GERMINATION- AND GROWTH-INHIBITING SUBSTANCES IN RICE GRAINS

I. Studies on the effect and properties of the inhibitors in the covering structures

by

M. VARGA

Institute for Plant Physiology, University of Szeged, Hungary
(Dir.: Prof. Dr. I. SZALAI)

Introduction

Experiments aiming at the physiological and biochemical examinations of the growth of rice varieties (VARGA 1963, 1964) drew the attention to the germination- and growth-inhibiting substances in the rice grains.

The presence and physiological role of the germination- and growth-inhibitors in the different dry seeds and fruits is well known from the literature. Numerous papers deal also with the examination of the effect and physiological significance of the inhibitors localized in the caryopsis — mainly in the layers covering the grains — of cereals such as wheat (MOSHEOV 1938, MIYAMOTO and EVENSON 1958, MIYAMOTO et al. 1961), oat (ELLIOT and LEOPOLD 1953, TUNG-FANG 1957, KÖVES 1957, PEERS 1958, FRITZGERALD 1959), wild oat (KOMMEDHAL et al. 1958, BLACK 1959), barley (POLLOCK 1959) and rye (FURSTE 1958). Some authors attempted to discover the chemical nature of the inhibitory substances too (MIYAMOTO and EVERSON 1958, MIYAMOTO et al. 1961, KÖVES 1957, VARGA and KÖVES 1961).

On the other hand, relatively few data are available concerning the germination- and growth-inhibiting substances in the rice caryopsis. ULALI et al. (1960), further NAIR and SHADEVAN (1962) on experimental basis concluded that rice covering structures contain inhibitory substances. MIKKELSEN and SINAH (1961) extracted substances, inhibiting the germination and the growth, from the rice grains. KÖVES and ÁCS (1963) studying the properties of rice grains of reduced germinating power demonstrated certain inhibitors in the husk and grain. ROBERTS (1961), however, found — neither in the rice husk nor in the grains — any germination- and growth-inhibiting substances extractible with water or ether which is at variance to other data. But authors demonstrating inhibitors from the rice grains carried out no detailed examinations concerning the effect and charachteristics of these substances.

In the first part of our paper we wish to elucidate the rate of the effect of the effect of the inhibitors in covering structures on the germination and growth as well as the principal properties of the inhibiting substances.

Material and method

As it is known from literary data and earlier examinations that the inhibiting substances of the rice grains are localized in the *pericarpium* and *testa*, further in the husk (*lemma* and *palea*); bran, resulting from dehusking and available in large quantities, was used for experiment. The bran — dehusked from *Dunghan Shali* harvested in 1962 — was obtained from the Dehusking Mill and Marketing Cooperative of Karcag. The bran contains the tissues of the husk, *pericarpium* and *testa*, moreover the *aleuron* layer of the endosperm — though in a smaller quantity compared with that of the former. The bran was stored in dry condition at 0° C.

Extraction of the inhibitors. 50 g of rice-bran with water (100 ml) was shaken at room temperature for 6 hours and the suspension was centrifuged. The supernatant fluid was completed with water to 100 ml and the obtained extract was considered as a stock solution. Then a serial dilution was prepared ranging from 2.5× to 100× and their germination- and growth-inhibitory effect and properties were examined with rice-embryo and lettuce seed germination test, further with rice and oat coleoptile section test as well as with lettuce hypocotyl test.

Rice-embryo test. The embryos were cut from *Dunghan Shali* grains of 96% germinating power, harvested in 1962. 20 excised embryos were placed with the endosperm side down in a Petri dish of 6 cm ∅ on a disc of double filter paper wetted with 3 ml of test solution. The dishes were then incubated at 25° C partly in dark, partly in light.

Lettuce seed test. 50 seeds (*Lactuca sativa* L. „Böttner") were germinated in an 8 cm ∅ Petri dish at 25° C in light, on double filter paper discs wetted with 4 ml test solution. Occasionally, beside the lettuce seeds — in the same way — intact or dehusked rice grains, mustard, millet, panicum and poppy seeds were also used for germination test. In every case the germination per cent, the germinating power as well as the length of the shoot and root of the seedlings were observed against the time and concentration.

Rice and oat coleoptile section test was made as described in our earlier papers (VARGA and FERENCZY 1957).

Lettuce hypocotyl test was carried out with FRANKLAND—WAREING's technique (1960) modified by WHEELER (1962).

Results and discussion

The cause of the effect of husk delaying germination

According to ROBERTS (1961) the husk of the rice grains contains no inhibitors; its germination delaying effect is solely due to its preventing the gas-exchange between the grain and environment. On the other hand, the above mentioned authors assumed i. e. found inhibitors in the husk.

Consequently first, as an informative experiment, we compared the germination of intact and dehusked grains and the growth of the seedlings. The germination of dehusked grains in the presence of the removed husks was also examined. In every case 50 rice grains were put in a 10 cm ∅ Petri dish on a double filter paper wetted with 5 ml water. Some of the dishes in dark, others in light were incubated at 25°C. Results are shown in Fig. 1 and 2. According to Fig. 1 the germination has been prevented by the husk not only mechanically but also by its watersoluble inhibitors, surely the husks removed from the grains and put in the Petri dishes exerted also a certain inhibitory effect. From our data it seems that the prevention of water- and gas-exchange by the husk and the inhibitor content of the husk play roughly equal part in delaying the germination. It is to be noted that no difference was between the germination of the grains incubated in dark and light.

The presence of the husk has also an effect on the initiate growth of the rice seedlings (Fig. 2): the coleoptile- and rootlength of the seedlings in the three samples shows the same differences as seen in the germination.

Therefore the husk of the rice grains — like other cereals — contains germination- and growth-inhibitory substances.

