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CHANGE IN THE ENDOGENOUS GIBBERELLIN CONTENT DURING SWELLING OF LUPINUS ALBUS L. SEEDS

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Abstract

The quantitative change in the various gibberellin forms occurring in *Lupinus albus L.* seeds was investigated during swelling with the lettuce hypocotyl and barley endospermium tests.

We demonstrated various gibberellin forms from dry seeds, including the free gibberellin-like substances. Our results show that probably not the appearance but the suitable level of the free gibberellins is necessary to germination. The level of the free gibberellin-like substances increased by leaps and bounds as early as in the firts few hours of swelling, achieving its maximum in the barley endospermium test in the sixth, and in the lettuce hypocotyl test in the eighteenth, hour of swelling. Considerable biological activity was observed in the butanol-soluble fraction, probably due to the action of nonspecific glucosidases. The amount of the gibberellin-like substances in the butanol-soluble fraction already showed a declining tendency in the early period of swelling. A change of similar character was observed in the case of the TCA-insoluble fraction, as well.

Between the endogenous gibberellin contents of the seeds swelled in the light and in darkness no difference was observed.

The quantitative increase in the free gibberellin-like substances is supposedly the result of the release of bound forms because the emergence of the radicula is not inhibited by CCC, the inhibitor substance of the biosynthesis of gibberellin.

Introduction

During maturing of the seeds, the transformation of free gibberellins into bound gibberellins can be observed (SEMBDNER et al., 1968). The results connected with the role played by bound gibberellins in the germination of seeds are not unambiguous. There are but few investigations of this character in the literature to be found, and these data are concerned with the conditions after the emergence of the radicula.

According to the statement of BARENDSE et al. (1968), the pea seedling is not sensitive to AMO—1618. For this reason they suppose that the increase in gibberellin content is not connected with synthesis. At the same time, seedling Pharbitis can be inhibited by AMO—1618 immediately after germinating; therefore, according to BARENDSE et al. (1968) it is not proved that the bound gibberellins play a role in the early development of Pharbitis. In the opinion of SEMBDNER et al. (1968), in the germination of beens gibberellin is de novo synthesized.

The change in the endogenous gibberellin content before the emergence of radicula was investigated in some cases in seeds in a state of deep rest (ROSS and BRADBEER, 1971; SZALAI and NAGY, 1974) but these statements only refer to a change in the free endogenous gibberellin content, without comprising the role of bound gibberellins.

If we take germination in a stricter sense of the word, meaning by that the activation of the cells of the seed, and regarding the observable germination, i. e., the protrusion of some part of the embryo from the seed as a result of growth (MAYER and POLJAKOFF—MAYBER, 1963), then we have to perform the investigation of the quantitative change in the endogenous gibberellin content, taking place in the germinating seeds, in the period preceding the emergence of radicula. This period is generally called swelling. But the physical processes of swelling and the physiological processes of germination cannot be isolated from each other with a sharp borderline.

The investigation of the change in the endogenous gibberellin forms, as well as of the problem of how the change of the endogenous free gibberellin level takes place before the radicula starts was carried out by using an object of a well-known high endogenous gibberellin content.

Materials and Methods

For our investigations we used Lupinus albus L. seeds obtained from the Gyulatanya-Station of the Agricultural Research Institute of Nyir (a district in North-Eastern Hungary).

Measurement of the change in weight of seeds during swelling

50—50 seeds were swelled, at 20 °C, with plenty of water. The seeds, removed from the water and dried and blotted with filter-paper, were weighed on an analytic balance in every four hours. The dry-matter content of seeds was determined in a desiccator at 105 °C, after being dried till achieving weight equilibrium.

Extraction and chromatographing of gibberellins

50 seeds were extracted after grinding with 80 per cent cold methanol of tenfold quantity in a refrigerator for 2×24 hours. The united methanolic extract was isolated according to the method of HARADA and YOKOTA (1970), into ethylacetate-soluble and butanol-soluble acid fractions. The ethylacetate-soluble acid fraction was distilled at reduced pressure and chromatographed on a silica gel G layer. For the development, a diisopropylether: acetic acid (95:5) solvent was used (REINHARD et al., 1964).

