

Endogenous growth regulators in embryonic shoots of Scots pine at the time of male and female flower primordia initiation*

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Abstract:

The initiation of Scots pine male flower primordia is connected with a high content of gibberellins and a low level of auxins, whereas the initiation of female flower primordia is correlated with a high content of auxins and a low level of gibberellins. There is lack of direct correlation between the content of cytokinins or abscisic acid and flower sex differentiation.

INTRODUCTION

Sex expression in both monoecious and dioecious plants can be modified by soil fertility, temperature, day length as well as exogenously applied chemicals of various nature. It has been also observed that monoecious cucumber plants have more endogenous gibberellin-like substances than gynodioecious ones (Atsmon et al., 1968), while hermaphrodite plants have more extractable auxins than andromonoecious plants (Galun et al., 1965). Consequently, the hypothesis has been proposed that environmental as well as chemical factors exert their effects on sex expression by manipulating the balance of endogenous hormones (Pharis and Morf, 1970).

There are only scarce information concerning the participation of growth regulators in the flower sex differentiation in conifers. Low or moderate levels of exogenous gibberellic acid given under long day to young seedlings of *Cryptomeria japonica*, *Chamaecyparis obtusa* and *Cupressus arizonica* will produce a preponderance of staminate strobili, but higher concentrations will result in an increase in a proportion of

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ovulate strobili (Hashizume, 1959; Pharis and Morf, 1970). Further, the transition of staminate strobili to ovulate strobili has been shown in *Cryptomeria japonica* by applying GA₃ immediately after the microsporangium is formed (Hashizume, 1960). It seems possible that higher levels of GA₃ are responsible for increasing endogenous auxin content (Hashizume, 1961), which in turn have enhanced femaleness. The exogenous application of auxins will increase the proportion of ovulate strobili produced by some *Pinus* species and also enhance femaleness in certain species in the *Cupressaceae* and *Taxodiaceae* (Hashizume, 1966). However, high levels of auxin reduced the number of ovulate strobili in lodgepole pine seedlings. (Wample et al., 1975) and enhance maleness in Douglas fir (Ross and Pharis, 1976). An analysis of endogenous auxin level in strobili of *Cryptomeria japonica* and certain *Pinus* species (Giertych and Forward, 1966) have shown to be highest in ovulate strobili and lowest in staminate strobili. Thus, while sexuality in nature is often correlated with situations indicative of gradients originating from the apex, and analysis of endogenous auxins of the strobili themselves point to auxin being associated with femaleness, exogenous application of auxin have, depending perhaps upon species and the adjunct treatment, promoted both maleness and femaleness. Where auxin has consistently promoted maleness gibberellins have been an adjunct treatment. It is thus possible that a balance between these two groups of substances is the most critical factor, however, the participation of other hormones cannot be excluded.

In the literature, however, there is lack of detailed information concerning the metabolism of endogenous hormones during the differentiation of male and female inflorescences in conifers. So the purpose of this work was to know the changes in the level of hormones in embryonic shoots of pine at the time of male and female flower primordia initiation.

MATERIAL AND METHODS

The studies were undertaken on the about 60 year old trees of *Pinus silvestris* L. growing on natural stands (Olek Forestry, Barbarka Wood near Toruń). Pine buds destined for preparation for both anatomical observation and extraction of growth hormones were sampled from the position that favours production of male inflorescences as well as from the position that favours the production of female cones. Embryonic shoots received from the buds taken from branches producing 90—95% of buds containing male inflorescence primordia are called male embryonic shoots. On the other hand embryonic shoots taken from branches producing 65—80% buds containing female cone primordia are called

female embryonic shoots. The term "embryonic shoot" is used after Gregory and Romberger (1974).

To compare the hormonal level of male and female embryonic shoots with that of vegetative ones some young 6-8 year old (not yet flowering) pine trees, growing in the same environment as adult trees were chosen.

Samples were taken at the following stages:

I — initial of 1-3 "whorls" of cataphyll primordia — it means at the stage just before the initiation of the axillary buds differentiating later into male inflorescences (May 28th, 1975),

II — initiation of the 5-8 "whorls" of cataphyll primordia — it means at the stage just before the initiation of the axillary buds that differentiate later as female inflorescences (June 16th, 1975),

III — ceasing of apical meristem activity — at this stage the mitotic activity could be observed in male and female inflorescence primordia (September 24th, 1975).

The term "whorl" is used here for determination of a turn of the genetic spiral of pine embryonic shoot.

For the determination of the development stages 20 embryonic shoots of each group (male, female and vegetative) were taken as a sample each time. These samples were fixed with uranyl-formaline (Podbielskowska, 1971) or Navashin's solution (Jensen, 1962) and stained with Ehrlich hematoxylin (Johansen, 1940) or azur B (Flax and Himes, 1952).