Examination of the effect of water extract

The rate of germination inhibitory effect of the dilutions (2,5×, 5×, 7,5×, 10×, 20×, 40×, 60× and 100× dilutions) prepared from the stock solution (0× dilution) considerably inhibit the germination. The higher dilutions influence somewhat less the germination per cent, but they retard the germination. The dilutions 60×, 80× and 100× proved to be ineffective. The results of the series incubated in dark and light were practically identical

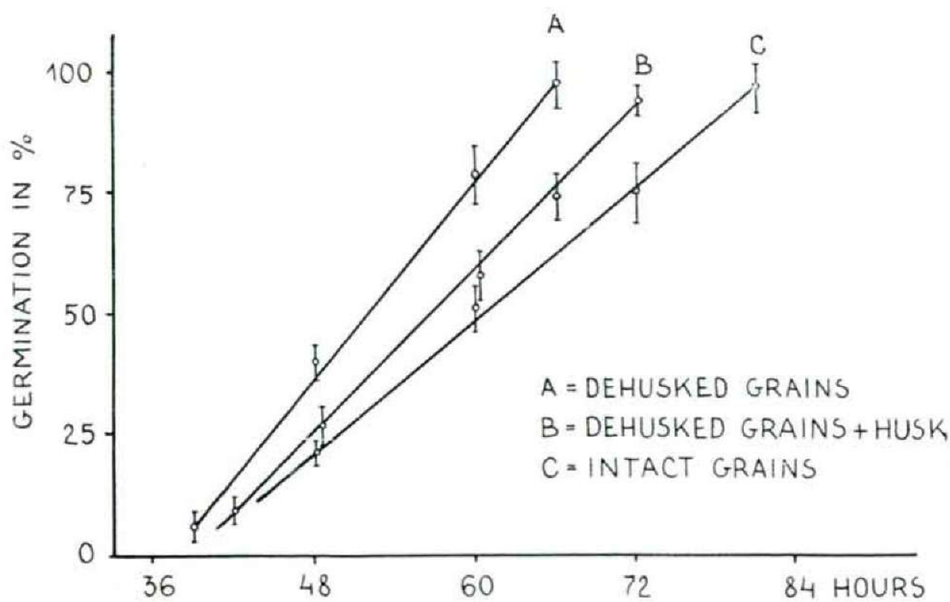

Fig. 1. Germination of rice grains with and without husk.

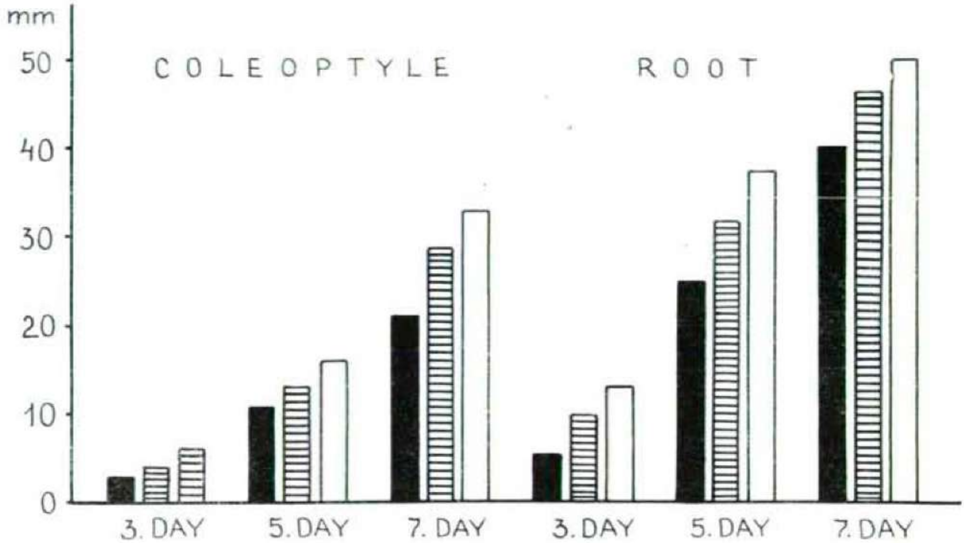

Fig. 2. Growth of rice seedlings obtained from intact and dehusked grains. (Black columns = intact grains, lined columns = dehusked grains + husk, white columns = dehusked grains).

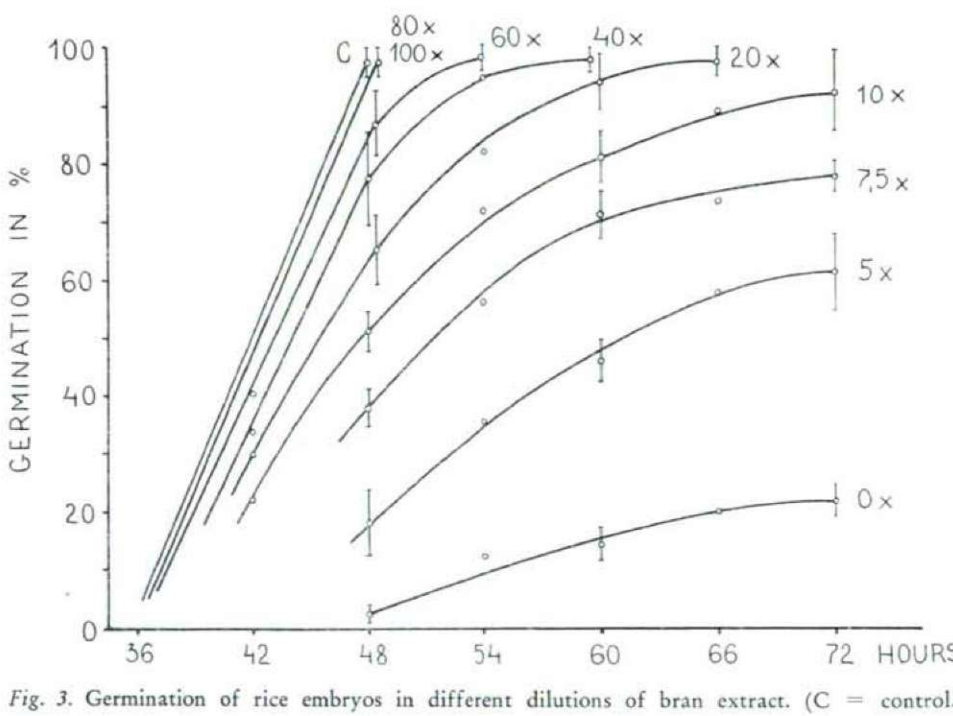

Fig. 3. Germination of rice embryos in different dilutions of bran extract. (C = control.)