The butanol-soluble acid fraction was divided into two parts. One of these was chromatographed after being distilled at reduced pressure. The solvent was chloroform: methanol: acetic acid: water (40:15:3:2) (HARADA and YOKOTA, 1970). The other part was hydrolysed with 1 N H_2SO_4, at 100 °C, for two hours (YOKOTA et al., 1969).

The gibberellins released during hydrolysis were extracted with ethylacetate, then the ethylacetatic extract was distilled at reduced pressure and chromatographed with thin layer. The tissue-homogenizate, a residue after the methanolic extraction, was suspended in phosphate-buffer (pH 8,0) after distilling the remains of the solvent, and centrifuged after standing for 24 hours. After being centrifuged, the supernatant was treated with 10 per cent trichloracetic acid (TCA). After being centrifuged again, the precipitate was hydrolyzed with 2 N NaOH, at 40 °C, for four hours. After being acidified with hydrochloric acid (pH 2,8), the gibberellins released were extracted with ethylacetate, distilled, and chromatographed with thin layer.

Lettuce hypocotyl test

After distilling the residue of the solvent, the chromatogram developed was divided into ten equal parts and the silica gel was scraped down into Petri dishes of 7 cm∅. The powder was covered with a filter-paper disk and wetted with 3 ml distilled water. The biological activity was measured by the method of Frankland and WAREING (1960). A layer, developed and tested, and containing no plant-extract, was used as control. For the investigations, the lettuce variety "King of May" was used.

Barley endospermium test

The silica gel scraped down from the chromatogram strips was eluated with ethylacetate, or n-butanol. Then after being centrifuged, it was distilled dry at reduced pressure, dissolved in acetate-buffer (pH 4,8) containing 2 ml 20 μ M $CaCl_2$, filtered through a bacterial filter, poured into a 5 cm ∅ Petri dish, and tested with barley endospermium under sterile conditions, according to the method

of JONES and VARNER (1967). After being incubated for 24 hours, to 1 ml of the incubation solution 1 ml of 1 per cent starch-solution prepared with acetate-buffer (pH 4,8) containing 1 ml 20 μM $CaCl_2$ was added. The incubation was carried out at 40 °C, for ten minutes. Then 1 ml was removed and we added to it 10 ml solution of 0,0003 N KJ—J_2, prepared with hydrochloric acid. The optical density of the solution was measured at 580 nm.

The amount of α-amylase was calculated by means of the formula given by JONES and VARNER (1967). As a control, a developed and tested layer was used, containing no plant extract.

Treatment of the seeds with a chlorcholine-chloride (CCC) solution

Seeds 50 per Petri dish were germinated in Petri dishes containing filter-paper with 500, and 1000 ppm CCC, respectively, in a 25 °C thermostat. Control seeds were placed a Petri dish containing some filter-paper wetted with distilled water.

Results and discussion

1. Change in weight of the seeds during swelling

The change in weight of the Lupinus albus seeds is illustrated in Fig. 1. As seen in the figure, the seeds swelled rapidly. They take up 69 per cent of the total water quantity taken during the whole length of swelling time by the fourth hour, and 78 per cent by the seventh hour.

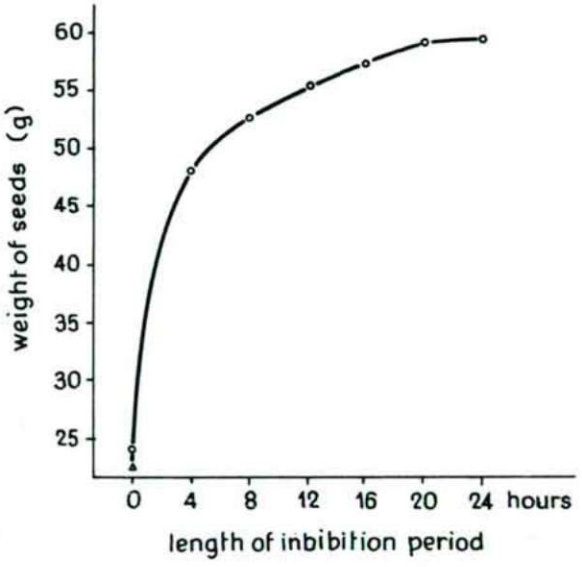

Fig. 1. Change in weight of 50 *Lupinus albus* seeds during swelling, at 20° C.

