Journal of
Plant Sciences

ISSN 1816-4951

Academic
Journals Inc.

www.academicjournals.com

Journal of Plant Sciences 3 (4): 260-265, 2008
ISSN 1816-4951

Variations in Seed Number per Fruit, Seed Physical Parameters and Contents of Oil, Protein and Phorbol Ester in Toxic and Non-Toxic Genotypes of *Jatropha curcas*

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Abstract: Aims of this research are to provide quantitative information on the variation in number of seeds per fruit and physical parameters and chemical composition of seeds and to compare these parameters between toxic and non-toxic genotypes. The fruits contained one, two, three and four seeds and the percentages of such seeds for the toxic genotype were 14.6, 25.7, 52.0 and 7.6%, respectively and these values for the non-toxic genotype were 8.1, 23.4, 58.3 and 10.2%. Within a genotype, these values differed significantly ($p<0.05$); however between genotypes the percentage of fruits containing only one seed was significantly lower. The seed, shell and kernel mass, oil content of seeds and kernels and protein content of kernel and seed did not differ amongst the four seed groups, both for the toxic and non-toxic genotypes. However, seed and shell mass of toxic genotype were significantly higher than those of non-toxic genotype. The contents of phorbol esters in oil and seed meal did differ significantly amongst the four seed groups for the toxic genotype and these esters were negligible in the non-toxic genotypes.

Key words: Jatropha, toxic genotype, non-toxic genotype, seed number per fruit, phorbol esters

INTRODUCTION

Jatropha curcas, a member of Euphorbiaceae family, is a drought-resistant multipurpose small tree of significant economic importance because of its several industrial and medicinal uses (Makkar *et al*., 2007a). This plant thrives on degraded and poor stony soils. The seed kernels are known to contain 55-60% oil, which can be used as fuel directly or as a substitute to diesel in the transesterified form. The oil is also used for making candles, soap, lubricants and varnishes and is used for illumination. Large scale plantations of this plant have been planned or developed in India, China, Madagascar, Myanmar, Philippines and many other developing countries, with the aim to use the oil as bio-diesel (Francis *et al*., 2005). The seeds of *J. curcas* are highly toxic and the toxicity is ascribed to phorbol esters (Makkar *et al*., 1997; Makkar and Becker, 1998; Goel *et al*., 2007). At present the seed meal is used as a fertilizer (Francis *et al*., 2005; Makkar *et al*., 2007a). Non-toxic genotypes which do not contain phorbol esters have been reported from Mexico (Makkar *et al*., 1998a, b). To our knowledge these genotypes are available only in Mexico.

Heller (1996) reported the fruits of *J. carcas* to contain three seeds. However, the present authors have observed fruits containing one to four seeds. No quantitative information is available on the variation in number of seeds per fruit and how this variation affects physical parameters and chemical

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composition of seeds in the species. The present study is an attempt to study this variation, its impact on physical and chemical parameters of the seeds and also to evaluate these characteristics in relation to the toxic and non toxic genotypes.

MATERIALS AND METHODS

Collection of Material

The yellow-staged ripe fruits from three mature trees each of the toxic and non-toxic genotypes were harvested from Mexico in August 2006. The non-toxic genotypes were collected from Papantla (LN 20′15°, LO 97′15°; altitude, 80 m above mean sea level; annual rainfall, 1500 mm; soil type, calcaric regosol), Veracruz State and the toxic genotypes from Coatzacoalcos (LN 18′09°, LO 94′26°; altitude, 10 m above mean sea level; annual rainfall, 2500 mm; soil type, eutric regosol) Veracruz State. The fruits were dried and brought to Germany for the study.

From one tree, between 300 and 400 fruits were cracked and number of fruits with one, two and three seeds counted. The seeds from the fruits containing one, two, three or four seeds were collected separately, forming four groups of seeds (one seed, two seeds, three seeds and four seeds) for analyses. This gives three replicates (three trees) each of the toxic and non-toxic *J. curcas*.

