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EFFECTS OF GROWTH REGULATIONS AND FERTILIZERS ON FLOWERING AND NUTRIENT ELEMENT OF *JATROPHA CURCAS* L.

WANG XIU-RONG, ZHAO YANG, DING GUI-JIE* AND LUPITA D MONTOYA[1]

Forestry College, Guizhou University, Guiyang-550025, China

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Abstract

To understand the effect of exogenous substances on flower bud differentiation and nutrient element of *Jatropha curcas* L. different levels of Kn + 2,4-D + fertilizer, P or K (treatments) were sprayed on the stem apex with orthogonal test. Results showed that the certain treatment could promote the flower bud formation. The optimum treatment was Kn (200 mg/l) +2,4-D (50 mg/l) + N (urea 1%) and 2,4-D was the most important factor in promoting flower bud formation. There were some obvious changes in the content of N, P, K, sugar and starch (nutrients) in the critical period of bud physiological differentiation. The carbohydrate content and C/N ratio came to a maximum in about 7 days after the treatment and then N content began to increase and reached maximum in about 15 days. More nutrients were consumed in flower bud morphodifferentiation and the content of N, sugar and starch reduced to a minimum gradually. The C and N content increased first and then remained stable until the stem apex stopped growing. Higher C/N ratio could benefit the flower bud differentiation. K had an important effect on the flower bud differentiation, flowering and fruiting of *J. curcas* and it was mostly absorbed in the critical period of bud physiological differentiation.

Introduction

Jatropha curcas L. is an important biodiesel tree species, belonging to Euphorbiaceae. The main obstacle to *J. curcas* industrialization is its low production of fruit. In recent years, more research has been done on bloom habits, biological characteristics of pollination and the flower anatomical structure of *J. curcas*. This research focused mainly on pollen viability, stigma receptivity, hand-pollination (Li *et al.* 2007, Luo *et al.* 2007, Pu *et al.* 2011, Abdelgadir *et al.* 2009), biological characteristics of pollination (Yang *et al.* 2007, Bhattacharya *et al.* 2005), morphological and anatomical structure (Wang *et al.* 2011), ratio, number and size of male and female flowers (He *et al.* 2011) and character of seeds (Abdelgadir *et al.* 2008, Kang *et al.* 2008).

Plant hormones play an important role in the growth and development of plants. At present, most studies on hormones regulation of blooming focus on agricultural plants, such as *Dimocarpus longan* Lour. (Liao and Peng 2009), *Litchi chinensis* L. (Xiao 2002), *Castanea mollissima* Bl. (Lei *et al.* 2009) and agricultural products such as *Cucumis sativus* L. (Wu 2009), *Cucumismelo* L. (Tong 2008) and *Saccharum officinarum* L. (Xiao *et al.* 2002). Studies have shown that NAA or ABT (ABT rooting powder are made of hormones, such as NAA and IBA, mainly to promote rooting.) can improve the seedling growth of *J. curcas* (Fan *et al.* 2011). Both pharmacy B (Borax) and MET (Metabolic equivalent of energy) can promote *J. curcas* flowering and fruiting (Liu *et al.* 2011). Suitable concentrations of GA_3 and Kn contribute to increasing the number of female flowers of *J. curcas* (Wang *et al.* 2010, He *et al.* 2011). It is still unknown, however whether other kinds of plant hormones can affect the bud differentiation of *J. curcas* and how the physiology substances change during exogenous applications of plant hormones. Therefore, in this paper, different plant hormones were sprayed on the shoot apices of *J. curcas* to find out effect of these changes in nutrient requirements in the tree during the bud differentiation.

*Author for correspondence: <gjdinggzu@126.com>. [1]ECCH, University of Colorado Boulder, Boulder, USA.

This paper has important theoretical and practical significance for understanding the bloom mechanisms of *J. curcas* at a system level. It also defines the time to regulate the flower, which can make the tree bloom in a short period and make it easier to harvest.

Materials and Methods

All the experiments presented here were conducted within a period of two years. Field experiments were made at the Forestry Center of Luodian County, Guizhou Province, China (25°43′58″N-106°42′56″E) at 500 m elevation. This region has a subtropical monsoon climate; annual average temperature of 20ºC, and average annual rainfall of 335 mm. Samples were collected and brought to the laboratory in Guizhou University, Guiyang city. At the beginning of the experiment, thirty samples *J. curcas* trees similar in age and growth condition were selected as three group. Eight branches on each tree were marked to record the number of leaves and other characteristics; every tree in a group was treated with a treatment separately. Three times repeated. Several plant hormone combinations were sprayed separately on the shoot apices of selected trees at early spring when they shot. The hormone combination Kn + 2,4-D + fertilizer (N, P or K) was selected after pre-experiments. The names and levels of hormones are shown at Table 1.

