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AMINO ACIDS AND β-AMINOPROPIONITRILE AS INHIBITORS OF SEED GERMINATION AND GROWTH

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Key Word Index—Amino acids. β-aminopropionitrile; lettuce; Leguminosae; germination; hypocotyl; radicle; growth

Abstract—The germination of lettuce fruits and legume seeds was affected by the imbibition of solutions of certain amino acids. Seedling growth was inhibited more markedly than germination by these compounds. Non-protein amino acids were, as a group, more effective inhibitors of germination and seedling growth than were protein amino acids, with the exception of lysine. Anomalous results were obtained with β-aminopropionitrile.

INTRODUCTION

Over 200 non-protein amino acids have been isolated from plants and we are concerned with their possible ecological roles. Evidence exists [1] that certain of these compounds may protect the species which contain them from vertebrate and/or insect predation [2–5]. It has also been shown that non-protein amino acids can be toxic to plant species other than those which synthesize them [6–9].

In this paper we present further evidence of the phytotoxic effects of certain amino acids and β-aminopropionitrile. β-Aminopropionitrile was included with the amino acids as it occurs with non-protein amino acids in *Lathyrus* species and is known to be toxic in animals [10].

At concentrations of 1 mM the non-protein amino acids which we tested were more effective inhibitors of seedling growth in lettuce than were the protein amino acids with the exception of lysine. This evidence adds weight to the view that non-protein amino acids produced by some plants may confer an evolutionary advantage on them by inhibiting germination and/or growth in competing species.

RESULTS AND DISCUSSION

The effects of various plant amino acids and β-aminopropionitrile on the germination of lettuce fruits are shown in Table 1. At a concentration of 1 mM only 3 of the 29 compounds tested reduced germination by more than 10%. All 3 were non-protein amino acids (canavanine, albizziine, α-amino-β-oxalylaminopropionic acid).

The effects on subsequent seedling growth of the imbibition of amino acid solutions by lettuce fruits are shown in Table 2. Of those tested at a concentration of 1 mM only the protein amino acids glycine, alanine, glutamic acid and aspartic acid and the common intermediary metabolite citrulline were without effect, the most significant inhibition was produced by canavanine. At a concentration of 1 mM more than 50% inhibition of hypocotyl and/or radicle growth was produced by 15 of the 31 compounds tested; of those, 12 were non-protein amino acids and 3, cysteine, lysine and serine were protein amino acids Of the 16 which had little or no effect on hypocotyl and/or radicle growth when supplied at this concentration, 9 were protein amino acids, 5 were

Table 1. The effects of amino acids and β-aminopropionitrile on the germination of lettuce fruits

Amino acidınopropionic acid	% Germination concentration 1 mM	10 mM
Non-protein		
Albizziine	82	54
α-Amino-β-acetylaminopropionic acid	100	53
α-Amino-β-methylaminopropionic acid	100	98
α-Amino-β-oxalylaminoprionic acid	84	70
α-Amino-γ-oxalylaminobutyric acid	96	78
Azetidine-2-carboxylic acid	100	100
Canavanine	86	63
α,β-Diaminopropionic acid	100	76
α,γ-Diaminobutyric acid	100	36
Homoarginine	95	95
Lathyrine	100	95
Mimosine	93	87
N-Methyltyrosine	100	94
Pipecolic acid	100	100
Protein		
Alanine	100	99
Arginine	100	98
Aspartic acid	100	100
Cysteine	98	90
Glutamic acid	100	10
Glycine	100	95
Histidine	100	90
Lysine	96	54
Methionine	100	85
Serine	100	75
Proline	100	100
Common intermediary metabolites:		
β-Alanine	99	92
Citrulline	100	100
Homoserine	92	40
Ornithine	98	76
Related compound:		
β-Aminopropionitrile	100	100

Table 2. The effects on seedling growth of the imbibition of amino acid and β-aminopropionitrile solution by lettuce fruits.

