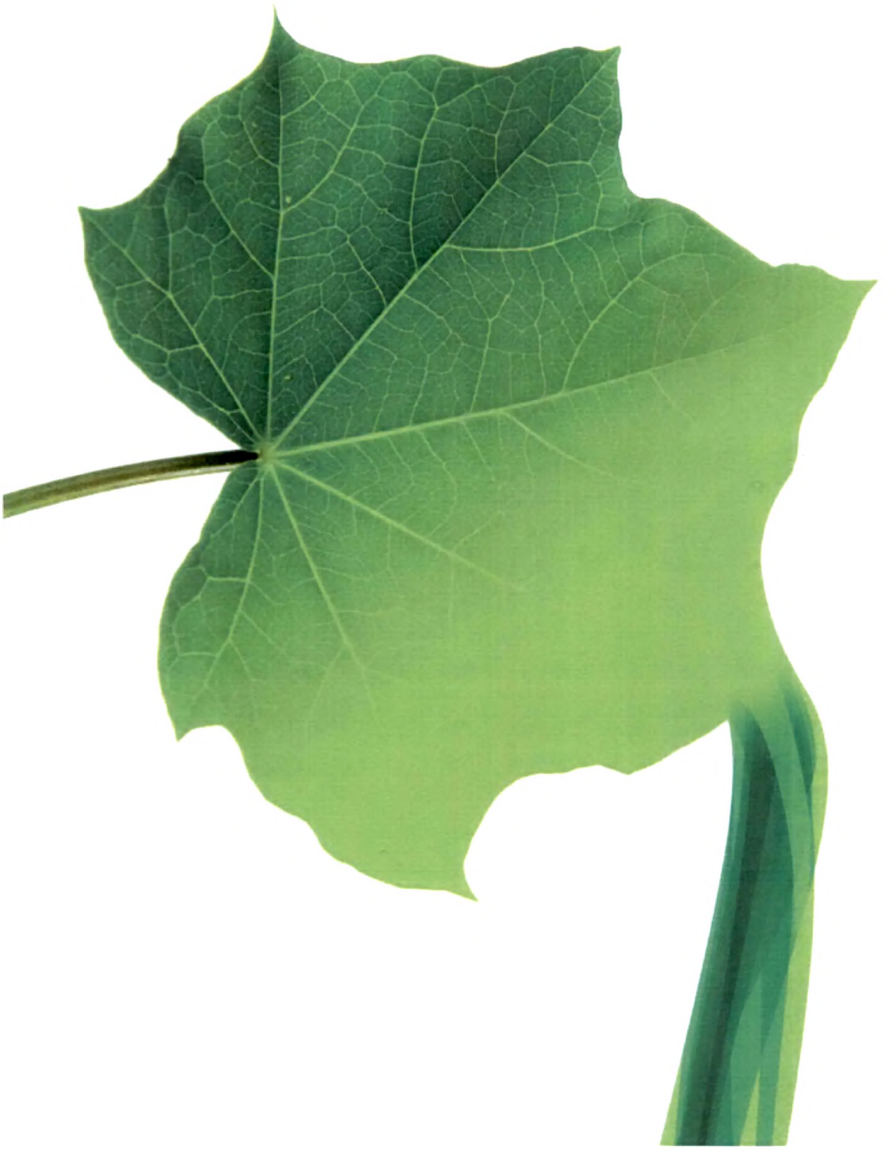
CHAPTER 4



Effect of exogenous application of Phytohormones on Sex alteration and Inflorescence development in *Jatropha curcas*

Effect of exogenous application of Phytohormones on Sex alteration and Inflorescence development in *Jatropha curcas*

4.1. Introduction

Plant hormones play an integral role in controlling the growth, development, metabolism and morphogenesis of higher plants. Plant hormones function as integrators of extracellular signals with the endogenous developmental program.

A change in flower sex ratio towards a greater number of female flowers could result into greater seed yield and hence greater oil yield. This would improve *Jatropha curcas* potential as a biofuel crop. Various factors regulate floral sex expression in plants (Irish and Nelson, 1989; Dellaporta and Calderon-Urrea, 1994; Stehlik et al., 2008). Among these factors, phytohormones play a very important role (Ghosh and Basu, 1983; Durand and Durand, 1991). Of various phytohormones Auxin, Ethylene and Gibbrellin (GA) play an important role in regulation of flower sex expression (Salman-Minkov et al., 2008; Tanimoto, 2007; Thomas, 2008). Effect of exogenous phytohormones on floral sex ratio depends on species of plant. GA has been shown to promote female flowers in maize and castor bean, on the contrary it increases masculine features in hemp, spinach and cucumber (Lazarte and Garrison, 1980; Pharis and King, 1985). Auxin feminizes hemp and cucumber, masculinizes mercury, and indirectly feminizes cucumber by raising ethylene levels in the plant (Heslop-Harrison, 1956; Rudich et al., 1972). In some plant species such as Arabidopsis and Tomato, GA deficiency leads to male sterility because of abnormal anther development (Nester and Zeevaart, 1988; Goto and Pharis, 1999). Though most studies have reported an increase in fruit yield in response to GA, Almeida et al., (2004), have reported decreased fruit yield in response to GA in oranges. They have attributed this to increased fruit withering because of endogenous release of hydrogen peroxide (H_2O_2) at the peduncle.

