DORMANCY IN FRUITS OF THE TILIA PLATYPHYLLOS SCOP. VI. POSSIBLE ROLE OF THE EXOGENOUS GA_3 IN THE BREAKING OF DORMANCY

MÁRIA NAGY, SZERÉN PATAKY and ÁGNES KÉRI

Department of Plant Physiology and Department of Botany, Attila József University, Szeged, Hungary

(Received September 30, 1981)

Summary

Intensity of the respiration of the chilled-stratified seeds increases more intensively than that of the warm-stratified ones. Intensity of the respiration was further increased by treatment with exogenous GA_3 at both temperatures. Change in the lipase activity was similar. Embryos of seeds treated with exogenous GA_3 were larger than the untreated ones at both temperatures investigated. Slight differences were, however, observed in the germination rate of the excised embryos and in the intensity of the growth of the seedlings.

Faster degradation of the endosperm cells around the radicle was promoted by the chilled-stratification. This process cannot be forced by the exogenous GA_3 alone.

Introduction

In the seeds of the *Tilia platyphyllos* the embryo increases during the chilled-stratification (NAGY et al., 1981) and all changes promoting the germination take place.

The germination is an energy-intensive process since it includes the cell-division and elongation. Therefore, at the swelling of the seeds mobilization of the stored nutriments, which are utilized as respiratory basic materials and for the synthesis of new substances, starts immediately. In the mobilization of the nutriment taking place during the swelling of the seeds have an important role the hormones, firts of all the gibberellins which are important in the start of the synthesis and the enhancement of the activity of the hydrolytic enzymes of decisive importance (LEWAK et al., 1975; ZARSKA-MACIEJEWSKA and LEWAK, 1976). This may explain that in the case of numerous species the chilled-stratification requirement, necessary for the germination, can be substituted for exogenous GA_3 treatment (AMEN, 1968; JUNTTILA, 1970; ROSS and BRADBEER, 1971; KOPCEWICZ and PORAZINSKI, 1973; BASKIN and BASKIN 1970, 1974).

Since in the case of the *Tilia platyphyllos* seeds the chilled-stratification cannot be substituted for exogenous GA_3 treatment (NAGY and SZALAI, 1973), the aim of the present studies was to get further data concerning the influence of the exogenous GA_3 treatment on the dormancy of the *Tilia platyphyllos* seeds. That's why the effect of the GA_3 treatment was investigated concerning the metabolism of the seeds, the growing intensity of embryos excised from the treated seeds and the mechanical resistance of the tissues surrounding the embryo.

Materials and Methods

Fruits used for these studies were obtained from the Forestry of Csongrád County from trees forming close stand, thus population material was investigated.

The pericarps were mechanically removed and the seeds treated with sulfuric acid for 8 min then thoroughly washed, dried and put into filter-papers moistened water and 3×10^{-4} M GA_3 solution (Phylaxia, Budapest) and stored in refrigerator (at +5 °C), while an other series in thermostat of 25 °C.

Measurement of the intensity of the respiration

Intensity of the respiration of variously treated seeds was measured with Warburg's apparatus (KEIL and SORMOWA, 1968) in 3 weeks periods. Test pots were put into water-bath of 25 °C. The manometers were read at 15 min intervals for 60 min. Amount of oxygen consumed by the seeds was calculated from the following formula:

$$\mu l\ O_2 = k \cdot K_{\theta_2}$$

where k is the pressure measured with the manometer and K_{θ_2} is the factor of vessel belonging to the corresponding manometer. Three parallel series were used for the measurements. .

Measurement of the lipase activity

The enzyme preparations were made according to COLOWICK and KAPLAN (1955). Lipolytic activity was measured by using the method of ORY et al. (1962) after the determination of the pH optimum.

Measurement of the growth intensity of the excised embryos

In the different periods of the stratification the embryos were excised and stored in semisterile conditions in Petri dishes on filter-papers moistened with White's culture medium (WHITE, 1943) and illuminated with 10 000 lx for 16 h per day at 25 °C daily and 20 °C night temperature. Size of the seedlings was measured on the 3rd day after the excision.

Histological examination of the seeds

For the histological examination of the seeds, longitudinal sections were prepared by means of the usual paraffine embedding technique from seeds chilled-stratified for 3 months and from seeds warm-stratified for 6 months and treated with GA_3. The sections were stained with Ehrlich's acidic haematoxilin, fixed in glycerine-gelatine and examined with light-microscope.

