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DISTRIBUTION OF CADAVERINE AND OTHER AMINES IN HIGHER PLANTS

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Key Word Index—*Pisum*; pea; *Phaseolus*; *Vicia*; bean; *Trifolium*; clover; *Lupinus*; lupin; Leguminosae; *Hordeum*; barley; Gramineae; cadaverine; putrescine; polyamines; amine oxidase; homoserine lactone.

Abstract—The distribution of the diamines cadaverine and putrescine and of the polyamines spermidine and spermine has been determined in pea seedlings at various developmental stages. In the axes cadaverine and putrescine increase on germination (× 9·5 and 5·7 resp.) while spermidine and spermine decline (× 0·19 and 0·12 resp.) on a fr. wt basis. The cadaverine declines from the 6th to the 18th day in the light-grown shoots. In the roots this decline is not significant and in the roots of 42-day-old light-grown plants the cadaverine level is 70-fold greater than in the leaves. The highest level of cadaverine occurred in the axes of dark-grown 6-day-old seedlings (2 μmol/g fr. wt) and the highest level of putrescine occurred in the roots of 6-day-old light-grown seedlings (2 μmol/g fr. wt). Addition of calcium carbonate to the sand, using an ammonium medium, reduced the putrescine level of the roots (× 0·12) and potassium deficiency increased the putrescine level of the shoots (× 6). There was no significant effect on the cadaverine content in either treatment. Cadaverine was detected in *Trifolium subterraneum* seedlings though not in seedlings of *Phaseolus aureus*, *P. vulgaris*, *Lupinus regalis* or *Vicia faba*. An additional compound found on dansylation of pea amine fractions was identified as the lactone of homoserine.

INTRODUCTION

The diamine cadaverine, $NH_2(CH_2)_5NH_2$, has been found in the genera *Pisum*, *Glycine*, *Lathyrus* and *Vicia*, all in the family Leguminosae [1–5]. Moreover, cadaverine acts as a precursor of the quinolizidine alkaloids formed in the legumes *Lupinus*, *Sarothamnus* [6] and *Goebelia* [7]. Cadaverine has been found only sporadically in other families of higher plants. Free cadaverine has been characterized in *Sedum* (Crassulaceae) [8] in which it is the precursor of the piperidine alkaloids. Cadaverine also occurs* in members of the Araceae [9], in tomato and tobacco plants [10], in the grains of rice [11] and of other cereals [12] and has been tentatively identified in the leaves of mature barley plants [13].

Although it has been suggested that the pea amine oxidase is concerned with the formation of indol-3-yl-acetic acid, this is now considered to be unlikely [14]. Anderson and Martin [1] have recently proposed that the reason for the high activity of this enzyme may lie in the occurrence of cadaverine in pea tissue. In the present work, the change in the concentration of cadaverine, of its lower homologue putrescine, and of the closely related polyamines spermidine and spermine has been determined in germinating pea (*Pisum sativum* L. cv. Meteor) seedlings in relation to the activity of the diamine oxidase, and the occurrence of these amines in other plants has been investigated.

* For earlier references, see Guggenheim, M. (1958) Die biogenen Amine in der Pflanzenwelt, in *Handbuch der Pflanzenphysiologie* (Ruhland, W., ed.). Vol. 8, p. 889. Springer, Berlin; Karrer, W. (1958) in *Konstitution und Vorkommen der organischen Pflanzenstoffe* Birkhauser, Basel.

Table 1. Di- and poly-amines in dark-grown pea seedlings

Tissue	Age (days)	Seedling units/ g fr. wt	Cadaverine	nmol/g fr. wt Putrescine	Spermidine	Spermine
Seed	1	4·1	<1	103	728	109
Axes	1	6·0	212	339	770	237
	2	2·9	1510	978	605	66
	3	2·0	1810	1530	183	24
	4	1·6	1600	1400	225	25
	6	0·7	2000	1920	148	28
Cotyledons	1	2·2	<1	82	279	41
	2	2·2	1	159	206	17
	3	2·2	1	217	195	16
	4	2·1	2	315	240	27
	6	2·1	2	592	136	23

Seed was germinated in Petri dishes and sampled at intervals for amine estimation by the dansyl method. The values for the 1- and 3-day-old seedlings were means of duplicate estimates made on material grown on separate occasions.

