METHODOLOGICAL EXAMINATIONS CONCERNING THE GROWING OF OAT SEEDLINGS FOR AUXIN-ASSAY

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Introduction

For determination of growth-substances, especially for auxin-assay, the most wide-spreading method is the use of the *Avena* straight-growth coleoptile test. According to our observations, the most critical factor in such examinations is to obtain oat coleoptiles of suitable size, i. e. 20 mm, at the time required. Therefore it is very important that the time needed for the growing of the oat seedlings should be standardized by keeping precisely all the external conditions.

The most favourable circumstances of the growing of oat coleoptiles were studied thoroughly by several authors (1, 3, 6, 7). However, no publication is known dealing with the role and effect of the inhibiting substances, occurring in the husks of the corns, on the growth and auxin-sensitivity of the coleoptiles.

The presence of growth- and germination inhibiting substances in the husks is known for a long time (2), however, the water-soluble inhibitors in husks were studied in detail only recently (4). With paper chromatography, KÖVES (4) separated six inhibiting substances from the water-extract of oat-husks and in the course of the identification they proved to be phenolic acids and their depsides and polydepsides, respectively. While growing the test seedlings, of course, we must reckon with their presence, their leaching into the medium, and therefore, with their biological effect on the germinating corns and on the developing seedlings. The aim of this paper is to analyse the role this factor.

Results and conclusions

Avena sativa L. of „FLEISCHMANN" variety, harvested in 1959 was used for experimental material.

To clear up the effect of the husk, experiments in PETRI dishes were made as preliminary work. 100—100 oat-corns were germinated on filter paper wet-

ted with water of equal quantity. The lst sample contained oats with husk, the 2d one oats without husk and the 3d the same but the husks were put beside the naked corns. The potency and the percentage of the germination obtained in these three samples are shown in Fig. 1. From the data we can draw the conclusion that the husks retard the germination only in part owing to their inhibitor content, since the potency of the germination observed in the 3d sample is greater than that of the intact oats (1st sample) but fall behind the germination of the dehusked corns (2d sample). In addition to the inhibitor content the husks delay the germination because they are a mechanical obstacle, further because they retard the swelling of the corns; that was demonstrated by measuring the swelling of oats with and without husk.

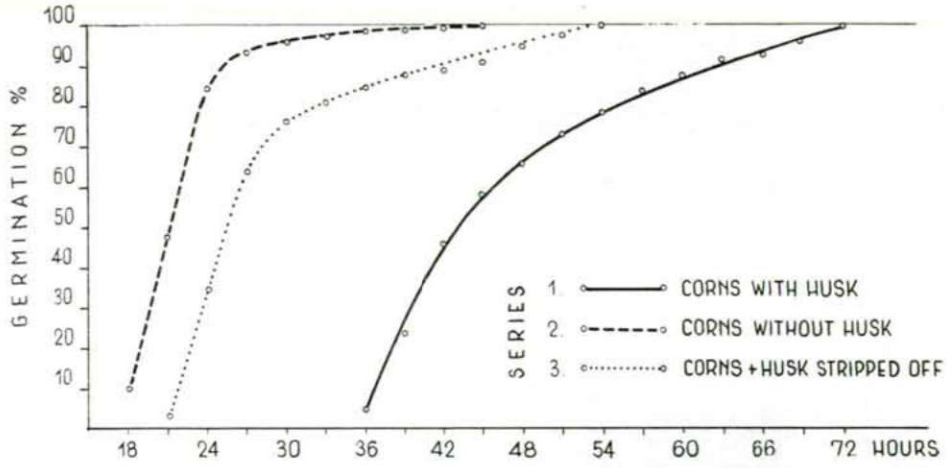

Fig. 1. Potency of germination of oats with and without husk at 23°C.
(Average of 3 parallels and 2 repetitions)

The results concerning the intensity of growth of the coleoptiles are indicated in Fig. 2. According to the data to attain the length of 20 mm, 102 hours are required for the seedlings grown from huskless corns and 139 hours for that of with husk; in the latter case the lag is one day and a half. Thus, the difference observed in the germination at the beginning is not equalized when the 20 mm length is attained.

