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DORMANCY IN FRUITS OF TILIA PLATYPHYLLOS SCOP. V. POSSIBLE ROLE OF CHILLING STRATIFICATION IN BREAKING DORMANCY

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Abstract

The sizes, germinative abilities and growth capabilities of embryos excised from lime seeds stratified in a chilled and warm state, respectively, were compared. Though the embryos could grow both during the warm and the chilled stratification, the elongation of the radicle proved to be more definite during chilled stratification. Embryos excised from seeds stratified in chilled state germinated quicker and grew more intensively than the embryos excised from seeds stratified in warm state. Chilled stratification plays a role also in the weakening of tissues surrounding the embryo.

Introduction

Lime seeds require for the breaking of their dormancy a chilled stratification for 150—180 days. Similarly to most seeds requiring stratification the decrease of the amount of inhibitors and the increase of the amount of endogenous gibberellin-like substances during stratification can be observed also in the case of lime seeds (SZALAI and NAGY, 1974; NAGY, 1980). However, stratification cannot be replaced by exogenous gibberellic acid, only the lenght of the stratification period can be shortened (NAGY and SZALAI, 1973). A further reduction of the stratification period is possible by the combined application of gibberellic acid and indole-acetic acid (IAA), (NAGY, 1976), which indicates that though these hormones may play an important role in the breaking of dormancy, during the stratification besides the rise of the level of endogenous hormones also other alterations important from the aspect of the breaking of dormancy take place.

On the effect of a hormonal stimulus arriving from the embryo a change may occur in the activity of metabolism, including the decomposition of the stored nutrients, ensuring in this way the nutrient supply of the embryo. Owing to the gluconeogenesis the water potential of the embryo will be more negative and thus it may represent a greater force against the surrounding tissues whose mechanical resistance remains unchanged. During the stratification also the mechanical resistance of the tissues surrounding the embryo may change or both processes of different direction may contribute to the breaking of dormancy.

In the course of our study into the changes occurring during stratification the following problems were investigated:

— observability of embryonal growth during stratification,
— existence of a difference in growth intensity between seedlings developed from embryos excised at various periods of stratification, and
— effect of the mechanical resistance of the surrounding tissues on the dormancy of the embryo.

Materials and Methods

Material of examination: Our investigations were carried out with fruits of Tilia platyphyllos Scop. obtained from the Forestry of County Csongrád.

Stratification and excision of embryos: After the removal of the pericarp the seeds were scarified with sulphuric acid then stratified in culture pots containing washed sand wetted to 80% of the full water capacity. Stratification was carried out in a refrigerator at 4—5°C and in a thermostat at 25°C, respectively. At various periods of stratification the seeds were disinfected with bromine water then the embryos were excised and kept in a Petri dish under semisterile conditions on filter paper wetted with White nutrient (WHITE, 1943) at a daily 16-hour illumination of 10 000 Lux, at a day-time temperature of 25°C and at a night temperature of 20°C.

Measurement of clorophyll content: the chlorophyll content of the leaves of the seedlings was determined by photometry, using the method of WINTERMANS and MOTS (1965).

Treatment of seeds with exogenous pectinase and cellulase

Also the seed-coat was removed from a part of seeds disinfected with bromine water and they were treated for 7 days with a 1% cellulase preparation (Fluka) prepared by a phosphate buffer of pH 5.0. Another part of the seeds was treated with a 1% pectinase preparation (Fluka) prepared by a phosphate buffer of pH 4.0 whereas a third part of the seeds similarly for 7 days with a preparation of 1% cellulase+1% pectinase.

During the treatment period the seeds were incubated at 25 °C, on exchanging each day twice the enzyme preparation. After the 7-day treatments with the enzyme preparation a part of the seeds was placed in a thermostat of 25 °C and another part in a refrigerator. In both cases also the influence of a treatment with 3.10^{-4} m GA_3 or with 3.10^{-4} m $GA_3 + 3.10^{-4}$ m IAA on the germination of seeds after the exogenous enzyme treatment was investigated (NAGY, 1976). The result was evaluated after the elapse of a month.

