BIOLOGIA PLANTARUM (PRAHA)

24 (1) : 1–6, 1982

Effect of Polyphenols on Shoot and Root Growth and on Seed Germination

D. J. STOM

Institute of Biology, A. A. Zhdanov State University, Irkutsk*

Abstract. The effect of high concentrations of the simplest polyphenols on extension growth of maize (*Zea mays* L.) shoot coleoptile segments, lettuce (*Lactuca sativa* L.) roots, and on radish (*Raphanus sativus* L.) seed germination, was studied. Quinone formation in the process of plant incubation in polyphenol solutions was proved. The data obtained are presumably explained by the important part that is played by the quinoid products of phenol oxidation in the ability of *o*- and *p*-biatomic phenols to inhibit growth.

Phenolic compounds in high concentrations suppress the growth processes in various biotests (KEFELI 1974). Plants are able to oxidize certain phenols (LUCKNER 1977). Some authors discussing the problems of interconnection between phenol oxidation and their inhibiting properties, often associate the oxidation process only with metabolic conversions of the natural inhibitors. But, side by side with these conceptions, a hypothesis exists according to which the inhibiting effect of the phenols is possibly connected with the action of their oxidation products — quinones (TOMASZEWSKI 1957, STOM 1969a, b).

The subject of the present work is the study of the role of quinoid products of phenol oxidation in inhibiting the plant growth by exogenic phenols.

MATERIAL AND METHODS

The following biotests were employed: extension growth of the coleoptile segments of maize (*Zea mays* L., cv. Krasnodarskaya 1/49), extension growth of the roots of lettuce (*Lactuca sativa* L., "Narrow leaf"), and germination rate of radish (*Raphanus sativus* L., "Pink with white tip") seeds.

Seven-millimetre long coleoptile segments (2 mm below the apex) of the four-day maize shoots were placed in Petri dishes with 15 ml of the test solution. The segment length was measured every 24 h with a horizontal microscope.

The radish seeds were germinated in test solutions in Petri dishes (50 seeds per dish). The germinated seeds were counted every 2 d. The lettuce roots

Received May 17, 1979; accepted February 27, 1981

* *Address:* 664003, Irkutsk-3, a/ya 24, USSR.

TABLE 1
Physico-chemical characteristics of aniline and phenyl sulfonic derivatives of quinones

Compound	Melting temperature [°C]	S content [%] Calculated	S content [%] Obtained	Absorption peaks in IR [cm^{-1}] ν	Absorption peaks in IR [cm^{-1}] Group	Absorption peaks in UV (dioxan) [nm] λ max	Absorption peaks in UV (dioxan) [nm] λ max
4,5-Dianiline-1,2-benzoquinone	192	—	—	1420 1619		307	416
o-Dioxydiphenyl sulfone	164—165	12.8	13.0	1140 1300	—SO_2	257	289
p-Dioxydiphenyl sulfone	196	12.8	13.0	1140 1300	—SO_2	221	320
Caffeic acid sulfone	132	10.0	9.6	1140 1300	—SO_2	257	305
Chlorogenic acid sulfone	129—130	6.3	6.1	1140 1300	—SO_2	254	309

were treated for 2 min with quinone solutions of pyrocatechol and chlorogenic acid, transferred after washing to 0.005 M phosphate buffer of pH 5.0 at 25 °C and kept in darkness for 24 h.

Quinones were preliminary analysed chromatographically as their stable derivatives (STOM 1975). This was followed by preparative isolation and identification of quinone derivatives (STOM 1975). Quinone derivatives were isolated from an acidified ether extract solution of a mixture of 5×10^{-3} M aniline and benzene sulfonic acid and of 5×10^{-3} M polyphenol after incubation of the maize coleoptiles and radish seeds in this solution. Pyrocytechol, hydroquinone, caffeic and chlorogenic acid (all in analytical grade) were used. Ussuri pear (*Pyrus ussuriensis*) leaves were employed in the experiments. Solution of polyphenol mixture with aniline and benzene sulfonic acid was used as control, without addition of plant material or with addition of plant material preliminary destroyed by heating (for 90 min at 100 °C).