During the length of swelling time we did not observe any essential change in the dry-matter content of the seeds.

2. Quantitative change in the endogenous free gibberellin—like substances during the swelling of seeds

The change in the endogenous gibberellin content during the swelling of Lupinus albus seeds was measured with two kinds of biological tests, because the biological activity of the various gibberellins was different in the different tests (REEVE, 1974). The barley endospermium test is sensitive to lower gibberellin concentrations than

the lettuce hypocotyl test; it is not impeded by the possible solvent residue in the extract. The lettuce hypocotyl test, on the other hand, is sensitive to a wider spectrum of gibberellins (JONES and VARNER, 1967).

The quantitative change of the endogenous free gibberellin-like substances during swelling is shown in Fig. 2 (under A and B).

With the barley endospermium test, the presence of three gibberellin-like substances of different Rf-values was demonstrated, showing maximum activity in the sixth hour of swelling. The presence of these substances was also indicated by the lettuce hypocotyl test (Fig. 2, B), as well as an active spot of the position Rf 0,3—0,4. To the gibberellin-like substance in the position Rf 0,3—0,4 the barley endospermium

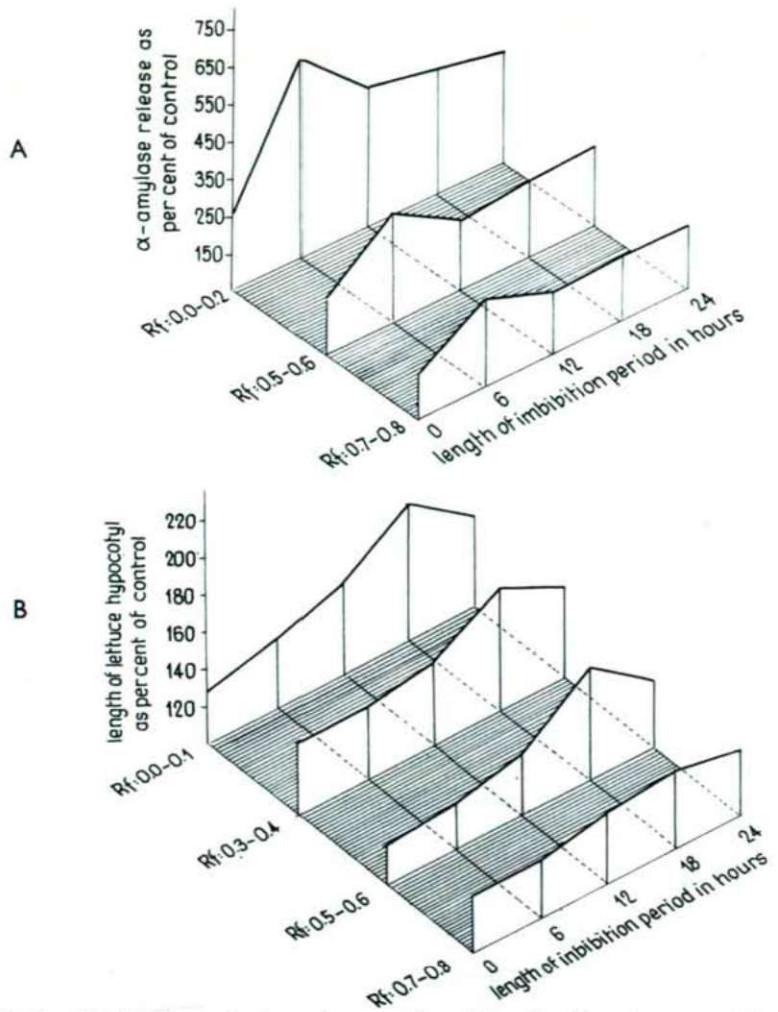

Fig. 2. Quantitative change in the endogenous free gibberellin-like substances of the *Lupinus albus* seeds, during swelling, measured as compared to 100 seeds, with the barley endospermium test (A) and the lettuce hypocotyl test (B).

test was not sensitive. We observed the maximum biological activity in the lettuce hypocotyl test in the 18th hour of swelling. The cause of the time difference between the maxima of the two tests may have been in connection with the different gibberellin-sensitivity of the tests. (In the case of the barley endospermium test the maximum enzyme activity was obtained at 0,1 ppm; in the case of the lettuce hypocotyl the maximum growing with extension was attained at a concentration of 10 ppm GA_3).

