

Article

# Productivity and Oil Content in Relation to *Jatropha* Fruit Ripening under Tropical Dry-Forest Conditions

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**Abstract:** *Jatropha* is promoted as a pro-poor bioenergy plant, while basic information about its productivity, age of maximum production, and oil content are missing. This study aims to determine the seed yield (dry weight) for three INIAP elite *jatropha* accessions, and to evaluate the changes in physical and chemical seed traits at the different fruit ripening stage in a split-plot design. Maximum seed production occurred four years after planting for the accessions CP041 and CP052, while for accession CP054, it occurred after the first year. CP041 was the most productive, with a mean of 316.46 g tree<sup>-1</sup> year<sup>-1</sup> ( $\pm 76.50$ ) over the 8-year study period. No significant differences in oil content were found among accessions, fruit ripening stage, and their respective interactions. Seed moisture content decreased drastically as the fruit ripening stage increased, from 40.5%  $\pm$  1.0% at fruit ripening stage 1 (greenish-yellow) down to 13.8%  $\pm$  0.4% at fruit ripening stage 4 (black-brown). No significant differences in seed weight were found among accessions, but it decreased as maturation progressed. Yellow fruits (stage 2) were the heaviest (62.4 g  $\pm$  1.5 g) and the black-brown fruits the lightest (44.3 g  $\pm$  1.9 g). The oil content (%) increased with seed weight up to the point of 58.3 g, but then decreased for heavier seeds.

**Keywords:** harvesting point; *jatropha* accessions; seed productivity; seed humidity; seed oil content

## 1. Introduction

*Jatropha curcas* L. (in the following: *jatropha*) is a shrub or small tree belonging to the tribe Joannesieae of the family *Euphorbiaceae*, adapted to arid zones, with potential use for biofuel production. *Jatropha* is commonly used in the Manabí province, Ecuador, as living fence for pasture division [1]. *Jatropha* had often been classified as an ideal “pro-poor” crop, due to its potential value for seed oil production in marginal and degraded areas. Consequently, substantial public and private investment has been made available for *jatropha* plantations in marginal lands [2]. Nonetheless, it has become clear that the requirements to obtain economically viable seed yields have been underestimated, and therefore, many investments have been withdrawn [3–5]. Despite this reversal, production derived from *jatropha* under favorable growing conditions continues to be a concept receiving considerable political and commercial interest [6,7], and efforts are being made to estimate the biomass production so that small *jatropha* producers could sell the carbon sequestration in the trees under the emissions trading scheme of the Clean Development Mechanism (CDM) of the Kyoto Protocol [8]. For example, in Ecuador, the Ministry of Electricity and Renewable Energy (Ministerio de Electricidad y Energía Renovable, MEER), with the support of private investors, implemented the “*Jatropha* for Galápagos” Project in 2011. The main objective of this project is to replace petro diesel by *jatropha* oil through the agro industrial development of *jatropha* in the continent [1]. The selected province is Manabí, which belongs to the tropical, dry forest zone [9], and focusing on smallholders.

Determination of seed productivity using long-term field data is crucial for the management of *jatropha* plantations [10]. When regional or local data on seed productivity is missing, information from the literature is commonly used to evaluate the economic and financial feasibility of *jatropha* plantations [10]. However, estimates of *jatropha* production used in several economic studies range between 3000–7000 kg ha<sup>-1</sup> year<sup>-1</sup> [10]. In Ecuador, Rade et al. [2] observed a huge seed production variation during seven years’ data collection of the INIAP accession CP041 planted in *jatropha* live fences in Manabí, Ecuador (average of 243.32 g tree<sup>-1</sup> year<sup>-1</sup>). In a *jatropha* pure plantation under tropical dry forest conditions, Cañadas et al. [1] reported an average *jatropha* dry seed productivity of 283.20 g tree<sup>-1</sup> year<sup>-1</sup> with 1677 trees per hectare for the INIAP accession CP041, but also with a broad variation in productivity from one year to the next. In addition to year-to-year variation in environmental factors, plantation age influences seed production. According to GTZ [11], *jatropha* plantations reach maturity at about eight years of age. Van Eijck et al. [10] highlighted that the *jatropha* production horizon is too short (10 years or less) to be able to reliably assess medium and long-term economic viability of *jatropha* plantations. However, a perspective on *jatropha* productivity in Ecuador was presented by Rade et al. [2], who found a negative covariance between *jatropha* dry seed production and time in a live fence for the INIAP accession CP041 (−991.35) and for traditional *jatropha* (−715.00) during seven years’ field observations.

