

## Auxins and gibberellins in embryonic shoots of Scots pine in relation to flower sex differentiation \*

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### Abstract

Flower sex differentiation in Scots pine is correlated with endogenous balance of auxins to gibberellins. Male flower primordia initiation is connected with high amounts of gibberellins whereas the initiation of female ones is associated with a high level of auxins and a low content of gibberellins.

### INTRODUCTION

There is only scarce information concerning the role of plant hormones in the flower sex differentiation in conifers (Pharis, 1975; Pharis et al., 1975). The results of our previous investigations (Kopcewicz et al., 1977) suggest that there is a lack of direct correlation between the content of cytokinins and abscisic acid-like inhibitors and flower sex expression. At the same time the results point out to the fact that endogenous balance of auxins to gibberellins is involved in the regulation of Scots pine sex differentiation.

The purpose of present experiments was to check the above mentioned suggestions by means of more detailed investigations of the changes in the level of auxins and gibberellins in embryonic shoots of Scots pine before and during the periods of male and female cone primordia initiation.

### MATERIAL AND METHODS

The investigations were carried out on the material from the same group of about sixty year old Scots pine (*Pinus silvestris* L.) trees as it had been done in the previous studies (Kopcewicz et al., 1977; Kop-

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cewicz et al., 1978). The embryonic shoots were taken from both: the branches producing about 90-95% of male cones (called male embryonic shoots) and from the branches producing 65-80% of female ones (called female embryonic shoots).

Samples were taken at the following stages:

- I. initiation of the earliest scale leaf primordia (May 16, 1977)
- II. initiation of the earliest axillary meristems which can be later changed into male inflorescences (June 1, 1977)
- III. initiation of further axillary meristems differentiating later into male inflorescences (June 10, 1977)
- IV. initiation of the upper axillary meristems which can be later differentiating into female cones (June 20, 1977)
- V. enlargement of both embryonic shoots and axillary meristems (June 27, 1977).

In potentially female embryonic shoots both the auxin and gibberellin contents, at all investigated stages, were determined. It was similar, as gibberellin contents were concerned, in male embryonic shoots. On the other hand the amount of auxins in male embryonic shoots was only examined during the time of female cones primordia differentiation (stage IV and V).

Ten both potentially male and female embryonic shoots were used for the determination of the development stages. These samples were fixed and stained as before (Kopcewicz et al., 1977).

Auxins and gibberellins were isolated from 500 embryonic shoots (average weight about 4 g) according to the methods previously described (Kopcewicz et al., 1977).

## RESULTS AND DISCUSSION

The previous investigations (Kopcewicz et al., 1977; Kopcewicz et al., 1978) showed that the remarkable difference in the level of auxins and gibberellins between potentially male and female embryonic shoots exists even for a long time (about four weeks) before the new embryonic shoots initiation (Kopcewicz et al., 1978). At the time just before the initiation of the axillary meristems of male embryonic shoots a high level of gibberellins and a low content of auxins are observed (Kopcewicz et al., 1977). Female embryonic shoots contain at the same time a high level of auxins and small amounts of gibberellins. Both male and female embryonic shoots have similar contents of cytokinins and abscisic acid-like inhibitors. At the period just before the initiation of the axillary buds differentiating later into female inflorescences a high level of auxins and a lack of gibberellins were found in potentially female

embryonic shoots (Kopcewicz et al., 1977). At the same time male embryonic shoots contain low amounts of auxins and gibberellins. Both male and female embryonic shoots have similar contents of cytokinins and inhibitors. So, previous results suggest that flower sex differentiation in Scots pine is connected with an endogenous equilibrium of auxins to gibberellins. At the same time cytokinins and abscisic acid-like inhibitors do not directly participate in the process of pine sex expression.

In the present work it was decided to study in a more detailed way the metabolism of auxins and gibberellins in embryonic shoots in order to learn whether the previously established dependences are of general value for the Scots pine flower sex differentiation. So the investigations of endogenous auxins and gibberellins were carried out before and during the periods of male and female cones primordia initiation.