RESULTS AND DISCUSSION

During the first 6 days germination there was an increase in cadaverine (× 9·5, $P < 1\%$) and putrescine (× 5·7, $P < 5\%$) in the axes, expressed on a fr. wt basis. The two polyamines spermidine (× 0·19, $P < 5\%$) and spermine (× 0·12, $P < 1\%$) declined over this period. In the cotyledons putrescine increased significantly (× 7·2, $P < 5\%$), but changes in the levels of the other amines over the 6-day period in the cotyledons were not significant (Table 1).

In the sand culture experiment (Tables 2 and 3) no significant difference could be found between the amine levels of the roots of plants growing in the light or dark, by contrast to the difference found between the light- and dark-grown shoots. No significant trend in amine level could be found for any amine in the roots in dark or light conditions for the periods under investigation. In the light- or dark-grown shoots no significant trend with time was found for the polyamines, nor for the cadaverine or putrescine in the dark-grown shoots. However, for the light-grown shoots there was a significant decline in cadaverine (× 0·012, $P < 0{\cdot}1\%$) and putrescine (× 0·1, $P < 5\%$). None of the amines showed significant trends in the cotyledons for 6–18 day's growth in light or dark. Expressing the results in terms of seedling units, a considerable increase was found over the initial 6-day period in cadaverine (× 81) and putrescine (× 48). By contrast, spermidine and spermine expressed on this basis remained roughly constant (Table 1). A continuation of these trends was found up to the 12th day in the sand-grown plants (Table 2). These results are similar to those found for *Lathyrus* sativus seedlings [4].

Table 2. Di- and poly-amines in pea plants grown in the dark with an ammonium based nutrient in sand

Tissue	Age (days)	Seedling units/ g fr. wt	Cadaverine	nmol/g fr. wt Putrescine	Spermidine	Spermine
Roots	6	6·0	1190	1430	120	4
	12	2·6	1160	1850	136	10
	18	1·7	867	1550	47	27
Shoots	6	6·6	1630	667	96	20
	12	1·0	1422	842	88	11
	18	0·9	859	686	58	9
Cotyledons	6	2·3	31	214	136	26
	12	2·7	29	298	70	6
	18	3·2	11	423	56	8

The values for the 12-day-old plants are means of duplicate estimates made on material grown on separate occasions.

Table 3. Di- and poly-amines in pea plants grown in the light with an ammonium-based nutrient in sand

Tissue	Age (days)	Seedling units/ g fr. wt	Cadaverine	nmol/g fr. wt Putrescine	Spermidine	Spermine
Roots	6	5·1	1520	2170	156	10
	12	2·0	939	1900	71	6
	18	1·7	770	1560	58	29
	42		880	1400	78	7
Shoots	6	8·0	1090	1290	163	54
	12	1·6	287	384	219	77
	18	1·2	10	156	50	110
	42		13	133	259	72
Cotyledons	6	2·4	67	324	119	24
	12	3·0	34	297	93	11
	18	5·0	43	167	78	14

The values for the 12-day-old plants are the means of duplicate estimates made on material grown on separate occasions. Estimates for the 42-day-old plants are the means of duplicate samples of the same material.

On the 6th day, the plants grown in the sand were about twice the size of those grown in the Petri dishes of the same age, probably due to the more favourable growth conditions in sand. The 6-day-old plants grown in the Petri dishes would probably correspond to 3- to 4-day-old plants grown in sand.

Table 4. Amine content of 20-day-old light-grown pea seedlings grown in an ammonium medium with and without 40 g calcium carbonate per pot

	nmol/g fr. wt Cadaverine	Putrescine	Spermidine	Spermine
Roots				
Control	381	1390	89	9
With $CaCO_3$	183	161	64	10
Shoots				
Control	69	111	230	66
With $CaCO_3$	51	125	260	85

Each value is the mean of two independent estimates.

