ACTIVITY OF AUXIN–LIKE SUBSTANCES IN RELATION TO FEMINIZATION OF CASTOR BEAN (*Ricinus communis* L.) INFLUENCED BY KINETIN AND MORPHACTIN[1] *

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Compendio

*La quinetina suprimió la tendencia a la masculinidad en el frijol de castor (*Ricinus communis. *L.) mientras que la morfactina no lo afectó. La quinetina indujo a un incremento en la femineidad y una disminución en la masculinidad, causando consecuentemente reducción en el número total de flores en comparación con el número de flores en las plantas testigo.*

La actividad de las sustancias endógenas parecidas a las auxinas se determinó en las terminales del tallo y las flores. El extracto de methanol fue dividido en fracciones ácida y neutral del etil acetato. Cada fase fue individualmente cromatografiada en papel cromatográfico. La actividad de la auxina fue medida con el bio ensayo de la clorigación del coleóptilo del trigo. La actividad de la auxina fue en su mayor parte vista en Rfs 0.3-0.4, la cual disminuyó conforme la planta avanzaba hacia el estado de reproducción. La actividad inhibitoria fue vista en Rfs 0.8-1.0.

Comparadas con el testigo, la quinetina y la morfactina aumentaron la actividad de las sustancias parecidas a la auxina en todos los períodos, más con el anterior que con el tratamiento posterior. Las flores femeninas mostraron una actividad más promotora y menos inhibitoria cuando fueron comparadas con flores masculinas. Ambos tratamientos aumentaron la actividad de substancias parecidas a la auxina en las flores femeninas más que en las masculinas. La quinetina y la morfactina causaron una general disminución en el contenido inhibidor de ambos sexos. El presente estudio, además, indicó que las sustancias parecidas a la auxina están asociadas con la floración de las plantas monoicas tales como el frijol de castor. Aún cuando las morfactinas son consideradas antagónicas a IAA, estimularon la actividad de substancias parecidas a las auxinas con el aumento hacia la tendencia femenina, simulando de este modo a la quinetina.

Introduction

The effect of various growth regulators on flower sex expression have been described for a number of plant species. Induction of feminization has been reported by treatments with cytokinins (8), benzyladenine in *Vitis vinifera* (24), zeatin and dihydrozeatin in *Vitis* sp. (14), 6-substituted adenine derivatives in grape (17) and 6-furfurylaminopurine in *Ricinus communis* (13). Morphactins significantly affect sex expression in some cucurbits such as *Cucumis sativus* (26) and *Cucumis melo* (3). Morphactin has been reported to promote the development of the ovary in some species (27, 23). Morphactin increase femaleness in *Carica papaya* (18) and male tendency in *Luffa acutangula* (6, 20).

Several reports have pointed out increase in female sex tendency with auxin treatment (21, 15, 11). Galun *et al.* (12) claimed that the local concentration and balance of auxin and gibberellin was important

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for sex differentiation in cucumber buds. Analysis of the auxin (11) and gibberellin (1) content of cucumber lines, that were related genetically but different in their sex expression, showed that auxin content was higher in hermaphrodite plants than in andromonoecious plants, and the levels of gibberellin were higher in monoecious than in gynoecious cucumber plants, indicating that these two hormones participate in the endogenous regulation of sex expression in cucumber. It was felt that the mechanism of sex expression might be independent of the endogenous level of auxins (29). The mode of action of growth regulators in flower sex expression has not been conclusively resolved, and hence, the present investigation has been designed to study the effect of kinetin and morphactin on sex expression in castor bean and changes in the activity of auxin-like substances during feminization.

Materials and methods

Seedlings of castor bean (*Ricinus communis* L. var. Aruna) were raised in plots. They flowered eleven weeks after sowing. Aqueous solutions of kinetin (6-furfurylaminopurine) and morphactin EMD 1 301 W (methyl ester of chlorofluerenol) were applied in two foliar sprays at a concentration of 20 ppm each, which was found to be more effective in increasing femaleness, till the point of run off. The sprays were made at 6 and 8 weeks after sowing. The shoot tips were collected after the second spray and at one week intervals thereafter. Second stage was the vegetative stage and third being the reproductive stage where the shoot tips possess minute female and male flower buds in the sheath. At the fourth stage female and male flowers formed were removed and their sex expression was determined. Shoot tips at different stages and flower buds at the last stage of control and treated plants were collected and were processed for the extraction of auxin-like substances by the following method.