Results and Discussion

Change in the intensity of the respiration of seeds under chilled- and warm-stratification

The intensity of the respiration measured with Warburg's apparatus with manometer is a good index of the activity of the metabolism.

Measure of the intensity of the respiration of the variously treated seeds at

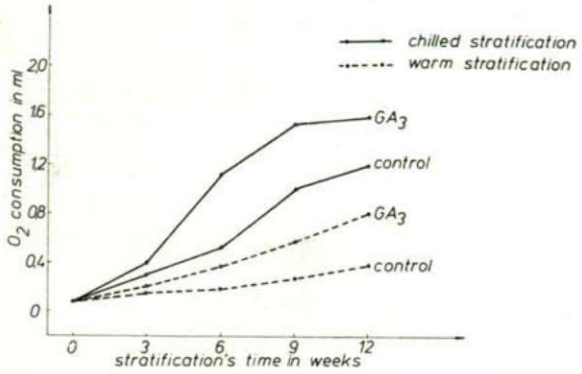

Fig. 1. Change of the respiration intensity of the *Tilia platyphyllos* seeds as a result of GA_3 treatment combined with chilled- and warm-stratification.

different times is demonstrated by Figure 1 showing the amount of oxygen (ml) consumed by 100 seeds during 1 h. As it can be seen from the Figure, intensity of the respiration of the warm-stratified seeds is much lower than that of the chilled-stratified ones.

The experiences are similar for the seeds of the pear (ALSCHER-HERMAN et al., 1981) and of the cherry too (POLLOCK and OLNEY, 1959). The intensity of the respiration is increased by the GA_3 treatment at both temperatures showing the metabolism increasing effect.

Change of the lipase activity of the seeds under chilled- and warm-stratification

In lipid-containing seeds the first step of the mobilization of the reserve materials is the degradation of the lipids with the help of lipases. Since the main reserve materials of the *Tilia* seeds are lipids (RADECKE, 1967), lipase activity was measured to characterize the metabolism activity.

Types of lipases present in the seed in dormancy and formed during the germination are question under dispute in the literature.

In the endosperm of the castor-oil bean a lipase with 4.3 pH optimum was found by ORY et al. (1962), while YAMADA (1957) described an other lipase system formed during the germination of the seed and possessing a pH optimum in the neutral range.

In the seeds, the presence of lipase system with different pH optima is contested by several authors. According to RAMAKRISHNAN and BANERJEE (1951) only one lipase system exists in the seeds pH optimum of which is in the acidic range, pH optimum observed in the neutral range is caused by the lipase activity of the microorganisms, invisible fungi, found on the seeds. This assumption was corroborated by the data of RIMON (1957) and ST ANGELO and ALTSCHUL (1964) as well.

However, lipase systems with different pH optima were found by SMOLENSKA and LEWAK (1974) in various seeds.

Determination of the pH optimum of the lipase activity of the *Tilia* seeds revealed two pH optima at 4.5 and 6.5, therefore, the activity was measured at both optima in all cases.

Change of the lipase activity of the *Tilia* seeds under stratification is shown by Figure. 2 (A and B). In the Figure, amount of the fatty acid (in oleic acid equivalent) released during 96 h by lipase extracted from 100 seeds is shown.

As it can be seen, during the 12 weeks investigation, the increase in the lipase activity of seeds stratified at room temperature was much lower than that of the chilled-stratified ones. The increase of the activity was observed at both pH as a result of the GA_3 treatment which is in accordance with the results of the measurements of the respiration intensity. The activity was higher at pH 6.5 (Fig. 2A) than at pH 4.5 (Fig. 2B).

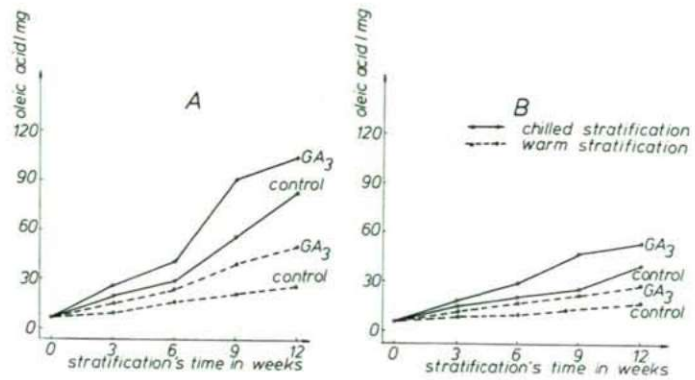

Fig. 2. Change of the lipase activity of the *Tilia platyphyllos* seeds as a result of GA_3 treatment at pH 6.5 (A) and pH 4.5 (B) combined with chilled- and warm-stratification.

