

GROWTH INHIBITING SUBSTANCES IN RICE-STRAW

By

E. KÖVES and M. VARGA

Institute for Plant Physiology of University, Szeged

(Received May 20, 1958)

Introduction

BÖRNER (1, 2), WINTER and SCHÖNBECK (10) demonstrated from water extract of straw of cereals such substances that considerably inhibit the root-growth of the young cereal seedlings. These examinations are important also from agricultural point of view because the inhibiting substances of the straw, getting deeper into the soil following the first ploughing after harvest, may influence the germination of the seeds of the second sowing and the growth of the seedlings. Such examinations however, have not been made on rice-straw, so, bearing in mind the great importance and the numerous problems of rice cultivation in our country, it seemed reasonable to study the question also from practical point of view.

In the present work the growth inhibiting substances contained in the straw of various rice varieties grown in our country, their effect and their chemical nature have been examined.

Material and method

The straw of four varieties of commonly grown rice-plants, harvested in 1957: *Oryza sativa* L. »Dunghan Shali«, »Dubowsky«, »Linia« and »Precoce«¹ has been subjected to examination.

The finely ground rice-straw placed in 1% NaHCO₃ solution has been shaken for 2 hours at room temperature, then extracted for 22 hours at temperature +2° C, and the aqueous extract acidified by hydrochloric acid to pH 2,8 was shaken into ether. From the condensed acidic fraction ascending chromatograms were prepared on Sch & Sch No. 2043b paper. In order to compare the quantitative relations the extract corresponding to 1 g dry material was dropped on each start point. In the biological tests isopropanol: ammonia: water (10:1:1) was used as solvent, while to identify the substances several other solvent-systems were employed. *Avena* coleoptile section test and Papaver seed test were carried out as described in our earlier papers (4, 7).

The inhibiting substances were chemically identified on the basis of R_f values shown in various solvents, fluorescent colours, spraying reactions as well as on

¹ Laboratory for Rice Breeding of the Institute for Irrigation and Rice Cultivation, *Kopáncs-Szarvas*.

that of simultaneous running and comparison of synthetic substances. Ferulic and p-coumaric acid were isolated from the bark of the trunk of *Catalpa bignonioides* Walt., caffeic and chlorogenic acid from coffee beans by the method of STEVENS and NORD (6).

Experimental results

1. *The biological effect of the chromatogram-spots.* The biological effect of the chromatograms made with isopropanol: ammonia: water (10:1:1) solvent is shown in Fig. 1, whose abscissa denotes the distance from the starting line and the ordinata indicates the elongation percentage of the coleoptile sections as compared to the control.

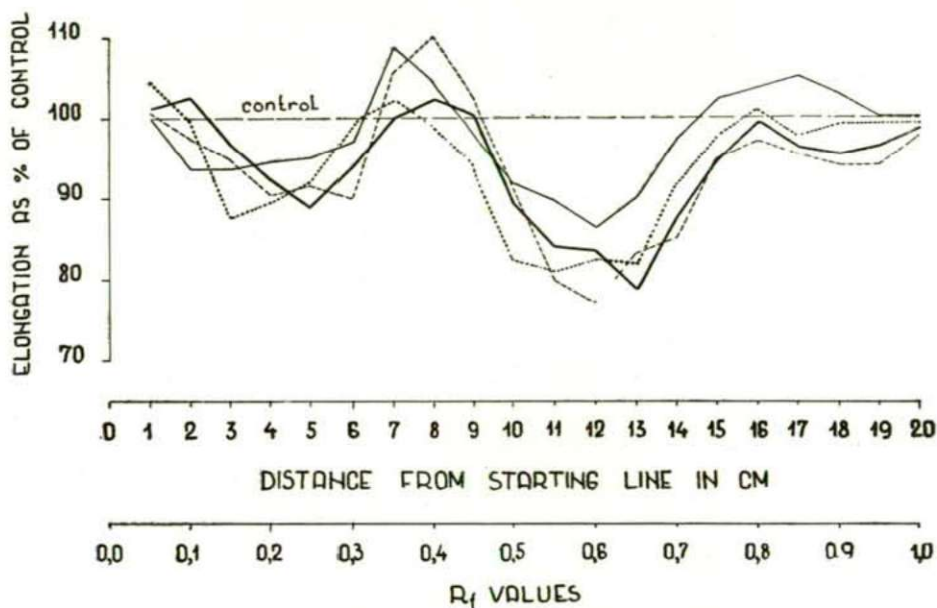


Fig. 1. Growth reactions of *Avena* coleoptile sections in eluate of 1 cm strips of chromatograms made from acidic fraction of the extract of rice-straw. Average of 3 parallels.
 — Dunghan Shali, ——— Linia, Dubowsky, - - - - Precoce

On the chromatograms of all the four varieties two significant, inhibitory spots were noted. The first was found between R_f 0,1—0,3 where the inhibition of the coleoptile sections is 10—15%. No significant difference is between the quantity of the substances localized here, of any of the four varieties. The second and the larger inhibitory zone is between R_f 0,49—0,74 where the extent of inhibition caused by the varieties of *Dubowsky*, *Linia* and *Precoce* is near each other, while the curve of *Dunghan Shali* shows somewhat less effect. The centre of the characteristic inhibiting spot, however, is observed on the same point (R_f 0,60) in all the four cases, the maximal value of the inhibition here is 15—23%.

The data of the root-growth of *Papaver* seeds germinated on the chromatogram-strips show the same results but owing to the higher sensitivity of the test an inhibition of 100 % may also be observed in the centre of the larger inhibiting zone.

