

The hormonal control of sex differentiation in dioecious plants of hemp (*Cannabis sativa*)

The influence of plant growth regulators on sex expression in male and female plants

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Abstract

The influence of GA₃, IAA, ethrel, ABA and kinetin on sex expression in male and female plants of hemp (*Cannabis sativa* cultivar LKC SD) was investigated. The growth regulators were applied separately and in combinations on stem apices of plant determined sexually and having the first flower primordia.

Gibberellic acid promoted masculinization, whereas IAA, ethylene and kinetin had a feminization effect on sex of hemp. Abscisic acid did not exert any direct effect on sex determination, it however acted antagonistically in relation to the effect exerted by GA₃ and IAA.

The results of combined application of IAA and ethrel with other growth regulators suggest, that the mechanism of action of auxin and ethylene in the control of sex expression in hemp is different. Auxins in this process cannot be regarded only as causing agents of ethylene production increase.

INTRODUCTION

The sex of plants may be, to a considerable extent modified by environmental factors such as day-length, temperature or mineral fertilization of soil (Nitsch, 1965) and the exogenous application of plant growth regulators. According to generally accepted view auxins and ethylene promote female tendency in numerous plant species, both mono and dioecious, whereas gibberellins cause masculinization (Heslop-Harrison, 1972).

There is considerably fewer information on the role of abscisic acid and cytokinins in the process of sex determination of hemp. The majority of data comes from investigations conducted on other plant species;

it follows from them that also these compounds participate in the endogenous control of sex differentiation. The feminization effect of cytokinins was observed in the experiment with *Vitis* (Hashizume and Iizuka, 1971) and *Luffa acutangula* (Bose and Nitsch, 1970) whereas the increase of female tendency under the influence of abscisic acid was established in squash (Abdel-Gawad and Ketellapper, 1969) and cucumber (Rudich and Halevy, 1974, Rudich et al., 1972).

In the present work the influence of growth regulators (IAA, GA₃, ethylene, ABA and kinetin), applied separately and in combinations, on sex differentiation in female and male plants of hemp was investigated.

MATERIAL and METHODS

The investigations were carried out on dioecious variety of hemp (*Cannabis sativa* cultivar LKC SD), being quantitatively a short-day plant.

Hemp seedlings were grown on 16-hr photoperiod at $25 \pm 2^\circ\text{C}$, at light intensity about 4000 lux. After 14 days the day-length was reduced to 8 hr in order to cause the transition of plants into the generative stage of development and their further cultivation was carried out under such conditions. After flower initiation, when the sex of plants was determined, selection was made and the application of growth regulators was commenced. 6 female and 6 male plants were taken for each treatment.

Growth regulators as water solutions were applied on shoot apices of plants. The following concentrations of growth substances were used: GA₃, IAA, ABA and kinetin — 25, 50 and 100 ppm, while ethrel, a compound releasing ethylene — 100, 250 and 500 ppm. The control plants received only distilled water.

Basing on preliminary investigations it was found that gibberellin does not influence the sex of male plants, whereas the other growth substances — female ones. So gibberellic acid was examined as far as its ability of stimulation the male tendency in female plants was concerned, whereas the other growth regulators — contrary to the above — were examined considering the stimulation towards the development of female generative organs on male plants.

The application was carried out on 5 consecutive days to a total of 25, 50 and 100 $\mu\text{g}/\text{plant}$ in the case GA₃, IAA, ABA and kinetin, and 100, 250 and 500 $\mu\text{g}/\text{plant}$ in the case of ethrel. In order to avoid the flow of applied solutions, cotton wicks were placed on shoot apices.

On the tenth day since the beginning of treatment, when plants reached the stage of flowering, the total number of flowers per plant and the percentage of flowers indicating sex reversion and the bisexual ones were recorded.

RESULTS and DISCUSSION

The obtained results point to the existence of distinct dependences between the applied growth regulators and sex expression in dioecious hemp.

