

# Selection of *Jatropha* full-sib families based on genotypic adaptability and stability via mixed models

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**ABSTRACT.** The objectives of this study were to estimate the genetic parameters for *Jatropha* full-sib families and to select superior genotypes based on grain yield, adaptability, and stability simultaneously to be used for cloning and crossings. Grain yield was evaluated in thirteen full-sib families in a randomized block design for 5 years. The harmonic mean of the relative performance of genotypic values and reaction norm were performed to estimate the adaptability and stability, and sequentially the 30 superior individual plants were selected based on the predicted additive genotypic value for the last crop season. Families 4, 8, and 9 may be used to several regions of Brazil since they have high genotypic adaptability and stability. Individuals belonging to families 9, 8, 2, 1, 6, and 5 should be used for cloning or crossing to obtain the next-generation individuals, and consequently to continue the *Jatropha* breeding.

**Key words:** *Jatropha curcas*; REML; BLUP; HMRPGV; *Jatropha* breeding

## INTRODUCTION

*Jatropha* (*Jatropha curcas* L.) has a huge importance due to its multiple uses (Laviola et al., 2013), such as a fence, phytoremediation, and medicinal use (Sharma et al., 2012). Moreover, *Jatropha* has become an interesting crop to biofuel production since it has a high seed oil content (Silva Junqueira et al., 2016; Montes and Melchinger, 2016; Peixoto et al., 2016).

*Jatropha* is spread out in almost all Brazilian regions, and it makes the genotype x environment interaction important in the selection process. Besides that, *Jatropha* has an enormous energetic potential, although this species still is in a domestication process in Brazil, which points out the importance of to choose specific selection strategies to develop new cultivars. Moreover, *Jatropha* is a perennial species that breeders spend a lot of time and resources to evaluate the selective cycles, which is one of the most challenge to turn this culture more sustainable.

Indeed, the application of accurate selection methods is essential to maximize the selection gain. Thus, one of the most important issues in the *Jatropha* breeding is to identify genotypes with high grain yield, good production stability, and high adaptability for several environments, simultaneously. Studies that consider all of these steps can be performed using mixed model concepts by harmonic mean to the relative performance of genotypic values (HMRPGV) method.

The HMRPGV method was proposed by Resende (2002) and has several advantages such as: the selection of genotypes based on three attributes mentioned above simultaneously, the consideration of genotypic effect as random, the consideration of correlated errors within local or years as well as the stability and adaptability to select individual within progenies, the output is genotypic values (GV) penalized by the instability, and the results are in the same unit of the evaluated trait (Resende et al., 2014).

This method has been applied for several species such as eucalyptus (Silva et al., 2013), cotton (Farias et al., 2016), rubber tree (Silva et al., 2014), and strawberry (Costa et al., 2015). However, few types of research have reported the use of this method in *Jatropha* breeding (Borges et al., 2014). Therefore, the objectives of this study were to estimate genetic parameters based on *Jatropha* full-sib families performance and to select superior genotypes for grain yield, adaptability, and stability simultaneously.

## MATERIAL AND METHODS

### Experimental design

The experiment was carried out in the experimental area of Embrapa Cerrado from 2010 to 2015, in the city of Planaltina, Distrito Federal (15°35'30"S, 47°42'30"W, at 1007 m asl). The climate is tropical with dry winter and rainy summer (Aw), according to the Köppen classification, with average annual temperature of 21°C, relative humidity of 68%, and average rainfall of 1100 mm/year. Management practices were based on Bahadur et al. (2012).

Thirteen full-sib families were obtained from the crossings between four accessions selected from the Germplasm Bank with the following characteristics: low-height (CNPAE-107), high grain yield (CNPAE-190), the absence of toxicity in the grains (CNPAE-170), and resistance to powdery mildew (CNPAE-259) according to Table 1. A randomized block design with five replications and three plants per plot spaced 4 x 2 m between rows was used. After

crosses, the seeds were germinated in plastic tubes of 500 mL filled with commercial substrate. Subsequently, the seedlings were transplanted to pit 40 x 40 x 40 cm, that was filled with soil, 5 L bovine manure and 500 g superphosphate. In the first year of cultivation, three topdressings with 80 g/plant fertilizer 20-00-20 were performed. Grain yield (g/plant) was evaluated in five crop seasons, corresponding to the second, third, fourth, fifth, and sixth year after planting.

**Table 1.** Genealogy of the 13 full-sib families evaluated.

Family	Male genitor	Female genitor
1	107	107
2	107	190
3	107	259
4	170	107
5	170	170
6	170	190
7	170	259
8	190	107
9	190	190
10	190	259
11	259	107
12	259	190
13	259	259

### Statistical analysis

REML (restricted maximum likelihood) method was used for estimating variance components, while the prediction of GV was performed using BLUP (best linear unbiased prediction). Initially, the parameters and GV were estimated for each crop season according to the following model:

$$y = Xm + Za + Wp + Tf + Qi + Rs + e \quad (\text{Equation 1})$$

where  $y$  is the phenotypic value vector;  $m$  is the crop season-block combination effect vector plus the overall mean assigned as fixed;  $a$  is the individual additive genotypic effect vector assigned as random;  $p$  is the plot effect vector assigned as random;  $f$  is the dominance effect vector for full-sib families assigned as random;  $i$  is the vector of the effect of family x crop season interaction assigned as random;  $s$  is the permanent effect vector assigned as random;  $e$  is the random error vector.  $X$ ,  $Z$ ,  $W$ ,  $T$ ,  $O$ , and  $R$  represent the incidence matrices for fixed effects, additive genotypic effects, plot effects, dominance effects, family x crop season interaction effects, and permanent effects, respectively.

By using this model, empirical BLUP predictors (eBLUP or REML/BLUP) of the GV without interaction effects was obtained, given by  $\hat{\mu} + \hat{g}_i$ , where  $\hat{\mu}$  is the mean of all crop season and  $\hat{g}_i$  is the genotypic effect of full-sib families without family x crop season interaction. For each season  $j$ , GV was predicted by:

$$\hat{\mu}_j + \hat{g}_i + (\hat{ge})_{ij} \quad (\text{Equation 2})$$

where  $\hat{\mu}_j$  is the mean of the season  $j$ ,  $\hat{g}_i$  is the genotypic effect of full-sib family  $i$  on the crop season  $j$ , and  $(\hat{ge})_{ij}$  is the family x crop season interaction.

The prediction of GV for each full-sib family that capitalizes the mean interaction in different crop seasons was given by  $\hat{\mu}_j + \hat{g}_i + \hat{g}e_m$  and was calculated by:

$$GV = \hat{\mu} + \frac{\left( \frac{\hat{\sigma}_g^2 + \hat{\sigma}_c^2}{n} \right)}{\hat{\sigma}_g^2} \hat{g}_i \quad (\text{Equation 3})$$

where  $\hat{\mu}$  is the overall mean of all environments; n is the number of crop seasons; and  $\hat{g}_i$  is the genotypic effect of full-sib family i.

The harmonic mean of the GV (HMGV) for the stability of each full-sib family was obtained by the equation:

$$HMGH_i = \frac{n}{\sum_{j=1}^n \frac{1}{Vg_{ij}}} \quad (\text{Equation 4})$$

where n is the number of crop seasons (n = 5) where the genotype i was evaluated;  $Vg_{ij}$  is the genotypic value of the family i on the crop season j, expressed by the ratio of the mean in this crop season. The values of the relative performance of the GV (RPGV) for adaptability were obtained according to the expression:

$$RPGV_i = \frac{1}{n} \frac{\sum_{j=1}^n Vg_{ij}}{M_j} \quad (\text{Equation 5})$$

where  $M_j$  is the mean of the yield grain in the crop season j.

Joint selection of full-sib families, simultaneously considering yield grain, stability and adaptability, was given by the parameter HMRPGV according to the equation below:

$$HMRPGV_i = \frac{n}{\sum_{j=1}^n \frac{1}{PRVg_{ij}}} \quad (\text{Equation 6})$$

Subsequently, linear equations of reaction norms of GV depending on the environmental gradient ( $\Delta e$ ) were estimated for each full-sib family, defined by:

$$\Delta e = \frac{\sum_j Y_j}{g} - \bar{Y} \quad (\text{Equation 7})$$

where  $\sum_j Y_j$  is the total grain yield in the crop season  $j$ ;  $g$  is the number of families;  $\bar{Y}$  is the general phenotypic mean over the crop seasons according to Finlay and Wilkinson (1963).

Finally, the selection of the 30 superior individual plants was performed based on the predicted additive genotypic value for the last crop season. Subsequently, the selection gain and the effective size were calculated for these plants (Resende et al., 2014). All analyses were performed with R (R Core Team, 2015) and the Selegen software (Resende, 2016).

## RESULTS AND DISCUSSION

### Estimation of genetic parameters

The estimation of genotypic variance between *Jatropha* full-sib families was greater than the estimate of environment variance, although it was much lower than the estimate of family x crop season interaction variance, and consequently the individual heritability had a low magnitude (Table 2). The individual heritability estimated in this study was lower than the estimates of this parameter in other studies (Laviola et al., 2010; Bhering et al., 2013; Laviola et al., 2013). However, the individual heritability reported in other articles was estimated based on one season, i.e., without family x crop season interaction. As the grain yield is a polygenic trait highly influenced by the environment and *Jatropha* is a perennial crop, which selection cycle takes a long time, the estimate of individual heritability calculated considering the family x crop season interaction is more accurate and may direct the breeders to choose the best selection strategy for this culture.

**Table 2.** Estimates of genetic parameters based on grain yield evaluation in 13 *Jatropha* full-sib families over five season crops.

Parameter	Estimate
Genotypic variance between full-sib families	12,502.92
Environmental variance between plots	12,085.88
Family x crop season interaction variance	40,953.75
Permanent effect variance	200.12
Residual variance	105,701.75
Individual phenotypic variance	171,444.42
Individual heritability	0.07
Coefficient of determination of plot effects	0.07
Coefficient of determination of family x crop season interaction effects	0.24
Coefficient of determination of permanent effects	0.01
Individual repeatability	0.14
Genetic correlation among crop seasons	0.23
Overall phenotypic mean	527.45

The coefficient of determination of the family x crop season effects revealed that approximately 24% of the individual phenotypic variance was explained by the family x crop season interaction variance. This finding indicates that it is suitable to perform adaptability and stability analysis to select superior full-sib families for grain yield. On the other hand, the coefficient of determination of the permanent effects was closed to zero (0.01), which indicates that the environmental variance across years can be unvalued for grain yield. The experimental precision was accessed by the estimate of the coefficient of determination of plot effects (0.07), which indicates low magnitude for this parameter, and consequently, there was no environment heterogeneity between plot within blocks (Resende, 2002).

The individual repeatability is a parameter that allows evaluating how many measures are needed to select superior genotypes with high accuracy. The evaluation of measures over the years is essential to characterize the productive behavior in perennial crops, such as *Jatropha*, which has a long, productive cycle and the traits are expressed in different ways over the years (Resende, 2002). The individual repeatability obtained in this study (0.14) was lesser than the repeatability reported by Laviola et al. (2013), which found 0.37 for yield grain evaluated for four crop seasons. These results may be explained by the low genotypic correlation between crop seasons (0.23) observed in this research, which demonstrates changes in the family rank over the years.

### Genotypic adaptability and stability of full-sib families

Predicted GV for each *Jatropha* full-sib family for yield grain ranged over crop seasons (Table 3). For instance, family 7 was the most productive in the first crop season. Meanwhile, it had low grain yield beyond the third crop season. This indicates that the family x crop season interaction in a complex way was significant, and consequently, it is needed to select families with high adaptability.

**Table 3.** Estimates of genotypic values for grain yield (g/plant) evaluated in 13 full-sib families in five crop seasons and the crop season average predicted by BLUP analysis.

Family	Crop season 1	Crop season 2	Crop season 3	Crop season 4	Crop season 5	Average
	$\hat{\mu}_1 + \hat{g} + \hat{g}e$	$\hat{\mu}_1 + \hat{g} + \hat{g}e$	$\hat{\mu}_1 + \hat{g} + \hat{g}e$	$\hat{\mu}_1 + \hat{g} + \hat{g}e$	$\hat{\mu}_1 + \hat{g} + \hat{g}e$	$\hat{\mu} + \hat{g} + \hat{g}em$
1	33.95	371.54	568.13	1417.66	1074.42	610.68
2	26.39	325.53	528.18	1512.62	1088.41	613.74
3	38.75	250.60	436.25	1263.13	1047.14	397.91
4	62.59	353.96	404.74	1665.82	1297.21	707.95
5	30.16	301.27	360.10	1205.30	1118.18	406.93
6	56.13	401.20	328.94	1452.65	1138.26	600.08
7	65.37	281.55	315.93	1104.01	1061.35	367.45
8	36.11	338.91	588.99	1573.65	1166.70	689.35
9	49.41	439.99	501.26	1477.92	1220.99	675.41
10	41.44	237.54	343.38	1155.49	1188.39	414.65
11	28.27	225.79	379.69	1332.18	1274.71	525.04
12	31.88	213.37	303.52	1046.83	1151.52	333.68
13	44.63	265.37	471.10	1370.40	1101.86	514.00

The parameter  $\hat{\mu} + \hat{g} + \hat{g}em$  refers to the average genotypic value for each crop season, and it capitalizes the average interaction for all crops (Resende, 2002). The superior full-sib families based on the average of the GV over crop seasons were 4, 8, and 9, which also were superior in the third and fifth seasons (Table 3). *Jatropha* is considered a species that has not domesticated in Brazil yet, which the economic viability depends on the identification of genotypes that maintain their grain yield high over crop seasons (Spinelli et al., 2015). Therefore, the selection of superior full-sib families that may be used in the vegetative propagation can contribute to increasing the commercial success of this culture.

Few studies have demonstrated the small genetic variability among Brazilian *Jatropha* genotypes (Rosado et al., 2010; Bhering et al., 2015). Thus, another strategy that can be used to increase the genetic variability is crossing among superior genotypes in an intrapopulation recurrent selection program, which allows exploiting a major proportion of the additive genetic variability available (Resende, 2002).

The full-sib families 4, 8, and 9 were also the three superior families according to

MHPRGV method (Table 4), i.e., these families have high grain yield, high stability, and great adaptability simultaneously over crop seasons. Despite the coincidence selection of the three superior full-sib families based on the average among all crop seasons, there were changes in the rank for the other full-sib families due to the significant low correlation between crop seasons and the family x crop season interaction in a complex way.

**Table 4.** Stability of genotypic values (HMGV), adaptability of genotypic values (RPGV and RPGV $\mu$ ), stability and adaptability of genotypic values (HMRPGV and HMRPGV $\mu$ ) for grain yield (g/plant) evaluated in 13 full-sib families in five crop seasons predicted by BLUP analysis.

Family	HMGV	RPGV	RPGV $\mu$ *	HMRPGV	HMRPGV $\mu$ *
1	74.81	1.20	630.63	1.05	551.41
2	18.57	1.02	536.55	0.49	257.68
3	84.61	0.76	399.53	0.73	387.45
4	191.96	1.44	761.50	1.25	660.55
5	55.18	0.70	367.23	0.67	352.16
6	151.46	1.25	658.81	1.09	572.28
7	161.30	1.02	538.96	0.74	391.67
8	78.77	1.27	671.22	1.12	592.56
9	123.60	1.39	731.23	1.31	691.97
10	88.89	0.75	397.78	0.70	368.43
11	33.21	0.72	382.10	0.55	291.72
12	49.26	0.54	282.97	0.45	236.35
13	97.38	0.94	498.37	0.91	478.51

\*Values of RPGV $\mu$  and HMRPGV $\mu$  were obtained by multiplying the genotypic value of the yield grain of the genotype i with the statistics RPGV and HMRPGV, respectively.

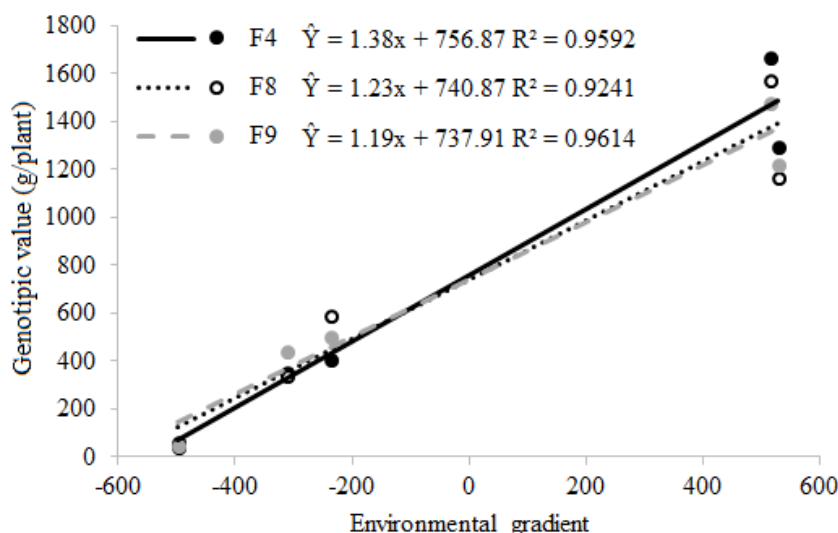
The interpretation of the performance of the perennial species over crop seasons is fundamental to help breeders recommend superior genotypes, especially for traits with low heritability such as grain yield (Resende, 2002). Estimates of the phenotypic averages for grain yield were higher in the fourth and fifth crop seasons (Table 5), which took for a positive environmental gradient for these crop seasons, and consequently, they can be considered as favorable environments according to Finlay and Wilkinson (1963) classification. These results are following the literature because according to Bahadur et al. (2012) the maximum productive potential of Jatropha plants is reached 4 or 5 years after planting.

**Table 5.** Phenotypic mean, environmental gradient, and classification of each season according to Finlay and Wilkinson (1963).

Crop season	Phenotypic mean	Environmental gradient	Classification
1	30.23	-498.06	Unfavorable
2	216.99	-311.30	Unfavorable
3	293.28	-235.01	Unfavorable
4	1043.34	515.05	Favorable
5	1057.60	529.31	Favorable

Figure 1 shows the reaction norm for the three superior families according to the average of GV over crop season and HMRPGV method. Reaction norm represents the genotypic performance of a specific genotype in function of the environmental gradient (Falconer and Mackay, 1996). The outcomes obtained by reaction norm for the 4, 8, and 9 full-sib families were similar than Eberhart and Russell (1966), although the regression coefficients are estimated using the predicted GV for each crop season. Superior families have regression coefficient ( $\beta_1$ ) greater than 1, which indicates an increase of GV with the

environment improvement (Eberhart and Russell, 1966). Moreover, these families have high stability ( $R^2 > 0.80$ ) of genotypic performance over environmental gradients.



**Figure 1.** Reaction norms of the three superior families selected by genotypic values and HMRPGV method.

Researchers investigating the effect of genotypic adaptability and stability on *Jatropha* genotypes are rare (Spinelli et al., 2015; Teodoro et al., 2016), which indicates the importance of this study. The selection of genotypes, which increases their performance with the environmental improvement, is an important factor to increase the grain yield and oilseed yield in *Jatropha* since these traits have large environmental influence. Therefore, findings reported in this research represent an advance to *Jatropha* breeding in Brazil and imply the utilization of the superior genotypes selected based on analyses presented for farmers by vegetative propagation.

### Selection of superior plants within full-sib families

Selection of superior plants is fundamental to increase grain yield in perennial species such as *Jatropha* with the minimum possible costs (Bahadur et al., 2012; Carels et al., 2013). In this study, the last crop season was considered to select superior individual plants within full-sib families since *Jatropha* has its grain yield stabilized beyond the fifth crop season (Laviola et al., 2013). Moreover, grain yield was highest in the fifth crop season for almost all evaluated full-sib families.

Two criteria were performed aiming to select the 30 superior individual plants based on grain yield: effective population size and selection intensity considering three plants per full-sib family defined by the maximization of the inferior limit of the confidence interval of genotypic value corrected by endogamy coefficient (Resende, 2002). The predicted genetic gain crossing the 30 superior individual plants was 31.05% (Table 6), that was superior to the estimates of predicted genetic gain performed by Spinelli et al. (2015).



**Table 6.** Estimates of additive genotypic values plus the overall mean of grain yield (g/plant) of Jatropha related to 30 plants selected to compose a recombination unit.

Order	Block	Family	Plant	Additive genotypic value predicted
1	4	9	3	1818.60
2	2	9	2	1600.80
3	3	9	2	1599.49
4	4	2	3	1568.86
5	4	8	1	1509.65
6	2	1	3	1500.47
7	1	1	1	1488.69
8	3	6	2	1480.13
9	4	2	2	1479.60
10	1	8	2	1457.92
11	5	5	1	1418.69
12	2	6	3	1391.63
13	5	5	2	1366.10
14	4	2	3	1360.08
15	5	8	2	1352.54
16	5	2	2	1351.12
17	3	1	3	1342.03
18	3	5	3	1298.70
19	1	12	2	1292.69
20	5	4	1	1267.42
21	5	4	3	1267.42
22	2	10	2	1247.22
23	4	10	1	1228.85
24	1	3	2	1226.21
25	4	12	3	1221.01
26	5	10	3	1220.78
27	5	7	1	1214.83
28	2	12	3	1214.75
29	4	4	3	1212.48
30	4	3	2	1187.51
Overall mean				1047.51
New mean				1372.81
Selection gain (%)				31.05%

Despite the effective population size considered in this study, other studies demonstrated the need to insert new accessions to increase the genetic diversity of the Brazilian Jatropha genotypes (Rosado et al., 2010; Bhering et al., 2013; Bhering et al., 2015).

Moreover, it was observed that the three superior plants belonged to the full-sib family 9, which has high genotypic adaptability and stability. Family 9 was obtained by the self-pollination of the genotype 190 characterized for high grain yield. Therefore, these findings reinforce the possibility to use clones for the family as commercial varieties. Furthermore, individuals belong to families 8, 2, 1, 6, and 5 may be used for crossing and cloning process.

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