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# Harvest date modifies seed quality and oil composition of *Jatropha curcas* growth under subtropical conditions in Argentina

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## ABSTRACT

One of the most currently promoted new crops for biodiesel productions the perennial species *Jatropha curcas* L. (Euphorbiaceae) due to its promising high seed yields with high oil concentrations (33–39%) and oil quality that reaching the international biodiesel standards. Although there have been different evaluations of genotypes growing under the same environmental conditions, revealing great variability for seed and oil quality, no reports are available about the effect environmental conditions during grain filling over such traits.

The objectives of this work were to determine (i) the effect of harvest dates on seed quality and oil concentration, (ii) if such changes can be explained by the average temperature during grain filling and (iii) how harvest dates could affect biodiesel quality. An experiment was carried out during 29 months in experimental plots located in Formosa, Argentina. A systematic fruit harvest was conducted during 15 different dates on 10 plants with similar height ( $\approx 1.80$  m). Environmental conditions during the experiment were appropriate to create a broad individual seed weight range (326–752 mg) and significant differences were found among harvest dates. Seed oil concentrations were significantly different among harvest dates with a maximum value of  $38.7 \pm 0.6\%$  on 08 August 2011 and a minimum of  $19.6 \pm 1.8\%$  on 17 March 2010. Oil concentration increased linearly as seed weight increased up to a value of 605 mg, after which higher seed weight was not associated with greater seed oil concentration. Oil concentration was largely ( $r^2 = 0.85$ ) explained by the kernel percentage through a linear regression ( $y = -25.9 + 0.967x$ ). Variations in seed weight and oil concentration were not associated with changes in average temperature during grain filling, suggesting that fluctuations in the source-sink relation through the growing season could explain the variations found among harvest dates. Temperature during seed filling period strongly affected oil composition and higher temperatures were associated with higher oleic acid and lower linoleic acid concentrations, although this effect generated only small effects on the biodiesel quality. Environmental conditions during grain filling modified seed quality and oil composition, while its concentration was not affected. This work reveals the existence of harvest dates effects on seed quality and oil concentration, although unrelated with the environmental conditions explored during grain filling period.

## 1. Introduction

Worldwide demand for vegetable oils used in biodiesel elaboration has markedly grown during the last years due to governmental decisions aiming to reduce greenhouse gas emissions and depen-

dence on fossil fuels. In this context, the interest for new oil crops in areas not used for agriculture has remarkably increased.

One of the most currently promoted new crops is the perennial species *Jatropha curcas* L. (Euphorbiaceae), due to promising high seed yields with high oil concentrations (33–39%; [Ginwal et al., 2004](#)) and oil quality reaching international biodiesel standards ([Achten et al., 2008](#)).

The real productive potential of *J. curcas* is controversial because the first plantations were performed assuming seed yield estimations which proved excessively optimistic ( $4\text{--}5$  t ha<sup>-1</sup>; [Francis et al.,](#)

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2005; Adil Edrisi et al., 2015), but experimental results showed great variability and lower yields than expected ( $0.4\text{--}2.0\text{ t ha}^{-1}$ ; Senger et al., 2016; Wani et al., 2016; Van Eijck et al., 2014; Laviola et al., 2014). These results are largely the consequence of low genetic improvement combined with poor development of crop management technology.

Another component that explains oil yield is seed weight and oil seed concentration, which for *J. curcas* is broadly assumed to range between 35 and 40% (Carels, 2009). However, different genotypes evaluations performed under the same environmental conditions revealed a great variability for oil seed concentration ranging between 29.8–37.1% (Rao et al., 2008); 17.1–38.8% (Subramanyam et al., 2010); and 28.0–38.8% (Kaushik et al., 2007).

Oil seed concentration does not only depend on the genotype, but it is also affected by the environmental conditions during grain filling, mainly by temperature that modifies seed oil concentration and the fatty acid composition through changes in grain filling dynamics and biosynthetic activity. In general, the higher the temperatures, the shorter the seed filling duration and the higher the seed filling rate, which determines lower seed weights than those obtained with lower temperatures (Singh et al., 2013; Singer et al., 2016).

In *J. curcas*, oil is accumulated during the last third of the seed filling period (Annarao et al., 2008; Sinha et al., 2015), so a shortening of grain filling duration should determine lower oil concentration. Furthermore, changes in seed weight generated by different temperatures can affect seed oil concentration through changes in the seed coat – kernel relationships in a similar way to that found by Rondanini et al. (2003) for the pericarp–seed relationship in sunflower (*Helianthus annuus* L.). Surprisingly, the effect of environmental conditions during grain filling in *J. curcas* has not been studied yet.

Regarding the composition of *J. curcas* oil, there is consensus that its quality is suitable for producing biodiesel that meets the international quality standards due to an adequate proportion of its two main fatty acids, oleic and linoleic, which represent 67–81% of total fatty acids (Rathbauer et al., 2012).

However, similarly to what happens to seed weight and oil concentration, there is abundant information indicating the existence of genotypic variability for oil composition, with extreme values that range from 21.8 to 49.0% for oleic acid and 47.4–29.7% for linoleic acid (Oliveira et al., 2009), but there is no information on the effect of environmental conditions on oil composition during grain filling.

It is generally demonstrated that low temperatures increase the accumulation of polyunsaturated fatty acids and decrease the monounsaturated oleic acid, which is explained by the optimal temperatures ranges for the activity of different enzymes involved in the biosynthesis of both types of fatty acids (Trémolières et al., 1982).

However, not all crops exhibit the same response (Canvin, 1965; Rondanini et al., 2014), which makes it necessary to determine how temperature during seed filling affects the composition of *J. curcas* oil in order to determine how biodiesel quality parameters could be affected.