3. Quantitative change in the gibberellin—like substances in the butanol—soluble fraction during the swelling of seeds

According to our results, the butanol-soluble fraction show significant biological activity in both tests.

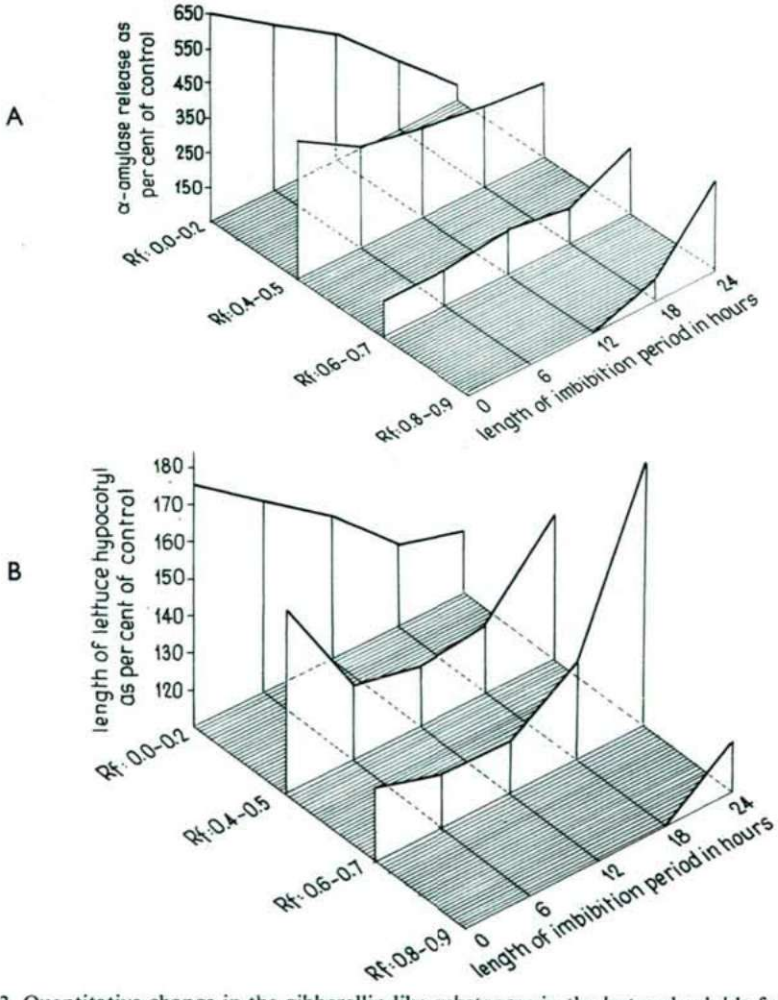

Fig. 3. Quantitative change in the gibberellin-like substances in the butanol-soluble fraction, during the swelling of *Lupinus albus* seeds, measured as compared to 100 seeds, with the barley endospermium test (A) and the lettuce hypocotyl test (B).

The biological activity observed may have been a result of the effect of gibberellins released by the non-specific glycosidases. Under normal conditions, these glycosidases do not encounter the endogenous gibberellin conjugata (Reeve, 1974).

The quantitative change in the gibberellin-like substances in the butanol-soluble fraction is illustrated in Fig. 3 (under A and B).

The quantity of the gibberellin-like substance of position Rf 0,0—0,2 tends to decline, according to the results of both barley endospermium and the lettuce hypocotyl test.

After an initial decrease the quantity of the gibberellin-like substance of positions Rf 0,4—0,5 and Rf 0,6—0,7 increased 24 hours after the beginning significantly.

