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Effect of plant growth regulators on floral differentiation and seed production in Jojoba (*Simmondsia chinensis* (Link) Schneider)

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ABSTRACT

The effect of foliar application of cytokinin (benzyl-adenine, 150 mg L^{-1}) and gibberellin ($GA_4 + GA_7$, 150 mg L^{-1}) on growth and flower development of 5-year-old plants of two jojoba clones was studied. The plant growth regulators were applied on October 5, 1999 (spring) and the plants were evaluated 120, 240 and 360 days after application. Shoot length, total number of nodes and number of nodes with branching were statistically different between clones but not between the growth regulator treatments.

The total number of flowers on both clones was significantly increased by treatment with benzyl-adenine (BA) and significantly reduced by treatment with gibberellin. The seed yields, evaluated 180 days after application, were not statistically different from the control due to an increase in flower abortion. One clone treated with gibberellin showed a significant decrease in number and weight of seeds, the other did not.

Histology of axillary buds revealed that BA application on one clone (4.11.32) enlarged the flower meristem, differentiating multiple flower production.

1. Introduction

Change from vegetative to flower buds is a complex process regulated by many factors, and can be influenced by application of plant growth regulators. Jojoba (*Simmondsia chinensis* (Link) Schneider) concentrates its flower production on new or current season's growth (Dunstone, 1980, 1986). Horticultural practices which favor production of new vegetative growth will produce an increase in flower production centers that will finally grow into fruits. Some background on jojoba demonstrate an effective response to applications of plant growth regulators, not only increasing or regulating flower production and fruit development, but also significantly increasing branch development (Ravetta and Palzkill, 1992).

Jojoba is a dioecious species, although hermaphroditic individuals can occur (Yermanos, 1979). Female flowers are usually solitary and have three pistils growing over an ovary with three ovules. Each of the three ovules can potentially develop into a seed, but usually only one ovule per ovary will develop (Ayerza, 1990).

Jojoba has two axillary buds at every node, but only one is normally activated in the growing season developing into a flower. It may occur that neither of these buds will develop into a flower; in this case flowers can be observed every 2–3 nodes. In other cases, both buds at the node differentiate into flowers (Botti et al., 2001). In some genotypes produce four flowers per node (a flower cluster), depending on both genetic and environmental factors (Cruz, 1995).

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In Chile, the reproductive cycle of jojoba begins with the vegetative growth in spring, and concurrently with the development of flower buds which generally remain dormant until the next winter (June–August), when anthesis and pollination occur. Fruit mature in March–April.

Dormancy in jojoba flower buds is associated with high abscisic acid (ABA) levels, a condition associated with elevated temperatures or dry seasons. In late autumn ABA levels drop significantly, flower buds break dormancy and anthesis occurs (Dunstone, 1988). In the field, flower buds formed during an active growing season can stay dormant for more than 3 years. In fact, the jojoba can produce a great number of flower buds per season, but only some of them will flower (Dunstone, 1980, 1988; Ayerza, 1990; Benzioni et al., 1992).

Ravetta and Palzkill (1992), after using growth regulators on jojoba plants during the first 3 years of growth, observed a significant increase in branching and flower bud production with some variation in clone responses. Clones AT1487 and AT1310 grown in pots under greenhouse conditions which were treated in August with 100 mg L^{-1} gibberellins ($GA_4 + GA_7$) caused an increase in extension growth in the branches, but significantly fewer tips and nodes, at 40 and 150 days post-application. Seventeen months after treatment, a significant increase in node and flower number was observed when compared to the control treatment (120 and 135% more, respectively). Application of 100 mg L^{-1} 6-benzyladenine (BA) resulted in branching stimulation and increased node number. Likewise, 17 months after application, a 75% increase in number of nodes and 74% increase in number of flower buds were observed in clone AT1310. In both cases, the increase was associated with an increase in the number of growing tips and node production.