Physical Parameters

From each of the four groups of the seeds (one seed, two seeds, three seeds and four seeds), forty seeds were selected and weighed separately to calculate seed mass. These 40 seeds were cracked to separate shells and kernel. The weights of shells and kernels were individually recorded and used for determination of shell and kernel mass.

Determination of Oil and Protein in Kernel

The kernels were ground using a coffee grinder. Oil content was determined using a Soxhlet apparatus and protein content as (N × 6.25) using Kjeldahl method (AOAC, 1990). Completely defatted kernel meal (designated as seed meal subsequently) after treatment of the kernel with petroleum ether (bp 40-60°C) in Soxhlet apparatus was collected and used for various analyses.

Determination of Phorbol Esters

The samples were extracted in methanol and phorbol esters were determined on a reverse phase C18 (LiChrospher 100, endcapped 5 μm) 250 × 4 mm I.D. column protected with a guard column containing the same material as the main column according to the procedure outlined in Makkar *et al.* (1997, 2007b). The four phorbol ester compound peaks that appeared between 26 and 31 min were identified and integrated at 280 nm. The results are expressed as equivalent to a standard, phorbol-12-myristate 13-acetate, which appeared between 34 and 36 min.

Statistical Analysis

The data were analyzed for ANOVA using Sigma Stat-Statistical Software Version 2.0 (Jandel Scientific, Sausalito, CA, USA). The significance of the differences between the means was tested using Tukey's test ($p<0.05$). The values are expressed as means ± standard deviation (SD).

RESULTS AND DISCUSSION

Seed Number per Fruit

The percentage of fruits containing one seed was significantly lower ($p<0.05$) in the non-toxic genotype compared to the toxic genotype (8.1 vs 14.6%). On the other hand, reverse was the case for fruits containing four seeds (10.2 vs 7.6%; $p<0.05$). The percentages of fruits containing two and three seeds did not differ significantly between the two groups of genotypes. Within a genotype, for both the genotypes, the highest percentage of fruits contained three seeds, followed by two seeds, which differed significantly. Within a genotype, the percentages of fruits with one and four seeds were statistically significant different (Table 1). Higher percentage of fruits with three and four seeds will give higher yield. In the non-toxic genotype the sum of the fruits containing three and four seeds were significantly higher for the non-toxic genotype (68.5 vs 59.6%; $p<0.05$) and also the variation (as is evident from the standard division) was higher for the non-toxic genotype (Table 2). These variations could be exploited in the breeding programmes to develop a line with desired traits.

Physical Parameters of Seeds

The seed, shell and kernel mass, shell/seed ratio (or in other words kernel to seed ratio) did not differ significantly ($p>0.05$), for both the genotypes, between the four groups of seeds. On the other hand, seed and shell mass of the toxic genotype was significantly higher ($p<0.05$) than that of the non-toxic genotype (Table 3); however, the kernel mass of the toxic and non-toxic genotypes was statistically similar (Table 3) suggesting that higher seed mass of the toxic genotype is contributed by its higher shell mass. From the same number of seeds from the toxic and non-toxic genotypes, the yield of kernels will be the same; however, from the same weight, the yield of shells will be higher for the toxic genotype. The kernel mass as percent of the seed mass was 61.5% for the toxic genotype and 63.9% for the non-toxic genotype. In our earlier studies, this value ranged from 54 to 64% for seeds obtained from India, Nicaragua, Cape Verde and Mexico. The seed and kernel mass and shell to seed ratio were also within the range observed earlier (Aderibigbe *et al*., 1997; Makkar *et al*., 1997, 1998a, b; Martinez-Herrera *et al*., 2006).

