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HASTENING GERMINATION OF CROP SEEDS AND SEEDLING GROWTH WITH GIBBERELLIC ACID

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Abstract

Crop seeds were treated with solutions of GA_3 at different concentrations, for hastening germination and the growth of seedlings. In general, the percentage of seeds germinating and the speed of germination increased proportionally with the concentration and at rates differing according to species.

The GA-concentration needed for the greatest stimulation of shoot growth was different according to species, but for most seeds, 500 ppm and 12 hours of treatment proved to be the most effective.

From seeds responding to GA treatment with a shortened germination period seedlings emerged earlier and more uniformly from the soil than those from untreated seeds.

The dry-weight loss of germinating seeds was increased by GA-treatment, and is an index of that.

Amylase and protease activity in the germinating seeds was considerably increased by GA-treatment and the quantity of starch and protein degraded is proportional to the germination period and the weight loss of the seeds. There seemed to be a parallel increase, induced by GA-treatment, of the activities of the hydrolytic enzymes and the elongation of seedlings.

GA-treatment is, therefore, suitable for hastening the germination of crop seeds. This is particularly valuable in the case of slowly germinating seeds where even the percentage of seeds germinating can be considerable increased by GA-treatment.

Introduction

Of the various physiological effects of gibberellins, one of the most important, both from the theoretical and practical points of view, is that they can considerably stimulate the germination of various seeds. In this way, they can assure a more rapid and more uniform emergence of the seedlings (WEAVER, 1972). In the case of crops, early germination and the rapid growth of seedlings are particularly advantageous, because of decreased susceptibility to insect and disease hazards, and also, owing to the possible earlier marketing of the yield. Germination may often be accelerated by soaking seeds in a GA-solution (HAYASHI, 1940; BURNS et al., 1966) or by incorporating GA in a seed protectant slurry (WITTWER and BUKOVAC, 1957; 1958).

The aim of our work was to select from the seeds of some cultivated crop plants, those species responding to GA-treatment by faster germination and growth and further, to establish the GA concentrations required for stimulated germination of seeds and optimum growth of the seedlings. If GA-treatment advances seedling emergence by even a few days, then the procedure may already be considered successful. The GA-induced hastening of germination is closely related to the effect on

synthesis and activity of the enzymes mobilizing storage reserves. Therefore, we have investigated, in a few cases, how much the hydrolytic enzyme activity was affected by our GA-treatment. In addition, we have examined the correlation between the activity of these enzymes and the GA-induced germination and growth.

Materials and Methods

Experiments were performed with seeds and seedlings of the following plants: wheat "Kompolti", barley "Sörárpa", corn "Arany mazsola", bean "Cherokee", pea "Chrestenens", cucumber "Rajnai fürtös", red pepper "Szentesi fehér" and tomato "Kecskeméti merevszárú".

The gibberellic acid used for the treatment of seeds was GA_3 (Phylaxia).

Pretreatment of seeds with GA and germination in Petri dishes

The seeds (50 pieces) were soaked at room temperature for twelve hours in GA_3-solutions at concentrations of 50, 100, 500 and 1000 ppm. (In previous experiments, a 12-hour soaking time proved to be most effective). As controls, some seeds were soaked in distilled water. Germination was carried out in large Petri dishes, in the dark at 25 °C with each seed being in identical conditions of moisture. The number of germinated seeds and the length of shoot and root were recorded daily for five days. Four replicate experiments were carried out.

Examinations of the emergence of seedlings from soil

The seeds (30 pieces in each case) were sown, after being soaked in a GA-solution at 500 ppm for 12 hours, into dishes containing an equal quantity of soil. Seeds sown in the same way and soaked in distilled water were used as controls. Germination and growth were performed in a phytotron (CONVIRON Cabinet Model EF 7, at 25/20 °C day/night temperature, 16-hour illumination, 10 000 lux, 64 percent relative humidity). The soil of dishes was identically watered with tapwater. The number and shoot growth of the emerged seedlings were followed carefully for two weeks. The experiments were also carried out as four replicates.

Measuring the loss of dry weight in germinating seeds

The dry weight of 6-day old germinating seeds was compared with the dry weight of non-germinated seeds as an initial value. The decrease in dry weight was considered as one of the indices of the utilization of food reserves.