In a similar experiment conducted under field conditions in Chile with different jojoba clones (3.57.43; 4.11.32; 4.13.1; 4.8 and AT1487), a significant increase in stem elongation, branching and flower bud number was observed following two applications of gibberellins ($GA_4 + GA_7$) in May and September (González, 1998). One year after applying 150 mg L^{-1} $GA_4 + GA_7$ increased stem elongation was observed in four of the five clones tested (3.57.43; 4.11.32; 4.13.1; 4.8.). Clone AT1487 quadrupled the response for the same treatment and duplicated the node number at the 150 mg L^{-1} dose. Four of the five clones studied had a significant response in flower number after growth regulator application. For clone AT1487 treatment with 150 mg L^{-1} $GA_4 + GA_7$ resulted in a 370% increase in flower buds, and treatment with 150 mg L^{-1} BA resulted in a 170% increase. In clone 4.8 a smaller, but significant, effect was detected using both growth regulators. In clone 4.11.32 flower number increased 340% with the application of 150 mg L^{-1} $GA_4 + GA_7$, but was not influenced by BA. For clone 3.57.43 there was a 280% increased in flower number with the BA application, but no increase with the GA application.

In the two studies reviewed above, no attempt was made to evaluate treatment effects on seed production and studies on histological changes occurring in jojoba buds from the action of these growth regulators were not conducted. The objectives of this study were to determine if BA and $GA_4 + GA_7$ applications had any effects on flower and seed production of two jojoba clones (4.8 and 4.11.32), and concurrently study histological changes in buds produced by these growth regulators.

2. Materials and methods

The experiment was carried out on 5-year-old plants at 'Las Cardas' Agricultural Experiment Station (30°13′S; 71°19′W) a facility of the Faculty of Agricultural Sciences of the University of Chile. Vegetative and reproductive development of jojoba was monitored in 48 plants, and a histological study of 12 plants was carried out. Clones tested were 4.8 and 4.11.32 which had been identified in earlier studies as being high seed-producers at Las Cardas Experiment Station and at Chaca Valley (Region I), respectively.

Plants were on a 4 m × 4 m spacing and were fertilized with 75 g triple superphosphate (TSP) and 50 g potassium sulphate per plant at the time of planting and 80 g urea per plant twice during growing season. The plants were drip-irrigated, the water requirements determined by the evaporation tray method, as described by Carrasco (2001), and calculated at 4.500 m^3 ha^{-1} year.

2.1. Growth regulators

The applications of plant growth regulators consisted of benzyladenine at 150 mg L^{-1} (BA 99% (w/w); Sigma–Aldrich, St. Louis, MO), $GA_4 + GA_7$ at 150 mg L^{-1} (Provide®, 21 g a.i./L) and water sprays (control treatment). All the treatments, including the control, contained Tween 20® at 0.2 cm^3 L^{-1}. Approximately 500 mL of solution per plant were applied to the foliage until solution dripped from the plant. Treatments were applied on October 5, 1999.

2.2. Vegetative and reproductive development

Prior to treatment applications, initial growth measurements were recorded for each plant, including height and two diameters (North–South axis and East–West axis). Three primary branches facing north were randomly marked in every plant. In each marked branch, its length, total number of nodes, branched node number and total number of flower buds were determined. The same evaluations were made 120, 240 and 360 days after applications. In addition, number and weight of total seed production per plant were recorded in April 2001.

2.3. Histological study

For the histological study of flower differentiation, treatments with growth regulators were applied in 12 jojoba plants (two plants per clone and treatment) on October 19, 2000. Once a month nodes above and below that in apical position at the time of plant regulator application were collected.

The collected buds were fixed in a FAA solution (4% formalin, 70% acetic acid, 70% alcohol, in a 5:5:90, v/v ratio) for at least 24 h. They were then dehydrated in a series of 70, 80 and 95% ethanol for 30 min each. After that, the resulting material was infiltrated with JB-4 plastic (Polyscience, Inc., Warrington, PA) according to the supplier's directions.

The already polymerized plastic blocks were cut to 4 μm thickness using a Leitz ultramicrotome model 1516. Sections were dyed with Schiff reagent and 1% blue aniline in 7% acetic acid (v/v), and then mounted on slides with Canadian bal-

sam. Slides were then observed through a microscope with magnifications of 10× and 40×.

2.4. *Experimental design and statistical analysis*

A completely randomized experimental design was used, with a 3 × 2 factorial design, with growth regulator treatment being the first factor and genotype the second.

Eight replications of one plant each per treatment were made, because the homogeneousness was observed in preview trials (González, 1998). The experimental unit was one plant with three marked branches. The results were analyzed by ANOVA and in cases with significant differences, Tukey's test of multiple comparisons at the 5% level of significance was applied.

When the analyzed data did not fulfill ANOVA, the results were evaluated using range variance analysis, and when significant differences were found the SNK multiple comparison test at 5% of significance was used.

No statistical analysis was needed for the histological study due to its descriptive nature.