Amino acid	Concentration (mM)	% Hypocotyl growth	% Radicle growth
Non-protein			
Albizziine	1.0	88	58
	10.0	88	25
α-Amino-β-acetylamino-propionic acid	0.01	85	100
	0.1	85	100
	1.0	70	55
	10.0	0	5
α-Amino-β-methylamino-propionic acid	0.01	100	100
	0.1	100	90
	1.0	70	40
	10.0	55	15
α-Amino-β-oxalylamino-propionic acid	0.01	100	95
	0.1	85	90
	1.0	45	35
	10.0	0	5
α-Amino-γ-oxalylamino-butyric acid	0.01	90	95
	0.1	90	95
	1.0	80	75
	10.0	15	5
Azetidine-2-carboxylic acid	0.1	75	85
	1.0	25	50
	10.0	0	10
Canavanine	0.01	45	65
	0.1	25	8
	1.0	17	5
	10	3	5
α,β-Diaminopropionic acid	0.01	85	95
	0.1	85	85
	1.0	55	40
	10.0	30	20
α,γ-Diaminobutyric acid	0.01	95	90
	0.1	90	90
	1.0	45	45
	10.0	0	15
L,-3,4-Dihydroxyphenylalanine	1.0	38	17
	10.0	13	8
Homoarginine	0.01	95	80
	0.1	85	70
	1.0	85	20
	10.0	40	15
Lathyrine	1.0	100	100
	10.0	50	25
Mimosine	1.0	13	13
	10.0	0	8
N-Methyltyrosine	1.0	100	95
	1.0	84	64
Pipecolic acid	0.1	95	95
	1.0	30	60
	10.0	0	8
Protein.			
Alanine	0.1	100	100
	1.0	100	100
	10.0	100	85
Arginine	0.01	100	95
	0.1	90	88
	1.0	75	75
	10.0	10	25
Aspartic acid	0.1	100	100
	1.0	75	75
	10.0	10	3
Cysteine	1.0	54	36
	10.0	0	5
Glutamic acid	0.1	100	100
	1.0	90	90
	10.0	25	45
Glycine	0.1	100	100
	1.0	100	95
	10.0	80	65
Histidine	1.0	64	62
	10.0	18	15
Lysine	0.1	90	80
	1.0	45	10
	10.0	0	5
Methionine	1.0	61	42
	10.0	32	29
Serine	1.0	85	40
	10.0	54	20
Proline	0.1	100	100
	1.0	100	100
	10.0	95	100
Intermediary metabolites.			
β-Alanine	1.0	75	20
	10.0	55	15
Citrulline	0.1	100	100
	1.0	85	85
	10.0	70	60
Homoserine	1.0	80	40
	10.0	60	25
Ornithine	0.1	95	95
	1.0	65	65
	10.0	45	35
Related compound.			
β-Aminopropionitrile	1	100	100
	10	100	100

non-protein amino acids, 2 were intermediary metabolites, citrulline and ornithine, and the last was β-aminopropionitrile.

The effects of certain of these compounds on lettuce seedlings which had been grown for 1, 2 and 3 days before treatment are shown in Table 3. The apparent effect of some, notably mimosine and azetidine-2-carboxylic acid was very much greater on the younger seedlings. It is also apparent that radicle and hypocotyl growth are affected differently by different amino acids. Canavanine for example affected hypocotyl growth in pre-grown seedlings more than radicle growth while α-amino-β-oxalylaminopropionic acid produced the reverse effect.

To establish whether the seedlings of legume species which synthesise non-protein amino acids are less sensitive to these amino acids than are lettuce seedlings, a number of legume seeds were allowed to imbibe amino acid solutions and the effect on subsequent seedling growth determined. The results are set out in Table 4. These results suggest that all the species studied are able to discriminate against potentially toxic amino acids which they themselves synthesise. A surprising result was the lethal effect of β-aminopropionitrile on seedlings of *Lathyrus aphaca* and *Vicia benghalensis* as the nitrile was without effect on lettuce seedlings.

Preliminary experiments show that lettuce seedlings are inhibited when grown together with certain legume species which accumulate high concentrations of non-protein amino acids in their seeds. Lettuce seedlings would not grow in the presence of germinating seeds of *Glycine wightii*, which contain canvanine, and were severely inhibited when grown in the presence of

Table 3. The growth of seedlings transferred after 1, 2 and 3 days growth to agar containing various amino acids

