Sex reversal in the female plants of *Cannabis sativa* by cobalt ion

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Abstract. Apical application of cobalt chloride to female plants (50 or 100 µg/plant) of *Cannabis sativa* caused drying of the shoot tip and formation of axillary branches. The latter bore male, reduced male and intersexual flowers, in addition to female flowers. Pollen in the flowers of altered sex were viable.

Keywords. *Cannabis sativa*; sex reversal; cobalt; sex expression.

1. Introduction

Ethephon, an ethylene releasing compound (Abeles 1973), promotes femaleness in cucurbits (McMurray and Miller 1968; Rudich *et al* 1969, 1970; Augustine *et al* 1973). In *Cannabis sativa*, it induces the formation of female flowers on male plants, or at male nodes (Mohan Ram and Jaiswal 1970). Mohan Ram and Jaiswal (1973) suggested that, in this plant, the expression of sex is controlled by a balance between the endogenous levels of ethylene and gibberellin. Elements like boron, calcium and iron have been shown to increase the number of female flowers in cucurbits (Choudhury 1967). Beyer (1976) reported that silver ion [Ag (I)] is a potent inhibitor of ethylene action. Recently, Sarath and Mohan Ram (1979) have demonstrated that application of Ag (I) also results in the production of male flowers on female plants of *Cannabis*.

The cobalt ion [Co (I)] has recently been shown to inhibit the synthesis of ethylene (Lau and Yang 1976; Beutelmann and Kende 1977). The present work was undertaken to ascertain whether or not Co (I) has any effect on sex expression. Preliminary findings are reported here.

2. Material and methods

Seedlings of *Cannabis sativa* L. growing naturally in the Delhi University Botanical Gardens were transplanted at the 3- or 4-leaf stage to 25 cm earthenware pots filled with garden soil. Female plants of uniform height, bearing 3 or 4 flowering nodes were selected for experimentation. Cobalt chloride solution
was prepared in distilled water. Tween-80 (0.02\%) was used as the surfactant. It was applied to the shoot tip as a 10 or 20 \( \mu l \) drop daily, for 5 days to make up the total amount. Ten plants were maintained for each treatment. The plants received either 50 or 100 \( \mu g \) of CoCl\(_2\) each, by the end of the fifth day. The controls received surfactant solution only. The plants were kept under outdoor conditions in the Botanical Garden. Viability of pollen in the induced male flowers was tested by germination in Brewbaker and Kwack's (1963) medium using the hanging drop method.

3. Results

The shoot apices treated with either dosage of CoCl\(_2\) dried up. The young leaves initiated before treatment failed to expand fully, turned pale yellow and became deformed. The control plants, however, continued to form new leaves and nodes (figure 1A). The small increment in height of the treated plants was due to elongation of the existing internodes (table 1). The lower lateral branches (which were already present at the time of the treatment) produced only female flowers, whereas those formed after treatment bore not only female flowers but also flowers with variously modified sex expression (figure 1B, C). The upper two axillary branches (Ax. I and Ax. II in figure 1C) showed maximum number of altered flowers. The induced male flowers were sessile to sub-sessile, were produced singly or in clusters and were similar to normal male flowers. They contained five stamens with a copious amount of viable pollen. The flowers which had both stamens and stigma(s) were classified as intersexual and those which had less than five stamens as reduced male flowers (figure 1D). The average number of different types of flowers formed on axillary branches are given in tables 1–2. The control plants bore only female flowers.

To determine whether flower sex inversion resulted from cobalt treatment or release of apical dominance, a few control female plants were decapitated. The forced lateral branches in these, however, produced only female flowers.

4. Discussion

In the present work treatment with Co(I) has been shown to cause masculinisation of flowers in the female plants of Cannabis. Cobalt promotes bean hypocotyl hook opening (Kang and Ray 1969a), leaf disc expansion in bean (Miller 1951; Loercher and Liverman 1964) and elongation of pea epicotyl (Miller 1954; Howell and Skoog 1955). These processes are inhibited by ethylene (Kang et al 1967; Kang and Ray 1969b; Abeles 1973). Lau and Yang (1976) have shown that Co(I) inhibits ethylene production by preventing the conversion of methionine to ethylene. Beutelmann and Kende (1977) have demonstrated the inhibition of ethylene-induced rolling up of rib segments, excised from the buds of Ipomoea by Co(I). The present finding suggests that Co(I) triggers male sex expression probably by preventing ethylene synthesis. Further evidence is needed to confirm the exact role of Co(I) in controlling sex expression in this dioecious plant.
Figure 1. A–D. Cannabis sativa L. A. Terminal portion of control female plant, \( \times 0.5 \). Inset shows a female flower, \( \times 5.0 \). B. A portion of the treated female plant (50 \( \mu g \)). Note the dried shoot tip and the axillary branch bearing male flowers, \( \times 1.0 \). C. A portion of treated female plant (100 \( \mu g \)). Note the dried shoot tip and axillary branches (I and II) bearing male flowers, \( \times 0.8 \). D. Modified flowers formed on the treated plant (100 \( \mu g \)), \( \times 5.0 \). a, b. Interssexual flowers bearing both pistils and stamens. c, d. Reduced male flowers. e. Normal male flower with five stamens. (Ax. I, axillary branch number one; Ax. II, axillary branch number two).
Sex reversal in Cannabis sativa by cobalt ion

Table 1. Effect of Co(I) on the height of the main axis and the number of nodes in female plants of Cannabis sativa.*

<table>
<thead>
<tr>
<th>Treatments**</th>
<th>0 (control)</th>
<th>50</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>x</em> ± Cl</td>
<td><em>x</em> ± Cl</td>
<td><em>x</em> ± Cl</td>
</tr>
<tr>
<td>Increment in height (cm)</td>
<td>34.9</td>
<td>10.9</td>
<td>14.4</td>
</tr>
<tr>
<td>Increment in number of nodes</td>
<td>7</td>
<td>0.7</td>
<td>D</td>
</tr>
</tbody>
</table>

* Mean values from 10 plants 48 days after treatment.
** Total amount of the chemical applied in μg/plant.
± Cl. Confidence intervals of mean values at P ≤ 0.01.
D, Shoot tip dried up.

Table 2. Effect of Co(I) on flower sex expression in female plants of Cannabis sativa.*

<table>
<thead>
<tr>
<th>Treatments**</th>
<th>0 (control)</th>
<th>50</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>x</em> ± Cl</td>
<td><em>x</em> ± Cl</td>
<td><em>x</em> ± Cl</td>
</tr>
<tr>
<td>Mean No. of flowers:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>I</td>
<td>9.4</td>
<td>9.4</td>
</tr>
<tr>
<td>II</td>
<td>7.4</td>
<td>12.8</td>
<td>15.5</td>
</tr>
<tr>
<td>Male</td>
<td>I</td>
<td>..†</td>
<td>7.9</td>
</tr>
<tr>
<td>II</td>
<td>..</td>
<td>7.9</td>
<td>4.5</td>
</tr>
<tr>
<td>Reduced Male</td>
<td>I</td>
<td>..</td>
<td>3.6</td>
</tr>
<tr>
<td>II</td>
<td>..</td>
<td>4.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Intersexual</td>
<td>I</td>
<td>..</td>
<td>3.4</td>
</tr>
<tr>
<td>II</td>
<td>..</td>
<td>2.9</td>
<td>3.4</td>
</tr>
</tbody>
</table>

* Mean values from 10 plants 48 days after treatment.
** Total amount of the chemical applied in μg/plant.
I, II, First and secondary axillary branches respectively.
† Only female flowers are found in control.

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