4.1.1 Molecular mechanisms of floral regulation

Multiple floral inductive pathways control the timing of flowering genes; they are long day photoperiod, GA, autonomous and vernalization. GA promotes flowering by inducing the floral organ identity and floral meristem pathway (Figure 3). *AP1* and *LFY* are the major floral meristem identity genes, which are regulated by GA. One of the key events in the development of flowers is the activation of *LFY* and *AP1*. *LFY* and *AP1* respond, either directly or indirectly, to outputs of flowering time pathways. Some of the outputs of the flowering time pathways are integrated by *LFY*, whereas *FLOWERING LOCUS C (FLC), SUPPRESSOR OF OVEREXPRESSION OF CONSTANS (SOC1),* and *FLOWERING LOCUS T (FT)* integrate others upstream or in parallel to *LFY*. Like *SOC1, LFY* is a key integrator of output signals from the long-day promotion and GA pathways (Blazquez et al., 1998; Nilsson et al., 1998; Blazquez and Weigel, 2000).

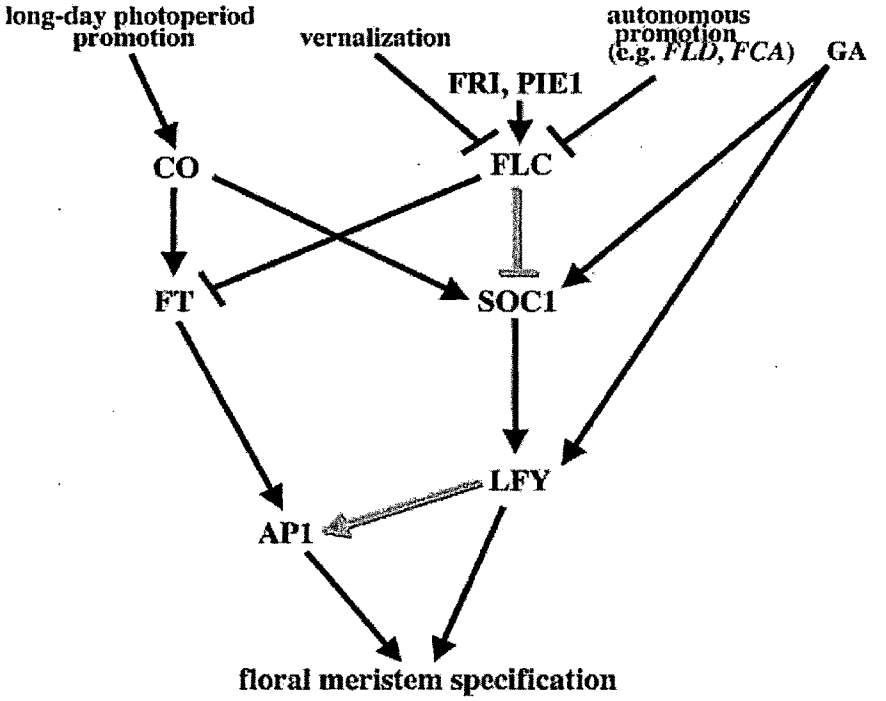


Figure 4.1: Major Floral Inductive Pathways.

Signals from the four major floral inductive pathways are integrated by *FLC, SOC1, FT,* and *LFY.*

AP1 and *LFY* are necessary to specify floral meristem identity; they do not function independently of one another. Instead, AP1 functions largely downstream of LFY. Another major integrator of flowering time pathways is FT (Kardailsky et al., 1999; Kobayashi et al., 1999). The *ft* mutants are late flowering in long days (Koornneef et al., 1991). The primary input to FT activation is long-day photoperiod promotion mediated via CO.

4.1.2 Floral Organ Identity Genes

The well-known ABC model has explained flowering mechanism. One of the important functions of the floral meristem identity genes is to activate the ABC floral organ identity genes. The A class genes specify the identity of sepals and petals that develop in whorls 1 and 2, respectively. Another function of A class genes is to repress C class activity in whorls 1 and 2. In Arabidopsis, there are two A class genes: *AP1* and *AP2*. The B class genes *AP3* and *PISTILLATA (PI)* are required to specify the identity of petals in whorl 2 and stamens in whorl 3. The C class gene *AGAMOUS (AG)* is necessary to specify the identity of whorl 3 stamens and whorl 4 carpels. The second major function of C class is to repress A class activity in whorls 3 and 4.

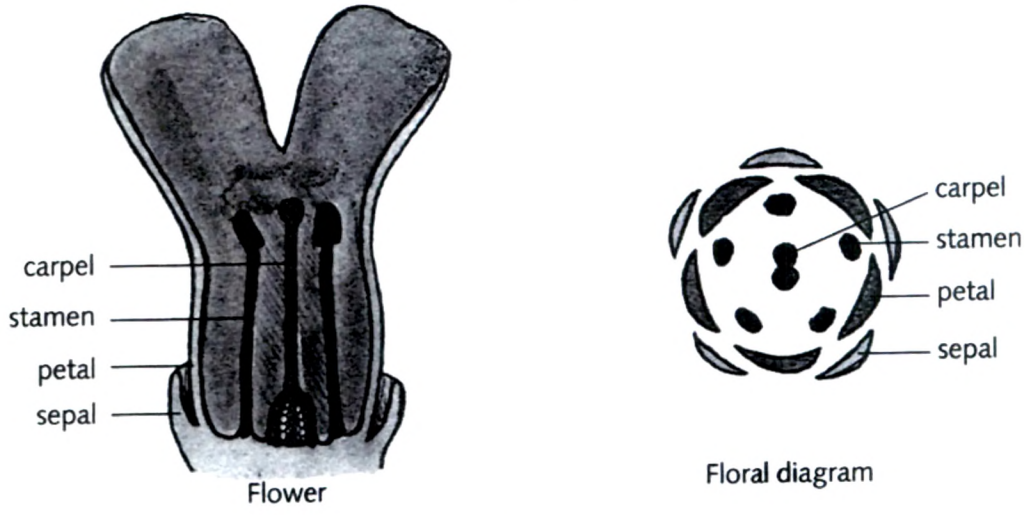


Figure 4.2: Section through a typical flower (left) showing organs arranged in concentric whorls of sepals, petals, stamens and carpels. The overall arrangement is shown in diagrammatic form on the right.

