

Research Article

Breeding and selection of elite lines combining high oil and seed characteristics in nontoxic background of *Jatropha curcas*

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Abstract

To develop high-yielding, nontoxic *Jatropha* cultivars suitable for biodiesel production, a study was conducted at Jawaharlal Nehru Krishi Vishwavidyalaya, Jabalpur, India. The background involved interspecific hybridization between *Jatropha integerrima* and elite lines of *J. curcas*, followed by backcrossing to eliminate seed toxicity while enhancing yield potential. The material under study comprised four provenances (NBj-1, JC11, MP55-1, and MP55-2); three spontaneous mutants, Dwarf (Dw), Green foliage (Gf), and Small leaf (Sl); and population crosses, viz., five single crosses (52-2, 52-3, NT-1, 61-3, and B1-11); eight three-way crosses (14-22, 9-1, 12-19, 13-11, 13-17, 13-28, 14-24, and 17-21); four four-way crosses (14-16, 21-8, 34-6, and 34-23); and one multiple cross (A9-1). Plants with a sufficient seed yield (>500 g) were analyzed for oil content and phorbol 12-myristate 13-acetate (PMA) content. The PMA was analyzed in different seed parts to understand the genetics of its nontoxic nature, which revealed its maternal inheritance. The four-way crosses indicated better assembly of genes with three-trait combinations; three-way crosses with three- and two-trait combinations; and single crosses with two combinations of traits. The plants derived from single crosses 52-3×34-6 and 61-3×52-2, as well as a four-way cross (52-2×13-11) × (61-3×52-2), emerged as the most promising through hybridization and require further evaluation in small-scale plantations for development and utilization in future breeding programs.

Keywords: Breeding, *Jatropha*, Nontoxic, Oil, Seed Characteristics, Selection

INTRODUCTION

The natural oil industry's primary challenges include rising prices, dwindling natural resources, escalating environmental damage, and rising temperatures. To overcome these limitations, scientists have explored plant biomass as a renewable energy source. As a consequence, biofuel has been produced from a variety of sources, including sugarcane, sweet sorghum, sweet corn, algae, and degraded food grains. In 2003, India launched the National Biofuel Mission with the goal of cultivating non-edible oils from trees and shrubs that can be easily grown on non-arable land (Das, 2020; Saravanan *et al.*, 2020). Based on their oil content and agronomic performance, five tree-borne oilseeds (TBOs) have been identified under this mission: *Pongamia pinnata*, *Azadirachta indica*, *Simmondsia chinensis*, *Vernicia fordii*, and *Jatropha curcas* (Dhyani *et al.*, 2015). *Jatropha curcas* is one of the TBOs with a reduced gestation period, along with a high amount of seed oil that can be used as an alternative to fossil fuels (Banerji *et al.*, 1985). Based on Net Present Value (NPV) and Benefit-Cost (BC) ratio analysis, Ntaribi and Paul (2019) suggested that, under current conditions, *Jatropha* cultivation is not economically feasible.

On the other side, it can be economically advantageous to begin horticulture on a wasteland with the lowest initial investment and annual maintenance expenditures. However, a study conducted by Baral *et al.* (2020) revealed that the economic viability of *Jatropha* could show beneficial outcomes when the output exceeds 5 tons per hectare, which could be achieved beyond 2030. The use of *Jatropha* cake and protein isolates derived from toxic genotypes is restricted due to the presence of antinutritional and harmful elements (Devappa *et al.*, 2010; Francis *et al.*, 2013). Makkar *et al.* (1998a) conducted a comparative examination of nontoxic and toxic varieties of *J. curcas* for their chemical composition and hazardous characteristics in cultivars from Nicaragua, Nigeria, and Mexico, and classified them into either toxic or nontoxic groups.

