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IAA-peroxidase relation in the microsporocytes and anther wall during successive stages of meiosis in *Larix europaea* L.

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Abstract

During anther meiosis in *Larix europaea* considerable variations in the level of peroxidase activity and endogenous auxin content occur both in the microsporocytes and in the anther wall. However, the IAA-peroxidase relations are different in each of these two parts of the anther. In the anther wall characterized by the occurrence of anodic isoperoxidases, the changes in peroxidase activity show a positive correlation with those in endogenous auxin content. In the microsporocytes containing almost only cathodic isoperoxidases the levels of endogenous auxin content and peroxidase activity show a reverse correlation. Thus a preponderance of isoperoxidases showing IAA-oxidase properties occur only in the microsporocytes. These results suggest the important role of the IAA-peroxidase system in the mechanism of differentiation of cells undergoing anther meiosis.

INTRODUCTION

In a previous study we noted that in the developing larch anther take place a considerable variations in the endogenous auxin content (Górska-Brylass et al., 1976). The present study reports the changes in the endogenous auxin content and peroxidase activity level and the peroxidase pattern in the microsporocytes and somatic cells of the anther wall during successive stages of meiosis in the larch.

MATERIAL AND METHODS

The male cones of *Larix europaea* L. collected for study directly from the trees, were subjected to a special procedure of separation of microsporocytes from the somatic cells forming the anther wall. The microsporocytes in different stages of meiosis were then separated by centrifugation in a non-linear density gradient of sucrose into cell fractions each of which included only one cytologically different stage

of development (Chwirot, 1980). The purity grade of the cell fractions was 94—100%.

In the microsporocyte fractions and in the fractions of the anther wall including 10 successive phases of meiosis, total protein content (Lowry et al., 1951), endogenous auxin content (Michalski, 1967), specific peroxidase activity (Luck, 1963) and peroxidase isoenzymes were determined.

The fractions of microsporocytes and of the anther wall were homogenized in 0.5 M acetate buffer at pH 5.5. The homogenate was then centrifuged for 30 min. at 20.00 × g at 0° C. The supernatant, after determination of protein content, was layered on gel tubes. Samples of 100 µm containing 100—150 µg protein with an addition of 20% sucrose and 0.001% bromophenol blue were layered on the gel surface. Electrophoretic separation of peroxidase isoenzymes was carried out on polyacrylamide gel in Tris-glycine buffer at pH 8.9. After electrophoresis the gel tubes were stained for 5—10 hours at 0—4° C in a mixture with 250 mM acetate buffer at pH 5.5, 5 mM EDTA, 0.1% p-phenylenediamine, 0.3 M hydrogen peroxide.

For each fraction 3—5 samples were checked.

RESULTS AND DISCUSSION

The results obtained revealed that during development of the larch anther in the period of meiosis considerable variations take place in the endogenous IAA content (Fig. 1). The variations occur both in cells

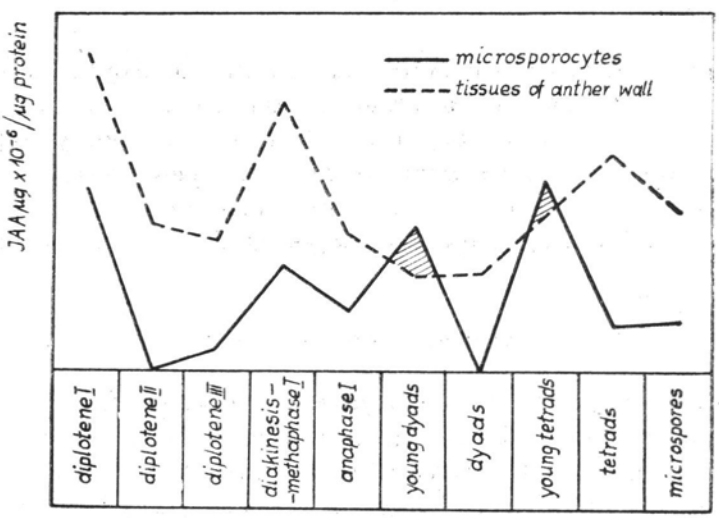

Fig. 1. Endogenous IAA content in microsporocytes and in the tissues of the larch anther wall during successive development stages

undergoing meiosis (microsporocytes) and in cells of the anther wall. The conspicuous differences in the endogenous IAA content in the meiotic cells are not connected with the topographic situation of the cell in the anther, but with its development stage. In the same anther, young tetrads may occur along with dyads. However, endogenous IAA contents in these two types of cells are widely different.

