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Kinetin and gibberellin reversal of the synergistic inhibition of germination of the seed of kale, *Brassica oleracea* L. var. *acephala*, by AMO-1618 and coumarin*

J. S. KNYPL

Three growth retarding chemicals, B995 (N,N-dimethylaminosuccinamic acid), CCC [(2-chloroethyl)trimethylammonium chloride] and Phosfon D (2,4-dichlorobenzyl-tributylphosphonium chloride) transiently delay germination of kale seeds (Knypl 1967). When applied in combination with coumarin, the well known inhibitor of germination and growth (Evenari 1957; Knypl 1966) which at the concentration of 100 mg/l like retardants also transiently delays germination of kale, they permanently inhibit germination. This synergistic inhibitory effect of anyone of the retardants and coumarin was shown to be initially reduced and finally reversed either by kinetin or gibberellic acid (Knypl 1967).

The aim of the present experiments was to see whether or not similar effect would be induced by other growth retardant, AMO-1618 [2′-isopropyl-4′-(trimethylammonium chloride)-5′-methylphenylpiperidine-1-carboxylate].

MATERIAL AND METHODS

Seeds of kale (*Brassica oleracea* L. var. *acephala* cv. Little Green Crinkled) of 1964 harvest were used. Seeds, in groups of 50, were sown on 2 discs of Whatman No. 2 blotting paper placed in 10-cm in diameter Petri dishes and moistened with 5 ml of distilled water (control) or solution of the substance(s) to be tested. The dishes were maintained in the dark at 25°. Germinated seeds were counted for 5 days in 24 hr. intervals. Other details were same as described previously (Knypl 1967).

If the effect of AMO-1618 on growth of kale seedlings was analysed, the seeds were germinated on water wetted blotting paper for 24 hr. Then the seeds which had ger-

* This is a paper No. 7 of the series „Growth retardants in relation to the germination of seeds".

minated were transferred, in groups of 15, into 12-cm Petri shales lined with 2 Whatman No. 2 blotting paper discs soaked with 6 ml of a test solution. Incubation was carried out in darkness at 25°. Length measurements were made 4 days from the time the seeds were placed in the test solutions.

The experiments were performed in summer 1967 with the same lot of seeds as had been used in previous tests with other growth retardants, carried out in 1965 (Knypl 1967).

The chemicals used were obtained from following suppliers: AMO-1618 from Calbiochem (Lucerne); coumarin and uracil from K and K Research Laboratories, Inc. (Plainview, N. Y.); kinetin from Dr. Th. Schuchardt (Munich); GA_3 and 2-thiouracil from Koch-Light Laboratories, Ltd. (Colnbrook); puromycin from Sigma Chemical Co. (DeKalb St., St. Louis, Mo.).

Solutions of all substances were prepared immediately before use; GA_3 was dissolved in 0.01 N KOH and kinetin in 0.02 N HCl, and neutralized.

RESULTS

AMO-1618 at 5×10^{-6} M to 10^{-4} M did not affect germination of kale seeds but at higher concentrations it delayed germination (Table 1). Growth of the seedlings was significantly reduced by 10^{-3} M and 2×10^{-3} M solutions of this compound (Table 2).

The retarding effect of AMO-1618 at 5×10^{-4} M on germination was completely reversed by gibberellic acid (GA_3) or kinetin (KIN) at 10^{-4} M. In accordance with the previous report (Knypl 1967), coumarin at 100 mg/l also transiently delayed germination, although in 1967 the seeds were markedly less sensitive to this inhibitor than in 1965. This fact indicates that sensitivity of kale seeds to coumarin changes

Table 1

Germination of seeds of kale under the influence of AMO-1618

Concentration of AMO-1618, M	Number of germinated seeds*				
	Days				
	1	2	3	4	5
O (Control)	22.8	39.6	43.0	44.5	45.5
5×10^{-6}	22.9	39.4	42.9	44.7	45.5
10^{-5}	22.8	39.6	42.8	44.7	45.5
5×10^{-5}	22.9	39.7	42.7	44.9	45.5
10^{-4}	22.2	39.9	42.0	43.2	43.7
5×10^{-4}	16.1	34.1	36.1	37.4	38.4
10^{-3}	13.5	29.2	31.7	33.2	34.9
2×10^{-3}	9.4	24.3	26.3	28.7	31.6

* 50 seeds = 100 per cent germination. L.S.D. between any two values at P = 0.01 = 4.1.