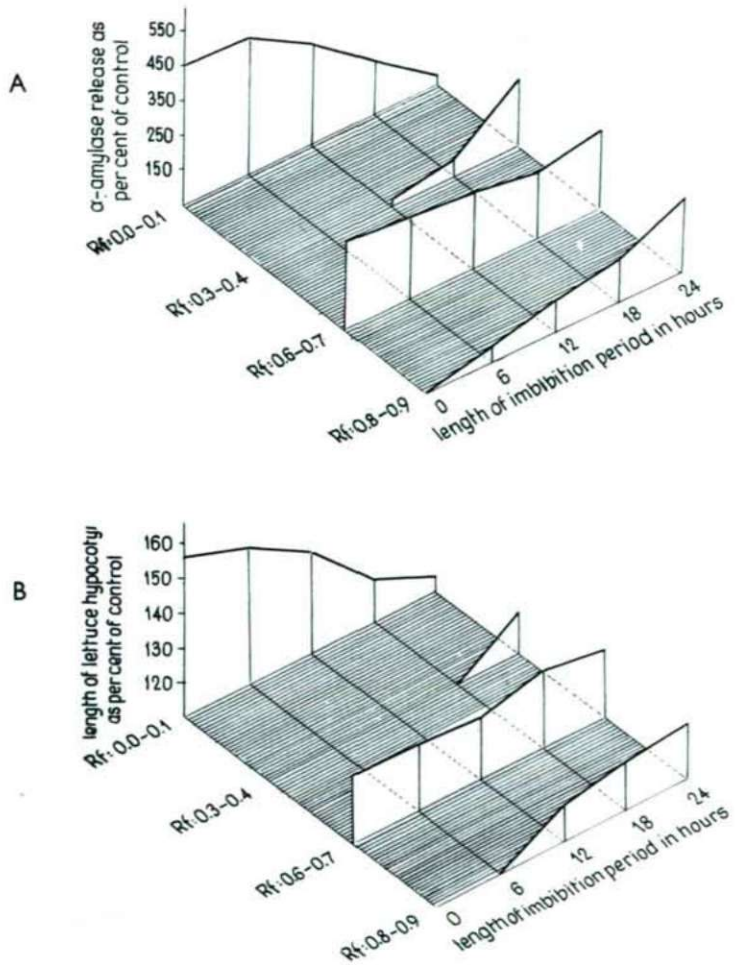

Fig. 4. Biological activity of the butanol-soluble fraction, after acid hydrolysis, measured as compared to 100 seeds, with the barley endospermium test (A) and the lettuce hypocotyl test (B).

The gibberellin-like substance in position Rf 0,8—0,9 could only be demonstrated from the 12th, and 18th hour of swelling respectively.

The tendency of the quantitative change in the gibberellin-like substances in the butanol-soluble fraction is not identical. The differences observed are difficult to interpret as we do not know if any of the gibberellin-like substances occuring in the seed act as regulators of germination.

The results of the biological testing after acid hydrolysis of the butanol-soluble fraction are shown in Fig. 4 (under A and B).

A quantitative change of similar tendency was seen in the results of testing before hydrolysis, but the biological activity was in any case lower. In the case of hydrolysing with β-glycosidase the results achieved were similar, as well.

The Rf-values of the biologically active substances obtained after the acid hydrolysis of the butanol-soluble fraction and of the free gibberellin-like substances in the ethylacetatic fraction do not always agree with one another. This, and the fact that with the α amylase test, after the hydrolysis of the butanol-soluble fraction, more gibberellin-like substance could be demonstrated than in the ethylacetatic fraction show that in the course of swelling we probably also have to reckon with interconversion. A convincing answer to this question can be given after the investigation of the qualitative change in the endogenous gibberellins.

4. The quantitative change in the gibberellin-like substances of the TCA-insoluble fraction during swelling

The presence of protein-bound gibberellins was established by a number of authors (McComb, 1961; Jones, 1964; Hayashi and Rappaport, 1962; Reinhard and Sacher, 1967). The nature of the gibberellin-protein complexes is not known, but the fact that gibberellin-like substances can be easily isolated from protein, shows that they are linked together with comparatively weak bonds.

These complexes precipitate as a result of the organic solvent applied during extraction. The amount of the gibberellins bound to the macromolecules can therefore be determined by using the tissue-remains after the evaporation of the solvent residues (Jones, 1964).

The biological activity, observed after the alkaline hydrolysis of the TCA-insoluble fraction and its extraction with ethylacetate at acid pH, is shown in Fig. 5 (under A and B).