TABLE 2
Effect of polyphenols and *p*-benzoquinone on extension growth of coleoptile segments of 4-day maize shoots and on oxygen consumption by their homogenates

Compound (10^{-2} M)	24-h extension growth [% of control] − IAA	24-h extension growth [% of control] + IAA	Oxygen consumption during 40 min exposure [% of control g^{-1} fr. m.]
Resorcinol	100 ± 14	195 ± 12	645 ± 73
Hydroquinone	74 ± 7	174 ± 10	609 ± 72
Pyrocatechol	37 ± 6	68 ± 9	7918 ± 681
p-Benzoquinone	dead	dead	*

* Not determined.

TABLE 3
Effect of polyphenols on radish seed germination

Compound (10^{-2} M)	Number of seeds germinated after 24 h [% of control]	
	10^{-3} M	10^{-2} M
Resorcinol	85 ± 8	70 ± 3
Hydroquinone	83 ± 5	75 ± 4
Pyrocatechol	42 ± 6	7 ± 1
p-Benzoquinone	12 ± 2	5 ± 1
Mannite	—	98 ± 8

UV-spectra were taken in dioxan solution with a spectrophotometer "SF-48" (USSR), IR-spectra (compressed tablets with KBr) were obtained on an IR-spectrometer "UR-10" (DDR). Molecular mass was determined by Rast's method (CHERONIS 1960).

To obtain *o*-quinones, pyrocatechol and caffeic acid were oxidized with $Ce(NH_4)_2(SO_4)_3$; Ce^{3+} was quantitatively precipitated by adding K_2HPO_4 and KH_2PO_4.0.005 M phosphate-citrate buffer was used for adjusting pH 4.0.

Quinoid products formed by oxidation with phenols were evaluated by iodometric titration (HOUBEN-WEYL 1967). The equivalence point was found potentiometrically. Oxygen consumption by homogenates was determined by Warburg's method. Maize coleoptiles were homogenized at 4 °C in 0.005 M phosphate-citrate buffer at pH 5.2.

RESULTS

Physico-chemical characteristics of phenyl sulfonic and aniline derivatives of quinones are given in Table 1. These derivatives were isolated from an incubation medium, containing the plants and a mixture of 5×10^{-3} aniline and benzene sulfonic acid and 5×10^{-3} M phenol. Physico-chemical charac-

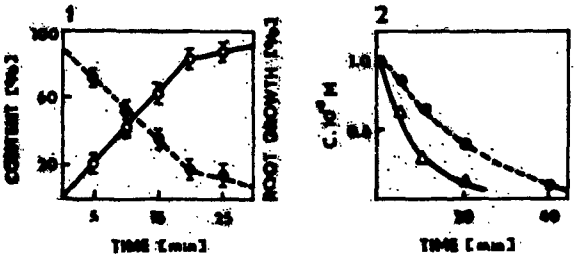

Fig. 1. *o*-Benzoquinone concentration alteration with time [% of control] and the effect of its solutions on the growth of lettuce roots. Initial *o*-benzoquinone concentration: 5×10^{-3} M; *o*-benzoquinone was obtained by oxidizing pyrocatechol with $Ce(NH_4)_2(SO_4)_3$. ● — *o*-benzoquinone; ○ — growth of lettuce roots in 24 h after 2 min treatment in *o*-benzoquinone solutions. Fig. 2. Kinetic curves of *o*-quinone dissociation at pH 4.0, and 19 °C. ● — *o*-benzoquinone; △ — *o*-quinone of caffeic acid.

TABLE 4

Extension growth of 4-day maize shoot coleoptile segments during 24 h incubated in mixture of different composition

Composition of incubation mixture	Growth [% of control]
Pyrocatechol (10^{-2} M)	39 ± 2
p-Benzoquinone (2.5×10^{-3} M)	46 ± 3
Glutathione (10^{-3} M)	98 ± 3
Natrium diethyl dithiocarbamate (5×10^{-3} M)	102 ± 6
Pyrocatechol (10^{-2} M) + glutathione (10^{-3} M)	91 ± 7
Pyrocatechol (10^{-2} M) + natrium diethyl dithiocarbamate (5×10^{-3} M)	65 ± 3
p-Benzoquinone (2.5×10^{-3} M) + glutathione (10^{-3} M)	109 ± 9

