Comparative effect of silver ion and gibberellic acid on the induction of male flowers on female *Cannabis* plants[1]

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Summary. Silver ion applied as $AgNO_3$ to the shoot tip of female plants of *Cannabis* induces male flowers. It is more effective than gibberellic acid (GA_3) in maintaining the induced state.

The silver ion (Ag^+) has recently been shown to be a potent inhibitor of ethylene action in ethylene-sensitive tissues[2]. In cucumber, higher ethylene levels are associated with femaleness[3,4] and Ag^+ induces maleness[5]. The present study was undertaken to test the effect of Ag^+ on the sex-expression in the dioecious *Cannabis sativa* L. and to compare its effect with gibberellic acid (GA_3). The preliminary findings are reported here. Earlier work on this plant had indicated that ethylene supplied as ethephon induces female flowers on male plants[6] and GA_3 application initiates male flowers in female plants[7].

Material and methods. Seedlings of *Cannabis sativa* L. were raised in earthen pots. The sexes were separated after the first few flowers had been formed. 10 female plants of uniform height, bearing 3 or 4 flowering nodes were selected for each treatment. The treated plants received either GA_3 or $AgNO_3$ dissolved in distilled water containing 0.02% Triton X-114 as the surfactant. Controls were given only distilled water and surfactant. The test compounds were applied to the shoot tip as a daily 10 µl drop for 10 days. The final amount of GA_3 or $AgNO_3$ received by each plant came to 100 µg. The test plants were maintained under natural conditions in the departmental garden. The number of plants showing conversion and the number of nodes bearing male flowers (including intersexual flowers) were recorded at 5-day intervals. Confidence inter-

Effect of GA_3 and $AgNO_3$ on the number of plants showing male flowers and the number of nodes per plant bearing male flowers in the female plants of *Cannabis sativa*

Treatment[a] (µg/plant)	Number of days after application															
	0		5		10		15		20		25		30		35	
	$\bar{x}$	CI	$\bar{x}$	CI	$\bar{x}$	CI	$\bar{x}$	CI	$\bar{x}$	CI	$\bar{x}$	CI	$\bar{x}$	CI	$\bar{x}$	CI
GA_3 (100)																
Number of plants showing male flowers	0		0		2		2		5		5		5		5	
Number of nodes bearing male flowers[b]	–		–		0.8	1.03[c]	1.1	1.4[c]	2.1	1.9[c]	2.6	2.3[c]	2.6	2.3[c]	2.6	2.3[c]
$AgNO_3$ (100)																
Number of plants showing male flowers	0		1		1		3		7		9		10		10	
Number of nodes bearing male flowers[b]	–		0.2	0.1[c,d]	0.2	0.1[c]	0.8	0.8[c]	2.8	2.2[c]	3.9	2.7[c]	4.6	2.9[c]	4.6	2.9[c]

[a] mean values of 10 plants; [b] nodes with at least 1 perfect male flower; ± confidence intervals (CI); [c] significantly different from controls at $p \leq 0.05$; [d] significantly different between treatments at $p < 0.05$.

vals and differences of means were calculated using Student's t-test at $p \leq 0.05$.

Results. Both the treatments caused sex inversion (table). Whereas $AgNO_3$ was able to cause male flower formation in all the female plants 30 days after treatment, GA_3 was able to do so only in half the number of plants. The difference in the number of nodes showing male flowers between the treatments was statistically insignificant on all days except day 5. The effect of treatment with $AgNO_3$ was more persistent. It delayed the resumption of production of female flowers by 10 days over GA_3 treatment. Normal male flowers are pedicellate and are borne on pedunculate cymose inflorescences. The tepals of the male flowers are typically reflexed at anthesis. The male flowers induced by $AgNO_3$ are sessile and form close clusters at each node as the inflorescence axis does not elongate. The flowers are otherwise similar to normal male flowers and set viable pollen. GA_3 causes elongation of the flower stalks and the flowers are smaller than normal male flowers, but set viable pollen. During the induction of male flowers, as well as at the time of formation of female flowers (true to the genetic sex at the expiry of the effect of treatment), numerous intersexual flowers were observed in the 2 treatments. GA_3 also caused a marked increase in shoot length over controls. Interestingly this was not observed with $AgNO_3$. The treated plants were equal to the controls in height.

Discussion. The induction of male flowers by Ag^+, in the female plants of *Cannabis* reported here, strengthens the concept that endogenous ethylene is probably responsible for female sex expression in this plant[8]. However, the mode of action of Ag^+ is still unclear. It has been proposed that Ag^+ can act at the receptor site of ethylene attachment, which is believed to contain a metal[9]. The direct action thus envisaged for Ag^+ possibly explains why the response to it is much greater than to GA_3 in inducing maleness in *Cannabis*. Although sex is genetically determined in *Cannabis*, sex-expression is influenced by several factors. As far as hormonal factors are concerned, there is evidence that sex expression is controlled by balance between levels of GA(s) and ethylene – higher ethylene levels favouring femaleness and higher GA levels favouring maleness[8]. When ethylene activity in female plants is blocked by Ag^+ or the relative GA levels are increased by exogenous GA, maleness is induced.

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