Histological investigations. After the removal of the seed-coat the seeds were fixed by the Juel-type fixina agent (SÁRKÁNY and SZALAI, 1968) and cross and longitudinal cuttings were prepared by embedment in celloidin. Hematoxylin of Ehrlich type was used for the staining of the preparations.

Results and discussion

Effect of chilled and warm stratification on the growth of embryos present in intact seeds

Since the embryos removed from dormant lime seeds are geminating quickly in a wet environment without any pretreatment and no signs of any morphological abnormity can be observed on the developing seedlings whereas at the same time a chilled stratification is required for the breaking of dormancy of the intact seed, it appeared to be of interest to examine whether a difference exists between the size and the growth intensity of embryos removed from warm stratified and chilled stratified seeds.

As shown by Fig. 1, the embryos are growing both during warm stratification and during chilled stratification. Though at the beginning the warm stratification appears to be more favourable for the growth of embryos, the elongation of the radicle

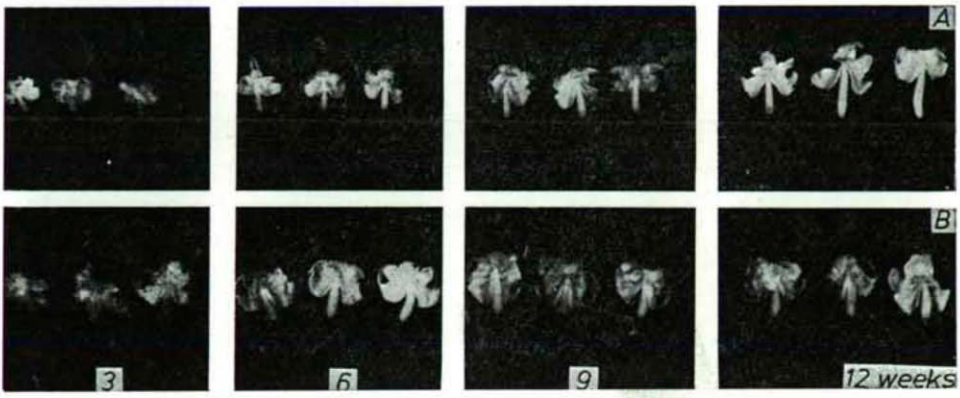

Fig. 1. Embryos excised from seeds chilled stratified (A) and warm stratified (B) for various periods.

is more intensive during chilled stratification. The difference in the size of embryos at the various periods of stratification is due not only to water uptake because also the dry matter content of the embryos increases with the length of stratification periods, indicating that an actual growth takes place (Table 1).

Table 1. Fresh mass and dry matter content of embryos excised from seeds stratified for various periods, referred to 100 embryos

Stratification period, weeks	5 °C		25 °C	
	Fresh mass, g	Dry mass, g	Fresh mass, g	Dry mass, g
3	0.8442	0.3862	0.8585	0.3855
6	1.2132	0.4341	1.1971	0.3909
9	1.6972	0.5963	1.4039	0.4876
12	1.8584	0.6616	1.6308	0.6011

The more vigorous development of embryos stratified at 5 °C is proved also by the histological investigations (Fig. 2). Striking differences appear in the thickness of the cotyledons and cell-walls on the effect of treatments at both applied temperatures. The more favourable effect of stratification at 5 °C manifests itself also at the shoot apex in the size and in the differentiated nature of the shoot apex. However, this difference is not as definite as in case of the cotyledones.