During the meiotic prophase, the changes in the level of endogenous IAA in the microsporocytes and in the somatic cells of the anther wall coincide. In the later period, i.e. in the period preceding the formation of young dyads and tetrads, this correlation undergoes some disturbance.

The level of endogenous IAA content is generally higher in the somatic cells of the anther wall than in the microsporocytes. An exception from this is the stage of young dyad and young tetrad: in these stages of development, the level of endogenous IAA content in the microsporocytes reaches a point exceeding the IAA level in the anther wall. Two peak points in the endogenous IAA level related with the formation of young dyads and young tetrads are separated by the dyad stage, in which the level drops to nearly zero. A characteristic feature

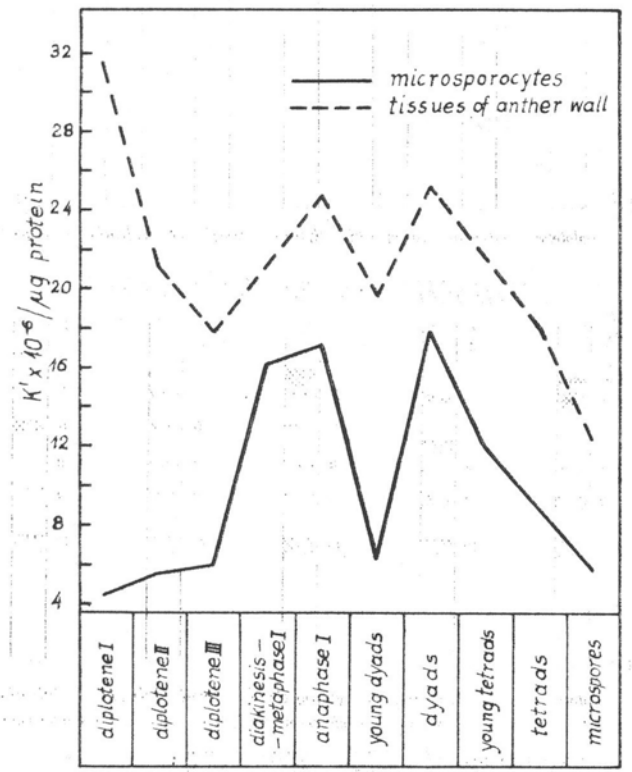

Fig. 2. Peroxidase activity level in microsporocytes and in the tissues of the larch anther wall during successive development stages

of the young dyad and young tetrad is the rapid and intensive synthesis of a callose wall. In the larch, synthesis of callose starts only in young dyad. The mature dyad does not synthesize a callose wall any more. Callose synthesis is resumed only as a result of the second cytokinesis, that is, in the young tetrad.

The extremely high level of endogenous IAA in the cells forming callose walls may suggest some participation of endogenous auxin in stimulating the synthesis of this polysaccharide.

Conspicuous variations in the level of peroxidase activity have been found in the developing anther during the period of meiosis (Fig. 2). From the end of diplotene the changes of enzyme activity in the microsporocytes and the cells of the anther wall are parallel. In microsporo-