Table 5. Effect of potassium deficiency on amine levels in 27-day-old plants

	nmol/g fr. wt			
	Cadaverine	Putrescine	Spermidine	Spermine
Roots				
Control	505	1130	60	<1
–K	519	1260	49	<1
Shoots				
Control	<1	79	142	57
–K	<1	447	185	34

Each value is the mean of two independent estimates.

Since putrescine formation may be influenced by the pH of the medium, it was of interest to modify the sand medium by incorporation of calcium carbonate to raise the pH. Using an ammonium-based nutrient solution, improved growth was found in these circumstances, as already demonstrated in other plants [15]. The putrescine content of the roots decreased significantly (× 0·12, $P < 0·1\%$) on addition of 40 g of powdered calcium carbonate per 10 kg pot of sand, but the other amines were not significantly affected (Table 4).

The effect of potassium deficiency on cadaverine level was also studied, since putrescine is known to increase in these conditions. In the –K shoots the following changes were significant (Table 5): putrescine (× 5·7, $P < 0·1\%$), spermidine (× 1·3, $P < 5\%$), and spermine (× 0·6, $P < 0·1\%$). No significant effect of K deficiency could be found on the cadaverine level in shoots, and there was no significant effect on any of these amines in the roots.

In the present work the cadaverine content of the pea seedlings ranged up to 2 μmol/g fr. wt (6-day-old dark-grown seedlings). Anderson and Martin [1] found 70 nmol/g fr. wt in the entire axes of 13-day-old light-grown seedlings. This is lower than in the 12-day-old light-grown tissues in the present study (0·3–1 μmol/g fr. wt). The explanation for this may be found in the higher growth-room temperature (up to 32°) and resultant greater physiological age of their seedlings. In soya bean seedlings Rudulier and Goas [2] found the highest concentration of cadaverine (9 μmol/g dry wt) in the roots of 15-day-old seedlings grown in the dark in ammonium based nutrient solution. In their work, highest levels of the diamines were found in the roots in both light- and dark-grown tissues, and in the present study this relationship was observed in the light-grown plants up to 42 days old.

In the present investigation cadaverine was found at *ca* 200 nmol/g in the embryo of barley (Table 6). This level is lower than that found in earlier work (2 μmol/g) [12]. After 2 days germination in the dark, the cadaverine was not detectable. In previous studies on the amines of the leaves of mature barley plants a peak was found on GLC with R_t corresponding to cadaverine at a maximal level of 5 nmol/g fr. wt [13].

Neither diamine oxidase activity nor cadaverine is widely distributed in the plant kingdom, but both occur together in pea seedlings. In view of this apparent correlation it would be of interest to determine whether the natural substrate of the

Table 6. Occurrence of di- and poly-amines in legumes and barley

Plant	Age and conditions of growth	Tissue	Cadaverine	Putrescine	Spermidine	Spermine
Trifolium subterraneum (clover)	15 days, light	Entire seedling	553	768	170	50
Vicia faba (broad bean)	8 days, light	Roots	<1	87	46	1
		Shoots	<1	30	22	<1
		Cotyledons	<1	82	100	16
Phaseolus vulgaris (runner bean)	14 days, light	Roots	<1	40	60	<1
		Shoots	<1	70	135	17
		Cotyledons	<1	74	84	4
Phaseolus aureus (mung bean)	3 days, dark	Axes	<1	40	366	101
		Cotyledons	<1	69	284	124
Lupinus regalis (Russell lupin)	3 days, dark	Entire seedling	<1	106	375	23
Pisum arvense (Maple pea)	6 days, dark	Axes	2650	1264	48	3
		Cotyledons	10	244	208	14
Hordeum vulgare (barley)	Ungerminated grain	Embryo	187	276	726	209
		Endosperm	50	57	88	47
	2 days, dark	Axis	<1	462	323	32
		Endosperm	<1	206	67	27

Concentrations expressed as nmol/g fr. wt were determined by the dansylation method (see Experimental).