Determination of amylase activity

The amylase activity of wheat grains, treated with GA and untrated, were measured on days 3, 6, 9 and 12 of germination, with the process used by VARGA et *al.* (1967) and MIERZWINSKA (1975).

The amylase was extracted from 1 g germinating wheat grain with 15 volumes of cold phosphate buffer (pH 5.3). The homogenate was centrifuged at 28 000 g for 30 minutes at 0 °C. The supernatant was made up to 20 ml. with the same buffer and this was used as a crude enzyme extract. The substrate was a 1 percent solution of soluble strach, made with phosphate buffer (pH 5.3).

The reaction mixtures (3 ml enzymatic extract+3 ml substrate; or, for the blank reaction, 3 ml buffer instead of enzyme) were incubated at 35 °C for 30 minutes, then 1 ml samples were taken into test-tubes containing 10 ml 0.05 N HCl. The

initial starch concentration was determined from the blank reaction mixture, from the sample withdrawn at zero time. 0.5 ml KI-KIO_3 solution was added to the test-tubes and the intensity of the blue colour produced was measured at 580 nm, with Spectromom 202 photometer. Amylase activity was expressed as the weight (in mg) of starch degraded in 30 min., by the enzyme extracted from 1 g of germinating seed.

The activity of α- and β-amylase was determined separately by heating a part of the enzyme extract in a water bath at 70 °C by which procedure β-amylase is inactivated. With this enzyme extract the α-amylase activity could therefore, be determined. Subtracting this value from the value obtained with the unheated preparation gave a measure of the β-amylase activity.

Measurement of protease activity

The protease activity of the GA-treated and control pea seeds was measured according to HARVEY and OAKS (1974a; 1974b), on the first five days of germination.

Fozen cotyledons were homogenized in a cooled mortar with cold acetate buffer (pH 3.8). The homogenate was centrifuged at 28.000 g for 30 min. at 0 °C. The supernatant was assayed for proteolytic activity with a 5 percent solution of albumin, prepared from dry pea seeds, as substrate. Each reaction mixture contained 2.0 ml of substrate, 0.5 ml of acetate buffer 0.05 M (pH 3.8), 2.5 mM EDTA, and 0.5 ml of enzyme in a total volume of 5 ml. The blank was made with boiled enzyme. The mixture was incubated for 15 min. at 40 °C, and the reaction was stopped by adding an equal volume of 5 percent TCA. Protease activity was measured as the increase in absorbance at 280 nm of the TCA-soluble fraction, and was arbitrarily calibrated against the absorbance as mg tryptophan released from protein per hr per seed.

The laboratory examinations were performed in triplicate.

Results and discussion

1. Effect of soaking seeds in GA-solution on germination and seedling growth

We have found only very limited data in the literature concerning the stimulation of germination and seedling growth by treating crop seeds with GA-solutions, and further, what GA-concentrations are effective on the diverse seeds. Taking into consideration the presumably different reactions of the individual species, we have chosen and tested, for our experimental seeds, a concentration range from 50 to 1000 ppm.

Results of germination in Petri dishes

By germinating the seeds in Petri dishes, we were able to examine the GA-effect on the speed of germination and percentage of seeds germinating as well as on the rapidity of seedling growth.

Examining the *speed of germination* (Table 1), it is obvious that hastening of germination is expressed in every species in days 1 and 2 after pretreatment. The increased speed of germination is very striking with cucumber, barley, corn, pea

Table 1. The speed of germination and germination percentage of seeds presoaked for 12 hours in GA-solutions of different concentrations. (n=4×50)