The A, B, and C genes are transcription factors. Different transcription factors are needed together to turn on a developmental gene program--such as A and B needed to initiate the program for petals. Each class of genes is required in two adjacent whorls. Class A genes are required in whorls 1 and 2, class B genes are required in whorls 2 and 3, and class C genes are required in whorls 3 and 4. Both class A and class B genes are required in whorl 2, and both class B and class C genes are required in whorl 3.

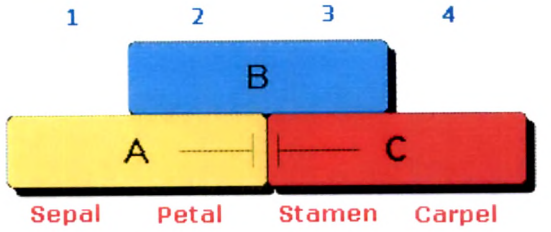


Figure 4.3: Molecular basis of differentiation:

An addition to the ABC model has been made. The *SEPALLATA (SEP)* genes, are necessary for proper floral organ identity (Pelaz et al., 2000, 2001a). The *SEP* genes played an important role in petal, stamen, and carpel identity due to the cosuppression of *SEP* ortholog FLORAL BINDING PROTEIN2 *(FBP2)* resulted in floral organ identity transformations in whorls 2, 3, and 4 as well as a loss of floral determinacy in petunia and tomato (Angenent et al., 1994; Pnueli et al., 1994).

GA and Auxin play an important role in flower growth and development. They regulate cell expansion and tissue differentiation. Auxin regulates GA signaling as well as its biosynthesis. Ethylene affects GA biosynthesis and its interaction with GA governs the stability of DELLA proteins, important modulators of GA action and hence flowering GA response is mediated by negative regulators of GA response called DELLA proteins. The DELLAs are named after the conserved N-terminal DELLA domain and also contain a C-terminal GRAS domain. GAs promote degradation of DELLA proteins, thus inducing the GA transcriptional response. It is reported that Ethylene via its effect on GA biosynthesis results in repression of two flowering genes, *LEAFY (LFY)* and *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1 (SOC1)* (Weiss et al., 2007;

Grauwe et al., 2008). Ethylene increases femaleness in cucurbits species such as cucumber and watermelon (Wang et al., 2010). The regulation of ethylene biosynthesis during inflorescence development has a pivotal role in deterrmining the sex of flower. Silver ion has been shown to interfere with ethylene action, presumbly at ethylene receptor sites (Beyer, 1976). Silver cross the biological membranes as the silver-thiosulfate complex, via an anion transporter (Fortin and Campbell, 2001). Silver ion applied as Silver thiosulfate was more effective than Silver nitrate in inducing flowers of altered sex (Mohan Ram and Sett, 1982). Silver thiosulfate has also been reported to be one of the chemicals for sex alteration in *Silene latifolia*. It shows its effect by partial staminization of female flower during its development (Theresa et al., 2002). Silver thiosulfate also leads to transient effect in which the effect of silver ions in cucumber and pea plants was of a limited duration (Atsmon and Tabbak, 1979).

In this chapter, we studied the effects of GA, 2, 4-D, Ethylene and Silver thiosulfate on the number of flowers, female: male flower ratio, flower development and fruit yield. Silver thiosulfate is an ethylene receptor inhibitor. Our results show that ethylene leads to abscission of flowers and fruits. So, in order to prevent abscission, Silver thiosulfate was used. The effects of phytohormones and silver thiosulfate on various stages of inflorescence development were also studied. Phytohormones regulate specific stages of inflorescence and hence alter the development of flowers and fruit. The results reported in this chapter helps in understanding the role of phytohormones in flower development.

4.2. Results

Two vastly different regions were selected for experimental purpose near Vadodara, Gujarat, which are Vishwamitri Railway colony and Padra region. Land, which was certified as non-agriculture was used for study. The soil was collected and an analysis was done using a kit as per guidelines mentioned.

Table 4.1: Soil Analysis from *Jatropha curcas* plantation region

Plantation region	pH	Organic Carbon	Phosphate kg / hectare	Potassium kg / hectare	Ammonical Nitrogen kg / hectare	Nitrate nitrogen kg / hectare
Vishwamitri Railway Colony	8. 5	0. 5 to 0.6 % Medium	Less than 22 kg / Hec Low	112 - 280 kg / Hec Medium	Less than 15 kg/ Hec Very Low	About 4 kg/ Hec Very Low
Padra	9. 0	0. 1 to 0.3 % Low	Less than 10 kg / Hec Very Low	Less than 112 kg / Hec Medium	Less than 15 kg/ Hec Very Low	About 4 kg/ Hec Very Low

As shown in table 4.1, pH of soil from both the regions was basic. Nitrogen and Phosphorus level were too low in soil while Carbon and Potassium level were medium. The amount of Nitrogen, Phosphorus, Potassium and Carbon present in soil is not compatible as per Fertilizer Control Order guidelines, Government of India. Thus, both regions used for *Jatropha curcas* plantation had soil, which is unsuitable for agriculture and hence could be used for a non-edible crop like *Jatropha curcas* plantation.