diamine oxidase is cadaverine as suggested by Anderson and Martin [1]. In previous work cadaverine and putrescine were found to be oxidized at similar rates by the diamine oxidase of pea seedlings [16–19] while for soya bean amine oxidase the oxidation of cadaverine is 1·8 × faster than the oxidation of putrescine [20]. For the diamine oxidase of *Lupinus luteus* putrescine is oxidized 1·7 × faster than cadaverine [21]. Kenten and Mann [16] showed that maximum activity of the amine oxidase in intact pea seedlings occurred after 8–12 day's growth, and Werle *et al.* [22] found maximum activity after 3 day's growth in the axes. On the basis of fr. wt Minář *et al.* [23,24] found that the activity declined in pea roots and shoots from the 5th day of germination, while increasing in cotyledons. In all tissues greater activity was found in dark-grown than in the light-grown seedlings [23]. In soya bean seedlings maximum activity occurred after 1 day in the roots and after 3 days in the hypocotyl, while none could be detected in the cotyledons up to the 6th day of germination [20]. In entire clover seedlings maximum activity occurred five days from germination [25].

The diamine oxidase activities found in the present work for up to 6 days from germination are shown in Table 7. Overall, the activity with cadaverine was 1·25 × that with putrescine. With both substrates the initial rate of increase of activity was greater in the axes than in the cotyledons, though after the 3rd day the activity in the axes was less than in the cotyledons. Combining these results with those of Minář *et al.* [23,24] it appears that the activity in the axes reaches a peak between the 4th and 6th days from germination and then declines. Amine oxidase activity in the axis therefore roughly parallels the increase in cadaverine and putrescine which both reach a peak at about the same time. It was not possible to establish a significantly better correlation for the rise in amine oxidase activity with either putrescine or with cadaverine, which are the potential diamine substrates.

Table 7. Diamine oxidase activity (as μl O_2/g fr. wt/hr) in the axes and cotyledons of pea seedlings grown for up to 6 days in the dark

Substrate	Cadaverine		Putrescine	
Days	Axes	Cotyledons	Axes	Cotyledons
1	17	7	n. .	<5*
2	400	88	390	110
3	390	535	360	440
4	432	695	410	490
6	560	832	450	520

* Intact seedlings. n.d. Not determined separately.

Two clovers (*Trifolium pratense*; *T. repens*) have a high amine oxidase level [16,25–27] and a third clover (*Trifolium subterraneum*) was shown to have a high cadaverine level for the first time in the present work (Table 6). The same correlation also occurs in soya bean seedlings [2,20,25]. Although amine oxidase activity has been detected in *Lupinus* [16,21,25,28], *Phaseolus* [25] and *Vicia* [25,29] in which cadaverine could not be demonstrated in the present work, this diamine, if it is formed, may be so rapidly oxidized in these species that its level is below the limit set by the sensitivity of the method of detection (1 nmol/g fr. wt). Certainly there is good evidence that cadaverine is utilized by lupin in the biosynthesis of alkaloids. Even so, definitive evidence linking either putrescine or cadaverine specifically with diamine oxidase *in vivo* is at present not available for any species.

Werle *et al.* [22] showed that the content of amine oxidase substrates (otherwise uncharacterized) in the pea seedling axes was highest on the third day after gemination when it rose to 5 μmol/g fr. wt. This agrees well with the value given in the present study for dark-grown seedlings (sum of putrescine, cadaverine, spermidine and spermine = 3·5 μmol/g fr. wt). Moreover, by the 12th day Werle *et al.* found the highest content of these substrates in the roots, as also shown in the present work, in which the sum of the substrate amines was *ca* 2 × greater in the roots than in the shoots on a fr. wt basis in both light- and dark-grown material. However, Růžička and Minář [24] found only *ca* 0·25 μmol amine oxidase substrates/g fr. wt and these somewhat lower values may be attributed to a difference in the method of extraction.

After germination for 3 days, the dry wts of the pea cotyledons and axes were 38 and 9·7% resp. Dry wts of 18-day-old roots, shoots and cotyledons were respectively 4·2, 5·4 and 5·2% in the dark, and 6·0, 10·4 and 8·4 in the light.

