Gibberellic Acid and Triacontanol Can Ameliorate the Opium Yield and Morphine Production in Opium Poppy (*Papaver somniferum* L.)

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Keywords: opium poppy, *Papaver somniferum*, medicinal plant, triacontanol, gibberellic acid, morphine

Abstract

A simple pot experiment was conducted at the Department of Botany, Aligard Muslim University, Aligarh, India, during 2004–2005 on opium poppy (*Papaver somniferum* L.) to study the effect of foliar spray of 10^{-6} M gibberellic acid (GA_3), 10^{-6} M of triacontanol (TRIA) as well as the combination of the two plant growth regulators (PGRs) on growth, yield, quality and morphine content of the respective plant. Among the three treatments, foliar spray of GA_3+TRIA significantly promoted the values of most of the physiological and biochemical parameters. The crude opium production was also enhanced by this treatment. Not only this, but the morphine content for which opium poppy is extensively valued, was also improved. However the individual effect of GA_3 was at par with that of TRIA. The later also induced branching in plants. Another important as well as interesting finding of this experiment was that the GA_3+TRIA treatment favored the development of capsules at uniform level of height in the branches which in turn will bring about a practical benefit of convenience in manual and/or mechanical lancing, collecting the latex and harvesting of opium poppy.

INTRODUCTION

In recent years, the rise in popularity of herbal medicines has put a great pressure on the only dependable, authentic alternative i.e. cultivation of medicinal plants (Li, 2000; Panda, 2005). The herbal medicines offer safe, natural remedies that are remarkably effective for maintaining health with minimum side-effects. The opium poppy (*Papaver somniferum* L.) is one of the most important medicinal plants worldwide due to its valuable alkaloids used in pharmaceutical industries since dawn of civilization. More than 40 alkaloids are found in the latex but only 5 are major ones viz. morphine, codeine, thebaine, narcotine and papaverine. Among these morphine (Fig. 1) is the most abundant and by far the most important alkaloid from the medicinal point of view. It is used as a pain killer in anxiety and insomnia. It is also invaluable in the treatment of renal colic, myocardial infarction, severe trauma, internal hemorrhage and congestive heart failure. Other alkaloids are also used as analgesic, hypnotic, sedative, antispasmodic and antitussive. Beside this, poppy seeds are highly nutritive (protein 24%) and devoid of any narcotic properties. These seeds are widely consumed as a condiment and in breads, curries, sweets and confectionary (Singh et al., 1995; Pushpangadan and Singh, 2001).

The global consumption of morphine has steadily increased during the last few decades from the level of 2 t (metric ton) in 1981–1983 to a level of more than 20 t per year in the period 1998–2000. Morphine apart from being used for medical treatment is predominately converted into other opiates (Shukla and Singh, 2004). Seeing the fast growing national and international demand of opium and its derivatives, India being world’s largest opium producing country, should trap this increasing demand by enhancing the opium yield and alkaloid content in the commercial cultivars of opium poppy.

Keeping it in mind, the prospects of plant growth regulators to enhance opium and alkaloid content directly or indirectly have been exploited in the present study. It has been a proven fact that the plant growth regulators (PGR) play a pivotal role in enhancing the growth and development of plants as well as boosting the alkaloid content in the herbs (Wareing and Philips, 1982). Keeping the effect of growth regulators in mind, two plant growth regulators viz. Gibberellic acid (GA_3), a very potent plant growth regulator (Fig. 2) (Hartmann et al., 1990; Zeevart, 1983; Harkness and Lyons, 1994), and n-Triacontanol (TRIA) a natural plant growth promoter (Fig. 3) (Ries, 1985, 1991; Oritani, 1993; Shripathi, 1996) and the combination of the two PGRs were used to work out the effect of foliar spray on growth, morphological, physiological and quality traits on the plants.