The toxic genotypes are prevalent worldwide, but the nontoxic genotypes of *J. curcas* and another nontoxic species, *J. platyphylla*, are found only in Mexico (Makkar *et al.*, 2009). Makkar *et al.* (2011) reviewed the potential of a nontoxic variety of *Jatropha* for developing alternative biofuels, as well as protein concentrates for livestock feed, and for producing value-added products that could enhance the economic viability of *Jatropha* oil-based biodiesel production. It is evident from the literature that very few breeding efforts have been made (Francis *et al.*, 2018, 2019) to genetically improve *Jatropha* in either toxic or nontoxic backgrounds, with high oil and stabilized seed yields.

A landmark approach, combining conventional breeding, genetic analysis of growth and seed traits with mo-

lecular markers, and the use of pleiotropic QTLs regulating plant growth and seed yield, has been described (Sun *et al.*, 2012) for *Jatropha* improvement. QTL mapping of *J. curcas* × *J. integerrima* hybrids indicated that favored alleles derive from two distinct parents- 2. Bidirectional transgressive segregation. 3. A complex genetic foundation for traits was proposed, implying that chromosomal areas were related to more than two qualities, indicating linkage or pleiotropy. This resulted in the adoption of elite *Jatropha* cultivars as recurrent lines for the transfer of desired alleles. The group underlined that *J. integerrima* alleles with pleiotropic roles, such as growth, seed yield, and female flower/fruit number, can be coupled via a backcross population. The present study aimed to work on interspecific hybrids involving *J. integerrima* to utilize the improved lines for development and to foster breeding programs to reintroduce *Jatropha curcas* with few traits of domestication, viz., number of seeds per plant, seed index, seed yield, and high oil content in a nontoxic background, to harness its full potential.

MATERIALS AND METHODS

Study area

The investigation was conducted to identify nontoxic lines in new crosses, old crosses, and backcross populations of *Jatropha curcas* at Jawaharlal Nehru Krishi Vishwavidyalaya, Jabalpur, India. The genotypes under study were derived from interspecific hybridization involving *Jatropha integerrima*, followed by backcrosses and repeated crosses with elite lines of *Jatropha curcas*. The existing plantation, comprising study material, was planted with a spacing of 3×3 m and 3×1.5 m. Genotypes under study were comprised of four provenances (NBJ-1, JC11, MP55-1, and MP55-2); three spontaneous mutants: Dwarf (Dw), Green foliage (Gf), and Small leaf (Sl); and population crosses, viz., five single crosses (52-2, 52-3, NT-1, 61-3, and B1-11); eight three-way crosses (14-22, 9-1, 12-19, 13-11, 13-17, 13-28, 14-24, and 17-21); four four-way crosses (14-16, 21-8, 34-6, and 34-23); and one multiple cross (A9-1). The single cross 61-3 involved nontoxic accession from ICAR-Indian Institute of Oilseeds Research, Hyderabad, India.

Data collected

The number of seeds per plant (g), seed index (100 seed weight in g), and seed yield (g) of 1024 plants were measured after four years of plantation. Only plants with seed yields exceeding 500 g were selected for nontoxic plant identification and oil estimation (Table 1). The shell, kernel, tegmen, endosperm, and cotyledon of seeds from direct and reciprocal crossings were also examined for phorbol 12-myristate 13-acetate (PMA) in order to comprehend the genetics of nontoxic nature.

Oil estimation

The oil content in the kernel on a dry basis was estimated using the Soxhlet extraction method, as described in AOAC (1980), with plants having sufficient seed.

Preparation of sample for Phorbol ester analysis

The seed samples were prepared according to the standardized protocol (Makkar *et al.*, 1998b): Each plant's five seeds were weighed and pulverized with a small amount of sterilized sand using a pestle and mortar before adding 20 mL of dichloromethane. The mixture was ground again using the mortar for approximately 5 minutes. The material settled, and the liquid phase was filtered. The residue on the filter paper and in the pestle was mixed with about 20 mL of dichloromethane and pulverized for 5 minutes in a mortar. The liquid phase was collected again, and the extraction procedure was performed three times. The filtrate from each extraction was then pooled. The residue (sand and kernels) went through treatment with ultrasonic waves (105 W) for 3 minutes in 50 mL of dichloromethane. This was followed by filtration and pooling the filtrate with previously extracted material. The filtrate was subjected to drying at about 400°C. The 5 mL of tetrahydrofuran is used to dissolve the dried residue and finally injected into the HPTLC using a 20 microliter tube.