Under our experimental conditions, seeds of *J. curcas* were produced in several cohorts throughout the growing season (usually three or four), extending from early summer to early winter, leading to uncertainty about how seed quality and oil composition could be affected by the environmental conditions explored during each seed cohort. Knowing how environmental conditions affect oil concentration and composition is important to develop agronomic practices aiming to concentrate seed production under the most favourable conditions. Thus, the objectives of this work were to determine (i) the effect of environmental conditions during grain filling on seed quality (seed weight, seed coat and kernel propor-

tion and seed oil concentration), (ii) if changes in seed quality and oil composition can be explained by the average temperature during grain filling and (iii) how harvest dates could affect biodiesel quality.

## 2. Material and methods

### 2.1. Experimental conditions

The experiment was carried out during 29 months, from March 2010 to August 2012, in experimental plots of *J. curcas* located in Siete Palmas, Formosa, Argentina ( $58^{\circ} 17' 59.67''\text{W}$ – $25^{\circ} 13' 21.04''\text{S}$ ), located in the Chaco region, sub-region of Humid Chaco. The climate is warm humid subtropical, with a rainy season that extends from late spring until early autumn, while during the winter rainfall is lower.

The plantation was established in 2008, with a 4 m inter-row distance and 2 m between plants ( $1.250\text{ pl ha}^{-1}$ ), with East-West row orientation. Plants were not pruned during the experiment. Weeds were mechanically eliminated and plants were fertilized every year (during October) with  $100\text{ kg ha}^{-1}$  of urea, under rainfed conditions. Insects were controlled using Dimethoate and preventive applications of systemic fungicide Amistar™ (Syngenta, active principle: Azoxystrobin) were performed twice a year. The applied doses were carefully chosen in order to avoid toxic effects.

Previous to starting the experiment, soil analysis was performed and results obtained were: pH in water (1:2.5) 6.8; electrical conductivity ( $\mu\text{S cm}^{-1}$ ) 5.9, organic matter by Walkley-Black method (%) 3.8; extractable phosphorus by Bray-Kurtz I ( $\text{mg kg}^{-1}$  as  $\text{P}_2\text{O}_5$ ) 25; total nitrogen by Kjeldahl method (%) 0.7; Exchangeable cations by Ammonium acetate method ( $\text{meq } 100\text{ g}^{-1}$ ), Na: 122; K: 0.25; Ca: 20.93 and Mg: 15.43.

A systematic fruit harvest was conducted on 15 different dates (between 4–6 harvests per year) on 10 plants with similar height ( $\approx 1.80\text{ m}$ ), using as criterion to harvest when more than 80% of each fruit cohort had the yellowish-brown colour characteristic of ripe fruit (Silva et al., 2012). On each harvest date, all ripe fruit was collected and separated into their components (the shell conformed by the exocarp and endocarp and the seeds), oven dried at  $70^{\circ}\text{C}$  until constant weight, and weighted.

### 2.2. Chemical analysis

#### 2.2.1. Seed oil concentration

Seeds samples (10–15 g) were oven dried at  $65^{\circ}\text{C}$  until constant weight and milled. Oil was extracted using a Soxhlet apparatus with hexane for 12 h. The extracts were evaporated under vacuum with a rotary evaporator until dry and weighted.

#### 2.2.2. Oil composition

Fatty acid composition was determined by gas-liquid chromatography of methylated fatty acids. Oil was dissolved in 1 ml of TBME (tert butyl methyl ether, approximately  $20\text{ mg ml}^{-1}$ ) and methylated using TMSH (trimethyl sulphonium hydroxide  $0.5\text{ M}$  in methanol) at  $103^{\circ}\text{C}$  for 3 min. Fatty acids components were identified with Varian 3900 gas chromatographer equipped with FID detector. The analytical conditions used were the following; capillary column Agilent CP-select CB for FAME fused silica WCOT ( $50\text{ m} \times 0.25\text{ i.d.} \times 0.25\text{ }\mu\text{m}$  film thickness; Agilent, USA); carrier gas: Helium; injector temperature:  $250^{\circ}\text{C}$ ; detector temperature:  $250^{\circ}\text{C}$ , initial oven temperature:  $185^{\circ}\text{C}$  (40 min); gradient:  $15^{\circ}\text{C min}^{-1}$  up to  $250^{\circ}$  (10.68 min).

The fatty acids myristic (14:0), palmitic (16:0), palmitoleic (16:1), heptadecanoic (17:0), heptadecenoic (17:1), stearic (18:0), oleic (18:1), linoleic (18:2), linolenic (18:3), arachidic (20:0),

arachidonic (20:1), behenic (22:0) and lignoceric (24:0) were identified in comparison with retention times of a known standard mixture (AOCS-1, Sigma-Aldrich, St. Louise, MO).

Glycerides composition was determined by gas-liquid chromatography of silylated fatty acids. Oil was dissolved in 1 ml of cyclohexane (approximately 1.5 mg ml<sup>-1</sup>) and silylated using Methyl-imidazole with MSHFBA (*N*-methyl-*N*-trimethylsilyl-heptafluorobutyramide) at 103 °C for 3 min. Fatty acids components were identified with Perkin Elmer Autosystem XL gas chromatographer equipped with FID detector. The analytical conditions used were the following; capillary column CP Sil 8 CB low bleed MS (15m × 0.32 i.d. × 0.25 μm film thickness; Varian, California, USA); carrier gas: Helium; injector temperature: 55 °C; detector temperature: 365 °C, initial oven temperature: 55 °C (0.5 min); gradient: 200 °C min<sup>-1</sup> up to 340° (40 min).

The glycerides were identified in comparison with retention times of a known standard mixture (AOCS-1, Sigma-Aldrich, St. Louise, MO) composed by the fatty acids palmitic (16:0) and stearic (18:0), monoglycerides and diglycerides of palmitic and stearic acids, and triacylglycerides conformed by 3 molecules of stearic acid, 2 molecules of stearic and 1 of palmitic, 1 molecule of stearic and 2 of palmitic acids and 3 molecules of palmitic acids.

