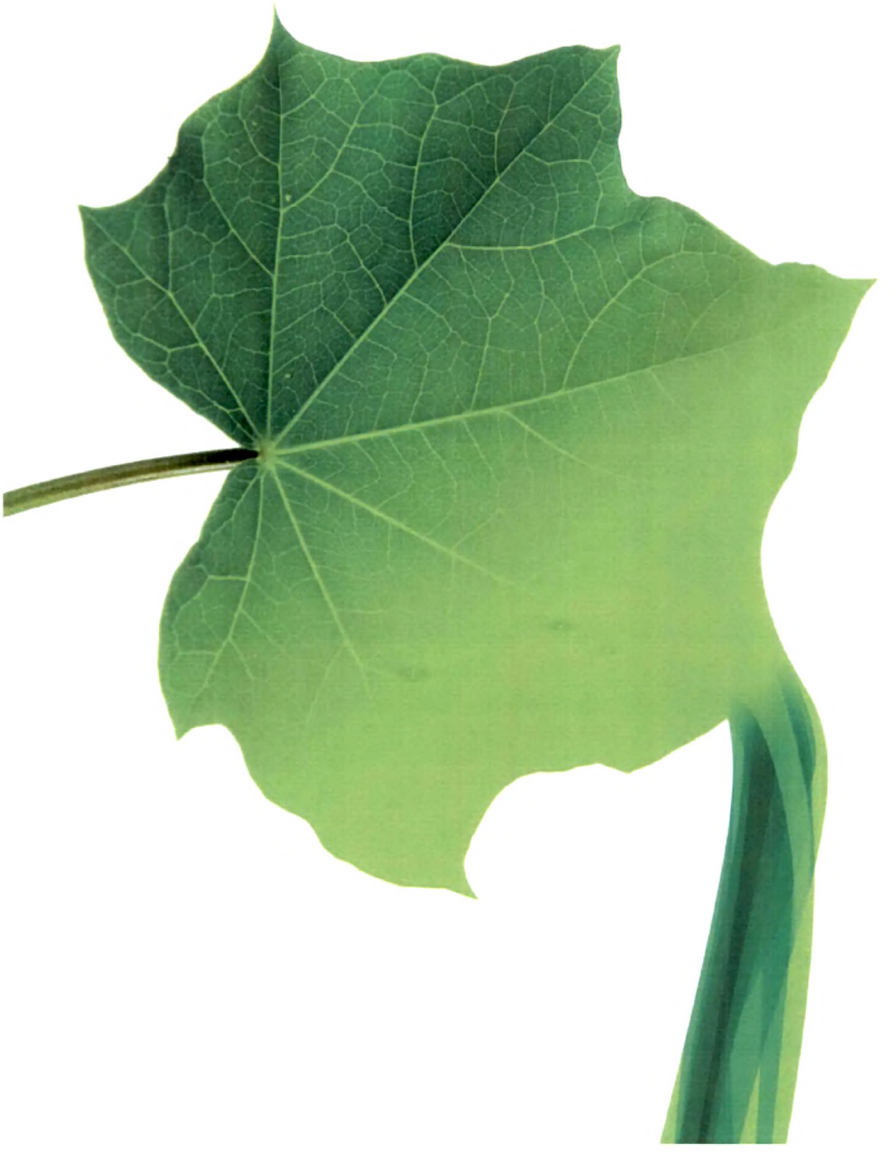
CHAPTER 5



Effect of exogenous application of Phytohormones on seed oil yield and Fatty acid composition in *Jatropha curcas*

Effect of exogenous application of Phytohormones on seed oil yield and Fatty acid composition in *Jatropha curcas*

5.1. Introduction

The oil from *Jatropha curcas* being non-edible is regarded as a potential fuel substitute. Conventional diesel is a mixture of hydrocarbon with 8-10 carbon atoms per molecule. Jatropha oil on the other hand has longer hydrocarbons (16-18). Thus, the nut oil is much more viscous than diesel and has a lower ignition quality (cetane number). For these reasons, using the oil directly in engines had not been fully tested over long periods. In Europe, plant oils are usually trans-esterified (with alcohol and hydroxide) to bio-diesel to obtain properties similar to mineral diesel. This process helps to reduce the viscosity and increases the cetane number of biodiesel. However, this requires considerable investment and currently it is not cost- effective.

