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THESIS OF DISSERTATIONS FOR CANDIDATE DEGREES

HORMONAL REGULATION OF THE DORMANCY AND GERMINATION OF SEEDS REQUIRING STRATIFICATION*

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Introduction

In the course of the few decades since the discovery of plant hormones, we have become familiar with several aspects of growth regulation, nevertheless, the regulation of the cessation in growth — the dormancy — is still unclear even nowadays.

In respect to the physiology of dormancy, the assumption is generally accepted that its development, maintenance and release are regulated by hormonal balance. As to the hypothesis regarding regulation by hormonal balance, the question arises what changes are brought forth by environmental factors in the metabolism and interaction of growth-regulating substances? Studies on the latter problem are of particular importance in case of the seeds manifesting deep dormancy which require special environmental conditions for dormancy release, for germination. From practical point of view, the most noteworthy from the special environmental requirements is low temperature.

A long known forestry experience is that most of the deciduous tree seeds of the forest do not germinate in the first spring following autumn sowing after harvest. During the course of adaptability to the environment, such a degree of inhibition develops in these seeds that only ceases on the effect of cold. This is when activation starts, ensuring the germination of these seeds in spring, after the cold period — the natural winter cold. However, the seed is only capable of perceiving the cold effect in swollen state; and the swelling is a rather slow process taking months owing to the presence of the fruit- and seed-coats.

By means of treatments substituting the natural effects, as well as studies in connection with the physiological role of stratification, numerous valuable data have been obtained during the past few years. Despite these, however, it cannot be said that the physiological processes taking place at low temperature have been clarified. It is assumable that many common features exist in the mechanism of the

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warming up phases in case of different seeds requiring stratification, but the data at hand do not make any generalizations possible.

Due to the lack and fractional character, resp., of the comparative studies (hormonal changes taking place in seeds being in deeply dormant and not deeply dormant state; changes taking place during cold- and warm-stratification, resp.), at present no essential differences can be concluded between the hormonal regulation of the germination of seeds with ordinary and special environmental demands, resp., furthermore, it cannot be revealed which are the physiological changes taking place only in case of low-temperature treatment.

Our studies aimed at investigating the role of gibberellin (GA) from germination-physiological point of view. The major questions which we wished to receive answer in our experiments were the followings:

— Is the breaking of dormancy and the start of germination in connection with the appearance of the endogenous free GA-s?
— Are there any differences in tendency in the changes of the endogenous GA-level during the preparation for germination of the seeds being in deeply dormant and those of in not deeply dormant state?
— What are the changes taking place in the endogenous GA-level of the seeds being in deeply dormant state under environmental conditions unfavourable for germination?
— What is the role of bound gibberellin in the changes of the endogenous GA-level in case of seeds being in deeply dormant state?
— Are there any essential differences in the endogenous gibberellin-spectrum of the different seeds?
— Does de novo GA synthesis have any significance in the early phase of germination?
— What is the possible role of abscisic acid (ABA) in the regulation of the endogenous GA-level?
— Which are the hormonal ratios and interactions regulating dormancy and its cessation?

To determine whether there are any differences between the hormonal changes taking place at the time of germination induced under special conditions and the hormonal changes in the seeds not requiring any special treatment for germination, comparative studies have been performed in accordance with the afore-listed viewpoints. Since from theoretical and practical points of view there are no literary data available on the germination-physiology of our most important study material — the linden seed —, the dissertation comprises the general physiological characterization of the dormant linden-seeds.

Materials and methods

Tilia sp., *Fraxinus excelsior*, *Phaseolus* sp. and *Lupinus albus* seeds were used in our comparative studies. The procumbent period of the deeply dormant, hard-coated seeds was shortened by means of

chemical scarification, performed after the fertilization of the seeds in heated sand moistened to 70 % of the total water capacity, at 25 and 4 — 6 °C, resp.

The methods of extraction and diffusion were used to study the changes in endogenous gibberellin-content. The gibberellins in the extract and diffusate, resp., were divided into fractions in dissolvents, according to their pH-dependent differing distribution, then were separated further by thin-layer chromatography. The biological activity measurements and the pertaining dosimetry were performed with barley endosperm and lettuce hypocotyl tests. The GA chromatogram-spots were identified on the bases of the Rf value measured on different adsorbents and several solvent-systems, the GA_3-related movement, induced fluorescence of the spots, colour-reactions, behaviour in various biological tests, as well as IV-spectrum; using authentic compounds.

The exogenous ABA content and the CCC amount absorbed by the seeds were determined with UV-spectrophotometry following thin-layer-chromatography, and Dragendorff reaction, resp. The possible interactions between the different hormones (GA, IAA, KIN, ABA) were modelled in embryo culture.

The leaf-pigments were measured by photometry, the protein content was determined according to Lowry et al. To follow the changes in the level of the growth inhibitors, straight-growth coleoptyl-tests, several germinating tests and linden embryo tests were used. The ABA-related instrumental measurement results were complemented with stoma-closing test, the GA-related ones with dwarf-pea and dwarf-maize tests. Several histological techniques were also applied in the course of the studies.

The mechanical role of the endosperm as well as the correlation effects between the different parts of the embryo were studied by means of operative interventions. Cellulase- and pectinase-activity measurements were made when studying the interaction between the embryo and the endosperm. The nuclear DNA-content of the embryonic cells was measured by cytophotometry following Feulgen-staining, the DNA + RNA contents following GCA-staining.

Summary of the new scientific results

The dormancy type of the *Tilia platyphyllos* seeds as well as the basic processes leading to the cessation of dormancy were clarified.