Extraction: Auxins were extracted following the method given by Thurman and Street (30). Twenty-five grams of the freshly harvested material was weighed and rapidly cooled in a deep freeze (-10°C). The samples were then macerated in a mortar with 80% cold methanol at low temperatures (all extractions were carried out in a walk-cooler maintanied at 0°C). The sample was immediately returned to the deep freeze and was stored in the dark at this temperature for 24 h. The methanol extract was decanted into another cooled beaker and fresh methanol was added to the sample, and this was repeated thrice during 24 h storage in dark. The combined methanolic extract was filtered and the methanol removed under low temperature to give an aqueous residue of pH 5.5. The aqueous residue was then adjusted to pH 3.0 with H_2SO_4 and shaken four times with twice its volume of ethyl acetate. The total ethyl acetate fraction obtained was separated into neutral and acidic fraction, following the method of Villiers and Wareing (31). The total ethyl acetate fraction was shaken with aliquots of 5% sodium bicarbonate solution. The bicarbonate solution was washed thrice with ethyl acetate and the three ethyl acetate fractions were pooled to give neutral ethyl acetate fraction. The sodium bicarbonate solution was then acidified to pH 3.0 with 5% H_2SO_4, shaken thrice with aliquots of ethyl acetate and the three fractions combined to give the acidic ethyl acetate fraction.

The neutral and acidic fractions were concentrated to small volume in vacuo, and the concentrated extracts were applied as streaks on Whatman No. 1 chromatography paper and developed in the solvent isopropanol: 0.88 N and ammonium hydroxide: water (8:1:1). The developed chromatograms were dried and cut transversely into ten equal strips. Each strip representing one Rf, was eluted in 2 ml of buffered 2% sucrose solution contained in specimen tubes by the method of Maheswari and Bhalla (21).

Bioassay: The biological activity of the eluates collected in the sucrose solution was determined by employing the wheat coleoptile straight growth test of Bonner (5). Seeds of *Triticum aestivum* L. (Samba wheat), were soaked in double distilled water for 4 h and the seeds were sown in 6" Petriplates containing wetted filter papers and were grown in a dark room maintained at 22-23°C. After 66 h of germination coleoptiles of uniform size were selected and were decapitated under dim light discarding 3 mm portions from the tips. The next 4 mm segments were cut and were floated for 2h on 0.1% solution of manganese sulphate in distilled water and were then used for bioassay. The coleoptile segments were transferred to each of the one inch diameter Petriplates containing 2 ml of test solution prepared earlier with sucrose in buffer at pH 5.4. Two ml of untreated buffer sucrose with 10 coleoptile segments served as control. The Petriplates were covered with wetted filter papers in a tray and they were incubated in darkness for 18 h at 20-22°C. Then the length of the coleoptile segments was measured. Five grams, fresh weight, of the material was employed for bioassay. The histograms show growth promotion (+) inhibition (–) of wheat coleoptile sections expressed as per cent of the sucrose buffer 'control' section.

Results and discussion

The proportion of pistillate to staminate flowers has been used as an index of sex expression. It was observed that the ratio of pistillate to staminate flowers in control plants was 1:4.75 (Table 1). The increase in femaleness over control was 78% with kinetin and 40% with morphactin treatment. The reduction in male flowers was 40% with kinetin and 3% with morphactin treatment. Kinetin caused reduction in maleness and increase in femaleness to greater degrees and hence the ratio of pistillate to staminate was significantly altered, whereas morphactin caused slight reduction in maleness and increase in femaleness and hence the ratio of pistillate to staminate flowers was not significantly altered. The values for critical difference (C. D.) of both the flowers were calculated at 5%, with differences being significant. The production of female flowers was highly significant between control and treatments. In the case of male flowers there was a significant difference between control and kinetin treated plants, but the results were not significant between control and morphactin treated plants. Morphactin simulates kinetin in increasing the femaleness. Bisaria (4) observed that morphactin increased the production of pistillate as well as staminate flowers in *Luffa acutangula*. Sankhla (27) reported suppression of maleness, whereas Krishnamoorthy (20) observed increase in maleness with morphactin treatment. Thus, there appears to be a difference in response to morphactin in different plants. Except for the significant changes in sex expression, the other morphological changes observed were insignificant.