The seed and oil yield from plant varies depending on different accessions and its geographical location. The oil yield from the seeds has been reported to be highly variable (25-45%). AFLP analysis revealed very little genetic variation in the seed samples collected from different sites, indicating that the variations in the fatty acid composition of these seeds were largely due to environmental differences (King et al., 2009). Both seed yield and its oil content are affected by various factors like soil quality, nutrients, water etc. The dominant fatty acids present in Jatropha oil are Oleic acid, Palmitic acid, Linoleic acid and Stearic acid. *Jatropha curcas* oil is known for its potential as a feedstock for biodiesel production. The ability of a biodiesel crop to meet the specified criteria is largely determined by the fatty acid composition. In particular, cetane number, cold-flow and cloud point properties, kinetic viscosity, and oxidative stability are all influenced by the fatty acid composition. The production of biodiesel involves the transesterification of the vegetable oil with methanol to produce fatty acid methyl esters (FAMES), which reduces the viscosity of the oil. The most significant variation between the different samples was between the relative amounts of oleate

(18:1) and linoleate (18:2). The actual cetane values of *Jatropha curcas* biodiesel has been determined by a number of groups, and has been within the range of 50–57 (Foidl et al., 1996; Senthil Kumar et al., 2003; Sarin et al., 2007). As biodiesel production from *J. curcas* becomes important, more information is necessary on the cetane values associated with oils produced with respect to its fatty acid compositions (Foidl et al., 1996; Knothe, 2008). The cold-flow and cloud point of biodiesels is determined largely by the concentration of saturated FAMES present in the blend (Imahara et al., 2006). The saturated fatty acid content of *Jatropha curcas* oils typically includes 14–16% palmitate (16:0), 5–8% stearate (18:0), and a trace of longer chain saturates (Foidl et al., 1996). The cloud point of Jatropha FAMES has been determined as 4–8 °C (Sarin et al., 2007; Krishnakumar et al., 2008). The kinetic viscosity of FAMES produced from Jatropha and most other plant oils (i.e. those comprising mainly C16 and C18 fatty acids) falls within these values (Sarin et al., 2007; Knothe, 2008; Krishnakumar et al., 2008).

Improvement in seed and oil yield could be achieved in a number of ways. The development of high-yielding crop varieties through plant breeding and sex alteration has significantly increased agricultural productivity (Evenson and Gollin, 2003). There are a number of traits, which could be targeted for improvement in *Jatropha curcas* including flowering, seed yield and oil content. As discussed in chapter 4, seed yield could be increased by phytohormone treatment. Yield increases in a number of plant species have also been obtained through the modification of plant architecture (Sakamoto and Matsuoka, 2004). Flowering architecture can be modified by pytohormones. Oil yield could also be increased by increasing the synthesis of fatty acid in a seed specific manner. None of the phytohormones have been reported to affect the fatty acid biosynthetic pathway. This has been an unexplored area of plant biochemistry.

In this chapter, an attempt has been made to study the effect of different concentration of phytohormones when applied exogenously on emerging floral bud. The outcome of these experiments was studied throughout flower development and until fruit maturation. The seeds were harvested and seed oil extracted. The oil was esterified and fatty acids were analysed by Gas Chromatography.

5.2. Results

5.2.1. Effect of phytohormones on Fruit yield

Phytohormones such as GA, 2, 4-D, Ethrel and Silver thiosulfate were used for this study. After application of phytohormones on emerging floral bud, the changes in flower sex ratio were monitored. We have discussed in chapter 4 that GA and 2, 4-D increases flowering and femaleness. The Jatropha fruits were harvested and seed weight, oil yield and fatty acid analysis were determined as mentioned in the methods and material section.

