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Short Communication

The Role of Roots in 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. When the shoots of young hemp (*Cannabis sativa* L.) plants were cut off the roots, cultured as cuttings, and regenerating (adventitious) roots were removed as soon as appearing, ca. 80–90% of the plants became male (had staminate flowers) whereas if the roots were allowed to develop a similar percentage became female (pistillate flowers). Treatment of de-rooted cuttings with 6-benzylaminopurine (15 mg/l) restored the percent of female plants to ca. 80. It is suggested that the root system plays an essential role in sex expression in hemp and that this role is related to cytokinin synthesis in the root.

Key words: *Cannabis* – Cytokinin – Flowers (sex) – Sex expression.

The role of the various organs in sex expression of plants with unisexual flowers is not well understood. Minina (1952, pp. 150–152), working with cucumber, showed that treatment of the leaves with CO caused a shift in sex expression, favoring formation of female (pistillate) flowers. She concluded that the leaves played a decisive role in the determination of sex expression, as sites of production of some "sexualizing" substance(s) (Minina and Kushnirenko, 1949). On the other hand, there are reports that the root system of adult female hemp plants is 3 times larger than that of male plants (see Senchenko et al., 1963, pp. 32–36) and that hemp plantlets with long roots produce more female individuals than do plantlets with short roots (Kubarev, 1966). The aim of the work reported in this paper was to study the role of the roots in sex expression in hemp (*Cannabis sativa* L.), by growing shoot cuttings in liquid culture and either allowing adventitious roots to develop, or removing them, and by studying the effect of cytokinin on de-rooted cuttings.

All experiments were carried out with the hemp stain US-6 (seeds from the Institute of Fiber Crops, Glukhov, USSR). In the first, preliminary experiment the plants were raised in the Botanical Garden of the Pedagogical Institute at Penza, USSR, in the field under natural daylength conditions; otherwise they were raised in the greenhouses of the Timiryazev Institute in flats with soil, under 18-h long days (extended with light from xenon-arc lamps). When the plants had formed 2–3 pairs of visible leaves they were cut off at the root-shoot juncture and placed with the cut shoot bases in containers with water. One day later they were transferred to 1/10-strength Knop nutrient solution, 2 more days later to 1/2-strength Knop, and after another 2 d to full-strength Knop. The nutrient solution was aerated daily. In the first experiment the plants were kept in a greenhouse under natural days; in the other experiments, they were kept in controlled-environment cabinets under 16-h photoperiods (light from fluorescent lamps, ca. 6000 lx), at 20° C and 80% relative humidity. In each experiment, the plants were divided into two groups: one in which adventitious roots were removed as soon as they were appearing, and another where they were allowed to develop. In the last experiment, the roots were removed in all plants, but half of the plants were placed in a solution of 15 mg/l 6-benzylaminopurine immediately after being cut off from the root systems and for a period of 28 h, while the other half was placed in water.

Table 1. The number of male and female hemp plants formed by shoot cuttings with and without roots

Expt. No.[a]	Roots	Male plants No. (%)	Female plants No. (%)
1	+	7 (20)	28 (80)
	–	20 (77)	6 (23)
2	+	5 (8)	59 (92)
	–	53 (88)	7 (12)
3	+	8 (9)	83 (91)
	–	81 (90)	9 (10)
4	–	34 (77)	10 (23)
	–; +BAP[b]	9 (19)	39 (81)

[a] Expt. 1: in the greenhouse, natural light conditions; Expts. 2–4: in controlled-environment cabinets, 16-h days

[b] Cuttings, with roots removed, treated 28 h with 15 mg/l 6-benzylaminopurine

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Fig. 1. Role of roots on sex expression in hemp. Left, cuttings with adventitious roots removed; right, with adventitious roots allowed to develop. During the growth of the plants, the containers were covered with light-proof material so that the shoot bases and roots were in darkness

