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HORMONE CONTENT AND HORMONE METABOLISM STUDIES IN MALE-STERILE SUNFLOWER

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Abstract

100% male-sterility may be induced in sunflower by 0.03 ppm GA_3 treatment at an appropriate time. By means of analysis of the hormone contents (IAA, gibberellin) of the treated inflorescences, it may be established that the maximum extent of male-sterility is not directly connected with a high endogenous concentration of gibberellin. In the most effective treatments (III, IV), the metabolization of IAA is very fast, but it is probable that that of GA_3 is also faster than in samples I and II, treated in a less developed state, in which the male-sterility percentage is low. This is supported by the high IAA oxidase activity in the male-sterile flowers compared to the control. It may be concluded from the results that the high GA_3/IAA ratio developing shortly after the treatment forms the hormonal base of the male-sterility. This is also suggested by the experience that the development of gibberellin-induced male-sterility can be partially inhibited by post-treatment with auxins.

Introduction

The influencing of the sex of flowers by exogenous hormones is an interesting procedure from both theoretical and practical aspects. One of the fundamental problems of hybrid breeding, for example, is the production of a male-sterile flower or inflorescence, a suitable method for wich may be treatment with certain hormones. Hormone treatment for such purposes was first applied to sunflower by SCHUSTER (1961, 1963, 1969).

In an earlier paper (FRANK et al., 1977) we reported experiments in which attempts were made to induce male-sterility in sunflower inflorescence by auxin and gibberellin treatment. In these experiments we also studied the changes in the gibberellin and auxin contents following treatment (KÖVES et al., 1978).

The subject of our present work was the study of the connection between the intensity of the hormone metabolism and the gibberellin-induced male-sterility.

Materials and Methods

In the outdoor experiments the shoot apices of 33, 35, 37 and 39-day-old plants of the species Helianthus annuus WNIIMK 6540 were treated with 0.033%, 0.016% and 0.0033% aqueous solutions of GA_3 (a product of Phylaxia). The method used for the treatment was uptake of the solutions via pipette. The sample included the tip bud and the uppermost leaf circle surrounding this (Fig. 1).

After every treatment, a sample was taken on the third day. Samples taken at four consecutive times were designated samples I–IV. Sampling was performed on the following days: 13 June 1975, 15 June 1975, 17 June 1975 and 19 June 1975 I–IV, respectively.

After paper-chromatographic separation, the amount of IAA (indole-3-acetic-acid) was determined by the method of HANCOCK and BARLOW (1952) and BENTLEY and HOUSLEY (1954) on the basis of the growth reaction of Avena coleoptile segments.

Gibberellin was determined on a methanolic extract by means of the barley endosperm test (JONES and WARNER, 1967) after ethyl acetate fractionation and layer-chromatographic separation (REINHARD et. al., 1964).

IAA oxidase activity was determined by colorimetric method of GALSTON and DALBERG (1954). Other conditions and procedures connected with the outdoor experiments were described previously (FRANK et al., 1977).

Fig. 1. Sunflower shoot apex used for sampling.

Results and discussion

A detailed account was given earlier (FRANK et al., 1977) of experimental results indicating that total male-sterility can be induced in sunflower by 0,033% GA_3 treatment (Fig. 4). Analysis of the auxin and gibberellin contents of the treated and untreated shoot apices led to the following results.

The endogenous IAA content of the control shoot apices varies with the time of examination according to an optimum curve, the maximum of which appears at the second sampling.

The endogenous gibberellin content displays a uniformly decreasing tendency in the examination period (Fig. 2).