Seeds	GA conc. ppm	Days					Germination percentage
		1	2	3	4	5	
		Number of germinated seeds					
Wheat	0	40	48	48	48	48	96
	50	43	49	50	50	50	100
	100	45	50	50	50	50	100
	500	46	50	50	50	50	100
	1000	45	50	50	50	50	100
Barley	0	5	37	43	43	43	86
	50	5	42	42	43	45	90
	100	16	42	43	45	46	92
	500	16	44	45	45	46	92
	1000	19	40	43	45	47	93
Corn	0	—	15	21	30	32	64
	50	—	19	24	38	40	80
	100	—	28	38	44	45	90
	500	5	27	36	42	42	84
	1000	3	26	41	45	45	90
Bean	0	20	39	42	45	45	90
	50	21	43	45	46	46	92
	100	22	42	44	46	47	93
	500	23	44	46	47	47	93
	1000	24	43	46	46	46	92
Pea	0	—	18	25	26	30	60
	50	—	20	28	30	32	64
	100	3	25	33	34	34	68
	500	5	27	35	38	38	76
	1000	5	27	36	39	40	80
Cucumber	0	25	41	47	47	47	93
	50	33	47	48	50	50	100
	100	32	46	50	50	50	100
	500	43	48	50	50	50	100
	1000	44	50	50	50	50	100
Red pepper	0	8	22	30	34	36	72
	50	7	25	34	37	37	74
	100	10	25	35	37	38	76
	500	10	24	37	40	41	82
	1000	12	26	36	40	40	80
Tomato	0	1	10	27	32	38	76
	50	—	13	30	38	38	76
	100	2	12	32	39	39	78
	500	3	17	38	42	43	86
	1000	3	15	33	41	41	82

and red pepper, but with wheat, bean and tomato seeds the effect is rather less obvious. In days 1 and 2 after pretreatment the effects of GA appear to be related to its concentration. In succeeding days the differences between the GA-treated and untreated, control, seeds become much less marked. The increase in germinating speed is greatest with barley, corn, pea and cucumber seeds.

Although indicated by previous reports (WEAVER, 1972) that the final *germination percentage* is not considerably increased by GA-treatment, we have nevertheless observed, in our experiments, a rise in the germination percentage of most species, with generally bears a direct relationship to GA-concentration (Table 1). As compared with the control, the rise in the final germination percentage is highest in the case of corn (+26 percent), pea (+20 percent), red pepper (+17 percent), and tomato (+10 percent). On the other hand, virtually no increase was observed with wheat, barley and bean seeds (+3 to 4 percent), wich germinated well, even without GA-treatment.

It can be ascertained on the basis of our results that the GA-treatment applied by us is suitable for hastening the germination of several crop seeds. This effect is particularly noticeable with seeds germinating comparatively slowly without any treatment, where an increased percentage of germination, ranging from +10 to +26 percent, could be achieved with GA pretreatment. The method is, therefore, noteworthy from practical point of view.

The *shoot growth* of seedlings originating from seeds treated with GA-solutions of different concentrations is shown in Fig. 1. The stimulative effect of GA on shoot growth begins to be conspicuous from the 4th day of germination and is the most vigorous between days 4 and 5. This is best observed in the case of wheat, barley, pea and cucumber. According to our data, cucumber, then barley, corn and bean seedlings respond most strongly to GA-treatment with an increased shoot growth. The GA-concentration needed for maximum stimulation of shoot growth is different according to species. For tomato, treating with a solution of 100 ppm of GA, for pea and bean with one of 1000 ppm, and for the other four species, treating with a solution of 500 ppm, proved to be the most successful. It can also be shown that the GA-concentrations stimulating maximal germination or maximal shoot growth are different, at least for the majority of species.

In our experience, *root growth* of seedlings is also stimulated by GA, apart from a few exceptions, in a contentration dependent manner.

Results of sowing into soil

For sowing into soil, the seeds were pretreated for 12 hours in a GA-solution at a concentration of 500 ppm, proved previously to have an optimum effect. In these experi mnts, the emergence of seedlings and the rapidity of their growth were followed closely.