### 2.2.3. Biodiesel quality parameters

Two oil parameters relevant for biodiesel quality (iodine value and cetane number) were estimated from the fatty acid profiles. Saponification number (SN) indicates the quantity of long-chain fatty acid whereas iodine value (IV) indicates the level of oil unsaturation, and both were estimated using Eqs. (1) and (2), respectively (Kalayasiri et al., 1996). Cetane number (CN) indicates the ignition speed of biodiesel after injection and was calculated using Eq. (3) (Krisnangkura, 1986).

$$SN = \sum (560 \times A_i) / MW_i \quad (1)$$

$$IV = \sum (254 \times D \times A_i) / MW_i \quad (2)$$

$$CN = 46.3 + 5458 / SN - 0.225 \times IV \quad (3)$$

where  $A_i$  is the percentage of each fatty acid,  $D$  is the number of double bonds of each fatty acid, and  $MW_i$  is the molecular mass of each fatty acid present in the oil.

Changes in IV significantly affects many biodiesel quality parameters as viscosity, density, cold behaviour, oxidative stability, CN and heat value, while the SN, affects cold behaviour, CN and heat value (Hoekman et al., 2012). There are six grades of biodiesel according to their cold behaviour (Grades A to F) that determine different prices in the European market (Garofalo, 2007)

### 2.3. Statistical analysis

Treatments were arranged in a completely randomized design and data were analysed by one-way ANOVA and LSD was considered at 5% significance level.

The percentages of fatty acids were transformed using arcsine transformation to correct for the non-normality in proportions. Means were separated with the Student–Newman–Keuls test (Keuls, 1952) at 5% significance level.

Seven plants were used for kernel and seed coat proportion in seeds and 10 plants for the other determinations. For each harvest date, all mature fruit was harvested, and random subsample consisted of 40 seeds of each plant taken to determine the seed and oil quality.

The nonlinear statistical routine of Prism (GraphPad, 2007) was used to fit bi-linear regression models with unknown cut-off to the relations of oil concentration and kernel proportion as a function of

seed dry weight. The model adjusted was:  $y = a + bx$  (for  $x \leq c$ ) and  $y = a + bc$  (for  $x \geq c$ ) where  $y$  is the oil concentration (%) or kernel proportion (%),  $a$  and  $b$  are the intercept and the slope, respectively,  $x$  is the seed dry weight (mg) and  $c$  is the unknown break point of the function indicating the end of the lineal association.

Multiple regression was used to predict seed weight and oil concentration through mean temperature 25 days before harvest (DBH), total radiation accumulated 25 DBH, accumulated rainfall 40, 50 and 60 DBH and the total number of hours during the 25 DBH with temperatures above 30 and 35 °C.

### 2.4. Meteorological information

Meteorological data, except precipitation measured in the experimental plots, were obtained from automatic meteorological station (Davis Vantage Pro 2 Plus, Davis Instruments, California, USA) located at the Universidad Católica “Nuestra Señora de la Asunción” (Asuncion, Paraguay) located 50 km from the experimental plot. This meteorological station was used because the original station installed in the experimental plot did not work properly and data was lost. So, there is no nearer station with data recording with an hourly frequency. Fortunately, temperature regimes in both sites are very similar because this area corresponds to a plain with the same isotherms throughout the year (Murphy et al., 2008).

## 3. Results

### 3.1. Meteorological conditions

The driest year was 2010, with an annual precipitation of 1043 mm, distributed 56% during summer, 17% in spring and 18% during fall, while 2012 was the most humid year with 1491 mm, distributed mainly during autumn (46%) and spring (42%). During 2011, a more concentrated distribution pattern was recorded, with 48% of the annual rainfall (1276 mm) during spring and 41% during early summer, and a long dry period of 5 months (March to August), in which precipitation was only 8 mm (Fig. 1). Winter rains were low, accounting for 9, 11 and 4% of the annual rainfall, occurring during 2010, 2011 and 2012, respectively.

For the 3 years analysed, the mean monthly maximum temperature increased from 22 to 23 °C in July up to 35–36 °C in January, while the mean monthly minimum temperature increased from 8 to 10 °C in July up to 21–23 °C in January. Light frosts were recorded every year, on 08 May, 2010 (3 h duration), 14 July, 2010 (1 h), 27 June, 2011 (6 h), 28 June, 2011 (3 h), 06 July, 2012 (1 h) and 06 August, 2012 (5 h) with temperatures that ranged between –0.1 and –1.9 °C. This caused the loss of all immature leaves and fruit, light damage to the branches, which involved the necrosis of between 10 and 20% at the top of the branches. During the next spring, no plant mortality was observed.

### 3.2. Seed quality

#### 3.2.1. Individual seed dry weight

Environmental conditions during the experiment were appropriate to create a broad individual seed weight range (326–752 mg) and significant differences were found ( $p < 0.05$ ) among harvest dates. The seeds harvested during late fall and early winter (April to July) were significantly ( $p < 0.05$ ) heavier than those harvested during summer and late winter, except for the seeds harvested on 09 August 2011 (Fig. 2).

Great variability for seed weight was found according to the harvest date, with seeds harvested on 05/20/2011 presenting the highest mean weight (695 ± 11 mg, mean ± standard error, respec-