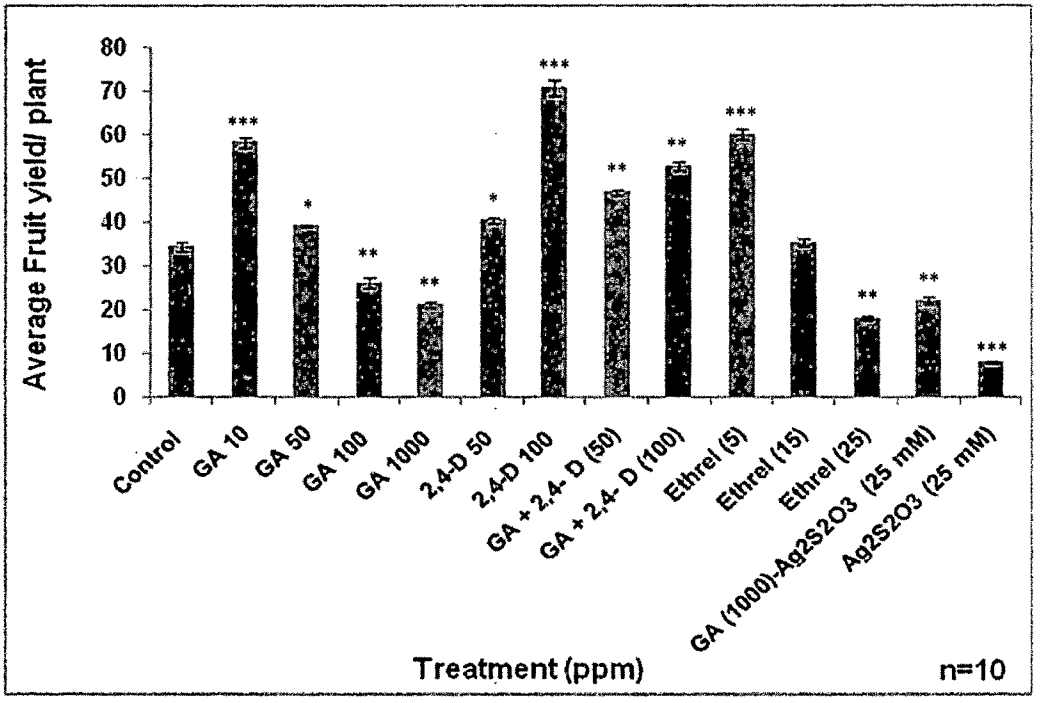


Figure 5.1: Effect of phytohormones on Fruit yield per plant

Average numbers of fruit yield was calculated until one month after last spray of phytohormone. All the plants having equal age and treat equally. Values represented is the mean of 10 replicates and bars indicate SE, *, **, * indicates significantly different at $p<0.05$, $p<0.01$, and $p<0.001$ respectively as compared to control.**

Though there was an increase in fruit yield with initial concentrations of GA, 2,4-D and Ethrel, the increase in concentrations led to a decrease in Fruit yield. This was because there was higher fruit fall in plants treated with 100 and 1000 ppm of GA as compared to 10 ppm. When fruit yield was calculated, the highest number of fruit yield is from plants treated with 2, 4-D (100 ppm), GA (10 ppm) and Ethrel (5 ppm) which is 65, 58 and 56 compared to other treatments and control (Figure 5.1). GA with 2, 4-D results in higher fruit yield as compared to GA 100 and 2, 4-D 50 ppm. 2, 4- D could restore the loss of fruit yield at higher GA treatment. Silver thiosulfate treatment alone or in combination with GA 1000 has a detrimental effect on fruit yield.