Moreover, *jatropha* oil content in seeds is known to vary between 40% to 60%, with protein content varying from 10% to 30% (although it is not usable due to the presence of phorbol ester and curcin contents) [12]. *Jatropha* fruits show different maturity degrees within the same tree as a result of a continuous fructification process [13]. Since physiological changes occur during the maturation stages [14], harvesting the fruits at an adequate maturation stage is an important factor determining seed quality and oil content. This information is essential for planning harvesting and processing, with the aim of optimizing oil extraction [15]. In addition, genetic variation of the lipid content of *jatropha* seeds has been found among genotypes [16]. Oil content and moisture change with time, and affect the seed quality and behavior in response to environmental changes, especially relative humidity [17]. Thus, it is important to evaluate changes in physical and chemical properties, including oil and moisture content, and dry weight of *jatropha* seed along the maturation process. These data are not only essential for harvest timing and equipment, but also for seed processing and storage [18].

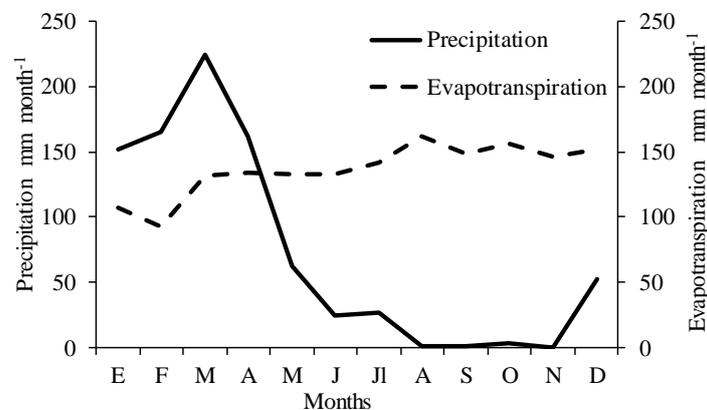
The productivity of mature *jatropha* stands is poorly described under tropical dry forest conditions. Moreover, determination of optimum seed quality in relation to oil content during the *jatropha* fruit ripening process is of utmost importance to establish the optimum time for fruit harvesting in *jatropha*.

The objective of this study was to evaluate the productivity and physical changes at four fruit ripening stages for three INIAP elite *jatropha* accessions.

## 2. Materials and Methods

### 2.1. Study Area

The study was conducted at the Portoviejo Experimental Station (EEP) of the National Institute of Agricultural Research (INIAP) during the period from August 2009 to December 2017. The geographical coordinates are 0°01' S and 80°23' W, sector Lodana, canton Portoviejo, province of Manabí. The EEP is located at 47 m.a.s.l, with an annual mean temperature of 26.4 °C and average annual rainfall of 798 mm, with a large year-to-year rainfall variability, 78% average relative humidity, and a total sun-light sum of 1159 h year<sup>-1</sup>. Figure 1 shows the monthly precipitation along with potential evapotranspiration, averaged over the experiment's period. A surplus of water generally occurs in the course of January to March, while there is a water deficit the rest of the year.



**Figure 1.** Water balance in the INIAP-EEP, 2009–2017. The solid line represents the monthly average of precipitation and the dashed line the potential evapotranspiration.

### 2.2. Soil Conditions

A soil analysis for the study area was carried out in the INIAP-EEP (Table 1). The soil has a neutral pH, with low levels of Nitrogen, Zinc, and Iron, medium levels of Boron, and high levels of Phosphorus, Potassium, Calcium, Magnesium, Copper, and Manganese.

**Table 1.** Soil chemical properties in the study area, INIAP-EEP, 2009.

pH	NH <sub>4</sub>	P	Zn	Cu	Fe	Mn	Zn	B	K	Ca	Mg
	(in ppm)								(in meq 100 mL <sup>-1</sup> )		
7.3	17.12	23.01	1.82	7.14	12.11	23.75	1.82	0.92	2.07	20.90	4.02

### 2.3. Preparation of Experimental Site

The land was prepared by mechanized clearing, ploughing, and harrowing. *Jatropha curcas* L. cuttings were collected from the mother trees. Three INIAP *jatropha* accessions (CP041, CP052 and CP054) were used in the present study. Diammonium phosphate fertilizer (18-46-0) was applied at a dose of 50 g per planting hole before cuttings were transplanted. The plantlets were planted in August 2009 as 70-day-old bare root transplants. Potassium chloride was subsequently applied, at a dose of 4 g per tree, 30, 90, and 120 days after transplanting. Weeds were cut manually, once a month with a machete, and Igran<sup>®</sup> liquid herbicide (Nufarm Australia Limited: Melbourne, VIC, Australia) was applied by spraying every 4 months, at a dose of 200 mL per 20 L of water. After *jatropha* establishment, no fertilization, or pest or mite controls were provided.