The experiments were continued with oats sown into sand. As a medium, river-sand was used after washing thoroughly and annealed at 700°C. Oats with and without husk were swelled for 8 hours in tap-water and sown into sand in glass dishes wetted with water corresponding to 80% water-capacity. The germination took place in dark, at 23°C and 95% relative humidity. In the course of the germination, repeated three times, it was seen that the coleoptiles of the seedlings from dehusked corns generally attained the length of 20 mm after 84 hours while the seedlings from intact corns required 93 hours for the same condition. Hence, the difference in development is not so much as in PETRI-dishes.

Further on we wanted to know how we can reckon with the washing out of the inhibitors from the husks into the sand and therein, with the effect on the development of the seedlings. So, the sand used earlier was dried at 105°C, sifted out, wetted again and used repeatedly as a medium. In one series husked corns were employed, while in the other one always unhusked ones, 9 times repeated, and the time needed to reach the 20 mm coleoptile length, i. e. the coleoptile length stated during the standard time in the basic experiment (84 hours for dehusked and 93 hours for husked corns) was recorded. Especial care has been taken of the proper identity of the experimental factors in order to

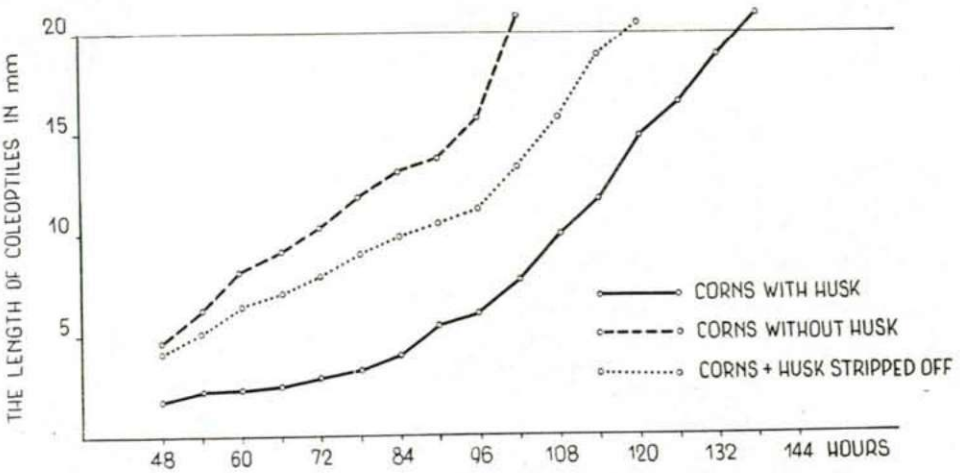

Fig. 2. Growth of coleoptiles from husked and dehusked oats at 23°C.
(Average of 3 parallels and 2 repetitions)

be able to ascribe the differences, occurring in the growth, alone to the biological effect of the substances accumulated in the sand. Results are shown in Fig. 3.

The average length of the coleoptiles, attained during the standard time, grown first in pure sand, is identical with that of the control. Sown the 2d and 3d time, in the case of both husked and dehusked corns, the coleoptiles grew longer during the same time, that is to say some sort of stimulatory effect was shown; later, however, the length of the coleoptiles gradually decreased. In the case of the husked corns, sown the 5th, 6th and 7th time, a definite inhibiting effect appeared which was in full agreement with our expectations on the basis of our previous experimental results (4). Accordingly, the stimulation observed in the 2d and 3d sowing is certainly caused for the most part by the stimulatory effect of the active substances being washed out from the husks and present in the sand still in low concentration; which, however, if accumulated in larger quantity, soon result a remarkable inhibition.