In a similar vein, specimens were generated from several seed components, including the shell, kernel, tegmen, endosperm, and cotyledon in selected crosses. The kernels were removed by pushing the hard shell (testa) with the thumb and first finger while holding the embryonic part of the seed and the other end with pliers. The testa broke when gently pressed. The divided testa was pushed open and removed to extract the kernel. The tegmen, which was very thin and closely attached to the endosperm, was extracted by immersing the seeds in water for 6 to 8 hours. The wet kernel was kept flat with the thumb and first finger while a light incision was made longitudinally on the tegmen. It was then carefully extracted by gripping it with forceps and rolling it around. To remove the cotyledon, two portions were pressed opposing the embryonic side to split the endosperm. The visible cotyledon was delicately lifted,

preserving the embryo, and then collected and dried in the shed for analysis.

Preparation of standard

The phorbol ester standard was prepared in methanol at a concentration of 1 mg/mL. Fifty microliters of the standard were diluted in 0.5 mL of methanol before analysis.

Phorbol analysis

The analysis was performed using 20 cm × 10 cm high-performance thin-layer chromatography (HPTLC) on silica gel G60F254 plates. The samples were put on 15 mm from the plate's base using the CAMAG Linomat 5 automated spray-on applicator. Plates were grown to an 80-mm distance using a phase with mobility of petroleum ether:acetone (6:4) (v/v) (Desmissie and Lele, 2010). Prior to development, the chamber was saturated with mobile phase for 20 minutes. The chromatogram produced by densitometric scanning at 254 nm was created using a CAMAG TLC scanner 3 and CAMAG WinCATS software. The standard phorbol ester peak was observed at $R_f = 0.67 \pm 0.02$, while the R_f value of the percentage matched that of the standard PMA.

RESULTS

Oil percentage

Oil estimations were performed in 164 plants based on the sufficient availability of seeds per plant in kernel on a dry basis. The percentage oil content was estimated in forty-three plants in Set 1 of crosses involved nontoxic female and male parents, varying from 43 to 52%. The cross (61-3×34-6) × (14-24×52-2) exhibited the highest mean value of 52%, followed by 51% in cross (9-1×61-3) × (61-3×52-2). The percentage oil content in Set 2 of crosses involving 28 new plants without a nontoxic parent varied from 41 to 51%. The highest mean oil content of 51% was obtained in cross (14-16×Dwarf) × Dwarf, followed by 50% in (GF×Dwarf) × (GF×34-23) and (Dwarf×13-11) × A-9-1. The percentage oil content in Set 3 across 44 plants varied from 33 to 53% within plants in backcrosses (old) with and without a nontoxic parent. The highest oil content (53%)

Table 1. List of selected one hundred sixty-four genotypes under study

Set No.	Selected crosses	No. of plants
1	Plants with new crosses involving nontoxic as female and male parent	43
2	Plants with new crosses without nontoxic parent	28
3	Plants as backcrosses (old) with and without nontoxic parent	44
4	Nontoxic as female and male parent	4
5	Nontoxic 52-2 (self)	2
6	Plants as clones of advanced line	35
7	Plants of advanced lines	8

was obtained in 9-1×52-2, followed by 52% in 21-8×52-2 and 52-2×13-11. The percentage oil content in Set 4 across four genotypes varied from 53 to 56% for the nontoxic plants used as female and male parents. The highest oil content of 56% was estimated in JC-11×NT-1, and the lowest (53%) in JC-13×NT-6. The percentage of kernel oil content ranged from 49 to 50% in two shelved plants of NT 52-2 (Set 5). The oil content in Set 6 varied from 33 to 54% for the plants as clones of the advanced line. The highest oil content (54%) was obtained in a clone plant of a cross 61-3×34-6, followed by 49% in a reciprocal cross 34-6×61-3.