teristics of quinone derivatives practically coincide with those of quinones obtained by counter synthesis (STOM 1975); the appearance of quinone derivatives in an incubation medium thus proves that the plants activate the formation of quinones from polyphenols. Quinones were not detected in the control. The extension growth of the maize shoot coleoptile segments and the radish seed germination were suppressed most by pyrocatechol, intermediately by hydroquinone, and least by resorcinol (Tables 2, 3). Presence of 5×10^{-5} M indolyl-3-acetic acid (IAA) in the incubation medium stimulated coleoptile segment growth, but did not alter the ratios of polyphenol activities (Table 2). A 10^{-2} M polyphenol solution was added to homogenate in order to evaluate the degree of polyphenol oxidation by the homogenate of the maize coleoptile tissues; solutions of 10^{-2} M phenols were added to them. Pyrocatechol caused the highest oxygen consumption (Table 2). 5×10^{-3} M natrium diethyl dithiocarbamate (DIECA) reduced inhibitory action of pyrocatechol on the growth of the coleoptile sections (Table 4). Glutathione (10^{-3} M) also weakened the pyrocatechol effect on the growth (Table 4).

Growth of the coleoptile segments and germination of the radish seeds was suppressed much more strongly under the action of *p*-benzoquinone than under that of polyphenols (Tables 2, 3).

The effect of *o*-benzoquinone (pH = 4.0) solutions on the growth of the lettuce roots is shown in Fig. 1. Beginning with the moment of formation, *o*-quinone concentration drops and simultaneously the ability of its solutions to suppress the lettuce root growth is reduced (Fig. 1).

Iodometric titration (Fig. 2) showed that the semi-decay period of the caffeic acid *o*-quinone is much smaller than that of *o*-benzoquinone. Therefore, considerable inhibition of the lettuce root growth (by 70%) is obtained only after treating the roots for 6 min with caffeic acid quinone, the solutions being replaced every 30 s. Control tests with equimolar solutions of caffeic acid and pyrocatechol did not demonstrate any inhibition of the root growth.

DISCUSSION

In the experiments with maize shoot coleoptile segments and in the tests with radish seeds pyrocatechol showed the greatest activity in growth suppression. Hydroquinone acted more weakly and the least inhibition of

the growth processes was caused by resorcinol. The same correlation of the inhibiting properties of pyrocatechol, hydroquinone and resorcinol has been obtained by other authors in tests on the ability of these polyphenols to suppress the extension growth of the coleoptile segments of the cereal shoots (PLOTNIKOVA *et al.* 1967). Resorcinol is the most stable polyphenol against oxidation. Its dehydration, contrary to hydroquinone and pyrocatechol oxidation, is not accompanied by formation of quinone (BLAJEI and SHOUTYI 1977). Therefore, weak growth suppression by resorcinol correlates well with an assumption according to which the increased ability of *o*- and *p*-diphenol to suppress growth processes is caused by the quinoid products of their oxidation (STOM 1969a, b).

The following facts are also in favour of this hypothesis. Pyrocatechol inhibited the maize shoot coleoptile segment growth more strongly than hydroquinone and caused a higher increase of oxygen consumption when added to the shoot homogenates.

The higher growth inhibition of the maize coleoptile segments and radish seed germination by quinones in comparison with phenols is in agreement with these ideas. The high ability of quinones to inhibit the plant growth has already been reported (CIASCA and LUCHETTI 1965, BRAGINSKIĬ 1972).

Prior oxidation of *o*-isomer polyphenols by coleoptile homogenates (Table 2) allows an assumption that in the tests described *o*-diphenol oxidase is basically responsible for oxidation of pyrocatechol. This assumption conforms with the absence of *o*-quinone formation in the course of incubation of coleoptiles in polyphenol solutions of coleoptiles and germinating radish seeds destroyed by heating.

The histochemical discovery of *o*-diphenol oxidase activity in the maize shoot coleoptiles and in the germinating radish seeds (STOM 1969a, b) is also in favour of the assumption. Earlier, HASKINS (1955) has reported on the presence of *o*-diphenol oxidase in maize shoots.

The weakening of pyrocatechol ability to impede the growth processes caused by the presence of diethyl dithiocarbamate, an inhibitor of *o*-diphenol oxidase (Table 4), and the compounds regenerating quinones is also an argument in favour of the connection between pyrocatechol effect and quinoid products of *o*-diphenol oxidase oxidation.

Polyphenol oxidation is accompanied by the formation of various combinations of radicals, dimers, polymer products of oxidation and condensation of phenols (BLAJEI and SHOUTYI 1977). The data in Fig. 1 indicate, however, that the basic inhibiting properties of pyrocatechol oxidation products are mainly connected with *o*-benzoquinone. Indeed, with the destruction of *o*-benzoquinone the ability of its solutions to inhibit the growth of the lettuce roots diminishes (Fig. 1). In this work, relatively high concentrations of polyphenol solutions have been used. Mannitol solutions, equimolar with polyphenol solutions, practically do not inhibit radish seed germination (Table 3). Therefore, inhibition of the seed growth by polyphenols, apparently, cannot be explained by the increased osmotic potential of the solutions.