#### 2.4. Plot Sizes and Determination of *Jatropha* Productivity

A split-plot design with three replications was used. The experiment covered a total of 2304 m<sup>2</sup> (48 m × 48 m). The three INIAP *jatropha* accessions (INIAP CP041, INIAP CP052, INIAP CP054) were assigned to the main plots (768 m<sup>2</sup>), and the fruit ripening stage was considered as four sub-plots (64 m<sup>2</sup>) which were located in each of the main plots. The experimental unit (each sub-plot) included a total of 16 trees at a spacing of 2 × 2 m. From October 2009 to December 2017, *jatropha* fruits were harvested monthly from four trees in each sub-plot. Seed weight was measured with a precision balance after removing the pulp from the yellow fruits, extracting the seeds and drying them in a convection oven at 60 °C until constant weight.

#### 2.5. Determination of Seed Weight, Moisture, and Oil Content at Different Fruit Ripening Stages

In April 2012, when *jatropha* stands were three years old, fruits at several fruit ripening stages were harvested in order to determine the relationship between fruit ripening and seed characteristics, such as seed weight, moisture, and oil content. Once collected from the research plots, fruits were taken to the laboratory, where they were separated visually according to their ripening stage. Four fruit ripening stages were distinguished in the analysis: (a) early maturity (greenish-yellow fruits); (b) physiological maturity (yellow fruits); (c) over maturity (mottled-yellow fruits); and (d) senescent (black-brown fruits). After fruit separation, the fresh weight of 100 seeds per fruit ripening stage and replication were weighed with a precision balance.

To determine seed moisture, a sample of 100 seeds from each replication was sun-dried for 30 days and then dehydrated by the standard hot air oven method at 105 °C ± 10 °C for 24 h to obtain the dry weight [19]. The moisture content was estimated as the weight loss (fresh weight - dry weight) of the sample, divided by the dry weight, expressed in percent. The oil content was determined for each seed sample by the solvent extraction method. The seeds were ground and placed in an extraction thimble. The oil was extracted using a Soxhlet apparatus with hexane for 6 h. The solvent material was evaporated with a rotary vacuum evaporator, and the remaining *jatropha* oil was weighed. The oil content of the seed was expressed as percent of dry matter mass. All the analyses were done in the seed laboratory of INIAP-EEP.

#### 2.6. Statistical Analyses

Analysis of variance (ANOVA) for seed dry weight, seed moisture, and oil content was performed with the MIXED procedure, using SAS software (V 9.4, SAS Institute Inc., Cary, NC, USA), and mean values were compared by a post-hoc Tukey's multiple comparison test. A significance level of 0.05 was assumed. The following linear model for the split-plot design was used:

$$Y_{ijk} = \mu + B_i + A_j + B_i \times A_j + R_k + A_j \times R_k + \varepsilon_{ijk}$$

where  $Y_{ijk}$  is the value of the seed sample in the  $k$ th sub-plot of the  $j$ th main-plot in the  $i$ th block;  $\mu$  is the population mean;  $B_i$  is the random effect of the  $i$ th block;  $A_j$  is the fixed effect of the  $j$ th accession;  $B_i \times A_j$  is the random effect of the main-plot error;  $R_k$  is the fixed effect of the  $k$ th ripening stage;  $A_j \times R_k$  is the fixed effect of the interaction between the  $j$ th accession and the  $k$ th ripening stage; and  $\varepsilon_{ijk}$  is the experimental error.

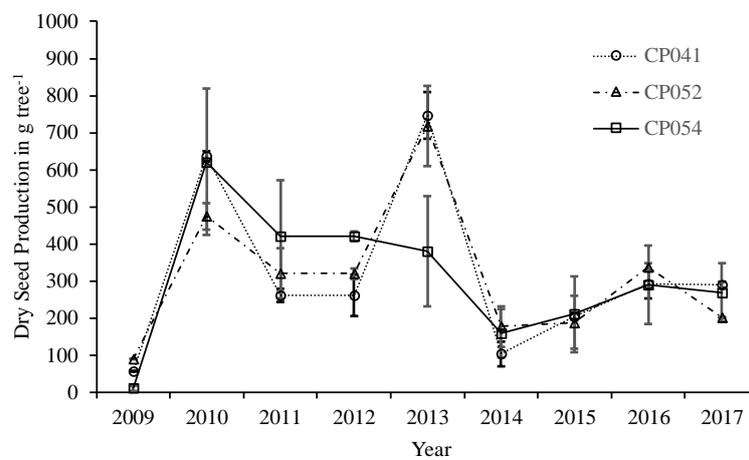
To determine the relationship between oil content (%) and seed weight for the seed samples harvested in April 2012 ( $n = 36$ ), linear, quadratic and two-segment piecewise linear regression models were evaluated with the GLM procedure of SAS software, considering their respective coefficient of determination ( $R^2$ ) and mean square error (MSE). For piecewise linear regression, the optimal break point model (with the lowest MSE) was found before comparisons were made with the linear and quadratic regression models.