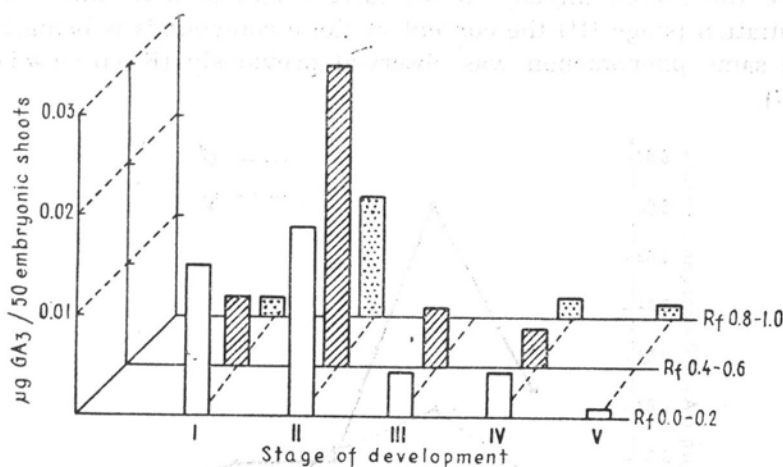


Fig. 1. Changes in the amount of gibberellins in male embryonic pine shoots

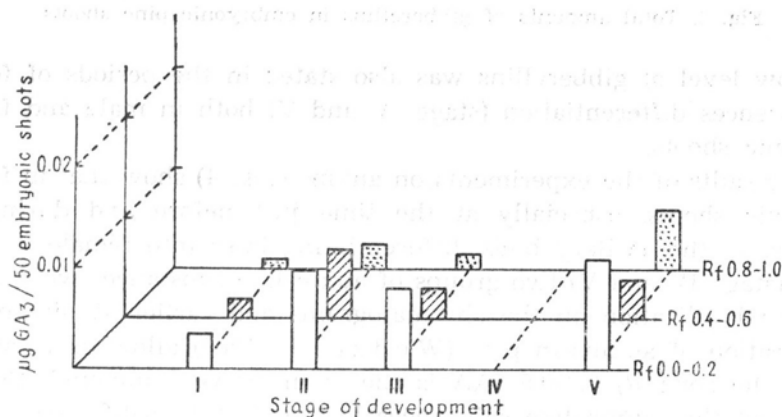


Fig. 2. Changes in the amount of gibberellins in female embryonic pine shoots

The results of the experiments on gibberellins show (Fig. 1 and 2) that in both male and female embryonic shoots three groups of these compounds occur. The biggest amounts of all three groups of gibberellins were found in male embryonic shoots at the stage just before the initiation of axillary buds differentiating later into male inflorescences (Fig. 1). A closer physico-chemical and physiological description of properties of the gibberellins occurring in pine shoots was presented previously (Kopcewicz, 1968). The comparison of the total amounts of gibberellins in male and female embryonic shoots (Fig. 3) shows that in the periods of the initiation of the earliest axillary primordia, that means before the initiation of male inflorescences (stage I and II) significantly more gibberellins occur in male embryonic shoots than in female ones. A high level of gibberellins is maintained, however, only during a short period of time, since already in the later stages of male inflorescences differentiation (stage III) the content of these compounds is being lowered. The same phenomenon was observed previously (Kopcewicz et al., 1977).

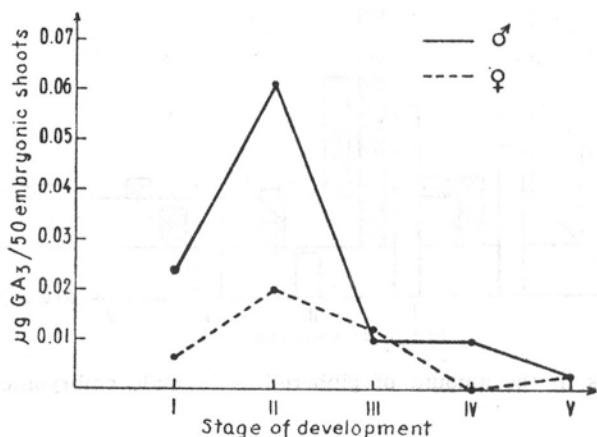


Fig. 3. Total amounts of gibberellins in embryonic pine shoots

A low level of gibberellins was also stated in the periods of female inflorescences differentiation (stage IV and V) both in male and female embryonic shoots.

The results of the experiments on auxins (Fig. 4) show that in female embryonic shoots, especially at the time just before and during the initiation of the axillary buds differentiating later into female inflorescences (stage IV and V) two groups of auxin-like substances were found. Both the localization on the chromatograms and earlier studies on the identification of auxins in pine (Wodzicki, 1968) allow us to assume that in the zone  $R_f$  0.3-0.5 IAA is the main active compound. Because of the fact that auxin-like substances localized at  $R_f$  0.0-0.2 occurred in the greater amounts only in the periods of female inflorescences dif-