Effect of the GA_3 treatment of the seeds on the growth of the embryos

Since in the case of the *Tilia platyphyllos* seeds the exogenous GA_3 treatment does not substitute the chilled-stratification, one can assume that amount of GA_3 necessary for the stimulation of the growth of the embryo cannot get into the seed. For this reason, embryos were excised from seeds treated with GA_3 for 2 months and compared with those excised from control seeds (Fig. 3). Figure shows the control embryos in the upper line while those excised from seeds treated with GA_3 in the lower line.

Although the embryos were larger than the control at both temperatures as a result of the exogenous GA_3 treatment, germination took place only in the case of the chilled-stratified seeds, and no germination was observed with the warm-stratified ones even on 6 months GA_3 treatment. The exogenous GA_3 treatment stimulated mainly the growth of the cotyledones. The same found for the Fraxinus excelsior seeds where, as a result of the GA_3 treatment, the cotyledones of the embryo increased to such an extent that they got crushed in the seed but no germination took place (SZALAI and NAGY, 1968).

Germination of the embryos excised from seeds treated with GA_3 under chilled- or warm-stratification was fast but the differences of the size of the seedlings did not justify the failure of the germination at room temperature. Figure 4 shows the size of the seedlings developed from the embryos obtained from variously treated seeds, 3 days after the excision. In the case of warm-stratification the resistance of the

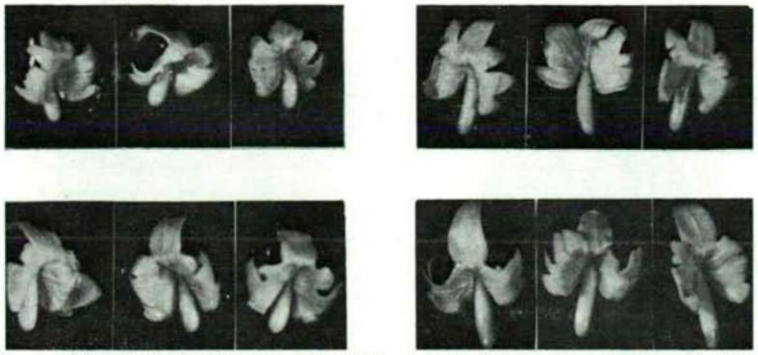

Fig. 3. Influence of the GA_3 treatment of the *Tilia platyphyllos* seeds on the growth of the embryos. Upper line is the control and the lower line shows the embryos excised from seeds treated with GA_3 for 2 months.

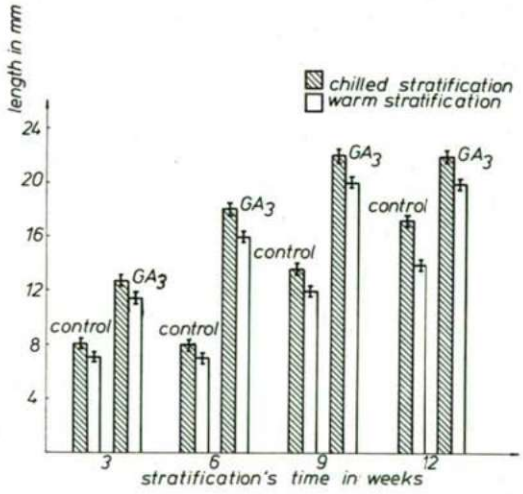

Fig. 4. Growing intensity of the embryos, excised from seeds treated with GA_3, at 25 °C and illuminated with 10 000 lx. Figure shows the size of the seedlings 3 days after the excision.

covering tissues seems to be the primary reason for the continuance of the dormancy and not the weaker growing ability of the embryo. This was corroborated by our histological observations as well.

Effect of the GA_3 treatment on the endosperm surrounding the embryo

At the end of the 3 months chilled-stratification the discharge and dissolution of the endosperm cells around the radicle were observed (Fig. 5). This change was not observed for warm-stratified and GA_3 treated seeds even after 6 months. This difference is essential concerning the cessation of the dormancy.