4.2.1 Effect of phytohormones on flowering and flower sex ratio in *Jatropha curcas*

Phytohormones such as GA, 2, 4-D, and Ethrel and Siver thiosulfate at specific concentration alone and combination were used for study purpose. There are several reports, which mentioned the application of these phytohormones for sex alteration in different plant species.

Table 4.2: Effect of GA and 2, 4-D on flower number and sex ratio in *Jatropha curcas*

Treatment (ppm)	Total flowers in inflorescences	Total Male flowers	Total Female flowers	Ratio Female: Male
Control	2058 ± 14.23	1978 ± 13.68	80 ± 0.55	1: 25
GA (10)	2084 ± 7.57	1990 ± 7.23	94 ± 0.34*	1: 21
GA (50)	2358 ± 8.05 ***	2226 ± 7.48***	132 ± 0.57 ***	1: 17
GA (100)	3117 ± 11.24***	2923 ± 9.82***	195 ± 0.26***	1: 16
GA (1000)	3342 ± 16.24***	3097 ± 2.95***	245 ± 0.28***	1: 13
2, 4-D (50)	2136 ± 4.48***	2025 ± 4.33***	112 ± 0.21***	1:18
2, 4-D (100)	3116 ± 2.09***	2920 ± 1.24***	196 ± 0.85***	1:15
GA + 2, 4-D (50)	1982 ± 8.91***	1881 ± 8.36***	101 ± 0.56***	1:19
GA + 2, 4-D (100)	1912 ± 8.49***	1788 ± 7.65***	124 ± 0.84***	1:15

n= 20 Inflorescences

Values are mean ± SEM, *, *** indicates significantly different at P<0.05 and P<0.001 as compared to the corresponding control. Five plants were used for observation from each treatment. In each plant at least, twenty inflorescences were used for observation. Results showed in table are twenty inflorescences, an average of five plants from each treatment.

Table 4.3: Effect of Ethrel and GA with Silver thiosulfate on flower number and sex ratio in *Jatropha curcas*

Treatment (ppm)	Total flowers in inflorescences	Total Male flowers	Total Female flowers	Ratio Female: Male
Control	2058 ± 14.23	1978 ± 13.68	80 ± 0.55	1: 25
Ethrel (5)	2692 ± 15.13***	2552 ± 11.21***	140 ± 2.51***	1: 18
Ethrel (15)	1947 ± 8.326***	1856 ± 7.24***	92 ± 1.23*	1:20
Ethrel (25)	1808 ± 6.237***	1728 ± 10.34***	80 ± 0.267*	1:22
GA (1000)	3341 ± 16.24***	3097 ± 12.95***	245 ± 0.28***	1: 13
$Ag_2S_2O_3$ (25 mM)	258 ± 3.13***	248 ± 1.28***	15 ± 0.13***	1:17
GA(1000) - $Ag_2S_2O_3$ (25 mM)	556 ± 4.81***	521 ± 3.12***	36 ± 0.12***	1: 15

n= 20 Inflorescences

Values are mean ± SEM, *, *** indicates significantly different at $P<0.05$ and $P<0.001$ as compared to the corresponding control. Five plants were used for each treatment.

Phytohormones were sprayed alone or in combination on emerging floral bud at different concentrations. Experiments were conducted on fourteen-month-old plants and method of spraying is as discussed in Materials and Methods (Chapter 2).

In a similar manner, plant growth regulators such as Ethrel and Silver thiosulfate were sprayed alone or in combination on floral bud at different concentrations.

Table 4.4: Effect of growth regulators on increase female flower in *Jatropha curcas*

Treatment	Plant sample size	Percentage increase in total flowering	Percentage increase in Femaleness in female: male flower ratio
Control	5	-	-
GA (10)	5	1.26	4.55
GA (50)	5	14.58	5.56
GA (100)	5	51.46	5.88
GA (1000)	5	62.40	7.14
2, 4-D (50)	5	3.80	5.26
2, 4-D (100)	5	51.41	6.25
GA + 2, 4-D (50)	5	-3.69	5.00
GA + 2, 4-D (100)	5	-7.09	6.25
Ethrel (5)	5	30.81	5.26
Ethrel (15)	5	-5.39	4.77
Ethrel (25)	5	-12.14	4.35
$Ag_2S_2O_3$ (25 mM)	5	-87.47	5.56
GA(1000) - $Ag_2S_2O_3$ (25 mM)	5	-72.98	6.25

(n=5 plants)

GA and 2, 4-D treatment resulted in an increase in total number of flowers and femaleness in a concentration dependent fashion and the best increase in flowering was observed in plants treated with GA (1000 ppm) and 2, 4-D (100 ppm) which is 62% and 51%. GA at 50 and 100 ppm resulted in an increase in total number of flowers by 15% and 51 % (Table 4.4). However, GA along with 2, 4-D significantly decreases the number of flowers and flower sex ratio (Table 4.2). This effect of GA and 2, 4-D on flower number and sex ratio was also concentration dependent. Ethrel treatment resulted in a decrease in total number of flowers and femaleness in a concentration dependent

manner (Table 4.3). Ethrel (25 ppm) showed significant decrease in total flowers and female: male flower ratio. A positive effect on flowering was observed in plants treated with Ethrel (5 ppm) which is 31% (Table 4.4). Silver thiosulfate alone or with GA shows very less flowering compared to control (Table 4.4). GA could decrease the drastic effect of Silver thiosulfate on flowering. Thus, from all the treatment the highest femaleness was observed in GA (1000 ppm).