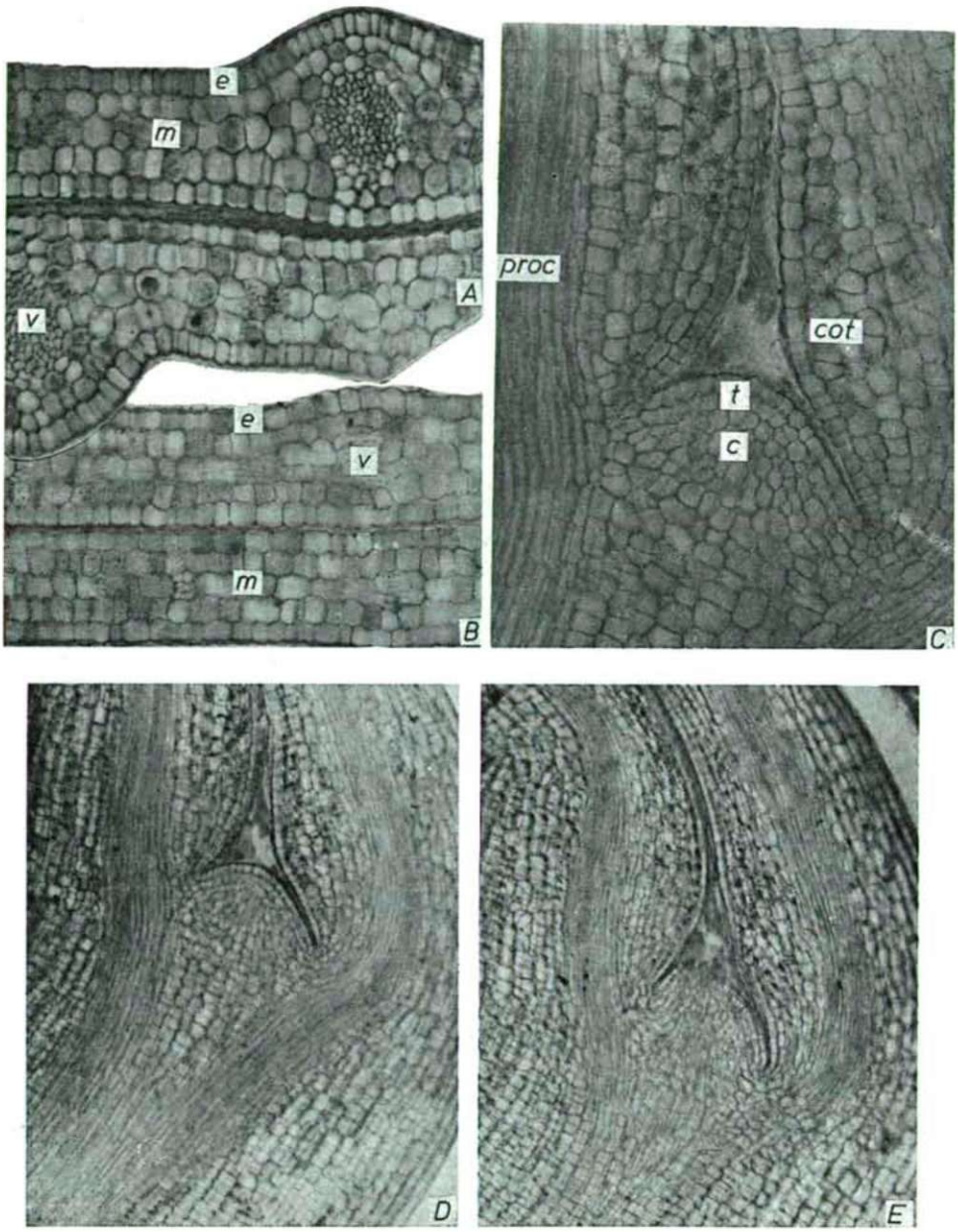

Fig. 2. State of development of embryos stratified for two months at various temperatures
A: cross section of the cotyledon of an embryo stratified at 5 °C. e=epidermis, m=mesophyllum, v=vascular tissue; x270
B: cross section of the cotyledon of an embryo stratified at 25 °C. x270
C—D: median longitudinal section of the shoot apex of an embryo stratified at 5 °C. c=corpus, cot=cotyledon, proc=procambium, t=tunica. C=x270, D=x140.
E: median longitudinal section of the shoot apex of an embryo stratified at 25 °C, x140

Growth intensity of embryos excised at various periods of stratification

Embryos excised at various periods of stratification differ from each other in the speed of germination (Fig. 3) (in the case of excised embryos the first geotropic curvature of the radicle is considered as germination). The longer was the period of chilled stratification, the higher is the percentage of germination in the first 24 hours. A parti-