2. *Chemical identification of the substances of the inhibitory spots.* On the basis of colour reactions obtained with different spraying reagents (FeCl_3 , diazotized benzidine, diazotized p-nitroaniline, 1% KMnO_4), R_f values observed in the different solvent systems, as well as of the fluorescent colours the following substances could be demonstrated from the lower inhibitory zone: protocatechuic acid, caffeic- and chlorogenic acid respectively, p-coumaric acid, p-oxybenzoic acid and ferulic acid. The properties of the spots, in all the cases, agreed with those of the synthetic chemicals run parallelly with the extract.

Salicylic acid could be similarly demonstrated from the upper, larger inhibitory zone. Here in the formation of the inhibitory zone there is an other yellowish-green fluorescent substance without visible reaction with the above reagents which, however, so far could not be identified.

Discussion

The experimental results indicate, in all varieties of rice examined, the presence of different cinnamic- and benzoic acid derivatives, in well-nigh identical quantity, that inhibit to a considerable extent the growth of the coleoptile setions as well as the germination of the *Papaver* seeds. The inhibiting substances are localized on the chromatograms in two zones well separated from each other. Of the substances of lesser effect, localized in the lower inhibitory spot, the presence of p-oxybenzoic acid, p-coumaric acid and ferulic acid was to be expected on the basis of BÖRNER's works (1, 2) and is in accordance with his experimental results. Here besides these substances caffeic- and protocatechuic acid could also be observed and from the upper inhibitory zone an other far more effective inhibitor than those mentioned above, salicylic acid was demonstrated. As the caffeic acid in the plants generally is present in form of chlorogenic acid, it may be supposed to be a hydrolized product due to alkaline or acidic extraction. It is probable that the other phenolic acids demonstrated from the acidic fraction in our experiments occur in the plants also in bound form, and getting into the stubble-field there they may easily decompose.

Most of the growth inhibiting phenolic compounds found in the straw of cereals have been demonstrated, as inhibiting agents, also from other parts of plant (3, 4, 5, 8). Peculiarly striking in the identity of the phenolic compounds occurring in the rice-straw and the germination inhibiting phenolic acids in various dry fruits (capsula, legumen, siliqua etc) (9).

Most of the substances demonstrated stimulatory in low concentration, while in higher concentration their effect is inhibitory. At any rate the experimental results suggest that *the effect of phenolic compounds demonstrated from the rice-straw has to be taken into account in the chemical and biological processes occurring in the soil.*

The position and the fluorescence of the stimulatory spot at R_f 0,35—0,45 agree with that of the indoleacetic acid, however, with GORDON-WEBER's reagent it did not give visible reaction. Therefore, it was undecided whether this stimulatory action is really to be attributed to IAA or not.

Summary

Examining the acidic fraction of the extract from the straw of four varieties of rice commonly grown in our country, with paperchromatographic method two well-distinguished inhibitory spots were obtained which inhibit the elongation of the coleoptile sections and the germination of the Papaver seeds.

From the lower of lesser effect zone (R_f 0,1—0,3) protocatechuic-, caffeic-, ferulic-, p-coumaric- and p-oxybenzoic acid, while from the upper of higher effect inhibitory zone (R_f 0,48—0,75) salicylic acid was demonstrated. In the formation of this upper inhibitory zone an other so far unknown cinnamic acid derivative plays also part.

Bearing in mind the experimental results the effect of phenolic compounds of rice-straw has to be taken into account in the chemical and biological processes occurring in the stubblefield.

References

- (1) Börner, H.: Untersuchungen über phenolischen Verbindungen aus Getreidestroh und Getreiderückständen. *Naturwiss.*, **42**, 583 (1955).
- (2) Börner, H.: Der papierchromatographische Nachweis von Ferulasäure in wässrigen Extrakten von Getreidestroh und Getreiderückständen. *Naturwiss.*, **129**, 43 (1956).
- (3) Griffiths, L. A.: Separation and identification of aromatic acids in plant tissues by paper chromatography. *Nature*, **180**, 286—287 (1957).
- (4) Köves, E.: Papierchromatographische Untersuchungen der ätherlöslichen Keimungs- und Wachstumshemmenden Stoffe der Haferspelze. *Acta Biol. Szeged*, **3**, 179—187 (1957).
- (5) Massart, L.: Inhibiteur de la germination dans des glomérules de la betterave a serce et dans d'autres fruit's secs et grains. *Biochimija*, **22**, 417—420 (1957).
- (6) Stevens, G., and Nord, F. F.: Natural phenylpropane derivatives. c. f. Peach, K., and Tracey, M. V.: *Moderne Methoden der Pflanzenanalyse III.*, 392—427 (1955).
- (7) Varga, M.: Examination of growth-inhibiting substances separated by paper chromatography in fleshy fruits. I. Results of the bio-assay of the chromatograms obtained from the ether extract of the fruits. *Acta Biol. Hung.*, **7**, 39—47 (1957).
- (8) Varga, M.: Examination of growth-inhibiting substances separated by paper chromatography in fleshy fruits. II. Identification of the substances of growth-inhibiting zones on the chromatograms. *Acta Biol. Szeged*, **3**, 213—224 (1957).
- (9) Varga, M., and Köves, E.: Examination of germination- and growth-inhibiting substances in dry fruits. Manuscript.
- (10) Winter, A. G., und Schönbeck, F.: Untersuchungen über den Einfluss von Kaltwasserextrakten aus Getreidestroh und anderer Blattstreu auf Wurzelbildung und Wachstum. *Naturwiss.*, **40**, 513 (1953).