### 3. Results

#### 3.1. *Jatropha* Seed Productivity

The dry seed production per tree from October 2009 to December 2017 is shown in Figure 2. Three months after the *jatropha* plantation, the fruit production began. The maximum *jatropha* dried seeds production occurred at four-year-old plantations for the accessions CP041 and CP052, while for accession CP054 it occurred after the first year.

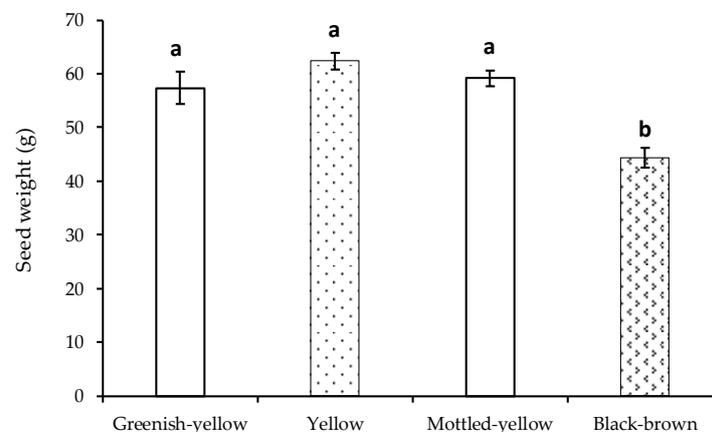
*Jatropha* accession CP041 was the most productive, with  $316.46 \pm 76.50$  g tree<sup>-1</sup> year<sup>-1</sup> (here and in the following: mean followed by its standard error), taking into account all harvests from the early fruits in the first year up to the harvest at 8 years of age. The *jatropha* accession CP052 obtained an average of  $314.02 \pm 63.19$  g tree<sup>-1</sup> year<sup>-1</sup> and CP054  $308.74 \pm 59.07$  g tree<sup>-1</sup> year<sup>-1</sup>.



**Figure 2.** Dry seed production per tree for three *jatropha* INIAP accessions during the years 2009–2017 at INIAP Portoviejo Research Station, Ecuador. Vertical bars indicate the standard error.

#### 3.2. Seed Dry Weight and Fruit Ripening Stage

There were no significant differences in seed weight among *jatropha* accessions or for the interaction accessions  $\times$  fruit ripening stage, but seed weight was significantly affected ( $p < 0.001$ ) by fruit ripening stage (Table 2). Tukey's (alpha value of 0.05) multiple test showed two distinctive group ranges of seed ripening. The first was the yellow fruits, being the heaviest, with  $62.4$  g  $\pm$  1.5 g (for 100 seeds), and the black-brown fruits the lightest, with  $44.3$  g  $\pm$  1.9 g (Figure 3).



**Figure 3.** Dry seed weight (100 seeds) at four *jatropha* fruit ripening stages. Vertical bars indicate the standard error. Different letters indicate a significant difference.

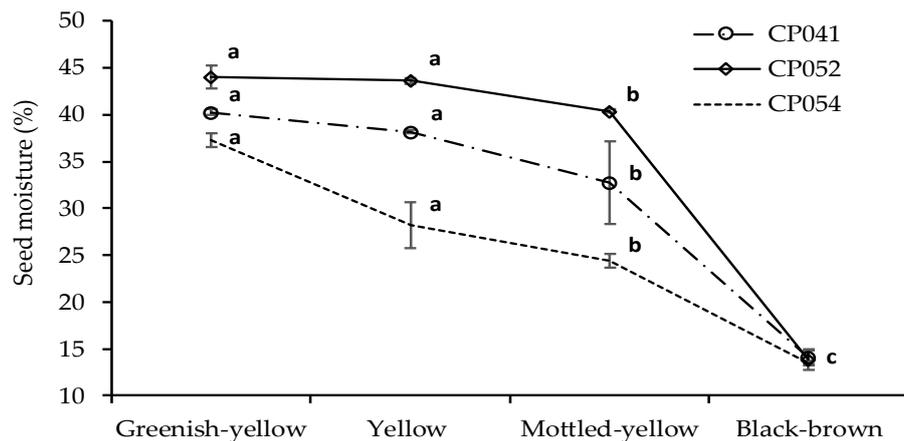
**Table 2.** Significance (*p*-value) of effects from *Jatropha* accessions and fruit ripening stage on seed dry weight, moisture content, and oil content in a *Jatropha* plantation at Portoviejo, Ecuador.

