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Assessment of the inheritance pattern of the novel "female only flower" trait in *Jatropha curcas* L. as potential for hybrid seed production

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***Jatropha curcas* L. (JLC) is a perennial shrub, originating in Central and South America and widely distributed in the tropical regions of the world. It is important in the bioenergy industries due to the characteristics of its oil which can be transformed into high quality biofuel for the substitution of diesel and jet fuel. The native gene, female only flower (FOF), is a desirable trait in the production of hybrid seed of *J. curcas*. L. In this study, inbreeding was induced in eight accessions from wild, S_1, S_2 to S_3 endogamy level, and two wild accessions naturally expressing the FOF trait were used as females to cross with male accessions (FM) in order to estimate segregation. The Chi-Square test was used to determine if the FOF trait follows a Mendelian inheritance. Results strongly support the assumption that FOF trait follows a recessive monogenic Mendelian inheritance pattern since, in the inbreeding process of the trait, it segregated a phenotypic frequency of 3:1 (FM:FOF) and in crosses, a frequency of 1:1 (FM:FOF) was segregated. This study potentially contributes to furthering the commercial production of JLC hybrid seed, given that the plants with FOF trait can be used as female parents to be naturally pollinated in isolated plots, thereby reducing labor and cost.**

Key words: Floral biology, heredity, inbreeding, *Jatropha curcas*, native gene.

INTRODUCTION

The physic nut *Jatropha curcas* L. (JCL) has gained attention in recent years as a feedstock source for biodiesel and biojet fuel, as its seed contains 30 to 40% oil (King et al., 2009). However, the crop is still being developed especially the production of commercial hybrids. Commercial hybrid seed production results from crossing two parental inbreds, homozygous or heterozygous (Johnson, 1966; McRobert et al., 2014). This raises the importance of understanding the flowering biology of JLC. The mating system of JLC is complex; however, it is considered to be a monoecious species, also presenting atypical inflorescences (gynoecious,

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androecious, andromonoecious, androgynomonoecious) (Adriano-Anaya et al., 2016), producing plants with female and male flowers: FM, plants with female only flowers: FOF and occasionally plants with hermaphrodite flowers: HF (Heller, 1996), or even producing seeds asexually by apomixes (Bressan et al., 2013; Ahoton and Quenum, 2012). The dioecious type (gynoecious plants) with the female only flower trait (FOF) has been naturally observed in JLC without any breeding process (Heller, 1996; Pecina-Quintero et al., 2011; Adriano-Anaya et al., 2016; Ovando-Medina et al., 2013) and can potentially be used as female parent for hybrid seed production. The production of hybrid seed is one of the most expensive and labor-intense processes in the seed industry (McRobert et al., 2014). The pollination for a cross, especially when homozygous inbreds are used, needs to be controlled as this guarantees the desirable combination of inbred traits and avoids pollen contamination on the female inbred parent by self-pollination or with pollen other than that of the desired male contributed by insects (Vaknin et al., 2012), this results in a uniform F1 population offspring, and expresses heterosis for yield (Birchler et al., 2010; Perez-Prat and van Lookeren, 2002). Pollination should be efficient and economical and depends on factors such as the synchronization of the flowering between the female and male parents in an interplanting row plot (Haque et al., 2012) and contamination of the female parent with foreign pollen (Masuda et al., 2010). Some strategies to reduce female pollen contamination include the emasculation by detaselling of the female parent, as in corn (Stevens et al., 2004), the usage of the cytoplasmic male sterility trait (CMS) (Yamagishi and Bhat, 2014) or the transgenic genes based on nuclear-encoded male sterility as the seed production technology (SPT) (Wu et al., 2016).

Although there are studies on JLC flowering biology (Heller, 1996; Pecina-Quintero et al., 2011; Adriano-Anaya et al., 2016; Costa et al., 2016; Tang et al., 2016; Li et al., 2017), including a published patent WO2011084867 A2 (Rotter, 2010), little is known about the type of inheritance patterns of the FOF trait. The objective of this work was to elucidate the heredity of the FOF native trait through the inbreeding and crossing process of JCL accessions for further potential usage as female parent for commercial hybrid seed production.