MATERIALS AND METHODS

A simple pot experiment was conducted during the years 2004–2005 at the Department of Botany, Aligarh Muslim University, Aligarh, UP, India. The authentic seeds of opium poppy were obtained from District Opium Officer, Afeem Kothi, Barabanki, UP, India. Seeds were sterilized in ethyl alcohol for half an hour and soaked in double distilled water for 12 hours after washing. Five seeds were sown in each clay pots (25 cm in diameter) on 30 October 2004, in the net-house of the department. After successful germination of the seeds, only one healthy plant was allowed to grow in each pot. The 10^{-6} M concentration of GA_3 and TRIA that has been proved the best dose for achieving the optimum yield potential in several crop plants (Devlin, 2001; Srivastava and Sharma, 1990; Alexander and Angelov, 1997) was also chosen for foliar spraying on poppy plants. The spraying was started at 90 days after sowing (DAS). Both growth regulators were sprayed on plants individually as well as in combination. A total of four sprays of each treatment at 15 days intervals were done. The Control plants were sprayed with de-ionized water. Each treatment was replicated five times. Irrigation, weeding, hoeing and other agricultural practices were followed as and when required. All plants started flowering after three weeks of spray of gibberellic acid and triacontanol. The flower petals fell off within 24–72 hours after the opening of the buds. The capsule took another 8–10 days to become fully mature for lancing (shallow incisions on capsules using sharp knife). The lancing operation was done in the afternoon and opium latex was collected in tarred glass vials the following morning, dried at room temperature, weighed and then the vials were stored in refrigerator at 5°C. Thus, the opium yield in mg/plant was assayed on dry weight basis. Data on plant height (cm), number of branches per plant, number of capsules per plant, dry weight per plant (g) and seed yield/plant (g) were recorded.

The fresh leaf samples were taken at 150 DAS to study various physiological and biochemical parameters including leaf carotenoids, chlorophyll *a*, chlorophyll *b* and total chlorophyll contents were estimated spectrophotometrically as suggested by Lichtenthaler and Buschmann (2001). Morphine content in opium was analyzed spectrophotometrically according to the method of Pride and Stern (1954). Morphine used as a reference was obtained from Sigma-Aldrich Company, St Louis, MO, USA. Duncan's Multiple Range Test (DMRT) was employed for statistical analysis of data using SPSS version 10.

RESULTS AND DISCUSSION

The effect of foliar sprays of GA_3, TRIA and their combination (GA_3+TRIA) was found to be significant for all the parameters studied (Fig. 4). However, among the three treatments, the combination (GA_3+TRIA) proved the best for most of the parameters. Foliar spray of 10^{-6} M GA_3+TRIA increased the plant height and dry weight by 36.7% and 71.7%, respectively, over their respective Controls. Treatment with TRIA also induced branching in plants which was not observed in Controls and GA_3-treated plants. However, the number of branches was more than doubled in GA_3+TRIA-treated plants than the Control. Interestingly, GA_3+TRIA triggered such development in plants that induced etiolation in the branches resulting in the capsules formation at the same height. It is a new finding and obviously, this feature of such plants could enable to realize a practical benefit of lancing, collection of the latex and harvesting of the capsules more conveniently (Fig. 5). Ries (1985, 1991); Shripathi (1996); Malabadi et al. (2005) have also reported the similar finding. Ries (1985, 1991) has proved the growth promoting effects of TRIA in various plants. Gorgiev and Cvetanovska (1987) showed the growth promoting effects of GA_3 in the opium plants. A conspicuous increase in the plant height and dry matter accumulation in GA_3+TRIA-treated plants are probably in agreement with the known fact that exogenous application of plant growth regulators evoked the intrinsic genetic potential of the plant causing increase in elongation of internodes as a consequence of cell division and cell wall extensibility (Moore, 1989; Taiz and Zeiger, 1998).

Furthermore, the combined treatment of GA_3+TRIA favoured capsules formation in plants. The treatment increased the number of capsules by 60.2% over the Control. Foliar spray of GA_3+TRIA also increased the biochemical and physiological processes of *Papaver somniferum*. The Chlorophyll *a*, *b*, total cholorophyll and carotenoid contents were increased by 68.1%, 64.9%, 68.5% and 57%, respectively, over their respective Controls. Similar findings were also noted in crop plants and in other plant species by Kumaravelu et al. (2000). The increases in these parameters were also noticed in GA_3-treated plants (Salisbury and Ross, 1992; Levy et al., 1986) and TRIA-treated plants (Trewavas and Gilory, 1991). The increase due to treatment with both these plant regulators probably enhanced the chlorophyll and carotenoids content in the combined application. The increased pigment content in GA_3+TRIA-treated leaves can be attributed to increases in number and size of chloroplasts, amount of chlorophyll/chloroplast and increase in grana formation. The beneficial effect of GA_3+TRIA was more prominent for crude opium yield per plant as it significantly increased yield by 27.7% as compared to the Control. The enhancement in opium yield could be due to the result of increased uptake of nutrients, enhanced photosynthesis and improved translocation of photosynthates and other metabolites to the reproductive parts (Miniraj and Shanmugavelu, 1987).

Surprisingly, the application of combined spray of GA_3+TRIA increased the morphine content (for which opium is valued generally) by 60.3% over the Control. However, the individual effect of GA_3 was at par with that of TRIA, which could be due to increase in phenol content in the plants by TRIA application since the precursor of morphine is a phenolic compound and it is well established that phenol content increases by TRIA application (Henry and Primo, 1979). It seems that increase in morphine content in GA_3 + TRIA treatment may be due to the triggering effect caused by combined dose of GA_3+TRIA on certain enzymes that are involved in the biosynthesis of morphine. Morphine yield (that is a computed value of opium yield per plant and morphine content in opium) was also significantly affected by this combination (GA_3+TRIA). The percent increase was 104.6% as compared to Control. This increase was due to the cumulative effect of opium yield and morphine content. Seed yield was also affected by GA_3 and TRIA application. However, it was the highest in GA_3 followed by GA_3+TRIA.

From the above results, it is clear that foliar spray of 10^{-6} M concentration of GA_3+TRIA proved considerably potent in increasing growth and biochemical characteristics of *P. somniferum*. The opium yield and other morphological and quality characters, viz. number of capsules, opium yield, morphine yield per plant and morphine content in opium can be enhanced by using the combined doses of GA_3+TRIA treatment. Furthermore, the combination (GA_3+TRIA) also proved useful in convenient lancing and harvesting as the capsules formation in each plant was almost at the same level. Obviously, this feature of the treated plants would facilitate the lancing of the capsules for opium latex and the latex collection as well as manual or mechanical harvesting of the poppies.

ACKNOWLEDGEMENTS

The authors are grateful to the District Opium Officer, Afeem Kothi, Barabanki, UP, India, for generous supply of authentic seeds of opium poppy. Mr. Tabarak H. Khan, M/s Samar Pesticides, Aligarh, UP is acknowledged for generous supply of TRIA.

Thanks are also due to Mr. M. Shakir for digital photography of *Papaver* plants. We are thankful to Dr. Sudhir Shukla, Scientist 'F', National Botanical Research Institute, Lucknow for critical examination of the paper.

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<u>Figures</u>

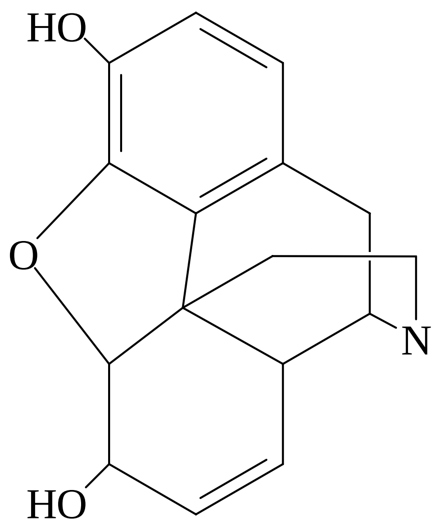

Fig. 1. Structural formula of morphine.

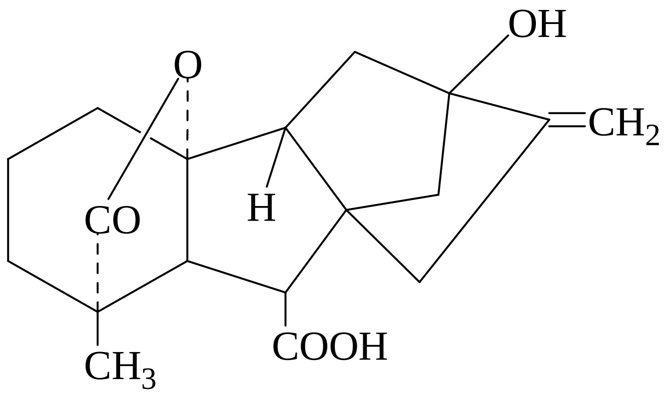

Fig. 2. Structural formula of gibberellic acid.