Phorbol estimation in seeds

Phorbol was analyzed in five different sets of crosses using HPTLC, and the estimated values are represented below.

Phorbol estimation in new crosses involving nontoxic female and male parent

The crosses with nontoxic genotypes 61-3 and 52-2 as male or female parents are shown in Table 2A. When PMA was absent, as shown by 'not detected' (ND), all fourteen crosses were categorized as nontoxic (NT). A cross using 61-3 as the female parent, involving seven plants (61-3 × 34-6) × (13-28 × 13-11), was deemed nontoxic. Plants that were nontoxic were also produced by using 61-3 and 52-2 in single and two-way crosses that were employed as either female or male.

Phorbol estimation in new crosses without nontoxic parent

All the eleven crosses (Table 2B) in the second set not involving a nontoxic source (61-3 or 52-2) exhibited non-detection of PMA or very low value (0.0127 mg/g) in (34-6 × Dwarf) × (Dwarf) or were not analyzed by the system and have been categorized as nontoxic.

Phorbol estimation in backcrosses (old) with and without nontoxic parent

All twenty-five crosses (Table 2C) among the back-cross population (old) with and without nontoxic parents were analyzed for PMA, and they were classified as nontoxic due to values below the toxicity level. The crosses at S. No. 3 and 8 to 22, involving 61-3, 52-2, and 52-3, whether used as a male or female parent once or in both directions, resulted in a nontoxic type. The remaining six crosses at S.Nos. 1, 2, and 4 to 7, not involving a nontoxic parent, also exhibited non-detection of PMA or very low levels, ranging between 0.0104 and 0.0505 mg/g, and are classified as nontoxic. The cross Dwarf × Small leaves at S.No. 4 and 5 with PMA values of 0.0145 and 0.0104 have also been identified as nontoxic.

Phorbol estimation in plants used as female and/or as male parent and selves

The three plants, viz. 61-3, 52-2, and 52-3, used as parents in crosses, also exhibited an absence of PMA; hence, they were classified as nontoxic (Table 2D). The two selfed plants, derived from 52-2, also showed the absence of PMA, which is classified as nontoxic.

Phorbol estimation in direct and reciprocal crosses involving toxic and nontoxic lines

The PMA content in the shell, kernel, tegmen, endosperm, and cotyledon (Table 3) was analyzed to determine its presence and extent of variation across various seed parts, based on the availability of seeds from direct and reciprocal crosses in F₁ toxic parents (P1 and P3) isolated from provenance MP55. Later, the population was derived from two plants, designated as MP55-1 and MP55-2, which were involved in direct and reciprocal crosses with 61-3 (P2).

The parent MP55-1 (P1) exhibited the highest content of PMA in the Tegmen (0.4090), followed by seed (0.0559) and endosperm (0.0030). The rest of the parts exhibited its absence, and therefore it was categorized as toxic, whereas 61-3 (P2), due to the absence of PMA, was identified as nontoxic. The direct cross MP55-1×61-3 (F₁) exhibited its presence in the seed (0.0576), Tegmen (0.5073), and endosperm (0.0058), whereas it was absent in other parts, such as the shell, kernel, and cotyledon, indicating toxicity. The reciprocal cross 61-3×MP55-1 was not detected in the shell, tegmen, or endosperm, and its absence in the remaining parts was not analyzed by the system.

The PMA in seeds and all parts of parent MP55-2 (P3) was either not available (NA) or not detected by the system and was identified as nontoxic. The second parent, 61-3 (P2), as cited above, is also nontoxic. Therefore, the cross MP55-2×61-3 also exhibited an absence of PMA, as indicated by ND. Similarly, its reciprocal 61-3×MP55-2 showed that PMA was neither detected nor analyzed by the system.

Overview of crosses for phorbol 12-myristate 13-acetate (PMA), oil content, and seed characteristics

The study was conducted on crosses to identify nontoxic and high-oil-bearing plants. Data for three seed characteristics — seed yield, number of seeds per plant, and seed index — were also included for the selection of elite plants. The tabulated information (Table 4) provides a comprehensive picture of quantitative traits, along with the status of toxicity and oil content for plants across the crosses.