The water extract inhibited the germination of the mustard, millet, panicum, poppy and lettuce seeds too, and considerably more than that of the rice embryos. Of the seeds used, the extract exerted the strongest effect on the lettuce, consequently later we employed lettuce seeds beside rice embryos to measure the inhibitory effect. Fig. 4 presents the effect of the serial dilutions

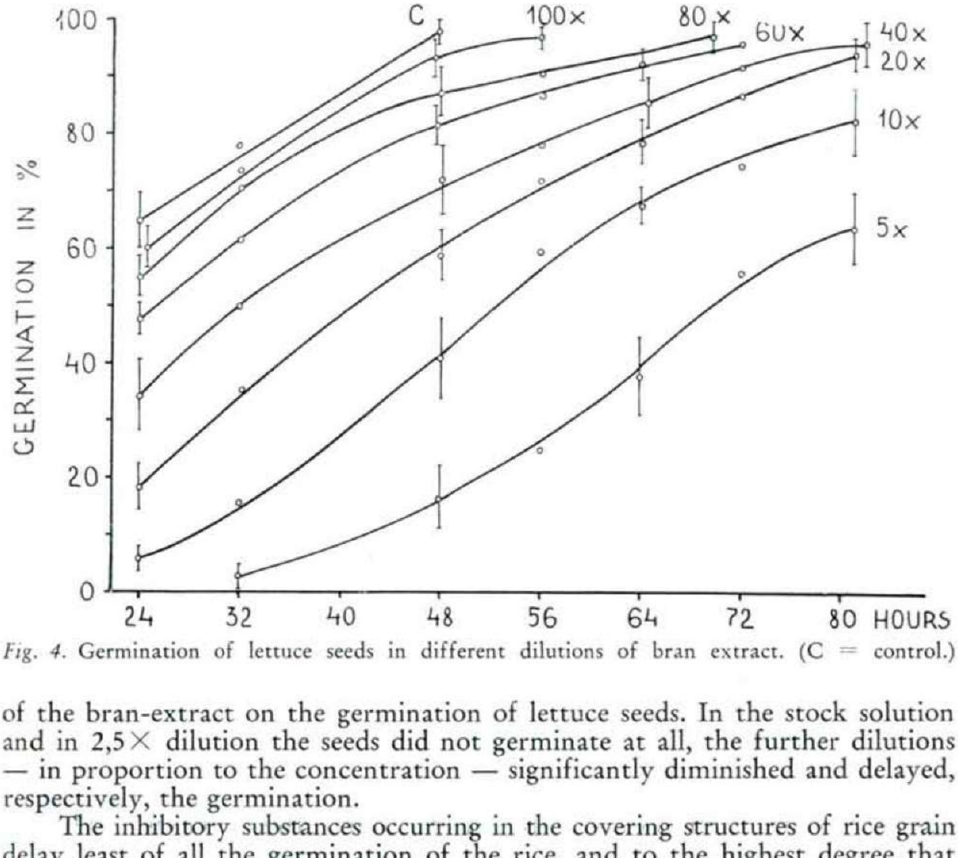

Fig. 4. Germination of lettuce seeds in different dilutions of bran extract. (C = control.)

of the bran-extract on the germination of lettuce seeds. In the stock solution and in 2,5× dilution the seeds did not germinate at all, the further dilutions — in proportion to the concentration — significantly diminished and delayed, respectively, the germination.

The inhibitory substances occurring in the covering structures of rice grain delay least of all the germination of the rice, and to the highest degree that of the lettuce seeds, of the seeds used as test in our experiments.

The water extract and its dilutions have reduced also the growth of the seedlings. Fig. 5 and 6 show the length of the root and shoot of the seedlings — rice and lettuce in water extracts — in the 72. hour. In the figures is shown also the rate of growth in percent of the length of the control. According to the results the inhibiting effect, in rice (Fig. 5) is more pronounced in the growth of the seedlings than in the germination; i. e. the length of the roots and coleoptiles is more sensitive test than the germination itself. It is worth mentioning that MIYAMOTO et al. (1961) experienced the same related to wheat.

It is remarkable that in the case of rice (Fig. 5) the root, while in the case of lettuce the hypocotyl is more sensensitive to the inhibitory effect; the different species, therefore, variously behave in this respect.