MATERIALS AND METHODS

Location of the experiment

The study was carried out in the Jatronergy Research Center, property of Lodemo Company, located in Espita, Yucatan, Mexico. The surrounding vegetation in the experimental areas is mainly tropical forest. The plantations are part of a breeding program aimed at improving JLC for biodiesel production. The soil types in the experimental areas are young soils with shallow depth, mostly stony soil with calcareous origin, with pH between 6.3 to 7.3 and poor organic matter content between 6.7 to 8.9% (Bautista et al., 2005; Bautista et al., 2015).

Genetic materials

A total of 23 accessions of JCL, all property of Jatronergy germplasm bank with origins from the Americas and Mexico, were included in the evaluation method. Table 1 shows the morphological description carried out from 2012 to 2014. All accessions were maintained *in vivo*, by planting cuttings in the field.

Determination of female only flower heredity by inbreeding

Inbreeding wild accessions

A total of eight wild accessions were included in the process of inbreeding to determine the segregation of FOF trait in different inbred populations (Table 2). In parallel, the FOF trait segregation was estimated in a single wild accession, ID 4, by self-pollination up to S_3 endogamy level by using only FM segregating plants (Table 3). In order to represent the endogamy process and differentiate between populations, a hypothetical pedigree was used; the first self-pollination was represented by the letter "X", the number position references a high level of inbreeding for example S_1: (4)X; S_2: (4)X6, and so forth. The number also represents the selected plant among all individuals in the populations.

Experimental design

The eight wild accessions were planted in the germplasm bank area of the company in 2012. Each individual accession was planted in rows of 15 plants per row with 3 m among rows and 2.5 m between plants. All replicated plants per accessions were cuttings. Subsequently, following Jatronergy protocols, inbred seeds were obtained by self-pollination from each wild accession with endogamy levels of S_1, S_2 or S_3. The inbreeding process S_1 was carried out and seeds were harvested in 2012 and S_2 and S_3 in 2013. All inbred seeds were planted in a separate plot in April to May 2014. An inbred population was composed of 10 or 30 plants, each planted at 3.0 m among rows and 2.5 m between plants. Some rows could have more than one population. The plants were drip irrigated, fertilized, and weeded according to the protocols of the company.

During the inbreeding process, the expected inheritance ratio was calculated assuming a Mendelian monogenic inheritance, where the FOF trait represents a homozygous recessive gene (*ff*) and the FM trait, both homozygous and heterozygous dominant gene (*FF or Ff*).

Heredity by crossing FOF female parental with FM male parental (F1 inheritance)

Genetic material

Two wild genotypes of JCL accessions, ID 20 and 24 (Table 1), were selected in the germplasm bank with previous knowledge of naturally expressing the FOF trait as observed by other authors (Adrano-Anaya., 2016; Ovando-Medina et al., 2013); both materials were used as female parents and crossed with 21 and 20 wild accessions, respectively (Table 1) with FM trait used as males to produce F1 hybrids (Tables 4 and 5).

Experimental design

The crosses were carried out and harvested according to Jatronergy protocols in 2012. All hybrid harvested seeds were

Table 1. Morphological description of 23 accessions of *Jatropha curcas* L. included in the evaluation of heredity Female Only Flower (FOF) trait.