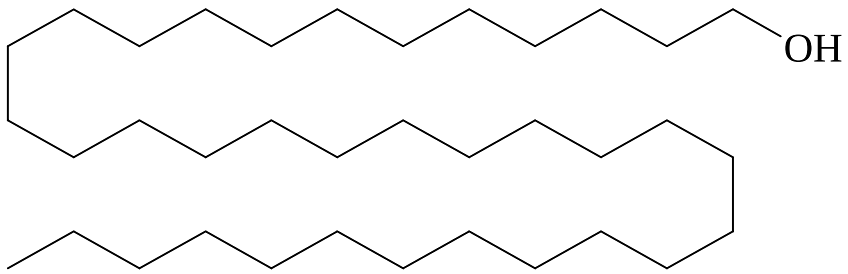

Fig. 3. Structural formula of n-triacontanol.

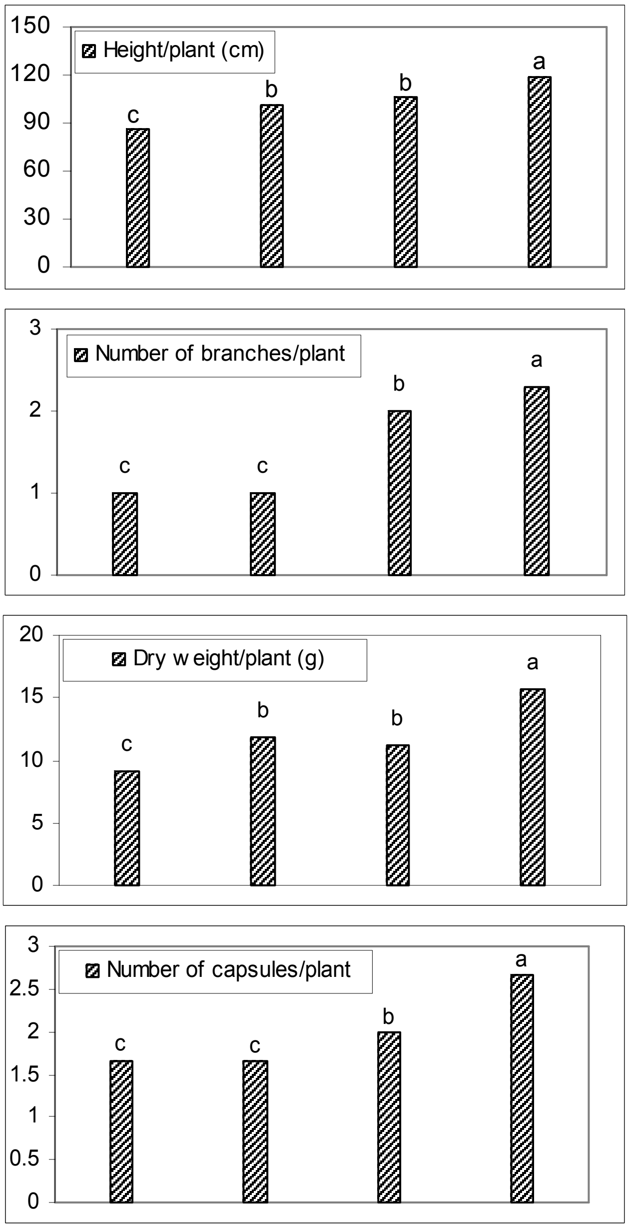

Fig. 4. The effect of sprays with 10^{-6} M GA_3, triacontanol or their combination, on the performance of poppy (*Papaver somniferum* L.). The vertical column represents the mean of 5 replicates. The values followed by the same letter are not significantly different at p = 0.05 significance according to DMRT.