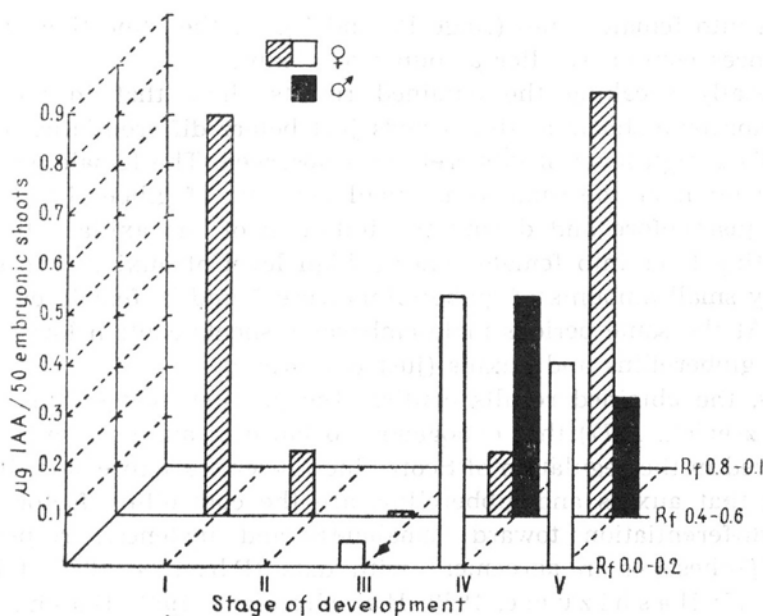


Fig. 4. Changes in the amount of auxins in embryonic pine shoots

ferentiation, it seems possible that these are the substances that are connected with the femaleness of initiated flowers. In male embryonic shoots at this time only auxins localized on the chromatograms at  $R_f$  0.3-0.5 are present (Fig. 4). Thus, it seems that in further investigations it would be very significant to make the more exact identification of auxin-like substances localized at  $R_f$  0.0-0.2. It would be also very useful to get additional data whether a rise in the content of this group of auxins is really related to female differentiation. The total amount of auxins in female embryonic shoots at different stages of development (Fig. 5) shows that the highest content of these substances occurs just before and during the initiation of the axillary buds which can be later

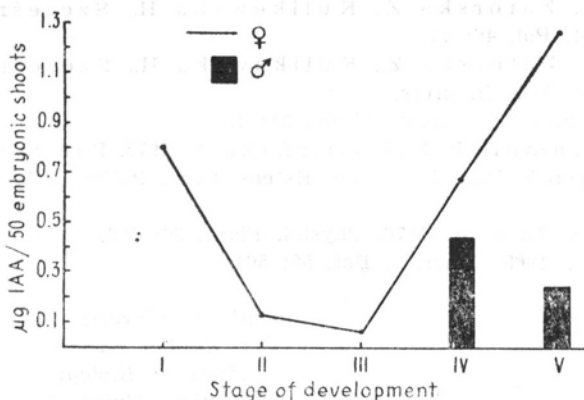


Fig. 5. Total amounts of auxins in embryonic pine shoots

changed into female cones (stage IV and V). At the same time male inflorescences contain smaller amounts of auxins.

Generally speaking the obtained results show that in potentially male embryonic shoots at the periods just before differentiation of male primordia a high level of gibberellins is observed. The female embryonic shoots contain at the same time small amounts of gibberellins. At the periods just before and during the initiation of the axillary buds differentiating later into female cones a high level of auxins (two groups) and very small amounts of gibberellins were found in female embryonic shoots. At the same periods male embryonic shoots contain low amounts of both gibberellins and auxins (just one group).

Thus, the obtained results confirm the previous suggestions (Kopcewicz et al., 1977) that endogenous balance of auxins to gibberellins is involved in the regulation of Scots pine flower sex expression. It seems possible that auxins and gibberellins are the controlling factors which direct differentiation toward femaleness and maleness, respectively. This hypothesis is in agreement with data (Pharis, 1975; Pharis et al., 1975; Hashizume, 1959; Hashizume, 1961; Hashizume, 1966; Ross and Pharis, 1976) concerning the influence of exogenously applied auxins and gibberellins on sex flower expression in conifers.

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*Dyferencjacja płci kwiatów a zawartość auksyn i giberelin w zawiązkach  
pędów sosny zwyczajnej*

Streszczenie

Dyferencjacja płci kwiatów u sosny zwyczajnej jest skorelowana z endogen-  
nym balansem auksyn do giberelin. Inicjacja męskich zawiązków kwiatowych jest  
związana z podwyższoną zawartością giberelin, podczas gdy inicjacja żeńskich za-  
wiązków kwiatostanów jest skorelowana z wysoką zawartością auksyn oraz niskim  
poziomem giberelin w tkankach sosny.