Experiments were conducted following the $L_9\ (3)^4$ orthogonal design with three replications. There were a total of 9 treatments, which were labeled as A1, A2……, A9. The program design is shown in Table 2. Treatments were sprayed on the shoot apices and the leaves around them. Spraying continued until the solution was dripping from the leaves. The treatment was done once a day (morning) and repeated a second day. A controlled experiment was designed and was labeled as A0, in which the trees were sprayed with an equal amount of water.

One week after treatment, the growth condition of flowers and leaves were checked and recorded. At the same time, the leaves around the 4th leaf from the top were gathered.

Leaves were dried first at 105ºC for 10 mins and then at 85ºC for 48 hrs when it reached a constant weight. The dried material was then grounded and blended to measure the content of N, P, K, soluble sugar and starch.

The nutrient content of each sample was determined using the H_2SO_4-H_2O_2 lixiviation method (Bao 2000). C/N= (Sug + Sta)/N.

All the data were processed by Excel and DPS (Data Processing System).

Table 1. The factors and levels of orthogonal test ($L_9(3)^4$).

Levels/factors	Kn (A)	2,4-D (B)	Fertilizers
Level (1)	50 mg/l	10 mg/l	N (Urea 1%)
Level (2)	100 mg/l	20 mg/l	P-K ($KH_2PO_4$0.3%)
Level (3)	200 mg/l	50 mg/l	B-Mn (Borax 0.3% + $MnSO_4$0.1%)

Results and Discussion

The analysis results about the number of inflorescence on each branch was shown in Table 2. The best treatment for improving blooming is A3B3C1, that is Kn (200) + 2,4D (50) + N, which exactly is the A9. The maximum of range value was 1.5556 for 2,4-D, which indicates that 2,4-D has the greatest influence on the number of inflorescence while the fertilizers have the smallest impact on it.

It can be seen from Fig. 1 that the number of the inflorescence increased with the increase of the 2,4-D concentration. One-way ANOVA (by DPS) showed that there were significant differences between level 3 and the other two levels. Therefore, in the future, increase the concentration of 2,4-D may be tried.

Table 2. The orthogonal test results.

Groups/factors	Kn (A)	2, 4-D (B)	A*B	Fertilizers (C)	No. of inflorescence/ branch/signifecance
A0	0	0	_	0	0
A1	50	10	_	N	0.00 b
A2	50	20	_	PK	0.20 b
A3	50	50	_	B-Mn	1.50 ab
A4	100	10	_	B-Mn	0.17 b
A5	100	20	_	N	0.00 b
A6	100	50	_	PK	0.83 ab
A7	200	10	_	PK	0.00 b
A8	200	20	_	B-Mn	0.33 b
A9	200	50	_	N	2.50 a
Level 1	0.6667	0.0556b	0.3889	0.8333	
Level 2	0.3333	0.2778b	1.0556	0.4444	
Level 3	0.9444	1.6111a	0.5000	0.6667	
Range	0.6111	1.5556	0.6667	0.3889	

Different lower case letters following the figures show the level of significance at 5% level.

There was no obvious correlation between the Kn concentration and the inflorescence number, but the maximum number of flowers were at the level 3. In the fertilizer treatment, N was the best level followed by B-Mn without a significant difference. This result demonstrated that certain amount of N can promote the differentiation of flower buds; and the nutrition management and fertilization of *J. curcas* trees should be strengthened.

Overall analysis of variance (Table 2) showed that there were 2.5 inflorescences on each branch in A9. The numbers of inflorescences in all treatments were significantly different except that in A3 and A6. No flower in control A0 was observed.

Statistical data showed that the variation of nutrient content in leaves of control A0 and the nine treatments is similar to each other, but only different in the time and content. Therefore, the nutrient content of A9, the treatment that showed the most significant effect, was analyzed to understand how the nutrients have changed and how they affected each other during blooming.

For the convenience of expression, the flower bud differentiation is divided into 4 stages according to the observation of phenological character and research on the exogenous growth regulators of *J. curcas* (on an other paper):

May 8 - 30, the physiological differentiation stage, the stem tips can be induced flowering by proper exogenous growth regulators; May 30 - June 29, the morphodifferentiation stage, inflorescences appear and the organs of florets form; June 13 - July 17, the blooming and fruiting stage, florets continue to bloom and fruit; July 17 - August 21, the stem tips stop growing stage, most of the stem tips growth arrest.