Oil and Protein Contents

Within a genotype, the oil content of seeds and kernels and protein content of seeds and kernels from the four groups of seeds did not differ significantly. Also there was no significant difference between the toxic and non-toxic genotype for these parameters (Table 4, 5). The values for the oil and protein are in the range observed earlier (Aderibigbe *et al*., 1997; Makkar *et al*., 1998b; Martinez-Herrera *et al*., 2006).

Table 1: Variation in seed number per fruit (% of total fruits) of toxic and non-toxic genotypes

	Percent fruits containing seeds			
Genotypes	1	2	3	4
Toxic	14.6±1.07^b	25.7±2.89^c	52.0±5.77^d	7.6±3.23^a
Non-toxic	8.1±3.80^a	23.4±3.83^c	58.3±5.77^d	10.2±5.61^e

Values with different superscripts within a column differ significantly ($p<0.05$)

Table 2: Percentage of fruits containing 1 seed plus 2 seeds and 3 seeds plus 4 seeds from toxic and non-toxic genotypes

	Percent fruits containing seeds	
Genotypes	1+2	3+4
Toxic	40.4±2.23^a	59.6±2.23^a
Non-toxic	31.5±6.13^b	68.5±6.14^b

Values with different superscripts within a column differ significantly ($p<0.05$)

Table 3: Seed, shell and kernel masses (g) of toxic and non-toxic genotypes

	Seed mass		Shell mass		Kernel mass	
Fruits with seeds	Toxic	Non-toxic	Toxic	Non-toxic	Toxic	Non-toxic
1	0.77±0.105^a	0.73±0.148^b	0.30±0.034^a	0.27±0.049^b	0.47±0.084	0.46±0.104
2	0.78±0.072^a	0.72±0.107^b	0.29±0.030^a	0.26±0.035^b	0.49±0.052	0.46±0.077
3	0.80±0.081^a	0.73±0.090^b	0.31±0.047^a	0.26±0.031^b	0.49±0.071	0.47±0.065
4	0.77±0.099^a	0.71±0.107^b	0.29±0.035^a	0.25±0.039^b	0.48±0.073	0.45±0.071

For seed mass and shell mass separately, values with different superscripts within a row or column differ significantly ($p<0.05$); for kernel mass, all values are statistically similar ($p>0.05$)

Phorbol Ester Contents

Within the toxic genotype, phorbol ester contents of the seed meal from one-seed group was statistically lower ($p<0.05$) than that from the other three seed groups (Table 6). For the non-toxic genotype, phorbol esters were virtually zero. In our earlier studies as well, the phorbol ester levels in the non-toxic Mexican provenances were near-zero (Makkar *et al*., 1997, 1998b). The levels obtained for the seed meal were similar to those obtained in our previous studies (Makkar *et al*., 1997, 1998b). There was no consistent pattern in phorbol ester content of oil. The phorbol ester content in oil from the two, three or four seed groups was statistically similar ($p>0.05$), whereas of the oil from one-seed group was statistically lower than that of the oil from the other three seed groups (Table 6). In our earlier studies, these levels of phorbol esters in the seed meal have been found to be toxic (Goel *et al*., 2007). Using the values obtained for phrobol esters in oil and seed meal and the oil content in kernel, the oil contained 70 to 75% of total phorbol esters (values for seed groups: one 71%, two 72.5%, three 74.5% and four 70.1%) and the rest (25 to 30%) was in the seed meal. Absence of oil in the seed meal and presence of considerable amount of phorbol esters in the seed meal suggest that phorbol esters are tightly bound to the matrix of the seed meal. The seed meal obtained has crude protein content of approximately 60% with good amino acid composition (Makkar *et al*., 1998a; Martinez-Herrera *et al*., 2006) and has the potential for use as animal feed. The removal of phorbol esters is imperative in order to make this by-product of biodiesel industry a valuable livestock feed. The potential toxicity to animals of the oil or seed meal obtained from the four groups of seeds, due to phorbol esters, is expected to be similar. While, the seed meal from the non-toxic genotype would be an excellent animal feed and the oil could possibly be used as an edible oil. Should the aim is to use the *Jatropha* oil as a source of phorbol esters for use as biopesticide, the potential of the two, three and four seed groups would be almost similar and that of the one-seed group the lowest.