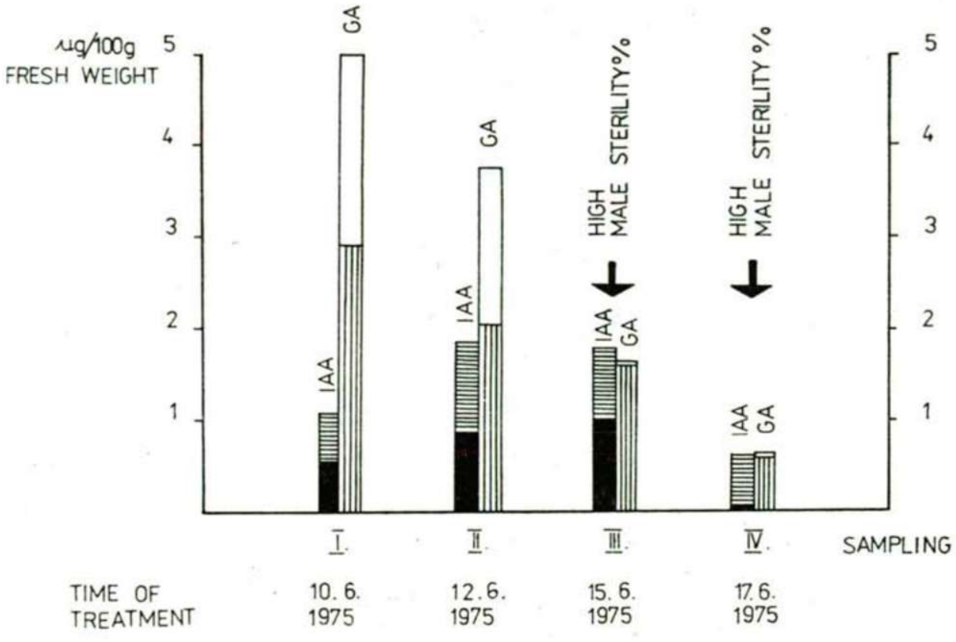

Fig. 2. IAA and gibberellin contents of sunflower shoot apex.
■ IAA content of plant treated with GA_3 (0.033%),
▤ IAA content of untreated plant,
□ Gibberellin content of plant treated with GA_3 (0,033%),
▥ Gibberellin content of untreated plant.

The extent of the change in the hormone level resulting from the treatment is decisively influenced by the time of treatment and, in connection with this, by the developmental state of the shoot apex (Fig. 2). The effectivenes of treatment increases progressively during the examination period, and is the greatest at the time of the third treatment and sampling, i.e. in the 37-day-old plants. The extent of the increase in the gibberellin content of the gibberellin-treated plants is the highest at the beginning of the examination period; it gradually decreases, and the highest male-sterility percentage is to be found in the inflorescence formed on the shoot apex with a similar gibberellin content to that of the control. The gibberellin content is also a function of the GA concentration used for treatment (Table 1).

The IAA content generally decreases compared to the control in objects treated with gibberellin. These hormone level variations resulting from the treatment vary the gibberellin/IAA ratio too (Fig. 2 and Table 1).

The most striking feature in the correlation of the male-sterility and the hormone level measured 3 days after treatment is that the extent of male-sterility is not proportional to the endogeneous gibberellin content. The highest gibberellin level observed in the first treatment caused male-sterility to only a slight extent. The high gibberellin level here is explained in that the metabolic activity is moderated in this developmental state, and the gibberellin administered during treatment is metabolized

Table 1. Correlations of the GA_3 concentration used for treatment and the endogenous gibberellin and IAA contents with the extent of male-sterility in sunflower shoot apex.
The Table shows the results obtained in the analysis of samples III. The data are the averages of the results of 3 parallel examinations

Treatment	Treated shoot apex				Total male-sterility*, %
	gibberellin content		IAA content		
	μg/100 g fresh. wt.	% of control	μg/100 g fresh wt.	% of control	
0,03% GA_3	1,6	100	1,06	59	100
0,016% GA_3	6,2	400	1,75	98	75
0,0033% GA_3	8,8	500	1,42	78	12

* The percentage value of the male-sterility refers to the treated plants.

slowly. Similarly, the slow metabolism is also the reason why the endogenous gibberellin level is relatively high at a lower exogenous gibberellin concentration. The intensity of the metabolism is enhanced in the course of the development of the shoot apex, and at the same time the measurable gibberellin content decreases. This is reflected in the phenomenon that the gibberellin content measured on the third day after treatment decreases in the samples taken consecutively every 3 days.

This is also the reason why a direct correlation is not found between the measurable quantity of endogenous gibberellin and the extent of male-sterility, but between the intensity of the gibberellin metabolism and the extent of male-sterility. What has been said refers to the IAA content too, and the IAA metabolism may be of similar importance in the development of the hormonal base of the male-sterility; The extent of this metabolism is probably enhanced by the gibberellin treatment at the concentration employed.

All of the signs indicate that the active IAA metabolism is one of the important factors inducing the male-sterility, presumable by decreasing the IAA level.

