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Effects of Abscisic Acid and Other Plant Growth Substances on Seed Germination

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The effects of abscisic acid, kinetin, indolacetic acid, gibberellic acid, testosterone, estradiol and estrone on the germination of lettuce, pea, radish and lupine seeds were studied. Abscisic acid was found to be a germination inhibitor in all the studied seeds. The abscisic inhibition was reversed by indolacetic acid, kinetin and gibberellic acid.

Abscisic acid (ABA) is now attracting a great deal of attention among plant physiologists. The qualifications of this substance to be considered as a plant growth regulator are, at the present, generally accepted, thanks largely to the initial work of WAREING and his colleagues. It has been found in the coat, endosperm or embryo of many seeds (7, 13, 19, 22) and it is an extremely potent inhibitor of germination. Non-dormant seeds of fescue, lettuce and rye grass *(Lolium multiflorum)* can be made dormant if they are placed on a solution of the inhibitor (20).

On the other hand, some substances as gibberellic acid (GA_3), kinetin (Kt) and indolacetic acid (IAA) have been shown to stimulate germination in several types of seeds, and also, there is a great evidence of an interplay of growth stimulators and inhibitors have been placed in the control of seed germination and dormancy (2, 4-6, 15-17).

This communication describes a sensitive method for studying the possible influence of natural inhibitors and promoters, on those seeds, which are very easy to find in our country. In this way, the effects of Kt, IAA, GA_3, estrone (E), estradiol (EL) and testosterone (T) on the germination of different types of seeds (lettuce, radish, pea and lupine) are reported, as well as the interaction between these promoters and ABA in those seeds germination.

Materials and Methods

Radish *(Raphanus sativus)*, pea *(Pisum sativum)*, lettuce (*Lactuca sativa*, non photo sensitive variety) and lupine *(Lupinus albus)* seeds were used in this study.

They were supplied by the Spanish Company of seeds of Madrid. The seeds were stored at room temperature and were used within 6 months.

Treatment of seeds with chemical substances or water was carried out as described BRADBEER and PINFIELD (3). The pea, radish and lupine seed surfaces were sterilised by immersion in a solution of sodium hypochlorite for 10 minutes, then washed with sterile distilled water and transferred to Petri dishes lined with filter paper and containing a total volume of 2 ml, one with growth stimulators, another, with ABA and chemical promoters and the last one with sterile distilled water.

All the substances used, except steroids, were applied in the form of water solutions. ABA (10^{-3} M) was solubilized in 4 ml of ethanol absolute for a total volume of 100 ml. A control was prepared in the same way. 10^{-5} and 10^{-7} M solutions (ABA and control) were obtained by dilution. The steroids (100 mg/l) were dissolved in a small amount of tween 80 before adding water. Steroid treatments (100, 1 and 0.01 mg/l) received an amount of tween 80 whose final concentration was 1, 0.01 and 0.0001 %.

Pea and lupine seeds, after being sterilised were placed in containers with vermiculite, each one with 50 seeds; every test was replicated at least 4 times. Germination was checked every day. Protrusion of radicle through the covering structure was used as a criterion for germination. The data have been expressed as germination percentage. Seeds were germinated in light at a constant temperature of 25-26° C.

Results and Discussion

Table I gives the data obtained from the germination of different types of seeds treated with ABA at 26±2° C in light. It can be appreciated that ABA reduces germination, most noticeably with high concentrations (10^{-3} M).

Table I. *Inhibitory effect of ABA on lupine, radish, pea and lettuce seeds germination, %.* This percentage of germinating seeds was determined after seven days of sown.

Type of seeds	Concentration of ABA 10^{-3} M	10^{-5} M	10^{-7} M
Lettuce			
Control	64 ± 2	98 ± 2	100 ± 1
ABA	0	66 ± 4	89 ± 3
Radish			
Control	40 ± 2	60 ± 3	72 ± 2
ABA	0	40 ± 6	50 ± 5
Pea			
Control	80 ± 1	88 ± 2	100 ± 3
ABA	44 ± 5	58 ± 6	74 ± 3
Lupine			
Control	100 ± 2	100 ± 2	100 ± 1
ABA	21 ± 3	81 ± 3	78 ± 4

When the ethanol concentration rises up (10^{-7}, 10^{-5}, 10^{-3} control), germination is reduced in lettuce, radish and pea, but this effect cannot be appreciated in lupine seeds.

The effect of GA_3, IAA, Kt, E, EL and T on germination is shown in table II. Treatment of seeds with GA_3, IAA and Kt improved germination considerably. These substances increase the total germination appreciably and have also a remarkable effect on the speed of seed germination (fig. 1). Only IAA reduces lettuce germination with high concentration 10^{-4} and 10^{-6} M, but an increase can be observed at 10^{-8} M.

A slight increase was obtained in seeds treated with either T, E or EL. The best results with steroidal hormones were observed at lower concentrations (1 and 0.01 mg/l).

Germination in pea, lupine, lettuce and radish seeds was fully or partly inhibited by ABA 10^{-3} M and this inhibition could be counteracted by the presence of Kt, GA_3 or IAA (fig. 2). Kt solution produces

Table II. *Effect of different growth regulators on lupine, radish, pea and lettuce seeds germination (%).*
This percentage of germinating seeds was determined after eight days of sown.

