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# Influence of plant growth regulators on flowering, fruiting, seed oil content, and oil quality of *Jatropha curcas*

H.A. Abdelgadir [a], A.K. Jäger [c], S.D. Johnson [b], J. Van Staden [a,*]

[a] *Research Centre for Plant Growth and Development, School of Biological and Conservation Sciences, University of KwaZulu-Natal Pietermaritzburg, Private Bag X01, Scottsville 3209, South Africa*
[b] *School of Biological and Conservation Sciences, University of KwaZulu-Natal Pietermaritzburg, Private Bag X01, Scottsville 3209, South Africa*
[c] *Department of Medicinal Chemistry, Faculty of Pharmaceutical Science, 2 Universitetsparken, 2100 Copenhagen O, Denmark*

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**Abstract**

Field experiments were conducted to determine the effects that plant growth regulators (PGRs) have on seed production of *Jatropha curcas* when they are used for chemical pruning. In the subsequent year, following a single foliar application of PGRs, flowering, fruit set, fruit characteristics, seed total oil content and oil free fatty acid (FFA) content were evaluated. The number of flowers per plant, number of fruits per bunch, fruit- and seed characteristics and seed oil content were significantly affected by the different treatments. However, there were no variations in the degree of fruit set or oil FFA content. A single foliar application of $N^6$-benzyladenine produced more flowers per plant, more fruits per bunch, heavier and bigger fruits and seeds with more oil compared to manual pruning. Treatment with 2,3,5-triiodobenzoic acid yielded more flowers per plant and heavier fruits with a higher oil content than the control and manually pruned plants. Treatment with 2,3:4,6-di-*O*-isopropylidene-2-keto-L-gulonic acid yielded similar results. More fruits per bunch and more seeds per fruit were also produced. Maleic hydrazide treatment yielded more flowers per plant, heavier and bigger fruits with more, heavier, oil rich seeds compared to the control and manual pruning. This study indicates that foliar application of PGRs as chemical pruners in *J. curcas* may have a sequential effect in boosting seed production, seed oil content and improves fruit quality.


*Keywords:* Benzyladenine; Dikegulac; Maleic hydrazide; Plant growth regulators; Seed oil content; TIBA

## 1. Introduction

The rise in the crude oil price and the uncertainty associated with ensuring uninterrupted supplies have compelled the need to look for renewable substitutes. Biofuels are technically feasible alternatives for crude oil (Srinivasan, 2009). Biofuels offer promise, but are controversial because of the large land area required for production, potential for competition with food production, and their marginal economic viability in the absence of subsidies (Gressel, 2008). These potential negative impacts could be reduced and profitability increased if production could be made more efficient. A crop with potential for biofuel production in arid and semi-arid regions is the physic nut, *Jatropha curcas* L (Heller, 1996; Augustus et al., 2002; Azam et al., 2005; Achten et al., 2008). Interest in using *J. curcas* as a feedstock for the production of biodiesel is growing rapidly. The properties of the crop and its oil are sufficiently persuasive to consider it as a substitute for fossil fuels and to help reduce greenhouse gas emissions. However, *J. curcas* is still an undomesticated plant in which many basic agronomic properties are not yet thoroughly understood (Achten et al., 2008). *Jatropha curcas* oil contains about 14% free fatty acid (FFA) which is beyond the limit of 1% level which can be efficiently converted into biodiesel by trans-esterification using an alkaline catalyst (Tiwari et al., 2007). The fatty acids that were reported in a previous study of *J. curcas* oil are palmitic acid (11.3%), stearic acid (17%), arachidic acid (4.7%), oleic acid (12.8%), and linoleic acid (47.3%) (Adebowale and Adedire, 2006).

*J. curcas* bears bunches of fruit at the apex of the branches. Therefore, limited branching is considered one of the major factors limiting yield in this species. Traditionally manual pruning

* Corresponding author. Tel.: +27 33 2605130; fax: +27 33 2605897.
*E-mail address:* rcpgd@ukzn.ac.za (J. Van Staden).

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(MP) is practiced to promote branching. This helps in producing more branches with healthy inflorescences and enhances flowering and fruit set that ultimately increases yield (Gour, 2006). However, cost, convenience and efficiency of MP in large-scale plantations still remain a major concern. A means of obtaining improved flowering, fruiting and seed oil content in *J. curcas* would be of enormous commercial benefit. Plant growth regulators (PGRs) may provide the means of bringing about required growth responses as there is abundant data indicating that their use can increase the yield of product per unit of time and land (Morgan, 1980). An increase in the seed hydrocarbon content in response to hormonal application to *J. curcas* has been reported (Augustus et al., 2002). In cottonseed, application of growth retardants increased seed protein content, oil yield, seed oil refractive index, unsaponifiable matter and total unsaturated fatty acid content (Sawan et al., 2001).