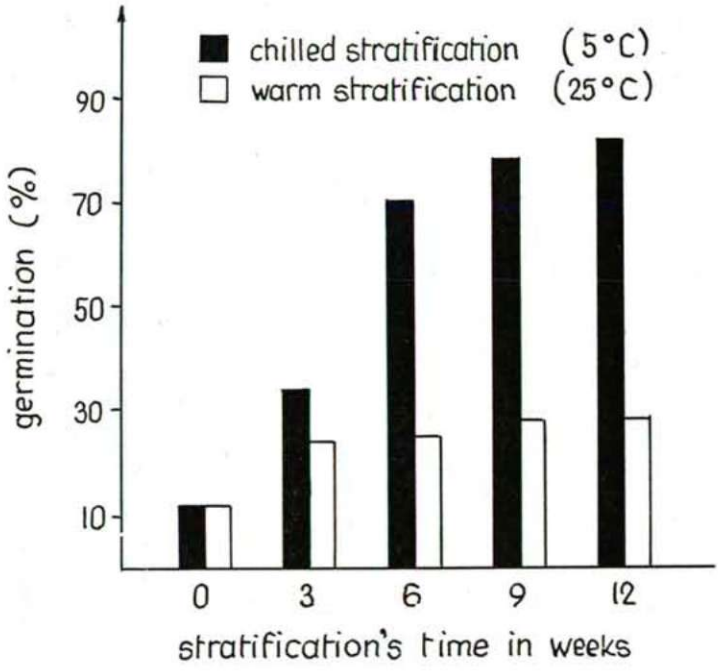

Fig. 3. Germination of embryos stratified for various periods, observed 24 hours after excision

cularly great change is observable after six weeks of chilled stratification. Embryos warm stratified for various periods are germinating much slower and hardly differ from each other from the aspect of their vigour of germination.

The growth activity of embryos excised at various periods of stratification is presented in Fig. 4. where the size of seedlings is shown at the seventh day after excision, expressed as percentages of the non-stratified controls.

Similarly to the results of germination tests, the growth intensity of embryos warm stratified for various periods is very similar but even in case of a stratification of the shortest duration higher than that of the non-stratified control.