In the 12-day-old light-grown seedlings a Sakaguchi-positive compound observed in the vicinity of agmatine on TLC was high in the roots and low in the cotyledons and shoots. Arginine was

high in the cotyledons and shoots and low in the roots. In 12-day-old dark-grown plants, presumed agmatine was high in the roots and shoots and low in the cotyledons. Arginine was high in the cotyledons and low in the roots and shoots. Homoarginine, which is a potential precursor of cadaverine [4] could not be detected in any of the extracts. Indirect evidence for the formation of cadaverine by a lysine decarboxylase in pea seedling extracts has been presented by Hasse *et al.* [30].

An additional prominent yellow fluorescent spot found on TLC of the amine fraction of pea tissues proved to be the lactone of homoserine. In acid solution homoserine is in equilibrium with its lactone [31] which behaves as a basic amino compound. For this reason the lactone was present in the amine fraction and separated with the amines on dansylation and TLC. Homoserine is known to be a major amino acid in the pea plant [32].

EXPERIMENTAL

Plants were grown from seed (10 g) in 9 cm Petri dishes on filter paper at 18° for up to 6 days in the dark. Water was given initially (10 ml), and after the first 24 hr (10 ml) (Table 1). Plants 6 days and older were grown in sand in the dark at 18° (Table 2) or under fluorescent lamps (6600 lx, 16 hr day) at 25° (Table 3) with nutrient treatment 7 of Ref. [33]. For K deficiency (Table 5) nutrient treatment 5 was used. Dark manipulations were conducted under a dim green light. The testa was removed from the pea cotyledons before analysis.

The amine fractions were prepared and dansylated according to the procedure given in Refs. [13] and [33]. The solvent for the quantitative estimation of the amines was cyclohexane–EtOAc (3:2) in which the mobilities relative to dans-NH_3 (R_{am}) were cadaverine 0·84; tyramine, 0·84; putrescine 0·77; spermidine, 0·55; spermine 0·42; ethanolamine, 0·32. Plates were removed from the tank when the dans-NH_3 was about to enter the pad. Dans-cadaverine and dans-putrescine were incompletely resolved in this solvent and areas under the curves were apportioned by drawing a perpendicular to the base of the trough between the two diamines to the base line. Standards with 4, 8 and 16 × 10^{-10} mol cadaverine per origin chromatographed with 8 × 10^{-10} mol of putrescine per origin gave mean estimates of 101% of the cadaverine run without added putrescine. Conversely, the mean estimates of putrescine under these conditions were 109% of the amine run without added cadaverine. Errors attributable to this overlap should be small, even when the ratio of these two amines is much greater than 2:1. The ratio of fluorescence for equimolar quantities of the dans-amines relative to putrescine (= 1) was 0·91, 1·28 and 1·38 for cadaverine, spermidine and spermine respectively. To confirm the presence of dans-tyramine and dans-cadaverine in barley extracts $CHCl_3$–NEt_3 (5:1) was used as solvent [13]. In this solvent dans-homoserine lactone in pea extracts had R_f 0·57 (streaked), and dans-tyramine (pink fluorescence) in barley extracts had R_f 0·90.

Amine oxidase estimations were made on pre-frozen pea seedling tissues extracted in 2 vol pH 7 NaPi buffer (0·1 M). After centrifuging (3000 *g*) and dialysis against the buffer, activity was estimated on 2 ml samples at 30° in a Warburg with 0·1 ml of catalase (1 mg/ml) and cadaverine (25 mM, 0·5 ml) as substrate. The vol of O_2 taken up in 20 min was used to calculate the activity.

Guanidino compounds were estimated semi-quantitatively on 20 μl samples of the amine fractions. These were separated by TLC on CC41 cellulose in *n*-BuOH–HOAc–H_2O (4:1:1). With the Sakaguchi reagent [34] the following R_f's (and colours) were found: homoarginine (orange), 0·2; agmatine (pink), 0·16; arginine (orange), 0·12.

Dans-homoserine lactone was identified by TLC (R_{am} 0·68 in cyclohexane–EtOAc, 3:2) and MS at 260° on comparison with the dans derivative of the authentic lactone prepared according to Ref. [31] and M^+ (334·098) confirmed the empirical formula. However, the spectrum obtained in the present study (*m/e* 171 (100), M^+ 334 (31), 170 (30), 168 (21), 172 (14), 154 (12)) did not show good agreement with that published in Ref. [35] (temp. 115°).