Table 4: Oil content (% in DM) in seeds and kernels from toxic and non-toxic genotypes

	Seeds		Kernels	
Fruits with seeds	Toxic	Non-toxic	Toxic	Non-toxic
1	34.8±1.79	35.30±1.56	56.5±1.03	55.5±0.76
2	35.8±0.78	37.00±1.09	57.2±0.27	57.6±0.94
3	35.2±1.81	37.10±0.97	57.4±0.50	57.5±0.69
4	35.5±1.01	36.01±1.57	57.1±0.42	56.2±0.77

For seeds and kernels separately, the values for toxic and non-toxic genotypes did not differ significantly ($p>0.05$)

Table 5: Crude protein content (% in DM) in seeds and kernels from toxic and non-toxic genotypes

	Seeds		Kernels	
Fruits with seeds	Toxic	Non-toxic	Toxic	Non-toxic
1	16.60±1.27	18.20±0.74	27.0±1.99	28.5±0.87
2	16.50±0.63	17.30±1.39	26.3±0.73	26.9±1.84
3	16.30±1.07	17.30±0.94	26.6±1.12	26.8±1.25
4	16.70±1.11	18.17±0.63	26.8±1.65	28.4±1.22

For seeds and kernels separately, the values for toxic and non-toxic genotypes did not differ significantly ($p>0.05$)

Table 6: Phorbol ester contents (mg/g) in seed meal and oil from toxic and non-toxic genotypes

	Seeds meal		Oil	
Fruits with seeds	Toxic	Non-toxic	Toxic	Non-toxic
1	0.62 ± 0.058^{a}	0.06 ± 0.024^{c}	1.17 ± 0.207^{c}	0.08 ± 0.084^{b}
2	0.78 ± 0.075^{b}	0.01 ± 0.001^{c}	1.54 ± 0.044^{ac}	0.04 ± 0.014^{b}
3	0.81 ± 0.087^{b}	0.04 ± 0.019^{c}	1.76 ± 0.189^{a}	0.03 ± 0.006^{b}
4	0.85 ± 0.064^{b}	0.03 ± 0.014^{c}	1.50 ± 0.132^{ac}	0.03 ± 0.007^{b}

For seed meal and oil separately, the values for toxic and non-toxic genotypes with different superscripts within a row or column differ significantly ($p<0.05$)

CONCLUSIONS

The hypothesis was that seeds from the fruits with higher number of seeds per fruit are likely to have lower seed size and mass because of physical limitation of space and will have lower oil and protein contents due to distribution of fruit nutrients amongst seeds. However, the work reported here does not support this hypothesis. The seed, shell and kernel mass of the seeds obtained from fruits containing one, two, three and four seeds were similar for both the toxic and non-toxic genotypes. Similarly, there was no significant difference in the contents of oil in kernels and seeds and of protein in kernels amongst the groups of seeds, both for the toxic and non-toxic genotypes. All the parameters studied, except seed and shell mass and phorbol esters, were statistically similar when compared between the toxic and non-toxic genotypes. The seed and shell mass were significantly higher for the toxic genotype and phorbol esters were absent in the non-toxic genotype. Fruits with three and four seeds were significantly higher for the non-toxic genotype than for the toxic genotype and also the variation was higher for the non-toxic genotype. These variations could possibly be used for breeding *Jatropha curcas* to obtain varieties with desired traits.

ACKNOWLEDGMENTS

The authors thank Mr. Herrmann Baumgartner for excellent technical help. The financial help of Bundesministerium für Bildung und Forschung (BMBF) and Bundesministerium für Wirtschaft und Technologie (BMWi), Berlin is also thankfully acknowledged.

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