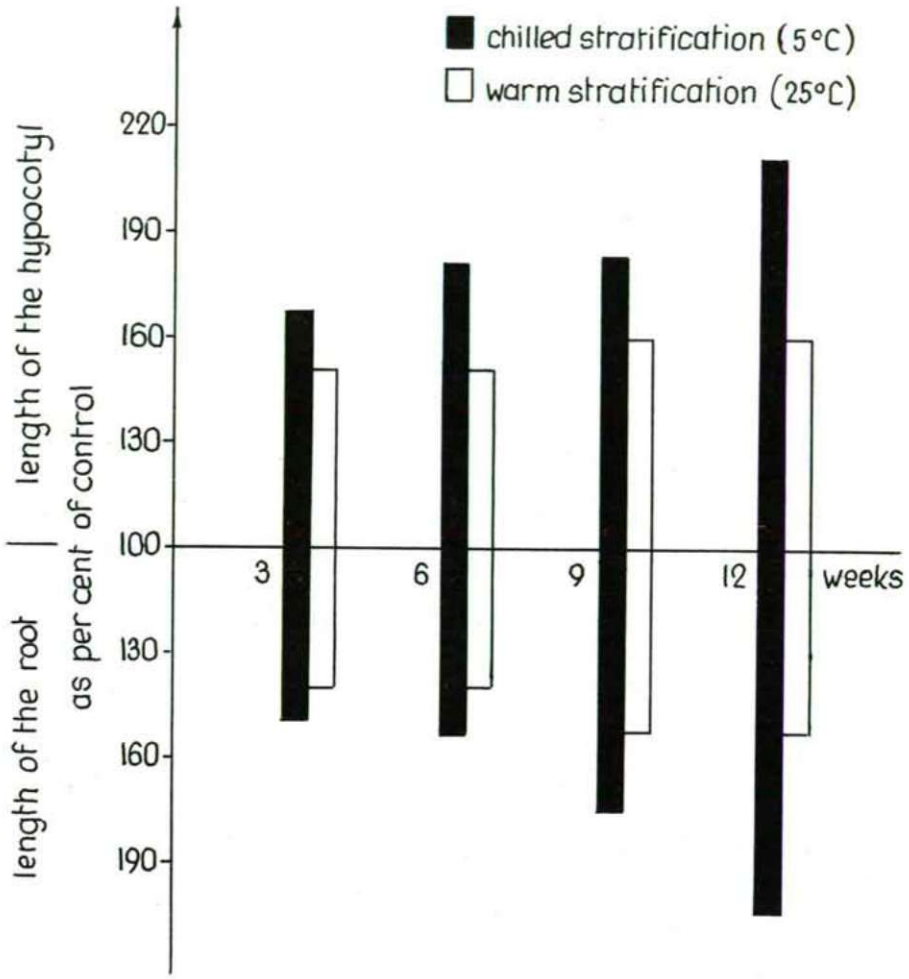

Fig. 4. Size of seedlings developed from embryos excised at various periods of stratification, observed on the seventh day after excision and expressed as percentages of the non-stratified control

Seedlings originating from chilled stratified embryos were at all investigated periods larger than seedlings developed from warm stratified embryos, indicating clearly that chilled stratification plays an important role in the increase of the capability of growth of the embryo. These statements are supported also by our results concerning the investigation of the degree of verdure (Fig. 5, A and B). As shown by these figures, in the leaves of the seedlings developed from chilled stratified embryos the formation of chlorophyll is more intensive than in the seedlings developed from warm stratified embryos.

Effect of the mechanical resistance of the surrounding tissues on the dormancy of the embryo

Low temperature may exert a favourable effect on the germination of seeds in two directions:

1. it may increase the capability of growth of the embryo which may represent at the end of the period of chilled stratification a greater stretching force against the surrounding tissues whose mechanical resistance remained unchanged, and thus the radicle will be capable of breaking through the surrounding tissues,

2. during the chilled stratification also the mechanical resistance of the surrounding tissues may decrease and this promotes the breakthrough of the radicle.

As indicated by our results presented thus far, the capability of growth of the embryo increases during the chilled stratification. However, the capability of growth