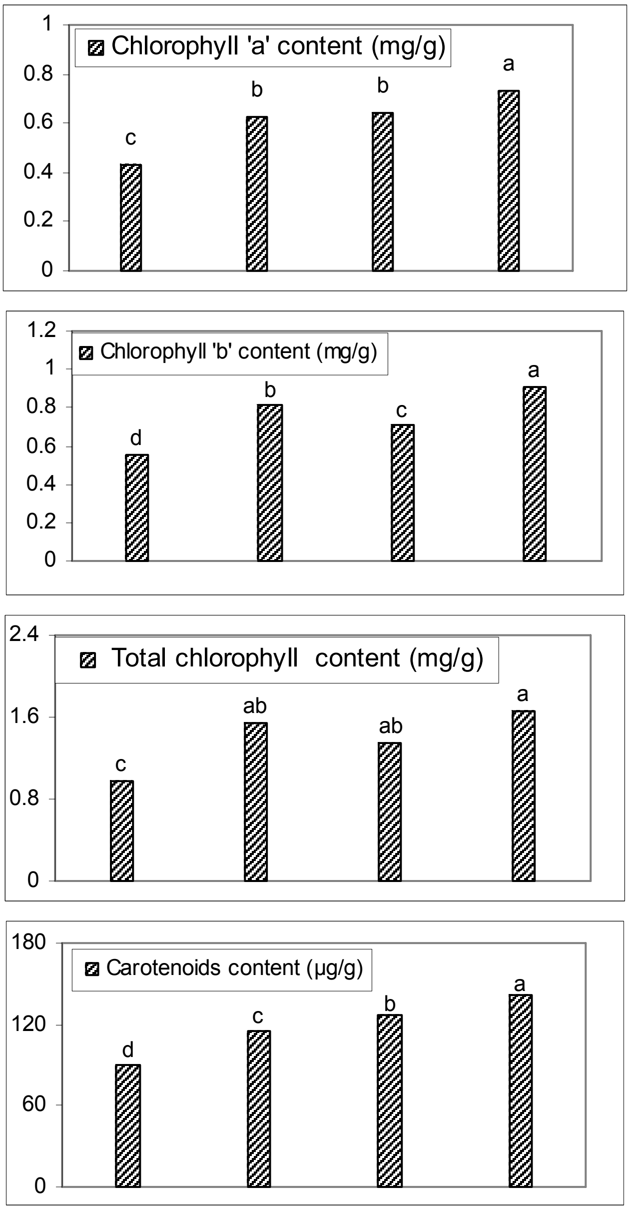

Fig. 4. (continued). The effect of sprays with 10^{-6} M GA_3, triacontanol or their combination, on the performance of poppy (*Papaver somniferum* L.). The vertical column represents the mean of 5 replicates. The values followed by the same letter are not significantly different at $p = 0.05$ significance according to DMRT.

