

INFLUENCE OF FERRULIC ACID UPON THE GERMINATION OF BARLEY AND THE FORMATION OF α -AMYLASE

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The author studies the influence of ferrulic acid upon germination in *Hordeum vulgare*, investigating both the morphological aspect of germinated seeds, as well as the α -amylase activity. After 48 and 72 hours of germination, ferrulic acid in 2.57×10^{-3} M and 5.14×10^{-3} M concentrations determines a special development of the radicle of the future plant, which is much elongated and thin, and with less developed absorbent hairs as compared with the control lot.

After the first 16 hours of germination, α -amylase activity is higher than that dosed for the control lot only in the case of 5.14×10^{-5} M, 5.14×10^{-4} M and 5.14×10^{-3} M solutions. One single ferrulic acid solution (2.57×10^{-3} M) has a slight inhibiting action. After 24 hours of germination all the employed ferrulic acid solutions have an inhibiting action, while after 48 hours of germination only the 5.14×10^{-3} M solution inhibits the formation and activity of α -amylase. In the case of 5.14×10^{-5} M 5.14×10^{-4} M solutions, α -amylase activity clearly higher than that recorded for the control lot.

All the utilized ferrulic acid concentrations had a stimulating action upon α -amylase activity after 72 hours of germination. Experimental results are discussed in the sense of the existence and of the formation of certain ferrulic acid compounds in the process of culture plants germination.

The evolution of biochemical processes during the germination of culture plants forms the object of investigations carried out lately by various research workers. The germination process is characterized by the formation and activation of a large number of enzymes, of which some accelerate the degradation process of reserve substances, while others participate actively in the biogenesis of substances necessary to

the growth and development of new seedlings. The main physiological aspects of the germination process are already generally known, but the biochemical mechanisms were less studied, and attempts to correlate the different phenomena in view of directing germination are rare.

The lately effected researches demonstrated the existence, in relatively minute quantities, of numerous compounds (giberilic acid, indole-3-acetic acid, cumaric acid, ferrulic acid, kinetin, vanillic, oxibenzoic acids, a.s.o.), to which an important role in germination is assigned.

In previous communications (1), (2), we demonstrated the influence of coumarin and giberilic acid on barley germination, on α -amylase formation, as well as the interaction between these two substances.

In the present work we propose to analyse the influence of ferrulic acid on α -amylase formation in *Hordeum vulgare* seeds, at different germination intervals. The results of preliminary experiments have previously been partially communicated.