It was fairly surprising the excessive elongation of the coleoptiles noted in the 8th and 9th sowing of husked corns. As these seedlings were thinner and curved, even in most cases deformed, the phenomenon may be attributed to the toxic effect of the inhibiting substances and other decomposition products

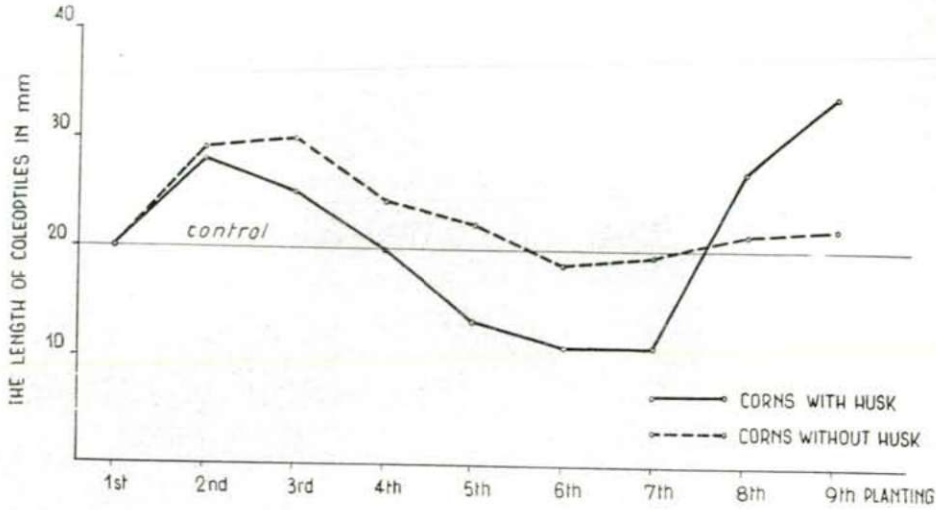

Fig. 3. The length of coleoptiles attained in the repeated sowing during the standard time (93 hours in husked and 84 hours in dehusked corns). Average of 3 parallels and 2 repetitions

(secreted by the roots or microorganisms) in the sand. It is noteworthy that even the germination itself appeared also uneven with repeated sowing of husked corns.

On the other hand, the mild stimulatory effect could be noticed up to the 5th sowing of the unhusked corns, however, no inhibition followed. In this case the biological effect of the substances washed from the grains or perhaps secreted by the roots may be assumed.

To support the above assumptions efforts have been made to demonstrate paper-chromatographically these substances from the sand. For this purpose the water-soluble substances of 10 g husks were extracted in 1% $NaHCO_3$ and acidified to pH 3 shaken into ether, then in order to separate the compounds chromatographed in isopropanol-ammonia-water 10 : 1 : 1 solvent on Sch & Sch 2043 b paper. In the same way the substances accumulated in the sand —used 5 to 7 times — were also extracted and likewise chromatographed. The spots developed on the chromatograms were marked in UV light and to identify them sprayed with reagents for acids and phenolic compounds (4, 5, 8). Comparing the chromatograms obtained from the extracts of the husks and sand could be stated as follows: In sowing husked corns primarily the presence of inhibitors released from the husks is considerable in the sand, namely, large molecular tannic acids, p-oxybenzoic acid, ferulic acid, p-coumaric acid and salicylic acid were found therein; besides, in smaller amount, some other substances — not originating from the husks — could be also demonstrated from the sand samples. These substances are presumably secreted by the roots or they may be the metabolic products of the germinating corns and of the microorganisms, respectively.

The results of the above experiments suggest that the most favourable conditions for oat-seedling cultivation would be to sow dehusked corns always in

pure sand. As the dehusking practically can not be carried out in serial experiments, good test-seedlings can be obtained even if the oat is sown husked but with the view to the standardization of the time for cultivation the sand is to be used but once.

An other important problem concerning the growing of the oat-seedlings is to obtain the maximal auxin-sensitivity of the coleoptiles which depends mainly on the variety and the circumstances of the growing of the seedlings. The question is, whether the inhibiting substances leached into the medium or secreted, have or not any injurious effect also on the auxin-sensitivity which may be expected on the basis of previous experimental results.