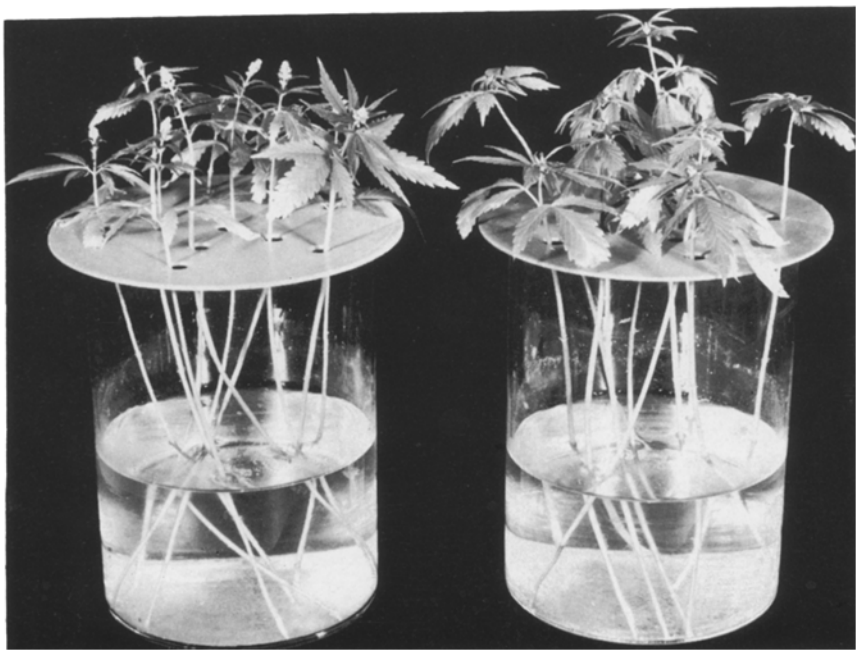

Fig. 2. Effect of cytokinin on sex expression in de-rooted hemp shoot cuttings. Left, controls; right, plants treated for 28 h with 15 mg/l 6-benzylaminopurine

An examination of the shoot apices of the plants at the start of the experiments whowed these to be still entirely vegetative, in agreement with previous reports (Heslop-Harrison and Heslop-Harrison, 1967; Khryanin and Milyaeva, 1977). Plants which were left to grow in soil developed into male and female plants (i.e. plants with staminate and pistillate flowers, respectively) in equal numbers. This was the case both under field and greenhouse conditions, under natural days and artificial long days, and regardless of whether the plants were grown under crowded conditions or were thinned out. For example, of 2970 plants grown on a 25-m^2 plot in the field, 1390 (46.8%) were male and 1580 (53.2%) female; in 80 plants grown in boxes with soil in the greenhouse on 18-h days, the ratio was 43:37 (53.7:46.3%).

When adventitious roots on shoot cuttings of young hemp plants were continuously removed the majority of the plants became male (between 77% and 90%); when they were allowed to develop a simi-

lar majority became female (80% to more than 90%). This result was again the same regardless of growing conditions (greenhouse vs. growth cabinet, natural light and photoperiod vs. 16 h artificial light per day). These results are summarized in Table 1 (experiments 1 through 3) and illustrated in Figure 1.

Thus, regeneration of a functional root system resulted in a striking increase in female hemp plants. This result is in accord with the suggestion, made by Sabinin as early as 1949, that substances of a hormonal nature which are derivatives of nucleic acids, are formed in the roots and affect the development of the shoots. Later, it was shown that roots produce cytokinins, which are substituted purines (see reviews of Mothes, 1964, and Kulayeva, 1973, pp. 9–34). On the other hand, treatment with cytokinin can modify sex expression in plants, promoting the differentiation of female flowers (Negi and Olmo, 1966; Chailakhyan and Khryanin, 1978). Based on these findings we tested the effect of a cytokinin, 6-benzylaminopurine, on sex expression in hemp shoot cuttings which were continuously de-rooted. It was found that treatment with 6-benzylaminopurine restored the percentage of female plants to about 80%, i.e. essentially to the percentage in rooted cuttings (Table 1, experiment 4; Fig. 2).

In conclusion, we suggest on the basis of the above results that the root system plays an essential role in sex expression in hemp, enhancing the formation of female plants, and that this role is based upon synthesis and export of cytokinins by the root.

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