Plant growth hormones (auxins, gibberellins, cytokinins and abscisic acid-like inhibitor) were extracted from 500 embryonic shoots with 80% methanol during 48 hrs at + 5°C. Evaporation at 35°C removed the methanol leaving the aqueous residue partitioned three times at pH 2.7 with equal volumes of ethyl acetate and kept for cytokinins determination. The combined ethyl acetate fractions were then partitioned three times with equal volumes of 4% sodium bicarbonate solution. The combined bicarbonate fractions were adjusted to pH 2.7 and partitioned three times with equal volumes of ethyl acetate. The acidic ethyl acetate soluble fraction thus obtained was taken to dryness in vacuo. Extracts were redissolved in 80% methanol and divided into three parts. One part was used for determination of gibberellins, the second for isolation of auxins and the third one for determination of abscisic acid — like inhibitor.

Gibberellins were partitioned chromatographically using TLC (silica gel G, solvent system: benzene/acetic acid 10:3 v/v/ and the lettuce hypocotyl test (Frankland and Wareing, 1960) was used for their quantitative determination.

Auxins were chromatographed on Whatmann 3MM paper — solvent system: isopropanol/ammonia/water 10:1:1 v/v and bioassayed by the *Avena* section straight growth test (Nitsch, 1956).

Absciscic acid-like inhibitor was chromatographed on Whatmann 3MM paper (solvent system: distilled water) and bioassayed using wheat coleoptile test (Bentley and Housley, 1954). Growth inhibition was expressed in activity units. As one activity unit 10% growth inhibition of the test plants in relation to control was taken.

Cytokinins were purified according to the method of Hewett and Wareing (1973). The aqueous phase at pH 2.7 (after partitioning with ethyl acetate) was absorbed on the cation exchange column (Dowex 50 W-X4 H^+ 50-100 mesh) which was washed with 70% methanol and distilled water. The active substances were then eluted with 2 and 5N NH_4OH . The soybean tissue test (Miller, 1968) was used for cytokinin determination.

RESULTS AND DISCUSSION

Investigations on the level of hormonal substances in embryonic shoots were carried out in the order to learn about hormonal balance during the initiation of male and female pine inflorescences. Male and female embryonic shoots were taken from 60 year old trees while vegetative ones from young 6-8 year old non-flowering trees. It seems to be important to underline that the comparison of the hormonal level between such two groups of plants is not entirely correct since the plants were at different age. On the other hand, however, it was impossible to find on adult trees such regions in the crown where only vegetative buds were being produced.

The results of the experiments on auxins (Fig. 1) show that in tissues of male and vegetative embryonic shoots there is only one group of these substances localized on chromatograms at R_f 0.3-0.5. Both the localization and earlier studies on the identification of auxins in pine (Wodzicki, 1968) allow to assume that IAA is the main active compound in this zone. In female embryonic shoots, especially at the second stage, it means at the time just before the initiation of the axillary buds differentiating later into female inflorescences, two additional groups of auxins were stated. The comparison of total amount of auxin-like substances in female, male and vegetative embryonic shoots in different stages of development (Fig. 2) shows that female embryonic shoots contained far higher amounts of these substances than the vegetative and male ones. It is interesting to note that at second stage, in spite of establishing the presence of three groups of auxins in female embryonic shoots, the general decrease in the content of these compounds was observed. Since, however the same was stated in male and vegetative shoots, it seems possible that the reason for that was some "stress" action of environmental factors. Thus the obtained results