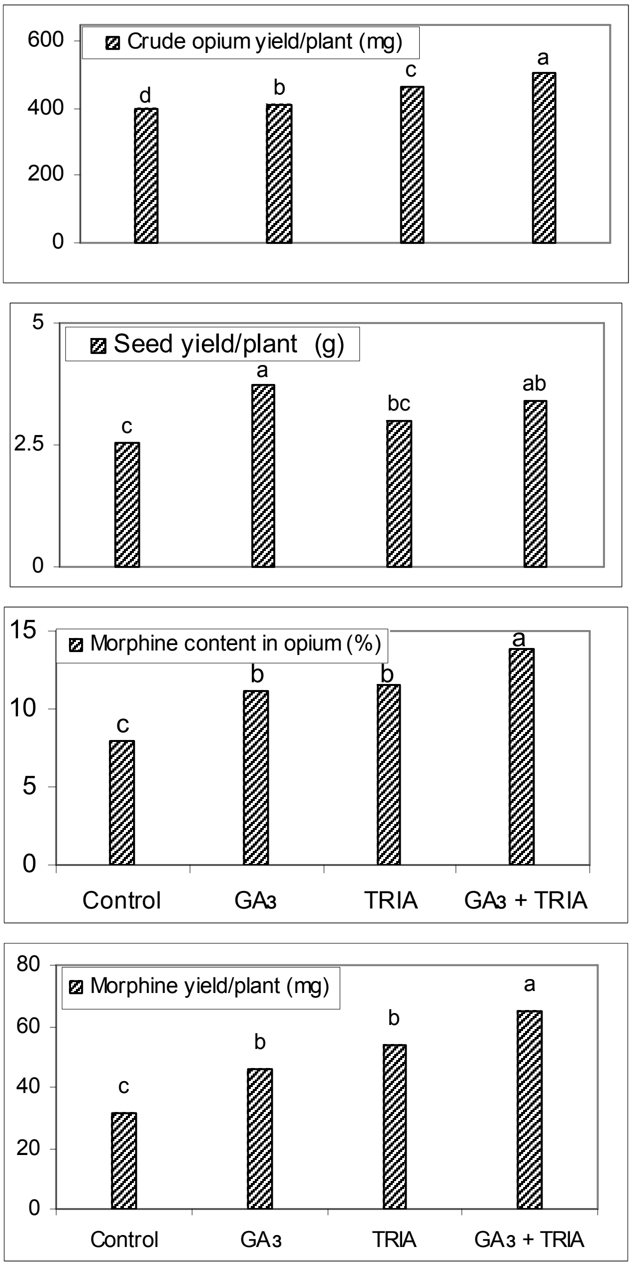

Fig. 4. (continued). The effect of sprays with 10^{-6} M GA_3, triacontanol or their combination, on the performance of poppy (*Papaver somniferum* L.). The vertical column represents the mean of 5 replicates. The values followed by the same letter are not significantly different at p = 0.05 significance according to DMRT.

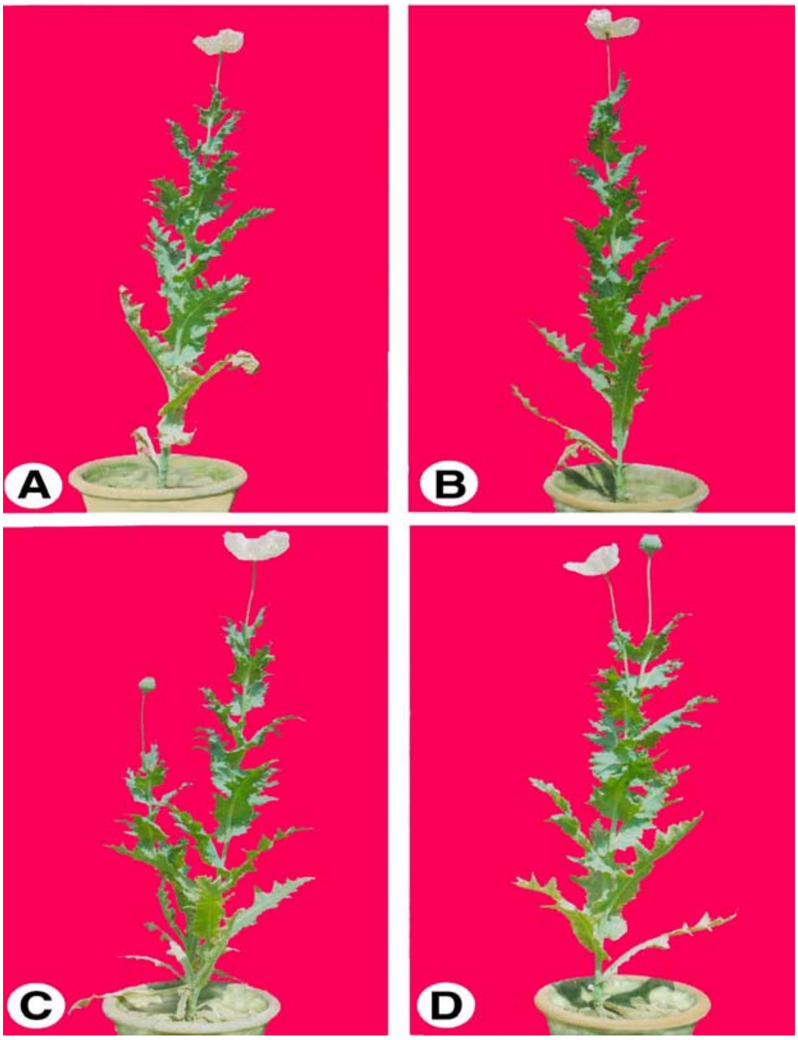

Fig. 5. Effect of spray of GA_3, Triacontanol (TRIA) and their combination on plant-height and branching of *Papaver somniferum* L. (A). Control, (B). GA_3 treated, (C). TRIA treated (D). GA_3+TRIA treated (produced tallest plant). TRIA triggered branching in plants. TRIA alone caused branching in the lower region of shoot, however, GA_3+TRIA did so in the above region of shoot. Additionally, the later also caused capsules formation in the branches at the same level (this feature would convene the lancing/ latex collection and capsules harvesting process of the crop).