The relationship between sex expression and the hormonal environment in the shoot tips has not been explored, although there is some evidence to suggest that auxin content was higher in hermaphrodite plants than in andromonoecious plants (11) and the levels of gibberellins were higher in monoecious than in gynoecious cucumber plants (1). The plant extracts were partitioned into neutral and acidic fractions. The results showed that auxin-like activity changes during the development of the shoot tips to the reproductive condition. Several workers have observed differences in the pattern of growth activity between ether extracts of neutral and acidic fractions. In the shoot apices growth promoting activity was observed at Rfs 0.1-0.7 mostly at Rfs 0.3-0.4. The inhibition occured at Rfs 0.9-1.0. In the control and treated shoot tips, there was a decrease in activity at Rfs 0.3-0.4 gradually up to the last stage (Figure 1). At all stages the growth promotion at Rfs 0.3-0.4 and 0.5-0.6 was higher in treated shoot tips than in those of the control. The increase in the levels of auxin-like substances induced by kinetin in the shoot tips was higher than that caused by morphactin. A further explanation for the greater activity of auxin-like substances with the treatments might have led to a reduced IAA oxidase activity or been due to the synthesis of more auxins. The growth promotion was more with kinetin treatment than with morphactin. The acidic fraction also showed the same trend as that of the neutral fraction, although it displayed a relatively lower growth promoting activity than neutral fraction. The results indicate a decrease in the activity of auxin-like substances from vegetative to reproductive growth.

In the present study female flowers showed more activity at growth promoting zones than male ones, both in neutral and acidic fractions. Female flowers showed higher activity at Rfs 0.3-0.4 than male flowers (Figure 2). These results can be corroborated by the observations made by Galun *et al.* (11) that endogenous auxin is a regulator of sex expression through which the control of genes concerned is mediated. A very large difference in auxin content

Table 1. Effect of kinetin and morphactin on changes in sex expression. (Mean of ten replications).

	Control	Kinetin	Morphactin
Female flowers	32	57	45
Male flowers	152	92	148
Ratio of female: male	1:4.75	1:1.61	1:3.29

	Female flowers	Male flowers
F. calculated	23.11*	31.14*
C. D. at 5%level	7.53	17.56

