Dynamics of endogenous auxins, cytokinins and abscisic acid-like inhibitor in embryonic shoots of young Scots pine (*Pinus silvestris* L.) at the time of flower primordia initiation*

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ABSTRACT

Auxins, cytokinins and ABA-like inhibitor were determined in the embryonic shoots of young pines.

It was stated that the transition from vegetative to reproductive stage of pine development is connected with the decrease of the IAA level. In the group of vegetative plants an unknown auxin-like substance was detected. This substance appeared in the period of generative development initiation.

It was also stated that differentiation of female inflorescences was connected with the increase of the level of ABA-like inhibitor. However the differentiation of male ones was connected with decrease of the amount of this inhibitor.

No correlation between sex determination and the level of auxins and cytokinins was observed.

INTRODUCTION

In our preliminary experiments (Galoch and Michniewicz, 1978) we found that transition from vegetative to the reproductive stage of pine development coincides with the increase of the level of gibberellins (GAs) in the embryonic shoots. It was also stated that differentiation of male inflorescences is connected with high level of these hormones. However the differentiation of female ones takes place in conditions of low level of GAs. In the present investigations the role

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of auxins, cytokinins and abscisic acid (ABA)-like inhibitor during the transition from vegetative to the reproductive stage of pine development as well as the role of these hormones in sex expression in such pines were studied.

MATERIAL AND METHODS

The subjects of our experiments were specially selected 15 years old pine trees from naturally occurring stands (Konstanciewo Wood near Golub). In 1977 the material for extraction of plant hormones was gathered: 1) from the trees which produced both, male and female inflorescences and 2) from the trees in vegetative stage of development (before blossoming). After one year, in the spring 1978 it was stated that 32% among the trees being in 1977 at vegetative stage, developed several male inflorescences, initiating in this way generative growth.

Auxins, cytokinins and ABA-like inhibitor were determined in embryonic shoots being in the following growth stages (Fig. 1):

I — stage of initiation of the earliest scale leaf primordia observed in about 40% of the buds (May 20, 1977).

II — stage of initiation of the earliest scale leaf primordia observed in about all the buds (June 2, 1977).

III — stage of initiation of the earliest axillary meristems which could be differentiated next into the male inflorescences (June 10, 1977).

IV — stage just before the initiation of the axillary meristems which could be differentiated next into female inflorescences (June 22, 1977).

V — stage of ending of the initiation of the axillary meristems which could be differentiated into female inflorescences. The axillary meristems start to enlarge (June 27, 1977).

VI — stage of enlargement of the axillary buds: the beginning of initiation of the first scale leaf primordia on the axillary buds (July 6, 1977).

Twenty embryonic shoots from each group of pines (i.e. vegetative and flowering) were used for determination of stages of their development. Plant hormones were extracted from 600 embryonic shoots.

Auxins and ABA-like inhibitor were partitioned chromatographically using the column chromatography with Sephadex LH-20 according to methods presented previously (Michniewicz and Galoch, 1974). Successive fractions (10 ml each) were bioassayed using *Avena* section straight growth test for auxins and by wheat coleoptile test for ABA.

The substances stimulating growth of *Avena* coleoptiles with higher elution volume than IAA (270—280 ml) were rechromatographed using paper and TLC chromatography in the following solvent systems: ethanol, water (7:1) and water. The fractions inhibiting growth of wheat coleoptiles at the elution volume of ABA were connected,

evaporated and partitioned using paper chromatography (Whatman 3) in two systems: isopropanol, ammonia, water (10:1:1) and isopropanol, ammonia, water (90:1:9). Synthetic ABA was used as a standard.

Cytokinins were isolated after Hewett and Wareing (1973). The aqueous extract (pH 2.7) was absorbed on an ion exchange column (Dowex 50 WX 8H⁺ 50—100 mesh). The active substances were washed out with 2 and 5 N NH₄OH. The ammonia fraction was concentrated and partitioned on Whatman 1 chromatographic paper using solvent system: isopropanol, ammonia and H₂O (10:1:1). The biological activity of cytokinins was determined by the soybean tissue test (Miller, 1968).

RESULTS AND DISCUSSION

The experiments presented here concern two aspects of generative differentiation in pines. One of them deals with the role of hormones in the latest stages of juvenility and with the process of transition of plants to flowering (experiments with vegetative group of pines among them 32% developed a few number of male inflorescences) and second aspect concerns the sex expression (experiments with flowering plants).

Fig. 1. Successive developmental stages of embryonic shoots of pine

As may be seen from the data presented in Fig. 2 in the group of vegetative pines the level of IAA (elution volume: 200—220 ml) was in the earliest stages of embryonic shoots development markedly higher than in flowering plants.

However, in further stages, the level of IAA in vegetative pines decreased so very much, that in the latest stages no auxins in embryonic shoots were detected. It must be underlined that the part of pines from the vegetative group of trees developed male inflorescences just in the period when the amount of auxins in embryonic shoots decreased (stage III).

Fig. 2. The amount of auxins in embryonic — shoots of vegetative* and flowering pines

*In this group of pines 32% of plants developed for the first time a few number of male flowers

Thus, these data allow to conclude that the transition from vegetative to the reproductive stage of pine development coincides with the decrease in the amount of IAA in plant tissues.

This conclusion is in accordance with our earlier results showing that pine trees in the stage of blossoming contain less auxins than not flowering trees (Galoch et al., 1978). It is also in accordance with the data of Łoboda et al. (1974) who suggested that the bud differentiation in apple trees is connected with the decrease of IAA and with the increase of the activity of IAA oxidase.

In embryonic shoots of vegetative pines (stages IV—VI) except IAA another substance with auxin activity was stated. This unidentified substance (elution volume 270—280 ml) was more active in *Avena* coleoptile test than IAA. It is possible that the appearance of this substance is connected with the ending of juvenility and with the transition to the generative stage in 32% of studied trees.