4.2.2 Effect of phytohormones on Inflorescence development in *Jatropha curcas*

Flower development was studied by categorizing the different stages with initial appearance of floral bud designated as stage I. The stages are as described in chapter 3. As shown in table 4.5, increased GA treatment hastens the process of flower development. GA (1000 ppm) significantly decreases the time duration of flower development from 36 to 21 days. 2, 4-D alone or in combination with GA also decreases the duration of flower development from 36 to 23 days. Application of Ethrel on the other hand leads to delayed flower development from 36 to 78 days (Table 4.6). There was a significant delay in the initial four stages of flower development by Ethrel treatment as compared to control. Silver thiosulfate alone delays the duration of flower development from 36 to 64 days compared to control or when spray with GA (Table 4.6). GA obliterates the harmful effect of Silver thiosulfate and enhances the flower development from 36 to 53 days.

Table 4.5: Effect of GA and 2, 4-D on time scale of inflorescence development in *Jatropha curcas*

Treatment (ppm)	Duration (in days) for inflorescence development					
	Initial appearance of floral bud (Stage I)	Distinct appearance of flower bud (Stage II)	Distinct Male and Female flower bud (Stage III)	Opening of Male flowers (Stage IV)	Opening of Female flowers (Stage (Stage V)	Complete bloom
Control	9.00 ± 0.577	6.333 ± 0.333	4.667 ± 0.315	6.160 ± 0.463	9.467 ± 0.235	36.20 ± 1.068
GA (10)	5.60 ± 0.509***	5.80 ± 0.583***	3.40 ± 0.748***	6.40 ± 0.719***	6.60 ± 0.812***	27.80 ± 0.812***
GA (50)	5.00 ± 0.447***	5.80 ± 0.365**	4.40 ± 0.509**	5.10 ± 0.871***	5.60 ± 1.02***	25.90 ± 1.02***
GA (100)	5.00 ± 0.487***	4.80 ± 0.374***	4.800 ± 0.274*	5.600 ± 0.254*	3.800 ± 0.374 ***	24.90 ± 1.02***
GA (1000)	4.20 ± 0.374***	3.60 ± 0.344***	3.20 ± 0.583***	5.60 ± 0.020**	5.20 ± 0.249***	21.80 ± 1.249***
2,4- D 50	3.80 ± 0.364***	4.800 ± 0.475**	5.800 ± 0.274**	4.800 ± 0.374***	4.600 ± 0.244***	23.80 ± 1.71***
2, 4-D (100)	4.80 ± 0.344***	3.800 ± 0.394***	7.200 ± 0.164***	3.800 ± 0.354***	3.400 ± 0.245***	23.10 ± 1.50 ***
GA + 2, 4-D (50)	3.80 ± 0.374***	6.800 ± 0.271*	3.800 ± 0.651*	4.400 ± 0.244***	3.600 ± 0.234***	23.40 ± 1.77 ***
GA + 2, 4-D (100)	3.20 ± 0.394***	9.00 ± 0.447**	4.20 ± 0.753**	3.60 ± 0.164***	3.20 ± 0.264***	22.10 ± 2.02 ***

n= 20 floral buds/treatments

Table 4.6: Effect of Ethrel and GA with Silver thiosulfate on time scale of inflorescence development in *Jatropha curcas*

Treatment (ppm)	Duration (in days) for inflorescence development					
	Initial appearance of floral bud (Stage I)	Distinct appearance of flower bud (Stage II)	Distinct Male and Female flower bud (Stage III)	Opening of Male flowers (Stage IV)	Opening of Female flowers (Stage (Stage V)	Complete bloom
Control	9.00 ± 0.577	6.333 ± 0.333	4.667 ± 0.333	6.160 ± 0.333	9.467 ± 0.333	36.20 ± 1.068
Ethrel (5)	22.00 ± 0.5774 ***	17.33 ± 0.333***	15.60 ± 0.467***	7.10 ± 0.583***	4.90 ± 1.365***	66.93± 1.171***
Ethrel (15)	30.00 ± 0.5774 ***	17.33 ± 0.333***	15.60 ± 0.467***	7.10 ± 0.583***	4.90 ± 1.365***	76.93± 1.171***
Ethrel (25)	24.00 ± 0.675 ****	26.33 ± 0.4333***	14.20 ± 0.365***	8.47 ± 0.361***	5.00 ± 1.447***	78.01± 1.198***
GA (1000)	4.20 ± 0.374***	3.60 ± 0.344***	3.20 ± 0.583***	5.60 ± 0.020**	5.20 ± 0.249***	21.80 ± 1.249***
$Ag_2S_2O_3$ (25 mM)	27.05 ± 0.4774 ***	15.21 ± 0.378 ***	7.65 ± 0.474 ***	9.28 ± 0.181 ***	4.10 ± 1.577 ***	64.30 ± 1.246 ***
GA(1000) - $Ag_2S_2O_3$ (25 mM)	22.07 ± 0.6894 ***	12.23 ± 0.245 ***	6.766 ± 0.348 ***	6.10 ± 0.245***	5.10 ± 1.614 ***	53.24 ± 1.577 ***

n= 20 floral buds/treatments

Emergence of floral bud was considered as day zero, Values are mean ± SE, **, *** indicates significantly different at $p<0.01$ and $p<0.001$ as compared to the corresponding control. Five plants were used per treatments and in each plant; at least four inflorescences were used for observation.