Experimental part

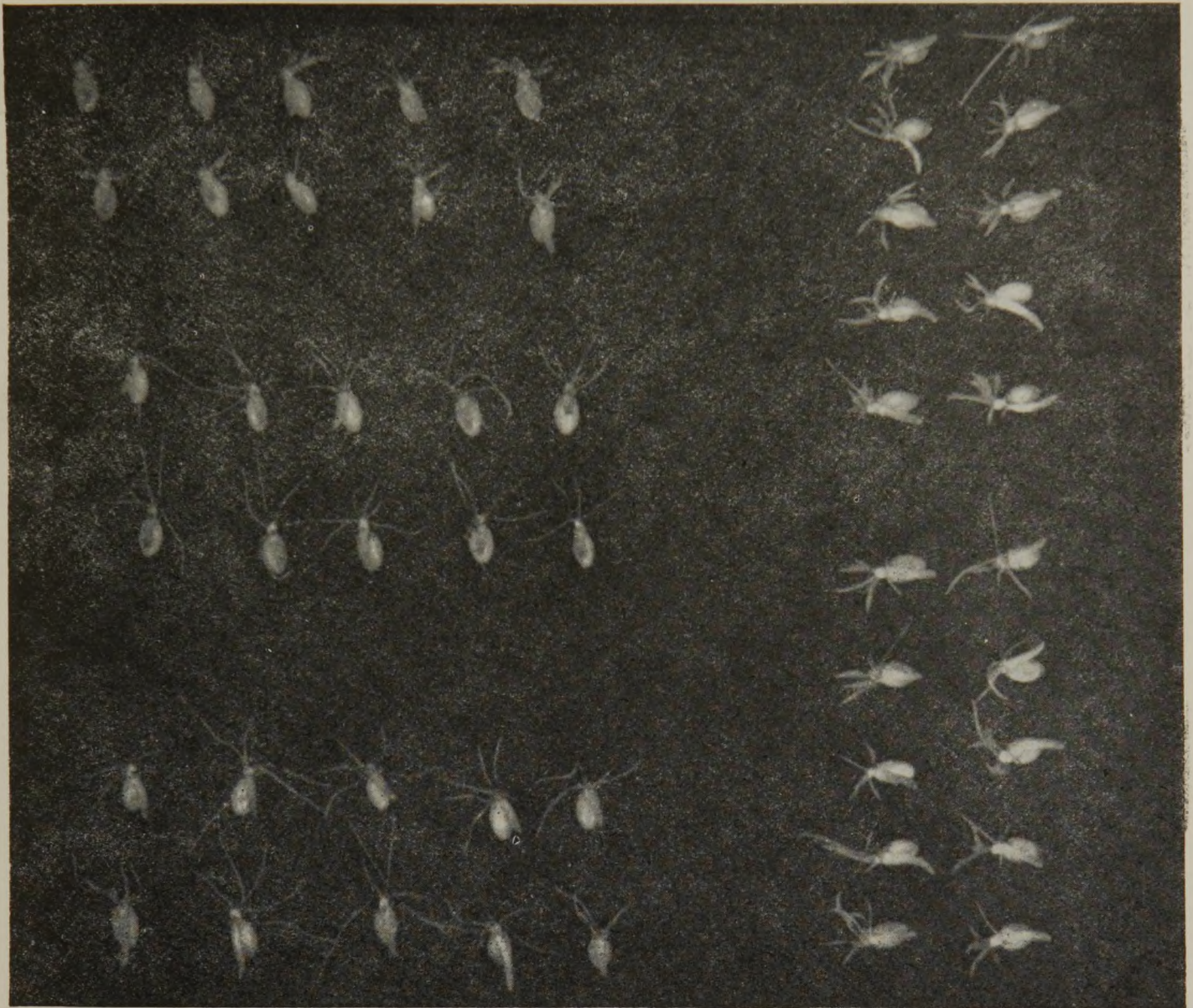
Hordeum vulgare seeds were sterilized for two hours in a 50% sulphuric acid solution, and subsequently washed in a tap water current to a neutral pH. Germination was effected in a camera obscura, at the temperature of 22°C, in Petri dishes containing 8 ml of ferrulic acid solution of different concentrations. Ferrulic acid solutions were prepared prior to their utilization in a $\text{Na}_2\text{HPO}_4/\text{KH}_2\text{PO}_4$, M/15, pH7, buffer. The following ferrulic acid concentrations were employed: $5,14 \times 10^{-5}\text{M}$; $5,14 \times 10^{-4}\text{M}$; $2,57 \times 10^{-3}\text{M}$ and $5,14 \times 10^{-3}\text{M}$. α -amylase activity was dosed after 8, 16, 24, 48 and 72 hours of germination of intact seeds. The control was achieved in the same experimental conditions, in M/15 phosphate buffer solution. After germination period seeds were taken out of the solution in which they germinated, washed with distilled water (twice or three times) and dried in a hot air current at the temperature of 25–30°C. After drying, the material was weighed and pounded in a mortar. A quantity of pounded material corresponding to 10 germinated seeds was extracted with 10 ml 2% calcium chloride (pH=5,3), for 24 hours, at the temperature of 20–22°C. Subsequently the samples were centrifuged at 10.000–12.000 r.p.m. in a centrifuge with cooling, and the aliquot part used for determining α -amylase activity.

α -amylase dosing was achieved by the method described by Briggs (4), the substrate being prepared after Kirsop's (6) prescription. After the development of colour reaction, samples were immediately re-read at the FEK-N-57, green filter (= 508 μm photoelectrocolorimeter, α -amylase activity was expressed in „I. D. C. units“ (4).