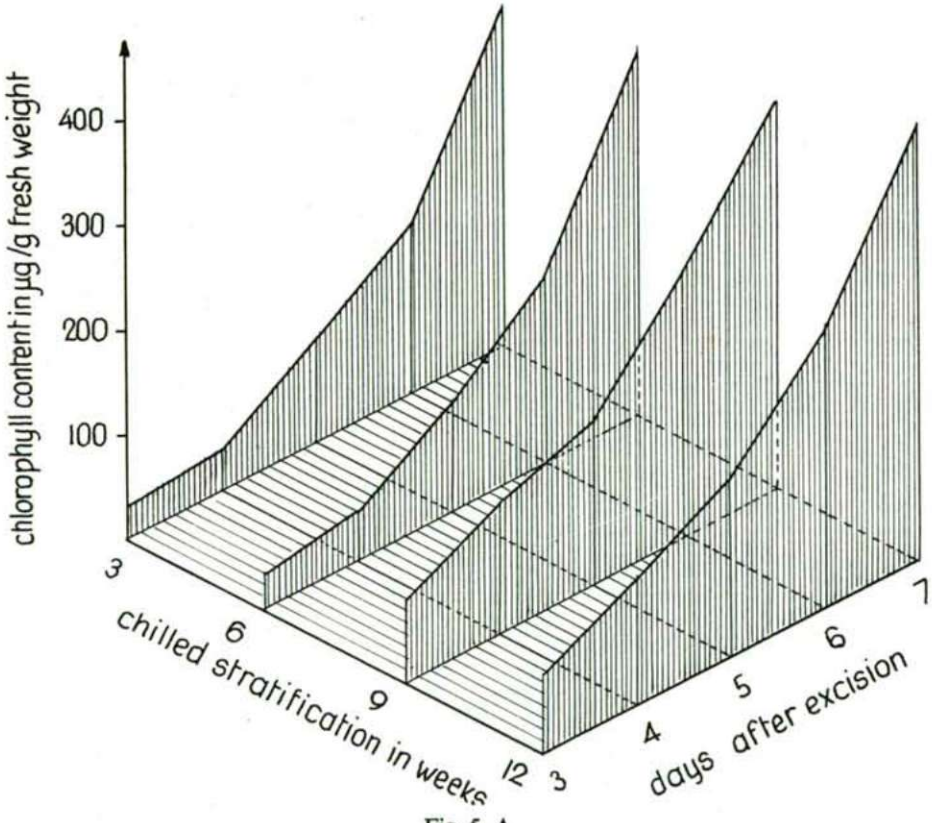

Fig. 5. A

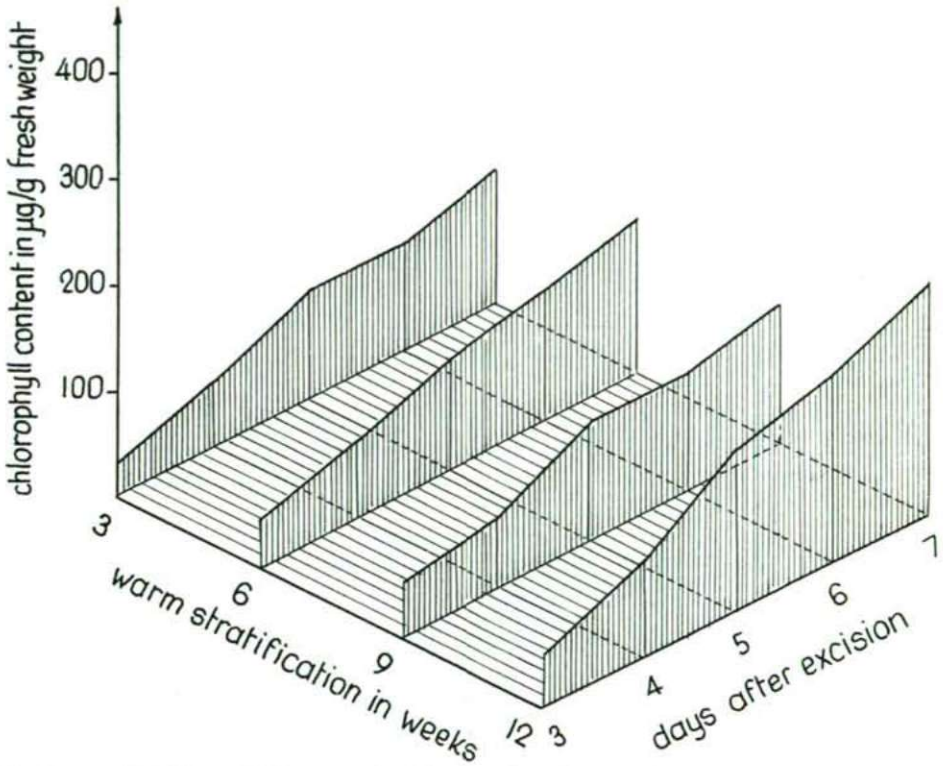

Fig. 5. (A and B) Chlorophyll content of the leaves of seedlings developed from embryos excised at various periods of stratification, determined for 7 days after excision

increases also during warm stratification in comparison to the capability of the non-stratified control, furthermore even on the effect of a treatment with exogenous GA_3, in certain cases to such an extent that the embryo gets crushed in the seed as an accordion (SZALAI and NAGY, 1968), and the radicle cannot break through the surrounding tissues.

In connection with the possible role of the mechanical resistance of the tissues surrounding the embryo in dormancy the effect of the breaking up of the ends of the seeds at the radicle or the apex, respectively, was investigated on the germination of seeds.