When the activity of IAA oxidase was measured in male-sterile sunflower inflorescence, it proved to be substantially higher than the IAA oxidase activity of normal inflorescence. This supports our assumption in connection with the important role of the IAA metabolism, all the more so since the IAA oxidase activity was higher than normal not only in the gibberellin-induced male-sterile inflorescence, but also in the Plasmopara halstedii-induced and the cytoplasmic male-sterile inflorescence (Table 2).

Table 2. IAA oxidase activities of cell-free extracts of male-sterile and normal sunflower tubular flowers

Sample	Decomposed IAA μg/g fresh weight/ hour
1. Control	43,0
2. Male-sterile induced with GA_3	333,3
3. Male-sterile infected with Plasmopara	250,0
4. Cytoplasmic male-sterile	200,0

The available data are not yet sufficient for a coherent picture to be obtained of the hormone metabolism changes caused by gibberellin treatment. The necessity of the relatively low IAA level, however, is supported not only by the development of the high IAA oxidase activity, but also by the fact that male-sterility could not be induced by IAA treatment in our experiments (FRANK et al., 1977), and the fact that the gibberellin-induced male-sterility can be partially reversed by post-treatment with auxin. On the action of 10^{-4} M IAA sprayed prior to flowering, some rows of normally developed hermaphrodite flowers appeared on the heads previously treated with gibberellin (Fig. 3).

The examination indicate, therefore, that a lastingly high gibberellin level is not necessary for male-sterility to be induced, since on third day after the most effective treatment the gibberellin content of the shoot apex no longer increases essentially above the control; flowering occurs only 2–3 weeks later.

The analysed tendency of the changes in the hormone content rather suggest that the gibberellin/auxin ratio developing within 3 days after the treatment may be responsible for the male-sterility.

As the data relating to this field are rare in the literature, and the sex-determining effects of the hormones on the individual species are different, it is hardly possible to compare the experimental and literature data. It may be mentioned that, in studies of flower-buds of various sexes of cucumber, MACIEJEWSKA—POTAPCZYKOWA et al. (1972) and RETIG and RUDICH (1972) found the IAA oxidase activity to be higher in the female ones. Similar investigations relating to hermaphrodite flowers are deficient, however.

Gibberellin is known to inhibit the IAA oxidase activity and thus lead to a high IAA level in certain objects (GALSTON, 1959; HOUSLEY et al., 1961; PILET and WURGLER, 1958; VARGA and BÁLINT, 1965).

Fig. 3. Sunflower inflorescences.
Control = untreated, normal; GA_3 = treated with gibberellinic acid, male-sterile; GA_3+IAA = treated with gibberellinic acid, post-treated with IAA — some rows of normally developed hermaphrodite flowers appeared.

VALDOVINOS et al. (1967) studied the effect of gibberellin on the decarboxylation of IAA in sunflower seedlings, but found no effect. In seedlings of other plants, however, e.g. wheat and barley, gibberellin treatment stimulated the activity of the enzyme (BOLDUC et al., 1970; GASPAR et al., 1967).

The above experimental results, therefore, indicate that the increase in the IAA oxidase activity as a result of gibberellin treatment may be an alternative reason for the devrease in the IAA content; however, it is also possible that the low IAA evel dvelops indirectly.

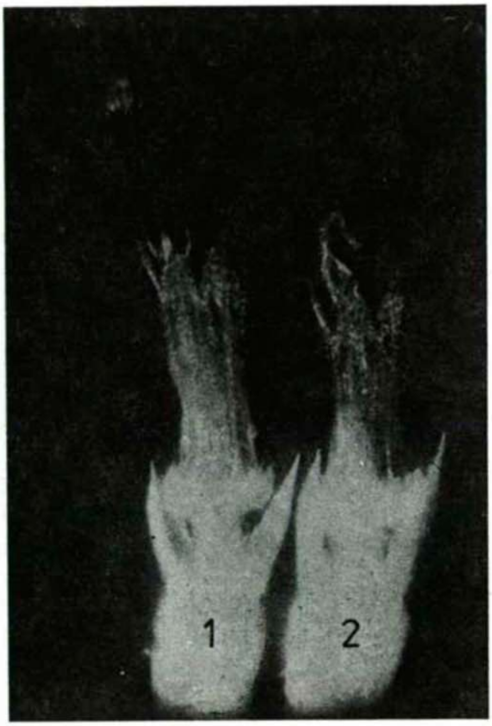

Fig. 4. Sunflower flower 1. normal = untreated, 2. male-sterile = treated with GA_3.

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