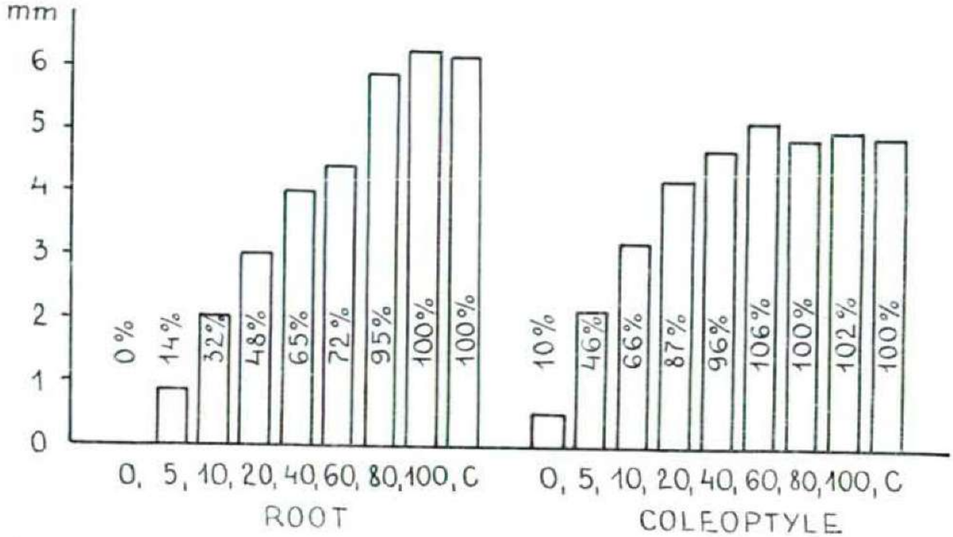

Fig. 5. Growth of the root and shoot of rice seedlings in the different dilutions of water extract, in the 72. hour.

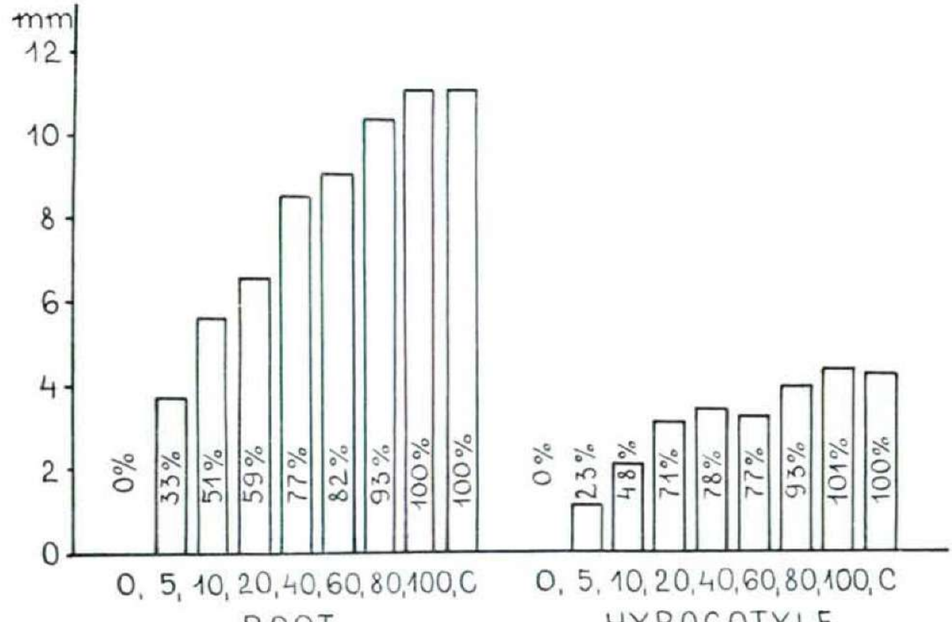

Fig. 6. Growth of the root and shoot of lettuce seedlings in the different dilutions of water extract, in the 72. hour.

Behaviour of the inhibitors to heat

The different dilutions of the water extract were kept at —15, +2, 20, 60 and 100°C for 24 and 48 hours following extraction, thereafter the inhibitory effect was examined with rice embryo and lettuce seed test. The results are summarized in Table I. and II. Accordingly, the activity of the extracts kept at —15°C did not change whereas the activity at +2, 20 and 60°C decreased in the ratio of the rise in the temperature, on the other hand at 100°C it increased. The average activity-change observed in the two tests following heat treatment was:

It follows that a part (the greater part) of the substances occurring in the covering structures of rice grains are thermostabile, the other part (the smaller one) is thermolabile. The rise of the inhibitory effect of the extracts kept at 100°C for 24 or 48 hours is likely to be attributed to the fact that other,

Temperature	Rice embryo	Lettuce seed	
— 15°C	0 %	0 %	Unchanged activity
+ 2°C	1,6%	2,3%	Change within the limit of error
+ 20°C	10,3%	11,0%	Decreased activity
+ 60°C	15,1%	17,3%	Decreased activity
+100°C	8,1%	6,2%	Rise of activity

Hours	Dilution 5×					Dilution 10×					Dilution 20×				
	—15°	+2°	+20°	+60°	+100°	—15°	+2°	+20°	+60°	+100°	—15°	+2°	+20°	+60°	+100°
36	—	—	—	—	—	—	—	—	8	—	—	3	10	15	—
42	—	—	4	11	—	20	25	29	35	10	30	35	40	46	15
48	19	24	33	50	—	40	40	47	60	21	48	52	60	65	35
54	33	40	51	62	20	56	63	66	72	40	69	74	80	80	50
60	45	43	60	71	40	78	68	85	85	52	80	80	83	85	73
66	60	70	80	80	56	84	86	90	90	78	90	90	91	90	80
72	63	72	80	90	65	87	92	95	96	85	95	97	96	96	90

Table I. Germination per cent of rice embryos in rice bran extracts previously treated at different temperatures. (Control is 97% in the 48. hour.)

Hours	Dilution 5×					Dilution 10×					Dilution 20×				
	—15°	+2°	+20°	+60°	+100°	—15°	+2°	+26°	+60°	+100°	—15°	+2°	+20°	+60°	+100°
24	—	—	—	3	—	8	11	14	25	18	18	23	58	50	15
32	—	7	5	9	8	23	37	35	51	23	38	49	72	68	33
40	8	14	13	20	11	49	56	57	68	42	55	67	79	82	47
48	15	24	24	32	16	54	67	72	77	54	62	72	80	85	60
56	31	35	41	49	24	64	75	79	84	59	77	85	90	94	75
64	48	51	61	66	37	72	81	84	89	66	82	90	92	95	83
72	62	64	73	76	51	77	86	88	94	75	90	93	95	96	89
80	72	78	82	87	65	80	89	89	95	81	93	96	94	97	95

Table II. Germination per cent of lettuce seeds in rice bran extracts previously treated at different temperatures. (Control is 96% in the 48. hour.)

more active inhibitory substances are produced due to the formation of certain decomposition products, i. e. to the reaction of some components. It is also possible that at 100°C the decomposition of some substances being able to counteract the inhibitory effect lead to the rise of the activity.