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REFERENCES

1. Anderson, J. N. and Martin, R. O. (1973) *Phytochemistry* **12**, 443.
2. Rudulier, D. and Goas, G. (1971) *C.R. Acad. Sci. Paris* **273**, 1108.
3. Wang, L. C. and Selke, E. (1973) *Plant Physiol.* **51**, 432.
4. Ramakrishna, S. and Adiga, P. R. (1975) *Phytochemistry* **14**, 63.
5. Strogonov, B. P. (1962) *Physiological Basis of Salt Tolerance of Plants.* Academy of Science, U.S.S.R. Transl. by Israel Program for Scientific Translations (1964).
6. Schütte, H. R. and Knöfel, D. (1968) *Z. Pflanzenphysiol.* **59**, 80.
7. Abdusalamov, B. A., Takanaev, A. A., Aslanov, K. A. and Sadykov, A. S. (1971) *Biochemistry (U.S.S.R)* (English transl.) **36**, 239.
8. Leistner, E. and Spenser, I. D. (1973) *J. Am. Chem. Soc.* **95**, 4715.
9. Smith, B. N. and Meeuse, B. J. D. (1966) *Plant Physiol.* **41**, 343.
10. Hohlt, H. E., Maynard, D. N. and Barker, A. V. (1970) *J. Am. Soc. Hort. Sci.* **95**, 345.
11. Umezu, M. (1961) *J. Ferment. Technol.* **39**, 247.
12. Moruzzi, G. and Caldarera, C. M. (1964) *Arch. Biochem. Biophys.* **105**, 209.
13. Smith, T. A. (1973) *Phytochemistry* **12**, 2093.
14. Smith, T. A. (1975) *Phytochemistry* **14**, 865.
15. Hewitt, E. J. (1966) *Sand and Water Culture Methods used in the Study of Plant Nutrition*, pp. 71, 222. Commonwealth Agricultural Bureaux, Farnham Royal, Bucks, U.K.
16. Kenten, R. H. and Mann, P. J. G. (1952) *Biochem. J.* **50**, 360.
17. Hasse, K. and Maisack, H. (1955) *Biochem. Z.* **327**, 296.
18. Hill, J. M. (1967) *Biochem. J.* **104**, 1048.
19. Hill, J. M. and Mann, P. J. G. (1964) *Biochem. J.* **91**, 171.

20. Suzuki, Y. (1973) *Plant Cell Physiol.* **14,** 413.
21. Schütte, H. R., Knöfel, D. and Heyer, O. (1966) *Z. Pflanzenphysiol.* **55,** 110.
22. Werle, E., Beaucamp, G. and Schirren, V. (1959) *Planta* **53,** 125.
23. Macholán, L. and Minář, J. (1974) *Biol. Plant.* **16,** 86.
24. Růžička, V. and Minář, J. (1975) *Biol. Plant.* **16,** 215.
25. Werle, E. and Pechmann, E. (1949) *Ann. Chem.* **562,** 44.
26. Hill, J. M. (1973) *J. Exp. Botany* **24,** 525.
27. Werle, E. and Hartung, G. (1956) *Biochem. Z.* **328,** 228.
28. Suzuki, Y. (1959) *Sci. Rep. Tohoku Univ. Ser. IV Biol.* **25,** 139.
29. Strogonov, B. P., Shevyakova, N. I. and Kabanov, V. V. (1972) *Fiziol. Rast.* **19,** 1098.
30. Hasse, K., Ratych, O. T. and Salnikow, J. (1967) *Z. Physiol. Chem.* **348,** 843.
31. Armstrong, M. D. (1949) *J. Am. Chem. Soc.* **71,** 3399.
32. Grant, D. R. and Voelkert, E. (1970) *Phytochemistry* **9,** 985.
33. Basso, L. C. and Smith, T. A. (1974) *Phytochemistry* **13,** 875.
34. Smith, T. A. (1963) *Phytochemistry* **2,** 241.
35. Seiler, N., Schneider, H. and Sonnenberg, K. (1970) *Z. Anal. Chem.* **252,** 127.