The first set of crosses with selected top five plants out of 43 reveals the highest seed yield (989 g) and number of seeds per plant (1496), coupled with a seed index (61 g) exhibited by a four-way cross (52-2×13-11) × (61

Table 2. Phorbol estimates in plants across four sets of crosses

A. Phorbol estimates in plants within new crosses involving nontoxic as female and male parent				
S. No.	Code	Cross details	PMA mg/g of seed	Classified as
1	34(18)01	(61-3×34-6) × (13-28×13-11)	ND	NT
2	34(18)04	(61-3×34-6) × (13-28×13-11)	ND	NT
3	34(18)05	(61-3×34-6) × (13-28×13-11)	ND	NT
4	34(18)06	(61-3×34-6) × (13-28×13-11)	ND	NT
5	34(18)08	(61-3×34-6) × (13-28×13-11)	ND	NT
6	34(18)14	(61-3×34-6) × (13-28×13-11)	ND	NT
7	34(18)18	(61-3×34-6) × (13-28×13-11)	ND	NT
8	36(15)01	(61-3×34-6) × (14-24×52-2)	ND	NT
9	38(15)03	(9-1×61-3) × (61-3×52-2)	ND	NT
10	39(34)09	(9-1×61-3) × B 1-11 (Dwarf)	ND	NT
11	41(25)01	(9-1×61-3) × (13-28×13-11)	ND	NT
12	45(34)26	(9-1×61-3) × B 1-11 (Dwarf)	ND	NT
13	47(01)01	(9-1×61-3) × (61-3×52-2)	ND	NT
14	49(03)03	(52-2×13-11) × (61-3×52-2)	ND	NT
B. Phorbol estimates in plants within new crosses without nontoxic Parent				
1	10(02)01	(Dwarf×13-11) × A 9-1	NA	NT
2	18(21)06	(14-16×Dwarf) × Dwarf	ND	NT
3	19(23)13	(14-22 × Dwarf) × Dwarf	ND	NT
4	19(23)15	(14-22 × Dwarf) × Dwarf	ND	NT
5	19(23)16	(14-22 × Dwarf) × Dwarf	ND	NT
6	19(23)20	(14-22 × Dwarf) × Dwarf	ND	NT
7	19(23)21	(14-22 × Dwarf) × Dwarf	ND	NT
8	22(09)01	(34-6×Dwarf) × Dwarf	0.0127	NT
9	22(09)04	(34-6×Dwarf) × Dwarf	ND	NT
10	11(17)10	(GF×Dwarf) × (GF×34-23)	ND	NT
11	26(18)03	(13-17×Small) × (13-17×Small)	ND	NT
C. Phorbol estimates in backcrosses (old) with and without nontoxic parent				
1	A1-5	GF×34-23	0.0108	NT
2	A7-2	13-17×?	ND	NT
3	A11-2	34-6×61-3	ND	NT
4	B1-6	Dwarf × Small-leaved	0.0145	NT
5	B1-8	Dwarf × Small-leaved	0.0104	NT
6	B5-9	NBJ-1×21-8	0.0505	NT
7	E9-7	17-21×21-8 (clone)	ND	NT
8	G2-3	52-3×34-6	ND	NT
9	G2-5	61-3×52-2	ND	NT
10	G2-6	61-3×52-2	ND	NT
11	G2-7	61-3×52-2	0.0068	NT
12	G3-3	52-3×34-6	ND	NT
13	H1-2	9-1 (old) × 52-2	ND	NT
14	H1-5	9-1 (old) × 61-3	0.0344	NT
15	H1-6	9-1 (old) × 61-3	0.0284	NT
16	H2-1	17-21×61-3	0.0704	NT
17	H3-12	21-8×52-2	ND	NT
18	H3-14	21-8×52-2	ND	NT
19	H4-15	21-8×52-2	0.0637	NT
20	H5-1	52-2×61-3	ND	NT
21	H6-7	52-2×13-11	ND	NT
22	H9-14	52-2×?	0.0208	NT
23	I10-1	34-6×12-19	ND	NT
24	I12-1	34-6×12-19	ND	NT
25	I14-4	34-6×12-19	NA	NT
D. Details of plants used as female and/or as male parent and selves				
D1. Nontoxic used as female and male parent				
1	61-3	Parent check	ND	NT
2	52-2	JC-11×NT-1	ND	NT
3	52-3	JC-11×NT-1	ND	NT
D2. Nontoxic 52-2 Plants as self				
1	H10-7A	JC-11×NT-1	ND	NT
2	H10-7B	JC-11×NT-1	ND	NT

