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INHIBITORY EFFECT OF THIOURACIL ON GERMINATION OF LEAF MUSTARD SEED AND ITS REVERSAL BY PYRIMIDINE DERIVATIVES

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1. Investigations were carried out to examine the effects of certain nucleic acid antimetabolites upon the germination of leaf mustard seed. Antimetabolites tested were 2-thiouracil, barbituric acid, various uracil analogues, azaadenine, azaxanthine, azaguanine and benzimidazol.

2. Among the antimetabolites tested, only 2-thiouracil showed a strong inhibitory action on the germination of *Brassica juncea* var. *foliosa* (BAILEY) Com. "Akatirimen".

3. This inhibition by 2-thiouracil was relieved markedly by such pyrimidine derivatives as uracil, thymine, orotic acid, uridylic acid and cytidylic acid, though these compounds did not show any promotive effect on the germination by themselves.

On the other hand, such a relieving effect was not shown by purine derivatives such as adenine, hypoxanthine and xanthine.

4. Among other kinds of cole-wort seeds tested, a similar thiouracil inhibition on seed germination was observed only in a limited number of species or varieties.

The dynamic aspects of the metabolism of nucleic acid in germinating seeds have hitherto been studied (*1–4*). The stimulatory and inhibitory effects of some compounds related to nucleic acid on the germination and the growth of several plants have also been reported.

ŠORMOVÁ, *et al.* (*5*) reported that a stimulatory effect on growth was displayed particularly by 5-nitrouracil and 5-bromouracil. It was also established that kinetin and other 6-(substituted) thio- and amino-purines stimulated germination of the seeds which require light for their germination (*6*, *7*).

In contrast with these reports, MAZZANTI (*8*) reported that 1, 3-bis (hydroxymethyl)-2-(3H)-benzimidazolethione and methylthiouracil inhibited the germination of wheat seeds and French bean seeds, and TOTTER (*9*) reported that cress seeds sown on a medium containing 2-thiouracil did not

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show normal germination.

In the present study the effects of a certain number of nucleic acid antimetabolites on plant seed germination were examined, and it was found that 2-thiouracil was a specific inhibitor for the germination of leaf mustard seed and that this inhibition was reversed by some pyrimidine derivatives.

MATERIALS AND METHODS

Seeds of leaf mustard (*Brassica juncea* var. *foliosa* (BAILEY) Com. "Akatirimen") were mainly used in the present investigation. In order to examine the effects of nucleic acid antimetabolites on the germination of other kinds of cole-wort, the following varieties were used: *Brassica juncea* (Coss) Com. "Okubagarashina" and Com. "Wasekigarashina", *Brassica japonica* (SIEB) Com. "Edogawakyona" and *Brassica pekinensis* (RUPR) Com. "Daibanseimana".

All the chemical compounds used in this study were obtained from the market.

These experiments were conducted in August and September, 1962.

General procedures of the seed germination were as follows. Fifty dry seeds of leaf mustard were spread on a double filter paper in a PETRI dish of 5.5 *cm* in diameter, and then 5 *ml* of tap water solution containing chemical compounds to be tested was placed into each dish. Unless otherwise mentioned, the counting of the germination percentage was done on the first and the second days after the beginning of incubation. Each germination percentage was the arithmetic mean of the counts of two replications. The incubation was carried out at room temperature. When the radicle emerged through the seed coat, it was considered that the seed had germinated.

In the investigation on the releasing effects of nucleic acid bases and related agents on the thiouracil inhibition, leaf mustard seeds were soaked in about five volumes of tap water in the presence or absence of 10^{-3} *M* 2-thiouracil, and left to swell for eight hours at room temperature with occasional stirring. After this treatment, the solution was decanted, and the seeds were rinsed once with tap water and then placed into each dish containing the solution tested.

RESULTS

Effect of some nucleic acid antimetabolites on leaf mustard seed germination

Effects of some nucleic acid antimetabolites such as 2-thiouracil (*10–12*), benzimidazole (*13*), azaguanine, azaadenine (*14*), and azaxanthine were tested. The results are shown in Table I.

Among the antimetabolites tested, only 2-thiouracil induced strong inhibition of seed germination. Azaxanthine, benzimidazole, azaadenine and

azaguanine did not show apparent inhibitory actions.

TABLE I

Effect of some nucleic acid antimetabolites on leaf mustard seed germination

Germination percentage was determined 44 *hr* after sowing.

Treatment	Concentration (M)	Germination percentage (%)
Water (Control)		72
Thiouracil	0.78×10^{-3}	13
	0.78×10^{-4}	23
	0.78×10^{-5}	65
Benzimidazole	0.85×10^{-3}	53
	0.85×10^{-4}	70
	0.85×10^{-5}	70
Azaxanthine	0.65×10^{-3}	68
	0.65×10^{-4}	60
	0.65×10^{-5}	73
Azaadenine	0.37×10^{-3}	65
	0.37×10^{-4}	60
Azaguanine	0.65×10^{-4}	65
	0.65×10^{-5}	65

Effects of various uracil analogues on the germination of leaf mustard seed

As the inhibitory action of 2-thiouracil on the seed germination was found, further investigation using barbituric acid (*15*), an antagonist of uracil as well as of 2-thiouracil was carried out. As shown in Table II, barbituric acid was neither inhibitory nor promotive. Only a slight inhibitory effect was observed at a concentration of $10^{-3}\,M$ after 25 hour incubation.

