academicJournals

Vol. 10(49), pp. 4406-4413, 3 December, 2015
DOI: 10.5897/AJAR2015.9710
Article Number: E201C9456258
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Genetic progress in seed yield of physic nut (*Jatropha curcas* L.)

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Received 12 March, 2015; Accepted 20 October, 2015

Physic nut (*Jatropha curcas* L.), a tropical shrub with medicinal properties has not undergone efficient selection for high seed yield or oil production over the years. Predictions on seed yield of this oilseed plant were based on isolated observations that did not consider the reductions in plant growth due to higher planting densities. The objective of this study was to quantify the genetic progress of seed yield using mass selection in a physic nut population at production age, evaluated in the Amazonic tropical climate. Genetic variability for seed yield exists among *J. curcas* genotypes, denoting the possibility of genetic progress through plant selection. There was also a tendency for the genotypes to maintain their genetic superiority over the time. The estimated genetic progress from the selection of 10 superior genotypes was 0.67 kg.plant^{-1}, which, compared to the overall mean, corresponds to a selection gain of over 99%. The new mean of these plants was 1.35 kg.tree^{-1}, equivalent to seed yield of 2.25 tons.ha^{-1}. For breeding, a minimum number of 30 genotypes were considered to ensure the genetic progress over the generations. The estimated mean of a recombination unit composed of the 30 superior genotypes was 1.17 kg.plant^{-1}, equivalent to a seed yield 1.95 tons ha^{-1}. Although accurate, the genetic progress estimated in this study does not appear to provide a qualitative increase in the yield of this oilseed. New breeding strategies may consider the use of crossings of divergent plants with better agronomic traits.

Key words: Oilseed, genetic parameters, selection, genetic gain.

INTRODUCTION

The use of new oilseed plants has potential to diversify the biodiesel production chain though it is associated with risks of cultivating non-domesticated species. The physic nut (*Jatropha curcas* L.) is a perennial plant of the Euphorbiaceae family, which has been surveyed in tropical and subtropical regions worldwide as an alternative source for oil production (Spinelli et al., 2015; Divakara et al., 2010). *J. curcas* is traditionally known as a hedge plant with medicinal properties and did not undergo efficient selection for seed yield over the years.

Despite the low genetic diversity that has been measure in accessions from India and Brazil (Rosado et al., 2010; Basha et al., 2009) sources of variability for seed toxicity rate of male/female flowers and resistance

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Table 1. Weather data recorded from 2005 to 2010 using an automatic weather stations (AWS) in Ariquemes – RO (latitude 9°55'34.81''S, longitude 63°01'09.10''O and 140 m altitude).

Years	Air temperature (ºC)			Relative humidity (%)		Precipitation (mm)	
	Mean	Max.	Min.	Mean	Min.	Total (mm)	Rain days
2005	25.5	32.1	21.3	86	68	2107.3	142
2006	25.5	31.7	21.4	84	70	2334.3	140
2007	25.5	31.7	21.3	84	72	2174.1	136
2008	25.4	29.8	22.3	80	77	1993.4	134
2009	26.2	31.1	22.4	82	62	2337.3	118
2010	26.0	32.4	21.5	84	59	1419.9	62

Source: SEDAM – Secretary of State for Rondônia Regional Development, INMET –National Institute of Meteorology. http://www.sedam.ro.gov.br/index.php/

to diseases have been characterized (Laviola et al., 2010). Other studies also observed high seed yield variability, ranging from 0.2 to 2 kg.plant^{-1} (Jongschaap et al., 2007). Although it is limited, previous work has indicated that there is genetic variability to be manipulated by selection and breeding (Heller, 1996).