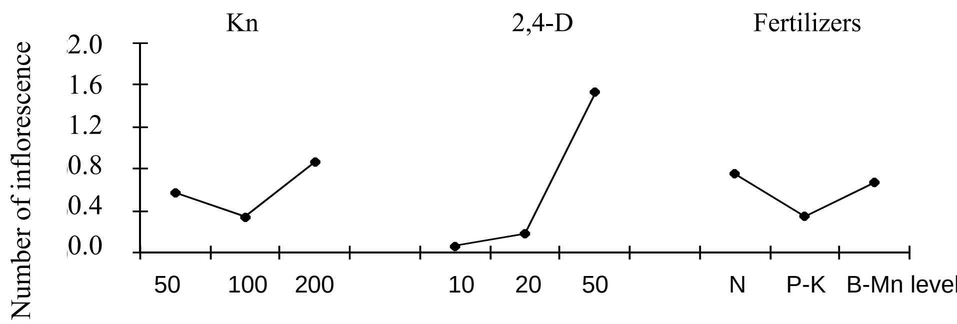


Fig. 1. The experimental curves of different levels and the number of inflorescence.

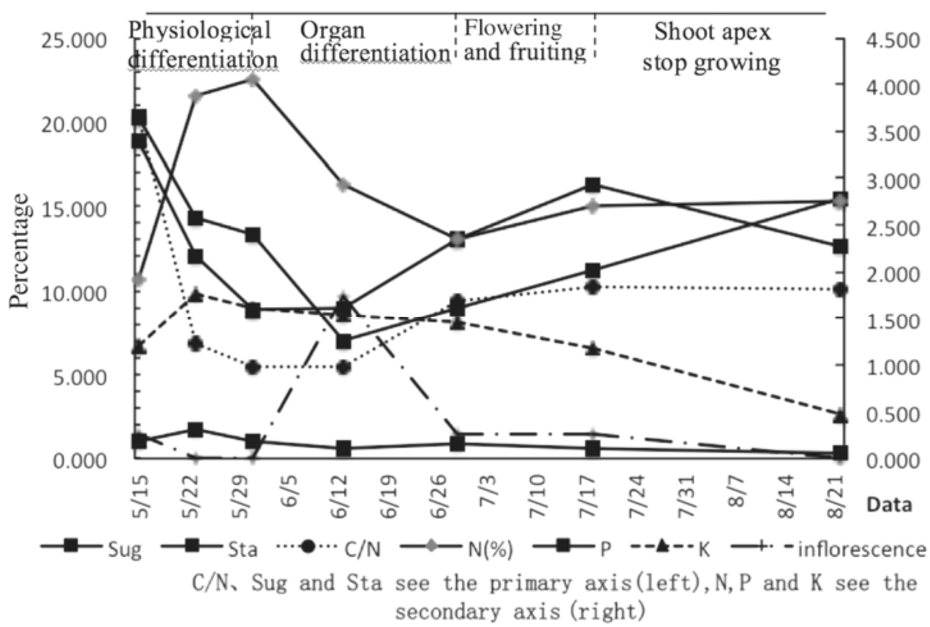


Fig. 2. The changes of amount of inflorescence and nutrient of A9 treatment.

It can be seen from Fig. 2 that from May 8 to 30, although there was no inflorescence, there was obvious change of the nutrients in leaves of A9, which means that the trees had come into the physiological differentiation stage: the Nitrogen (N) level increased quickly and reached the peak of 4.067% before the inflorescence emerged, and it decreased after the inflorescence bloomed.