Effects	Source of Variation ( <i>p</i> -Value)		
	Seed Weight	Seed Moisture	Oil Content
Accession	0.9688	<0.0001	0.1497
Fruit ripening stage	0.0002	<0.0001	0.3239
Accession × fruit ripening stage	0.9206	<0.0001	0.2575

### 3.3. Seed Moisture Content and Fruit Ripening Stage

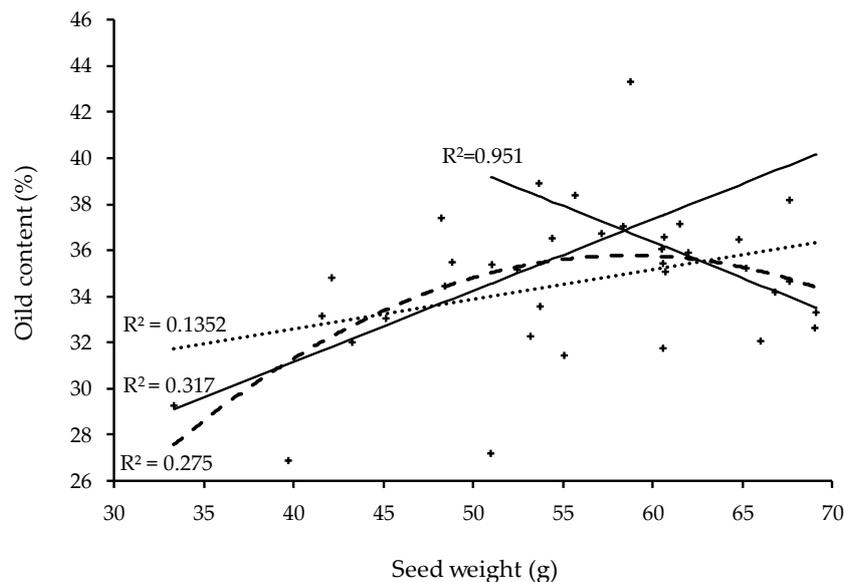
Highly significant differences ( $p < 0.001$ ) in seed moisture content were found among *Jatropha* accessions, fruit ripening stage, and the interaction of these factors (Table 2). A Tukey's multiple comparison test showed three ranges of fruit moisture content among accessions. Accession CP052 had the highest average moisture content with  $37.2\% \pm 4.1\%$ , followed by CP041 with  $31.3\% \pm 3.3\%$ , and CP054 with  $25.8\% \pm 3.0\%$ .

As expected, fruit moisture content decreased as fruit ripening advanced, from an average value of  $40.53\% \pm 1.0\%$  at the initial stage (greenish-yellow fruits), down to  $13.81\% \pm 0.4\%$  at the senescent stage (black-brown fruits). Physiologically mature and over-mature fruits still have moisture contents over 30%, on average (Figure 4).

**Figure 4.** *Jatropha* seed moisture variation between *Jatropha* INIAP accessions along the fruit ripening stages. INIAP Portoviejo Research Station, Ecuador. Vertical bars indicate the standard error. Different letters indicate a significant difference.

### 3.4. Oil Content and Fruit Ripening Stage

No significant differences in oil content were found among accessions, fruit ripening stage, or the interaction of these factors (Table 2). Mean oil content varied from  $36.54\% \pm 1.45\%$  (accession CP041) to  $33.48\% \pm 1.08\%$  (accession CP052). Among fruit ripening stage, it varied from  $36.01\% \pm 1.60\%$  (yellow stage) to  $33.31\% \pm 1.10\%$  (black brown stage). The coefficients of variation (a measure of the experiment's validity) for main plots (9.92%) and subplots (8.64%) are acceptable for field experiments. One component that explains the variation in seed oil content in *Jatropha* is seed weight (Figure 5). However, the relationship was not as simple as expected; the linear regression model based on seed weight explains only about 13.5% of the variation in seed oil content expressed as percentage of seed mass (Table 3). The quadratic regression model showed a somewhat better fit, with an  $R^2 = 0.275$ , indicating that seed oil content (%) has a maximum value around 58 g of seed mass, decreasing at both sides of this point. The optimal two-segment piecewise linear regression model, however, showed the best fit, with an  $R^2 = 0.317$  and the lowest MSE (2.72).