* Significant at P = 0.05

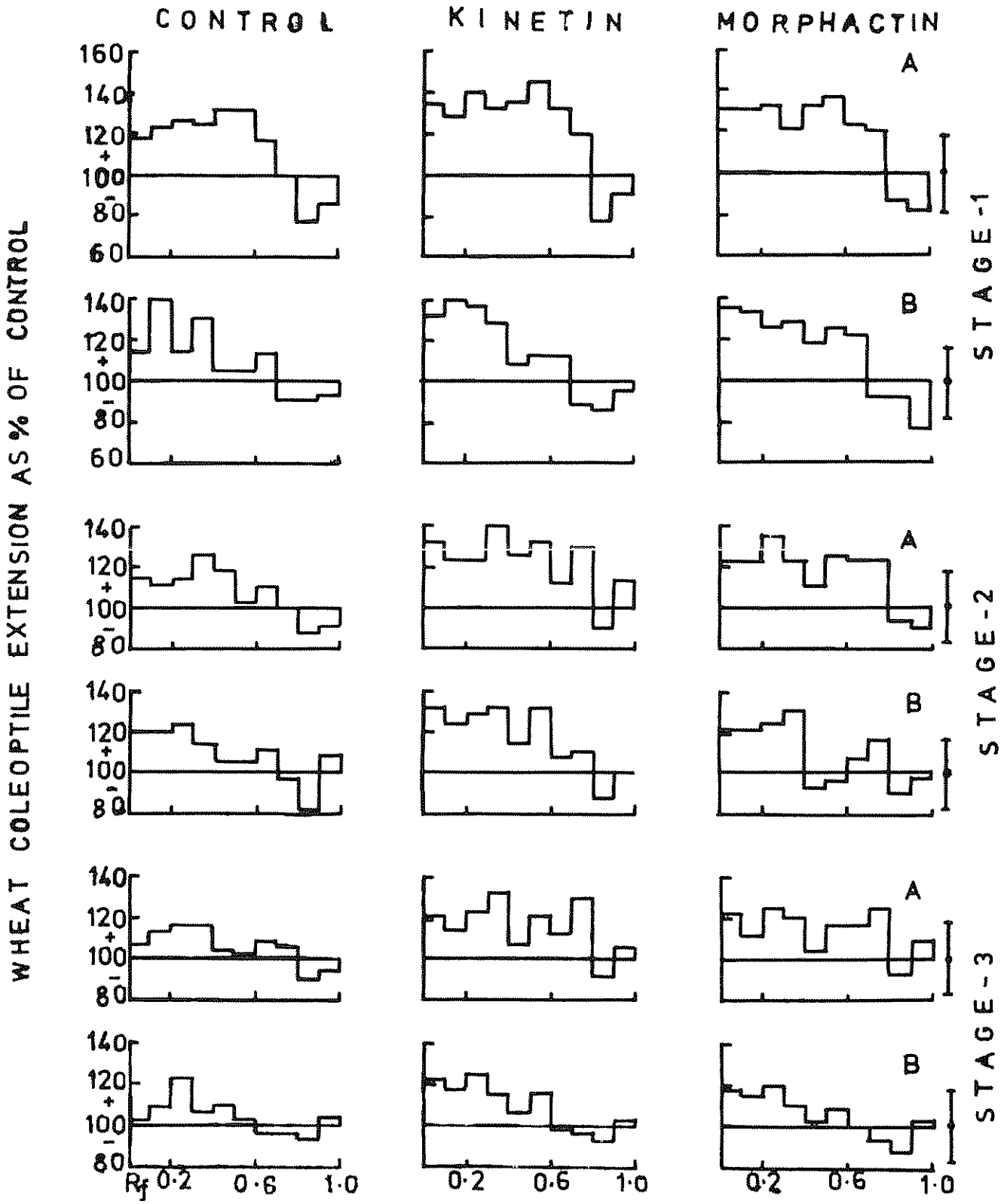

Fig. 1. Activity of treated and control shoot tips in wheat

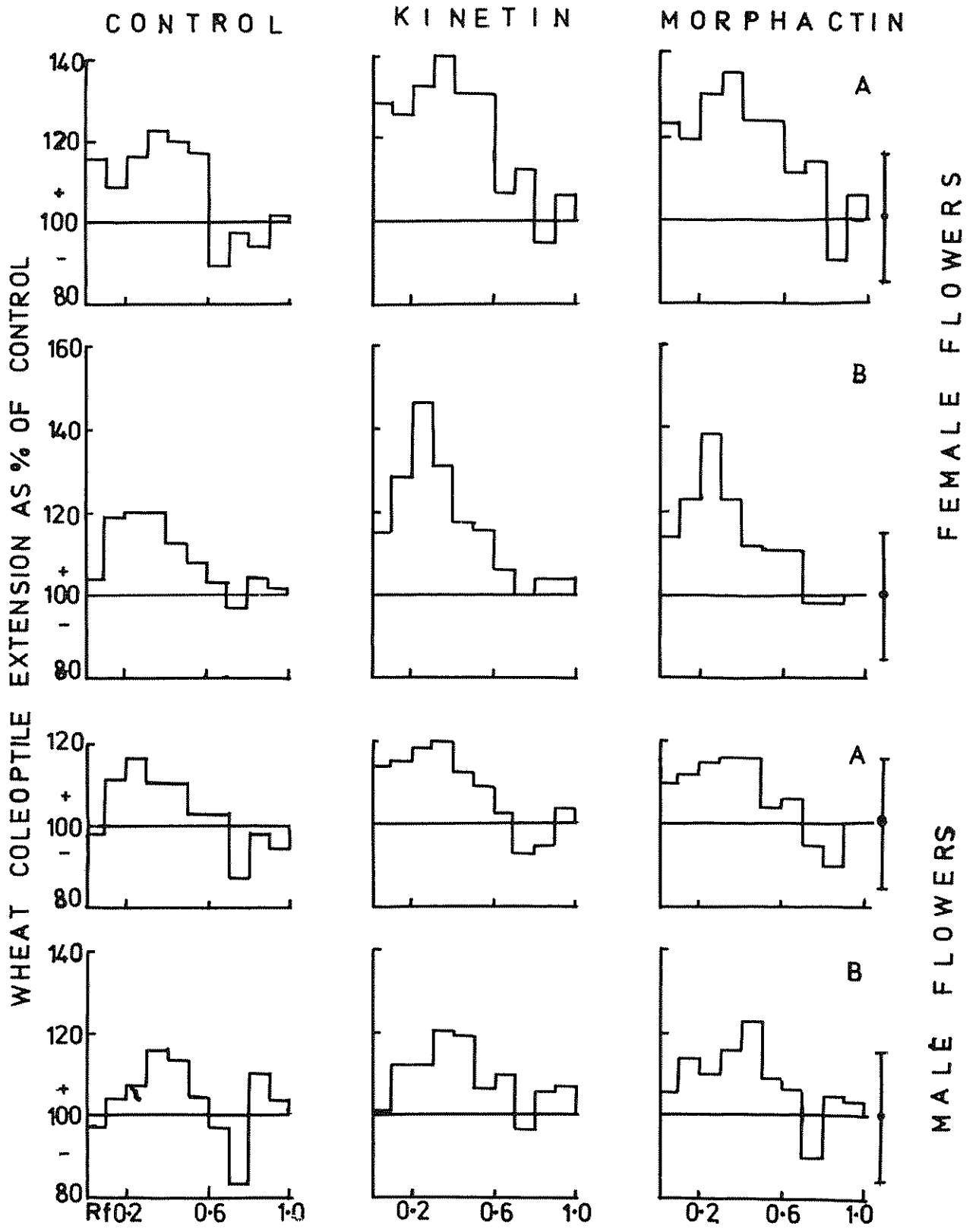

Fig 2. Comparison between female and male flowers activities

between male and female of *Cannabis* plants was observed by Conrad and Mothes (10). Female plants contained 30 times more auxin than male plants. An increase in the number of female flowers induced by exogenous application of auxin (21) indicates an association of auxin with femaleness. Kinetin and morphactin treatments showed a decrease in the inhibitor zones in both male and female flowers. Kinetin could increase the auxin-like substances content during vegetative phase, in particular. According to Chailakhyan (9), the role of kinins in vegetative growth is highly significant, though it is much less clear for processes of transition of plants to a flowering state. According to Schneider (28), morphactins inhibit the transport of IAA, although the possibility of a stimulation of enzymatic breakdown was also considered. Surprisingly enough, results of the present study indicate that morphactin stimulates auxin activity quite similar to kinetin.

In the present study, the growth inhibitory zones were observed at Rfs 0.9-1.0. This may be different in different plants. In the acidic ether extracts of etiolated broad bean and pea shoots and roots, potato shoots and tubers and immature maize kernels, Bennet-Clark and Kefford (2) and Kefford (19) observed an inhibitory zone at Rf 0.6, which extended up to Rf 1.0, thus exhibiting a broad zone of inhibitors. Also, in the present study, the growth promotor zones were observed at Rfs 0.1-0.7, mostly at 0.3-0.4. This is not uncommon and in most of the plants it ranges from 0.1-0.2. In the ether extract of tomato and cabbage the growth promotion zone was observed at Rf 0.1-0.2 (7, 16). Thurman and Street (30) observed promotory activity at Rf 0.2 in tomato root when chromatographed with ammoniacal isopropanol. According to Phillips (25) a rough estimate of the total auxin in the different tissues can be obtained by adding the curvatures from the eluates at the appropriate Rfs and by converting the total curvature into IAA equivalents.

