pericarp color as following: EMF1 (green); EMF2 (yellow); EMF3 (yellow-brown) and EMF 4 (brown) in an adjusted proportion of 19:31:36:14, respectively.

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