**Figure 5.** Linear, quadratic, and two-segment piecewise linear regression models for oil content on seed weight of *jatropha* seed from three elite accessions. INIAP Portoviejo Research Station, Ecuador.

**Table 3.** Parameter estimates for linear, quadratic, and two-segment piecewise linear regression equations of oil content ( $y$ ) on seed weight ( $x$ ) of *jatropha* seed samples from three elite accessions. INIAP Portoviejo Research Station, Ecuador.

Model	Equation	R <sup>2</sup>	CV (%)	MSE	p-Value	b <sub>0</sub> ± (se)	b <sub>1</sub> ± (se)	b <sub>2</sub> ± (se)
Linear	$y = b_0 + b_1x$	0.135	8.695	3.012	0.02	27.441 (3.164)	0.129 (0.056)	n.a.
Quadratic	$y = b_0 + b_1x + b_2x^2$	0.275	8.082	2.800	0.01	−8.218 (14.444)	1.499 (0.546)	−0.013 (0.005)
Piecewise <sup>§</sup>	$y = b_0 + b_1x + b_2(x - t)(x_2)$	0.317	7.845	2.718	0.001	18.797 (4.082)	0.309 (0.079)	−0.617 (0.208)

<sup>§</sup> For piecewise regression model,  $t = 58.8$ , is the optimal break point and  $x_2$  is an indicator variable ( $x_2 = 0$  when  $x \leq t$ ;  $x_2 = 1$  when  $x > t$ ).

The optimal piecewise linear regression model had a break point at 58.8 g of seed mass. Below this point, seed oil content is positively related to seed mass, but beyond the break point, the relationship becomes negative, so seed oil content decreases as seed mass increases (Figure 5).

## 4. Discussion

### 4.1. *Jatropha* Maturity and Productivity

The early study undertaken by Jongschaap et al. [20] established that the maturity of *jatropha* as a crop depends on its geographical location, i.e., radiation and temperature levels, resulting in higher net primary production potential in some in comparison to other latitudes. In general, these plantations can reach their maturity and maximum production 3–4 years after planting. This information on *jatropha* stand maturity is comparable to our results in this study. Under dry forest conditions in the province of Manabí-Ecuador, seed production of *jatropha* declined after four years for INIAP *jatropha* accessions CP041 and CP052. Nevertheless, INIAP *jatropha* accession CP054 reached maximum production 1.4 years after planting. Such *jatropha* behaviour was observed in rain fed marginal lands of Uttar Pradesh (India), where the best dry seeds production was in the first year, with a yield between 3.2 to 4.1 t seeds ha<sup>−1</sup> [21].

These results do not agree with data provided by Euler and Gorriz [22], and Achten et al. [23], who observed that *jatropha* plantations show considerably lower dry seeds production at younger ages due to the inefficient interception of radiation, and therefore, the distribution of dry matter to permanent biomass instead of harvestable parts such as fruits and seeds. Our results also differ from the eight years required by *jatropha* to reach maturity in Kenya, as observed by GTZ [11].

Jongschaap et al. [20] emphasized that a decrease in *jatropha* seed productivity has been reported for aging plantations. What is still unclear is whether or not this is a general phenomenon. The study conducted by Cañadas et al. [1] and Rade et al. [2] showed a decreasing production of seed for INIAP *jatropha* mono-plantation and a local *jatropha* material in live fences, Manabí, Ecuador.

The seed production estimated in this study for *jatropha* is similar to that obtained for *jatropha* stands under 6 years of age in other regions of the world (Table 4). Cañadas et al. [1] showed that, after having reached a maximum, *jatropha* production decreases over time. These results were accompanied by a high inter-annual variation in dry seed production, which was not related to the mean annual precipitation. Rade et al. [2] calculated a 7-year average of 0.24 kg tree<sup>-1</sup> year<sup>-1</sup> ( $\pm 0.06$ ) for *jatropha* INIAP CP041 accession in live fences, while the local *jatropha* varieties reached an average production of 0.18 kg tree<sup>-1</sup> year<sup>-1</sup> ( $\pm 0.03$ ) with comparatively little variation in dry seed production. Site-specific knowledge about the development of seed production of *jatropha* trees is fundamental for estimating their economic viability. Projections of seed production in the literature for more mature *jatropha* stands often lack sound scientific bases, or contain wrong assumptions [2].