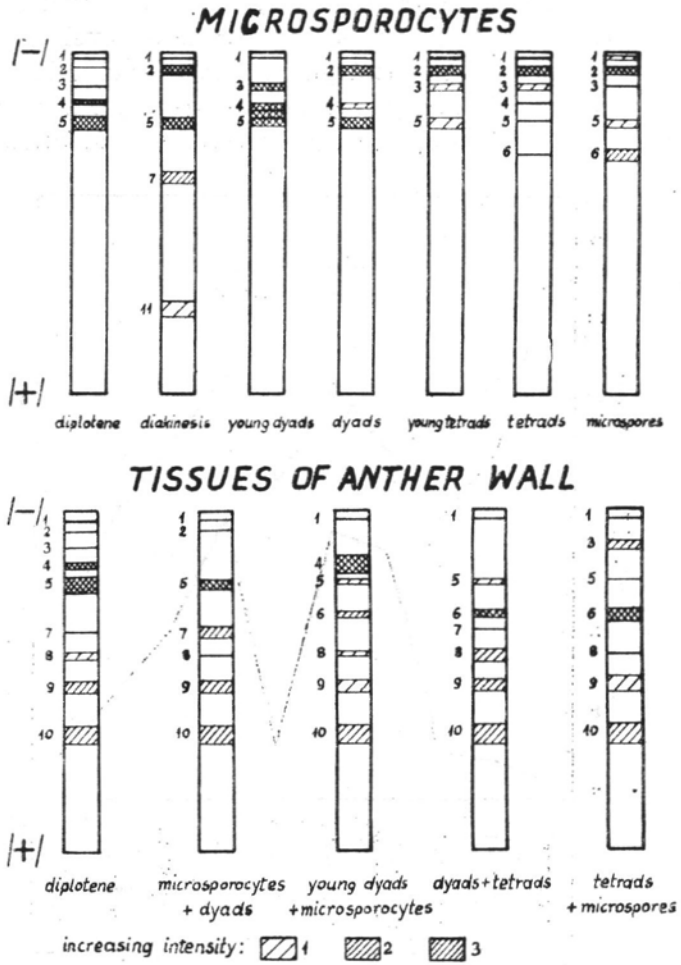

Fig. 3. Isoperoxidase patterns in microsporocytes and in the tissues of the larch anther wall during successive stages of development

cytes, as well as in somatic cells, the highest level of enzyme activity is observed in the period preceding the formation of young dyads and young tetrads. During all stages of meiosis, the anther wall shows a higher level of peroxidase activity than the microsporocytes.

Electrophoretic separation of peroxidases from the microsporocytes and the anther wall revealed considerable differences in the zymograms obtained from these two parts of the anther (Fig. 3). These differences concern in the first place electrophoretic mobility of the isoenzymes. In the microsporocytes there are almost solely cathodic isoenzymes. In the anther wall also anodic isoenzymes and isoenzymes with intermediate Rf value occur. There are also qualitative differences: one isoenzyme (No 11) is present only in the microsporocytes and three others (Nos 8, 9, 10) are present only in the anther wall.

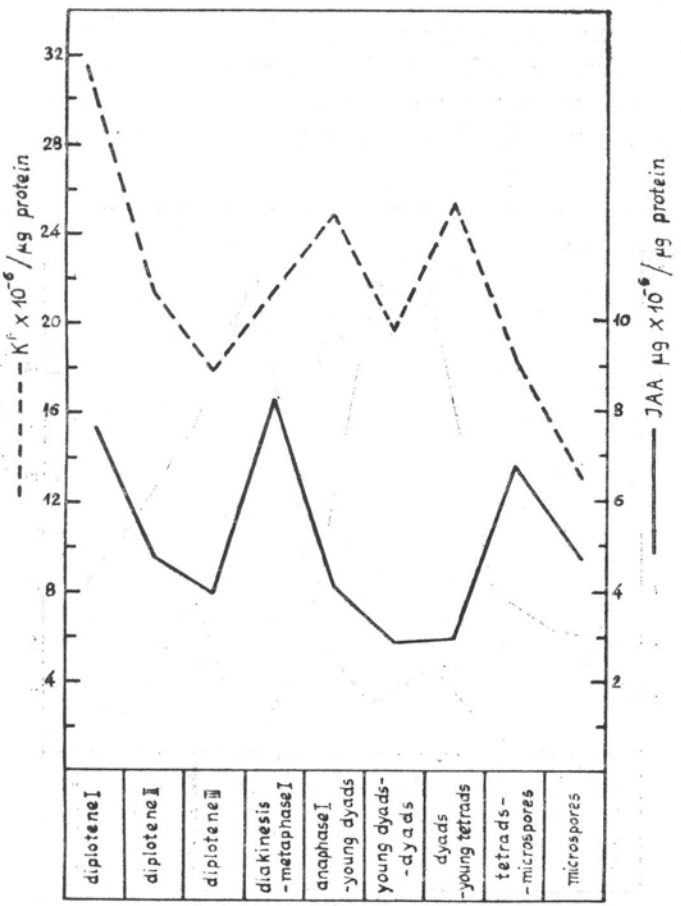

Fig. 4. Positive correlation between the level of peroxidase activity and endogenous IAA content in the larch anther wall in the period of meiosis