The effects of other uracil analogues were also tested. The results are also given in Table II.

No noticeable effect was observed after 40 hours of incubation except in the case of dithiothymine which was slightly inhibitory at a concentration of $0.63\times10^{-3}\,M$, while 2-thiouracil tested simultaneously showed a marked inhibition even after 14 hours of incubation.

Reversal of thiouracil inhibition by pyrimidine bases and nucleotides

Effects of various pyrimidine bases and nucleotides on the germination of leaf mustard seeds which had been treated with 2-thiouracil were examined. Each compound was used at concentrations of $10^{-3}\,M$ and $10^{-4}\,M$. The results are shown in Table III.

Noteworthy was the observation that thiouracil inhibition was overcome to a great extent by treating the seeds with $10^{-3}\,M$ of uracil, thymine,

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TABLE II

Effect of various uracil analogues on leaf mustard seed germination

Experiments 1 and 2 were performed on different dates in August 1962.

	Treatment	Concentrations (M)	Germination percentages (%)	
			A[a]	B[a] (hr after sowing)
Expt. 1	Barbituric	10^{-3}	18	83
		10^{-4}	33	75
	Water (Control)		28	75
Expt. 2	5-Bromouracil	0.52×10^{-3}	6	57
		0.52×10^{-4}	10	52
	5-Nitrouracil	0.64×10^{-3}	3	56
		0.64×10^{-4}	14	56
	6-Methyluracil	0.79×10^{-3}	7	51
		0.79×10^{-4}	5	40
	1,3-Dimethyluracil	0.71×10^{-3}	8	49
		0.71×10^{-4}	14	61
	Dihydrothymine	0.79×10^{-3}	9	66
		0.79×10^{-4}	6	58
	Dithiothymine	0.63×10^{-3}	1	34
		0.63×10^{-4}	6	64
	Thiocytosine	0.79×10^{-3}	7	49
		0.79×10^{-4}	13	52
	Methylcytosine	0.80×10^{-3}	9	52
		0.80×10^{-4}	8	39
	2-Thiouracil	0.78×10^{-3}	2	4
		0.78×10^{-4}	6	17
	Water (Control)		10	54

[a]: The counting of the germination percentages was done at 25 (A) and 41 (B) hr after sowing in experiment 1, and at 14 (A) and 40 (B) hr after sowing in experiment 2.

TABLE III

Reversal of thiouracil inhibition of leaf mustard seed germination by pyrimidine bases and nucleotides

Pre-treatment	Treatment	Concentration (M)	Germination percentage (%)	
			24	40 (hr after bedding)
Soaking in water	Water		28	60
Soaking in 10^{-3} M of 2-thiouracil	Thymine	10^{-3}	8	30
		10^{-4}	3	33
	Uracil	10^{-3}	25	58
		10^{-4}	8	35
	Orotic acid	10^{-3}	15	54
		10^{-4}	5	20
	Uridylic acid	10^{-3}	13	45
		10^{-4}	5	23
	Cytidylic acid	10^{-3}	15	42
		10^{-4}	5	32
	Water (Control)		2	15

orotic acid, uridylic acid and cytidylic acid. These compounds showed some reversing effect even at a lower concentration (10^{-4} *M*).

Then the effect of the same compounds on the germination of leaf mustard seeds which had not been treated with 2-thiouracil was tested. However, as shown in Table IV, neither promotive nor inhibitory action was shown by those compounds.

TABLE IV

Effect of pyrimidine bases and nucleotides on leaf mustard seed germination

Treatment	Concentration (*M*)	Germination percentage (%)	
		26	52 (*hr* after sowing)
Thymine	10^{-3}	22	65
	10^{-4}	15	65
Uracil	10^{-3}	15	70
	10^{-4}	10	80
Orotic acid	10^{-3}	12	70
	10^{-4}	20	75
Uridylic acid	10^{-3}	22	65
	10^{-4}	20	75
Cytidylic acid	10^{-3}	22	72
	10^{-4}	25	78
Water (Control)		20	75

Effects of purines on the germination of leaf mustard seeds inhibited by thiouracil

Releasing effect of purines such as adenine, xanthine and hypoxanthine on the thiouracil inhibition was also tested. The results are shown in Table V.