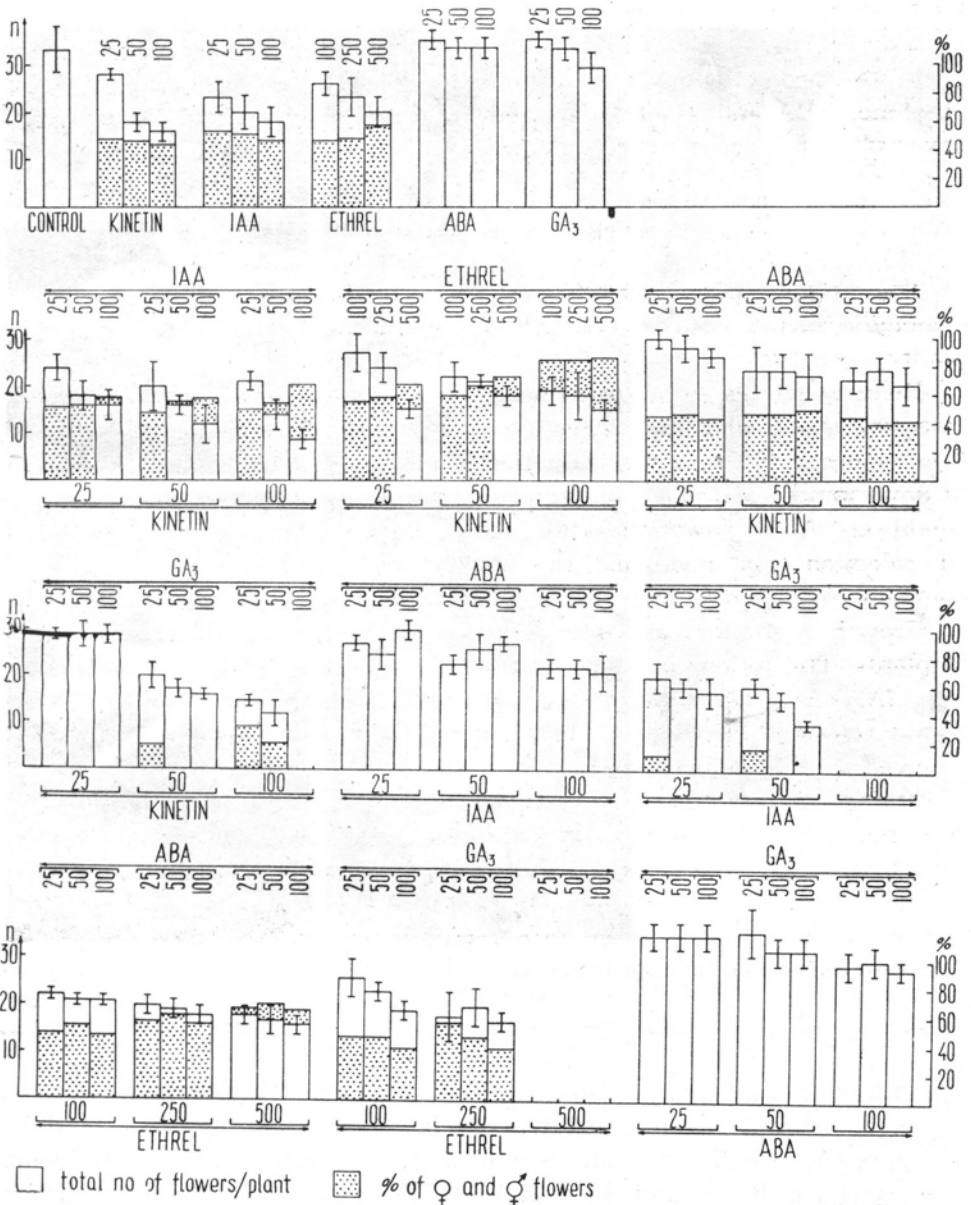


Fig. 1. Effect of plant growth regulators on flower sex expression in male plants of *Cannabis sativa*

The auxins increase feminization in plants. Under the influence of IAA normal female flowers or transitory forms having both male and female elements of flowers (Fig. 1, 2) are formed on male individuals of hemp. This statement is in accordance with the generally accepted view concerning the feminization influence of auxins on the sex of plants (Heslop-Harrison, 1972; Lang, 1961).