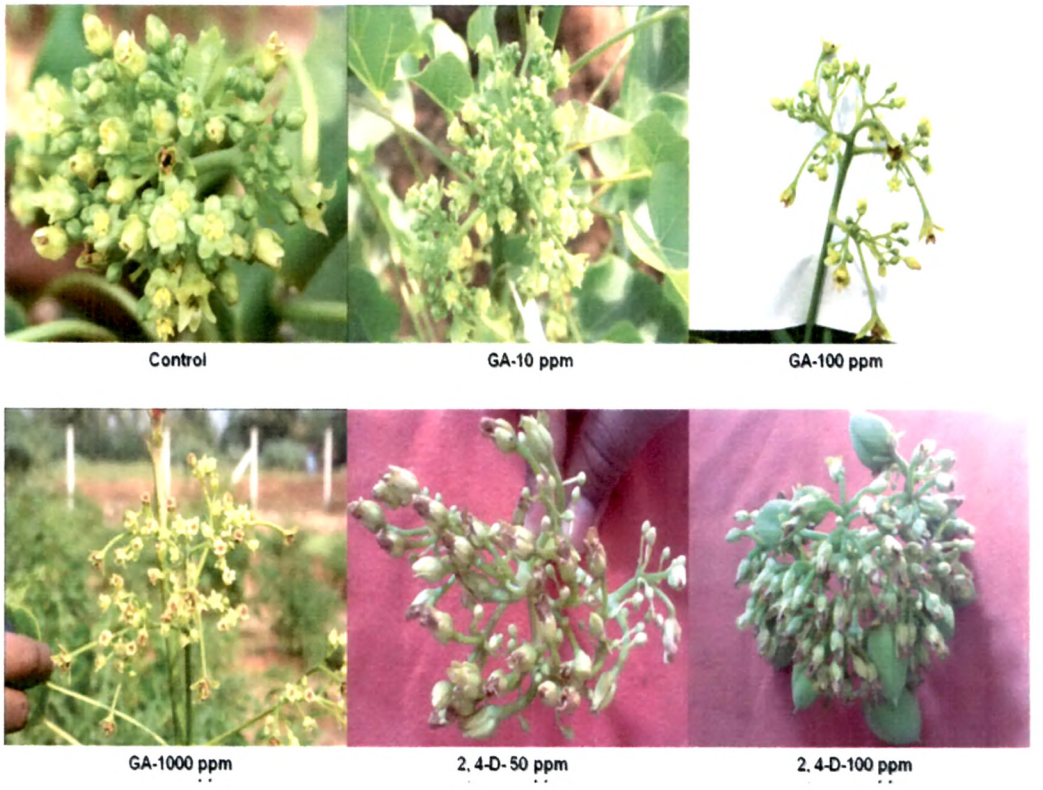


Figure 4.4: Effect of GA and 2, 4-D on Inflorescence pattern in *Jatropha curcas*

As shown in figure 4.4, Inflorescence pattern at stage V were observed after GA and 2, 4-D treatment. Photographs were captured at 5X zoom with digital camera. In GA (100 and 1000 ppm) treatments, there is an increase in female flower peduncle length than male flower. There are large numbers of female flowers than male flowers in GA treatment. With 2, 4-D treatment, male and female flowers are still present at stage V inflorescence though female flowers are open and pollination had occured. GA with 2, 4-D treatment increases the peduncle length and delay the withering of male and female flowers at stage V.

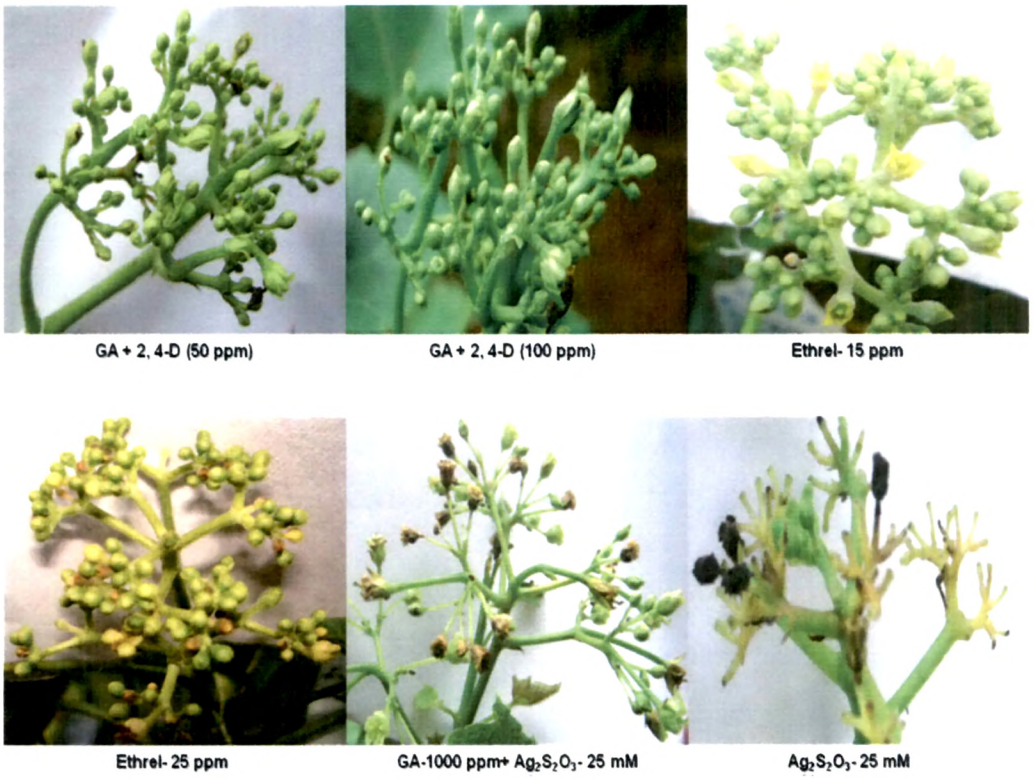


Figure 4.5: Effect of GA, Ethrel and Silver thiosulfate on Inflorescence pattern in *Jatropha curcas*