Table 2

Growth of kale seedlings under the influence of AMO-1618

	Concentration of AMO-1618, M						
	0	10^{-5}	5×10^{-5}	10^{-4}	5×10^{-4}	10^{-3}	2×10^{-3}
Hypocotyl length, mm	21.4	21.5	21.5	21.1	19.6	18.9*	16.3*
Radicle length, mm	41.9	40.0	40.0	38.0	38.0	34.0*	30.9*

* Significantly different from control, P = 0.01.

on storage; similar phenomenon has been reported to occur in the case of *Lepidium sativum* L. seeds (Libbert 1961).

Kinetin markedly reduced the inhibition brought about by coumarin, the effect being especially evident after the initial 24 hours of incubation; GA_3 initially was without effect, but in subsequent days it also slightly increased the number of germinated seeds in the series treated with coumarin (Table 3).

Table 3

Germination of kale seeds under the influence of AMO-1618, coumarin, GA_3 and kinetin in sole and combined solutions

Treatment**	Number of germinated seeds*				
	Days				
	1	2	3	4	5
Water (dist.)	23.1	39.4	42.0	44.2	45.5
AMO-1618	16.0	34.0	36.0	37.2	38.2
AMO-1618+GA_3—I	23.4	38.5	42.0	45.0	46.0
AMO-1618+GA_3—II	25.7	41.0	45.0	46.0	47.0
GA_3-I	24.9	39.3	43.1	45.6	46.9
GA_3-II	27.3	40.3	44.3	47.0	48.0
AMO-1618+KIN-I	22.4	39.4	42.4	44.9	46.6
AMO-1618+KIN-II	17.0	36.5	39.0	43.0	45.0
KIN-I	27.5	40.4	43.7	45.5	46.6
KIN-II	26.8	36.0	40.5	43.5	45.0
COU	5.2	29.6	34.9	38.2	40.6
COU+GA-I	5.7	33.5	38.3	43.5	45.5
COU+GA-II	6.3	35.0	39.0	43.0	46.0
COU+KIN-I	9.5	34.5	38.6	32.3	44.0
COU+KIN-II	13.5	35.7	36.5	41.5	44.0
L.S.D., P = 0.01	2.0	3.7	3.5	3.5	3.5

* 50 seeds = 100 per cent germination.

** Concentrations in sole and combined solutions: AMO-1618, 5×10^{-4} M; Coumarin (COU), 100 mg/l; GA_3-I and GA_3-II, 10^{-4} and 5×10^{-4} M respectively; kinetin: KIN-I and KIN-II, 10^{-4} and 5×10^{-4} M respectively.

Table 4

Synergistic inhibition of kale seed germination by AMO-1618 and coumarin, and reversal by kinetin or gibberellic acid

Treatment**	Number of germinated seeds*				
	Days				
	1	2	3	4	5
AMO+COU	2.1	14.8	18.8	23.1	27.1
AMO+COU+GA_3-I	4.5	29.3	36.5	40.6	43.2
AMO+COU+GA_3-II	6.7	31.3	39.7	44.0	47.3
AMO+COU+KIN-I	7.8	29.5	35.1	41.1	44.9
AMO+COU+KIN-II	10.0	29.1	33.0	36.8	42.0
L.S.D., P = 0.01	1.2	2.7	3.0	2.9	2.8

* 50 seeds = 100 per cent germination.
** Concentrations of the compounds and germination in sole solutions the same as noted in Table 3.

AMO-1618 at 5×10^{-4} M in a mixed solution with coumarin at 100 mg/l strikingly decreased germination: In the first day of incubation germinated only 2 seeds and in the fifth one 27 seeds in comparison with 23 and 46 in the water treated control respectively (Table 4). This synergistic inhibition was reduced and finally completely reversed by either GA_3 (10^{-4}, 5×10^{-4} M) or kinetin (10^{-4} M). But in the first day the most effective in this respect was kinetin at the supraoptimal concentration, inhibitory for growth, of 5×10^{-4} M: In the series treated with combined solution of AMO-1618 plus coumarin plus kientin, the latter at 5×10^{-4} M, germinated 10

Table 5

Effect of 2-thiouracil and uracil on germination of kale seeds, treated with AMO-1618, coumarin, kinetin and GA_3

Treatment**	Number of germinated seeds*				
	Days				
	1	2	3	4	5
AMO+COU	2.1	14.8	18.8	23.2	27.1
AMO+COU+KIN	8.0	29.2	35.0	41.0	45.0
AMO+COU+KIN+TU	2.0	10.7	16.7	26.7	38.7
AMO+COU+KIN+TU+U	4.3	15.0	19.7	28.7	41.7
AMO+COU+GA_3	4.3	28.0	36.0	40.7	44.0
AMO+COU+GA_3+TU	2.0	8.7	14.3	24.3	38.0
AMO+COU+GA_3+TU+U	4.3	11.3	18.3	29.3	42.7
TU	4.3	17.3	27.8	34.6	39.3
U	18.0	37.7	42.5	44.9	45.7
L.S.D., P = 0.01	1.2	2.9	2.8	2.8	2.7

* 50 seeds = 100 per cent germination
** Concentrations: AMO-1618, 5×10^{-4} M; COU (coumarin), 100 mg/l; GA_3, 10^{-4} M; KIN (kinetin), 10^{-4} M; TU (2-thiouracil), 10^{-3} M; U (uracil), 10^{-3} M.

seeds in comparison with 2 ones in the combination AMO-1618 plus coumarin. Similar results were observed in experiments with other growth retardants, B995, CCC and Phosfon D (Knypl 1967).

The reversing effect of kinetin or GA_3 on the synergistic inhibition of germination by AMO-1618 and coumarin was antagonized by 2-thiouracil (2-TU) at the concentration of 10^{-3} M and again restored by uracil (Table 5). Uracil, kinetin and GA_3 reduced also the inhibition of germination caused by thiouracil alone (data not shown). It is thus evident that 2-TU competes here with uracil, and is probably incorporated into RNA in place of uracil (cf. Matthews 1958).

Puromycin (PMC), the specific inhibitor of protein synthesis acting as the analogue of the aminoacyl-tRNA (Yarmolinski and de la Haba 1959), did not affect germination in the series treated with AMO-1618 and coumarin applied alone; it did also not decrease germination in the control. However, PMC slightly but significantly increased germination in the series treated with combined solution of AMO-1618 and coumarin (Table 6).

Table 6

Effect of puromycin on germination of the seeds of kale, treated with AMO-1618 and coumarin

Treatment**	Number of germinated seeds*				
	Days				
	1	2	3	4	5
Water (dist.)	23.0	38.5	41.0	43.8	45.5
PMC	24.7	37.3	40.1	41.7	44.8
COU	5.5	30.0	35.0	38.5	41.0
COU+PMC	6.2	28.7	37.0	38.5	43.0
AMO-1618	16.3	34.3	36.0	37.2	38.3
AMO-1618+PMC	17.3	35.3	36.5	38.0	39.0
AMO+COU	2.3	15.0	18.3	23.0	27.0
AMO+COU+PMC	4.0	20.0	25.0	30.0	33.7
L.S.D., P = 0.01	2.2	3.6	3.9	3.6	3.3

* 50 seeds = 100 per cent germination.

** Concentrations: PMC (puromycin), 100 µg/ml; COU (coumarin), 100 mg/l; AMO-1618, 5×10^{-4} M.

DISCUSSION

Only a few experiments on the influence of AMO-1618 on germination have been done as yet. Cathey and Stuart (1961) reported that this growth retardant delayd germination of the light induced seeds of *Lepidium*, being without effect on germination in complete darkness. In contrast, Lacoppe and Gaspar (1968) have recently found that AMO-1618 at 5×10^{-4}—5×10^{-3} M inhibits germination of *Lens culinaris* seeds, both in the dark and in light. It is of interest that CCC did not

affect germination of *Lens* seeds (Lacoppe and Gaspar 1968). Seeds of other genera are sensitive to Phosfon D and insensitive to CCC, and *vice versa* (Knypl and Słupek 1968, Knypl and Bilecka 1969). It is thus evident that seeds of different plant genera can selectively respond to different growth retardants. Nevertheless, seeds of other plant genera are sensitive to action of all retardants tested; to this group belongs kale.

The data of Tables 1—6 are similar with the results of experiments with other growth retardants (Knypl 1967). In summary, [1] each of the growth retardants delays but not inhibits germination of kale seeds, the effect being completely (B995, CCC, AMO-1618) or partially (Phosfon D) reversed by gibberellin; and [2] each of them acts synergistically with coumarin, strikingly decreasing the germination percentage — the effect being initially most effectively reduced by kinetin applied at the supraoptimal concentration, inhibitory for growth.

Growth retardants are belived to be inhibitors of gibberellin biosynthesis (Kende et al. 1963; Dennis et al. 1965; Baldev et al. 1965, Harada and Lang 1965). Since exogenous GA reversed their effect on germination in kale, it seems that they temporary reduce the content of gibberellins in the seed; gibberellins are possibly necessary here for the last phase of germination, namely the phase of early growth (Evenari 1957).

There is no doubt that coumarin, in contrast, acts on a quite different site of metabolism in the germinating seed, on the process being controlled by cytokinins. This conclusion is supported by several lines of evidence on the coumarin-kinetin antagonism in growth and germination (Khan and Tolbert 1965; Khan 1969 and 1967a). Similar antagonism has also been shown to occur between cytokinins and other germination and growth inhibitors like abscisic acid, morphactins, IAA and others (Khan 1968; Khan and Dowing 1968; Sankhla and Sankhla 1968a,b). It would seem reasonably to suggest that kinetin and coumarin act on the phase of activation in the germinating seed of kale. In this phase synthesis of RNA and protein (enzymes) is accelerated. This synthesis is controlled by kinetin, and inhibited by coumarin (and other germination inhibitors), possibly indirectly. Results of the tests with 2-thiouracil (Table 6) which is commonly held to be an inhibitor of RNA synthesis, suggest that kinetin reversal of the growth retardant-coumarin synergistic inhibition of germination is dependent on the synthesis of ribonucleic acids, directing the synthesis of proteins. Recently it was found that coumarin inhibits synthesis of α-amylase in germinating barley seeds; this effect was reversed by kinetin (Khan 1969). It was also found that coumarin and growth retardants markedly inhibited incorporation of labelled leucine into proteins of barley and kale leaves (Knypl 1969; Knypl and Kulayeva 1969). It is thus possible that cytokinin-coumarin physiological interaction in the germinatin of kale seed may be at the site of protein assembly.

Results of the tests with puromycin (Table 6) seem to contradict validity of this suggestion. It has, however, been shown that PMC can act as the enzyme inducer, and the induction occurs even though there is a depression of the total protein synthesis (Venis 1966); it has also been shown that PMC had no effect on GA_3-induced

dark germination of lettuce seed (Khan 1967b). Thus, it is necessary to study the protein (and RNA) pattern in the seed, germinating under the influence of inhibitors and phytohormones.

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Department of Plant Physiology
University of Lodz
Łódź, ul. Nowopołudniowa 12/16

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SUMMARY

AMO-1618 (5×10^{-4} M) and coumarin (100 mg/l) inhibited germination of the seed of kale; when applied alone, these compounds retarded germination.

The synergistic inhibitory effect of the retardant and coumarin was reversed by kinetin or GA_3; it was also slightly decreased by puromycin. Kinetin or GA_3 reversal was antagonized by 2-thiouracil and restored again by uracil.

It is suggested that GA_3 (stimulator) and AMO-1618 (an inhibitor of gibberellin biosynthesis) act on the phase of early growth, whereas kinetin as the stimulator and coumarin as an inhibitor act on the synthesis of RNA and protein in the germinating seed.

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Odwrócenie przez kinetynę i giberelinę hamującego synergistycznego działania AMO-1618 i kumaryny na kiełkowanie nasion jarmużu, Brassica oleracea L. var. acephala

STRESZCZENIE

AMO-1618 (5×10^{-4} M) i kumaryna (100 mg/l) opóźniają kiełkowanie nasion jarmużu; obie substancje podane w mieszaninie silnie hamują kiełkowanie. Ten ostatni efekt odwraca kinetyna lub giberelina; zmniejsza go również w pewnym stopniu puromycyna. Tiouracyl (10^{-3} M) znosi odwracające działanie zarówno kinetyny, jak i gibereliny; uracyl zmniejsza skutki działania tiouracylu.

Przypuszcza się, że GA_3 i AMO-1618 (inhibitor biosyntezy GA) działają na ostatnią fazę kiełkowania, mianowicie na fazę wczesnego wzrostu. Natomiast kinetyna jako stymulator i kumaryna jako inhibitor działają na syntezę RNA i białka w fazie aktywacji.

Katedra Fizjologii Roślin
Uniwersytetu Łódzkiego
Łódź, ul. Nowopołudniowa 12/16