Experimental results and their discussion.

The morphological examination of barley seeds germinated during the first 24 hours evidences the fact that the different ferrulic acid concentrations do not influence the germination process, the control lot

presenting the same characteristics as the test lots. An altogether peculiar effect of the influence of ferrulic acid appears after 48 and 72 hours of germination, and solely for concentrations of $2,57 \times 10^{-3} \text{M}$ and $5,14 \times 10^{-3} \text{M}$ ferrulic acid: the future plant root is much elongated and thin, and the absorbent hairs less developed in comparison with the control lot. The effect was ascertained in all the experiments and is



easily reproduced in the experimental conditions mentioned in the present paper. The $5,14 \times 10^{-5} \text{M}$ and $5,14 \times 10^{-4} \text{M}$ ferrulic acid solutions stimulate the barley germination process, the lots treated with these solutions being characterized by an evident development of plants as against the control lot (Fig. 1).

The influence of different ferrulic acid concentrations upon α -amylase activity is summarized in table 1. During the first 48 hours of germination, the utilized ferrulic acid concentrations have an irregular

effect upon α -amylase formation. If in the first 8—10 hours of germination, no α -amylase enzymatic activity can be detected, by the method utilized by us, in the test material, the effect of the different ferrulic acid concentrations is difficult to be interpreted after 16 hours of germination. Solutions containing $5,14 \times 10^{-5}M$ and $5,14 \times 10^{-3}M$ have a stimulating effect upon α -amylase formation, while a $2,57 \times 10^{-3}M$ concentration inhibits enzyme activity.

It is noteworthy that in a $5,14 \times 10^{-3}M$ concentration, α -amylase activity is twice higher as compared with that detected in the control lot.

After 24 hours of germination all ferrulic acid solutions, except the one containing $5,14 \times 10^{-4}M$ have a clear inhibiting effect upon α -amylase activity. At an 48 hours germination interval, in conditions of total darkness, ferrulic acid concentrations of $5,14 \times 10^{-5}M$ and $5,14 \times 10^{-4}M$ stimulate α -amylase formation as compared with the control lot. Ferrulic acid solution containing $2,57 \times 10^{-3}M$ has no tangible influence upon α -amylase formation, enzyme activity being close, as value, to the activity recorded for the control lot. A single ferrulic acid concentration equal to $5,14 \times 10^{-3}$ has a slight inhibiting action upon α -amylase.

This peculiar action of ferrulic acid upon barley germination and α -amylase formation determined us to carry out a series of 72 hours experiments on a test lot of germinated seeds. From the experimental results shown in table 1 the fact can be recorded that all the ferrulic acid concentrations employed have, after 72 hours of germination, an evident stimulating effect upon α -amylase formation as compared with the control lot, enzyme activity diminishing with the increase of ferrulic acid concentration, the values of α -amylase activity remaining much higher than the corresponding values obtained for the control lot, even in the case of the most concentrated ferrulic acid solution ($5,14 \times 10^{-3}M$).

The experimental results obtained by us can be correlated with El-Basyouni and Towers' experiments (5) which showed that during the germination of culture plants, endogenous ferrulic acid concentration increases, ferrulic acid being found in seeds, under a combined form. The action of the different ferrulic acid concentrations used by us can be thus interpreted: as germination time increases and the concentration of internal ferrulic acid of the seed grows, the action of external ferrulic acid (from the solution) is probably depending on the seed potentiality of neutralizing the action of external ferrulic acid by the formation of the latter's compounds with the glucides. The different effect which one and the same ferrulic acid concentration, e.g. the one containing $5,14 \times 10^{-5}M$, has at different germination intervals, depends, probably, on the evolution of internal ferrulic acid fractions and on the relationships between them.