This operation was carried out after disinfecting the seeds by bromine water. This was done manually at diffuse light, with the use of a scalpel, a lance-shaped needle and a dissecting needle, then the seeds were placed in a Petri dish on sterile filter paper wetted with water containing 100 μg/ml streptomycin and incubated in a 25 °C thermostat. The result visible on the fourth day after this operation is shown in Fig. 6.

Seeds operated at their end near the radicle germinated in all cases, quite independently of being treated previously by chilling. The higher growth intensity observable in chilled stratified seeds is quite in accordance with our earlier results ob-

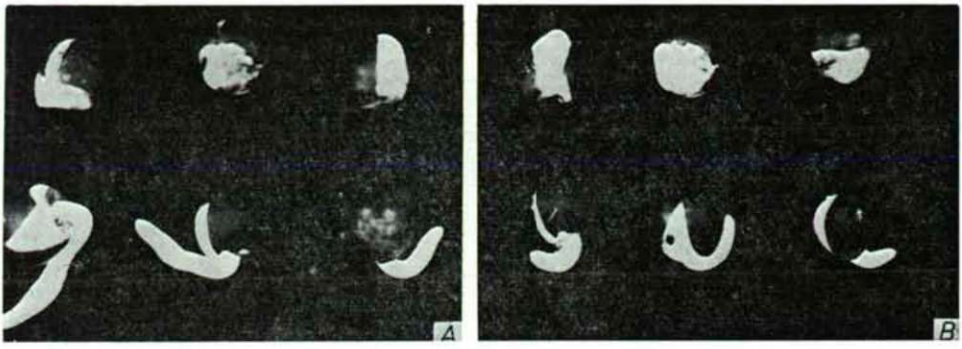

Fig. 6. Effect of operative treatment on the germination of seeds
A: seeds stratified at 5 °C for 2 months
B: seeds stratified at 25 °C for 2 months.

tained with excised embryos. In contrast to that, germination was not observed at all in seeds operated at their ends near the apex.

Attempts were made to clear up the role of the mechanical resistance of the tissues surrounding the embryo by experiments where the seeds were treated with exogenous cellulase and pectinase. Our aim was here to weaken the mechanical resistance of tissues surrounding the embryo and to observe the germination of seeds at 5 °C and at 25 °C.

The best results were obtained at the treatment with 1% cellulase. Experimental data are shown in Fig. 7, where the dark columns refer to seeds with intact seed-coat and the light columns to results obtained with seeds whose seed-coat was previously removed.

It can be seen in Fig. 7 that 100% germination could be observed in case of seeds whose seed-coats were removed and which were kept after their treatment with the cellulase preparation for one month at 5 °C and were subsequently treated with GA_3+ +IAA. The percentage of germination was in case of seed-coat-less seeds in all the cases higher than in tests with intact seeds. The results indicated as well that at low temperature the treatment with hormones is more efficient than at higher temperature. This may be in correlation with the conditions of the entrance of the hormones.

Thus, the primary role of chilled stratification may be likely not the ensuring of the growth of the embryo but rather the increase of the capability of germination of the embryo. Simultaneously with this process a weakening of the mechanical resistance of the tissues surrounding the embryo occurs on the effect of enzymes of key importance whose synthesis or activation starts at the impulse of chilled stratification. This presumption is confirmed also by the results obtained with other species requiring chilled stratification (OLNEY and POLLOCK, 1960; VILLIERS and WAREING, 1965; VANSTONE and LA CROIX, 1975), in experiments wherein the growth of embryos could be experienced during warm stratification but germination started only after chilled stratification. It can be imagined that similarly to the observations made in case of celery (JACOBSON et al., 1976) also in the lime seeds a quicker degradation of the cells located in the environment of the radicle occurs at low temperature.