Non-toxic parents highlighted in bold; NA: Not analyzed by the system; ND: Not detected; NT: Non-toxic; ?: unknown parent

Table 3. PMA content (mg/g) analyzed over seed, shell, kernel, tegmen, endosperm, and cotyledon in direct, reciprocal crosses and parents

Parents and	Generation	Seed	Shell	Kernel	Tegmen	Endosperm	Cotyledon
MP55-1	P1	0.0559	ND	ND	0.4090	0.0030	ND
MP55-1×61-3	F ₁ (P1×P2)	0.0576	ND	ND	0.5073	0.0058	ND
61-3×MP55-1	F ₁ (P2×P1)	NA	ND	NA	ND	ND	NA
61-3	P2	NA	ND	NA	ND	ND	NA
MP55-2	P3	ND	ND	ND	ND	ND	ND
MP55-2×61-3	F ₁ (P3×P2)	ND	ND	ND	ND	ND	ND
61-3×MP55-2	F ₁ (P2×P3)	ND	ND	NA	ND	ND	NA
61-3	P2	ND	ND	NA	ND	ND	NA

NA: Not analyzed by the system; ND: Not detected

-3×52-2) with low oil (50%), followed by a four-way cross (61-3×34-6) × (14-24×52-2) with seed yield (570 g), the next highest number of seeds per plant (1336), a very low seed index (45 g), and slightly higher oil content (52.9%).

The crosses in the second set with five top plants out of 28 exhibited the highest seed yield (970 g), number of seeds per plant (1772), and lower seed index with 51% oil in the three way cross (14-16×Dwarf) × Dwarf, followed by a three-way cross (34-6×Dwarf) × Dwarf, which recorded the second-highest seed yield (695 g) and number of seeds (1238) and a slightly higher seed index (52.5 g). The other four-way cross (GF×Dwarf) × (GF×34-23) exhibited a higher seed index (65 g) and a lower seed yield (650 g) and number of seeds per plant (1001).

The crosses in the third set, with the top eleven plants out of 44, exhibited the highest seed yield (1,180 g) and the highest number of seeds per plant (2,014) in a single cross, 52-3×34-6, with a 49% oil content. A single cross, NBJ-1×21-8, exhibited the second-highest seed yield (993 g) with 1,630 seeds and 44% oil content.

The nontoxic female and male parents, along with their seeds, exhibited yields ranging from 510 to 630 g, with seed numbers per plant varying from 747 to 918, and a seed index of 59.3 to 63.5 g.

DISCUSSION

Although *Jatropha* produces fruit within the third month of transplantation, it becomes financially feasible only after the third year. Pant *et al.* (2006) employed composite seed samples from six trees in Himachal Pradesh, India, to estimate seed oil variability under various situations, including arable (T₁), non-arable (T₂), and altitude ranges E₁ (400-600m), E₂ (600-800m), and E₃ (800-1000m). Both elevation and site characteristics had a major impact on oil content.

Oil recovery was best in T₂ (42.34%) and E₁ (43.19%), while it was lowest in T₁ (34.97%) and E₃ (30.66%). As a

result, it was found that seed properties and oil content varied depending on the geographical location. Later, Kaushik *et al.* (2007) explained that the fluctuation in oil content was due to the crop's allogamous nature. Singh *et al.* (2016, 2024) identified plants with a high number of seeds and an inadequate seed index as having seeds missing kernels or kernels with poor density, resulting in low oil content.