In the TCA-insoluble fraction, a small amount of gibberellin-like substance was demonstrated with both tests. The tendency of the quantitative changes is similar to that of the gibberellin-like substances in the butanol-soluble fraction.

5. The effect of CCC-treatment on the germination of seeds

In order to decide if the increase in the endogenous free gibberellin content observed during the swelling of *Lupinus albus* seeds results from the release of bound forms, or from de novo synthesis, we germinated seeds in CCC solutions of 500, and 1000 ppm concentration. CCC is a biosynthesis retardant often applied to both lower and higher plants (Harada and Lang, 1965; Jones and Phillips, 1966). It impedes the cyclization process of geranyl-geranyl-pyrophosphate. Our results are illustrated in Fig. 6. According to our investigations, the emergence of the radicula is not impeded.

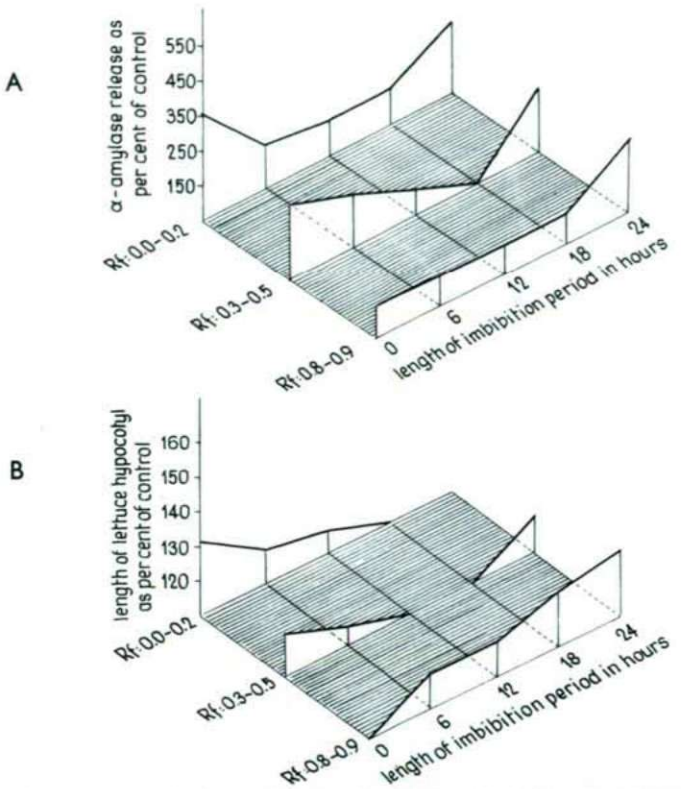

Fig. 5. Quantitative change in the gibberellin-like substances of the TCA-insoluble fraction, during the swelling of *Lupinus albus* seeds, measured as compared to 100 seeds, with the barley endospermium test (A) and the lettuce hypocotyl test (B).

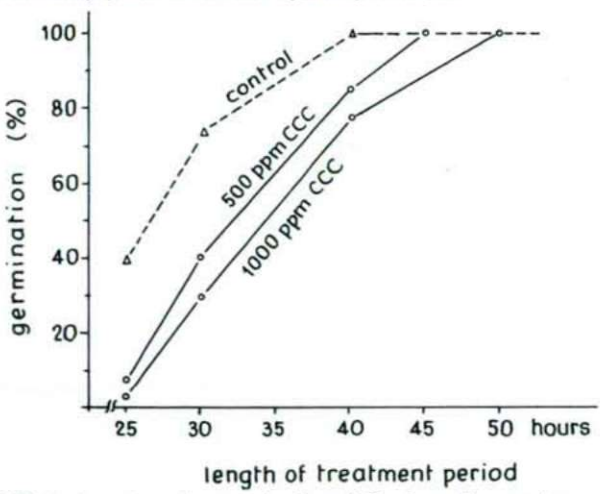

Fig. 6. Effect of CCC treatment on the germination of *Lupinus albus* seeds.

This means that the increase in the endogenous free gibberellin content is probably due to being released, and that is the main moment during swelling. As germination is retarded by the CCC-treatment as compared to the control, we possibly have to also reckon with minor de novo synthesis, and the speed of germination depends upon that.

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