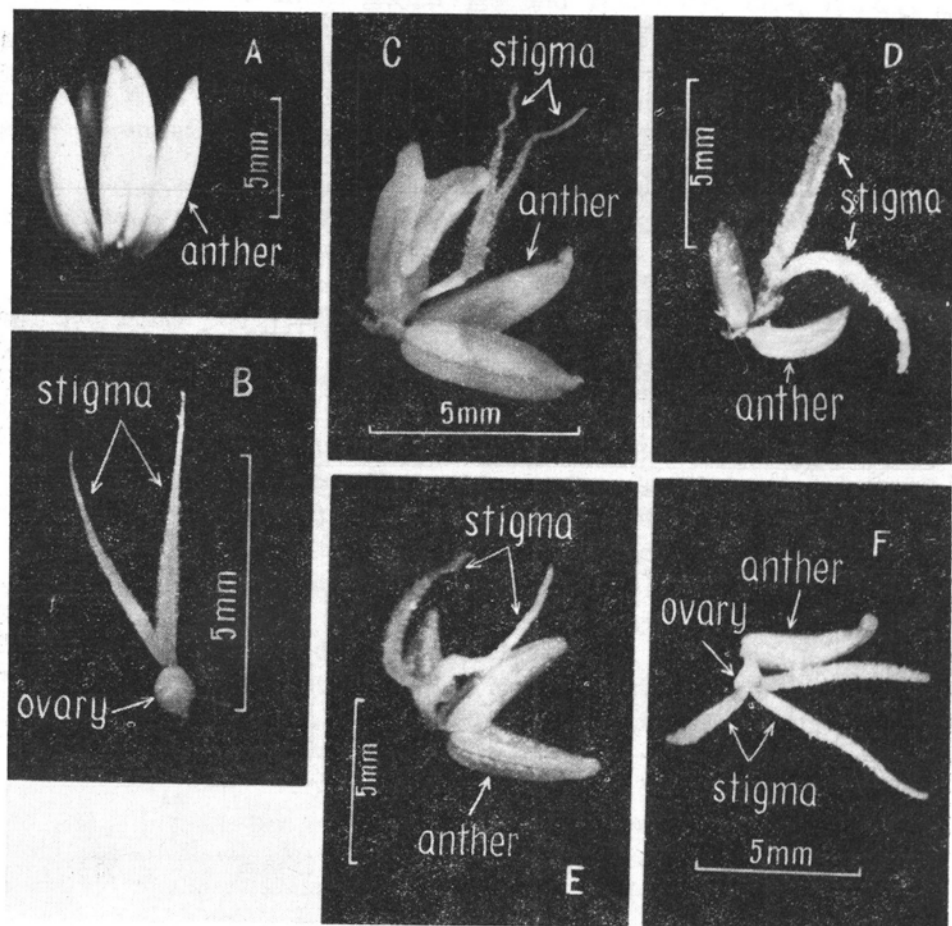


Fig. 2. Feminization of male plants of *Cannabis sativa* as an effect of IAA treatment (50 ppm)

A — Male flower; B — Female flower; C-F — Stages of transformation into female flowers

Similarly as auxins, also ethrel — a compound releasing ethylene (Fig. 1) induced the feminization of plants. So, these results would be in agreement with the data obtained, during experiments with hemp, by Mohan Ram and Jaiswal (1970) and with *Cucurbitaceae* by Abdel-Gawad and Ketellapper (1969), Shannon and De La Guardia (1969), Iwahori et al. (1970), Chromiński and

Kopcewicz (1972). This similarity in the action of ethrel and auxin on sex differentiation could be easily explained, assuming after some investigators (Abeles and Rubinstein, 1964; Burg and Burg, 1966, 1968; Fuchs and Lieberman, 1968) that in many physiological processes the role of auxins consists in enhancing ethylene production in plant tissue. Accepting this outlook it may be assumed that auxins promote feminization, increasing ethylene production.

The fact of a complete inhibition of female plants flowering caused by ethrel (Fig. 3) would also be understandable then. Namely it may be accepted that this negative effect is caused by the surplus increase in the level of ethylene, which is high in female plants in any case.