Accession ID	TF	PH (m)	SGF[1]	LR[2]	LL[2]	SS[3]	SR[2]	SW (g)	ER[4]
1	FM	3.68	2	0.876	5.00	2	1.730	0.914	7
2	FM	3.31	1	0.916	5.00	2	1.678	0.800	7
3	FM	3.15	2	0.834	5.00	2	1.687	0.931	7
4	FM	2.42	3	0.855	2.50	2	1.664	0.752	5
5	FM	3.40	2	0.984	5.00	1	1.572	0.818	5
6	FM	3.05	2	0.857	5.00	1	1.574	0.826	7
7	FM	2.77	2	0.839	4.75	2	1.655	0.837	7
8	FM	2.10	3	0.899	5.00	2	1.760	0.740	5
9	FM	3.55	1	0.926	4.75	2	1.691	0.837	7
10	FM	2.35	2	0.830	4.75	1	1.627	0.904	7
11	FM	1.96	3	0.822	5.00	2	1.753	0.671	5
12	FM	1.64	3	0.866	5.00	2	1.783	0.712	5
13	FM	1.65	2	0.893	5.00	3	1.736	0.648	7
14	FM	2.59	2	0.934	5.25	2	1.825	0.805	7
15	FM	2.78	2	0.878	4.00	2	1.596	0.858	2
16	FM	2.16	3	1.014	5.00	2	1.757	0.748	5
17	FM	2.92	2	0.877	4.75	2	1.616	0.788	5
18	FM	2.03	2	0.870	5.00	2	1.734	0.802	7
19	FM	1.78	2	0.901	4.75	2	1.773	0.766	7
20	FOF	2.99	2	0.869	5.00	2	1.578	0.846	7
22	FM	2.92	2	0.904	5.00	2	1.726	0.887	7
23	FM	2.45	2	0.933	4.00	2	1.605	0.774	7
24	FOF	2.93	2	0.877	4.00	1	1.517	0.981	7
Average	-	2.63	-	0.889	4.75	-	1.680	0.811	-
SD ±	-	0.60	-	0.05	0.59	-	0.08	0.080	-

TF, Type of flowering; FM, Female and male flowers; FOF, Female Only Flowers; PH, Plant height; SGF, Stem growth form; LR, leaf relationship (length/width); LL, leaf lobes; SS, seed shape; SR, seed relationship; SW, seed weight; ER, endocarp rugosity; [1]qualitative, 1, Erect; 2, Semierect; 3, Open; [2]Quantitative numerical; [3]Semiqualitative; 1, Oblong; 2, elliptical; 3, obovate; [4] Qualitative; 1, Absent; 5, medium; 7, High. Accession ID 21 was not included in the morphological description analysis.

Table 2. Segregation of FOF plants of several populations per accessions of *Jatropha curcas* L. under process of inbreeding planted in Yucatan, Mexico, during 2014 and 2015.

Accession ID	Inbreeding level by population Endogamy level	N	Number of FM plants	Number of FOF plants	FOF trait (%)	E (FM/FOF)	χ^2	*P<F*
4	S_1	35	25	10	29	18.7/6.25	3.0	0.083
4	S_2	148	127	21	14	111/37.0	9.2	0.002
4	S_3	199	152	47	24	149.2/49.7	0.20	0.653
6	S_2	4	3	1	25	3.0/1.0	>0.0	0.99
7	S_2	125	101	24	19	93.7/31.2	2.24	0.134
9	S_2	16	15	1	6	12.0/4	3.0	0.083
13	S_2	13	11	2	15	9.75/3.2	0.64	0.423
16	S_2	21	20	1	5	15.75/5.25	4.87	0.032
19	S_2	16	14	2	25	12.0/4.0	1.33	0.248
22	S_2	24	22	2	8	18.0/6.0	3.55	0.059

N, Total plants in the population; FM, plants with female and male flowers; FOF, plants with female only flowers.

Table 3. Segregation of FOF plants by tracking one accession of *Jatropha curcas* L. under process of inbreeding planted in Yucatan, Mexico, during 2014 and 2015.

[1]Endogamy Population	Endogamy level	N	Number of FM plants	Number of FOF plants	FOF trait (%)	E (FM/FOF)	χ^2	*P<F*
[2](4)	S_0	5	5	0	0	-	-	-
(4)X	S_1	9	-	-	-	-	-	-
(4)X1	S_2	10	9	1	10	7.5/2.5	1.200	0.273
(4)X11	S_3	5	4	1	20	3.7/1.2	0.067	0.796
(4)X2	S_2	44	35	9	20	33/11	0.485	0.486
(4)X21	S_3	4	4	0	0	3.0/1.0	1.330	0.248
(4)X22	S_3	4	4	0	0	3.0/1.0	1.330	0.248
(4)X23	S_3	30	18	12	40	22.5/7.5	3.600	0.058
(4)X24	S_3	5	5	0	0	3.75/1.25	1.660	0.197
(4)X25	S_3	5	5	0	0	3.75/1.25	1.660	0.197
(4)X3	S_2	34	27	7	21	25.5/8.5	0.353	0.552
(4)X31	S_3	27	27	0	0	20.25/6.75	9.000	0.003
(4)X32	S_3	5	5	0	0	3.75/1.25	1.660	0.197
(4)X33	S_3	56	37	19	34	42/14	2.381	0.123
(4)X34	S_3	4	4	0	0	3.0/1.0	1.330	0.248