In the interuption of the dormancy the exogenous gibberellin would be effective if its role were double in the *Tilia platyphyllos* seeds: supply of the embryo with soluble nutriments and the stimulation of the synthesis and/or the activity of enzymes

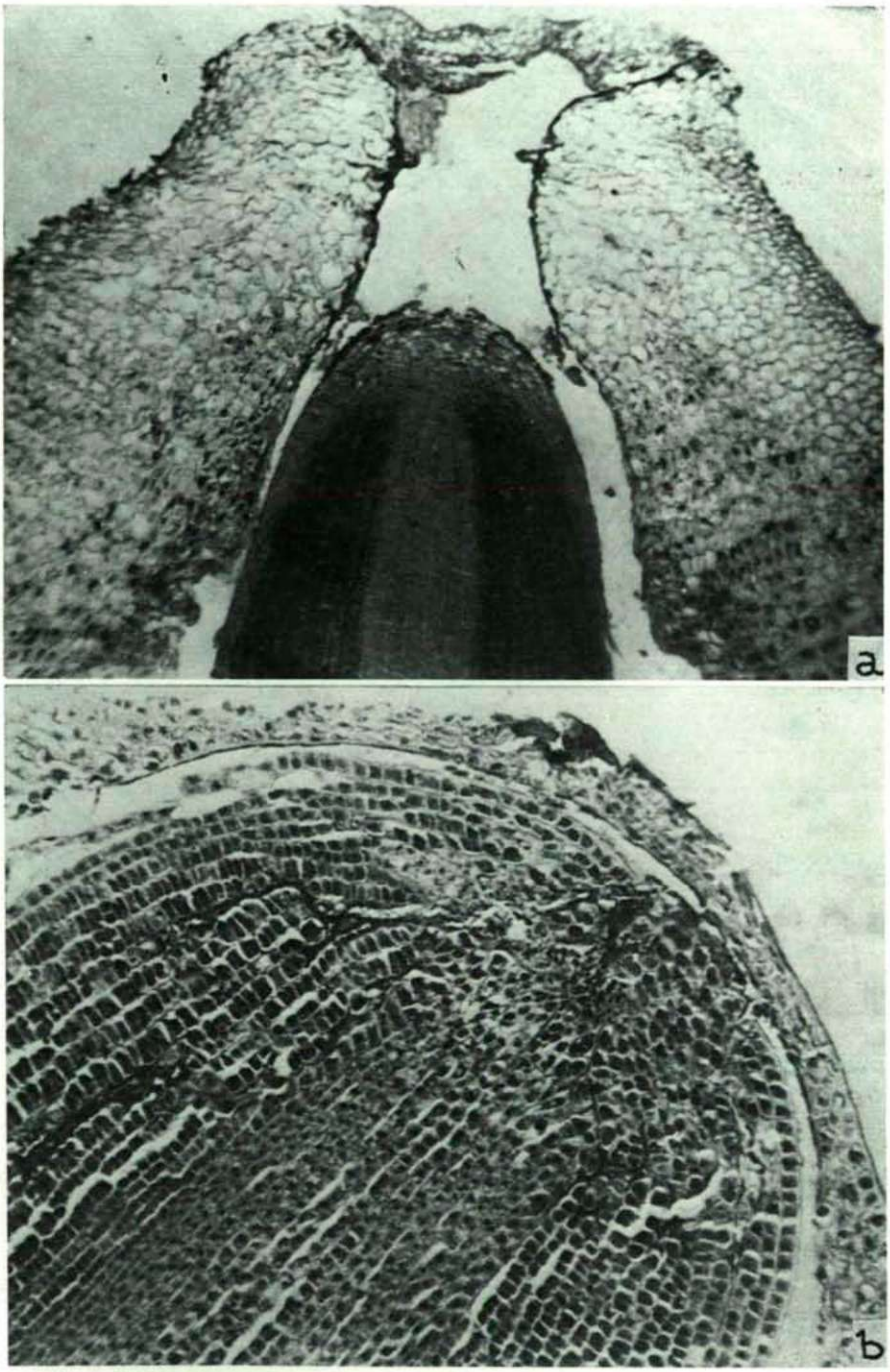

Fig. 5. Effect of GA_3 treatment on the endosperm around the radicle.
a) seed chilled-stratified for 3 months, x50.
b) seed treated with GA_3 and warm-stratified for 6 months, x123.

decreasing the mechanical resistance of tissues surrounding the embryo. As it is shown by our results, the enhancement on the gibberellin level is not enough for the cessation of the dormancy of the *Tilia* seeds since it can promote only the nutriment supply and the growth of the embryo. Probably the enhancement of the level of other hormones are also required for the weakening of the mechanical resistance of the endosperm.