In this study, oil estimations performed on kernels generated reliable data due to the absence of shells, and the kernel oil percentage across various sets ranged from 33% to 56%. Ewunie *et al.* (2021) identified variations in kernel oil content among Ethiopian *Jatropha curcas* genotypes at different altitudes, ranging from 47.10% to 59.32%. Similarly, studies on *jatropha* kernel oil percentage showed variation ranging from 47.08 to 58.12% (Ginwal *et al.*, 2004), 13 to 58.20% (Gohil and Pandya, 2009), 35.50 to 51.10% (Rao *et al.*, 2009), 57.40 to 57.50% (Makkar *et al.*, 2011), 23.44 to 52.58% (Ghatak and Gaur, 2014), 35.60 to 66.08% (Francis *et al.*, 2018), 33.80 to 44.20% (Andrianirina *et al.*, 2019), 50.60 to 60.30% (Salazar *et al.*, 2020), and 47.10 to 59.32% (Ewunie *et al.*, 2021).

The commercial utilization of toxic genotypes is limited due to the presence of anti-nutritional and toxic factors (Devappa *et al.*, 2010; Francis *et al.*, 2013; Alherbawi *et al.*, 2021; Jonas *et al.*, 2021). The phorbol content in nontoxic *J. curcas* is below the critical toxicity threshold of 0.1 mg/g (Makkar *et al.*, 1998a; Rodrigues *et al.*, 2023). Makkar and Becker (1997) studied a variation in phorbol content, ranging from 0.87 to 3.32 mg/g, in the kernels of 18 toxic provenances from West and East Africa, North and Central America, and Asia. In the present study, 55 genotypes were identified as nontoxic, including 21 elite genotypes with high kernel oil percentages, seed yields, and seed indices. The involvement of 61-3 and 52-2 in single and two-way crosses, using either female or male plants, also resulted in nontoxic plants. Yi *et al.* (2014) reported improvement of the *Jatropha* variety (JO S2) through selective breeding.

Table 4. Identification of elite lines from crosses based on PMA estimates, seed yield, number of seeds per plant, seed index, and oil content

S. No.	Code	Cross Details	PMA Seed (mg/g)	Oil Content (%)	Seed Yield (g)	Seeds Per Plant (No.)	Seed Index (g)
Plants within new crosses involving nontoxic as female and male parent							
1	34(18)18	(61-3×34-6) × (13-28×13-11)	ND	47.2	586.7	952	57.6
2	36(15)01	(61-3×34-6) × (14-24×52-2)	ND	52.9	570.8	1336	45.0
3	39(34)09	(9-1×61-3) × B 1-11 (Dwarf)	ND	50.4	571.2	1303	44.4
4	47(01)01	(9-1×61-3) × (61-3×52-2)	ND	51.5	586.3	1292	46.5
5	49(03)03	(52-2×13-11) × (61-3×52-2)	ND	50.0	989.0	1496	61.0
B. Plants within new crosses without nontoxic Parent							
1	10(02)01	(Dwarf×13-11) × A 9-1	ND	50.0	610.0	1247	50.2
2	18(21)06	(14-16×Dwarf) × Dwarf	ND	51.2	970.0	1772	44.0
3	22(09)01	(34-6×Dwarf) × Dwarf	0.0127	47.0	695.0	1238	52.5
4	11(17)10	(GF×Dwarf) × (GF×34-23)	ND	50.8	650.0	1001	65.0
5	26(18)03	(13-17×Small) × (13-17×Small)	ND	47.7	-	-	-
Plants within backcrosses (old) with and without nontoxic parent							
1	A1-5	GF×34-23	0.0108	45.0	-	-	69.0
2	A7-2	13-17×?	ND	44.0	-	1734	-
3	B1-6	Dwarf×Small leaved	0.0145	45.5	903.5	1285	70.5
4	B5-9	NBJ-1×21-8	0.0505	44.0	993.0	1630	61.9
5	E9-7	17-21×21-8 (clone)	ND	47.0	873.0	-	-
6	G2-3	52-3×34-6	ND	49.5	1180.0	2014	59.4
7	G2-5	61-3×52-2	ND	51.2	970	1442	66.8
8	H1-2	9-1×52-2	ND	53.0	-	-	70.0
9	H3-12	21-8×52-2	ND	52.6	977.0	-	-
10	H5-1	52-2×61-3	ND	45.5	-	-	71.5
11	H6-7	52-2×13-11	ND	51.0	924.0	1450	63.9
Plants used as female and/or as male parent and self							
D1. Nontoxic plant used as female and male parent							
1	61-3	JC 13×NT 6	ND	53.0	580.0	918	63.0
2	52-2	JC-1×NT-1	ND	56.5	510.0	769	59.3
D2. Nontoxic 52-2 plant as self							
1	H10-7A	JC-11×NT-1	ND	49.0	634.0	747	63.5