In a previous study we used $N^6$-benzyladenine (BA); 2,3,5-triiodobenzoic acid (TIBA); 2,3:4,6-di-*O*-isopropylidene-2-keto-L-gulonic acid (dikegulac; DK); and 1,2-dihydro-3,6-pyridazine-dione, coline salt (maleic hydrazide; MH) as chemical pruners to increase production of lateral shoots (Abdelgadir et al., 2009). We now report on the effects of foliar applications of PGRs and MP, 1 year after application on flowering, fruit set, fruit characteristics, total seed oil content, and oil free fatty acid content of *J. curcas*.

## 2. Materials and methods

### 2.1. Study site and experimental design

The experiments were conducted in a monoculture plantation at the University of KwaZulu-Natal Agricultural Research Station (Ukulinga) Pietermaritzburg, South Africa, (30° 41′ E, 29° 67′ S and 781 m a.s.l). The plantation was established from seeds obtained from plantations at the Owen Sithole College of Agriculture, South Africa. The original seeds were imported from Malawi. The trial had an irrigation system installed to ensure survival of the plants during early establishment period. These plants were irrigated twice a week in the morning for 3 h with sprinkler system. This irrigation was only for the first three weeks with no fertigation. Shortly after planting, the trial was invaded by weeds which were controlled by a broad-spectrum herbicide Roundup (Monsanto, St. Louis, US) to clear all the unwanted vegetation. The mulch (grass cuttings) was thereafter applied to the base of the plants to reduce weed regrowth and conserve moisture. In the early stage, ten months from planting, the solution of Prev-Am (Citrus Oil Products (SA) (Pty) Ltd., Somerset West, SA) and Cyperfos 500 EC (Gouws and Scheepers Pty Ltd., Witfield, SA) insecticides in combination was sprayed onto plants to control the infestation of beetles (*Aphtona* species). The foliar treatments of one-year-old plants consisted of BA (3, 6, 9 and 12 mM), TIBA (0.5, 1.0, 1.5 and 2 mM), DK (2, 4, 6 and 8 mM) and MH (1, 2 and 4 mM). Three millilitre of 0.1 M sodium hydroxide was used to solubilize the PGRs before adding water. A few drops (2 ml) of Tween® 20 (Merck) were added as a surfactant. Each plant was treated once with 200 mL of respective PGR solution using a 1 L plastic spray bottle. Early in the morning, entire plants were sprayed covering leaves, stems and meristems (Abdelgadir et al., 2009). The adjacent plants were covered temporarily with a sheet of transparent plastic film to avoid drift of PGR. Plants sprayed with distilled water plus an equivalent amount of 0.1 M NaOH served as control. Each treatment consisted of twelve uniform plants considering a single plant as one replicate selected randomly. The spacing between the plants was 2.5 × 2.5 m. Manual pruning was done using a lopping shear on the same day as that of the foliar treatment. The cut was made about 1 cm above an active bud to prevent dieback of the stem and to encourage a new branch to develop. The minimum and maximum temperature during the experiment was 15 and 27 °C respectively. In the subsequent year (May 2008) following the foliar spray in 2007, data for the number of flowers per plant, fruit set percentage and the number of fruits per bunch were collected. In August 2008 fruits were harvested and fruit characteristics: number of fruits per plant, number of fruits per bunch, fruit weight, fruit size, number of seeds per fruit and seed weight were recorded (Fig. 1). Fruit set was considered the percentage of flowers that sets fruit per plant and was normalized by using the angular transformation. SPSS® release 15 (SPSS Inc., Chicago, USA) statistical software was used for data analysis and one-way ANOVA was performed for significant differences.

### 2.2. Extraction of oil

The same seed samples used to determine the fruit characteristics were ground using an A11 BASIC analytical mill. Distilled n-hexane was used as solvent to extract the oil, using a Soxhlet apparatus (Araújo and Sandi, 2006). Three samples (3 g each) of the seed meal from each treatment were placed in Whatman single-thickness cellulose extraction thimbles. Empty round-base glass flasks were weighed for initial mass. Hexane (150 mL) was transferred to each flask and these placed on the Soxhlet plate. The Soxhlet system was connected firmly to a cooling system and ran for 2 h. The solvent was then removed from the extract using a Rotary Evaporator (Büchi).

### 2.3. Oil analysis

Oil was analysed (Martínez-Herrera et al., 2006; Jäger et al., 2008; Halim et al., 2009) as follows: oil (5 µL), 1000 µL MeOH (HPLC-grade), 200 µL (trimethylsilyl) diazomethane (Aldrich); were mixed and shaken for 15 min. Glacial acetic acid (250 µL), 2.5 mL heptane (HPLC-grade), 3 mL saturated NaCl solution; were added, shaken for 30 min, and 1 mL of the heptane fraction was transferred into a GC–MS vial. The sample (1 µL) was injected into the GC–MS. An Agilent 6890N Network GC system coupled to a 5973 Network Mass Selective Detector was used. GC conditions: injector temperature: 250 °C; temperature programme: start 70 °C hold for 4 min, 40 °C/min to 160 °C, 3 °C/min to 270 °C; column: HP5MS; and carrier gas: He.

## 3. Results

### 3.1. $N^6$-benzyladenine

Foliar application of BA at 3 mM significantly ($P < 0.05$) increased the number of flowers per plant compared to the control