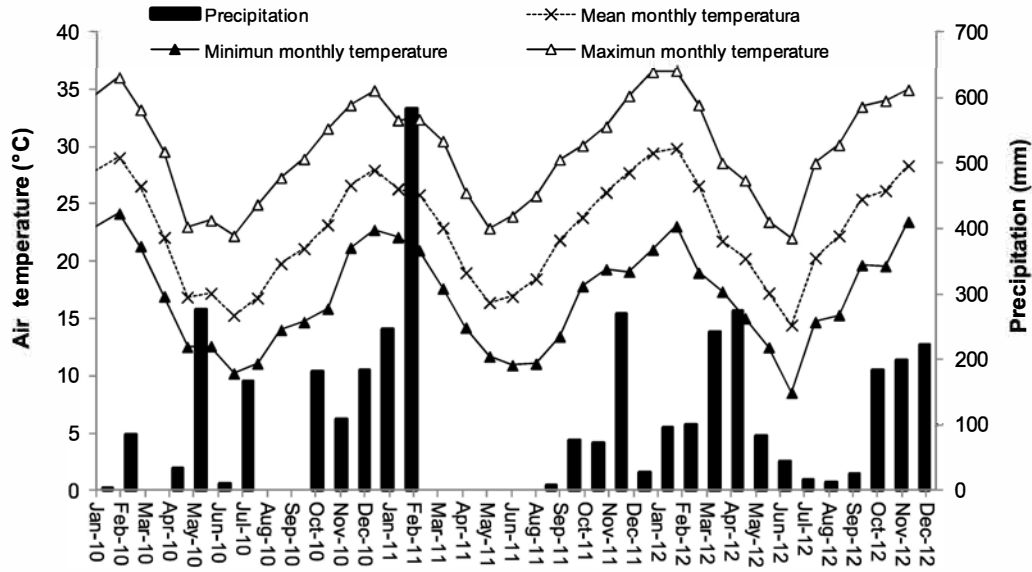


Fig. 1. Meteorological conditions during the experiment from 2010 to 2012.

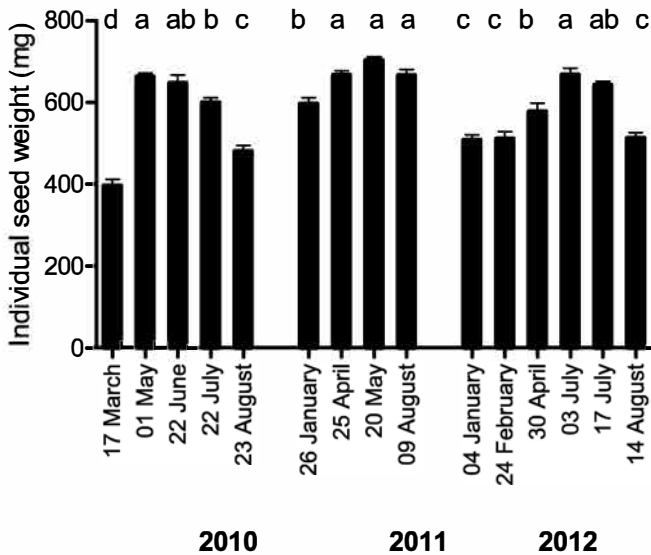


Fig. 2. Mean individual seed weight of *Jatropha curcas* plants harvested on different dates during 3 years. Different letters indicate significant differences between means at  $p < 0.05$ , vertical lines represent standard errors,  $n = 10$ .

tively) and the seeds harvested on 17 March, 2010 the lowest ( $395 \pm 16$  mg).

### 3.2.2. Relationship seed coat – kernel

The seed mass partition between seed coat and kernel was significantly different ( $p < 0.05$ ) among harvest dates (data not shown) and were significantly related to seed weight. Thus, the slope of seed coat weight as a function of individual seed weight was significantly ( $p < 0.001$ ) lower (0.17) than the slope of kernel weight (0.83, Fig. 3).

### 3.2.3. Seed oil concentration

Seed oil concentration was significantly ( $p < 0.01$ ) different among harvest dates. Seeds harvested on 17 March 2012 had the lowest oil concentration while the seeds harvested on 22 June, 2010, 26 January, 2011, 20 May, 2011, 09 August, 2011, 30 April, 2012, 03 July, 2012 and 17 July, 2012 had the highest values (Fig. 4). The mean maximum value was  $38.7 \pm 0.6\%$  on 09 August,

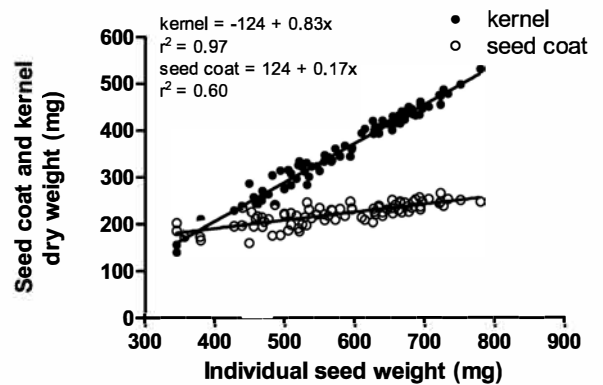


Fig. 3. Linear regression between hull and kernel dry weight with seed weight. The slopes are significantly different from zero at  $p < 0.001$ ,  $n = 84$ . Data were taken from all harvest dates evaluated.

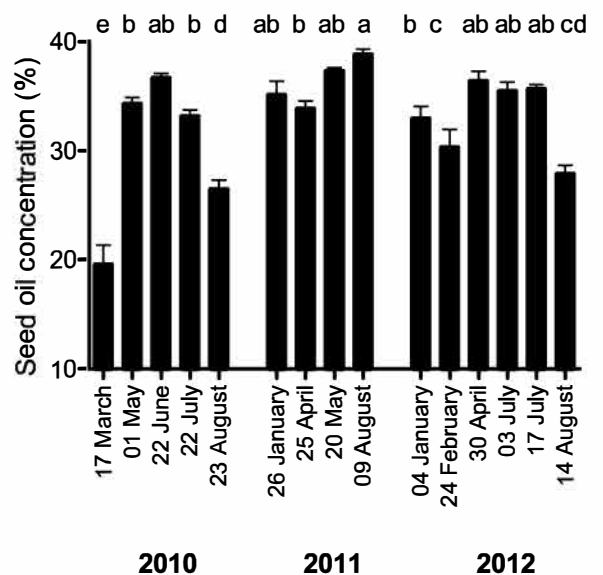
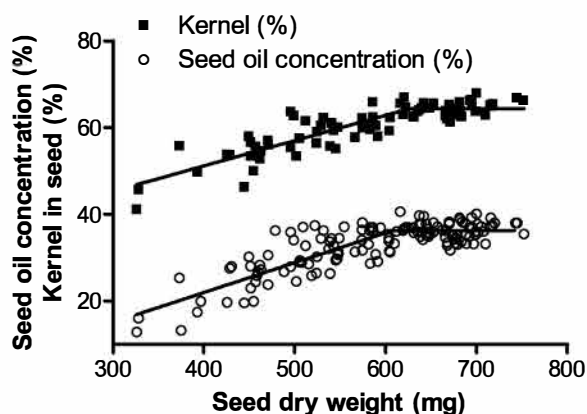
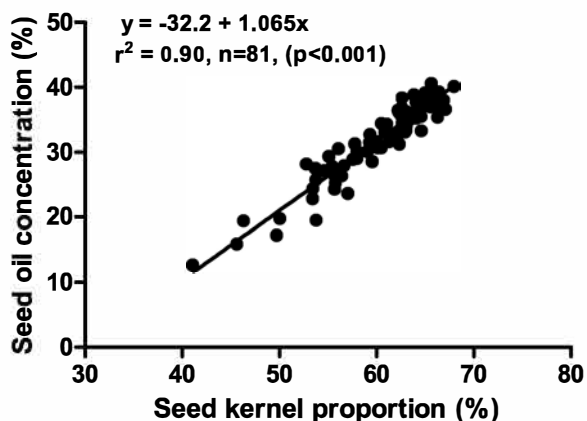


Fig. 4. Mean oil concentration in seeds of *Jatropha curcas* harvested on different dates during 3 years. Different letters indicate significant differences between means at  $p < 0.05$ , vertical lines represent standard errors,  $n = 10$ .



**Fig. 5.** Bilinear regressions between oil concentration (%) ( $y = -8.68 + 0.0742x$ ); up to 595 mg, the slope was significantly different from zero at  $p < 0.001$ ,  $n = 65$ ; and  $n = 71$  for values higher than 594.9 mg, with slopes not significantly ( $p > 0.05$ ) different from zero) and kernel percentage (%) of total seed dry weight ( $y = 26.89 + 0.0592x$ ; up to 624 mg, the slope was significantly different from zero at  $p < 0.001$ ,  $n = 47$ ; and  $n = 33$  for values higher than 624 mg with slopes not significantly ( $p > 0.05$ ) different from zero).



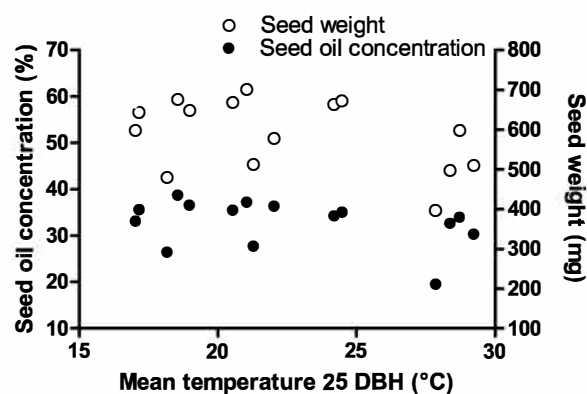
**Fig. 6.** Linear regression between seed oil concentration (%) and kernel percentage in seed (%).

2011 (means  $\pm$  standard error, respectively) whereas the lowest was  $19.6 \pm 1.8\%$  on 17 March, 2010. Seasonal variations in seed oil concentration presented the same pattern that was found for seed weight only in 2010, while for the remaining years, seed oil concentration tended to be more stable among harvest dates.

The relationship between oil concentration and seed weight can be explained through a bilinear regression. Oil concentration increased linearly as seed weight became heavier, up to a value of 605 mg, after which higher seed weight was not associated with increases in seed oil concentration (Fig. 5). In addition, the relationship between kernel percentage and seed weight presented the same pattern that seed oil concentration, where linear increases were detected up to a value of 625 mg ( $p < 0.01$ ), after which increases in seed weight were not associated with changes in kernel percentage (Fig. 5). No significant differences ( $p < 0.05$ ) were found between the values from which both relationships begin to have a slope equal to zero (605 and 625 mg).

Oil concentration was largely ( $r^2 = 0.85$ ) and significantly ( $p < 0.01$ ) explained by the kernel percentage through linear regression ( $y = -25.9 + 0.967x$ ,  $n = 156$ ,  $p < 0.001$ ; Fig. 6).

No significant relationships were found between mean temperature 25 DBH and individual seed weight ( $p = 0.107$ ) or seed oil concentration ( $p = 0.204$ ; Fig. 7). In addition, no relationships were



**Fig. 7.** Relationships between mean temperature 25 DBH and seed weight (mg) and oil concentration (%),  $n = 15$ .

found with other environmental variables, such as solar radiation or water availability (data not shown).

### 3.3. Oil characteristics

#### 3.3.1. Fatty acid composition

The main fatty acids present were linoleic and oleic, which ranged from 28.9 to 47.5% and from 31.7 to 47.1%, respectively; with a minor proportion of palmitic (13.1–14.1%) and stearic (7.2–4.1%) and minimal proportions of palmitoleate (0.72–1.04%), linoleic (0.18–0.312%) and arachidonic (0.14–0.24%; Table 1). Linoleic and oleic concentrations were strongly affected by harvest date, while the proportion of minor components tended to be stable.

#### 3.3.2. Glycerides composition, cetane number and iodine values

The proportions of free fatty acids, mono- and diglycerides in oil were very small, and the total amount of the three compounds was always lower than 3% (Table 2). The harvest date significantly affected ( $p < 0.05$ ) the proportion of free fatty acids and monoglycerides. The proportion of free fatty acids on 17 March 2010 was significantly higher than that found on 22 June 2010 and 23 August 2010, while among the rest of the harvest dates there were no significant ( $p > 0.05$ ) differences. The proportion of monoglycerides was significantly ( $p < 0.05$ ) higher on 01 May 2010 and 25 April 2011 compared with those found on 17 March 2010, 26 January 2011 and 20 May 2011, while among the remaining harvest dates there were no significant differences (Table 2). The amounts of diglycerides were very low and significant differences ( $p < 0.05$ ) were found among seed collected on 01 May 2010 and 25 April 2011 compared with those harvested on 17 March 2010, 26 January 2011 and 20 May 2011. No significant differences ( $p < 0.05$ ) were found for the proportion of triglycerides on any of the harvest dates evaluated. The CN and IV had values in the range of 47.1–52.1 and 96.6–116.1, respectively.

#### 3.3.3. Relationship between temperature during grain filling and fatty acid composition

For each harvest date, we calculated the thermal environment under which seed filling occurred, considering the mean daily temperature during 25 DBH. The oil fatty acids proportion, especially for oleic and linoleic acids, was strongly affected by mean temperature 25 DBH (Fig. 8). Under the cool mean temperatures of 19.0, 17.0 and 18.2 °C registered for the harvest dates on 22 June, 2010, 22 July, 2010 and 23 August, 2010 respectively, we found the highest proportion of linoleic acid (47.5–45.4%) and the lowest oleic acid proportion (31.7–33.4%, Fig. 8). Under warmer temperatures (27.9 and 28.7 °C), for the harvest dates on 17 March, 2010 and 26 January, 2011, respectively, we found the opposite response pattern, with

**Table 1**

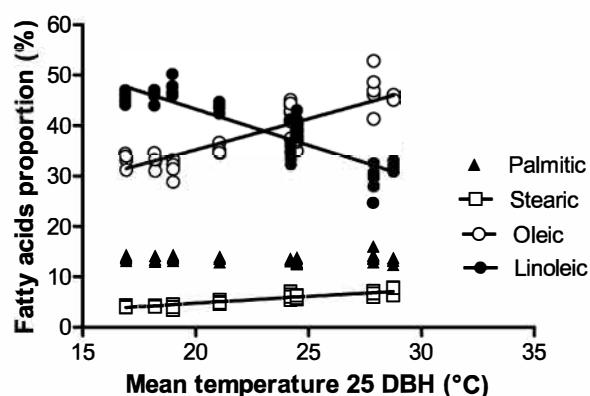
Fatty acid composition in oil obtained from seeds harvested on different dates during two years (2010 and 2011) from an experimental plantation of *Jatropha curcas* in Formosa, Argentina. Means  $\pm$  standard errors, n = 10. Different letters indicate significant differences between means at  $p < 0.05$ .

Harvest date	Fatty acids						
	Palmitic (C16:0)	Palmitoleate (16:1n7c)	Stearic (C18:0)	Oleic (C18:1n9c)	Linoleic (C18:2n6c)	Arachidonic (C20:0)	Linolenic (C18:3n3a)
2010							
17 March	14.1 $\pm$ 0.4a	1.04 $\pm$ 0.10a	6.6 $\pm$ 0.1b	47.1 $\pm$ 1.5a	28.9 $\pm$ 1.1f	0.24 $\pm$ 0.005a	0.31 $\pm$ 0.016a
01 May	13.4 $\pm$ 0.1ab	0.80 $\pm$ 0.02b	6.3 $\pm$ 0.2b	42.4 $\pm$ 0.9b	35.4 $\pm$ 1.0d	0.20 $\pm$ 0.005c	0.20 $\pm$ 0.005c
22 June	13.8 $\pm$ 0.2ab	0.88 $\pm$ 0.03ab	4.1 $\pm$ 0.1e	31.7 $\pm$ 0.6e	47.5 $\pm$ 0.6a	0.14 $\pm$ 0.004f	0.23 $\pm$ 0.009b
22 July	13.9 $\pm$ 0.1ab	0.97 $\pm$ 0.02a	4.2 $\pm$ 0.1e	33.4 $\pm$ 0.3e	45.4 $\pm$ 0.3ab	0.14 $\pm$ 0.002f	0.24 $\pm$ 0.003b
23 August	13.5 $\pm$ 0.2ab	1.03 $\pm$ 0.05a	4.2 $\pm$ 0.1e	32.9 $\pm$ 0.7e	46.0 $\pm$ 0.7ab	0.15 $\pm$ 0.002ef	0.30 $\pm$ 0.010a
2011							
26 January	13.2 $\pm$ 0.3ab	0.72 $\pm$ 0.03b	7.2 $\pm$ 0.4a	45.4 $\pm$ 0.2a	31.8 $\pm$ 0.4e	0.22 $\pm$ 0.008b	0.19 $\pm$ 0.006c
25 April	13.1 $\pm$ 0.2b	0.79 $\pm$ 0.03b	5.9 $\pm$ 0.1c	38.1 $\pm$ 0.6c	40.3 $\pm$ 0.6c	0.18 $\pm$ 0.002d	0.19 $\pm$ 0.006c
20 May	13.5 $\pm$ 0.2ab	0.87 $\pm$ 0.02ab	4.9 $\pm$ 0.1d	35.2 $\pm$ 0.4de	43.7 $\pm$ 0.4b	0.16 $\pm$ 0.003e	0.18 $\pm$ 0.004c

**Table 2**

Glycerides composition and biodiesel quality of oil from seeds harvested on different dates during 2010 and 2011 from a plantation of *Jatropha curcas* in Formosa, Argentina. Mean  $\pm$  standard error, n = 10. Different letters indicate significant differences between means at  $p < 0.05$ . Limits values for ASTM D 6751-15a for grade N°1-B15 biodiesel and EN 14214 (in parentheses): Acid number (mg KOH g<sup>-1</sup>) 0.5 (0.5); Cetane number: 47 (51); Monoglycerides: 0.4 (0.8); Diglycerides: 0.2; Iodine value: (120 max).