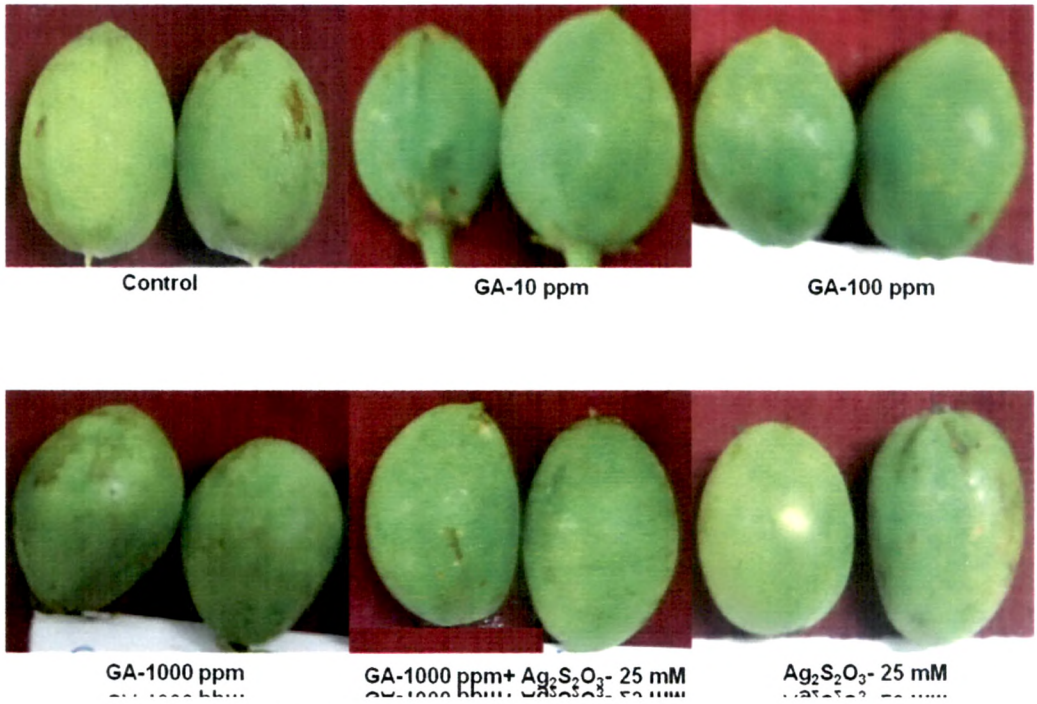


Figure 5.2: Effect of phytohormones on Fruit morphology

The fruit morphology was also observed as shown in Figure 5.2. It was seen that increased GA treatments led to increase in elongation of fruit. Silver thiosulfate treated plants showed black spots on the fruits. GA with Silver thiosulfate treatment protects against the effect of silver ion and shows lesser black spots on fruits, it also shows elongation of fruits as compared to control.

Table 5.1: Effect of phytohormones on Fruit development in *Jatropha curcas*

Treatment (ppm)	Duration (in days) for fruit development				
	Emergence of fruit after pollination (Stage I)	Appearance of Green colour immature big fruit (Stage II)	Yellow colour mature fruit (Stage III)	Brown colour dry fruit (Stage IV)	Total days for fruit maturation
Control	22.80 ± 1.030	48.60 ± 1.077	25.80 ± 1.020	30.10 ± 1.022	129.8 ± 1.414
GA (10)	22.14 ± 2.011	44.50 ± 1.275**	23.60 ± 1.040*	29.20 ± 1.031*	120.1 ± 1.112**
GA (50)	21.60 ± 0.927	39.98 ± 1.287***	21.40 ± 1.435*	24.20 ± 1.121**	104.6 ± 1.536***
GA (100)	20.60 ± 0.812**	36.71 ± 1.364***	19.00 ± 1.414***	22.25 ± 1.121***	102.0 ± 1.164***
GA (1000)	19.70 ± 0.632***	33.60 ± 0.509***	17.60 ± 1.166***	19.85 ± 1.357***	91.00 ± 1.000***
2, 4-D (50)	26.14 ± 2.011**	49.50 ± 1.275*	28.60 ± 1.040*	32.20 ± 1.031*	137.1 ± 1.112**
2, 4-D (100)	22.75 ± 1.041	52.60 ± 1.067**	28.80 ± 1.029**	36.10 ± 1.012**	139.3 ± 1.234**
GA + 2, 4-D (50)	24.20 ± 1.010*	48.20 ± 1.024	22.90 ± 1.170**	31.10 ± 1.050*	128.3 ± 1.426*
GA + 2, 4-D (100)	21.41 ± 1.126*	46.60 ± 1.082*	26.90 ± 1.080*	32.70 ± 1.045*	126.7 ± 1.114*

Values are mean ± SEM; five plants were used for observation in each treatment. In each plant at least, four fruit bloom immediately after pollination until to maturation were used for observation. Results showed in table are twenty blooms of fruits from each treatment. Numbers of fruit bloom used for observation were twenty.

As shown in table 5.1, increased GA treatment enhances the process of fruit development. GA (1000 ppm) significantly decreases the time duration of flower development from 130 to 91 days. 2, 4-D in combination with GA decreases the duration of fruit development as compared to 2, 4-D alone treatments.

5.2.2. Effect of phytohormones on Seed weight

After the maturation of fruits, the seeds were harvested and weighed before oil extraction.