The growth of the emerged shoots was stimulated, in the majority of species, by GA-treatment, but in different ways and to different degrees. In the case of wheat and bean, where no considerable hastening of germination is induced by GA, an increased shoot growth could only be observed after days 4 to 6 and thus, emergence from the soil was not enhanced considerably. The degree of growth stimulation was +17 percent and +14 percent, respectively, at the end of the experimental time (Fig 2). On the other hand, the seedlings from barley, corn, pea and cucumber

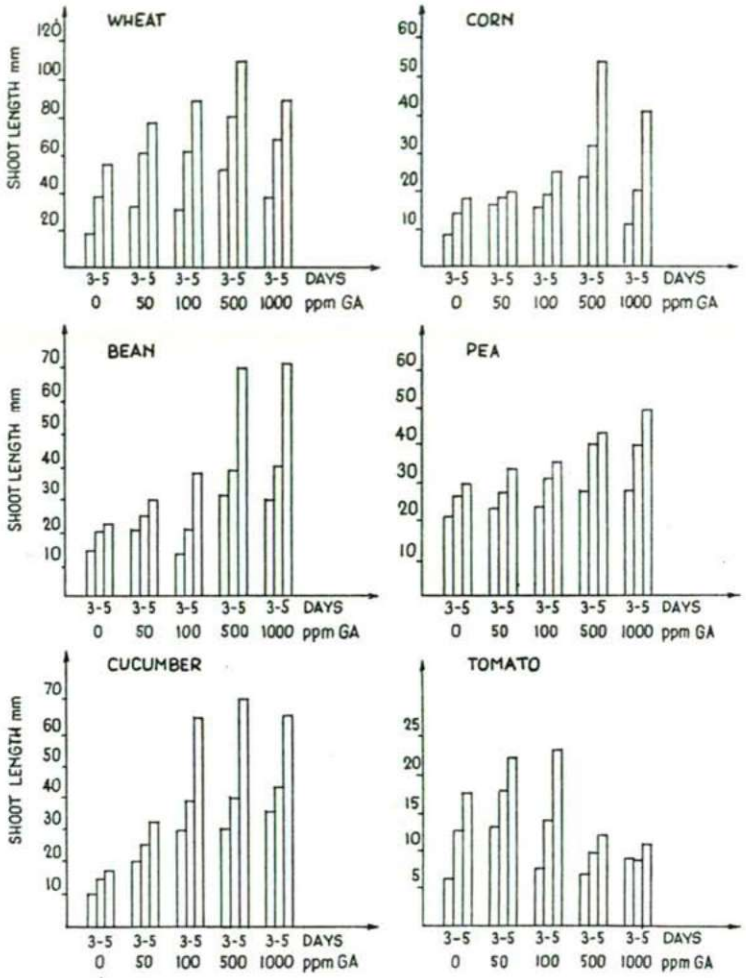

Fig. 1. Shoot growth of seedlings growing from seeds treated with GA-solutions of different concentrations.

seeds, which responded with accelerated germination to GA-treatment, emerged earlier than controls and, in addition, their shoot was longer from the time of emergence. In this group we have observed a growth stimulation ranging from +28 to +40 percent at the end of the experimental period (Fig. 3). These results partly agree with HAYASHI's report (1940) who experienced a more rapid germination and stimulated shoot growth of barley and rice grains soaked in GA-solution.

The emergence of seedlings originating from treated seeds is therefore correlated with the GA-effect on speed of germination. The stimulation of shoot growth is, on the other hand, considerable for every species. Enhanced stem elongation is also

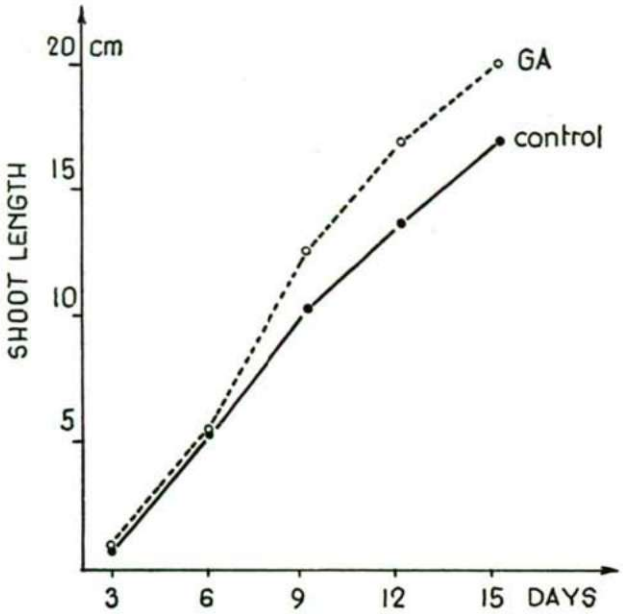

Fig. 2. Shoot growth of bean seedlings, from GA-treated and untreated seeds, after emergence from soil.

