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Evaluation of Plant Bio-Regulators (PBR's) application on the fruit and seed yield of *Jatropha curcas*: A bio-fuel plant

Abstract

Jatropha curcas is a potential bio-fuel plant with highly variable fruit and seed yield. Female flowers are far lesser in number as compared to male flowers which is the main reason behind the low fruit yield. Effect of four different Plant Bio-Regulators (PBRs) viz. 6-Benzyladenine (BA), Inole-3-butyric Acid (IBA), Indole-3-Acetic Acid (IAA) and Gibberelic acid (GA_3) were studied on the male: female flower ratio, fruit yield and seed yield of *Jatropha curcas* at DIBER Project site, Mhow (MP), India. The PBR's concentration range was kept from 50 ppm to 250ppm.This experiment was conducted during 2013, 2014 and repeated in 2015 to confirm the results. Exogenous application of Plant Bio-Regulators had a positive effect on the number of female flowers, total number of flowers and number of fruits per plant. BA (100ppm) application modifies the male: female flower ratio significantly from 13:1 to 5:1. Consequently, 3 to 3.5 fold increases in fruit numbers and a 3.1 fold increase in final seed yield were observed. IAA (100ppm), IBA (100ppm) and GA_3 (50ppm) were also found enhancing the fruit and seed yield as compared to control plants. The study revealed that 3 to 3.5 times seed yield of *Jatropha curcas* can be increased by exogenous application of PBRs, which is a significant finding to improve the availability of feed stock for biodiesel production from the *Jatropha* plantation.

Keywords: *Jatropha curcas*, plant bio-regulators, BA, GA_3, IBA, IAA

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Ranjit Singh, Tiwari S, Sanjay Mohan Gupta, Dwivedi SK
Defence Institute of Bio Energy Research, India

Correspondence: Ranjit Singh, Defence Institute of Bio Energy Research, Haldwani, Distt-Nainital (Uttarakhand), India, Email sangmanranjet@rediffmail.com

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Abbreviations: PBRs, plant bio-regulators; BA, 6-benzyladenine; IBA, inole-3-butyric acid; IAA, indole-3, acetic acid; GA_3, gibberelic acid; PPM, part per million; mg, mili gram; kg, kilogram

Introduction

Jatropha curcas is a drought tolerant, oil seed bearing shrub of family *Euphorbiaceae,* widely distributed in Central and South America, Africa, India and south East Asia.[1,2] It is found growing in tropics and subtropics.[3–5] *Jatropha* is a monoecious plant and flowers are unisexual, occasionally hermaphrodite flower also occur.[5] There is a huge variation in seed yield of this plant (Figure 1). It varies from 0.1 to15 tonnes/ha/year.[7] Different researchers have reported different amount of oil content ranging from 24% to 60 %.[8–13] Low yield is the main constraint in successful utilization of this oil yielding plant as biodiesel feedstock.

Studies of exogenous applications of various plant Bio-regulators (PBRs) and analysis of endogenous phyto-hormones showed that PBRs play important roles in floral development.[14,15] Exogenous cytokinin (CK) application has been shown to increase inflorescence meristem activity and promote floral initiation in several species).[16–18] Srinivasan & Mullins[19,20] reported that the tendrils of grape (Vitis vinifera) were converted into inflorescences by application of various CKs. Ohkawa[21] found that 6-benzyladenine (BA, a synthetic compound with CK activity) treatment had a significant influence on increasing flower numbers of Lilium speciosum, particularly when combined with gibberellins GA4 and GA7. Chen[22] showed that flower bud differentiation of Litchi (Litchi chinensis) was significantly promoted by exogenous kinetin application after bud dormancy. The total number of flowers on Jojoba (Simmondsia chinensis) was also significantly increased by treatment with BA.[23,24] Recently, Li & others[25] reported that the flower-specific elevation of cytokinin through transgenic expression of an Arabidopsis cytokinin biosynthesis enzyme gene (ATP/ADP isopentenyl transferase 4, AtIPT4) under the control of the APETALA1 (AP1) promoter led to a three-fold increase of flowers in the transgenic plants. PBRs are also important regulators of floral sex determination, which depends on the plant species.[26–28] CKs have been shown to have a feminizing effect on a number of plant species.[26,27] For example, CK induced bisexual (hermaphroditic) flowers of grape (Vitis vinifera)[29,30] and also female flowers of Luffa acutangula[31] and Luffa cylindrical.[32]

To find ways to increase the total number and proportion of female flowers of *Jatropha*, which may result in increased seed yield.

Figure 1 *Jatropha* fruit bunch.

Materials and methods

Yield enhancement studies were carried out at DIBER Bio-fuel Park at Harsola, Mhow (latitude of 22° 55′ N and longitude of 75° 75′E and at an elevation of 556m above mean sea level in District, Indore (Madhya Pradesh) India. Average temperature of the region ranges from 18.1°C in winter to 32.4°C in summer and average rain fall of the region is around 304mm. Four different plant Bio-regulators *viz.* Gibbrellic acid (GA_3), Benzyl Adenine (BA), Indole Butyric acid (IBA) and Indole acetic acid (IAA) were evaluated on five year old *Jatropha curcas* plants at DIBER Bio-fuel Park during April-May 2013-2015 to study their effect on the fruit and seed yield of *Jatropha curcas*. Exogenous spray of these plant bio-regulators at three growth stages *i.e.* just before the emergence of flowering buds, during flowering and at the time of fruit setting was done (Figure 2). Each PBR was first dissolved in organic solvent or water whichever is applicable and then stock solutions were prepared in 50ppm, 100ppm, 150ppm, 200ppm and 250ppm concentrations for spray. Solutions were sprayed with knapsack sprayers. Data were recorded for flower and fruit intensity in the PBR's treated and control plants. Data were collected regarding Male: female flower ratio, number of female flowers per bunch, fruit/flowering branch, fruits/plant, seed/fruit and seed yield per plant and hence per hectare.

Figure 2 Prepation for PBR's spray on field.

Results and discussion

Plant Bio-Regulators (PBRs) application

It was observed that plants sprayed with PBR's showed 2 to 3 fold increase in the flowering intensity as compared to control plants and higher fruit and seed yield was recorded in these plants as compared to control (Figure 3). Plant bio-regulators (GA_3, BA, IBA and IAA) were found to increase the seed yield as compared to the control plants. But application of BA, significantly modifies the M:F ratio from 21:1 to 5:1. Consequently, 3 to 3.5 fold increase in fruit numbers and 3.1 fold increase in final seed yield were observed in the plants sprayed with 100mg/L of BA. This study indicated that the seed yield of *Jatropha curcas* can be increased by exogenous application of PBRs especially BA (100ppm).

Effect of gibberellic acid (GA3)

It was also sprayed in 50, 100, 150 and 200ppm concentrations and data regarding male: female ratio, fruits per flowering branch, seeds/fruit and seed yield per plant were recorded. Data revealed that 50ppm GA_3 resulted in highest seed yield per plant as comparison to all other PBRs, though other concentrations also resulted in higher yield per plant as compared to control but 50ppm GA3 significantly enhanced the seed yield per plant (Table 1).

Table 1 Effect of different concentrations of GA_3 on fruit and seed yield of *Jatropha*

Conc.	Fruits/flowering branch	Seeds/fruit	Seed yield/plant
50mg	52.11±14.76*	2.59±0.34*	1.121kg*
100mg	41.23±11.26	2.55±0.46	0.913kg
150mg	34.11±10.18	2.57±0.13	0.822kg
200mg	32.72±17.19	2.33±0.43	0.752kg
Control	11.63±2.78	2.25±0.71	0.102kg

Figure 3(A) Effect of Plant Bio- regulators on flower and fruit numbers.

Figure 3(B) Effect of Plant Bio- regulators on flower and fruit numbers.

Effect of benzyl adenine (BA)

BA was sprayed in 50,100,150 and 200ppm concentrations and data regarding male: female ratio, fruits per flowering branch, seeds/fruit and seed yield per plant were recorded. Data revealed that 100ppm BA resulted in the highest seed yield per plant though other concentrations also resulted in higher yield per plant as compared to control but 100ppm BA significantly enhanced the seed yield per plant. The results are shown below in the Table 2.

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Table 2 Effect of different concentrations of BA on fruit and seed yield of *Jatropha*

Conc.	Fruits/flowering branch	Seeds/fruit	Seed yield/ plant
50mg	34.18±15.78*	2.28±0.19	0.947kg
100mg	58.41±18.67	2.25±0.71	1.352kg
150mg	33.26±13.19	2.75±0.15	0.932kg
200mg	31.17±18.61	2.53±0.43	0.852kg
Control (Water only)	11.63±2.78	2.25±0.71	0.102kg

*Data are mean of three years data ±SD

Indole-3- butyric acid (IBA)

Indole-3-butyric acid was also sprayed in 50, 100, 150 and 200ppm concentrations and data regarding male: female ratio, fruits per flowering branch, seeds/fruit and seed yield per plant were recorded. Data revealed that 100ppm IBA resulted in highest seed yield per plant though other concentrations also resulted in higher yield per plant as compared to control but 100ppm IBA significantly enhanced the seed yield per plant (Table 3).

Table 3 Effect of different concentrations of IBA on fruit and seed yield of *Jatropha*

Conc.	Fruits/flowering branch	Seeds/fruit	Seed yield/plant
50mg	38.41±18.67	2.25±0.71	0.962kg
100mg	44.21±12.71	2.85±0.13	1.022kg
150mg	34.21±12.71	2.85±0.13	0.931kg
200mg	32.71±19.60	2.49±0.53	0.902kg
Control	11.63±2.78	2.25±0.71	0.102kg

*Data are mean of three years data ±SD

Indole-3-acetic acid

It was also sprayed in 50,100,150 and 200ppm concentrations and data regarding male: female ratio, fruits per flowering branch, seeds/fruit and seed yield per plant were recorded. Data revealed that 100ppm IAA resulted in highest seed yield per plant though other concentrations also resulted in higher yield per plant as compared to control but 100ppm IAA significantly enhanced the seed yield per plant (Table 4).

Table 4 Effect of different concentrations of IAA on fruit and seed yield of *Jatropha*

Conc.	Fruits/flowering branch	Seeds/fruit	Seed yield/ plant
50mg	31.41±17.72	2.15±0.76	0.928kg
100mg	38.17±18.61	2.53±0.43	0.952kg
150mg	32.21±11.91	2.81±0.18	0.931kg
200mg	30.72±16.92	2.81±0.33	0.812kg
Control (Water only)	11.63±2.78	2.25±0.71	0.102kg

*Data are mean of three years data ±SD

A comparative performance of all the plant bio-regulators on the fruit and seed yield is depicted below in Figure 4.

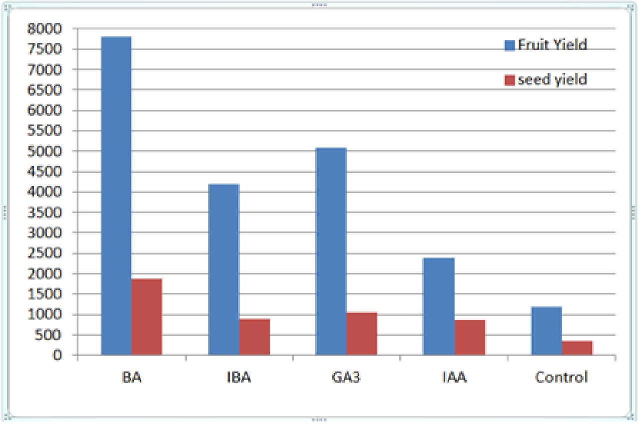

Figure 4 Effect of different PBRs on fruit and seed yield (g) per plant.

PBRs mainly act on the newly developing branches and it mainly changes the male: female ratio. It also affects the seed setting in fruits. Generally three seeds are found in each *Jatropha* fruit but in PGRs applied plants around 5% fruits with 4 seeds in a fruit were also reported and thus enhancing the seed yield (Figure 5).

Figure 5 Change in seed number per fruit due to PBRs spray.

Sex determination in unisexual flowers is a complicated process that is achieved by selectively arresting or aborting pistil or stamen development within a bisexual floral meristem.[33] A number of studies have shown that phytohormones play a pivotal role in the process of the selective arrest or abortion of pistils or stamens in female and male flowers, respectively.[14,15,26] The availability of GA plays an essential role in the expression of feminizing *An1* (*Anther earl*) and *D* (*Dwarf*) genes in maize flowers.[33,34] In cucumber, ethylene is the key hormone involved in sex determination.[16,35] The expression of two genes, *CS-ACS1* and *CS-ACS2*, encoding the ethylene biosynthetic enzymes (1-aminocyclopropane-1-carboxylic acid synthase), correlated with sexual phenotypes.[35] Recently, Martin & others[36] proposed an integrated model of sex determination in melon plants in which the andro-monoecious gene *CmACS-7*, encoding an ethylene biosynthesis enzyme,[37] and the gynoecious gene *CmWIP1*, encoding a zinc-finger transcription factor, interact to control the development of male, female, and hermaphrodite flowers. The expression of the pistil repressor *CmWIP1* causes the arrest of carpel development and the repression of the expression of the stamen repressor *CmACS-7*, leading to the formation of male flowers. The inactivation of *CmWIP1* by promoter hyper methylation, which also indirectly leads to the activation of *CmACS-7*, permits the development of female flowers. Hermaphrodite flowers resulted from *CmWIP1* repression and the presence of a non functional *CmACS-7* gene.[36,37] These results demonstrated that genes encoding metabolic enzymes for different phyto-hormones and the related transcription factors play important roles in the sex determination of various plant species. Thus by the application of plant growth regulators particularly, BA and GA_3 on the *Jatropha curcas* plantation, its production can be enhanced from 2 to 3.5 fold depending upon their concentration. 50mg of GA_3 and 100mg of BA were found to enhance the fruit and seed yield significantly and hence the availability of raw material for biodiesel production.[38–42]

Conclusion

The possible reason of failure of *Jatropha* as a bio-fuel crop is due to mass plantation of low yielding germplasm which resulted in low yield per plant and per hectare. Therefore, application of PBRs at right time and in optimum quantity (50mg of GA_3 and 100mg of BA) can result in higher yield (more than one kg per plant & <1 ton per hectare) which will surely enhances the *Jatropha* production and may result in its consideration further as a viable source for Bio-diesel in future because this plant has potential due to low water requirement and its ability to grow in marginal lands and is also not consumed by stray and wild animals.

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Conflict of interest

Author declares there is no conflict of interest towards the manuscript.

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