Stability of the inhibitors in vitro

Examining the stability of the inhibitory substances of water extract, its dilutions were kept at +2°C for 6 weeks measuring their activity weekly. (The extracts can not be kept at room temperature for a longer period without infection, administration of antibiotics influences also the germination of the test-seeds and autoclaving, according to the earlier experimental results, is not possible without activity-change). At +2°C, in aqueous solution, in 6 weeks, $5{,}5 \pm 4{,}2\%$ of the activity was lost which being about equal with the standard error can not be taken into account.

The quantity of the inhibitory substances extractable with water from the dry bran practically did not change for 8 weeks following dehusking, measuring weekly.

Interaction between the inhibitors and indoleacetic acid

As the results show that the inhibitory substances of the rice-bran retard the growth of the seedlings to a greater extent than the germination, we examined the interrelationships in the action of these substances and of IAA. The effect of the different concentrations of the bran-extract on the elongation — in the presence of 10^{-3}—10^{-6} M IAA — was measured with rice and oat coleoptile section test. Fig. 7 shows the results obtained with rice coleoptile

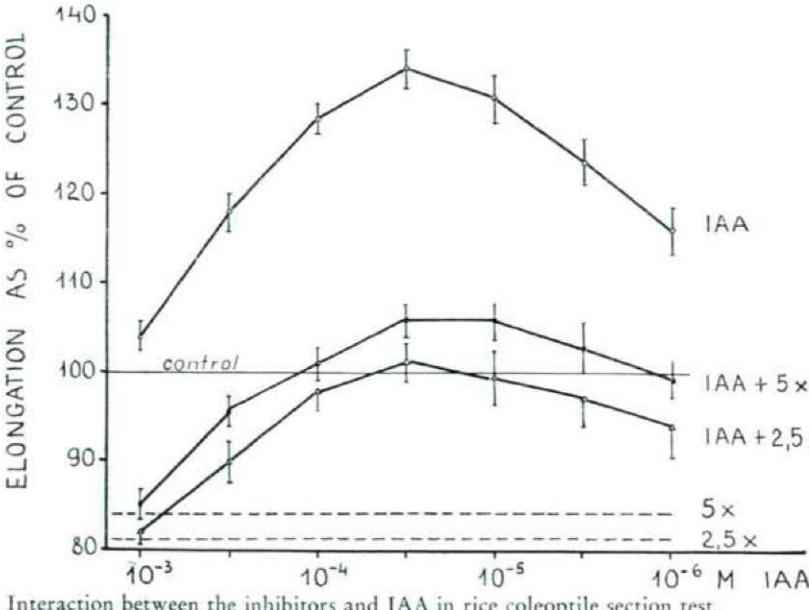

Fig. 7. Interaction between the inhibitors and IAA in rice coleoptile section test. ($s = \pm 4{,}2$; $s_x = \pm 0{,}59$; $s\ \% = 5{,}5$; $n = 50$).

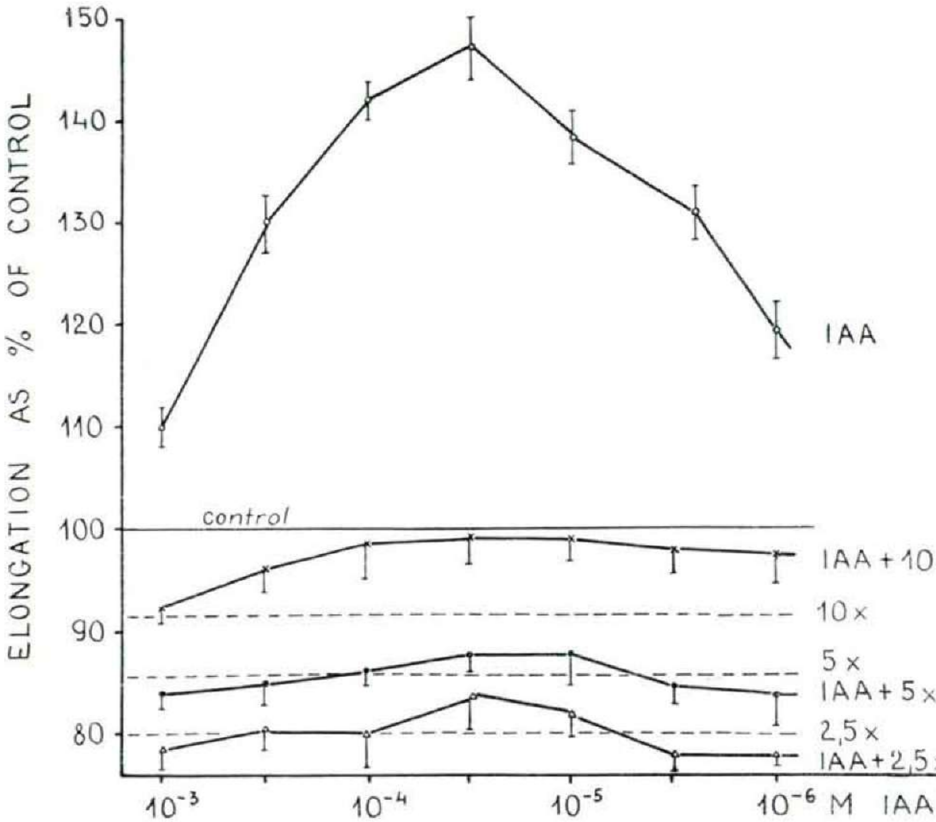

Fig. 8. Interaction between the inhibitors and IAA in oat coleoptile section test. ($s = \pm 4{,}2$; $s = \pm 0{,}63$; $s\ \% = 5{,}02$; $n = 50$).