The intensity of changes in the isoenzyme pattern, expressed by the ratio of the number of the stable isoenzymes to the number of those appearing periodically, is decidedly higher in the microsporocytes than in the anther wall. Out of ten isoenzymes of the anther wall, five occur permanently, whereas out of eight isoenzymes of the microsporocytes only two occur permanently. The isoenzyme pattern shows more intensive differentiation in the microsporocytes than in the anther wall cells.

The differences found between the isoperoxidases in the cells of the anther wall and those occurring in the microsporocytes are reflected in the different IAA-peroxidase relations in each of these two parts of the anther (Figs 4, 5). In the anther wall characterized by the occurrence of anodic isoperoxidases, the changes in the peroxidase activity level show a positive correlation with the changes in the level of endogenous auxin (Fig. 4). However, in the microsporocytes containing almost solely cathodic isoenzymes, the levels of endogenous auxin and peroxidase activity show a reverse correlation (Fig. 5). Starting from the meiotic prophase, each increase in peroxidase activity is associated with a drop in the endogenous auxin level. The two peaks in endogenous auxin level

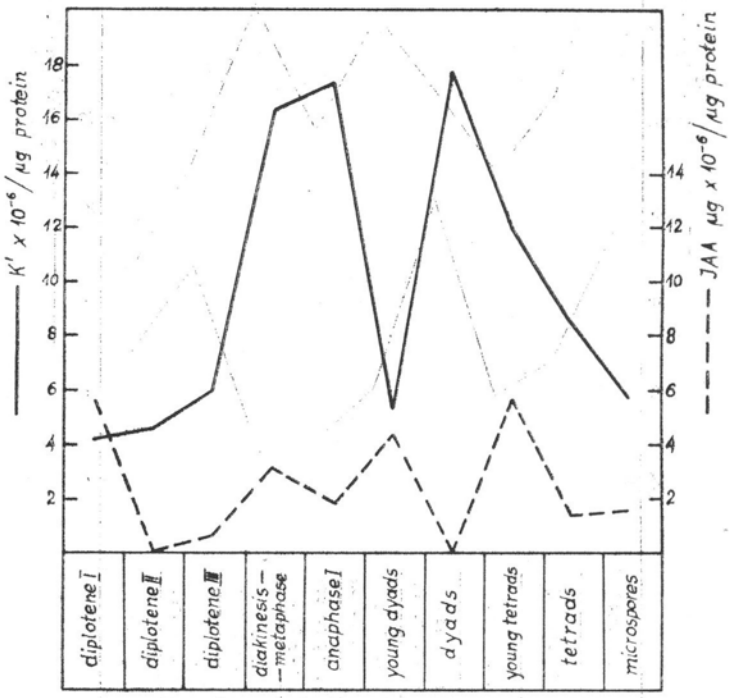

Fig. 5. Reverse correlation between the level of peroxidase activity and endogenous IAA content in the larch microsporocytes during successive stages of meiosis

connected with the young dyad and the young tetrad stages are preceded by a rapid drop in peroxidase activity. These results suggest that there is a preponderance of isoenzymes showing IAA oxidase properties only in the microsporocytes.

The endogenous system: IAA-peroxidase occurrence in the developing microsporocytes of the larch, seems to confirm the results obtained in different experimental systems. The latter results show that the most cathodic (basic) peroxidases are the most powerful auxin-oxidases *in vivo* and, consequently, their activity is reflected in the endogenous auxin level (Mazza et al., 1970; Gaspar et al., 1975; van Hoff, Gaspar, 1976).

The dynamic character of the endogenous system IAA-peroxidase found in developing microsporocytes and the surrounding anther wall tissues of the larch seems to be evidence of the important part of this system in the mechanism of differentiation of cells undergoing anther meiosis.

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