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The Variation of ADH and Catalase Activity during the Germination of the Green Pea *(Pisum sativum)*

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Earlier work by different authors *e.g.*[1,2] has shown that during the germination of the pea, great variations in the activity of the enzymes ADH (alcohol dehydrogenase), formic acid dehydrogenase and catalase occur.

The reasons for these variations are obscure. In this paper, the above mentioned variations in enzymatic activity are confirmed. The variations of protein, nonprotein nitrogen, alcohol production and of readily titrated SS and SH groups are followed, and a possible connection between these variations in the cotyledon during the germination is discussed.

METHODS

The experiments were performed with green peas of the trade mark "Fenomen". The peas were placed in water overnight, and then sowed out in a box with soil, and placed in a window. The temperature averaged 15° C. For obtaining samples from the first few days of germination, peas were also germinated between moist filter paper in Petri dishes. Every day, between 3 and 10 seedlings were taken for analysis. After washing and weighing, they were stored at − 15° C until the analyses were performed.

All analyses were made on extracts of the seedlings, either whole or dissected into embryos, cotyledons and seed shells, made by grinding in a mortar with $M/100$ K_2HPO_4. The amount of phosphate solution added, was approximately ten times the fresh weight of the sample. After standing an hour, the suspension was centrifuged for ten minutes at 3 500 r.p.m. in a small angle centrifuge, and the clear, yellowish or greenish solution used for analysis.

Protein in the extract was determined by coagulation by heating after the addition of 1 ml 0.1 N HAc per 5 ml of solution, filtering and careful washing on a small filter paper, drying at 110° C for half an hour, and weighing.

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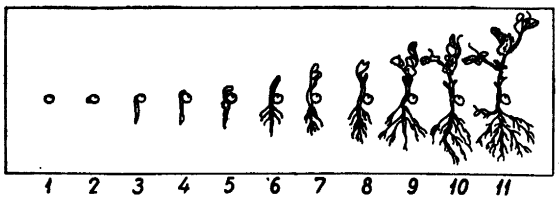

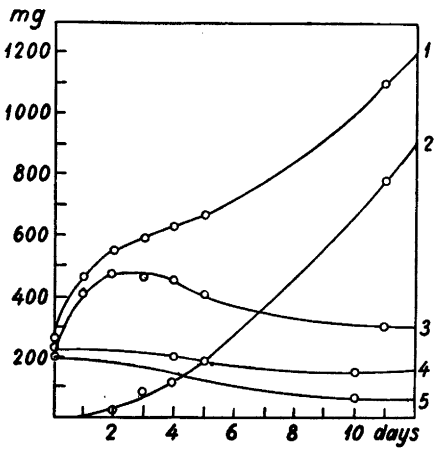

Fig. 1. The development of the pea plant during germination. Curve no. 1, shows total fresh weight, curve 2, the fresh weight of the plants without the cotyledons, curve 3, the fresh weight of the cotyledons, curve 4, the dry weight of the whole plant, and curve 5, the dry weight of the cotyledons.

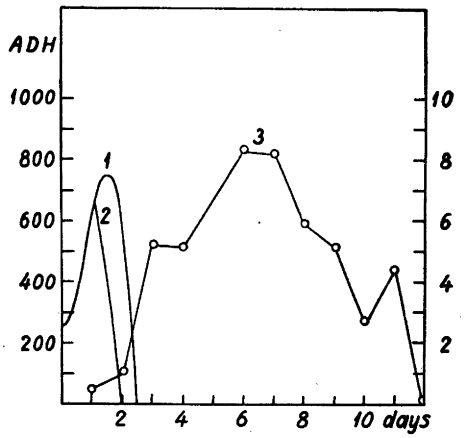

Fig. 2. ADH and catalase activity during germination. Curve no. 1, shows the ADH activity in the cotyledons, units per gram dry matter, curve 2, the ADH activity in the embryos, and curve 3, the catalase activity as Kat.f.

Total N was determined according to Kjeldahl, on 1 ml samples of the solution. Non-protein N was determined as the difference between the total N and the protein N, assuming the N content of the protein to be 17 %.

Total readily titratable SS plus SH groups in the extract were determined amperometrically according to Kolthoff and Stricks [3], and SH amperometrically according to Kolthoff and Stricks [4]. The former method involves a titration with copper salt and the latter an argentometric titration.

The activity of ADH was determined spectrophotometrically with ethanol and DPN at pH 9.7 [5,6], and the alcohol content in the peas enzymatically with ADH and DPN [7]. The catalase activity was determined by titration with permanganate [8].

By means of the results from the analyses of the extracts, the content per gram fresh weight was calculated, and by means of the curves for fresh and dry weight in Fig. 1, the content per gram dry weight found.

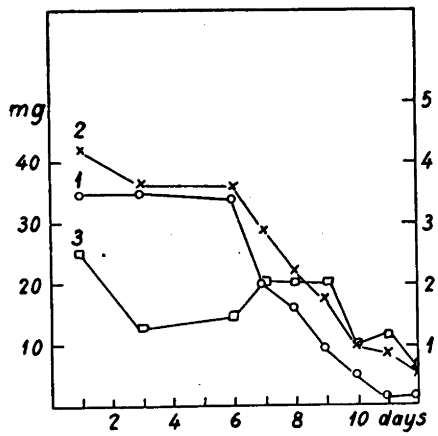

Fig. 3. Nitrogen in the cotyledons during germination. Curve no. 1, the protein content, scale to the left, curve 2, total N scale to the right, and curve 3, nonprotein N, scale to the right. The amounts are given in mg per pea (mean values).

