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EFFECT OF PHENOLICS ON PLANT AMINE OXIDASES

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Key Word Index—*Pisum sativum*; Leguminosae; pea; *Hordeum vulgare*; Gramineae; barley; diamine oxidase; polyamine oxidase; phenolics.

Abstract—Phenolic compounds at low concentrations decrease pea diamine oxidase activity without affecting growth, but they have no effect on barley polyamine oxidase in spite of a decrease in growth.

INTRODUCTION

Plant amine oxidases, which may be involved in the regulation of intracellular polyamine concentration, have been extensively characterized from pea [1–3] and barley [4–7]. Pea diamine oxidase (DAO) (EC 1.4.3.6), which has a broad specificity [8], is absent in ungerminated seeds and appears during the early period of germination. The enzyme activity is greatest in the growing regions and decreases with the maturity and senescence of the plant [9]. It is regulated by a feed-back mechanism through auxins and is induced by its substrates, suggesting that it may have an important role in the growth and development of plants by regulating indoleacetic acid (IAA) biosynthesis [10]. The polyamine oxidase (PAO) (EC 1.5.3.3) from barley seedlings oxidizes spermidine and spermine [11] but has no action on diamine substrates and nothing is known about its regulation. Since certain phenolic compounds appear to control growth by regulating IAA levels, [12], it was of interest to study the effect of phenolics on DAO and PAO in pea and barley.

RESULTS AND DISCUSSION

Pea and barley seeds were imbibed for 8 hr in the presence of phenolics (100 ppm) and germinated for various lengths of time. No significant effect on growth was observed with the pea seedlings at 100 ppm, however, increasing the concentration to 200–400 ppm caused a 25–40% decrease in growth. In the imbibition of barley seedlings with phenolics (100 ppm) the result was a considerable decrease in germination ($>50\%$). DAO of pea cotyledon and embryo decreased during the early periods but was reversed to the control level at later periods (Table 1). The inhibitory effect was more pronounced for the cotyledon enzyme. In the embryo, the inhibition was mainly evident on day one, but increasing the concentration to 200 ppm prolonged the inhibition to later periods of germination (data not given). The barley root PAO was not affected by phenolics in spite of a decrease in growth. Prolonging the period of imbibition from 8 to 20 hr or increasing the concentration to 200 ppm had no effect on PAO activity.

The effect of phenolics (1 mM) was also tested in homogenates from the seeds imbibed and germinated in water for 3 days. In the case of DAO activity (Table 2), quercetin, naringenin and phloridzin caused significant inhibition (60–80%). The inhibition by rutin, naringin and *p*-coumaric acid was less pronounced (30–40%). Coumarin, *trans*-cinnamic acid, rotenone and nonanoic acid gave no significant *in vitro* inhibition even though these compounds caused a considerable decrease in enzyme activity *in vivo* (Table 1). Barley root PAO was, however, not inhibited by any of the phenolics even at 10 times higher concentration. The results indicate that in the case of the pea seedlings some of the phenolics may decrease the enzyme activity as a result of their binding to enzyme protein, as reported for some enzyme systems [13], whilst others may exert their effect by decreasing the level of enzyme synthesis. The compounds which inhibited pea DAO *in vitro* were also tried at different concentrations and the results (Table 3) show that a major fraction of the inhibition was obtained at 0.2 mM. Addition of catalase to the assay system along with the phenolics had no effect on the inhibition pattern, indicating that the inhibition may not be due to the accumulation of H_2O_2.

Phenolic substances thus appear to control pea DAO activity by binding with the enzyme as well as by affecting its synthesis. The effects are not related to the growth since the concentrations used had no effect on germination of seedlings. In the case of barley, even though the growth was decreased by phenolics the PAO of roots was not affected, indicating that the amine oxidases present in pea and barley, in addition to different substrate specificities, also differ in their mode of control.

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Table 1. Effect of phenolics on amine oxidases on days 1–4 after germination

Compounds*	DAO activity (nkat/g fr. wt tissue)								PAO activity (nkat/g fr. wt tissue)		
	Cotyledon				Embryo				Root		
	1	2	3	4	1	2	3	4	2	3	4
—	0.4	5.3	11.2	14.0	2.6	11.6	13.1	13.6	1.8	2.3	2.5
Rutin	0.2	3.0	13.4	13.1	1.2	9.2	13.6	13.6	2.0	2.4	2.6
Quercetin	0.3	2.9	8.9	10.3	0.8	10.6	14.8	14.9	1.9	2.0	2.5
Naringin	0.1	1.9	12.3	13.5	1.0	9.7	13.6	13.1	1.8	2.1	2.4
Naringenin	0.3	1.6	6.3	13.6	0.4	9.7	12.3	13.3	1.7	2.4	2.4
Phloridzin	0.1	0.7	4.6	12.4	0.3	7.5	12.8	12.5	2.0	2.2	2.6
p-Coumaric acid	0.1	1.3	6.8	9.7	0.1	9.9	11.7	12.1	1.7	2.0	2.3
Coumarin	0.2	2.7	10.0	12.3	0.3	10.2	10.9	10.4	1.8	2.0	2.1
t-Cinnamic acid	0.1	3.1	10.3	13.1	0.9	9.4	11.2	11.3	2.1	2.1	2.5
Rotenone	0.2	2.7	13.1	14.0	1.0	11.3	14.1	13.7	1.9	2.3	2.4
Nonanoic acid	0.1	0.7	5.5	10.9	0.3	9.7	9.7	12.7	1.3	2.0	2.2

*Seeds were imbibed with phenolics (100 ppm) for 8 hr.

Table 2. *In vitro* inhibition of amine oxidases by phenolics*

Compounds	% Inhibition of DAO		% Inhibition of PAO
	Cotyledon	Embryo	Root
Rutin	39	43	0
Quercetin	81	76	0
Naringin	28	36	4
Naringenin	77	80	0
Phloridzin	60	57	0
p-Coumaric acid	38	40	2
Coumarin	3	7	0
t-Cinnamic acid	7	14	3
Rotenone	8	13	0
Nonanoic acid	4	5	10

*Homogenate from 3 day germinated seeds was used for enzyme assay with 1 mM phenolics as inhibitor.

Table 3. Effect of 0, 0.2, 0.6 and 1.0 mM concentrations of phenolics on pea DAO activity*

Compounds	DAO activity (nkat Δ^1-pyrroline formed)							
	Cotyledon				Embryo			
	0	0.2	0.6	1.0	0	0.2	0.6	1.0
—	0.23				0.28			
Rutin		0.21	0.18	0.15		0.18	0.17	0.16
Quercetin		0.08	0.06	0.05		0.14	0.08	0.06
Naringin		0.23	0.20	0.17		0.20	0.19	0.17
Naringenin		0.16	0.07	0.05		0.17	0.11	0.06
Phloridzin		0.17	0.14	0.10		0.16	0.14	0.12
p-Coumaric acid		0.18	0.16	0.15		0.17	0.16	0.15

*Homogenate from 3 day germinated seeds was used for enzyme assay.

EXPERIMENTAL

Plant material. Pea (*Pisum sativum*) and barley (*Hordeum vulgare*) seeds were soaked in H_2O or phenolics for 8 hr or longer and germinated in light at 25° in Petri dishes over one layer of filter paper. Growth was measured by taking the fr. wt/seed of the embryo in the case of pea and of the roots in the case of barley.

Enzyme activity. DAO of pea cotyledons and embryos and PAO of barley roots were determined as described in ref. [14].

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