Several traits such as seed size, seed oil content, seed yield and plant branching determine the final oil production of this oilseed plant. However, evaluations of these oil yield components showed that more than 90% of the oil yield variability is related to the seed yield variability, indicating the major importance of the biomass production for this crop (Spinelli et al., 2010). Compared with optimistic seed yield prognosis of over than 3 tons.ha^{-1}, smaller seed yields of 1 ton.ha^{-1} or less, have been reported in different environmental conditions (Raiger et al., 2011; Bhering et al., 2013). Natural limitations such as hydric deficit, incidence of diseases and attack of pests limit the production of this crop. *Oidium* spp. incidences in savannah regions and severe *Empoasca* spp. attacks have been observed worldwide, including in Brazil (Laviola et al., 2012).

Two main strategies are considered for increasing the seed yield: the plant management and the selection of superior genotypes (Cruz and Carneiro, 2003; Islam et al., 2013). Pallet and Sale (2006) observed an additive relationship between these factors due to the expression of superior genotypes in appropriate environmental conditions. The physic nut is still considered a non-domesticated plant and in many instances, the crop seed yield is too low to be commercialized. Few studies have quantified the seed yield of plants with more than 36 months of planting (Spinelli et al., 2015). Raiger et al. (2011) reported genotypes with 48 months of planting that produced approximately 1 ton.ha^{-1} at four different sites from India. More accurate seed yield prognoses that consider higher planting density, repeated measures and plants at production age are important for this crop, since optimistic prognosis stimulated *J. curcas* plantations worldwide.

Different strategies have been used for the early selection of superior genotypes. Drumond et al. (2010) selected responsive genotypes in irrigated conditions that produced 2.12 kg.plant^{-1} at 12 months of cultivation. In regards to other environments, Laviola et al. (2010) observed that seed yield ranged from 0 to 0.18 kg.plant^{-1} at 12 months of cultivation in savannah conditions. These authors also concluded that observations over multiple agricultural years were necessary to effectively evaluate seed yield.

It is reasonable that genetic improvements to seed yield will rely on intra-population recurrent selection and manipulation of genetic variance, as well as the vegetative propagation of superior genotypes. Evaluations over multiple agricultural years also allow the estimation of the repeatability coefficient, which measures the maintenance of genetic superiority over time, and may be used to estimate the minimum number of evaluations required to predict the breeding value to minimize costs and labor (Resende, 2002).

The objective of this study was to quantify the genetic progress of seed yield using mass selection with repeated measures in a physic nut population at production age, evaluated in the Amazonic tropical climate.

MATERIALS AND METHODS

Field experiment

In March 2005, an experiment was established using environment stratification of a *J. curcas* plantation of three hectares located in Ariquemes, RO − latitude 9°55'24.50''S, longitude 63°7'15.58''O and 142 m altitude. The field trial was established in a wild seed population of 550 plants of unknown genetic origin from Ariquemes, RO, Brazil. The climate is tropical Aw, hot and humid with a well defined dry season in which hydric deficit occurs from June to September. The mean annual temperature is 25°C with an average annual rainfall of 2.354 m and average annual evapotranspiration of 851 mm, according to the Climatological Means (Brasil, 1992). The climate data was recorded from 2005 to 2010 (Table 1). The soil in the experimental area is classified as Distrophic Red Yellow Latosol, of clayey texture.

Table 2. Chemical analysis of the soil at the location of the field experiment located in Ariquemes – Rondônia, Brazil.

Date (month/year)	Depth (cm)	pH	P (mg/dm^3)	K	Ca	Mg	Al+H	Al	O.M.	V
				($mmol_c/dm^3$)					(g/Kg)	(%)
08/08	0-20	4.2	0.4	1.1	7.0	6.0	41.0	20.0	13.0	21.0
08/08	20-40	4.5	2.0	0.3	5.1	4.0	61.0	10.1	9.2	13.0
09/09	0-20	5.2	16.0	1.2	27.0	17	57.8	0.0	19.6	44.0
09/09	20-40	4.2	1.0	0.4	5.0	4.1	57.8	9.1	9.5	13.0
09/10	0-20	5.4	9.0	1.0	33.3	15.0	52.8	0.0	17.6	49.0
09/10	20-40	4.6	1.0	0.3	5.0	4.5	62.7	10.7	11.1	14.0

Depth: Depth of the soil samples collected (cm); P: exchangeable soil phosphorous, (Mielich1), K: exchangeable soil potassium (Mielich1), Ca: exchangeable soil calcium, Mg: exchangeable soil magnesium, Al+H: exchangeable cations, Al: exchangeable soil aluminum , O.M.:organic material content, V: base saturation.

The experiment was arranged with a plant spacing of 2 x 3 m using 1-month old rooted plants produced with seeds of unknown genetic origin from the Northen region of Brazil. Each plant pit received 100 g of P_2O_5 and in the second year, 4 tons of lime (PRNT 60%) was distributed per hectare on the soil surface. Fertilization initiated in the second year, with the application of 50 g of N, 60 g of P_2O_5 and 40 g of K_2O three months before the two main harvests in the region, in June and December. Soil samples were collected for analysis in August 2008 and September 2009. Physical and chemical soil analyses were made on air-dried soil samples passed through a 2 mm sieve and determined according to Embrapa 2011 (Table 2).

Seed yield evaluation

The seed yield of 550 plants was assessed at 36 and 48 months after planting in two agricultural years (2008/2009 and 2009/2010). Uneven ripening is a characteristic of this oilseed plant that is constantly producing in the region from the beginning through the end of the rainy season (from November to June) comprising two concentrated productions in June and December. Seed yield was evaluated in two agricultural years including four harvests in the periods of concentrated production (first: June, 2008; second: December, 2008; third: June, 2009; fourth: December, 2009). Fruits at their final maturity stage were harvested from trees and crown projections. After the harvest, fruits were dried in the shade for 15 days when they were processed. After processing, the seed moisture was measured in a moisture determiner Dole 500 (Gehaka), and seed with moisture content lower than 9% were weighed using electronic balance Mark 4100 (BEL Engineering).

Estimates of genetic parameters

Repeated measures are important to quantify the maintenance of genetic superiority over time and may be interpreted using models that consider the permanent environmental effects (PE) caused by the non-random evaluation of plants in the same position over the years. To evaluate the significance of the random genotype effect (which measures the diversity among the genotypes) the following model was considered:

$$Y_{ij} = u + gp_i + m_j + \varepsilon_{ij} ,$$

$Y_{ij} =$ observation of the ith genotype in the jth measurement (agricultural year), $u =$ overall mean, $gp_i =$ random ith genotype effect added the permanent environmental effect (PE), $m_j =$ fixed jth measurement effect (agricultural years), $\varepsilon_{ij} =$ random temporary environmental effect (TE). Distributions and structures of means and variances: $E(gp_i) = 0$; $E(gp_i^2) = \sigma_{gp}^2$; $E(m_i) = m_i$; $E(m_i^2) = \phi_{mi}$; $E(\varepsilon_{ij}) = 0$; $E(\varepsilon_{ij}^2) = \sigma^2$.

The variance components were estimated using Restricted Maximum Likelihood (REML) methods, according to the model:

$$y = Xm + Wp + e$$

y: seed yield data vector, m: fixed measure (agricultural years) effect vector added to the overall mean, p: random genotype vector added to permanent environmental effects, e: random temporary environmental (TE) vector. Capital letters stand for the incidence matrices for each one of these effects.

Repeatability and genetic progress

The individual repeatability is estimated by the phenotypic correlation of the repeated measures of the same individual:

$$\hat{\rho}_i = \frac{\hat{\sigma}_{gp}^2}{\hat{\sigma}_{gp}^2 + \hat{\sigma}^2}$$

$\hat{\rho}_i =$ individual repeatability, $\hat{\sigma}_{gp}^2 =$ variance of the genotype added the permanent environmental influence, $\hat{\sigma}^2 =$ residue variance.

The mean repeatability of the population may be estimated as:

$$\rho_{im} = \frac{m\rho_i}{1 + (m-1)\rho_i}$$

$\rho_{im} =$ mean repeatability, $\rho_i =$ individual repeatability, $m =$ number of measurements.

The correlation between parametric genetic values and phenotypic evaluations is the selection accuracy that can be

Table 3. ANOVA results of the seed yield evaluated in a *J. curcas* population at 36 and 48 months after planting in the Amazonic tropical climate.

Source	df	MS	F
m	1	908081.8	3.59**
gp	500	248564.8	
Residue	500	70033.7	
Total	1001	160047.3	
Mean	619.30		
CV	30.96		

Source: Sources of variation, df: degrees of freedom, MS: mean square, F: F test, **: significance at a 1% of probability, M: fixed effect of the j´th measure, GP: random effect of the i´th genotype added the permanent environment influence, Residue: random environmental effect, CV: coefficient of variation.

interpreted as a measure of the selection efficiency (Resende, 2002):

$$r_{\hat{p}p_{12}} = \left[\frac{m\rho_i}{1+(m-1)\rho_i}\right]^{1/2}$$

$r_{\hat{p}p_{12}} =$ selection accuracy, $m =$ number of measurements, $\rho =$ individual repeatability

The genetic progress estimates of the individual selection were estimated as:

$$G_{S_{12}} = k.r_{\hat{p}p_{12}}.\sigma_{fp}$$

$G_{S_{12}} =$ genetic progress, $k =$ standard selection differential, $r_{\hat{p}p_{12}} =$ selection accuracy, $\sigma_{gp} =$ variance of the genotype added the permanent environmental influence.The efficiency of the use of repeated measures was estimated as:

$$E = \left[\frac{m}{1+(m-1)\rho_i}\right]^{1/2}$$

$E =$ efficiency related to the use of several measurements, $\rho_i =$ individual repeatability, $m =$ number of measurements.

RESULTS

The selection of genotypes that are responsive to environmental improvement is an important step to increase the seed yield of Jatropha. Liming and plant nutrition resulted in better environmental conditions as determined by soil analyses from August 2008 and September 2010 (Table 2). The F test of the ANOVA indicated significant genetic variability in the *J. curcas* seed yield at 1% of probability (Table 3). The genetic variability is essential for the selection of genetic progress, which primarily depends on significant genotypic contributions to the trait expression.

The physic nut requires three to four years to stabilize its production and just few studies evaluated plants with more than 36 months of planting (Brittaine and Lutaladio, 2010). The seed yield difference of the 10 most productive genotypes and the overall mean was higher in the first agricultural year (1.16 kg.tree^{-1}) than in the second year (0.68 kg.tree^{-1}). An increase in the seed yield due to growth of the plants from 36 to 48 months after planting has not been verified (Figure 1).

The repeatability estimate, which represents the upper limit of the individual heritability, indicates the possibility of selecting materials with superior adaptability and stability, that is, genotypes that are responsive to environmental improvements and are able to maintain their superiority over time. The individual repeatability estimate indicates a general trend of the genotypes to maintain their superiority (Table 4).

The selection accuracy, defined as the correlation between the parametric genetic values and the phenotypic evaluations, is one of the most important parameters for evaluating the efficiency of the selection. A larger increase in this estimate was observed when utilizing two repeated measures (agricultural years) compared to only one measure (Table 5). Selective accuracies higher than 80% are considered adequate to accomplish gains with mass selection.

The estimates of genotypic and temporary environmental effects support the genetic progress prediction considering the vegetative propagation or the breeding of superior genotypes. The larger influence of the genotype effect on the expression of this trait indicates the chance of genetic progress through selection. The estimated genetic progress through selection of the 10 superior genotypes is 0.671 kg.plant^{-1}. Compared to the overall mean, this value represents a selection gain of 99.32% (Table 6).

DISCUSSION

Since optimistic seed yield projections stimulated *J.*

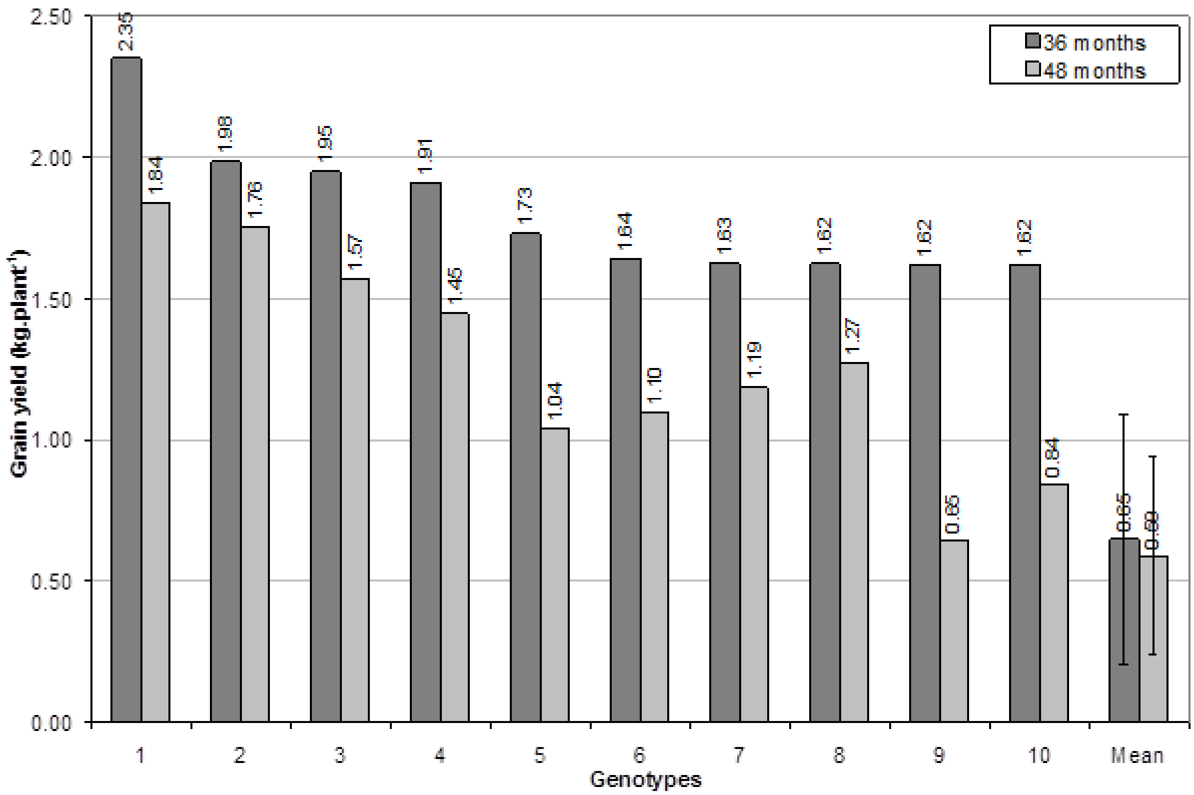

Figure 1. Seed yield of the 10 more productive genotypes at 36 and 48 months after planting compared to the overall mean of the population ($kg.plant^{-1}$). Bars over the mean values represent the standard deviation.

curcas plantations worldwide it is important to consider the production of this oilseed plant at higher planting density, with genotypes at production age and evaluation over, at least, two agricultural years. These results demonstrate the existence of genotypic variability for seed yield, it may be inferred that increases in yield of *J. curcas* may rely on the manipulation of genetic variance by breeding or propagation of superior genotypes.

In contrast to the low genetic diversity measured by molecular markers (Rosado et al., 2010; Resende et al., 2008), agronomic evaluations have shown variability of several seed and oil yield components (Basha et al., 2009; Raiger et al., 2011; Costa et al., 2010; Drumond et al., 2010; Mishra, 2009). Although the use of molecular markers allows a fast quantification of genetic diversity, the evaluation of random genomic variations is often indicative of sequence polymorphisms not related to traits of agronomic interest (Resende et al., 2008).