It was determined that the linden seeds represent the type of morpho-physiological dormancy state where the fruit- and seed-coats do not have any decisive role, but the cotyledone and endosperm play an active regulatory role; and the cold effect cannot be substituted by GA. The cold-stratification is necessary for the induction of the conditions ensuring harmonious embryo-growth as well as for launching the synthesis of the hydrolases responsible for the cytolysis of the part of the endosperm around the radicle. Accordingly, we have characterized a special type of the seed dormancy, which has not been described yet.

We have demonstrated that a certain amount of the GA-s is in free form in booth the deeply dormant and non-deeply dormant type dry seeds. Thus, from the viewpoint of germination, not the appearance of the free gibberellins is the decisive motif.

In the course of preparations for germination we have experienced the elevation of the endogenous free GA-level in both seed types. Therefore, the free GA-s should be present in a determined quantity in a determined phase of the swelling process for the biochemical and physiological processes of germination, furthermore, for ensuring normal growth of the seedlings. The change in GA-level according to the maximum-curve is a general observation in the early phase of

germination. The time-point of the development of the GA-maximum, however, is not identical in case of the seeds in deeply dormant state.

The differences manifesting in the sequence of the maxima of the promotor hormones during cold-stratification can be correlated with the success of the exogenous hormone-treatments. Since in the *Tilia* seeds the GA-maximum develops only at the end of the cold-stratification, exogenous GA-treatment applied during warm-stratification is not effective in breaking of dormancy

The raise in the endogenous GA-level is measurable under non-inductive circumstances, too (during warm-stratification), it also indicates the fact that elevation of the GA-level in itself is not sufficient for stopping the dormant state.

It has been demonstrated that the raise in the amount of free GA-s is a process parallel with the decrease in the amount of bound GA-s. The genetic relationship between the free and bound GA-s is also referred to by the fact that upon the hydrolysis of the bound fraction, active GA-s were obtained with the same Rf-value as the free GA-s found in the acidic ethyl-acetate fraction.

From the linden seeds, we have isolated six kinds of free GA-s, among which four have been identified (GA_1, GA_4, GA_9, GA_{16}) and their succession according to concentration was defined. A definite difference exists in the endogenous gibberellin spectrum of the different types of seeds studied by us, but in every type the endogenous gibberellins showing the greatest biological activity and changing the most intensively during inductive treatment were found to be the GA_1 and the GA_4.

The diffusion method — also giving an idea of the GA transmembrane movement — was introduced to characterize the GA-supply of the tissues treated with the growth retardants.

It was proved that the results obtained with the antigibberellins (CCC, AMO—1618) cannot be applied for the purpose of judging the significance of the de novo GA-synthesis, either in the early phase of germination, or in the course of later development, since these retardants exhibit a complex effect on the metabolism of the GA-s: apart from inhibiting their biosynthesis they also have effect on the interconversions, they influence the ratio of the free and bound GA-s as well as the symplastic motion of the GA-s in the plant.

We have demonstrated that the GA-supply of the tissues treated with the growth retardant can be satisfactory even besides a free GA-level lower than the control, since the retardant enhances the GA movement in the plant.

We have concluded that the dormancy-maintaining effect of ABA, besides its direct effect of inhibiting embryo growth, can also be attributed to its role of hindering the release of the gibberellins from bound forms.

It has been proved by the studies on the changes during stratification of the endogenous ABA content, by model-experiments with excised embryos as well as by treatments of intact seeds with exogenous hormones that the ABA and other growth inhibitors play an important role in the maintenance of dormancy.

We have pointed out the significance of hormone-interactions and allosteric effects in the breaking of dormancy of the linden embryos and in the development of the germination ability. Dormancy release in the linden seeds is a process consti-

tuted of several phases in which the increase to optimal level of the promotor hormones (GA, IAA, cytokinin) and the optimal sequence of their appearance are of extreme importance, besides the decrease of the inhibitor levels caused by cold-effect. In this sequence, gibberellin is not a hormone inevitably standing in the first place in every seed; our data have otherwise refuted the generally decisive role of the GA-s played in the state of dormancy.

According to our results the dormancy of the *Tilia* seeds is firstly regulated from the direction of the inhibitors. Therefore, the role of the cold-effect is not considered to be the optimalization of the endogenous GA-level, but in the decrease in inhibitor-content and development of the ensuing new, favourable hormonal ratios.

Practical importance of the results

When selecting the topic of our study and setting the experimental targets, it was expected to gain such experiences in the course of characterizing the dormancy of the linden seeds and clarifying the hormonal regulation of dormancy, in the possession of which a proposal can be made for elaborating a method of fast germination without stratification, applicable also on a large scale. By means of comprehensive studies, however, it was proved that the characteristic anatomic structure and special nature of dormancy of the linden seeds/linden fruits do not make it possible to omit cold-stratification and have it substituted with chemical methods. This is so because the application of promotor exohormones for breaking of dormancy of the linden seeds is not successful without cold-effect.

The speeding up the germination of the linden seeds is possible by removing the fruit-coat hindering swelling and by the scarification of the seed-coat. These, however, are operations requiring much manual work; thus are only expediential for shortening the duration of experiments in case of laboratory studies. Therefore, our experimental data obtained with the linden seeds verify the practice of stratification.

Nevertheless, our results obtained upon treatment of the intact seeds with hormone combinations provide possibility for shortening the procumbent period.

The dissertation may provide useful data to the better understanding of the physiological processes taking place during vernalization, too; and our results regarding the mechanism of effect of the growth retardants as well as the interactions between the phytohormones can be utilized indirectly, in the practical use of the growth-regulating substances.

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