# The Influence of Growth Regulators Absorbed by the Root on Sex Expression in Hemp Plants

**M.Kh. Chailakhyan and V.N. Khryanin K.A.**

**Timiryazev Institute of Plant Physiology, Academy of Sciences of USSR, Botanicheskaya ul. 35, Moscow, USSR 127273**

**Abstract.**
Application, through the root system, of growth regulators to hemp (Cannabis sativa L.) plants having ~3 pairs of visible leaves caused pronounced shifts of sex expression in the adult individuals. Treatment with gibberellic acid (25 mg/1) resulted in more than 80% of the plants being male, i.e. having staminate flowers (controls, ca. 30%). Treatment with 6-benzylaminopurine and with indole-3-acetic acid (ineither case, 15 mg/1) resulted in all plants being eitherfemale (pistillate flowers) or intersexes (bisexual flowers); treatment with abscisic acid (10 mg/1) had a similar but somewhat less pronounced effect.

Key words: Abscisic acid - Auxin - Cannabis -Cytokinin - Flowers (sex) - Gibberellin - Sex expression

**Fig. 1.** The influence of growth regulators, absorbed by the roots, on growth and sex expression of hemp plants. From left to right: controls, gibberellic acid, 6-benzylaminopurine, indole-3-acetic acid, abscisic acid (ABA)

**Table 1.** Effects of growth regulators, absorbed by the roots, on growth, development and sex expressions in hemp plants

| Treatment | Height of plants (cm)[a] | | | Appearance of flower buds[b] | Percent of plants[a] | | |
|---|---|---|---|---|---|---|---|
| | Male | Female | Intersexes | | Male | Female | Intersexes |
| Control | 31 ± 1.7 | 16 ± 1.2 | 25 ± 2.1 | 12 | 29 ± 1.2 | 37 ± 2.3 | 34 ± 1.1 |
| $GA_3$ | 46 ± 2.3 | 39 ± 1.8 | 41 ± 1.6 | 8 | 84 ± 2.6 | 7 ± 0.4 | 9 ± 0.7 |
| BAP | – | 7 ± 0.3 | 8 ± 0.2 | 12 | 0 | 47 ± 1.8 | 53 ± 3.2 |
| IAA | – | 17 ± 1.0 | 27 ± 1.3 | 16 | 0 | 40 ± 2.0 | 60 ± 2.8 |
| ABA | 27 ± 1.4 | 15 ± 0.7 | 22 ± 2.7 | 12 | 20 ± 1.3 | 39 ± 1.4 | 42 ± 1.8 |

[a] Mean ± standard error
[b] Days after beginning of hormone treatment

It is well-known that sex expression in plants with unisexual flowers can be modified by hormone treatment. In hemp (Cannabis sativa L.), spraying with gibberellin solutions increases the number of male plants, i.e. plants with staminate flowers (Atal, 1959; Zhukov et al., 1963; Chailakhyan et al., 1969; Khryanin, 1969) while spraying with auxin enhances the appearance of female plants (pistillate flowers) and "intersexes" (bisexual or hermaphroditic flowers)(Heslop-Harrison and Heslop-Harrison, 1956). Cytokinin treatment promoted formation of female flowers in a male grapevine (Negi and Olmo, 1966).

Sex expression in hemp was found to be determined quite early, when the third leaf pair became visible (Khryanin and Milyaeva, 1977). We studied the effect of growth regulators, applied to the roots of hemp plants of this age (2-3 leaf pairs), on subsequent sex expression.

Seeds of hemp, strain US-6, obtained from the Institute of Fiber Crops, Glukhov, USSR, were germinated for 3 d in the dark on filter paper soaked with water, at 25 ~ C. Plantlets selected for roots of equal length were then placed with their roots in 1- or 3-1 containers with water for 2 d, and after this for 24 h in solutions of growth regulators. The treatments, with 70 plants each, were: 1) control (water); 2) 25 mg/1 gibberellic acid (GA3); 3) 15 mg/1 6-benzylaminopurine (BAP); 4) 15 rag/1 indole-3-acetic acid (IAA); and 5) 10 mg/1 abscisic acid (ABA). After treatment, the plants were first placed into 1/10-strength Knop nutrient solution, and after 2 d transferred to half-strength Knop and after another 2 d to full-strength Knop. The nutrient solution was aerated daily. Before, during and after treatment the plants were kept in the greenhouse, on 8-h short days, until sex expression was clearly apparent.

An overall view of the plants near the end of the experiment is shown in Figure 1. The results are summarized in Table 1. At the concentrations used GA 3 promoted the growth of the shoots while BAP inhibited it; in the other treatments growth did not differ from that of the controls.

The leaves of the BAP-treated plants were dark green, those of the GA3-treated ones yellowish-green. After 15 d of growth, swellings appeared in the roots of the BAP-treated plants; a microscopic examination showed that they were caused by overgrowth of the parenchymatous cells of the root.

Plants treated with GA3 formed flower buds 4 dearlier than the controls whereas IAA treatment delayed the appearance of flower buds; in the other treatments, flower buds appeared at the same time as in the controls. In all plants, flower buds appeared when the plants had three pairs of visible leaves, except in the BAP treatment where they appeared after the formation of two such leaf pairs.

As to sex expression, GA3 treatment resulted in the formation of more than 80% male plants, while in the controls the percentage was ca. 30. In both the BAP and the IAA treatments, no male plants were formed at all, the plants becoming either female, or intersexes, instead.

Representative plants from the GA3, BAP and IAA treatments are shown in Figure 2. ABA application also caused a reduction of the percentage of male plants, but the effect, at the concentrations used, was smaller than that of either BAP or IAA.

Thus, growth regulators applied through the root system cause considerable changes in the sex expression of hemp plants, GA 3 inducing predominantly mate plants and BAP, IAA, and to a somewhat lesser extent ABA, inducing female plants and plants with bisexual flowers. This effect of growth regulators on sex expression occurs at quite an early developmental stage of the plants.

a

**Fig. 2a–c.** Effect of growth regulators, applied through the roots, on sex expression in hemp. **a** Effect of $GA_3$: left, 6 control plants (3 male, 3 female); right, 5 treated plants (all male). **b** Effect of BAP: 6 treated plants, 3 female ones (left) and 3 intersexes (right). **c** Effect of IAA: 4 treated plants, 2 female ones (left) and 2 intersexes (right)

2 b

2 c