References

ALSCHER-HERMAN, R. and M. MUSGRAVE and A. C. LEOPOLD and A. A. KHAN (1981): Respiratory changes with stratification of pear seeds. — Physiol. Plant *52*, 156–160.

AMEN, R. D. (1968): A model of seed dormancy. — Bot. Rev. *34*, 1–31.

ANGELO, ST. A. J. and A. M. ALTSCHUL (1964): Lipolysis and the free fatty acid pool in seedlings. — Plant Physiol. *39*, 880–883.

BASKIN, J. M. and C. C. BASKIN (1970): Replacement of chilling requirement in seeds of *Ruellia humilis* by gibberellic acid. — Planta *94*, 250–258.

BASKIN, J. M. and C. C. BASKIN (1974): Breaking dormancy in seeds of *Isanthus brachiatus* (Labiatae) with gibberellic acid. — Fiton *32*, 159–165.

COLOWICK, S. P. and N. O. KAPLAN (1955): Methods in enzymology I. New York 640–642.

JUNTTILA, O. (1970): Effects of stratification, gibberellic acid and germination temperature on the germination of *Betula nana*. — Physiol. Plant. *23*, 425–433.

KEIL, B. and Z. SORMOWA (1968): Laboratorni technika biochemie. — Nakladatelstvi Ceskoslovenské Akademie, Praha.

KOPCEWICZ, J. and Z. PORAZINSKI (1973): Influence of low temperature on germination and endogenous growth regulator contents in scots pine (*Pinus silvestris* L.) seeds. — Acta. Soc. Bot. Pol. *43*, 233–240.

LEWAK, S. and RYCHTER and B. ZARSKA-MACIEJEWSKA (1975): Metabolic aspects of embryonal dormancy in apple seeds. — Physiol. Vég. *13*, 13–22.

NAGY, M. and SZ. PATAKY and Á. KÉRI (1981): Dormancy in fruits of *Tilia platyphyllos* SCOP. V. Possible role of chilling stratification in breaking dormancy. — Acta Biol. Szeged 27, 127–137

NAGY, M. and I. SZALAI (1973): Dormancy in fruits of *Tilia platyphyllos* SCOP. I. — Acta Biol. Szeged, *19*, 71–77.

ORY, R. L. and A. J. ST. ANGELO and A. J. ALTSCHUL (1962): The acid lipase of the castor bean. Properties and substrate specificity. — J. Lipid. Res. *3*, 99–105.

RADECKE, H. R. (1967): Beiträge zur Entwicklungsphysiologie und Biochemie der Samen. — Pharmacie *22*, 97—109.

RAMAKRISHNAN, C. Y. and B. N. BANERJEE (1951): Destruction of the lipase in the oil seeds as the molds grow on them. — J. Indian Chem. Soc. *28*, 591–594.

RIMON, D. (1957): Lipase activity of germinating lettuce seeds. — Israel. Res. Council Bull. *6*, 53–55.

ROSS, J. D. and I. W. BRADBEER (1971): Studies in seed dormancy .The effects of growth retardants on the gibberellin content and germination of chilled seeds of *Corylus avellana* L. — Planta *100*, 303–308.

SMOLENSKA, G. and S. LEWAK (1974): The role of lipases in the germination of dormant apple embryos. — Planta *116*, 361–370.

SZALAI, I. and M. NAGY (1968): The causes of dormancy in *Fraxinus excelsior* L. fruits and the role of gibberellic acid in breaking dormancy. — Acta Bot. Acad. Sci. Hung. *14*, 425–433.

WHITE, P. R. (1943): A handbook of plant tissue culture. — Catell Press, Lancaster.

ZARSKA-MACIEJEWSKA, B. and S. LEWAK (1976): The role of lipase in the removal of dormancy in apple seeds. — Planta *132*, 177–181.

YAMADA, M. (1957): Studies on fat-metabolism in germinating castor beans. — Scientific Papers of the College of General Education. University of Tokyo, Japan. *7*, 94–104.

Address of the authors:
DR. MÁRIA NAGY
DR. ÁGNES KÉRI
Department of Plant Physiology
DR. SZERÉN PATAKY
Department of Botany, A. J. University,
H-6701 Szeged, P. O. Box 428, Hungary