sections. On the figure the degree of the elongation of rice coleoptile sections can be compared in pure IAA solutions, in the dilutions (2,5× and 5×) of the bran-extract, moreover in simultaneous application of inhibitors and of IAA. The dilution 2,5× and 5× considerably diminishes the effect of IAA; whereas the auxin counterbalances to a certain extent the growth-inhibitory activity of the extracts. On the basis of these results the reduced growth of the shoot of rice seedlings treated with bran-extract, can be explained.

In a still higher degree the auxin-counterbalancing effect of the extracts is shown in the oat coleoptile section test (Fig. 8). Here the dilutions 2,5×, 5× and 10× diminish the effect of the IAA concentrations to such an extent that the elongation of the sections remains below that of the control in every case; and the effect of the auxin can be observed only in the case of dilution 10× to the extent over the limit of standard error. Consequently the oat test is more sensitive to the inhibitory substances of the rice-bran than the rice itself. It may be mentioned that according to GRACZA's results (1957) the rice coleoptiles have the smallest auxin-sensitivity among the cereals.

Interaction between the inhibitors and gibberellins

It is known that the GA generally stimulates the seed germination and the elongation of the stem cells, i. e. the longitudinal growth of the shoots. Now the question arises whether the inhibitors in rice bran extract are able to reverse also this effect of GA.

Therefore we studied the rate of the germination of the rice embryos in the presence of these inhibitors and of the GA together, in different concentrations (Fig. 9). According to the results the water extract, in 2,5×, 5× and 10× dilutions, significantly retards the germination stimulatory effect of the GA; whereas the GA — in the proportion of the concentration — reduces the germination inhibitory effect of the inhibitors. On the basis of our data the inhibting effect due to 10× dilution is reversed only by 100 ppm GA.

Interaction of similar character was noted also in the germination of lettuce seeds (Fig. 10), with the difference that, the lettuce seed being more sensitive test, here the germination retarding effect of the inhibitors is still more strongly shown against the GA. In this case the inhibition due to dilution 20× is ceased by the presence of 100 ppm GA.

The inhibitors of the rice bran prevent also the elongation stimulatory effectiveness of the GA on stem cells. It is seen in Fig. 11 how much is reduced the GA-induced elongation of the rice coleoptiles by the 2,5×, 5× and 10× dilutions of the extract; and this inhibitory effect can be completely reversed only by a relatively larger quantity of the GA. In an earlier work (1964) we demonstrated that the native GA content in rice seedlings is insignificant related to the GA quantity added from outside.

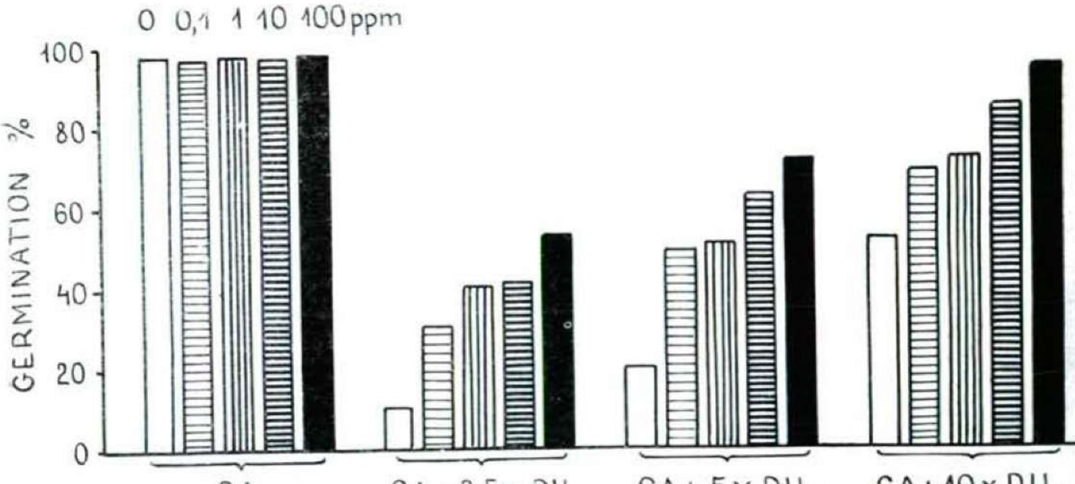

Fig. 9. Germination of rice embryos in simultaneous application of different dilutions of the inhibitors and of GA, in the 48. hour.

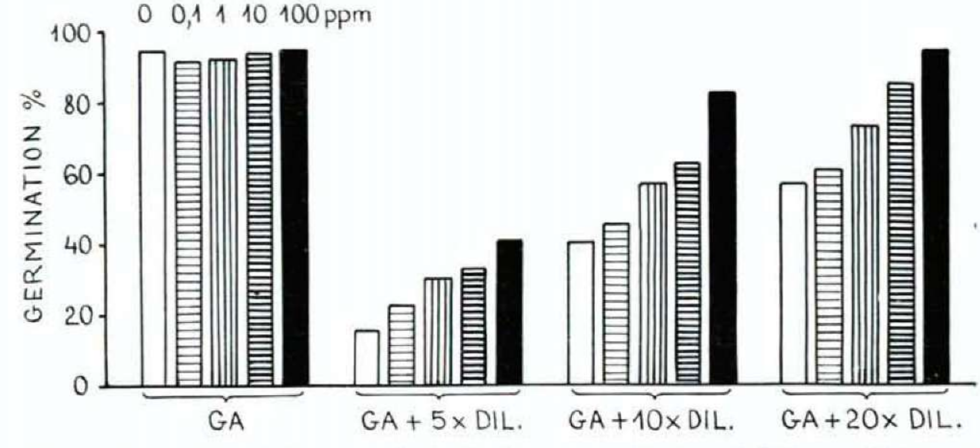

Fig. 10. Germination of lettuce seeds in simultaneous application of different dilutions of inhibitors and of GA, in the 120. hour.