Amino acid	Concentration (mM)	Hypocotyl growth* in seedlings transferred after			Radicle growth* in seedlings transferred after		
		1 day	2 day	3 day	1 day	2 day	3 day
Non-protein.							
Albizziine	1.0	100	100	100	50	70	90
	10.0	90	100	90	40	70	70
α-Amino-β-acetylaminopropionic acid	1.0	80	100	100	25	45	55
	10.0	5	10	15	10	35	50
α-Amino-β-oxalylaminopropionic acid	1.0	25	60	80	10	30	55
	10.0	0	10	20	10	30	70
α-Amino-γ-oxalylaminobutyric acid	1.0	75	95	95	50	75	95
	10.0	30	70	20	20	70	100
Azetidine-2-carboxylic acid	1.0	20	30	75	20	50	80
	10.0	10	10	50	20	45	70
Canavanine	1.0	10	15	40	20	50	85
	10.0	5	10	30	15	50	70
α,β-Diaminopropionic acid	1.0	75	75	100	30	50	75
	10.0	15	25	55	30	40	55
α,γ-Diaminobutyric acid	1.0	50	75	85	28	58	95
	10.0	10	20	50	20	60	90
L-3,4-Dihydroxyphenylalanine	1.0	54	69	84	30	68	60
	10.0	25	44	80	28	64	64
Homoarginine	1.0	90	85	100	20	35	95
	10.0	40	30	65	15	30	85
Mimosine	1.0	10	10	75	20	50	90
	10.0	10	10	60	10	20	80
N-Methyltyrosine	1.0	90	100	100	100	88	100
	10.0	85	100	82	90	100	100
Pipecolic acid	1.0	25	40	ND	15	50	ND
Protein:	10.0	10	15	ND	5	40	ND
Arginine	1.0	70	90	85	30	80	80
	10.0	40	60	85	20	60	75
Cysteine	1.0	42	68	78	15	53	64
	10.0	5	11	35	12	46	62
Histidine	1.0	80	89	100	33	56	85
	10.0	65	61	80	19	55	70
Lysine	1.0	69	55	ND	13	35	ND
	10.0	8	10	27	8	35	63

*Expressed as % of control seedlings.
ND—not determined.

germinating seeds of *Lathyrus aphaca*, which accumulate α-amino-β-oxalylaminopropionic acid, and homoarginine or in the presence of germinating seeds of *Vicia benghalensis* which contain canvanine.

Table 4. The effect of non-protein amino acids and β-aminopropionitrile on seedling growth in Legume species which do and do not synthesize these compounds.

	Concentration (mM)	% Hypocotyl growth	% Radicle growth
Vicia benhalensis (synthesizes canavanine)			
with canavanine	1	93	100
		(17)	(5)
with homoarginine	1	85	20
		(85)	(20)
with β-aminopropionitrile	1	21	33
		(100)	(100)
Lathyrus aphaca (synthesizes homoarginine)			
with homoarginine	1	88	60
with canavanine	1	10	5
with β-aminopropionitrile	1	0	0
Lathyrus odoratus (synthesizes β-aminopropionitrile)			
with β-aminopropionitrile	1	100	100
with homoarginine	1	51	55
with canavanine	1	15	12

Figures in brackets refer to the equivalent value for lettuce fruits, see Table 2.

EXPERIMENTAL

Germination and growth of lettuce fruits. Fruits of lettuce (*Lactuca sativa*, var. Great Lakes) were sown in 0.5% agar containing a measured amount of amino acid and incubated at 30° for 3 days. Germination was indicated by the emergence of the radicle, however small, from the fruit. The figures quoted for germination represent the percentage of the control. Each determination was performed on 100 fruits in batches of 20. Growth was calculated after 3 days by taking measurements of the hypocotyl and radicle lengths and determining a mean for 100 seeds. This value is expressed as a percentage of the control.

Growth of lettuce seedlings. Fruits of lettuce were sown in 0.5% agar and grown at 30° for 1, 2 and 3 days respectively. At the end of this period they were transferred to 0.5% agar containing the amino acid undei investigation. 100 seedlings per experiment in batches of 10, and grown at 30° for a further 3 days. The hypocotyl and radicle lengths were measured as before.

Growth of legume seeds. The optimum temp. for growth of each species was determined by incubating the seeds in 0.5% agar on a thermal gradient bar [11] for up to 5 days. It was found that 25° was a suitable temperature for all the species under investigation. 100 seeds were sown in batches of 20 on 0.5% agar containing the aminoacid and grown at 25° for 5 days. At the end of this period the mean hypocotyl and radicle lengths were determined as before

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