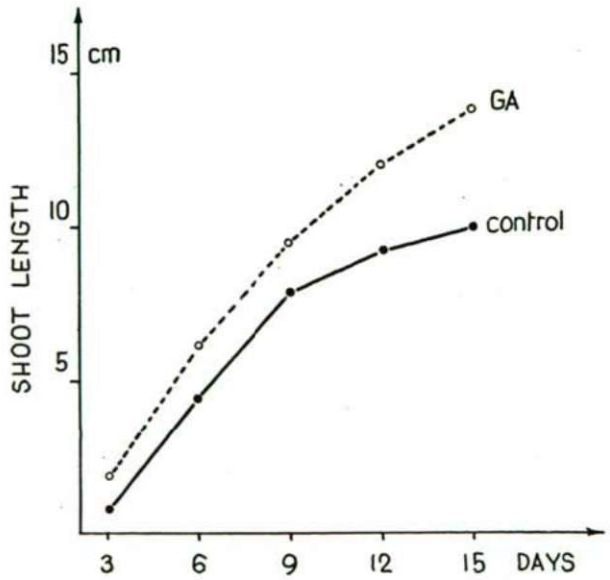

Fig. 3. Shoot growth of cucumber seedlings from GA-treated and untreated seeds, after emergence from soil.

accompanied by a noticeable leaf expansion and the treated plants generally seem to be more vigorous than untreated controls. GA-treatment does not result, therefore, in an etiolation-type shoot growth.

2. Effect of GA on dry-weight loss of germinating seeds

According to our results, the percentage of dry weight of wheat and pea seeds, germinated in GA-solution at a concentration of 500 ppm, is considerably smaller on day 6 of germination than that of the control germinated in tapwater (Table 2). This observation reflects more rapid and intensive germination and an increased seedling growth. The mobilization of endosperm reserves and their utilization by the seedling was increased by GA by 30–33 percent. The weight loss taking place during germination is proportional, both with wheat and pea, to the intensity of germination and growth, i.e., it is a good indicator of these processes.

Table 2. Effect of GA-treatment on the loss of dry weight of germinating seeds

Species	Seeds germinated in tapwater	Seeds germinated in GA-solution	Effect of GA on dry weight loss %
	dry weight % on the 6th day		
Wheat	8.8	6.1	30.7
Pea	7.2	4.8	33.4

3. Effect of GA-treatment on hydrolysis of starch reserves

From those seeds where the main storage product is starch, we have chosen wheat for the present experiments. The effect of the 12-hour long GA-pretreatment of 500 ppm, on the $\alpha+\beta$ amylase activity of germinating wheat grains is shown in Fig. 4. According to the data, during the 3–12 days of germination more starch reserves were degraded in the endosperm of the GA-treated wheat grains; that is, amylase activity was more increased than in control seeds. It is obvious, therefore, that the dry-weight loss of the GA-treated wheat grains is a result of a more intensive mobilization of the reserved starch.

The difference between the amylase activity of controls and the GA-treated grains was the greatest at the beginning of germination (on the third day +35 percent). As germination and growth advanced, amylase activity declined. The greater initial enzyme activity of GA-treated seeds corresponds to the hastened germination observed in the first days.

According to our present knowledge, GA stimulates germination at a genetic level, by inducing the synthesis of the enzymes that hydrolyze the endosperm reserves. The hormone derepresses the genes responsible for the hydrolytic enzyme synthesis. The increase of the GA-induced enzyme activity is, therefore, based upon a *de novo* protein synthesis. A great number of publications concerning the enzyme inducing effect of GA have appeared to date. These results have been reviewed recently by MARCUS (1971), JONES (1973), MAYER (1974), and JACOBSEN (1977).