[1]Underlined represents founder population at a certain endogamy level; [2]Wild plant; N, Total plants in the population; E, expected value; FM, plants with female and male flowers; FOF, plants with female only flowers.

planted in a separated plot in August 2013. A hybrid population composed of 10 or 30 plants; each one planted at 3.0 m among rows and 2.5 m between plants. Some rows could have more than one population. The plants were drip irrigated, fertilized, and weeded according to the protocols of the company.

Data collection

Traits recorded were those relating to flowering biology at a single plant level; type of inflorescence per plant (FM or FOF plants). The data were recorded from August to October in 2014 and 2015 for methodologies of inbreeding and crossings.

Genetic diversity

For assessment of the genetic differences between accessions, eight morphological traits (Table 1) were included in a cluster analysis using Euclidean distance and UPGMA method, with Primer v6 (Clarke and Goyler, 2006). This was to demonstrate evidence of genetic diversity for the crosses to produce F1 and parents used to produce F1 were no siblings.

Data analysis for segregation of FOF trait

For the two methodologies described above, in order to estimate proportions of segregation of FOF plants, the χ^2 (Chi-Square) test was used to validate the existence of Mendelian inheritance of the FOF trait; this is a simple method of quantifying the various deviations expected from observed frequencies, or how well the observed data fits the predictions values if a hypothesis is true. A statistical significance of p=0.05 was used.

RESULTS

FOF heredity by inbreeding of several accessions

All ten populations from eight different *J. curcas* L. accessions in S_1, S_2 and S_3 levels of endogamy showed segregation of FOF plants ranging from 5 to 29% in proportion to all their number of plants. The values of χ^2 ranged from 0.0 to 9.2 and only two populations out of ten in S_2 level of endogamy had values less than *P* <0.05, indicating that mating is random and that segregation and independent assortment resulted in a substantial statistical deviation between observed and expected values of FOF traits plants. In the rest of the populations there was not rejection of the null hypothesis, indicating that observed values were the same as the expected values (no substantial statistical deviations). The hypothesized phenotypic ratio of FM:FOF traits was 3:1 (75%:25%), with eight out of ten populations of JCL accession confirming a monogenic Mendelian inheritance of the recessive homozygous allele *ff* . Given that nine populations segregated the FOF trait in less than 29%, this indicates that the alleles of the plants which were self-crossed were in a heterozygous state *Ff*, and none of them had homozygous dominant alleles *FF* for FM trait (Table 2).

FOF heredity by tracking the inbreeding process of one accession

The wild JCL accession, subjected to an inbreeding process from wild to S_3 endogamy level, showed segregation of plants with FM and FOF traits in their offspring. The founder S_1 population (4)X is a group of bulk plants subjected to an inbreeding process; however, in S_2 and S_3 endogamy levels it was possible to track the segregation (Table 3).

Table 4. Hybrids F1 from crossing a wild accession ID 20 of *Jatropha curcas* L. with naturally occurring FOF trait with other 21 accessions with FM trait.

Hybrid (F1) (Acc. ID)	N	Number of FM plants	Number of FOF plants	% FOF plants	E FM plants	E FOF plants	χ^2	*P<F*
A1 (20x1)	10	0.00	10	100.00	5.00	5.00	10.0	0.002
A2 (20x2)	9	3.00	6	66.67	4.50	4.50	1.0	0.317
A3 (20x3)	10	5.00	5	50.00	5.00	5.00	0.0	1.000
A4 (20x4)	10	6.00	4	40.00	5.00	5.00	0.4	0.527
A5 (20x5)	10	7.00	3	30.00	5.00	5.00	1.6	0.206
A6 (20x6)	10	7.00	3	30.00	5.00	5.00	1.6	0.206
A7 (20x7)	10	7.00	8	20.00	5.00	5.00	3.6	0.057
A8 (20x8)	10	7.00	3	30.00	5.00	5.00	1.6	0.206
A9 (20x9)	10	6.00	4	40.00	5.00	5.00	0.4	0.527
A10 (20x10)	10	9.00	1	10.00	5.00	5.00	6.4	0.011
A11 (20x11)	10	10.00	0	0.00	5.00	5.00	10.0	0.002
A12 (20x12)	10	10.00	0	0.00	5.00	5.00	10.0	0.002
A13 (20x13)	10	10.00	0	0.00	5.00	5.00	10.0	0.002
A14 (20x14)	10	10.00	0	0.00	5.00	5.00	10.0	0.002
A15 (20x15)	10	10.00	0	0.00	5.00	5.00	10.0	0.002
A16 (20x16)	10	10.00	0	0.00	5.00	5.00	10.0	0.002
A17 (20x17)	10	10.00	0	0.00	5.00	5.00	10.0	0.002
A18 (20x18)	9	9.00	0	0.00	4.50	4.50	9.0	0.003
A19 (20x19)	10	10.00	0	0.00	5.00	5.00	10.0	0.002
A20 (20x22)	10	6.00	4	40.00	5.00	5.00	0.4	0.527
A21 (20x23)	10	10.00	0	0.00	5.00	5.00	10.0	0.002

N, Total plants; FM, plants with female and male flowers; FOF, plants with female only flowers.

From all 15 populations with different endogamy levels S_1, S_2 and S_3, only six populations segregated FOF plants. The percentage of FOF segregation ranged from 0 to 40% and the χ^2 values ranged from 0.067 to 3.60. According to the statistical significance for χ^2 test only two populations showed values of P <0.05 indicating that that observed values differ from expected values (substantial statistical deviations).

The S_2 population (4)X1 and the next endogamy level S_3 population offspring (4)X11 segregated FOF plants. The S_2 population (4)X2 segregated FOF plants, however, only one population out of 5 of its S_3 endogamy level offspring segregated FOF plants. The S_2 population (4)X3 segregated FOF plants, however, only one population out of 4 of its S_3 endogamy level offspring segregated FOF plants.

The hypothesized phenotypic ratio of FM:FOF plants of 3:1 (75%:25%) was achieved in 13 out of 14 populations of JCL, according to the χ^2 values and P<0.05, confirming a Mendelian inheritance of a recessive homozygous allele *ff*.

The populations in S_3 level of endogamy without segregation of FOF plants may have homozygous dominant allele *FF* or perhaps the small sample size or number of plants produced was too low, even though the self-pollinated plant had heterozygous alleles *Ff* (Table 3).

Crossing accessions naturally expressing FOF trait accession with FM accessions

The cluster analysis showed phenotypic and genotypic differences among the accessions by using all traits; twenty-tree accessions were grouped in two clusters (“a” and “b”), and two accessions separated (“4” and “15”) from all evaluated accessions, with a range of distances of 8, 7, 13 and 20, respectively (Figure 1). Cluster “b” grouped the maximum number of accessions (total of 15) and the accessions with the FOF trait. The dendrogram showed a genetic contrast between all evaluated accessions, even among accessions with FOF trait within the same group (Figure 1). The results indicated high genetic variability.

The F1 hybrids of JCL, product of crossing a female accession ID 20 with FOF trait x 21 males, showed FOF segregations ranging from 0 to 100%. However, only eleven out of twenty-one F1 hybrids showed segregation of FOF plants (Table 4).

Three F1 hybrids had values ≥ 50% of FOF segregation

Table 5. Hybrids F1 from crossing a wild accession ID 24 of *Jatropha curcas* L. with naturally occurring FOF trait with 20 other accessions with the FM trait.

Hybrid (F1) (Acc. ID)	N	Number of FM plants	Number of FOF plants	% FOF plants	E FM plants	E FOF plants	χ^2	*P<F*
B1 (24x1)	1	1	0	0.00	0.50	0.50	1.00	0.317
B2 (24x2)	8	7	1	12.5	4.00	4.00	4.5	0.034
B3 (24x3)	10	2	8	80.00	5.00	5.00	3.60	0.058
B4 (24x4)	4	2	2	50.00	2.00	2.00	0.00	1.000
B5 (24x5)	3	0	3	100.00	1.50	1.50	3.00	0.083
B6 (24x6)	10	7	3	30.00	5.00	5.00	1.60	0.206
B7 (24x7)	10	8	2	20.00	5.00	5.00	3.60	0.058
B8 (24x8)	7	5	2	28.57	3.50	3.50	1.29	0.257
B9 (24x9)	6	5	1	16.67	3.00	3.00	2.66	0.120
B10 (24x10)	3	3	0	0.00	1.50	1.50	3.00	0.083
B11(24x11)	9	9	0	0.00	4.50	4.50	9.00	0.003
B12 (24x12)	10	10	0	0.00	5.00	5.00	10.0	0.002
B13 (24x13)	10	10	0	0.00	5.00	5.00	10.00	0.002
B14 (24x14)	10	10	0	10.00	5.00	5.00	10.00	0.002
B15 (24x15)	10	8	2	20.00	5.00	5.00	3.60	0.058
B16 (24x16)	10	10	0	0.00	5.00	5.00	10.00	0.002
B17 (24x17)	10	10	0	0.00	5.00	5.00	10.00	0.002
B18 (24x18)	10	10	0	0.00	5.00	5.00	10.00	0.002
B19 (24x19)	9	9	0	0.00	4.50	4.50	9.00	0.003
B20(24x21)	10	10	0	0.00	5.00	5.00	10.00	0.002

N, Total plants; FM, plants with female and male flowers; FOF, plants with female only flowers.

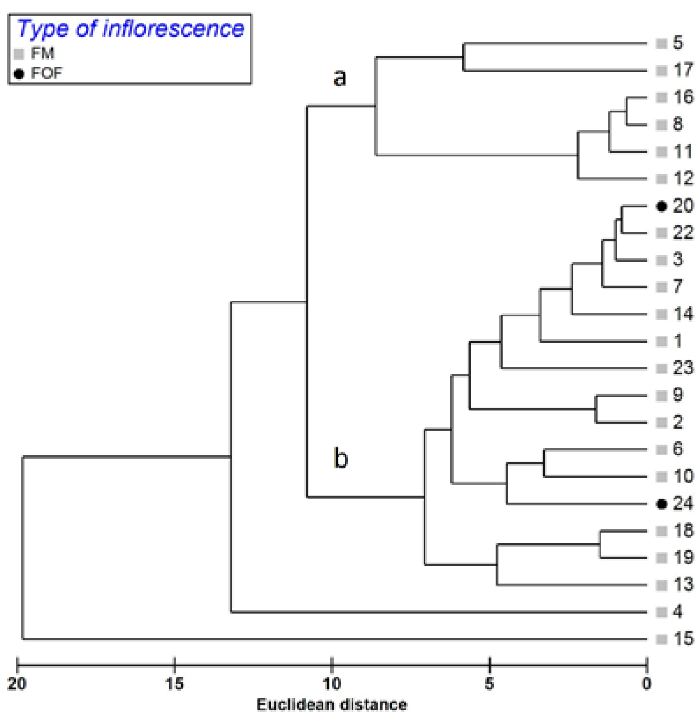

Figure 1. Dendrogram UPGMA showing the morphological diversity between the 23 accessions of *Jatropha curcas* L. evaluated. FM, Female and male flowers; FOF, female only flowers.

plants. The values of χ^2 including all F1 hybrids ranged from 0.0 to 10.0 and the *P*<0.002 to 1.0. Of eleven F1 hybrids that segregated FOF plants, only two (A1 and A10) had a *P*<0.05, indicating that observed values differ from expected values (substantial statistical deviations). The nine remaining hybrids with *P* <0.05 presented a FOF segregation less than 50% (Table 4).

The F1 hybrids that segregated FOF plants reached values from 10 up to 100% which confirmed the hypostatized phenotypic ratio of FM:FOF plants of 1:1 (50%:50%) respectively and also confirmed the Mendelian inheritance of a recessive homozygous allele *ff* in the female parent when crossed with a male with heterozygous alleles *Ff.* However, in the case of F1 hybrids without segregation of FOF plants, this could be due to the small sample size or perhaps the number of plants produced was too low, even though the male parent may have heterozygous alleles *Ff* or the male parent had homozygous dominant allele *FF* (Table 4).

The F1 hybrids of JCL, product of crossing the accession ID 24 with FOF trait with 20 males, showed FOF segregations ranging from 0 to 100%. However, only nine out of 20 F1 hybrids showed segregation of FOF plants (Table 5). Three F1 hybrids had values ≥ 50% of FOF segregation plants. The values of χ^2 including all F1 hybrids ranged from 0.0 to 10.0 and the *P* from 0.002 to 1.0. From nine F1 hybrids that segregated FOF plants only one cross, B12, had a P<0.05, indicating that observed values differ from expected values (substantial statistical deviations). The remaining eight hybrids with P<0.05 had FOF segregation less than 50%.

The crosses that segregated FOF plants reached values from 10 up to 100%, confirming the hypothesized phenotypic ratio of FM:FOF plants of 1:1 (50%:50%), respectively, and thereby confirming the Mendelian inheritance of a recessive homozygous allele *ff* in the female parent when crossed with a male with heterozygous alleles *Ff.* However, in the case of crosses without segregation of FOF plants, this could be due to the sample size or the low number of plants produced, even though the male parent may have heterozygous alleles *Ff* or the male parent had homozygous dominant allele *FF* (Table 5).

DISCUSSION

Inbreeding several populations

Segregation of FOF trait naturally occurred in almost all wild populations of JCL when induced to an inbreeding process. Segregation of less than 30% of FOF plants in S_1, S_2 and S_3 levels of endogamy, confirmed that this trait is inherited at a phenotypic ratio of 75% of FM and 25% FOF, according to Mendelian genetics, since monogenic inheritance implies segregation at a single locus (Elston et al., 2012). Similarly, in tobacco, the native gene *fw* expressing the double-flowering follows an inheritance of a monogenic recessive trait when it is homozygous (Zainol and Stimart, 2001). However, for the FOF segregation, more than 25% in S_1 could be due to the law of maximum segregation according to Mendel's laws. For all S_2 populations FOF segregation was ≤ 25%. The FOF trait variability ranged between 5 to 29%, which may also be due to factors relating to sampling size, since the seeds for planting, which were selected randomly from one single plant or seed number and produced by self-pollination, were not enough. It is likely therefore that the protandry and protogyny reported in JLC may play a role in the amount of seed production, as reported by Negussie et al. (2014). Plants expressing FOF trait for two consecutive years give support to the belief that this trait is not environment dependent, as in the case of cytoplasmic male sterility (CMS) (Fan and Stefansson, 1986).

Inbreeding of one population

Segregation of FOF traits occurred in one wild population of JCL, when induced by the inbreeding process; it also occurred in S_1, S_2, and S_3 endogamy levels and the segregation of less than 40% of FOF plants in all endogamy levels confirmed that this trait is inherited at a phenotypic ratio of 75% of FM and 25% of FOF plants according to Mendelian laws. However, not all plants of a population segregated FOF plants; this could be due to a selection of plants with FM trait to continue the inbreeding process that have heterozygous alleles *Ff* for the FOF trait or even homozygous alleles *FF* for FM trait that may not segregate in the further endogamy dose and relating to sampling size. Occurrence of FOF trait has been observed in wild accessions of JLC (Adrano-Anaya et al., 2016; Ovando-Medina et al., 2013); this may occur due to the type of JLC reproduction which can produce seed by self-pollination (geitonogamy) or even by xenogamy when crossing half or full sibling plants (Ahoton and Quenum, 2012). Inbreeding depression in JCL did not affect survival and fertility although the possible presence of recessive deleterious mutations has been reported by Charlesworth and Willis (2009).