TABLE V

Reversing effect of purines on 2-thiouracil inhibition of leaf mustard seed germination

Treated afterwards with	Concentrations (*M*)	Germination percentage (%)			
		Soaking in water		Soaking in 10^{-3} *M* thiouracil	
		21	38 (*hr* after bedding)	21	38 (*hr* after bedding)
Adenine	10^{-3}	23	57	2	28
	10^{-4}	30	59	3	35
Hypoxanthine	10^{-3}	29	63	5	38
	10^{-4}	33	64	4	39
Xanthine	10^{-3}	30	58	2	33
	10^{-4}	35	65	2	35
Uracil	10^{-3}	27	55	13	55
	10^{-4}	27	66	2	31
Water (Control)		30	60	3	30

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However, no releasing effect was observed with these compounds in contrast to uracil which showed a marked releasing effect at a concentration of $10^{-3}M$.

Inhibition of seed germination of several cole-worts by some nucleic acid antimetabolites

Effects of 2-thiouracil and other nucleic acid antimetabolites on the seed germination of four related kinds of cole-wort were examined. The results are summarized in Table VI.

TABLE VI

Effect of some inhibitors of nucleic acid metabolism on seed germination of some cole-worts

Varieties	Hours after sowing	Germination percentage (%)		
		Thiouracil $0.78\times10^{-3}M$	Benzimidazole $0.85\times10^{-3}M$	Azaxanthine $0.65\times10^{-3}M$
B. juncea (COSS) Com. "Okubagarashina"	22	2[a]	14	49
	44	21[a]	98	99
B. juncea (COSS) Com. "Wasekigarashina"	22	14[a]	38	56
	44	71[a]	84	87
B. japonica (SIEB) Com. "Edogawakyona"	22	14[a]	20	48
	44	88[a]	90	92
B. pekinensis (RUPR) Com. "Daibanseimana"	22	21[a]	58	78
	44	73[a]	93	95

Varieties	Germination percentage (%)		
	Azaadenine $0.74\times10^{-3}M$	Azaguanine $0.65\times10^{-3}M$	Water (Control)
B. juncea (COSS) Com. "Okubagarashina"	32	54	54
	97	99	97
B. juncea (COSS) Com. "Wasekigarashina"	58	68	56
	94	92	85
B. japonica (SEIB) Com. "Edogawakyona"	29	38	42
	82	89	82
B. pekinensis (RUPR) Com. "Daibanseimana"	78	72	88
	95	97	94

[a] Growth of the radicle and cotyledons was apparently inhibited.

Among the cole-worts tested, only *Brassica juncea* (Coss) Com. "Okubagarashina" was found to be inhibited from germinating by 2-thiouracil to about the same extent as that observed with *Brassica juncea* var. *foliosa* (BAILEY) Com. "Akatirimen".

In the case of the other three cole-worts, the seed germination had been considerably suppressed by 2-thiouracil and benzimidazole when observed

22 hours after sowing, but 44 hours after sowing, the germination percentage was almost the same as in the control. In these cases, however, it was noticed that the growth of radicle and cotyledons had been markedly inhibited, particularly in the presence of 2-thiouracil.

DISCUSSION

Among the nucleic acid antimetabolites tested, only 2-thiouracil was found to be markedly inhibitory against the seed germination of leaf mustard. At present, no adequate explanation is found to account for this specific action of 2-thiouracil.

The reversal of thiouracil action on germination by pyrimidine bases and nucleotides suggests that 2-thiouracil may compete in the plant with uracil as has been reported by several authors (*10–12*).

ŠORMOVÁ, *et al.* (*5*) reported that a strong stimulative effect on the growth and development of plants was displayed particularly by 5-nitrouracil and 5-bromouracil which has been known to stimulate the mitotic activity of some plants. It has also been established that kinetin and other 6-(substituted) thio- and amino-purines stimulate germination of seeds which require light for their germination, by replacing the action of red light (*6*, *7*).

On the other hand, MAZZANTI (*8*) has reported that 1, 3-bis (hydroxymethyl)-2(3H)-benzimidazolethione and methylthiouracil inhibited the germination of wheat seeds and French bean seeds. TOTTER (*9*) also reported that cress (*Nasturtium officinalis* R. BROWN) seeds sown on a medium containing 2-thiouracil did not germinate normally, and that, if the seeds were sown on a medium containing both 2-thiouracil and uracil, or were treated with uracil before sowing on thiouracil-medium, germination was virtually normal. In TOTTER's experiments, germination was defined by the extension of cotyledons. In the present experiments, however, it was considered that germination took place when the radicle had emerged from the coat. Though germination percentage was not affected by thiouracil in "Edogawakyona", "Wasekigarashina", and "Daibanseimana" as shown in Table VI, the growth of the emerged radicles was abnormal forming dwarfs. It was interesting to note that a bad smell was generally perceived after soaking of seeds of *Brassica juncea* (BAILLEY) var. *foliosa* Com. "Akatirimen" in tap water for 8 hours, but such a smell was scarcely perceived when the seeds were soaked in tap water containing $10^{-3}M$ of thiouracil. This fact suggests that thiouracil affects the metabolism in the leaf mustard seeds long before the seed germination takes place.

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