For this purpose the husked and dehusked corns were repeatedly sown in the same medium in the identical way as described above, and following the 1st, 3d, 5th and 7th sowing, the growth of the 5 mm sections cut subapically from the coleoptiles of 20 mm long, were determined in IAA concentration-series. No definite correlation could be found between the auxin-sensitivity of the coleoptiles of the dehusked corns and the quantity of the biologically active substances leached into the medium or secreted; on the other hand, in the case of the husked corns the correlation could positively be recognized. These results are in Fig. 4. Thus our supposition proved to be right, that is, the sensitivity of the coleoptiles to IAA is proportionately decreasing with the increasing concentration of the substances washed out from the husks and accumulated in the medium, which is manifested in the gradual decrease of

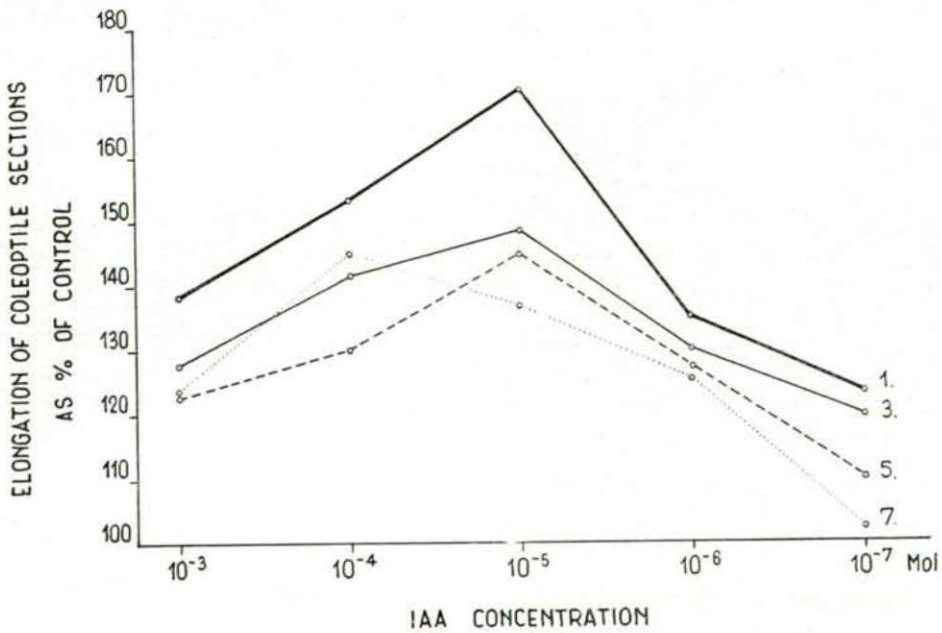

Fig. 4. Growth of coleoptile sections in IAA observed in 1st (_____), 3d (— — —), 5th (— — — —) and 7th (.....) sowing in the same medium

the growth reactions observed in the consecutive sowings. Consequently to use the same medium repeatedly is to be avoided, even for the sake of securing the maximal sensitivity of the test.

Summary

In connection with the growing of oat-seedlings for auxin-assay, the potency and percentage of the germination, the role and effect of the substances washed out into the sand, especially of the inhibiting substances of the husks on the growth and auxin-sensitivity of the seedlings, have been demonstrated.

The presence of the husk — beside other factors — delays the germination of the corns as well as the time required to attain the length of 20 mm of the coleoptiles, chiefly due to the inhibiting content. This delay is considerably greater in PETRI-dishes than in sand.

The inhibiting substances of the husks are for the most part washed out by water and are accumulated in the medium, whence they can be demonstrated with paper chromatographic method (large molecular tannic acids, p-oxybenzoic acid, p-coumaric acid, ferulic acid, salicylic acid, etc.). Using repeatedly the medium, the washed out substances first somewhat stimulate, later — in higher concentration — remarkably inhibit the germination of the corns, the growth and development of the seedlings, further they decrease also the auxin-sensitivity of the coleoptiles. Thus to standardize the time required for the growing of the *Avena* test seedlings and to secure the maximal auxin-sensitivity the medium must not be used more than once.

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