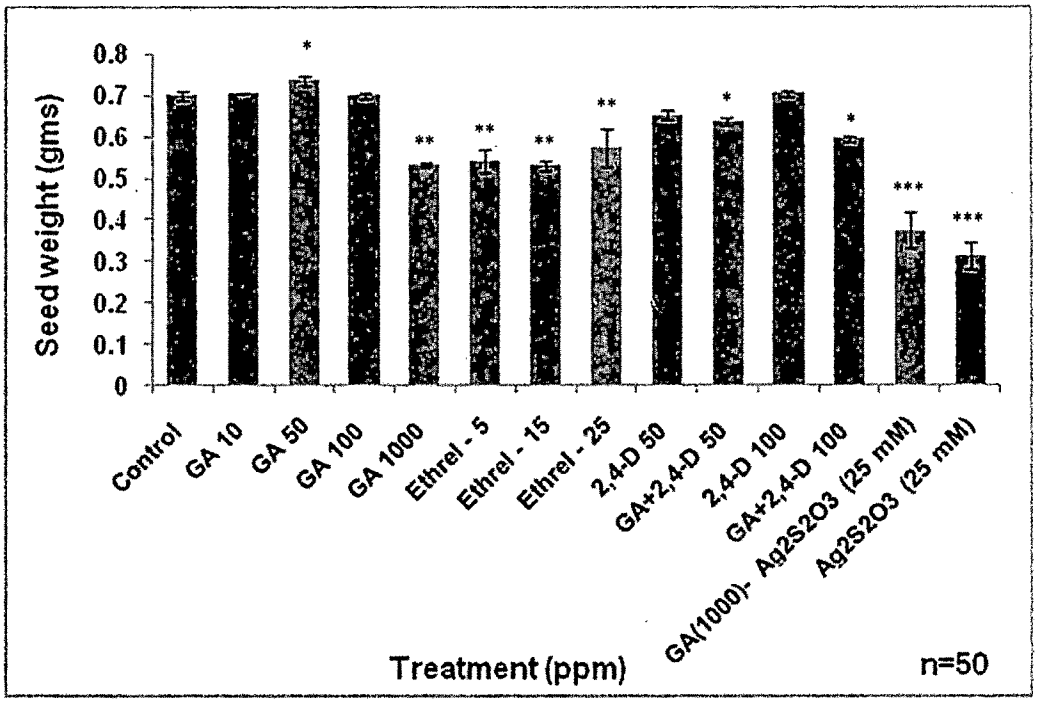


Figure 5.3: Effect of phytohormones on Seed weight

The fruits were dried and seeds were collected after the complete maturation of fruits. Values represented is the mean of 50 replicates and bars indicate SE, *, **, * indicates significantly different at $p<0.05$, $p<0.01$, and $p<0.001$ respectively as compared to control.**

There was a significant decrease in seed weight with GA (1000 ppm), 2, 4-D (50 ppm), Ethrel and Silver thiosulfate treatments. Seed weight was significantly higher only with GA 50 ppm. There was no significant change in seed weight with GA and 2 4-D (100 ppm) treatments when compared with control. There is a significant reduction in seed weight in GA with 2, 4-D and GA with Silver thiosulfate treated plants (Figure 5.3).

Figure 5.4: Effect of phytohormones on Seed morphology

The seeds morphology of the mature dried seeds was observed. As shown in figure 5.4, it was seen that, there was an increase in elongation of seed as the GA treatment increases. Seeds from GA (1000 ppm), Silver thiosulfate alone or in combination were affected as they showed a decrease in weight.

5.2.3. Effect of phytohormones on Seed oil yield and Fatty acid composition

As shown in figure 5.5, there was a significant decrease in seed oil content with GA and Ethrel treatment. The oil content in the seed is significantly higher in 2, 4-D alone and in combination with GA compared to control. Silver thiosulfate treatment significantly decreases oil content.