3. Results and discussion

3.1. *Vegetative and productive development*

BA and $GA_4 + GA_7$ applications had no effect on plant height or diameters in any of the evaluation dates. No differences were found in the factors between the clones. Table 1 shows the total growth increment at 360 days post-application.

Increase in branch length is the result of cell division and elongation. One of the most important functions of gibberellins is the induction of cell division in the subapical meristem, resulting in stem elongation (Reid et al., 1992; Ross, 1994; Talón, 1998). In this experiment, little effect on branch length and plant growth was observed for the $GA_4 + GA_7$ application. This could be due, as Forshey (1991) points out, to an insufficient growth regulator dose that is diluted among the different growing points in the plant. Therefore, in this study, the effect of gibberellins measured only on three branches of 4-year-old plants was inappreciable. There exists a quantitative relationship between the endogenous levels of gibberellins and stem height. Consequently, the efficiency of the response depends directly on factors such as concentration of the applied hormone (Lenton, 1984) and the quantity of gibberellins applied (Agustí and Almela, 1991). González (1998) in her study conducted on the same jojoba clones found significant branch elongation in response to $GA_4 + GA_7$ application; this discrepancy may have resulted from the fact that she used small jojoba plants of only 1.5 years of age with fewer growing points and probably more sensitive to this regulator, so the concentration used was enough. On the other hand, the application time also appears as a critical factor (Agustí and Almela, 1991), which is difficult to determine since weather conditions vary year to year and a same phenological state can be reached with differences of several days in 2 consecutive years (Moseline, 1979).

Significant differences were not observed either between the growth regulators in increase of branch length, total number of nodes and branched nodes. However, significant differences appeared between the clones, with clone 4.8 showing more branch lengthening, and greater number of total nodes and branched nodes (Table 2) than clone 4.11.32. This difference could be explained by the natural growth habits and branching of clones, concordant with the experience of Ravetta and Palzkill (1992) and González (1998).

Since an interaction was detected for the total number of flowers and solitary flowers per branch and seed number per plant between clones and treatments, a separate analysis was made for each clone.

BA application to clone 4.11.32 resulted in the development of flower clusters containing as many as nine flowers per node.

Table 1 – Plant height and North–South and East–West plant diameters for growth regulators (R) and jojoba clones (C) 360 days post-application

Factor	Plant height (cm)	Plant diameters (cm)	
		N–S	E–W
Regulator (R)			
Control	24.8 a*	17.3 a	11.6 a
$GA_4 + GA_7$	26.8 a	13.5 a	16.0 a
BA	18.6 a	15.8 a	18.8 a
Clones (C)			
4.8	25.5 A**	17.9 A	15.3 A
4.11.32	21.2 A	13.2 A	15.7 A
R × C	NS	NS	NS

R × C: Interaction between treatment and clones with a significance level of $p \leq 0.05\%$.

* Different lowercase letters in the column indicate significant differences of $p \leq 0.05\%$ (Tukey) in the growth regulator factor.

** Different capital letters in the column indicate significant differences of $p \leq 0.05\%$ (Tukey) between clones.

Table 2 – Branch length-increase and total node number and branched nodes for different growth regulator combinations (R) and jojoba clones (C) at 360 days post-application

Factor	Branch length increase (cm)	Number of nodes (cm)	
		Total	Branched
Regulator (R)			
Control	8.7 a*	88.3 a	23.8 a
$GA_4 + GA_7$	9.0 a	73.2 a	18.1 a
BA	12.3 a	80.6 a	19.6 a
Clones (C)			
4.8	14.2 B**	106.7 B	28.6 B
4.11.32	5.8 A	54.7 A	12.4 A
R × C	NS	NS	NS

R × C: Interaction between treatment and clones with a significance level of $p \leq 0.05\%$.

* Different lowercase letters in the column indicate significant differences of $p \leq 0.05\%$ (Tukey) in the growth regulator factor.

** Different capital letters in the column indicate significant differences of $p \leq 0.05\%$ (Tukey) between the clones.

This clone has been observed to produce up to four flowers per node naturally (without the application of any growth regulator).