**Table 4.** Published data for seed productivity of *jatropha* plantations from different countries.

Country, Region	Age of <i>Jatropha</i> Plantation in Years	Productivity (in kg tree <sup>-1</sup> )	References
Brazil	1	0.4	Saturnino et al. [24]
Guatemala	1	0.8	Ouwens et al. [25]
Indonesia	1	1.6–2.0	Ouwens et al. [25]
Indonesia	1	1.8	Manurung [26]
India, Pradesh	1	2.4	Lal et al. [21]
India	1.25	1.7	Achten et al. [27]
India	2	1.5	Ouwens et al. [25]
Mali, Digin	2	0.3	Achten et al. [27]
India	2.5	0.8	Ghosh et al. [28]
India, Rajasthan	2.5	0.1–0.5	Achten et al. [27]
Kenya	2–3	<1.0	Iiyama et al. [29]
Tanzania, Arusha	3	0.5–2.0	Messemaker [30]
Nicaragua	5	4.5	Heller [31]
Ecuador, CP052, 041, 054	6	0.2–0.3	Cañadas et al. [1]
Ecuador, CP041	7	0.2	Rade et al. [2]
Ecuador, CP041, 052, 054	8	0.1–0.3	Present study

*Jatropha* stands in the present study had received minimal care in relation to plantation maintenance. This could significantly affect the productivity potential and sustainability of the *jatropha* plantation. However, most small farmers do not have alternatives, and apply sub-optimal management practices [29], so the productivity shown in this research would be a real scenario for *jatropha*, when used in a pro-poor crop program.

#### 4.2. Seed Dry Weight

No statistical significance was detected in relation to seed weight of the three INIAP *jatropha* accessions investigated in the present study. This result contrasted with those reported by Ginwal et al. [32], who found statistical differences ( $p < 0.05$ ) in relation to seed weight and seed size of diverse *jatropha* provenances of Central India. Similar results were obtained by Kaushik [33] in Andhra Pradesh, India. In the present study, however, a high statistical difference in seed weight was found across the various fruit ripening stages. This corresponds with the results obtained by Kaushik [33].

Rondanini et al. [34] emphasized the need to determine how temperature during seed filling could affect several characteristics of *jatropha* fruit. Not only the fruit ripening stage influences seed weight; the environmental conditions throughout the various fruit ripening stages also do

so, and for this reason, a broad variation in seed weight (from 32.6 g to 75.2 g) was found in Argentina [13]. Seed weights found in our study are similar to those obtained in the province of Andhra Pradesh, India by Rao et al. [16] in accessions CRDJ1 (77.4 g), CRDJ20 (78.3 g) and CRDJ6 (79.1 g). While Subramanyam et al. [35] reported a seed weight between 49.3 g and 74.2 g and Rathbauer et al. [36] between 49.3 g and 74.2 g and this broad variation was attributed to the diverse agro-ecological growth conditions and genetics. Wani et al. [37] found that seed weight for 100 seeds in Indian *jatropha* accessions varied between 44 g and 77 g. In Ecuador, Cañadas et al. [1] recorded an average weight of  $72.6 \text{ g} \pm 0.77 \text{ g}$  for 100 seeds in seven *jatropha* elite accessions of INIAP in Portoviejo-Manabí.

#### 4.3. Seed Moisture Content

According to Silva et al. [14] seed moisture content is not a good indicator of physiological ripening, since this parameter can vary among genotypes. In fact, seed moisture content varied widely among the *jatropha* accessions tested, from 25.8% in INIAP CP054 to 37.2% in INIAP CP052. Statistical differences were also identified in seed moisture contents among the fruit ripening stage, which was negatively and significantly correlated. A clear gradient of moisture content as ripening advanced was established. A similar seed moisture content decline was described by Dias et al. [38] and Dias et al. [39]. The rapid humidity reduction is proportional to the reserve deposit compounds, which to a large extent replace the space occupied by water. Finally, water reduction occurs by the search for hygroscopic equilibrium between the seed water content and the environmental relative humidity [40]. The moisture content varies in relation to the maturation and ripening of the *jatropha* fruit with high water content in the physiological maturity stage and low moisture content in the senescent stage [40].

#### 4.4. Oil Content

The best *jatropha* INIAP accessions were collected from Manabí province (Ecuador) for further testing [1]. *Jatropha* local accessions had adapted to a wide range of edaphic and ecological conditions, suggesting that a considerable amount of genetic variability exists to be exploited for potential oil production. Osorio et al. [41] found a higher genetic variability of *jatropha* in Central American accessions compared to Asian, African, and South American accessions. Nevertheless, a variation of oil seed concentration was reported by Rao et al. [16] ranging between 29.8%–37.1%, by Subramanyam et al. [35] between 17.1%–38.8%, and by Kaushik et al. [42] 28.0%–38.8% from different genotypes evaluated under the same environmental conditions. Among INIAP's *jatropha* accessions, no statistical differences were found in seed oil content; mean values varied by only 3% among the evaluated accessions. The small variation in *jatropha* oil content found in the present investigation is not sufficient as a viable selection option at a very early stage (germplasm collection) form seed base material.