Brittaine and Lutaladio (2010) and Jongschaap et al. (2007) evaluated accessions that produced 0.2 to 2 $kg.tree^{-1}$. According to Jongschaap et al. (2007), this high variability is mainly caused by environmental conditions at the *J. curcas* plantations, which were established at a wide range of latitudes between 30° N and 35° S. However, even in the same environment, it was observed that cultivating non-selected genotypes is an important factor of the seed yield variability. Compared to the low mean production evaluated in these two years (1.03 $ton.ha^{-1}$), it is noteworthy the genetic progress that may be achieved through plant selection.

Improvements were made to circumvent natural cultivating limitations by increasing the soil pH and reducing the soil aluminum, providing better conditions for plant development that reflected in the levels of available phosphorous, calcium and magnesium (Table 2). However, out of the 550 plants evaluated, 80 plants (15%) did not produce or produced seed less than 0.1 $kg.plant^{-1}$, and showed no response to the environmental improvements, obtained by manipulating the plant nutrition and liming the Amazonic soil. The coefficient of variation quantifies the variability of this trait in a population of unknown genetic origin (Table 3).

One of the strategies that have been considered to reduce harvesting costs of the physic nut is the increasing the plant production through adjustments of planting density (Laviola et al., 2010). Optimistic predictions for seed yield were based on isolated observations that did not consider the reduction in plant growth due to higher planting densities, caused by increased competition for natural resources, such as radiation, water and nutrients. The limited plant growth observed at within-plant spacing of 2 m indicates that

Table 4. Genetic parameter estimates of a *J. curcas* population evaluated at 36 and 48 months after planting in the Amazonic tropical climate.

Genetic parameter	Estimates
$\hat{\sigma}^2_{gp}$	89261.18
$\hat{\sigma}^2$	70036.94
$\hat{\sigma}^2_f$	159298.12
$\hat{\rho}_i$	0.56 +- 0.07
$\hat{\rho}_{im}$	0.75
$r_{\hat{p}p_{12}}$	0.85
CV_{exp}	30.96
Overall Mean	619.30

$\hat{\sigma}^2_{gp} =$ variance of the genotype added the permanent environment influence, $\hat{\sigma}^2 =$ residue variance, $\hat{\sigma}^2_f =$ phenotypic variance, $\hat{\rho}_i =$ individual repeatability, $\rho_{im} =$ mean repeatability, $r_{\hat{p}p_{12}} =$ selection accuracy.

Table 5. Selection accuracy of a *J. curcas* population evaluated at 36 and 48 months of planting in the Amazonic tropical climate considering different numbers of repeated measures.

m	Selection accuracy	Efficiency
1	0.748	1.000
2	0.847	1.132
3	0.890	1.189
4	0.914	1.220
5	0.930	1.242
6	0.940	1.256
7	0.948	1.267
8	0.954	1.275
9	0.959	1.281
10	0.962	1.286

m = number of measurements.

lower planting densities may increase the productivity per plant 36 or more months after planting.

Compared to other perennial oleaginous trees, such as oil palm (*Elaeis* spp.), the individual repeatability estimate may be considered high and suggests the maintenance of genetic superiority over time. Two measures allowed the selection of plants, at production age, with an accuracy of 85% (Table 4). For the selection of hybrid progenies of *E. oleifera* and *E. guineensis*, for example, at least four measures are necessary to obtain similar selection accuracies (higher than 80%) (Chia et al., 2009) (Table 5).

In addition to the selection progress that may be achieved through breeding, it is important to consider the vegetative propagation of superior genotypes. Promising results have been reported concerning the propagation of this oilseed plant, either by tissue culture techniques or minicuttings production (Datta et al., 2007). The mean production was estimated through selection of 10 superior genotypes to be 1.35 $kg.tree^{-1}$, equivalent to seed yield of 2.25 $tons.ha^{-1}$ (Table 6).