BA application to clone 4.11.32 resulted in a significant increase in flowers per branch, including solitary and individual flowers in each cluster. Conversely, the application of $GA_4 + GA_7$ resulted in a significant reduction in this parameter. As to the number of solitary flowers, the control treatment bore significantly more of them, because most of the flowers in plants treated with BA occurred in flower clusters. The inhibitory effect of gibberellins on flowering is well known in citrus species (Monselise and Halevy, 1964), *Prunus* (Bradley and Crane, 1960) and blueberry (Retamales et al., 2000.) It has been observed that the applications of gibberellins inhibit flowering in woody angiosperms (Pharis and King, 1985.) The study of the role of gibberellins in flowering is complex because the various species respond differently to them (Levy and Dean, 1998; Pharis and King, 1985). For example, in *Arabidopsis*, a facultative long-day length plant, gibberellins affect both the time of flowering and flower morphology. Exogenous gibberellins bring about early bloom particularly in short-length days (Langridge, 1957; Wilson et al., 1992).

Control plants of clone 4.11.32 produced a mean of 5.5 clusters per branch (clusters may have up to four flowers per node); while plants treated with BA allowed to have 18.3 clusters per branch (clusters with as many as nine flowers per node). Treatment with $GA_4 + GA_7$ caused the formation of only four clusters per branch (never with more than four flowers per node).

In clone 4.8 BA application induced the development of flowers in clusters, a phenomenon that does not occur naturally in this clone. Plants treated with BA had a mean of 1.9 clusters per branch, with a maximum of seven flowers per cluster. Differences in number of solitary flowers were not detected between the treatments. Since the treatment with $GA_4 + GA_7$ reduced the total flowers per branch with respect to the control and while the treatment with BA increased them, the difference in this parameter was significant between these treatments.

The histological study corroborated the effect of these plant growth regulators on the formation of flower clusters. Jojoba flowers originate in meristems located in the axillary buds. In some clones, such as clone 4.11.32, more than one flower can develop from each axillary bud. In other clones, such as 4.8, flower-bud production is individual in each axillary meristem. With BA application to clone 4.11.32 a marked enlargement (from 500 μm to more than 1200 μm) of the axillary meristematic zone was observed, in only one direction (Fig. 1). This meristematic zone, later on, differentiated in many flowers resulting in clusters of as many as nine flowers per inflorescence.

In clone 4.8 treated with BA application, no distinct histological change was observed, except for an increase in the number of clusters which was considerably less than that in clone 4.11.32. As this clone produced few clusters it is probable that the bud chosen for the histological study would not present a cluster. In plants treated with $GA_4 + GA_7$, the histological sections were very similar to those of the control. Axillary buds appeared with their meristems fully formed. Then, it is likely that some of these would not continue to flower differentiation due to the effect of the gibberellins, causing a decrease in the number of flowers.

The significant increase in the number of clusters caused by BA application would be due to the cytokinin action on the axillary meristems, reflected in an enlargement of the axillary meristematic zone. This growth would allow the differentiation of more than one flower per axillary bud, resulting in flower clusters rather than solitary flowers, and also resulting in an increase in total number of flowers produced. This BA effect on meristems was described by Werner et al. (2001), who found that cytokinins had an important regulatory effect on *Nicotiana tabacum* meristem morphogenesis, enlarging the meristem, which gave a greater probability for the development of flower meristems.

Later, seeds developed and as seen in Fig. 2 some flower clusters grew into fruits with many seeds.

Although the total number of flowers was significantly greater in the BA treatment than in the control, when evaluating the number and total weight of seeds per plant, in clone 4.11.32 significant differences were not detected between plants treated with BA and the control. There was only a significant reduction from $GA_4 + GA_7$ application when comparing it with the control and BA treatment. This situation was due to a great abortion of flowers and, even, of flower clusters in the BA treatment. These differences remain when analyzing the number and total weight of seeds per plant in clone

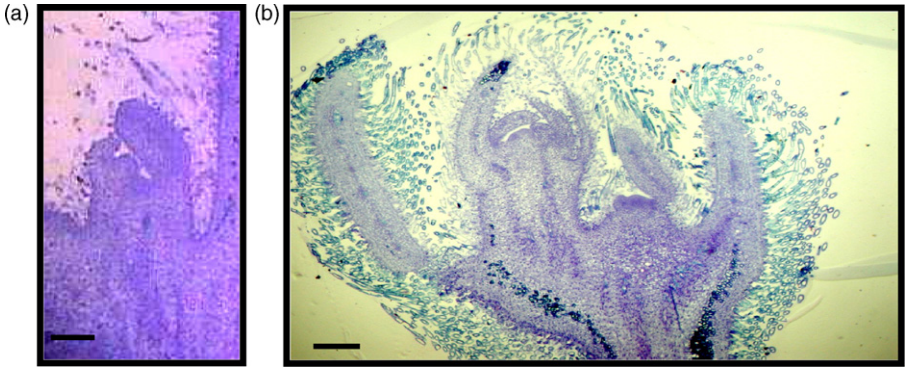

Fig. 1 – Lengthwise section of axillary meristem in jojoba shoot: (a) left axial of clone 4.11.32 without regulator application; (b) left axial of clone 4.11.32 with BA. The great enlargement of the meristematic zone that will produce a cluster can be observed. - - - - represents 100 μm.

