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*Short Communication*

# Induction of Male Flowers in a Pistillate Line of *Ricinus communis* L. by Silver and Cobalt Ions

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**Abstract.** Aqueous solutions of silver nitrate (10–100 μg/plant) and cobalt chloride (125–500 μg/plant), injected into the main stem of plants of the pistillate cv. 240 of *Ricinus communis* when the vegetative shoot apex was beginning to become reproductive, induced the formation of staminate (male) flowers with viable pollen in the normally strictly pistillate (female) terminal inflorescence, their number increasing with the dose of $Ag^+$ and $Co^{2+}$. No formation of bisexual flowers was noted. Female flowers pollinated with pollen from the induced male ones produced fruits and viable seeds.

**Key words:** Cobalt – Flowers (sex expression) – *Ricinus* – Sex expression – Silver.

Sex expression in monoecious and dioecious species is one of the many growth and developmental processes that can be regulated by hormones, with ethylene promoting "femaleness". Treatment with 2-chloroethylphosphonic acid (Ethrel, Ethephon), a compound releasing ethylene, causes formation of female (pistillate) or perfect (bisexual) flowers in place of male (staminate) ones in cucurbits (Rudich et al. 1970; Augustine et al. 1973); and induces the formation of female flowers in staminate plants of *Cannabis sativa* (Mohan Ram and Jaiswal 1974). In contrast treatments which are known to reduce the ethylene level in the tissue (hypobaric conditions, treatment with benzothiodiazole) or to antagonize the action of ethylene ($CO_2$) have the opposite effect, causing formation of male or bisexual flowers in place of female ones (Byers et al. 1972). A higher ethylene production has been found to be associated with femaleness in *Cucumis* species (Byers et al. 1972; Rudich et al. 1972).

Recently, it has been shown that cobalt and silver ions are antagonistic to the effects of ethylene, $Co^{2+}$ inhibiting ethylene formation from methionine (Lau and Yang 1976) and $Ag^+$ interfering with ethylene action, presumably at the ethylene receptor sites in cells (Beyer 1976). Treatment with $Ag^+$ enhanced the production of male flowers in cucumber (Beyer 1976) and treatment with $Ag^+$ and $Co^{2+}$ induced the formation of male flowers on female plants of *Cannabis* (Sarath and Mohan Ram 1979; Mohan Ram and Sett 1979).

In this communication we report the effects of $Ag^+$ and $Co^{2+}$ on sex expression in a female line (cv. 240) of castor bean, *Ricinus communis* L., since even marginal sex reversal can be easily detected in plants of this cultivar.

Seeds of cv. 240 of *Ricinus* were procured from the Indian Agricultural Research Institute, Regional Research Station, Hyderabad, where this cultivar has been developed (Ankineedu and Rao 1973). Plants were raised in the Botanical Garden of the Department of Botany, University of Delhi. All treatments were given when the plants had 10–13 nodes and the shoot apex had begun the transition from the vegetative to the reproductive phase. Earlier and later treatments had no effect on sex expression. Since a few male flowers appear spontaneously in the secondary and tertiary inflorescences of cv. 240 plants, observations were restricted to the primary (terminal) inflorescence which in this cultivar bears only female flowers.

The plants were treated with three concentrations of $AgNO_3$ (5, 25, 50 μg/0.5 ml) or $CoCl_2$ (62.5, 125, 250 μg/0.5 ml) containing ca 0.01% Tween-80 (polyoxyethylenesorbitan monooleate; Sigma Chemical Comp., St. Louis, Mo., USA) as wetting agent, twice at an interval of 2 d, the total amount received by each plant thus being 10, 50, 100 μg of $AgNO_3$ or 125, 250, 500 μg $CoCl_2$. Control plants received distilled water containing Tween-80. The treatment was done by injection of the solutions into the hollow internode (3rd or 4th from top) of the plants with the help of a 1-ml syringe. This method was found to be superior to foliar spray at least under monsoon conditions.

The viability of the pollen grains produced by $Ag^+$- or $Co^{2+}$-induced male flowers was tested by the hanging-drop technique using Brewbaker and Kwack's (1963) medium, and by staining in a trimethyltetrazolium-chloride solution (Stanley and Linskens 1974).