Crossing accession naturally expressing FOF trait with FM accessions

Wild accessions of JCL (ID 20 and 24) naturally express the FOF trait and, when crossed with wild accessions with the FM trait, can segregate FOF plants. However, the percentage of that segregation can range from 0 up to 100%. Segregation of >50% of FOF trait indicates a phenotypic ratio of 50% of this trait according to Mendel's laws. The range of the variable may be due to factors such as sampling size and zygosity of the alleles, either

Ff or *FF,* in the male parents (Fairbanks and Rytting, 2001).

Although JCL is a monoecious plant (Noor Camellia et al., 2012), the origin of those two JLC accession natural expressing FOF trait in this study, may be occurred by natural inbreeding by geitonogamy or by xenogamy (sibling cross or cross with two genetically different plants) (Fresnedo-Ramirez, 2013; Rincón-Rabanales et al., 2016). Sibling crosses may occur when an indehiscent fruit produces three sibling plants which germinate and develop in close proximity and cross with each other. Ahoton and Quenum (2012) also reported that JLC can produce seed by self-pollination, inducing natural /spontaneous autogamy (geitonogamy). If two wild plants have the FOF trait in a heterozygous allele they can also produce FOF trait plants. Kaur et al. (2011) reported up to 72.2% of natural self-pollination and 36.3% of apomixes which is associated with a high level of endogamy in JCL.

In nature, it is likely that plants do not produce the FOF trait due to the fact that at the moment of crossing, one parent has homozygous dominant FM trait alleles (*FF*) and the other heterozygous FOF trait alleles (*Ff*), thus, the offspring will never phenotypically express the FOF trait. There is also evidence of seed produced by apomixes; in this case, the plant produced under this mechanism could affect the expression of FOF trait (Ahoton and Quenum, 2012).

The inheritance of this trait, therefore, is nuclear and not cytoplasmic, as with the CMS in other crops, which is an environment-dependent trait. Some reports mention that sexual ratio in Jatropha flowering is influenced by temperature (Kaur et al., 2011) or by the mode of reproduction (Nietsche et al., 2014) however, there is-no strong data available to support this statement. Nietsche et al. (2014) reported that extreme high temperatures produced flower abortions. The FOF trait, therefore, has the potential to produce JCL hybrid seed without the risk of compromising sterility in the female parent or causing contamination, while also reducing the cost by avoiding detasseling.

Fresnedo-Ramirez and Orozco-Ramirez (2013) has reported that flower sex ratio in JCL is genetically controlled and can be altered by photoperiod, relative humidity, temperature, soil nutrient availability, exogenously applied growth regulators and by an epigenetic control. Plant growth regulators such as benzyladenine and gibberellic acid could affect number of flowers and branching but would not modify the genetics (Costa et al., 2016). Also, some genes play a role in regulating flowering; the gene *jcLFY* in JLC regulates flower identity, flower organ patterns and fruit shape (Tang et al., 2016), *JcTFL1* genes play redundant roles in repressing flowering in *Jatropha* (Li et al., 2017). Flower sex ratio, therefore, is not a conservative trait. The inbreeding process of JCL could be a method to derive FOF trait plants used as female plants in a hybrid production.

The genetic diversity observed in this study formed two clusters that could be divergent for a hybridization program, similar to that reported by Gohil and Pandya (2008) in India, although they evaluated nine genotypes for a breeding program, which were grouped into five clusters. They proposed that only clusters III and IV were the most divergent. The results in this study may be attributed to a wide diversity among groups, since all accessions proceed from America, center of origin for JCL (Heller, 1996), where the possibility to naturally express a homozygous recessive allele for FOF trait is high, as reported (Adriano-Anaya et al., 2016; Ovando-Medina et al., 2013). This genetic diversity is a prerequisite to achieve a successful breeding program (Chikara et al., 2013).

A morphological study of seed traits, based on non-hierarchical Euclidean cluster analysis, in 24 accessions from India, revealed 6 clusters; the maximum inter-cluster distance was 5.1 and the minimum was 2.4 (Kaushik et al., 2007); the American and Mexican accessions evaluated in this study revealed greater genetic diversity, as was also reported by Fresnedo-Ramírez and Orozco-Ramirez (2013). Based on our results, FOF is a monogenic homozygous recessive trait with potential for use in the production of commercial volumes of JCL hybrid seed.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest.

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