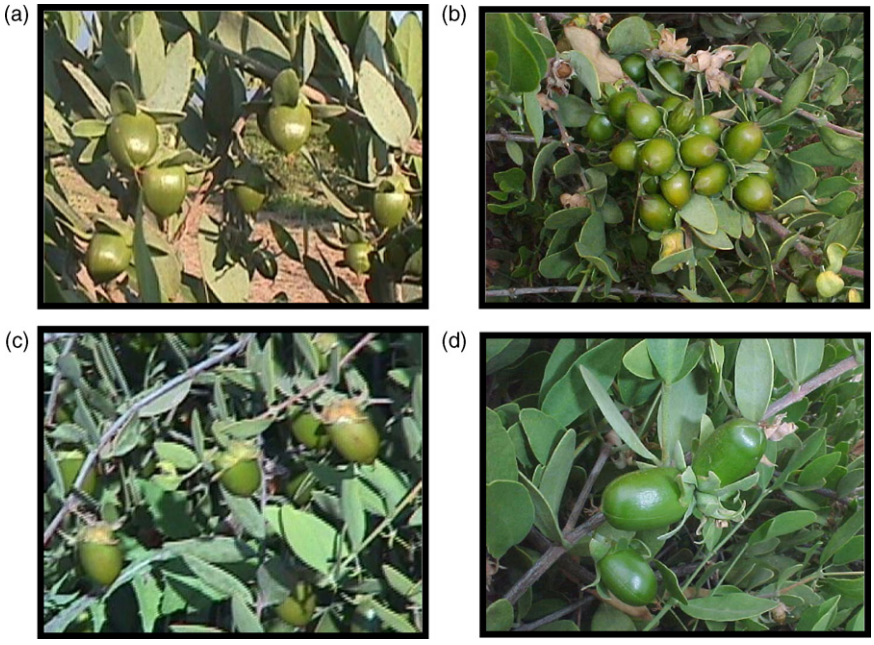

Fig. 2 – Seed production in jojoba plants 16 months after treatments: (a) Clone 4.11.32, control; (b) Clone 4.11.32, BA application; (c) Clone 4.8, control; (d) Clone 4.8, BA application.

4.8. In this clone, flower and cluster abortion caused by BA application was also observed. Such flower abortion could be a consequence of the inability of the plant to support a greater development of seeds in spite of having significantly increased its number of flowers, as seen in citrus where the number of harvested fruits is less than 10% of the developed flowers (Agustí, 2000) and in avocados, where the proportion between flower produced and fruits harvested can be even less than 0.2% (Gazit and Degani, 2002). Fruit drop can be observed in many of the cultivated species and is more noticeable when bloom intensity is greater (Agustí et al., 1982). For example, in some citrus varieties an increase in flowering results in less weight of flowers at anthesis (Agustí and Almela, 1989). This fact can be interpreted in terms of competition, so when flowering intensity is greater, there is more metabolite demand and less weight gain by the flowers. If flowering intensity is so great high that its demand exceeds the plant's possibility of nourishing the developing fruits with metabolites, the plant activates a self-control mechanism that adjusts the number of organs to its capacity to nourish them, with only the best situated fruits remaining and the rest abscising, with which final fruit setting decreases (Agustí and Almela, 1989; Guardiola et al., 1984).

4. Conclusions

Findings from this study on floral differentiation and seed production in jojoba plants as affected by growth regulators were as follows: (1) the response of jojoba plants to $GA_4 + GA_7$ and BA applications is clone-dependant; (2) $GA_4 + GA_7$ application has an inhibitory effect on flower production; (3) BA application induces a greater number of flowers per node (flower cluster) by increasing the size of the axillary meristem; (4) the greater production of flowers resulting from BA application did not result in increased seed production because of more flower and seed abortion.

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