Naturally occurring pea diamine oxidase inhibitors obtained from *Sorghum vulgare* during germination

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Summary. During germination of *Sorghum vulgare* seeds, inhibitors of pea diamine oxidase appeared in the embryos. One was heat labile, dialysable and inhibited the enzyme in vitro, while another was heat stable and inhibited the enzyme synthesis when pea seeds were soaked and allowed to germinate in the extract containing the inhibitors.

Pea diamine oxidase (diamine-oxido-reductase (DAO) EC: 1.4.3.6) is known to have a broad substrate specificity and oxidises many primary amines[4]. The enzyme, which may be involved in the regulation of intracellular polyamine concentration, is distributed widely[5] and oxidises these compounds by a variety of mechanisms[4]. This enzyme has been purified extensively and characterized[6-8], but its functions in vivo are not understood fully. The role assigned to it was once thought to involve the formation of auxins like indolyl-acetic acid (IAA) from tryptamine in plants[9,10] though later it was believed to be unlikely[11]. More recent studies indicate that auxins whose synthesis may be controlled by DAO seem to inhibit their own synthesis in a feed-back manner which in turn results in increased levels of di- and polyamines which would again induce the synthesis of the enzyme[12,13]. The DAO enzyme activity has been detected in *Phaseolus ocontifolium, Lens esculenta, Cicer arietinum, Vigna catjung* and *Cyamopsis tetragonoloba* seeds; while its inhibitors have also been detected in seeds belonging to the graminae family during germination[15].

The absence of DAO in the extracts of cotyledons of groundnut seedlings has been reported to be due to the presence of a natural inhibitor of this enzyme which is a lipid-protein complex[16]. The present communication presents data on the natural inhibitors of pea DAO from *S. vulgare* seeds during germination and their properties.

Methods and materials. The seeds were purchased from the local market. They were surface-sterilized with lysol and washed thoroughly with water. They were then soaked in water for 16 h in the dark at 22 °C. For germination the seeds were placed in a petri dish over moistened filter papers and this was considered as the zero day of germination. The reference DAO enzyme was obtained from the cotyledon of pea seeds *(Pisum sativum)* germinated for 2 days. At specified intervals, the cotyledon and the embryo were separated and homogenized by grinding with 10 mM phosphate buffer at pH 7.0 with a pestle and mortar previously kept on crushed ice. The extract obtained after passing the homogenate through 2 layers of cotton cheese cloth was used as the enzyme or the inhibitor source. The DAO activity was measured as described by Holmstedt et al.[14].

The enzyme unit is defined as the amount of the enzyme required to liberate 1 μM of Δ^1-pyrroline per 30 min at 37 °C under the assay conditions. 1 unit of the inhibitor is the amount of the inhibitor required to inhibit 1 unit of DAO completely. The action of the inhibitor was studied under in vivo conditions by soaking pea seeds in *S. vulgare* shoot supernatant containing inhibitors for 16 h at 22 °C in the dark. The seeds were then allowed to germinate in a petri dish over filter papers previously moistened with the same extract.

The nature of the inhibitors was investigated by heating the shoot supernatant (SS) first in a boiling waterbath for 15 min or 30 min. The SS was then deproteinized by the addition of equal amounts of cold 10% TCA. The supernatant obtained after centrifuging at 11,500 × g for 20 min at 4 °C was neutralized while the residue was suspended in 0.025 M phosphate buffer and both the supernatant and the

Table 1. Nature of the inhibitor of Pea DAO in *Sorghum vulgare* embryo

Addition	Pea DAO units/g fresh tissue	Inhibition units/g fresh tissue
None	22.6	–
Root:		
Supernatant	20.1 ± 1.02	2.5 ± 1.02
Residue	16.1 ± 1.02	6.6 ± 1.02
Shoot:		
Supernatant	0.9 ± 0.89	21.7 ± 0.08
Residue	22.6	0.0
Heat treatment:		
15 min	11.3 ± 0.78	11.3 ± 0.83
30 min	16.7 ± 0.82	5.9 ± 0.82
TCA treatment:		
Supernatant (neutralized)	1.3 ± 0.37	21.3 ± 0.36
Residue	22.6	0.0
Dialysis:		
None	22.3	–
Dialysed	21.8 ± 0.95	0.5 ± 0.08
Undialysed	3.8 ± 0.42	18.5 ± 0.41
Dowex 50 eluate:		
Charcoal treated	0.0	22.6
Dowex 50 eluate	22.1 ± 0.94	0.5 ± 0.20

Each observation is expressed as mean ± SD.