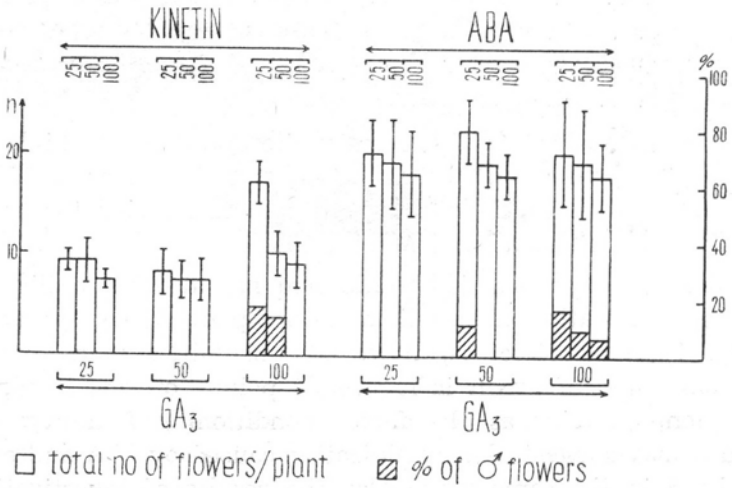
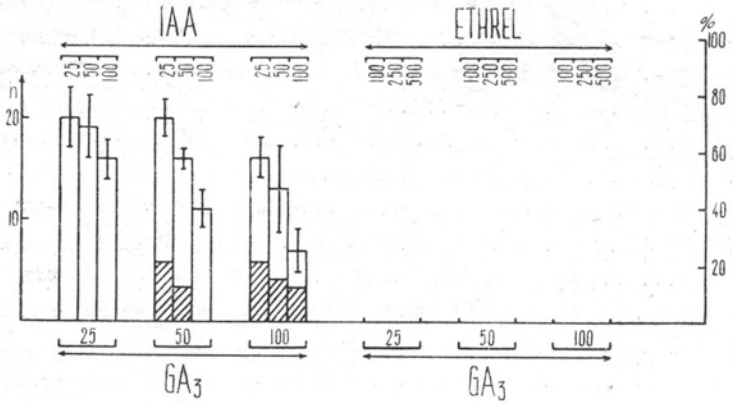
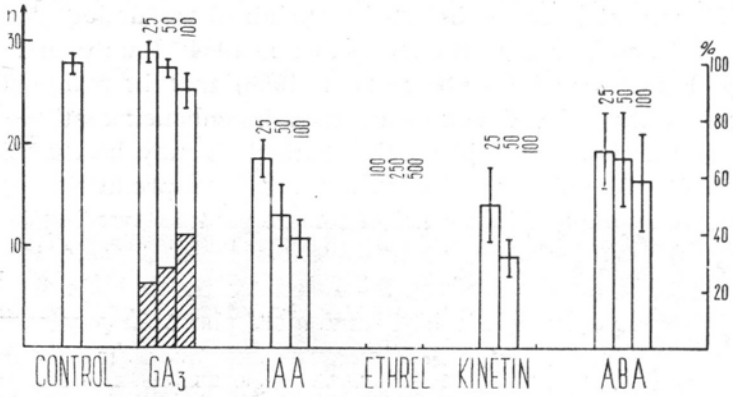
The results presented in Fig. 3 suggest that male flowers differentiation is connected with the metabolism of gibberellins. As a result of treating the plants with gibberellic acid the normal male flowers were formed on female plants which is in agreement with reports of other authors (Atal, 1959; Mohan Ram and Jaiswal, 1972). The experiments conducted on some species of *Cucurbitaceae* also confirmed the above data (Galun, 1959; Bukovac and Wittwer, 1961). The application of gibberellin caused in these plants an increase in the number of male flowers on monoecious plants, and in gynoecious plants the staminal flowers were formed.

As it follows from the experiments during which GA_3 and IAA were applied together (Fig. 1 and 3) antagonism of these compounds in the process of sex expression occurs.

Depending on the applied concentration, auxin inhibits partially or completely the inducted by gibberellin male flower formation on female individuals, whereas gibberellin efficiently counteracted the female flower formation induced by indolacetic acid. Similar dependences were observed in cucumber (Galun et al., 1962, 1963).

Gibberellic acid, which did not influence significantly the flowering intensity, promoted the inhibitory effect of auxins on this process until it caused a complete lack of flowering while applying the highest dose of IAA (Fig. 3) together with GA_3 . The cause of this fact could be the undue increase in the level of endogenous auxins under the influence of gibberellins (Kuraishi and Muir, 1963).

As it could be expected, gibberellic acid has also the ability of decreasing the feminization caused in male hemp plants by the action of ethrel (Fig. 1). This effect is, however, insignificant in comparison with the conversion of auxin influence. It points, on one hand, to the strong ethylene activity as the factor conditioning feminity; on the other hand it may suggest, that feminization caused by IAA and ethylene is not realized in the same way. Also the results of investigations on vernalization obtained by Chromiński and Banaszak (unpublished) do not suggest the existence of interaction between auxin and



□ total no of flowers/plant ▨ % of ♂ flowers

Fig. 3. Effect of plant growth regulators on flower sex expression in female plants of *Cannabis sativa*

ethylene. This suggestion would be contradictory to the views of many authors who link the mode of action of auxins with ethylene production.

The next investigated compound, abscisic acid, proved to be completely ineffective as far as it concerns the sex tendency both of male (Fig. 1) and female (Fig. 3) hemp. It influenced the sex of a plant only when this hormone was applied together with other growth regulators.

It follows from the experiment in which the plants were treated simultaneously with gibberellic and abscisic acids that ABA inhibits the formation of male flowers induced by gibberellin (Fig. 3). This effect depended on the applied concentration of both compounds; male flowers formation could be inhibited partially while applying higher concentrations of gibberellin, or completely when gibberellin was applied in smaller doses. On the basis of these results and in connection with works published by other authors (Mohan Ram and Jaiswal, 1972, Rudich and Halevy, 1974) it should be assumed that ABA participation in sex expression consists in the decrease of gibberellin activity by this compound. It was observed, for example (Nadeau et al., 1972), that abscisic acid promoted the binding of free GA and the formation of GA glycosides and other unidentified conjugants.

Interesting results were observed when ABA and IAA were applied together. Abscisic acid, which itself did not influence the sex, at all concentrations completely inhibited the formation of female flowers induced by auxin (Fig. 1). The observed effect remains undoubtedly in connection with the inhibitory influence, which is exerted by abscisic acid on the transport of indolacetic acid (Pilet 1971; Nagvi, 1972; Nagvi and Engvild, 1974).

The simultaneous application of abscisic acid and ethrel indicates that ABA increases although not significantly, the effect of ethylene (Fig. 1). It follows from the literature concerning the mutual interaction of these two compounds that it is very differentiated. It has been observed, for example, that ABA increases the ethylene production in orange peel plugs but decreases it in three-day-old pea seedlings (Gertman and Fuchs, 1972). The suppression of ethylene evolution as an effect of ABA treatment was also observed in rose petals (Mayak and Halevy, 1972) and in tissue cultures of *Ruta* (Gamborg and LaRue, 1971).

Kinetin is also a growth regulator participating in sex control of plants causing the feminization of male individuals (Fig. 1). The results obtained in this work confirm earlier observations conducted on other plant species (Bose and Nitsch, 1970; Hashizume and Iizuka, 1971; Krishnamoorthy and Bhatia, 1976). The confirmation of feminization effect of cytokinins on sex can also be found in the re-

sults of endogenous growth regulators analysis in dioecious plant *Mercurialis annua* L. (Kahlem et al., 1975). It was observed that the level of active cytokinins was higher in female generative organs than in the male ones. Moreover the presence of 2 specific cytokinins connected only with female sex was established.

The participation of kinetin in sex control is obvious from the presented in this work results of experiments in which this substance was applied together with other growth regulators (Fig. 1). They show that interaction of kinetin and IAA or kinetin and ethylene is of synergistic character. These results are understandable after the consideration of mutual dependences between kinetin and auxin. Kinetin influences the metabolism of auxins, increasing their level in plant tissues (Hemberg, 1972; Syono and Furuya, 1972). Also the increased uptake of ^{14}C IAA under the influence of kinetin (Lagerstedt and Langston, 1967) and the promotion of basipetal transport of IAA in petioles of young bean leaves (McCready et al., 1965) were stated.

The data obtained with simultaneous application of kinetin and gibberellic acid show, that the interaction between these two compounds is of antagonistic character, and the final effect depended on the concentrations of applied substances. The feminization effect of kinetin was decreased or completely nullified by gibberellic acid and vice versa — the masculinization effect of gibberellic acid was decreased or completely nullified in some combinations with kinetin.

No interaction between kinetin and abscisic acid in the regulation of sex expression in hemp has been observed (Fig. 1). Abscisic acid as rule, has not influenced the flowering intensity, or feminization resulting from kinetin action.

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Rola czynnika hormonalnego w procesie różnicowania płciowego u dwupiennych konopi (*Cannabis sativa*)

I. Wpływ regulatorów wzrostu roślin na determinację płci u żeńskich i męskich osobników konopi

Streszczenie

Zbadano wpływ GA_3 , IAA, etrelu, ABA i kinetyny na determinację płci u żeńskich i męskich roślin konopi (*Cannabis sativa*, odm. "LKC SD"). Regulatory wzrostu podawano oddzielnie i w kombinacjach na wierzchołki pędów roślin zdeterminowanych płciowo i posiadających już pierwsze zawiązki kwiatowe.

Kwas giberelowy wywoływał maskulinizację, zaś kwas 3-indoliloctowy, etylen i kinetyna działały feminizująco na płeć konopi. Kwas absycynowy nie miał bezpośredniego wpływu na determinację płci, działając jednakże antagonistycznie w stosunku do efektu, jaki wywierały GA_3 i IAA.

Rezultaty łącznego podawania IAA i etrelu z innymi regulatorami wzrostu sugerują, że mechanizm działania auksyny i etylenu w kontrolowaniu płci u konopi jest różny. Roli auksyn w tym procesie nie można zatem sprowadzać jedynie do zwiększania produkcji etylenu.