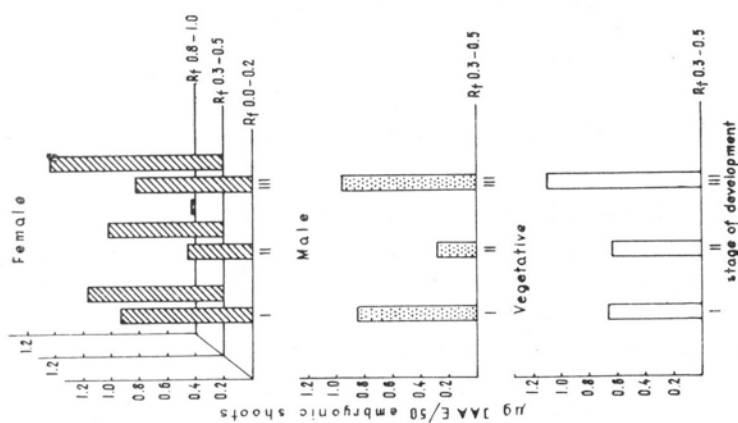


Fig. 1. Changes in the amount of auxins in embryonic pine shoots

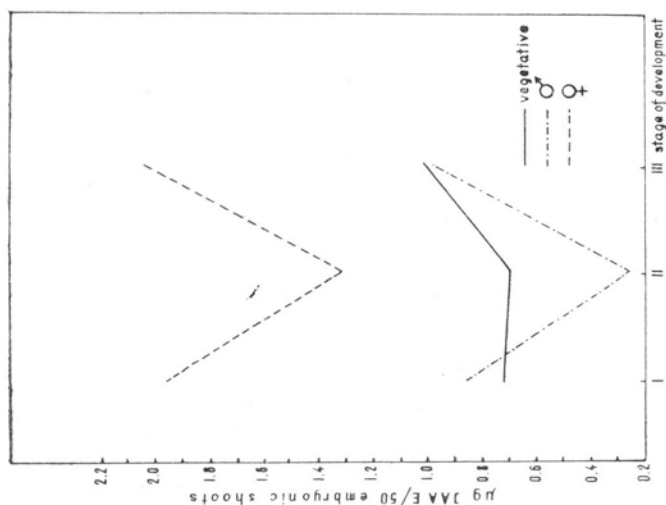


Fig. 2. Total amount of auxins in embryonic pine shoots

suggest the participation of endogenous auxins in sex expression of pine confirming at the same time the earlier suggestions concerning the role of these substances in female flower formation in conifers (Hashizume, 1966; Giertych and Forward, 1966).

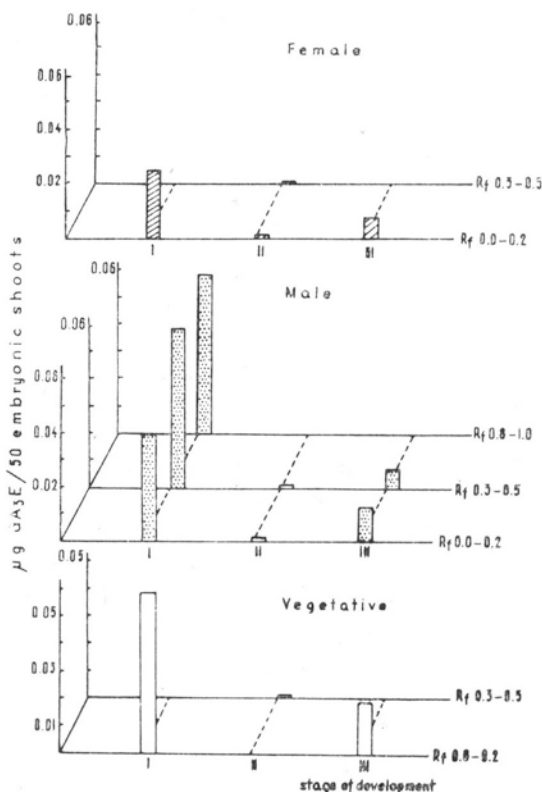


Fig. 3. Changes in the amount of gibberellins in embryonic pine shoots

The results of the experiments on gibberellins show (Fig. 3) that female and vegetative embryonic shoots contain small amounts of these compounds. In male embryonic shoots big amounts (three groups) of gibberellins were found at the stage just before the initiation of the axillary buds differentiating later into male inflorescences. A closer physico-chemical and physiological description of properties of the gibberellins occurring in pine shoots was presented in the previous paper (Kopcewicz, 1968). The comparison of total amounts of gibberellin-like substances in male, female and vegetative embryonic shoots (Fig. 4) shows that in the period just before the initiation of male inflorescences in potentially male embryonic shoots there occur many times more gibberellins than in female and vegetative ones. Later periods are characterized by a distinct decrease in gibberellin content. So the results

suggest the participation of endogenous gibberellins in flower sex differentiation towards maleness confirming at the same time the data concerning the influence of exogenously applied gibberellins on male flower formation in conifers (Hashizume, 1959, 1960; Pharis and Morf, 1970).

The results of investigations on cytokinins (Fig. 5) show that both at the stage just before the initiation of male (stage I) and female (stage II) flower primordia the amounts of cytokinins in male and female