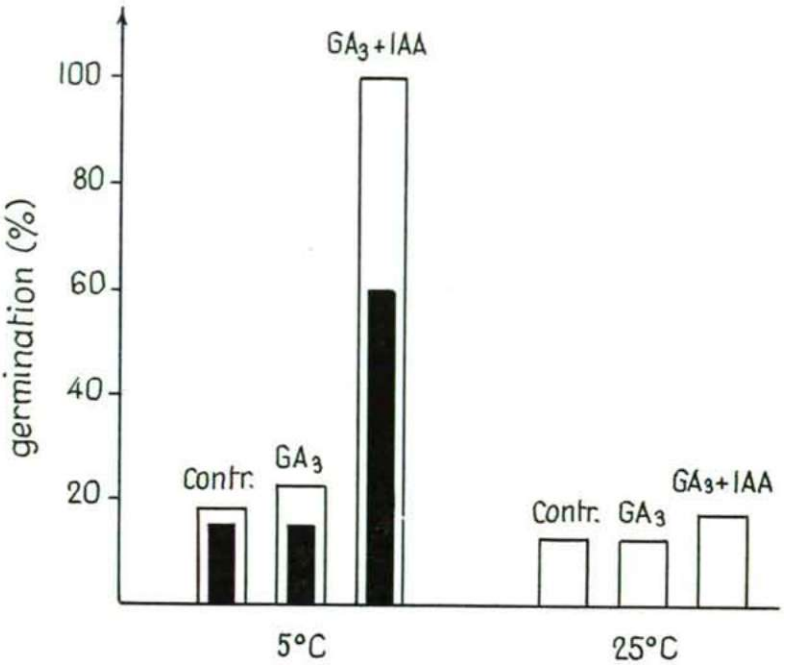

Fig. 7. Effect of exogenous cellulase treatment on the germination of seeds. Dark columns indicate the results obtained with scarified seeds whereas light columns show the results obtained with seeds whose seed-coats were removed previously.

References

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

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

FRANKLAND, B. (1961): Effect of gibberellic acid, kinetin and other substances on seed dormancy. — Nature, *192*, 678.

FRANKLAND, B. and WAREING, P. F. (1962): Changes in endogenous gibberellins in relation to chilling of dormant seeds. — Nature *194*, 313—314.

FRANKLAND, B. and WAREING, P. F. (1966): Hormonal regulation of seed dormancy in hazel and beech. — J. Exp. Bot. *17*, 596—61.

JACOBSEN, J. V., PRESSMAN, E. and PYLIOTIS, N. A. (1976): Gibberellin-induced separation of cells in isolated endosperm of celery seed. — Planta *129*, 113—122.

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

NAGY, MÁRIA (1976): Dormancy in fruits of *Tilia platyphyllos* Scop. III. The possible role of auxin and cytokinin in the breaking of dormancy. — Acta Biol. Szeged. *22*, 47—55.

NAGY, MÁRIA (1980): Dormancy in fruits of *Tilia platyphyllos* SCOP. IV. Changes in the endogenous gibberellin content during the stratification of the seeds. — Acta Agron. *29*, 1—11.

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

OLNEY, H. O. and POLLOCK, B. M. (1960): Studies of rest period. II. Nitrogen and phosphorus changes in embryonic organs of after-ripening cherry seed. — Plant Physiol. *35*, 970—975.

SÁRKÁNY, S. and SZALAI, I. (1957): Növényszervezettani gyakorlatok (Phytomorphological exercises) (in Hungarian). — Tankönyvkiadó, Budapest, Hungary, pp. 524—577.
SZALAI, I. and NAGY, MÁRIA (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.
SZALAI, I. and NAGY, MÁRIA (1974): Dormancy in fruits of *Tilia platyphyllos* SCOP. II. The inhibitor substance content of dormant fruits. — Acta Bot. Acad. Sci. Hung. — *20*, 389—394.
VANSTONE, D. E. and LA CROIX, L. J. (1975): Embryo immaturity and dormancy of black ash. — J. Am. Soc. Hort. Sci. *100*, 630—632.
VILLIERS, T. A. and WAREING, P. F. (1965): The possible role of low temperature in breaking the dormancy of seeds of *Fraxinus excelsior* L. — J. Exp. Bot. *16*, 519—531.
WHITE, P. R. (1943): A handbook of plant tissue culture. — Catell Press Lancaster.

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