Since the genetic progress is inversely proportional to the intensity of selection, the plant breeding may also consider the number of non-related selected individuals. The intensity of selection was estimated to maximize the lower limit of the genetic progress confidence interval, adjusted for inbreeding (Rocha et al., 2009; Basha et al., 2009); which indicated the selection of 12 superior genotypes for breeding. The estimated mean seed yield obtained through breeding of these genotypes is 1.23 $kg.plant^{-1}$, which is equivalent to a seed yield of 2.05 $tons.ha^{-1}$. To ensure genetic progress over the selection generations, it was also considered a minimum effective number of genotypes (Resende et al., 2008). The estimated mean of a recombination unit composed of the 30 superior genotypes is 1.17 $kg.plant^{-1}$, equivalent to seed yield 1.95 $tons.ha^{-1}$ per hectare (Table 6).

The estimated genetic progress indicates the importance of planting superior genotypes that were previously selected for higher biomass production. Aside from the selection gain estimated in this study, *Jatropha* has a potential to reach higher productivities. The advance of plant management practices also allows the expression of the genetic potential in improved environmental conditions. Abdelgadir et al. (2010) and Gouveia et al. (2012) reported promising results on the use of growth regulators in *J. curcas* and Ghosh et al. (2010) observed an increase in the seed yield of this crop through application of paclobutrazol 24 months after planting.

Conclusions

It is determined that there is indeed genetic variability among the *J. curcas* genotypes, denoting the possibility of genetic progress through plant selection. Mass selection over multiple agricultural years provided accurate seed yield estimates. There was also a tendency of the genotypes to maintain their genetic superiority over time. Aside from the selection progress estimated in this work, it is considered that this oilseed plant has potential for higher productivities.

Conflict of Interests

The authors have not declared any conflict of interests.

Table 6. Genetic progress estimates of the 30 superior genotypes evaluated in *J. curcas* population evaluated at 36 and 48 months in the Amazonic tropical climate.

Position	gp	u + gp	Genetic progress	New mean (kg.plant^{-1})	New mean (tons.ha^{-1})
1	949.76	1625.69	949.76	1625.69	2.71
2	799.67	1475.60	874.72	1550.65	2.58
3	726.70	1402.63	825.38	1501.31	2.50
4	687.29	1363.22	790.86	1466.79	2.44
5	671.59	1347.52	767.00	1442.93	2.40
6	640.16	1316.10	745.86	1421.79	2.37
7	607.43	1283.36	726.09	1402.02	2.34
8	589.88	1265.81	709.06	1384.99	2.31
9	552.37	1228.31	691.65	1367.58	2.28
10	488.96	1164.89	671.38	1347.31	2.25
11	475.27	1151.21	653.55	1329.49	2.22
12	464.93	1140.86	637.84	1313.77	2.19
13	443.18	1119.11	622.86	1298.79	2.16
14	436.35	1112.28	609.54	1285.47	2.14
15	430.07	1106.01	597.57	1273.51	2.12
16	419.63	1095.56	586.45	1262.38	2.10
17	417.80	1093.73	576.53	1252.46	2.09
18	415.94	1091.87	567.61	1243.54	2.07
19	413.59	1089.52	559.50	1235.44	2.06
20	404.94	1080.87	551.78	1227.71	2.05
21	386.63	1062.56	543.91	1219.84	2.03
22	386.17	1062.10	536.74	1212.67	2.02
23	385.71	1061.64	530.17	1206.11	2.01
24	373.69	1049.62	523.65	1199.59	2.00
25	372.60	1048.53	517.61	1193.54	1.99
26	367.96	1043.89	511.86	1187.79	1.98
27	367.71	1043.65	506.52	1182.45	1.97
28	356.35	1032.28	501.15	1177.09	1.96
29	355.46	1031.40	496.13	1172.06	1.95
30	355.33	1031.26	491.44	1167.37	1.95

gp: genotype effect added the permanent environment influence (PE), u: overall mean.

ACKNOWLEDGEMENTS

The authors thank the Brazilian research founding agencies for the support that was fundamental for this work: The National Counsel of Technological and Scientific Development (CNPq) and the Research and Projects Financing (FINEP).

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