For studying fruit set, female flowers were hand-pollinated

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**Table 1.** Effect of $AgNO_3$ and $CoCl_2$ on shoot growth, number of plants bearing male flowers, number of male flowers, female flowers, and per cent fruit set[a]

| Treatment (μg/plant) | Increment in height (cm) | Increase in number of nodes (over initial number) | No. of plants bearing staminate flowers | No. of staminate flowers per plant | No. of pistillate flowers per plant | Fruit set (%) |
|---|---|---|---|---|---|---|
| Control | 103±15.4 | 9±1.5 | – | 0.00±0.00 | 191.9±13.0 | – |
| 10 $AgNO_3$ | 100±31.2 | 8±1.5 | 4 | 6.5 ±5.6* | 175.5± 9.8* | 11.1 |
| 50 $AgNO_3$ | 103±31.0 | 7±0.9 | 9 | 6.8 ±2.4* | 184.7± 6.5 | 12.0 |
| 100 $AgNO_3$ | 87±12.8* | 7±2.3 | 10 | 12.0 ±4.7* | 176.9± 9.6* | 24.0 |
| 125 $CoCl_2$ | 98±21.1** | 8±2.7 | 6 | 6.8 ±3.4* | 173.7±16.5* | 14.0 |
| 250 $CoCl_2$ | 91±17.8* | 7±2.9 | 8 | 14.3 ±3.8* | 165.1±14.3* | 19.7 |
| 500 $CoCl_2$ | 92±19.7* | 8±1.5 | 10 | 16.7 ±6.3* | 147.0±13.9* | 30.9 |

[a] Average of 10 plants; ±values = confidence interval at $P \leqslant 0.05$; * significantly different from control at $P \leqslant 0.01$; ** significantly different from control at $P \leqslant 0.05$