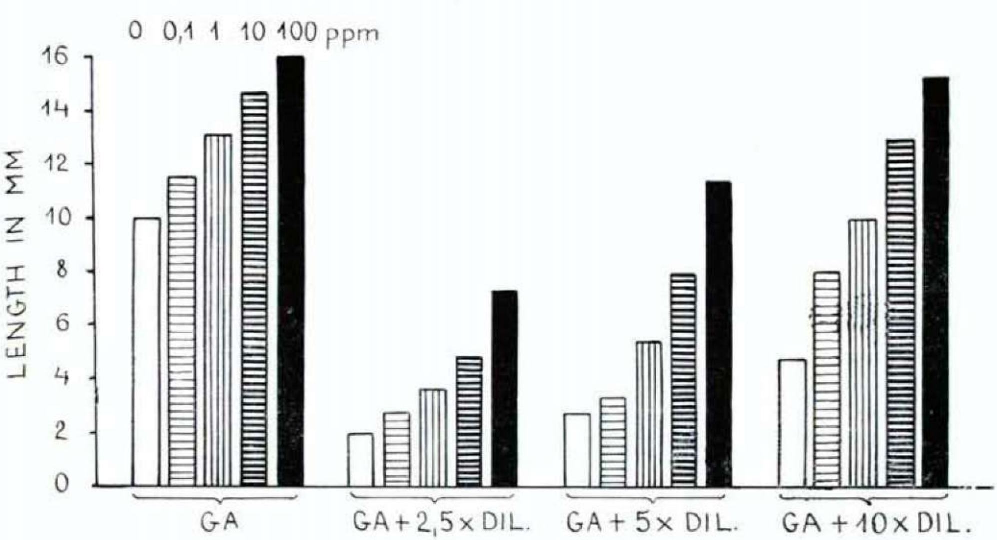

Fig. 11. Growth of the shoot of rice seedlings in the presence of different dilutions of the inhibitors and of GA, in the 120. hour.

The inhibitory substances are able to detain the growth stimulatory effect of the GA, in the highest degree, in the lettuce hypocotyl test (Fig. 12). This is one of the most sensitive tests, so it is very frequently used to demonstrate the gibberellins (FRANKLAND and WAREING 1960, WHEELER 1962, VARGA 1964). The growth reaction of the lettuce hypocotyls to GA — as shown in Fig. 12 — is, to a considerable extent, reduced by the inhibitors of the rice bran and no doubt by other natural inhibitory substances too.

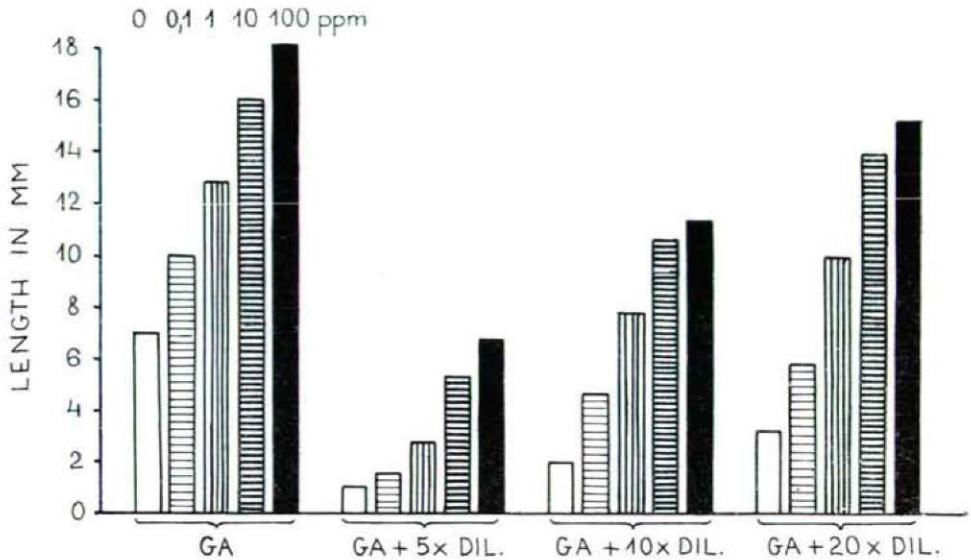

Fig. 12. Interrelationships in the effect of rice bran inhibitors and of GA, in lettuce hypocotyl test. ($s = \pm 1{,}84$; $s_{\bar{x}} = \pm 0{,}33$; $s\,\%_0 = 16{,}3$; $n = 75$.)

Our data are confirmed by CORCORAN et al. (1961) who demonstrated that the extract of some seeds and fruits (e. g. *Ceratonia siliqua*) is able to detain the elongation induced by GA. These facts indicate that the GA content of natural plant extracts can be really determined with biological tests only by a careful separation of the gibberellins from other substances.

Solubility of the inhibitors in organic solvents

50 g of rice bran previously soaked in water was extracted with 100 ml of natural plant extracts can be really determined with biological tests only by were evaporated and the residue was solved in water. The inhibitory effect of the obtained solutions was examined with lettuce seed and lettuce hypocotyl test. In every case smaller or larger germination- and growth-inhibition was observed suggesting that the inhibitory substances, or at least some, are solubile in these organic solvents.