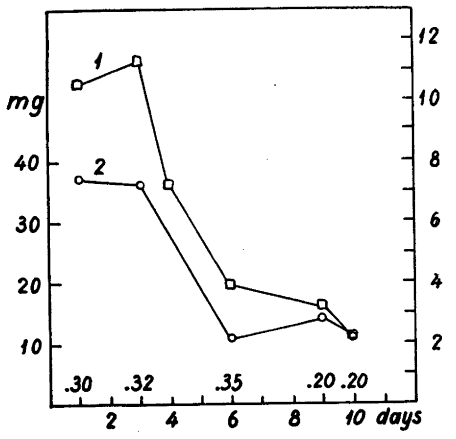

Fig. 4. Readily titratable SS and SH groups in the cotyledons during germination. Curve no. 1, RSH, scale to the right, and curve 2, RSSR, scale to the left. The values are given as µmol per gram dry weight. The figures above the abscissa show the quotient RSH/RSSR.

RESULTS

Fig. 1. shows the physical development of the peas during the experimental period.

On Fig. 2 is seen the ADH activity and the catalase activity in the pea extracts. These results show good conformity with earlier works. It might be mentioned that the ADH activity showed great variability from pea to pea, as is seen in Table 1.

Table 1. The ADH activity in single, whole peas during germination.

At start of germination (dry peas)	1st day		2nd day		3rd day		
Act./pea	Act./g	Act./pea	Act./g	Act./pea	Act./g	Act./pea	Act./g
93	279	135	245	203	335	0	0
108	240	110	205	51	103	0	0
104	207	81	160	17	29		
46	87	106	265	54	87		
47	111	164	315	155	259		

Table 2. The ethanol content in peas, kept for 12 hours in nitrogen at room temperature, at the germination stage indicated. The alcohol content given as pro mille of the fresh weight of the peas.

1st day	2nd day	4th day
0.80	1.96	0
0.74	3.50	0
0.93	2.74	0
	3.22	

The reason for the great differences between individual results may be due to a not quite simultaneous development of the peas. Thus it is quite evident that this must be the reason for the variations in the results from the second day, during which there must be a very rapid fall of ADH activity. The curves given in Fig. 2 are, we believe, the most probable ones for the ADH activity during the germination. We see from these curves also that ADH first disappears from the embryo, and thereafter from the cotyledon. The seed shell does not at any time contain any ADH.

The ability of the pea to produce alcohol under anaerobic conditions is closely correlated with the ADH activity. This is seen from Table 2. The alcohol concentrations given there, were obtained in single peas by keeping them in nitrogen for 12 hours at the development-stage indicated. It should be stressed that the alcohol contents are determined enzymatically, and thus are presumably true values of the ethanol concentration, not obtainable by non-specific methods such as the Widmark technique.

In Fig. 3 are given the curves for the variations in the cotyledons of total N, protein dry weight and nonprotein N.

It seems as if the pea during the first six days of germination, utilizes the non-protein components of the cotyledon. Then, there is a rapid fall in the protein content, together with a slight rise in the non-protein N, showing that after the sixth day the hydrolysis of proteins has started, and that the products of the hydrolysis are being removed from the cotyledon. In this period of germination an increase in the activity of proteases and peptidases should be expected. After the 12th day, the store of nitrogen, soluble in $M/100$ K_2HPO_4 in the cotyledons, is practically depleted.

In Fig. 4, are seen the changes in the titratable RSSR and RSH groups in the cotyledon extracts during the germination. It is seen that both SS and SH groups disappear quickly during the first 6 days after which time the amount

becomes more constant. The ratio SH/SS is slightly decreasing after the sixth day of germination, but not to such an extent that it would mean a significant change in the redox potential of the extract.

DISCUSSION

The connection between the alcohol producing ability of the pea and the ADH activity, seem to leave no doubt that the enzyme ADH is responsible for the alcohol production.

The protein content of the pea keeps nearly constant till the 6th day, so the drop in ADH activity is hardly due to utilisation of protein in the cotyledon. The drop of catalase activity, however, falls together with the utilisation of protein beginning at the 6th day. The fall in the amount of SH groups as shown in Fig. 4 goes together with the disappearance of the ADH and the increase in catalase activity. In this connection it is interesting to notice that the ADH is depending upon SH groups for its activity while catalase is inhibited by SH groups [9,10] and at the same time it slowly oxidizes the SH groups to SS [11,12].

However, it should also be remembered that there is a lack of knowledge of the changes in the metabolism during the germination. A number of biologically active substances investigated, showed marked variations during the germination period. Important in this connection is the finding of Van Herk [13] that the DPN content falls rapidly between the 4th and the 7th day of germination. Changes have also been observed in the ascorbic acid content [14], and of a number of the B vitamins [15]. This might indicate a fundamental change in the metabolic pattern and thus in the enzyme composition of the pea during the germination.

From the 7th day of germination, the variation of the enzymes seems to be dependent upon the disappearance of protein as shown in Fig. 3.

SUMMARY

The variations of the enzymes ADH (alcohol dehydrogenase) and catalase, and of the concentrations of protein, total N and readily titratable SH and SS groups, extractable in $M/100$ K_2HPO_4 are followed during the first 12 days of germination of the green pea.

The ADH activity parallels the decrease in titratable SH groups during the first three days. At the same time the catalase activity is increasing.

The alcohol producing ability of the peas is closely correlated with the ADH activity. The significance of these findings is discussed.

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