Nontoxic parents highlighted in bold; NT: Nontoxic

The discovery of harmless lines in *Jatropha curcas* from Mexico, along with the addition of a new species, *Jatropha platyphylla* (Makkar *et al.*, 2011), which contains 60% oil in the kernel, has provided a new avenue for transforming *Jatropha*'s potential in a nontoxic setting. Molecular techniques have successfully identified and classified toxic and nontoxic *Jatropha* (Sujatha *et al.*, 2005; Basha and Sujatha, 2009; Tanya *et al.*, 2011; King *et al.*, 2013; Trebbi *et al.*, 2019; De Souza *et al.*, 2021), as evidenced by practical breeding for genetic improvement.

The first direct cross, MP55-1×61-3 (F_1) exhibited its presence in seed (0.0576), Tegmen (0.5073), and endosperm (0.0058), whereas it was absent in other parts, viz., shell, kernel, and cotyledon, hence toxic; whereas, in the reciprocal cross 61-3×MP55-1, it was neither detected nor analyzed by the system in all the parts, confirming its maternal inheritance. The pattern of inheritance in the present study is in agreement with previous propositions (He *et al.*, 2011) and is known to

be present in both endosperm and tegmen, but is most concentrated in the tegmen (Corner, 1976). This is likely due to a monogenic trait that may be under maternal control. The research findings revealed maternal inheritance (Tanya *et al.*, 2011), and it is now confirmed that this trait is maternally controlled and monogenic dominant (Trebbi *et al.*, 2019), controlled by a locus on linkage group 8, which is responsible for PMA biosynthesis. The presence of PMA in endosperm (0.0058) could be attributed to the non-excision of a complete layer due to the 26-30 cell thickness of the tegmen or soaking of seeds (8 h) and the permeability of phorbol from the inner layer of the tegmen to the cotyledon. The second direct cross, MP55-2×61-3, also exhibited an absence of PMA, as indicated by ND. Similarly, in its reciprocal, 61-3×MP55-2, PMA was neither detected nor analyzed by the system. The plant isolated from MP55, designated as MP55-1 and MP55-2 as parents (P3), reveals the existence of nontoxic material in the indigenous collection, with the former being toxic and the lat-

ter nontoxic. The analysis of large accessions in indigenous *Jatropha* collections may provide nontoxic lines.

Conclusion

Authors resorted to interspecific hybridization due to the limited variability in the indigenous collection of *J. curcas*. The derivatives from crosses between *J. curcas* × *J. integerrima* were repeatedly backcrossed with elite lines to combine and develop plants with high seed yield and high oil content in a nontoxic background. The PMA analysis of different seed parts in direct and reciprocal crosses revealed its maternal inheritance under genetic control. The result proves the existence of nontoxic plants in provenance MP 55. The nontoxic lines had a high kernel oil percentage, ranging from 33 to 56%. The plants of cross JC11×NT-1 recorded the highest oil content (56%). The plants derived from crosses 52-3×34-6, 61-3×52-2, and 52-2×13-11 × 61-3×52-2 were identified as most promising, characterized by a high number of seeds per plant, high seed index, and high oil content in a nontoxic background.

Conflict of interest

The authors declare that they have no conflict of interest.

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