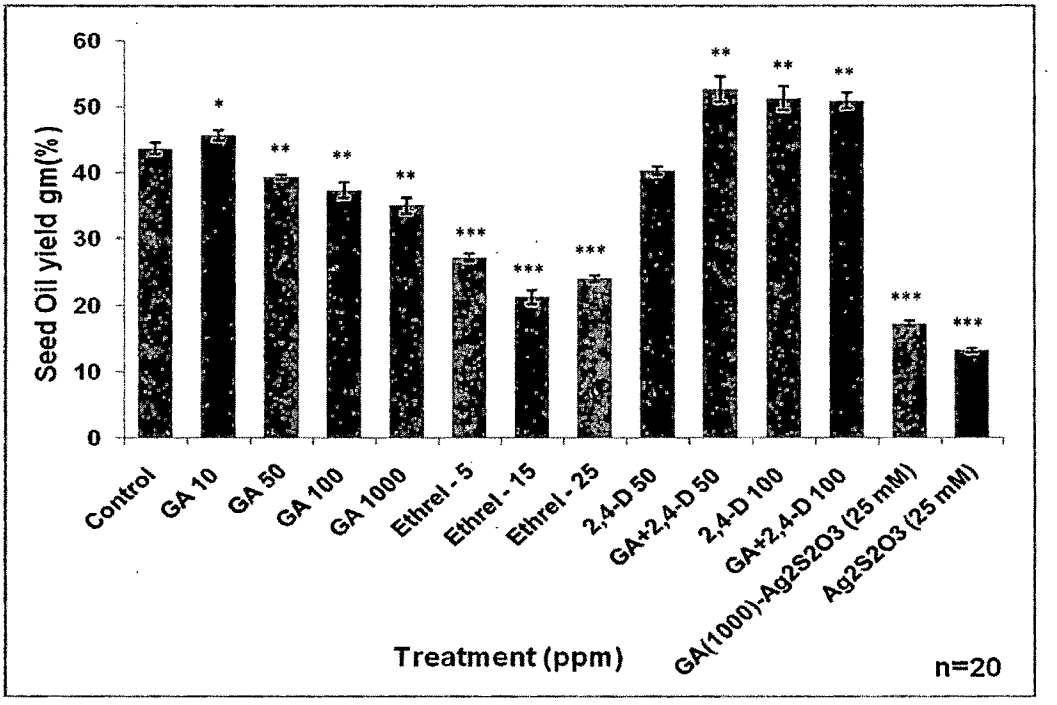


Figure 5.5: Effect of phytohormones on Seed Oil yield

Dry mature seeds were analysed for oil content after removal of seed coat using the method of Bligh and Dyer, 1959 as mentioned in Materials and Method section. Values represented is the mean of 20 replicates and bars indicate SE, *, **, * indicates significantly different at $p<0.05$, $p<0.01$, and $p<0.001$ respectively as compared to control.**

Table 5.2: Effect of GA and 2, 4-D on Fatty acid composition of Seed oil

Fatty Acids	Control	GA-10 ppm	GA-50 ppm	GA-100 ppm	GA-1000 ppm	2, 4-D-50 ppm	2, 4-D-100 ppm
Myristic acid	0.29	---	---	---	---	0.24	---
Palmitic acid	11.29	10.58	10.41	11.62	9.53	13.28	12.28
Palmitoleic acid	0.70	0.64	0.58	0.68	1.0	0.78	0.92
Stearic acid	4.56	4.41	3.89	4.23	4.36	4.80	4.51
Oleic acid	45.15	45.92	45.44	46.39	47.23	48.83	49.13
Linoleic acid	37.81	38.23	39.35	36.92	37.38	31.74	32.75
Linolenic acid	0.20	0.17	0.18	0.16	0.29	0.19	0.25

The seed oil was subjected to fatty acid analyses. This was done by Gas Chromatography as mentioned in chapter 2. The values represented are as g (%). There were no significant changes seen in fatty acid composition in phytohormone treated and control plant seeds.

Table 5.3: Effect of GA, and Silver thiosulfate on Fatty acid composition of Seed oil

Fatty Acids	Control	GA + 2, 4-D- 50 ppm	GA + 2, 4-D- 100 ppm	GA(1000) – $Ag_2S_2O_3$ (25 mM)	$Ag_2S_2O_3$ (25 mM)
Myristic acid	0.29	---	0.07	---	---
Palmitic acid	11.29	13.28	14.07	14.16	13.78
Palmitoleic acid	0.70	0.78	0.62	0.76	0.81
Stearic acid	4.56	4.8	4.76	4.35	4.85
Oleic acid	45.15	48.84	47.31	47.71	48.64
Linoleic acid	37.81	31.74	32.99	32.79	31.87
Linolenic acid	0.20	0.19	0.19	0.19	---

Table 5.4: Effect of Ethrel on Fatty acid composition of Seed oil

Fatty Acids	Control	Ethrel- 5 ppm	Ethrel- 15 ppm	Ethrel- 25 ppm
Myristic acid	0.29	0.12	0.19	0.15
Palmitic acid	11.29	14.27	14.18	14.38
Palmitoleic acid	0.70	0.87	0.89	0.92
Stearic acid	4.56	4.36	4.13	4.26
Oleic acid	45.15	48.32	46.73	45.25
Linoleic acid	37.81	31.71	33.64	34.81
Linolenic acid	0.20	0.25	0.21	0.22