The growth stimulator (elution volume 270—280 ml) was also tested using thin layer and paper chromatography. This substance was localized at R_f corresponding to IAN however it did not react neither Salkowski's nor with Prohazka's reagents. Unfortunately, we obtained only small amount of this substance, insufficient for its identification. Further experiments are necessary for explanation of this question.

Flowering pines contained only one kind of auxin i.e. IAA and only in low amount. We did not observe any significant changes in the level of this hormone during the embryonic shoots development. The amount of IAA in studied organs was similar in the stage of differentiation of male (stage III) as well as in female (stage IV) inflorescences.

The reason for the lack of differences could be the very poor flowering of young pines. On the other hand it may be also concluded that sex determination in young pines does not depend on the level of auxins in embryonic shoots.

This last statement is in discordance with the general opinion based on the results of experiments with herbaceous plants assuming that auxins play an essential role as a factor increasing feminization of plants (Heslop-Harrison, 1972).

The results concerning this question obtained in experiments with conifers are divergent. According to Giertych and Forward (1966), Hashizume (1969) and Kopcewicz et al. (1977) the development of male inflorescences is connected with the low level of auxins and the development of female inflorescences coincides with high level of these hormones. However Bocharova et al. (1975) in experiments with *Populus balsamifera* and *Pinus silvestris* found more auxins in male plants or branches producing male inflorescences in comparison to female ones.

It was also stated that the treatment of *Pinus densiflora* with NAA increased the number of female inflorescences (Saito, 1957) but in *Picea sitchensis* treatment with these substance reduced the ratio of female to male inflorescences (Tompsett, 1977).

Interesting are also the results of experiments with *Pseudotsuga mensiensii* which show that the treatment of plants with NAA at high concentration increased the production of staminate strobili stimulated by GAs but NAA used in low concentration increased the development of ovulate strobili in some combinations with GAs (Pharis, 1975; Pharis et al., 1976).

Taking these facts into consideration it must be concluded that the role of auxins in sex expression in conifers is not yet elucidated.

The results of experiments presented in Fig. 3 show that high activity of apical meristems almost in all buds (stage II) coincided with the increase of the level of cytokinins. In subsequent stages (II—VI) a decrease of these hormones was noted. No significant differences in the level of cytokinins between flowering and vegetative pines was stated. Thus, it must be concluded that cytokinins are not involved in the processes of generative differentiation in pines.

Fig. 3. The level of cytokinins in embryonic shoots of vegetative* and flowering pines

*Explanation — see Fig. 2

The lack of correlation between the level of cytokinins and flower sex expression in pines was also stated by Kopcewicz et al. (1977).

Contrary to conifers, in Angiosperms cytokinins promote the female tendency of plants. This was stated by Negi and Olmo (1966) and by Hashizume and Iizuka (1971) in experiments with *Vitis*, by Bose and Nitsch (1970) in experiments with *Luffa acutangula* and in experiments with hemp by Galoch (1978).

The results of analyses of ABA-like inhibitor in embryonic shoots of pines in different stages of their development are presented in Fig. 4.

Fig. 4. The amount of abscisic acid in embryonic shoots of vegetative* and flowering pines

*Explanation — see Fig. 2

It is clear that the stage of development when about 60% of buds were dormant (stage I) was characterized by very high level of this inhibitor. Breake of bud dormancy (stage II) coincided with the decrease of ABA both in flowering and in vegetative pines. Thus, these results are in accordance with the general opinion about the role of ABA in buds dormancy.

In flowering plants being in the stage of development when the initiation of male inflorescences took place (stage III) further decrease of ABA-like inhibitor was found. However, in the stage when the initiation of female flowers took place (stage IV) the amount of the inhibitor increased. Thus, these results suggest that ABA is involved in the control of sex determination in pines.

The data concerning the role of ABA in sex determination in plants are very scarce and deal mainly with the herbaceous species.

The feminizing influence of this inhibitor was stated in experiments with cucumber (Rudich et al., 1972; Rudich and Halevy, 1974), squash (Abdel-Gawad and Ketellapper, 1969) and hemp (Galoch, 1978; Mohan Ram and Jaiswal, 1970).

It must be underlined that in the group of vegetative pines significant increase of ABA-like inhibitor was stated in embryonic shoots being in the stage III i.e., when 32% of plants initiated inflorescences. In further stages of development (IV and V) the progressive decrease of this inhibitor was observed.

These results show that the transition of pines from the juvenile stage to generative one may be connected with the increase of the ABA level. This increase of growth inhibitor preceded the appearance of unidentified auxin-like stimulator (compare Fig. 4 and Fig. 2).

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Zmiany poziomu endogennych auksyn, cytokinin i ABA-podobnego inhibitora w pędach generatywnych młodych sosen (Pinus silvestris L.) w czasie zawiązywania się pąków kwiatowych

Streszczenie

Badano zawartość auksyn, cytokinin i inhibitora o właściwościach ABA w pędach embrionalnych młodych drzew sosny.

Stwierdzono, że przejście roślin ze stanu wegetatywnego do generatywnego związane jest z obniżeniem poziomu IAA.

W grupie roślin wegetatywnych wykryto obecność nieznanej substancji auksyno-podobnej. Pojawienie się tej substancji było zbieżne z okresem inicjacji generatywnego rozwoju.

Stwierdzono również, że dyferencjacja żeńskich kwiatostanów była związana ze zwiększeniem poziomu ABA, zaś różnicowanie kwiatostanów męskich związane było ze zmniejszeniem zawartości tego inhibitora. Nie stwierdzono korelacji między determinacją płci a poziomem auksyn i cytokinin.