The present results show that these growth regulators may act in the control of auxin-related phenomenon: increase in auxin biosynthesis or inhibit IAA-oxidase activity. The similarity between the effects of kinetin and morphactin on sex expression of castor bean suggests that these two growth regulators increase the femaleness, and that the degree of increase corresponds to the degree of auxin-like substances. A striking point to be noted is that although morphactin is antagonistic to IAA, the present study indicates that it stimulates auxin activity.

Summary

Kinetin suppressed male tendency in castor bean (*Ricinus communis* L.) while morphactin did not affect it. Kinetin elicited an increase in femaleness and decrease in maleness, consequently causing reduction in the total number of flowers, compared to the number of flowers in the control plants. The activity of the endogenous auxin-like substances was determined in shoot tips and flowers. Methanol extracts were partitioned as acidic and neutral ethyl acetate fractions. Each phase was individually chromatographed on Whatman No. 1 chromatography paper. The auxin activity was measured with the wheat coleoptile elongation bioassay. Auxin activity was mostly seen at Rfs 0.3-0.4, which decreased as the plant proceeds towards the reproductive stage. Inhibitory activity was seen at Rfs 0.8-1.0. Compared to control, kinetin and morphactin increased the auxin-like activity at all stages, more with the former than with the latter treatment. Female flowers showed more promotory and less inhibitory activity when compared to male flowers. Both the treatments increased auxin-like activity in female flowers than in male ones. Kinetin and morphactin caused a general decrease in the inhibitor content of both sexes. The present study also indicated that auxin-like substances are associated with flowering in monoecious plants such as castor bean. Although morphactins are considered to be antagonistic to IAA, they stimulated auxin-like activity in relation to increased female tendency, thus simulating kinetin.

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