	Lupine	Radish	Pea	Lettuce
Control	90±6	50±6	50±7	76±4
GA_3 3×10^{-4}	100±1	100±2		100±1
3×10^{-6}	100±1	90±1		100±1
3×10^{-8}	100±2	78±2		100±1
IAA 3×10^{-4}	100±1	62±2	100±1	68±1
3×10^{-6}	100±1	70±1	100±2	32±2
3×10^{-8}	100±1	64±2	100±1	100±1
Kt 3×10^{-4}	100±1	68±3	92±1	100±1
3×10^{-6}	100±1	68±2	84±3	56±2
3×10^{-8}	100±1	70±3	80±2	60±1
Tween				
(mg %) 1,000	45±5	32±3	40±1	46±3
10	43±2	43±2	39±2	43±6
0.1	70±2	46±2	40±2	69±8
E. (mg/l) 100	58±4	33±4	25±2	34±6
1	71±4	61±8	50±1	52±6
0.01	56±1	58±6	54±2	76±7
El. (mg/l) 100	70±4	28±8	52±2	60±8
1	64±2	47±6	62±1	67±9
0.01	73±3	58±1	58±2	92±6
T. (mg/l) 100	62±4	37±2	37±3	79±6
1	66±2	44±8	47±2	44±8
0.01	64±4	65±7	53±1	79±9

a nearly complete reversal of inhibition caused by 10^{-3} M. ABA in lettuce seeds. However, GA_3 and IAA appear to be less effective in reversing the ABA inhibition. These results strongly suggest that IAA, Kt and GA_3 act in a different manner, which is consistent with other reports on these substances comparative effectiveness (8, 9, 18).

At present it is known that estrogens are found in small amounts in plant tissues and that they have influence on the growth and development processes of plants. Previous reports (12, 14) showed that estrone, estradiol and testosterone

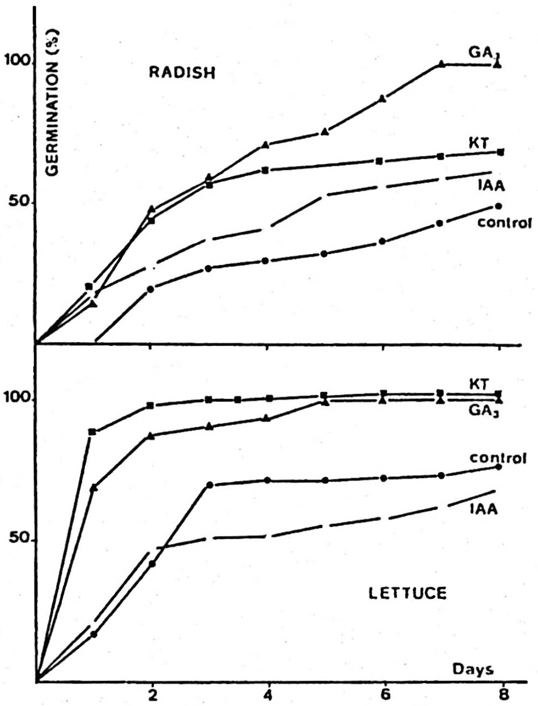

Fig. 1. *Effects of GA_3, IAA and Kt 3×10^{-4} M on seeds germination.*

increase pine seeds germination in darkness or in light. We have only observed a slightly positive influence of these substances on radish and lettuce seeds germination in light; this effect is not appreciable in darkness. There is a possibility when the positive influence of estrogens on germination results from an increase in the content of endogenous growth regulators (GA_3 and auxin) (10, 11). However, since the available information concerning the mechanism of steroidal hormone action in plant is scant, this problem remains opened for further investigations.

The results reported here strongly suggest that there is an interaction between natural inhibitors and stimulators acting as regulators of germination and growth.

Our results show a different interaction between ABA-GA_3, ABA-IAA, ABA-Kt on seed germination. Similar observations

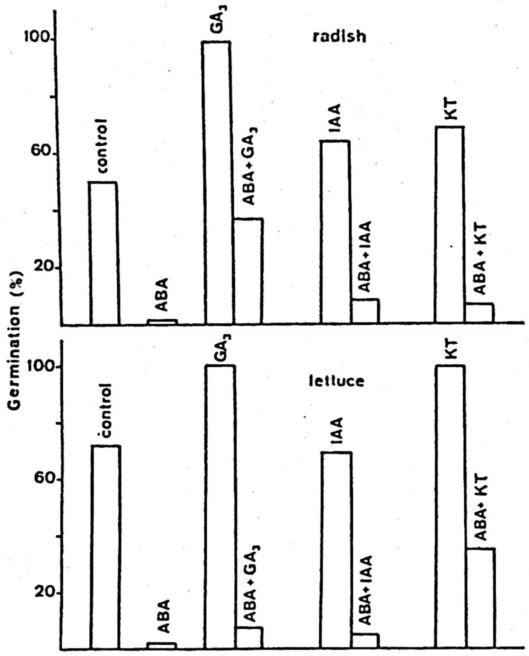

Fig. 2. *Effects of growth substances on radish and lettuce seeds germination.*

were shown in other reports (1, 8, 18). ABA might specifically antagonize gibberellin in plants (18), or, in some other cases act as an inhibitor of gibberellin synthesis (21).

However, other investigations have shown that the action of ABA is also opposed by cytokinins and that the effect of the promoter-inhibitor combination may vary (1). In some cases the interactions become complex.

Resumen

Se ha estudiado el efecto de algunas hormonas vegetales (IAA, GA_3, Kt y ABA), así como el de un segundo tipo de sustancia cuyo carácter es de naturaleza esteroidica E, EL y T, sobre la germinación de semillas de lechuga, rábano, guisante y altramuz. Se ha observado que el ácido abcísico es un gran inhibidor de la germinación en todas las clases de semillas estudiadas y cómo la inhibición producida por ABA podía ser contrarrestada por GA_3, IAA y Kt.

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