Harvest date	Glycerides composition (% mass)				Biodiesel quality	
	Free fatty acids	Mono-glycerides	Diglycerides	Triglycerides	Cetane number	Iodine value (g/100 g)
2010						
17 March	0.85 $\pm$ 0.10a	0	1.60 $\pm$ 0.07b	97.54 $\pm$ 0.14a	52.1	96.6
01 May	0.76 $\pm$ 0.05ab	0.005 $\pm$ 0.02	1.93 $\pm$ 0.06a	97.30 $\pm$ 0.05a	50.4	103.6
22 June	0.44 $\pm$ 0.03b	0	1.78 $\pm$ 0.05ab	97.77 $\pm$ 0.07a	47.7	116.1
22 July	0.55 $\pm$ 0.02ab	0	1.78 $\pm$ 0.05ab	97.66 $\pm$ 0.07a	48.2	113.8
23 August	0.48 $\pm$ 0.03b	0	1.70 $\pm$ 0.02ab	97.82 $\pm$ 0.05a	48.0	114.9
2011						
26 January	0.82 $\pm$ 0.13ab	0	1.54 $\pm$ 0.07b	97.65 $\pm$ 0.20a	51.3	99.8
25 April	0.63 $\pm$ 0.10ab	0	1.95 $\pm$ 0.07a	97.42 $\pm$ 0.87a	49.3	108.7
20 May	0.58 $\pm$ 0.11ab	0.004 $\pm$ 0.02	1.63 $\pm$ 0.10b	97.79 $\pm$ 0.21a	48.4	112.3



**Fig. 8.** Fatty acids composition of *Jatropha curcas* oil obtained from seed filled under different mean temperatures 25 days before harvest (DBH). Linear regression between mean temperature during 25 DBH and fatty acids percentage (stearic acid =  $-0.60 + 0.269$  mean temp 25 DBH ( $^{\circ}$ C),  $r^2 = 0.84$ ; oleic acid =  $10.34 + 1.24$  mean temp 25 DBH ( $^{\circ}$ C),  $r^2 = 0.78$ ; linoleic acid =  $72.39 - 1.44$  mean temp 25 DBH ( $^{\circ}$ C),  $r^2 = 0.79$ ; n = 50,  $p < 0.001$ ).

the lowest linoleic proportion (28.9 and 31.8%, respectively) and maximum oleic proportion (47.1 and 45.4%, respectively, Fig. 8). The proportion of stearic acid increased significantly with higher temperature 25 DBH, but showed lower sensitivity (regression slope: 0.27) than that found for oleic (slope: 1.24) and linoleic (slope;  $-1.44$ ) acids while the proportions of minor oil fatty acids were more stable and their slopes were not statistically ( $p > 0.05$ ) different from zero (Fig. 8).

#### 4. Discussion

The environmental conditions during grain filling corresponding to seeds from different cohorts which were exposed, created a broad individual seed weight that ranged between 326 and 752 mg (Fig. 2), and resulted higher than the ranges reported for different genotypes evaluations under the same environmental conditions (569.8–790.9 mg, Rao et al., 2008; 491–683 mg, Subramanyam et al., 2010 and 493–742 mg, Rathbauer et al., 2012). In coincidence, a similar pattern was observed for oil seed concentration, which ranged between 12.8 and 40.6% (Fig. 4). These results clearly revealed that harvest date has the same importance as the genotype for determining both individual seed weight and oil seed concentration in *J. curcas*.

The results clearly revealed that the partitioning between kernel and seed coat depends on the wide range of individual seed weight generated by the different harvest dates. Thus, the kernel weight was strongly associated ( $r^2 = 0.97$ ) and showed a high sensitivity (linear regression slope = 0.83) to changes in seed weight (Fig. 3), while the seed coat weight was less sensitive (linear regression slope 0.17). The average kernel proportion in seeds found in this work ( $60.43 \pm 0.07\%$ , mean  $\pm$  standard error, respectively) was similar to the mean of 63.6% found by Montes et al. (2013) and the ranges reported by Naresh et al. (2012) and Makkar et al. (1997) for genotypes evaluation trials (53.9–64.2%).

As a consequence of the above described, a bilinear relationship was found between the proportion of kernel in seed (expressed as% of total seed weight) and seed weight, with a threshold around 625 mg, where no increases were produced with higher seed weights (Fig. 5). Since oil concentration was strongly associated with the proportion of kernel in seed (Fig. 6), a similar pattern



was also observed for the former as a function of seed weight (Fig. 5), which oil concentration increases up to a similar seed weight (605 mg) than for the proportion of kernel in seed. Clearly, this pattern limits the usefulness of seed weight as a tool to identify genotypes with high oil seed concentration. Consistently with our results, a linear relationship between seed weight and oil concentration was found by Karaj and Müller (2010). As explained above, seed oil concentration was better explained (90% through a linear relationship) by the kernel proportion (Fig. 6), which could be useful as a rapid genotypes selection criterion for genotypes with high oil concentration, in the same way as was performed during the sunflower breeding process (López Pereira et al., 1999).

Variations in seed weight and oil concentration were not associated with changes in average temperature (or radiation, rainfall or exposure time to temperatures above 30 and 35 °C either) during the grain filling period (Fig. 7). This results are inconsistent with the general pattern of temperature response observed in other oil crops such as sunflower (Rondanini et al., 2003), castor bean (*Ricinus communis* L.; Vallejos et al., 2011), jojoba (*Simmondsia chinensis* L.; Wardlaw and Dunstone, 1984) and flax (*Linum usitatissimum* L.; Green, 1986), where the lower rate of grain filling under cool temperatures is overcompensated by the higher grain filling duration, resulting in heavier seeds with higher oil concentration due to longer grain filling duration (Rotundo and Westgate, 2009; Triboi and Triboi-Blondel, 2002). Likewise, no interactions were found between temperature 25 DBH and other environmental factors that affect seed weight and oil concentration, such as solar radiation accumulated during grain filling, as found in sunflower for water status (Seiler, 1986; Aguirrezábal et al., 2003). This apparent temperature insensitivity during seed filling could be caused by the temperature ranges explored in these experiments, which that may not have been high enough to reduce seed weight and oil concentration. In this experiment, average temperatures explored were between 17–29.2 °C. However, on some harvest dates the seeds were exposed to extended periods of heat stress (i.e on 24 February, 2012, 248 h with temperatures > than 30 °C and 86 h > 35 °C were recorded. This represents 41 and 14%, of the filling period considered (25 DBH) respectively, while on 01 April, 2012, 243 h > 30 °C and 74 h > 35 °C were registered (40 and 12%, respectively). This fact allows arguing that on some harvest dates thermal stress conditions were more severe than those reflected by average temperatures.