5.3. Discussion

5.3.1. Effect of phytohormones on Fruit yield

When fruit yield was calculated, the highest yield was from plants treated with GA (10 ppm), 2, 4-D (100 ppm) and Ethrel (5 ppm). This was unexpected as the female: male flower ratio as well as total number of female flowers was higher in GA (1000 ppm) treated plant (as results shown in chapter 4). GA with 2, 4-D results in higher fruit yield as compared to GA (100 ppm) and 2, 4-D (50 ppm) alone. Thus, when 2, 4-D with GA was given, it inhibits the process of senescence and prevents withering of fruit and thus increase fruit yield and maturation. Almeida et al., 2004 used similar strategy; to

increase fruit yield in GA treated plants. Auxins are well known to be used for the development of parthenocarpic fruit. The 2, 4-D (100 ppm) treatments during development of fruit triggered an increase in endogenous IAA and enhanced its activity in the ovary, which was responsible for promoting the increased fruit set and enhanced development. This interpretation is consistent with that of Gustafson, (1939) and Kim et al., (1992). This result is also supported by report which mentioned that, in teasel gourd, higher percent of fruit set was also observed by applying 2, 4–D and IAA (Vijay and Jalikop, 1980). Larger fruit size was obtained when the ovary was sprayed with 2, 4–D. Tomato ripening was characterized by regulatory genes such as *RIPENING INHIBITOR (RIN), NON-RIPENING (NOR)* and *COLORLESS NON-RIPENING (CNR),* which have been found to control early stages of fruit development and maturation (Bartley and Ishida, 2003).

5.3.2. Effect of phytohormones on Seed weight

When Seed weight was calculated, it was significantly higher in GA (50 ppm) and 2, 4-D (100 ppm) treatments when compared with control. Kim et al., 1992 have showed that an increased 2, 4-D treatment, triggers an increase in endogenous IAA level and enhanced its activity in the ovary during embryogenesis, which was responsible for high seed weight. Seed weight was significantly lower in GA (1000 ppm), Ethrel and Silver thiosulfate treatments. In the case of *Jatropha curcas* GA may have increased the activity of sugar mobilization up to the flowering and fruiting stages, however a subsequent decline in sugar levels reduced the formation of fruits or improper formation of fruits and thus seed weight is decrease (Bernier et al., 2002; Bernier and Perilleux, 2005). Ethylene is a senescence hormone, which decreases the seed weight due to abscission of immature fruits during embryogenesis. This could be due to limitation of nutrients to sustain the developing seeds or generation of hydrogen peroxide during embryogenesis, which leads to abscission. *Jatropha curcas* is grown as a hedge plant with no due attention given to its nutrient requirement. Addition of fertilizers could help circumvent this problem. However, there is report, which mentions that no significant differences were found in the Seed weight of Olives between control and phytohormones

treatments (Rugini and Panelli, 2003). The results reported here show that the seed weight could be altered by phytohormone treatment in *Jatropha curcas*.

5.3.3. Effect of phytohormones on Seed oil yield and Fatty acid composition

The oil content in the seeds showed appreciable decrease in GA at higher concentration and Ethrel treated seeds indicating that the fatty acid synthesis pathway is not up regulated. The oil content in the seeds increases with 2, 4-D 100 ppm. GA with 2, 4-D shows higher oil yield indicating that 2, 4-D negates the negative effect of GA and increases the flux of fatty acid pathway. This result is in agreement with the result that was obtained by Pandrangi et al., 1992 and Sawan et al., 2001. They studied the effect of Nitrogen on Seed and Oil yield in Cotton. In conjunction with the oil content, the fatty acid profile did not change by phytohormones application (Table 5.1, 5.2, 5.3 and 5.4). Only four free fatty acids were found in our study. In control plant, the seed oil contained saturated fatty acids mainly palmitic acid (16:0) with 11.29% and stearic acid (18:0) with 5%. Unsaturated fatty acids consisted of oleic acid (18:1) with 45.15%, and linoleic acid (18:2) with 37.81%. The oil contained a high percentage of monounsaturated oleic and polyunsaturated linoleic acid indicating it has a semi-drying property (Bailey, 1951). In addition, the oil also contained a higher concentration of unsaturated fatty acids than the saturated ones (Kramer et al., 1979). There was no variation detected in the free fatty acids content between treatments. Similar results were obtained in report, which mentioned the influence of Benzyladenine, which shows no significant changes on oil quality of *Jatropha curcas* (Abdelgadir et al., 2010).