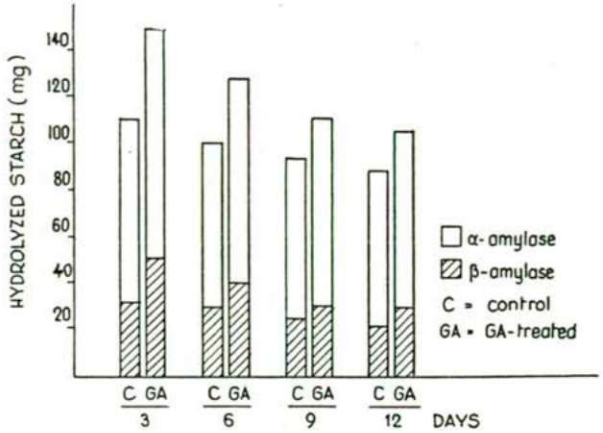

Fig. 4. Effect of GA on the amylase activity in germinating wheat grains.

4. Effect of GA-treatment on the mobilization of protein reserves

The GA-effect on the formation of protease activity was measured in the germinating pea seeds, which contain a considerable quantity of protein, for six days. The treated seeds were soaked for 12 hours in GA-solution at a concentration of 500 ppm. Controls were soaked in tapwater. The rate of the protein-reserve degradation was considerably increased during germination by the GA-treatment of pea seeds (Fig. 5). The GA-induced increase in protease activity was greatest on days 2 and 3 of germination (+75 and +73 percent). This coincides with the increased rate of germination as observed in treated seeds on the same days. As germination advanced, the difference between the protease activity of treated and untreated pea seeds decreased to some extent but the increased mobilization of the protein in the treated seeds could be distinctly observed even on the sixth day (+33 percent).

5. Connection between the GA-induced enzyme activity and the shoot elongation

The stimulating action of GA on the synthesis and secretion of some hydrolytic enzymes has been noted many times. Much less is known about the relation between the enzyme activity and seedling elongation induced by GA.

KATSUMI and FUKUHARA (1969), after treating seedlings of dwarf corn mutants with GA_3, found that amylase activity increased in parallel with shoot elongation. This was, however, not observed in isolated bean hypocotyls exhibiting a high amylase activity, stimulated by GA-treatment (CLUM, 1967). Incubation of alfalfa seeds in a GA_3-solution induced parallel increases in proteolytic activity and hypocotyl elongation (CONEN et al., 1969). On the other hand, MICHNIEWICZ and KAMIENSKA (1969) did not observe a direct correlation between the stem elongation and the activity of hydrolytic enzymes in bean seedlings growing on a medium with added GA. It is, therefore, worth comparing how, in our own experiment, the

GA-induced amylase and protease activity is connected with the shoot elongation of seedlings.

In the case of germinating wheat, amylase activity was increased by GA-treatment by 18 to 25 percent (Fig. 4). Comparing these results with the simultaneous shoot elongation (Fig. 1), we observe a definite correlation.

In germinating pea seeds, protease activity was increased by GA-treatment by 33 to 65 percent (Fig. 5). The GA-induced increased degradation of protein reserves, and the stimulated transport of amino acid components into the seedlings are in full agreement with the shoot elongation data displayed in Fig. 1.

Thus, from our experiments, it would appear that there is a direct correlation between the increased activity of hydrolytic enzymes and the elongation of seedling shoots induced by GA-treatment.

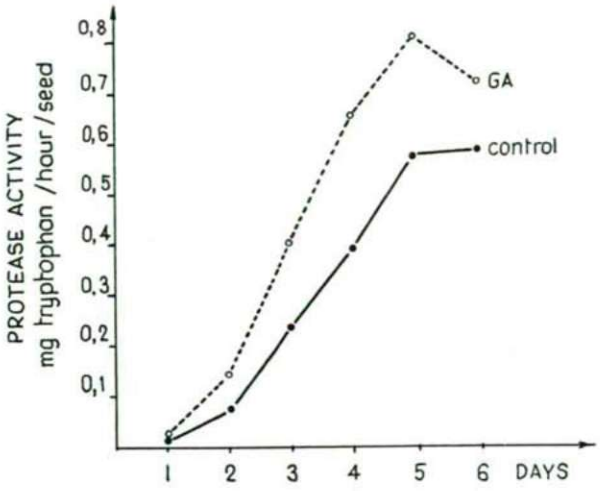

Fig. 5. Effect of GA-treatment on the protease activity in germinating pea seeds.

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