On the other hand, changes in seed weight and oil concentration observed for the different harvest dates could be explained by fluctuations in the plants source-sink relation through the growing season generated by a trade-off between seed number and seed weight (Sadras, 2007; Pallas et al., 2013). In perennial plants, it becomes more important to consider the role of carbohydrate reserves, which can be partitioned toward seeds when availability of assimilates is restricted, or to be a sink of photoassimilates when the plant accumulates reserves. In *J. curcas* there is no information on carbohydrate dynamics, the organs where reserves are accumulated, or their contribution to grain filling. Further research is needed in order to test this possibility.

The information available about *J. curcas* oil fatty acid composition was mainly focused on the genotypic effect, and differences were found for the proportions of their main fatty acids (oleic and linoleic), which ranged between 35.1–42.7% and 31.6–38.2%, (Rathbauer et al., 2012), 36.7–42.6 and 37.5–43.4% (Rodrigues et al., 2013), respectively. As a complement of this approach, we found that environmental conditions during seed grain filling strongly affected oil composition.

Environmental conditions during grain filling strongly affected oil composition. Temperature response pattern found for *J. curcas* oil was similar to that found for traditional oily crops like sunflower and rapeseed (*Brassica napus* var. *oleifera* L.), where high temperature during oil synthesis period increases the oleic/linoleic

acid ratio (Rondanini et al., 2003; Green, 1986). However, this contrasted with the response found for the olive fruit (*Olea europaea* L.), where increases in temperature during fruit filling determine reductions in the oleic acid proportion (García-Inza et al., 2016).

Despite the fact that the main saturated fatty acids present in *J. curcas* oil (palmitic and stearic) showed significant differences among harvest dates, the magnitude of differences was considerably small, while oleic and linoleic showed high sensitivity to temperature during grain filling. We found that for every 1 °C increase in mean temperature during the 25 DBH oleic acid increased by 1.24% and stearic acid by 0.26% while linoleic acid decreased by 1.44%. In this sense, Demurin et al. (2000) found increments of 2% in the oleic acid percentage for every degree increased during sunflower fruit filling.

In general terms, no important changes were produced by the different harvesting dates over the quality of biodiesel. Thus, FFA found on all harvest dates were very low (0.44–0.85%) and similar to the values range (0.42–2.31%) reported by Rodrigues et al. (2013) and Akminul Islam et al. (2013). Nevertheless, higher FFA values, ranging between 2.24–4.5%, were found by Nzikou et al. (2009) and Adebawale and Adedire (2006) while Huerga et al. (2014) reported the highest FFA values for *J. curcas* oil obtained in Argentina (8.7–20.5%). The large variation of FFA values reported in the literature suggests that this parameter should be carefully considered. There is no evidence to relate FFA with genotype or environmental conditions during grain filling, but it was demonstrated that in early stages of grain filling the proportion of FFA is high and then drops abruptly (Annarao et al., 2008). Thus, the incorporation of immature seeds during harvesting could be the cause of FFA increases.

In addition, changes in oil composition produced by different temperatures during grain filling could have changed the quality of biodiesel and in this sense triacylglycerols were found to comprise the major oil component ( $\approx 97.5\%$ ) followed by diacylglycerols and free fatty acids (FFA), with practically absent monoacylglycerols on all harvest dates (Table 2).

Finally, the iodine values found in this study ranged between 96.6 and 116.1, and they were similar to values reported in the literature (Akintayo, 2004; Nzikou et al., 2009) and broadly being close to the limits set by the rather restrictive maximum value of 120 (EN 14214, 2003). Cetane number has specified minimum values of 47 for the USA (ASTM D6751, 2008) and 51 for the European Organization (EN 14214, 2003). The estimated CN for *J. curcas* oil obtained in this work was between 47.7 and 52.1 and, for all harvest dates the quality standards used in USA were met. However, only on 2 of the 8 harvest dates evaluated, values set by European regulations were reached. The higher CN values were obtained in oil from seed filled during the warm months as a consequence of their higher oleic and stearic acids proportions and lower linoleic acid proportion.

## 5. Conclusions

This paper makes a contribution in determining the existence of harvest dates effects on seed quality and oil concentration unrelated with the environmental conditions explored during grain filling period, which suggests that plant internal balance could play an important role in the regulation of seed quality. The variations for seed weight and oil concentration found in this work have similar magnitude to those found for genotypic evaluations, which highlights the importance of working in order to understand the ecophysiological mechanisms involved in generation of good quality seeds.

The relationship between seed weight and oil concentration was described by a bi-linear regression, which limits its usefulness as

a rapid selection tool for genotypes with high oil concentration. The strong relationship between seed kernel proportion and oil concentration offers a rapid tool to identify genotypes with high oil concentration. The functional relationship that explains this is the lower seed coat weight sensitivity to changes in seed weight compared with the kernel weight.

Fatty acid oil composition was affected by mean temperature during seed filling in a similar way to the reported for most oil crops, where warmer conditions increased the oleic acid proportion and decreased linoleic, but the magnitude of changes observed generated non important effects on the biodiesel quality. On the other hand, no sensitivity to temperature was detected for oil concentration and individual seed weight. This trades could be of great interest in the context of global warming, because they open the possibility of introducing more stable oilseed crops in order to generate more stable grain yield and quality.

More controlled experiments should be conducted in order to expand the temperature ranges explored during grain filling under field conditions, as well as for understanding the carbohydrate dynamics in the plant and characterize whether variations in the sink-source relationships throughout the growing season may affect *J. curcas* seed quality.

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