As shown in figure 4.5, Inflorescence pattern at stage V were observed after Ethrel and Silver thiosulfate treatment. Photographs were captured at 5X zoom with digital camera. Increased Ethrel treatments yellowing some of the female flowers and finally leads to browning of inflorescence before pollination. Silver thiosulfate shows the browning of inflorescences, which finally wither. We observed that this effect was deleterious and transient. In GA with Silver thiosulfate treatment, GA obliterates the toxic effect of Silver thiosulfate and increases the length of peduncle in female flowers.

4.2.3 Effect of GA on peduncle length in *Jatropha curcas*

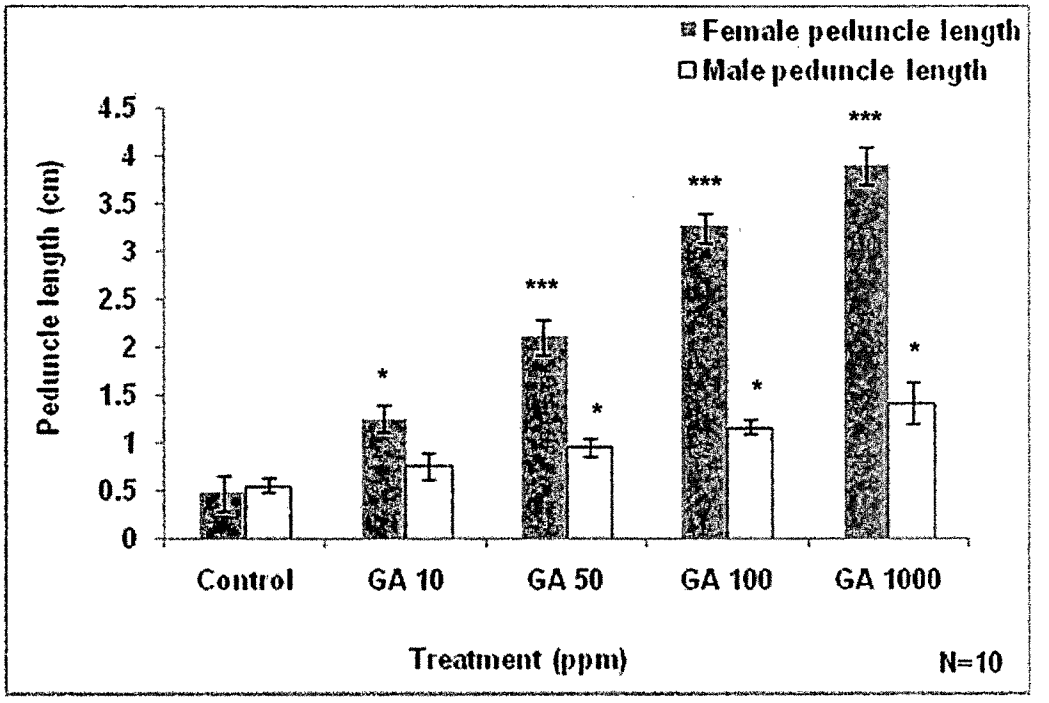


Figure 4.6: Effect of GA on peduncle length of *Jatropha curcas*

Values reported are mean ± SE; *, *** indicates significantly different at $p<0.05$ and $p<0.001$ respectively as compared to control.

There is an increase in length of peduncle immediately after first spray. The length of peduncle was measured after the end of third spray. In plants treated with 50, 100 and 1000 ppm GA, the length of peduncle of female flowers increased significantly by 3, 5 and 6 folds, respectively (Figure 4.6); whereas the increase in peduncle length of male flowers was only 0.5, 1 and 2 fold. This showed that GA effect on peduncle length was more prominent in female flowers compared to male flowers. GA at a lower concentration (10 ppm) showed negligible effect on peduncle length.

4.3. Discussion

4.2.1 Effect of phytohormones on flowering and flower sex ratio in *Jatropha curcas*

There are several reports which mention the role of phytohormones in alteration of sex ratio and flower development in different plant species (Thomas, 2008; Tanimoto, 2007). In maize it was shown that increased GA levels in the developing pistils arrest stamen development (Dellaporta and Calderon-Urrea, 1994; Irish et al., 1994). Ethylene has been reported to be a mojor hormone for sex expression in cucumber (Yamasaki et al, 2005). In an attempt to maximize *Jatropha curcas* yield by exogenous phytohormone treatement we studied the effect of various phytohormones alone or in combination and we observed that phytohormone treatment alters the ratio of female: male flowers in all groups. The highest effect being observed in plants treated with GA at 1000 ppm. The total number of flowers increased by 62% on 1000 ppm GA treatment. Number of female flowers increased by almost three fold and this reflected in an appreciable and desirable increase in ratio of female: male flowers from 1:24 to 1:13. The role of GA in regulation of flowering has been well studied and molecular events involved have been deciphered. A clear modification of sex expression in *Sagittaria latifolia* from male to female was induced by a foliar spray of GA (Tanimoto, 2007). In vitro application of GA increases femaleness in bitter melon (Thomas, 2008). GA also regulates the development of flowers by activation of *LFY* and *AP1* genes. GA activates floral meristem *LFY* signal which up regulates *AP1* promoter, responsible for flowering (Jack, 2004).