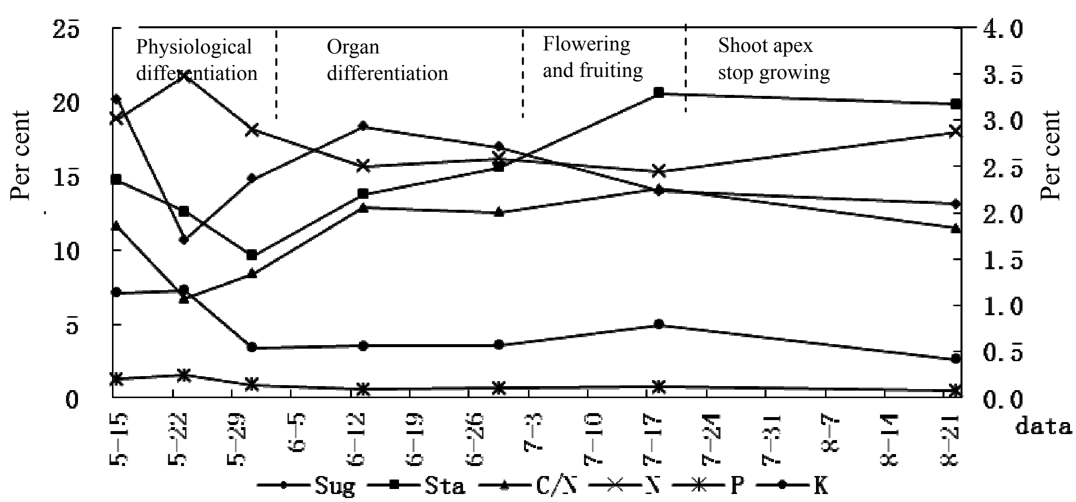
Nitrogen exists in many forms in plants: protein, nucleicacid, chlorophyll, etc. Nitrogen in proteins accounts for 80 - 85% of total nitrogen in plants. Therefore, the rapid increase of N content could result from increased synthesis of proteins and enzymes at the physiological differentiation stage. The contents of soluble sugar and starch reached the maximum value18.808 and 20.260%, and started to decline afterward. By May 30, the content of soluble sugar began to level off but the starch content continued to fall, which means that starch is decomposed continuously. The starch content reached the minimum at June13 when trees have the most number of inflorescences, and then started to increase. In this period, the demand of soluble sugar decreased. Thus it can be seen that the demand of carbohydrates for the flower bud development was centered around the physiological differentiation stage.

C/N ratio reached the maximum point (20.316) at one week after the treatment, and decreased rapidly to the value 6.777 at May 23, then gently decreased. The contents of P and K displayed an increasing trend after thetreatment and continued to drop after May 23 when they peaked. The content of P reached the minimum at June 12, when there were the most inflorescences, and still decreased after a slight recovery. Therefore, the P content showed an overall decreasing trend. The K content only went down.

The control group A0 did not form any inflorescences and the trend of the nutrients change was similar to that of A9. However, there are still some differences between them. For example, the highest N content span in A0 was shorter than those of the treatment groups, and the contents of soluble sugar and starch had a short-time decline at May 22 and then continued to increase. At the same day, the maximum of C/N ratio was 9.325, which was far below the value 20.316 of A9. All of these show that the change of tree nutrients have a close link to the number of inflorescence, and a higher C/N ratio is beneficial for the differentiation of flower bud.

The appearance of flower bud morphodifferentiation stage is the time when inflorescences appear and flower lets form. The changes of A9 were divided into two phases by June 13, when trees have the most number of inflorescence: from May 31 to June 13, the contents of N, P, K, Sta declined and Sta content drops to the minimum value in the period of duration, 7.001%, but that of sugar showed a little increasing trend. After June 13, some of the flower lets on the earlier inflorescences finished the development of organs and began to bloom. At the same time, the reduction rate of N content slowed down gradually, and the contents of P, Sta and Sug increased. It might be because the demand for nutrient has changed with the forming of flowers.

A0 was different from A9 in substances changing. The contents of all kinds of substances began to increase after May 31 except that of the N and P, which is possible because there is no inflorescence in A0 and trees have a low demand for nutrient. Additionally, with the leaf area increasing and photosynthesis strengthening, the material accumulation gets faster.



C/N, sugar and stach see the primary axis (left), N, P and K see the secondary axis (right)

Fig. 3. Change of the content of nutrient in leaves of A0.

Blooming and fruiting happened simultaneously in this period. Some of the female flowers bloomed earlier, finished pollination and then developed into fruits; while at the same time some flowers which formed later still were not open or were opening. In regards to the nutrient of A9, the contents of N, Sta and Sug increase in this stage. By July 18, the content of Sug reached the second peak (16.352%) and then started to drop. The content of N and Sta kept on increasing, but that of the P and K declined. The changing processes of A0 were similar to that of A9.

The continuous investigation results of the number of leaves (Fig. 4) showed that the rate of the nutritive growth has decreased. From July 7 to August 21, shoot apices almost stopped growing by only 0.4 leaves increasing on each branch per week. During this period, the contents of nutrient remained stable, which indicates a balance between the consumption and the supply of the trees nutrients with the increasing of leaves area.