Singer et al. [43] mentioned that seed oil concentration does not only depend on the genotype, but is also affected by the environmental conditions during grain filling, i.e., mainly temperature, that modifies seed oil concentration and the fatty acid composition through changes in grain filling dynamics and biosynthetic activity. *Jatropha* oil is accumulated during the last third of the seed filling period [44], so a shortening of grain filling duration should lead to lower oil concentrations. Furthermore, changes in seed weight generated by different temperatures can affect seed oil concentration through changes in the seed coat-kernel relationship. Such seed weight variation was detected, but only through the fruit ripening stage.

Fruit color has been associated as a visual indicator of the fruit ripening stage. In the current research, no statistical differences were found in oil content between fruit ripening stages. This fact has a practical application. The difference in price between a quintal (45 kg) *jatropha* fruit for US \$10 and *jatropha* seed for US \$12, as paid in Manabí province, is currently not a sufficient incentive to invest more labor which is necessary for the sale of seeds, because this would increase production

costs excessively [2]. The harvest timing of small producers within the project “*Jatropha* for Galápagos” would be the yellow fruit ripening stage, due to the greater weight.

The oil content fluctuated from 33.3% in the dry fruits (fruit ripening stage 4) to 36% in yellow fruits (fruit ripening stage 2). Nevertheless, Silva et al. [14] had associated the *jatropha* fruit color with the oil content and germination power of seeds. The oil contents from *jatropha* INIAP accessions is comparable with the values reported by Wassner et al. [13] in Argentina, under subtropical conditions and precipitation of 1005 mm year<sup>-1</sup>, with a maximum seed oil content of 38.7% ± 0.6% and a minimum of 19.6% ± 1.8%. Wani et al. [37] for Jammu and Kashmir State in Northwest India obtained an oil concentration from 27.8% to 37.9%. Kaushik et al. [41] found an oil content range from 28% to 39% in the Haryana-India accession. Rao et al. [16] established between 30% to 37% of oil contained in Andhra Pradesh, India accessions. Other referential data on oil content is reported by Srivastava et al. [45], who observed values between 17.1% for accession IC5660864 to 34.5% for the accession IC558231 in New Delhi and China, and between 34.1% for accession S1 and 55.5% for accession S2 [46]. Higher oil content (61%–64%) was found in *jatropha* seeds coming from Malaysia, Indonesia, and Thailand [47]. In the Latin-American context, the *jatropha* oil content reported in Colombia with *jatropha* variety Brazil tested in two areas, varied just slightly from 37% in Vichada to 38% in Santander [48].

## 5. Conclusions

Our study revealed that there are no differences in oil content among the three compared *jatropha* accessions, nor do oil content changes occur during the fruit ripening process. This means that, for oil content in the Ecuadorian *jatropha* accessions, the genotype and collection date of fruits under tropical dry forest conditions are not relevant. Under subtropical conditions, the harvest timing is as relevant as the *jatropha* genotypic selection [13].

Studies conducted by Rao et al. [16] and Karaj and Müller [49] pointed out that the significant statistical correlation between seed weight and oil content can be considered as an important trait for early selection of seed sources. A bilinear relationship for oil content and seed weight was observed in the present study, even though the coefficient of determination was not high. Better adjustment of the bilinear relationship between these variables was reported by Wassner et al. [13], where the harvest time was at the mottled-yellow color stage, characteristic of ripe fruit. In both studies the bilinear relationship showed similar trends, but the break point, where the linear relationship between oil content and seed weight changes in slope, was 58.8 g in our study, while in the subtropical zones of Argentina, the break point was at 62.5 g [13]. Additionally, the particular relationship between seed weight and oil content limits the usefulness of a rapid selection for genotypes with high oil concentration based on seed weight.

Finally, it is safe to state that site-specific data on *jatropha* is necessary when aiming at promoting it, since reported variation is high. Basing management plans on literature data bears the risk of overestimating potential returns to farmers. Some authors reported rather high yields, which might be due to studies being undertaken under “optimal” conditions, which are different from usual farm management, as simulated in our study, where caring for *jatropha* trees is just one duty among many others. Our results provide important building blocks for a comprehensive cost-benefit analysis essential for realistic planning of *jatropha* plantations at farm, regional energy, and national levels.

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