The stimulating effect of ferrulic acid at 72 hours of germination upon α -amylase activity, which is easily reproducible and preserves permanently the same characteristics (morphological aspect of the material employed, evident stimulation of α -amylase formation) is investi-

gated at present by us in a series of experiments in which the quantity of ferrucic acid present in seeds under combined form is likewise simultaneously dosed. The preliminary results evinced the existence of some direct quantitative relationship between internal ferrucic acid and the effect of the ferrucic acid from the solution in which seeds germinate.

Table 1

EVOLUTION OF α -AMYLASE ACTIVITY DURING BARLEY GERMINATION
(EXPRESSED IN "I. D. C." UNITS/10 SEEDS/10 ML.)

Duration of germination (hours)	Control lot	Different ferrucic acid concentrations			
		$5.14 \times 10^{-5}M$	$5.14 \times 10^{-4}M$	$2.57 \times 10^{-3}M$	$5.14 \times 10^{-3}M$
8*	0	0	0	0	0
16	0.50	0.50	0.66	0.38	0.85
	0.45	0.65	0.63	0.35	0.83
	0.39	0.90	0.60	0.45	0.90
	0.52	0.82	0.70	0.42	0.87
	0.48	0.78	0.68	0.37	0.93
24	4.10	2.30	3.42	2.20	3.10
	3.80	3.04	3.32	2.24	3.07
	3.64	2.90	3.38	2.12	2.96
	4.60	2.75	3.76	2.80	2.30
	4.90	2.48	3.42	3.08	2.42
48	48.92	65.00	66.00	47.10	43.40
	50.80	67.20	65.80	48.56	45.02
	50.00	69.20	64.40	54.00	42.80
	49.86	80.00	69.00	53.56	43.60
	49.86	82.40	65.60	50.64	41.80
72	74.40	103.6	119.2	101.2	112.8
	83.20	128.0	117.6	118.0	90.80
	80.60	98.4	120.0	104.0	112.4
	75.00	108.0	117.2	104.8	92.8
	81.60	109.6	120.6	118.4	94.4
	82.59	108.9	118.6	105.2	95.62
	86.72	113.4	115.9	103.4	92.30
	78.24	112.6	114.9	108.2	90.24
	72.58	121.2	119.0	100.3	93.00

8*. In all the experiments in which α -amylase activity was dosed after 8 hours of barley germination, no enzyme activity could be ascertained either in the control lot or in the test lots.

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INFLUENȚA ACIDULUI FERULIC ASUPRA GERMINĂRII LA ORZ: (HORDEUM VULGARE L.) ȘI FORMĂRII α -AMILAZEI

Autorul studiază influența acidului ferulic asupra germinării la *Hordeum vulgare*, urmărind atât aspectul morfologic al semințelor germinate cât și activitatea α -amilazei. După 48 și 72 ore de germinare acidul ferulic în concentrații de $2,57 \times 10^{-3}M$ și $5,14 \times 10^{-3}M$ determină o dezvoltare particulară a radiceii viitoareii plante, care este mult alungită și subțire și cu peri absorbantți mai puțin dezvoltați în comparație cu lotul martor.

După primele 16 ore de germinare activitatea α -amilazei este superioară celei dozate pentru lotul martor numai în cazul soluțiilor $5,14 \times 10^{-5}M$; $5,14 \times 10^{-4}M$ și $5,14 \times 10^{-3}M$. O singură soluție de acid ferulic ($2,57 \times 10^{-3}M$) are o ușoară acțiune inhibitoare. După 24 ore de germinare toate soluțiile de acid ferulic utilizate au o acțiune inhibitoare, în timp ce după 48 ore de germinare numai soluția de $5,14 \times 10^{-3}M$ inhibă formarea și activitatea α -amilazei. În cazul soluțiilor de $5,14 \times 10^{-5}M$; $5,14 \times 10^{-4}M$ activitatea α -amilazei a fost net superioară celei constatate pentru lotul martor.

Toate concentrațiile de acid ferulic utilizate au avut o acțiune stimulatorie asupra activității α -amilazei după 72 ore de germinare.

Rezultatele experimentale sînt discutate în sensul existenței și al formării unor compuși ai acidului ferulic în procesul germinării plantelor de cultură.