The separation of fractions of different solubility from the water extract and the detailed examination of the effect of single fractions and of their properties is being treated in a subsequent paper.

Summary

Examining the germination- and growth-inhibitory effect and properties of the water extract of the covering structures of rice grains the following results were obtained:

1. The rice husk — alike other cereals — contain germination- and growth-inhibitory substances. The inhibitor content of the paleae play a significant part in retarding the seed germination by the husk.

2. The water extract of the bran and its dilutions, depending on the concentration, inhibit the germiation of the rice embryos and other seeds. Of the seeds used as test object the inhibitory substances inhibit the least the germination of rice and in the highest degree that of lettuce seeds.

3. Inhibitors in the bran of rice reduce the growth of the root and shoot of rice and lettuce seedlings too. In the case of rice the root is more sensitive to the inhibitory effect while in the case of lettuce the hypocotyl.

4. The greater part of the inhibitory substances is thermostabile and the smaller one is thermolabile. At 100°C in the extract, due to certain decompositions or certain reactions of the components, other inhibitors, more active than the original ones, are formed.

5. The inhibitors are stabile at room temperature and at +2°C; some of them are solubile also in methanol, ethanol, chloroform and ethylether.

6. The inhibitors are able, to a considerable extent, to retard the effect of IAA and of GA on the cell elongation and on the stimulation of the germination respectively; whereas IAA and GA, in a certain degree, reduce the germination- and growth-inhibitory activity of these substances.

Literature

1. Black, M.: Dormancy studies in seeds of *Avena fatua*. I. The possible role of germination inhibitors. Canad. J. Bot. 37, 393—398. 1959.
2. Corcoran, M. R., West, C. A. and Phinney, B. O.: Natural inhibitors of gibberellin-induced growth. Am. Chem. Soc. 28, 152—158. 1961.
3. Elliot, B. B. and Leopold, A. C.: An inhibitor of germination and amylase activity in oat seeds. Physiol. Plant. 6, 65—77. 1953.
4. Frankland, B. and Wareing, P. F.: Effect of gibberellic acid on hypocotyl growth of lettuce seedlings. Nature 185, 255—256. 1960.
5. Fritzgerald, P. H.: Germination induced by excision of the endosperm of immature wheat grains. New Zealand J. Agric. Res. 2, 735—742. 1959.
6. Furste, K.: Möglichkeiten zur Abkürzung der Keimruhe bei Keimlingen von Getreidearten durch Entfernen der Kornhülle. Saatgut-Wirtsch. 10, 247—276. 1958.
7. Gracza, L.: Vergleichende Untersuchungen über die Auxin-Empfindlichkeit der Koleoptyle verschiedener Getreide-Sorten. Acta Biol. Szeged, 3, 145—149, 1957.
8. Kommedhal, J. E., De Vay, J. E. and Christensen, C. M.: Factors affecting dormancy and seedling development in wild oats. Weeds, 6, 12—19. 1958.
9. Köves, E.: Papierchromatographische Untersuchungen der ätherlöslichen keimungs- und wachstumshemmenden Stoffe der Haferspelze. Acta Biol. Szeged, 3. 179—187. 1957.
10. Köves, E. and Ács, I.: Csökkent csírázóképességű rizsszemek növekedésszabályozó anyagainak vizsgálata. Kézirat.
11. Mikkelsen, D. S. and Sinah, M. N.: Germination inhibitor in *Oryza sativa* and control by preplanting soaking treatments. Crop. Sci. 1, 332—335. 1961.
12. Miyamoto, T. and Everson, E. H.: Biochemical and physiological studies of wheat seed pigmentation. Agron. J. 50, 733—734. 1958.
13. Miyamoto, T., Tolbert, N. E., Everson, E. H.: Germination inhibitors related to dormancy in wheat seeds. Plant Physiol. 36, 739—746. 1961.
14. Mosheov, G.: The influence of the water extract of wheat seeds upon their germination and growth. Palestine J. Bot. Jerusalem Ser. 1, 86—92. 1938.
15. Nair, N. R. and Shadevan, P. C.: An instance of bran layer influencing seed dormancy in rice. Curr. Sci. Bang. 31, 72—73. 1962.
16. Peers, F. G.: Germination inhibitory substances in oat husk. W. African J. Biol. Chem. 2, 9—16. 1958.

17. POLLOCK, J. R. A.: Studies in barley and malt. XV. Growth substances and other compounds in relation to dormancy in barley. J. Inst. Brew. 65, 334—340. 1959.
18. ROBERTS, E. H.: Dormancy in rice seed. II. The influence of covering structures. J. Exp. Bot. 12, 430—445. 1961.
19. TUNG-FANG, C.: Dormancy of wheat grains harvested at different stages of maturity and treated with various methods. Acta Bot. Siniaca 6, 80—90. 1957.
20. ULALI, D. L., BARKER, M. B. and DUMLAR, R. C.: A preliminary study on the cancellation of dormancy period of rice seeds. Philippine Agric. 44, 279—289. 1960.
21. VARGA, M. and FERENCZY, L.: Quantitative changes in growth-promoting and growth-inhibiting substances in Rindite-treated and untreated potato tubers. Acta Bot. Hung. 3, 111—121, 1957.
22. VARGA, M. and KÖVES, E.: Methodological examinations concerning the growing of oat seedlings for auxin-assay. Acta Biol. Szeged, 7, 39—44. 1961.
23. VARGA, M.: Növekedésélettani vizsgálatok rizsen. Növénytermelés, 12, 33—42. 1963.
24. VARGA, M.: Rizs csíranövények növekedése különböző magasságú vízborítás alatt. Bot. Közl. 50, 167—173. 1963.
25. WHEELER, A. W.: Growth activity of the gibberellins of dwarf French bean, potato and lettuce. J. Exp. Bot. 13, 36—44. 1962.