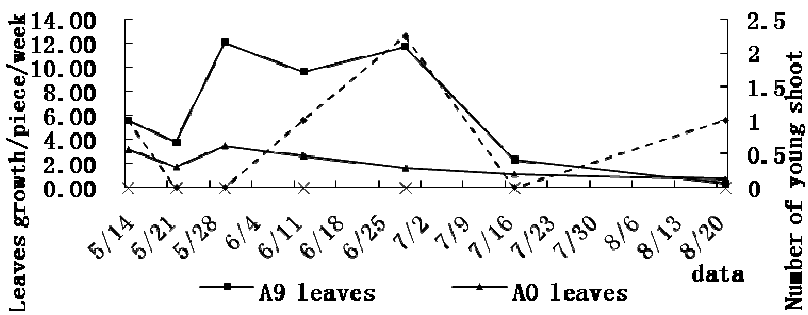


Fig. 4. The number of leaf growth weekly and the number of young shoot of A0 and A9.

However, the nutritive growth of trees in A0 was always in a slow rate and there was almost no new bud, which might be one of the reasons why there was no inflorescence. The rate of leaves increasing was 3.5 leaves per week, which was only one third of the highest rate 12.08 in A9.

Throughout the growing period, the contents of K between treatments were greatly different. It can be seen from Fig. 5 that 7 days after treating, the contents of K were close to each other, but that of A9 reached the maximum value, 1.766% by May 23. The K content of A9 was always higher than that of other treatments and it indicated that K plays a significant role in the forming of flower and fruit of *J. curcas*. In A2, A6 and A7, trees were sprayed with P and K-fertilization, but the K content of these three treatments were not higher than that of others in the physiological differentiation stage, which showed that the external K content was not the main factor affecting the content of K in the leaves. K can promote the transport of carbohydrates to the storage organs as well as the synthesis of polysaccharides. It is an activating agent of enzymes which can improve protein synthesis and nitrogen metabolism. Plants often absorb N and K at a certain proportion. *J. curcas* is a kind of high-potassium tree and the proportion of N and K in *J. curcas* tree is 9 : 5 (Wang *et al.* 2012). The possible reason for the high content of K in leaves of A9 was that hormones treating stimulated trees into the physiological differentiation stage and then turned on the absorption of N and K.

Certain substances can improve *J. curcas* trees into the differentiation of flower buds. Exogenous growth substances play an obvious role in improving blooming after treating the stem tip with them. The best treatment of exogenous growth substances has been A9, Kn (200) + 2,4-D (50) + N, and 2,4-D has been the key factor of them in improving the flower bud formation.

The flower buds at physiological differentiation stage lasted for about 20 days and the nutrients in leaves had dramatically changed in this period: the content of carbohydrates reached the peak in one week after being treated by exogenous substances and the C/N ratio reached at the maximum at the same time. After that, the content of N began to increase and reached the maximum value at the 15th day. The flower bud at morphological differentiation stage was about 30 days and then blooming and morphological differentiation proceeded simultaneously at the

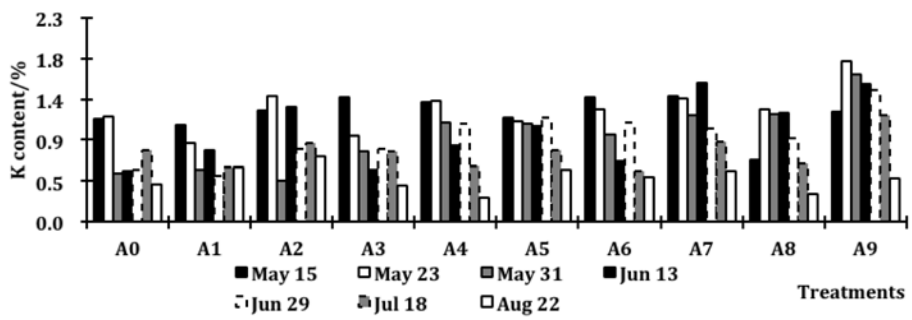


Fig. 5. Changes of the contents of K in leaves of different treatments.

later stage of it. The demand for nutritions was increasing in morphological differentiation stage, so was the contents of sugar, starch and N dropped down to the lowest point successively and then rose up slowly. Flowering and fruiting proceeded simultaneously and the contents of C and N increased at first and then remained stable. Stem tips were not growing for about one month and the number of leaves was not increasing. In this period, the content of N had a little variation, the content of soluble sugar declined, the starch content rise up and C/N ratio remained largely unchanged. The external concentration of K was not the key factor affecting the content of K in leaves. K had an important influence on the flower bud differentiation, blooming and fruiting and it reach to the peak at the physiological differentiation stage and then always declined.

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