In the tests *in vitro*, resorcinol accelerated destruction of IAA by oxidase of indolyl-3-acetic acid (o-IAA). Contrary to resorcinol, pyrocatechol and hydroquinone delayed IAA destruction caused by o-IAA (WADA 1961, BARABOĬ 1976). From this it was to be expected that if the polyphenol

effect is connected with its influence on o-IAA, then resorcinol should inhibit the growth processes more strongly than pyrocatechol and hydroquinone. But, as is seen from the above data and also from the experiments of PLOTNIKOVA *et al.* (1967), the inhibiting properties of pyrocatechol and hydroquinone, both in the presence and in the absence if IAA, are higher than those of resorcinol. Therefore, the effect of polyphenols on the growth cannot be explained by their influence on o-IAA.

The following fact also speaks in favour of the absence of direct relationship between the effect of phenols on growth and their interaction with IAA. Although IAA stimulated the maize shoot coleoptile section growth, it did not change the relation of polyphenol toxicity (Table 2).

Thus, the materials obtained and their analysis allow a conclusion to be made that intermediate products of phenol oxidation, close to quinones, play an important role in the ability of *o*- and *p*-diphenols to inhibit the maize shoot coleoptile section growth and radish seed germination.

REFERENCES

BARABOĬ, V. A.: [Biological Effect of Vegetative Phenolic Compounds.] In Russ. — Naukova Dumka, Kiev 1976.

BLAJEI, A., SHOUTYI, L.: [Phenolic Compounds of Vegetative Origin.] In Russ. — Mir, Moskva 1977.

BRAGINSKIĬ, L. P.: [Pesticides and Life of Water Basins.] In Russ. — Naukova Dumka, Kiev 1972.

CHERONIS, N.: [Micro- and Semimicromethods in Organic Chemistry.] In Russ. — I. L., Moskva 1960.

CIASCA, M. A., LUCHETTI, M. A.: Impiego di culture di tessuti vegetali "*in vitro*" per lo studio dei sistemi enzimatici. — Ann. Ist. super sanita **1** : 533—539, 1965.

HASKINS, F. A.: Changes in the activities of several enzymes during germination and seedling development in corn (*Zea mays* L.). — Plant Physiol. **30** : 74—78, 1955.

HOUBEN-WEYL, H.: [Methods of Organic Chemistry. Vol. 2.] In Russ. — Khimiya, Moskva 1967.

KEFELI, V. I.: [Natural Growth Inhibitors and Phytohormones.] In Russ. — Nauka, Moskva 1974.

LUCKNER, M.: [Secondary Metabolism of Microorganisms of Plants and Animals.] In Russ. — Mir, Moskva 1979.

PLOTNIKOVA, I. V., RUNKOVA, L. V., UGOLIK, L. A.: [Polyphenol influence on growth of the wheat — couch-grass hybrid No. 1 coleoptiles.] In Russ. — Byull. glav. bot. Sada **64** : 71 to 76, 1967.

STOM, D. J.: Use of thin-layer and paper chromatography for detection of ortho- and para-quinones formed in the course of phenol oxidation. — Acta hydrochim. hydrobiol. **3** : 39 to 45, 1975.

STOM, D. J.: [Quinones as a possible active form of polyphenolic growth inhibitors.] In Russ. — Izv. Akad. Nauk SSSR **186 (3)** : 714—716, 1969a.

STOM, D. J.: [Oxidation of phenols by plant tissues in the extreme conditions.] In Russ. — In: [Plant Growth, Development and Stability. Proc. 3rd Conference of Plant Physiologists and Biochemists of Siberia and the Far East.] In Russ. — Pp. 155—158, Irkutsk 1969b.

TOMASZEWSKI, M.: Das Phenol-Phenoloxydase-System der Blätter einiger Obstgehölze und seine Beziehung zur Winterruhebereitsschaft. — Flora **145** : 146—166, 1956.

WADA, S.: IAA-oxidase inhibitors contained in rice coleoptiles. — Sci. Rep. Tohoku. IV Ser. B **27** : 237—249, 1961.