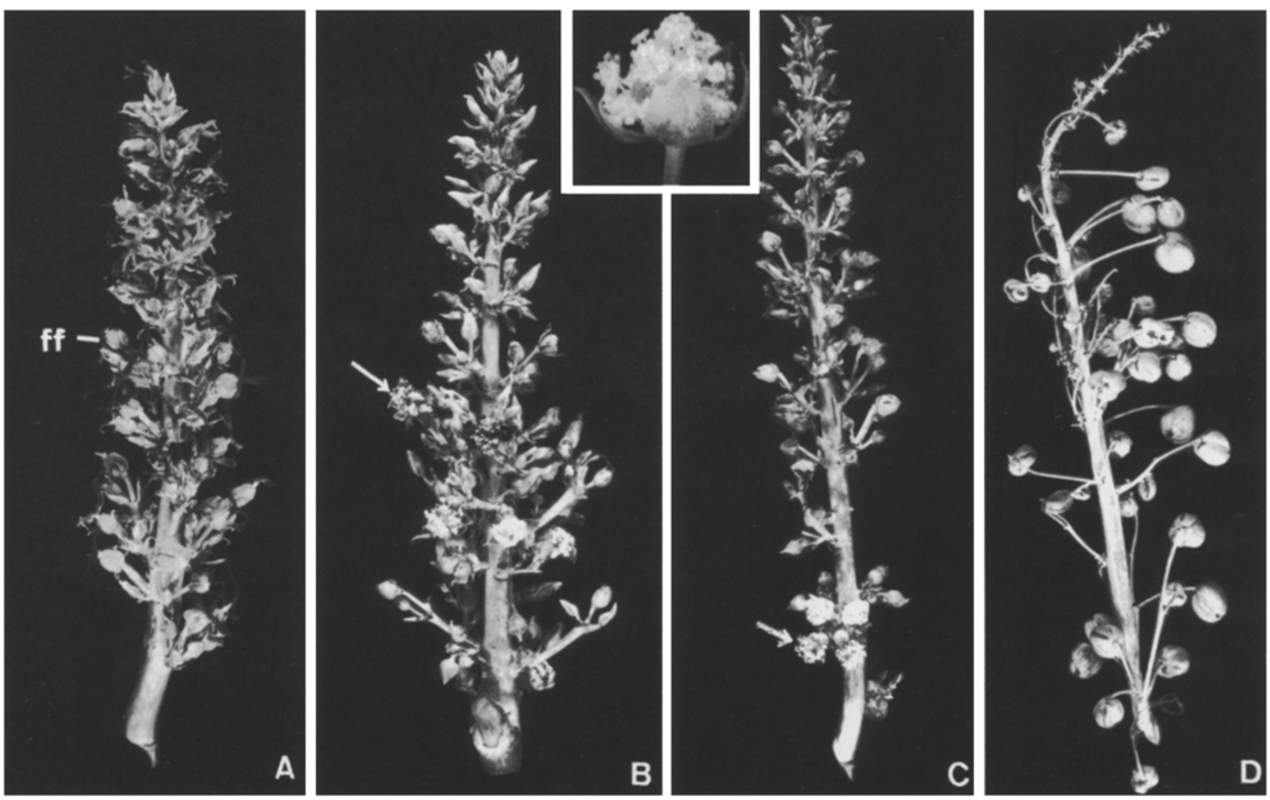


**Fig. 1 A–D.** Inflorescences of *Ricinus*, pistillate cv. 240, **A** of control plant bearing only female flowers (ff), ×0.5; **B, C** of plants treated with 100 μg $AgNO_3$ and 500 μg $CoCl_2$ respectively, ×0.6, ×0.5. Arrows indicate induced male flowers, inset shows a male flower, ×2; **D** mature inflorescence from plant treated with 500 μg $CoCl_2$ bearing fruits, ×0.5

using pollen from induced male flowers in the same inflorescence and bagging the latter. For verifying pollen germination in vivo, pollinated stigmatic lobes were excised, softened in 0.1 N HCl for 15 s, stained with 1% cotton blue (BDH, poole, U.K.) in lactophenol or 1% acetocarmine (BDH) in 45% acetic acid for 5 s, and mounted in glycerin jelly (1 g of phenol crystals added to 100 ml of a 1:1 mixture of gelatine (17%) and glycerin, after Johansen 1940, p. 25).

Standard deviation and confidence intervals of the mean values were calculated as described in Spiegel (1972).

Injection of $AgNO_3$ or $CoCl_2$ solutions into the stem of cv. 240 plants resulted in the formation of male flowers in the terminal inflorescence; the effect increased with the dose (Table 1). The greatest number of male flowers was observed after treatment with 500 μg of $CoCl_2$ (Table 1, Fig.1). To verify whether the effect of $CoCl_2$ was caused by the Co or the Cl ion, some plants were injected with an NaCl solution (1 ml containing 500 µg). No change in the sex of the flowers occurred in response to this treatment. The plants treated with $Ag^+$ or $Co^{2+}$ generally produced fewer female flowers than the controls (Table 1).

The $Ag^+$- and $Co^{2+}$-induced male flowers had viable pollen grains which germinated within 20–30 min of incubation in Brewbaker and Kwack's medium with an average percentage of ca. 70. These pollen grains also stained positively with a tetrazolium solution. Pollen tubes were observed on the stigmatic surface 2 h after pollination. Female flowers pollinated with pollen from induced male flowers set fruits with seeds, but fruit set was low (11–30%) because of flower abscission. The average number of fruits formed was dependent on the concentration of $Ag^+$ and $Co^{2+}$; it was greatest in plants that had been trated with 100 µg $AgNO_3$ and 500 µg $CoCl_2$. The seeds germinated on moist filter paper within 72 h.

Thus, staminate flowers can be induced in the primary inflorescence of the pistillate cv. 240 of castor bean, by $Ag^+$ and $Co^{2+}$. These ions presumably lower the effective level of ethylene in the plant and thus reduce the "femaleness", resulting in a shift of sex expression in the male direction. Both these ions also induced male flowers on female plants of *Cannabis* (Sarath and Mohan Ram 1979; Mohan Ram and Sett 1979). In *Ricinus* gibberellin is reported to promote femaleness (Shifriss 1961), in contrast to its action in most other plants with unisexual flowers, although there is also a report of increased "maleness" in response to gibberellin in the same plant (Juneja 1971). The effect of ethylene in this plant does not differ from that in other dioecious and monoecious plants, in that it enhances female sex expression.

A point of interest is the absence of bisexual flowers in the primary inflorescence of the treated plants; such flowers were found after $Ag^+$ and $Co^{2+}$ treatment of *Cannabis* (Sarath and Mohan Ram 1979, Mohan Ram and Sett 1979). One probable explanation for this differential behaviour is that in *Ricinus*, the sex of each flower is pre-determined early in ontogeny and can be altered only if the treatment is given before inflorescence initiation. In *Cannabis*, in contrast, the developmental pattern of the flowers is flexible and can be changed at later stages of development by manipulating the relative endogenous hormonal balance by exogenous supply (Mohan Ram and Jaiswal 1974).

Because of their ability to keep the plant height nearly unaltered (see Table 1) and stimulate male flower formation, $Ag^+$ and $Co^{2+}$ are more advantageous than gibberellin which causes increased shoot elongation in addition to inducing maleness (Atsmon et al. 1968; Splittstoesser 1970). This technique of inducing male flowers for selfing can be useful for maintaining the pistillate castor bean cv. 240, a useful genotype for breeding and genetic improvement.

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