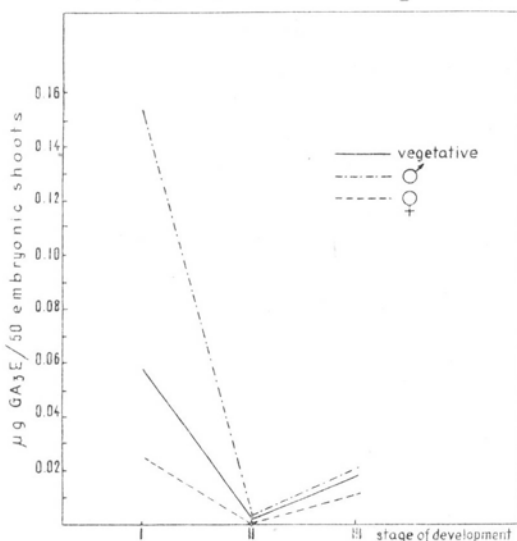


Fig. 4. Total amount of gibberellins in embryonic pine shoots

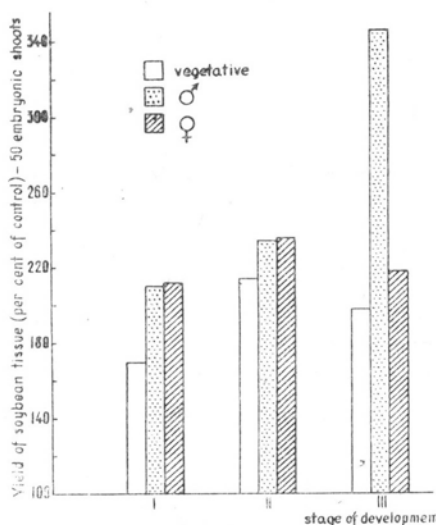


Fig. 5. Cytokinins in embryonic pine shoots

embryonic shoots are identical. The considerable increase in the level of cytokinins in male embryonic shoots in September (stage III) is probably connected with the mitotic activity of male archesporial cells. Although male and female embryonic shoots contain at the first two stages similar amounts of cytokinins, the content of these compounds is, however, higher in June. It seems that it may be connected with enlarging of the peripheral tissue zones in shoot apex and increasing of mitotic activity of this tissue at that time. The obtained results seem to point out to a close correlation between the amount of cytokinins and intensity of mitotic activity of embryonic shoot cells, suggesting at the same time the lack of direct interrelations between the level of cytokinins and flower sex differentiation in pine. The results also show that in conifers the situation is different than in herbaceous plants in which cytokinins seem to be related with femaleness (Hashizume and Iizuka, 1971).

The results of investigations on abscisic acid (Fig. 6) show that both in May and in June embryonic shoots contain similar amounts of this substance. A general increase in the level of abscisic acid was found

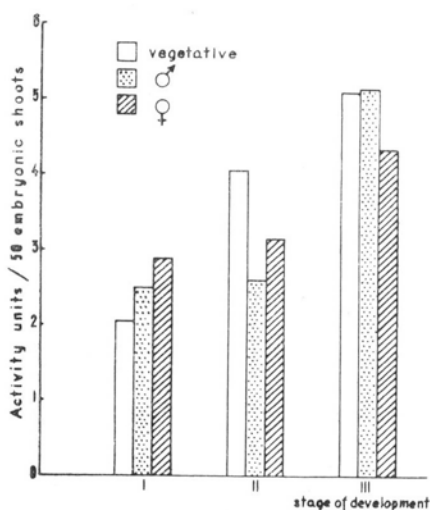


Fig. 6. Absciscic-like inhibitor in embryonic pine shoots

in September, that is in period of ceasing of apical meristem activity connected with approaching dormancy. The obtained results show clearly that the level of abscisic acid is correlated with seasonal changes in the rate of growth and development of pine shoots, while there is no interrelation with flower sex expression.

Generally speaking the obtained results show that in potentially male embryonic shoots at the time just before differentiation of male primordia the high level of gibberellins and low content of auxins are

observed. 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 amounts of cytokinins and abscisic acid.

At the stage just before the initiation of the axillary buds differentiating later into female inflorescences a high level of auxins and lack of gibberellins were found in potentially female embryonic shoots. 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 abscisic acid.

In September at the stage of ceasing of apical meristem activity there occur only enlargement of female cone primordia and mitotic activity of male archesporial cells, female embryonic shoots contain great amounts of auxins and low level of gibberellins. At the same time male embryonic shoots are characterized by a low level of auxins and gibberellins and a high content of cytokinins. Male, female and vegetative shoots contain at that time a high level of abscisic acid.

Vegetative embryonic shoots contain at all the investigated stages small, not undergoing considerable changes, amounts of auxins, gibberellins as well as cytokinins and increasing contents of abscisic acid.

Thus the obtained results suggest that flower sex differentiation in pine is connected with endogenous equilibrium of auxins to gibberellins similarly as in the case of herbaceous plants (Atsmon et al., 1968). At the same time cytokinins and abscisic acid, undoubtedly taking part in the regulation of the whole metabolism, do not directly participate in the process of pine sex expression.

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*Endogenne regulatory wzrostu w zawiązkach pędów sosny zwyczajnej
w czasie inicjacji męskich i żeńskich kwiatostanów*

Streszczenie

Inicjacja kwiatów męskich sosny zwyczajnej zbiega się z wysoką zawartością giberelin i niskim poziomem auksyn w zawiązkach pędów. Powstawanie kwiatów żeńskich jest natomiast skorelowane z wysoką zawartością auksyn i niskim poziomem giberelin. Nie wykazano bezpośredniego związku między inicjacją zawiązków kwiatostanów a zawartością cytokinin i kwasu abscysynowego.