Table 2. Effects of the inhibitors of pea DAO from *S. vulgare* embryo on pea seeds germination and DAO synthesis

Pea seeds soaked in	Germination on		Pea DAO activity units/g fresh weight			
			Cotyledon		Embryo	
	2nd day	4th day	2nd day	4th day	2nd day	4th day
Water	G (++)	G (++++)	22.6 ± 0.94	24.7 ± 0.97	24.1 ± 0.51	24.2 ± 0.42
Shoot supernatant	SG	G (+)	6.6 ± 0.37	5.6 ± 0.55	13.2 ± 0.90	9.5 ± 0.55
Heated shoot supernatant	SG	G (+)	7.0 ± 0.71	5.8 ± 0.68	12.0 ± 2.28	10.8 ± 0.42
TCA supernatant	SG	G (+)	0.0	0.5 ± 0.14	1.0 ± 0.14	1.0 ± 0.14
Bowex-50 eluate	SG	G (+)	0.5 ± 0.14	0.9 ± 0.06	0.50 ± 0.14	0.9 ± 0.25
Charcoal treated Dowex-50 eluate	SG	G (+)	2.8 ± 0.25	3.2 ± 0.19	3.2 ± 0.52	3.0 ± 0.14

SG, Slightly germinated; G, germinated. Each observation is expressed as mean ± SD.

residue were assayed for inhibitor activity. In the next step, the SS was dialyzed extensively against 0.0001 M phosphate buffer pH 7.0 at 10 °C overnight. The SS was passed through a column (15 cm × 1.1 cm ø) of Dowex 50 W × 8 (200–400 mesh) and the inhibitor eluted with 1 column-volume of distilled water at about 3 °C. The eluate thus obtained was treated with activated charcoal (0.25 g/ml eluate) at 2 °C. This was filtered and the filtrate was assayed for the inhibitors.

Analysis of data was done by repeating all the experiments in series 5 times. Each observation was made in duplicate keeping its control and blank side by side, and each datum was expressed as mean ± SD. Almost all observations were found to fall within ± 2 SD.

Results and discussion. Pea DAO is reported to be absent from resting seeds in general[15] and it is said to appear in the cotyledon of pea seeds on the 2nd day of germination[12, 17, 18] In the present study, it was also detected on the 2nd day. *Sorghum vulgare* shoot and root homogenates obtained from seeds germinated for 5 days were incubated with pea DAO, showing the presence of the inhibitors. The shoot supernatant of the embryo obtained from 50 mg of the tissue showed the maximum inhibition of 21.7 units/g fresh tissue; while the shoot residues had practically no inhibitor (table 1). The inhibitor seems to be thermolabile as 15 min of treatment destroyed about 50% of the inhibitor, and a further treatment gave a final reduction to about 25% of the original value. The inhibitor does not seem to be a protein since after trichloroacetic acid treatment the TCA supernatant, after neutralization, contained all the detectable inhibitor activity (21.3 units). Shoot supernatant, after dialysis overnight, no longer contained the inhibitor whilst the control solution had the same initial activity. The inhibitor could be eluted with water from Dowex-50 but was adsorbed on activated charcoal.

The action in vivo was then studied by soaking pea seeds in the shoot supernatant and germinating them on filter papers moistened by the same extract in a petri dish. The germination of the seeds was found to be diminished in the extract-treated seeds which also had reduced enzyme activities. Heated extract could not show any reversal effect on the germination or on the enzyme synthesis. Various fractions at different stages of purification could not restore the normal germination nor the enzyme synthesis (table 2); this indicates the presence of more than one inhibitor - one acting in vitro blocking the enzyme activity and the other acting in vivo blocking the germination of the seeds and the enzyme synthesis.

The 2 types of inhibitors, one active under in vitro conditions and the other on germinating seeds could be separated by adsorption on active charcoal. The latter inhibitor is heat stable and is neither inactivated by TCA nor adsorbed on activated charcoal.

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Influence of the feeding conditions on competition between *Drosophila melanogaster* and *Drosophila simulans* for their oviposition site

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Summary. Under low feeding conditions, the number of eggs laid by the female *Drosophila melanogaster* or the female *Drosophila simulans* is frequency dependent: it increases simultaneously with the increase of the frequency of the species to which the female belongs. This result could be explained by the role of scents in the choice of the oviposition site in *Drosophila*.

Populations of both sibling species, *Drosophila melanogaster* and *Drosophila simulans*, are often sympatric[1]. An important component of competition between *Drosophila* is the amount of eggs laid by each female per unit of time. Both species employ the same kind of oviposition sites which are also the feeding sites of the adult flies. Owing to the limited food supply available at certain periods of the year, these closely related species may compete for the use of a common resource. This work will be devoted to an experimental study dealing with competition for an oviposition under low feeding conditions, at various frequencies of the two species.

Material and methods. A *sepia* mutant strain of *D. melanogaster* and a wild strain of *D. simulans*, captured in 1971 and 1977 respectively, have been kept in the laboratory by mass-replications at 20 °C. 6 populations of 1000 fertilized, 3-day-old females were initiated with these strains, the relative frequencies of each species in each population being respectively 0.00, 0.20, 0.40, 0.60, 0.80, 1.00. Each population was placed in a population cage with 12 vials containing 30 cm^3 of Pearl's food medium[2]; the age of each population was 2 days in order to suppress the effects of anaesthesia. The cages were stored under conditions commonly recognized as optimal for both species[3–5]: