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On the influence of the auxins and the anti-auxin upon vernalization[1]

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INTRODUCTION

Many researches have already been made on the relation between the auxins and photoperiodism (Dostál and Hošek (2), Leopold and Thimann (11), Bonner and Thurlow (1), Harder and van Senden (5), Fisher and Loomis (3), etc.), and not a few works have also been published on the relationship of anti-auxin and flower initiation (Zimmermann and Hitchcock (12), Galston (4), Leopold and Thimann (11), Bonner and Thurlow (1), Fisher and Loomis (3), etc.) but concerning the correlation between the low-temperature treatment and the auxin as well as the anti-auxin there are found a comparatively small number of investigation besides those conducted by Leopold (Leopold and Guernsey (8, 9), etc.).

The authors, who took an interest in the study of vernalization, carried out researches on the influence of the auxins and anti-auxin, given at various stages of the low-temperature treatment, upon flower initiation.

MATERIALS and METHODS

As material, seeds of the "Minowase-Daikon," a race of Japanese radish plant (*Raphanus sativus* L. var. *raphanistroides* Makino), were used. This plant needs almost strictly a low temperature treatment for its flower initiation.

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The seeds were first cleansed with water; then soaked in water, or on some occasions in the auxin or anti-auxin solution, they were left in room temperature or placed in an incubator of 30°C; after 20–30 hours, when the radicles had elongated to be about 2–3 mm long, the seedlings were selected and divided into 2 lots; the first lot was treated with low temperature and the second lot was left in the room temperature or placed in an incubator of 30°C, and during these thermal treatments some parts of each lot were drenched respectively in auxin and in anti-auxin solution and one part in water.

The growth hormones used were α-naphthalene acetic acid (NAA) and potassium salt of β-indole acetic acid (abbreviated: KIA); and the anti-auxin was triiodobenzoic acid (TIBA) (prepared by Ishihara Sangyo Kaisha, Ltd.).

Experiment I

The solutions of NAA and of KIA were prepared in four degrees of concentration, namely 0.1, 0.02, 0.01 and 0.002%, and in each of these

Table 1. Application of NAA and potassium indole acetate to the seedlings during the low-temperature treatment.

Growth substance	Concentration (%)	Days of low-temperature treatment	Number of individuals planted[1]	Bolting percentage: Number of days from the last day of low-temperature treatment to the day of observation		
				23	28	31
Water (control)	–	6	44	0	7	8
		8	46	13	43	64
NAA	0.02	6	14	0	0	0
		8	2	0	0	0
	0.01	6	15	0	0	0
		8	4	0	0	0
	0.002	6	17	0	0	0
		8	13	0	17	22
KIA	0.1	6	17	0	0	0
		8	28	0	15	40
	0.02	6	25	0	5	10
		8	37	5	34	61
	0.01	6	20	0	5	6
		8	31	6	27	50
	0.002	6	20	0	5	6
		8	25	0	17	36

[1] Six-day-treated seedlings were planted on July 26, and 8-day-treated on July 28, 1952.

and in water a part of the seedlings were dipped during the period of low-temperature treatment (Table 1).

At the end of 6-day or 8-day low-temperature treatment they were transplanted into gardens; several weeks later the bolting individuals were counted.

Of the individuals treated 6 days in NAA solutions none bolted, while the controls which were treated in water for 6 days, showed only a small bolting percentage.[1] Those treated 6 days in solutions containing less than 0.02% of KIA bolted at a nearly equal rate to the controls, but those in 0.1% KIA solution did not bolt.

Several bolted among those under the 8-day treatment in water, but the 8-day treatment in NAA solution failed, except with individuals in 0.002% solution, which showed a low bolting percentage. On the other hand individuals treated in KIA solutions showed bolting percentages of 36~61, that is, nearly the same percentages as shown by the controls.

Experiment II

Besides the intact seedlings the materials from which cotyledons were cut off were also examined by the use of 0.01% or 0.05% NAA solution during the low-temperature treatment (Table 2).

Table 2. Application of NAA to the intact seedling as well as the cotyledonless one during the low-temperature treatment.

Concentration of NAA (%)	Preliminary treatment	Days of the low-temperature treatment	Number of individuals planted[1]	Bolting percentage: Number of days from the last day of low-temperature treatment to the day of observation						
				33	36	39	42	46	52	72
Water	Cotyledons left intact	5	20	0	0	20	40	65	100	100
		8	20	0	35	75	100	100	100	100
0.01	〃	5	40	0	0	43	60	60	70	100
		8	40	0	18	58	70	98	98	100
0.05	〃	5	19	0	5	11	16	47	94	100
		8	14	0	0	0	0	64	100	100
Water	Cotyledons cut off	5	19	0	0	11	11	57	78	100
		8	19	0	0	0	37	68	89	100
0.01	〃	5	14	0	0	0	0	14	50	100
		8	7	0	0	0	0	0	29	100

[1] Five-day-treated seedlings were planted on April 8, and 8-day-treated on April 11, 1953.

1 Bolting percentage: "number of individuals bolting or shooting into a flowering stalk" : "total number of the plants under test" × 100.

As to the intact seedlings there was no remarkable difference in the bolting percentage between those treated in 0.01% solution and those in water; but comparing those treated in 0.05% solution with the water-treated, it could be seen that the former was behind in bolting in respect of time.

Cotyledonless seedlings, when treated in 0.01% NAA, likewise required more time for bolting than when treated in water.

On the other hand the cotyledonless seedlings treated in 0.01% NAA fell in time of bolting behind the intact seedlings similarly treated.

Experiment III

Seedlings were immersed in KIA solution during the low- as well as the high-temperature (30°C) treatment (Table 3).

Table 3. Application of potassium indole acetate to the seedlings during the period of low-temperature treatment or during the same lapse of time in an incubator of 30°C.

Duration of the treatment and the temperature	Concentration of the solution (%)	Number of individuals planted[1]	Bolting percentage: Number of days from the last day of the treatment to the day of observation						
			33	36	39	42	46	52	72
5 days 5±2°C	Water	30	2	13	43	57	80	93	100
	0.005	31	0	0	19	59	65	90	100
	0.01	30	0	0	10	30	70	93	100
5 days 30°C	Water	16	0	0	0	0	0	14	43
	0.005	17	0	0	0	0	0	0	29
	0.01	22	0	0	0	0	0	5	27
8 days 5±2°C	Water	35	0	17	63	86	100	100	100
	0.005	33	0	12	55	76	100	100	100
	0.01	27	0	0	37	70	100	100	100
8 days 30°C	Water	29	0	0	0	0	0	7	31
	0.005	31	0	0	0	0	0	20	39
	0.01	23	0	0	0	0	0	4	14

[1] Five-day-treated seedlings were planted on April 8 and 8-day-treated on April 11, 1953.

Of the materials of the 5-day low-temperature treatment, those immersed in KIA solution were somewhat later in bolting than those

in water. As to the materials treated with low-temperature for 8 days, there was scarcely any difference in bolting between those immersed in KIA and those in water; but there was a tendency that the higher the percentage of KIA-solution the lower was the bolting percentage.

The other set, which was held at 30°C during KIA treatment, showed a much lower percentage of bolting, similar to the material which was subjected to the same temperature in water.

As stated above, this plant essentially cannot bolt without low-temperature treatment, but it did bolt in this experiment, though in a very low percentage. The reason might be that as the materials were transplanted into the garden in early April, they were affected by a low temperature of less than 8°C several times, and thus some of the materials were vernalized.

Table 4. Application of TIBA to the seedlings during the low-temperature treatment or during the same period of time in an incubator of 30°C.

Duration of the treatment with the solution and the temperature	Concentration of the solution (%)	Number of individuals planted[1]	Bolting percentage: Number of days from the last day of the treatment to the day of observation						
			33	36	39	42	46	52	72
5 days 5±2°C	Water	86	0	15	23	33	63	83	100
	0.01	36	0	0	6	19	47	89	100
	0.02	34	0	3	29	38	68	94	100
	0.05	40	0	0	15	28	30	85	100
5 days 30°C	Water	34	0	0	0	0	0	0	24
	0.01	35	0	0	0	0	0	0	20
	0.05	46	0	0	0	0	0	0	22
	0.1	52	0	0	0	0	0	0	22
8 days 5±2°C	Water	83	0	16	59	78	94	100	100
	0.01	30	0	13	57	83	87	100	100
	0.05	38	0	0	21	45	92	97	100
	0.1	38	0	3	21	66	90	92	100
8 days 30°C	Water	50	0	0	0	0	0	0	45
	0.01	46	0	0	0	0	0	0	38
	0.05	34	0	0	0	0	0	0	38
	0.1	43	0	0	0	0	0	0	33

[1] Five-day-treated seedlings were planted on April 8 and 8-day-treated on April 11, 1953.

Experiment IV

TIBA solution was used during both the low- and high-temperature treatment (30°C) (Table 4). Materials both for 5-day and for 8-day low-temperature treatment, immersed in TIBA solution, differed very little in bolting percentage respectively from those immersed in water, but there could be seen a tendency that the solution of higher percentages retarded bolting to some extent.

Low percentages of bolting were observed on the 72nd day after the transplantation into the garden in all the lots of materials which had been immersed in TIBA during the 5-day or 8-day high-temperature treatment, as well as in the lots of those immersed in water during the same respective treatments; only the former showing rather lower bolting percentages than the latter. Here also the fact of bolting observed, though scantily, in the plant not artificially subjected to low temperature, might be explained by the same circumstance already mentioned in Experiment III.

Experiment V

Materials were immersed in NAA or in TIBA for ten days, that is, 3 days before plus 7 days during the low-temperature treatment (Table 5). It should not be said that there existed any remarkable

Table 5. Application of NAA or TIBA to the seedlings, prior to as well as during the low-temperature treatment.

Seeds were soaked, prior to the treatment, for 3 days with:	Germinated seeds were treated with low-temperature (6±1°C) for 7 days, drenching in:	Number of individuals planted [1]	Bolting percentage: Number of days from plantation to observation							
			21	24	27	30	35	39	45	49
Water	Water	26	0	0	0	0	12	21	41	54
NAA (0.002%)	NAA (0.002%)	27	3	3	3	7	22	31	52	56
TIBA (0.002%)	TIBA (0.002%)	19	0	0	0	4	7	11	14	16
Water	NAA (0.002%)	31	0	0	0	0	6	11	31	61
Water	TIBA (0.002%)	26	0	0	0	6	11	11	19	31

[1] Planted on March 25, 1954.

difference in the bolting percentage between seedlings treated in NAA (0.02%) and those treated in water. Seeds immersed in TIBA (0.02%) showed a lower bolting percentage than those immersed in water. In another case, materials were treated with solutions merely for 7 days,

namely, during the low-temperature treatment only. In this case, seedlings immersed in NAA (0.002%) solution were almost equal in bolting percentage to those in water, while materials drenched in TIBA (0.002%) turned out inferior to those in water. As to the NAA solution treatment, there was scarcely any difference between the material which was subjected to it for a period of ten days including 3 days preceding the 7 days of the low-temperature treatment and the material which underwent it only during the 7 days of the low-temperature treatment. Comparison between the plants immersed in TIBA during the period covering the 3 days prior to and the 7 days of the low-temperature treatment and those immersed only for the period of the low-temperature treatment showed that the former were somewhat lower than the latter in bolting percentage, though not remarkably so.

Experiment VI

In this experimentation a lot of the material which was treated

Table 6. Application of TIBA to the seedlings during as well as after the low-temperature treatment.

Germinated seeds were treated with low temperature for 13 days, being drenched in:	After-treatment	Number of individuals planted	Bolting percentage: Number of days from the finish of the low-temperature treatment[1] to the observation					
			28	33	38	43	50	58
Water	Seedlings were trasplanted into gardens immediately after the low-temperature treatment	30	0	3	40	63	100	100
NAA (0.002%)		32	6	16	70	78	100	100
TIBA (0.002%)		25	0	0	0	0	24	58
Water	Seedling were drenched in NAA (0.002%) for 4 days, after the finish of the low-temperature treatment until the transplantation	32	0	6	56	78	97	100
Water	Seedlings were drenched in TIBA (0.002%) for 4 days after the finish of the low-temperature treatment until the transplantation	31	3	13	42	62	97	100

[1] The low-temperature treatment was finished on Oct. 10, 1954.

with TIBA or NAA during the low-temperature treatment of 13 days, was compared with a lot which was similarly treated for 4 days after the close of the low-temperature treatment (Table 6). No difference was noticed between the results of the two lots of material treated with NAA; but concerning the material treated with TIBA, the first mentioned lot exhibited a tendency by far stronger than the other to inhibit flower-initiation.

It may also be said that, as to the material treated with solutions during the low-temperature treatment, the NAA-treated was equal to the water-treated, while the TIBA-treated was far inferior to the water-treated, and that concerning the material treated with solutions after the low-temperature treatment, the bolting percentages were approximately the same whether the material was treated with NAA or TIBA or in water.

Discussion

On a general survey of those experimentations it may be said that the bolting percentage was not increased by KIA or NAA applied prior to, in the course of, or after the low-temperature treatment, excepting the cases where, by the application of NAA (0.002%) prior to or in the course of the treatment as seen in Exper. 5 (Table 5) and of NAA (0.002%) in the course of the treatment as seen in Exper. 6, the bolting percentage was somewhat promoted in terms of time. This, however, should not be stated with certainty as the number of individuals treated was small, whereas it was generally noticed that the higher concentrations of those auxins very likely induced the lower bolting percentages.

Concerning TIBA, it was said that its application after the low-temperature treatment gave an almost equal result with the controls (without TIBA); nevertheless the application prior to or during the low-temperature treatment led to a bolting percentage lower than in the controls; only in the case of Exper. 4 the inhibiting effect was scarcely observed.

In the absence of low-temperature treatment, or rather in a treatment with high-temperature (30°C), the application of NAA showed a very poor bolting percentage, as bad as with the controls (with no NAA application). Leopold and Guernsey (9) succeeded in hastening flower initiation by using the low-temperature treatment and auxin application jointly. In the present experiments, though the degree of concentration of KIA and of NAA, as well as the duration of treatment, was not sufficiently varied to make it reasonable to draw a general conclusion, yet it might be said at least that NAA or KIA application could neither be substituted for the low-temperature treat-

ment nor have a power to strengthen the effect of the treatment as far as the present experimentation was concerned. From the fact that several materials used by Leopold and Gurnsey flowered to some extent without either auxin application or low-temperature treatment, whereas the plant which was used in the present experiments almost completely fails to bolt without low-temperature treatment, it might be assumed that the former were qualitatively different in flowering maturity from the latter.

It was mentioned that in Exp. III and Exp. IV the materials which were not intentionally treated with low-temperature bolted to some extent, but that by nature this plant does not flower unless treated with low temperature; yet in these cases the materials were transplanted into the garden in early April and so were exposed several times to temperatures lower than 8°C, and perhaps it was for this reason that some of them vernalized and bolted.

In the present experiments, the TIBA-application caused some inhibiting effect upon flower initiation. If the auxin-level has a delicate action upon flower-initation as stated by Lang (7), it will be considered that an application of TIBA disordered the balance of auxin-level and inhibited bolting.

The length of the period of low-temperature treatment was 6 or 8 days in this experiment. This material was on the whole vernalized completely by the 8-day treatment, but 6 days were somewhat too short for vernalization, being just the length of time for the incipient stage; if an exogenous application of auxin or anti-auxin had had an influence upon flowering, the most evident result could have been noticed; however, the auxin application showed practically no visible modification; only TIBA induced an inhibitory effect. The effect of TIBA was probably produced at the very time of low-temperature treatment and seemingly not as an after-effect upon the later stage of development of the seedling, because TIBA, given after the end of the low-temperature treatment, indicated no inhibitory effect.

The results of the previous work of Kojima, Yahiro and Inoue (6) which demonstrated the fact that the bolting percentage of cotyledonless plants was lower to some extent led to the comparative experimentation on the intact and the cotyledonless seedlings, in Exp. II. If auxin be made in cotyledons, and by cutting off the cotyledon the amount of auxin be reduced to scarcity or zero, the exogenous application of auxin will be expected to have a positive influence upon flower initiation; yet the experimental result in question did not answer to this expectation.

RÉSUMÉ

1) The material used was the seed of the Japanese radish plant which in almost all cases fails to flower-initiate, without being subjected to low temperature.

It was examined whether the bolting percentage was changed or not by application of solutions of several grades of concentration of NAA, potassium indole acetate, or TIBA, prior to, in the course of, or after the low temperature treatment.

2) The application of NAA or KIA to young seedlings sometimes lowered the bolting percentage and in other cases showed no difference from the bolting percentage of the seedlings left without application; yet there was scarcely found any case where the application exactly induced an increase in bolting percentage.

3) The exogenous application of NAA to cotyledonless seedlings could not increase the bolting percentage; it showed rather an inhibiting influence upon bolting.

4) In general, materials treated with TIBA were lower in bolting percentage than those without TIBA treatment; but application of TIBA after the close of the low-temperature treatment showed no inhibitory effect.

5) The materials without low-temperature treatment or those subjected to 30°C instead of low temperature, did not bolt within 46 days after the transplantation. After applying KIA or TIBA, those materials still did not improve their bolting percentage.

6) Though it is too hasty to draw general conclusions from the present experimentations, yet it may be said that the application of NAA, KIA or TIBA to seedlings in their very early stages neither had the effect to induce flower initiation of plants which were not subjected to low-temperature, nor to promote the bolting of plants already vernalized.

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