In 2, 4-D (100 ppm) treatment the total number of flowers increased by 51%. Number of female flowers increased by almost two fold, thus ratio of female: male flowers from 1:24 to 1:15. Auxin activates the *LFY* and *AP1* genes via regulation of DELLA. The enhancement of femaleness by auxin possibly occurs through the induction of Ethylene and GA biosynthesis (Takahashi and Jaffe, 1984; Trebitsh et al., 1987). Specifically, it has been reported that auxin regulates the expression of the genes that encode ACC synthase, a key enzyme in the ethylene biosynthesis payway (Arteca and Arteca, 1999; Botella et al., 1992; Nakagawa et al., 1991).

In the GA with 2, 4-D group a decrease in total flowering and number of inflorescence was seen. It suggests that combination of phytohormone treatment increases the endogenous level of ethylene which hamper the regulation of *LFY* and *SOC1* and hence decreases flowering. The inhibition of flower induction by 2, 4-D in *Pharbitis nil* is caused by stimulation of ethylene production. Similar results had been obtained by Wijayanti et al., 1997, as well as Kulikowska-Gulewska and Kopcewicz (1999). Thus, from this study it can be concluded that GA can be used as a potent phytohormone to increase flowering and femaleness in *Jatropha curcas*.

Silver thiosulfate decreases total flowering but it increases pistillate flowers and thus it changed the flower sex ratio from 1:24 to 1:17. Silver thiosulfate is an inhibitor of ethylene release which decreases flowering (Ockendon and Clenaghan, 1993; Ghaemi et al., 1994). It suggests that, basal level of ethylene is responsible for flowering via regulation of *AGAMOUS 1* and *AP1* promoter (Yamasaki et al., 2005). Similar result is also reported where in vitro modification of sex expression in mulberry was achieved by Silver nitrate (Thomas, 2004).

Increased Ethrel treatment decreases total flowering. Ethylene has been shown to be responsible for delaying flowering by acting as a repressor of *LFY* and *SOC1* via DELLA regulation (Archard et al., 2007). Ethrel treatment increases the femaleness which supports that Ethylene plays a central role in the control of sex determination in different species of cucurbits (Rudich, 1990; Papadopoulou et al., 2005). Ethylene is a major hormone for sex expression in cucumber (Yamasaki et al, 2005). Earlier studies have shown that, in vivo and in vitro applications of Ethrel induces significantly higher female flowers in bitter melon (Thomas, 2008). Ethylene has been shown to induce female flowers in dioeciouus male plants of *Cannabis sativa* (Mohan Ram and Jaiswal, 1970). It regulates ACS genes to cause femaleness in cucurbits. It is also reported that, Ethylene promotes female flower development via organ specific induction of DNA damage in primordial anthers (Wang et al., 2010).

In flowering plants the sex expression is controlled by the balance between endogenous Auxin and Gibberellin and or between Gibberellin and Ethylene. Ethylene

and Auxin promote the formation of female floweres, whereas Gibberellins promote the formation of male flowers in cucurbits (Mohan Ram and Jaiswal, 1970; Saito and Takahashi, 1987). It is believed that Ethylene acts more directly than GA on sex expression in some plants (Yin and Quinn, 1995). GA and Ethylene evolution is highly correlated with sex expression in plants and dioecious plants produce more ethylene than monoecious ones (Rudich et al., 1972; Trebitsh et al., 1987). From this study we can conclude that GA seems to be the predominant phytohormone promoting femaleness in *Jatropha curcas.*

4.2.2 Effect of phytohormones on Inflorescence development in *Jatropha curcas*

Apart from increasing, total number of flowers and female: male ratio, GA, 2, 4-D, Ethrel and Silver thiosulfate treatments also alters the time taken for flower development. GA hastened the process of flower development. GA is a well-known phytohormone that breaks the dormancy of seeds (Rudich et al., 1972). It also acts to enhance the flower development process. Ethylene could have delayed the flowering process via DELLA regulation. 2, 4-D alone and GA with 2, 4-D treatment showed a similar result. Silver thiosulfate also delays the flower development process. Silver nitrate inhibits receptor mechanism of ethylene action and supress the development of flowers (Beyer, 1976; Atsmon and Tabbak, 1979; Takahashi and Jaffe, 1984). Silver ions interfere with *LFY* and *SOC1* genes via inhibiting ethylene action and thus it delay the process of flower development and flowering (Yamasaki et al., 2003). In Silver thiosulfate with GA treated group, there is restoration of flower development which suggests that GA rescues the toxic effect of Silver ions. Thus, GA not only increased flowering but also enhanced the rate of flower development.

4.2.3 Effect of GA on peduncle length in *Jatropha curcas*

The other obvious morphological change brought about by GA application was a pronounced increase in length of peduncle. In plants treated with 50, 100 and 1000 ppm GA, there was an increase in peduncle length of female and male flowers compared to control. GA at a lower concentration (10 ppm) showed negligible effect on peduncle length. However, increase in length of peduncle was accompanied by greater withering of immature fruits. It was observed that there was higher fruit fall in plants treated with 100 and 1000 ppm of GA as compared to 10 ppm. It has been reported that fruit fall is a consequence of senescence and programmed cell death and one of the important initiators of senescence is endogenous release of Hydrogen peroxide (Strother 1988). There have been several reports which have demonstrated that GA treatment leads to release of endogenous hydrogen peroxide, a factor responsible for cell death (Bethke et al. 1999; Steffens and Sauter 2005).