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PEROXYDASE CONTENT OF DWARF TYPES AND GIANT TYPES OF PLANTS

BY

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1. INTRODUCTION

Several publications report an abnormal content of the enzyme peroxydase in dwarf types and giant types of plants. In the former case the content is said to be greatly increased, in the latter greatly decreased. In most cases however, the quantity of the enzyme was only approximately estimated.

As the Genetic Laboratory of the university at Groningen had *Phaseolus* dwarf plants available it seemed desirable to determine the enzyme content in a more quantitative way. The researches took place at the Botanical Laboratories at Groningen and Leiden.

In 1935 VAN OVERBEEK published a study on the dwarflike growth of maize seedlings. The dwarfs differed from the normals in that the mescotyl remained short. The coleoptile was in both cases of the same length. His material was exceedingly suitable for a comparative physiological study because he worked with samples of seedlings which produced 50 % normals and 50 % dwarfs. This was due to the fact that the dwarf property was based on one genetically recessive factor. The research led van Overbeek to conclude that an equal quantity of growth substance was formed in either race, but that in the case of the dwarfs more destruction took place during the transport in basal direction. The cell elongation in the mesocotyl was thus differently influenced. Besides, van Overbeek found a higher catalase and peroxydase content in dwarfs than in normals. The author suggested that these enzymes were responsible for the inactivation of the growth substance, the latter being oxidized.

The reverse, i.e. giant growth, has been described and examined closer by DE HAAN and GORTER (1936). From *Pisum* crosses a so-called slender pea could be grown which was more than three metres tall. The genetic condition for this was that a certain two recessive factors should be homozygously present. So here too the phenomenon was genetically determined. Researches as to the catalase activity showed that it was subnormal in the slender pea. Moreover they thought it probable that less growth substance was inactivated in the slender pea than in normal types. Like van Overbeek they did not prove that the inactivation was caused by the oxidizing enzymes.

Dwarf types of *Epilobium hirsutum* L. have been described and

examined e.g. by MICHAËLIS (1943). Beside dwarf types there were examples of growth stimulation to be found in the *Epilobium* crosses which may be ranged under the genetic phenomenon of heterosis. So the symptoms were also genetically controlled, though here it was a question of so-called plasmatic heredity. The physiological behaviour of these plants has been investigated by Ross (1941, 1942, 1948). His work clearly appears to have been inspired by van Overbeek's train of thought. In his investigations correlation was found between the inhibition or stimulation of growth and the peroxydase content. In plants with inhibited growth the peroxydase content was higher, in plants with stimulated growth it was lower. From the research it has not become clear whether there was a causal relation between peroxydase content and rate of growth.

The lack of a bacterium symbiont in *Ardisia crispa* (Thunb.) A.DC. results in a cripple of the same plant. This has been described in detail by DE JONGH in 1938. He showed that, very probably, the catalase content is higher in the cripple than in the normal plant and that peroxydase is only demonstrable in the dwarf.

2. METHOD OF DETERMINATION OF PEROXYDASE

To determine the peroxydase content the method of DERX (1942 *a* and *b*) has been used. His method is based on the oxydation of the colourless o-tolidine, which has a blue reaction product. Adding an extract containing the enzyme peroxydase to a mixture of solutions of o-tolidine and hydrogen peroxide the blue oxidation product of o-tolidine will appear. If to this solution ascorbic acid is added the blue reaction product will be reduced to the colourless substance, which will remain colourless until the whole of the ascorbic acid will have been oxidized. Then the solution will suddenly turn blue. The time necessary for the appearance of the blue colour is, with a certain quantity of ascorbic acid, inversely proportional to the activity of the peroxydase enzyme. So the method is chronometrical.

Standardizing the conditions DERX has used this reaction for a quantitative peroxydase test. To start the reaction we always added a temperature-conditioned mixture of 1 ml H_2O_2 (0.1 n), 0.5 ml o-tolidine (0.05 %) and 22.5 ml citrate-buffer solution to a mixture of 0.5 ml ascorbic acid (0.01 n), 1 ml enzyme solution and 4.5 ml buffer solution. According to the activity of the enzyme solution the quantity of the ascorbic acid solution and the enzyme solution may be varied, provided that the total volume remains 30 ml. From the time the blue colour appears one may calculate the quantity of the enzyme solution necessary for reducing one millimol H_2O_2 in one minute. This unit Derx calls "Unité Normale de Peroxydase" (U.N.P.). As in the preparation of the enzyme solution a definite quantity of the leaves is used, it is possible to calculate the enzyme activity on the fresh weight of the plant material.

The standardizing conditions for the Derx reaction were: 1. limitation to no more than 5 % conversion of the added H_2O_2. 2. ph 5.

3. temperature 25° C. 4. certain definite concentrations of H_2O_2 and o-tolidine.

Derx's method has considerable advantages over other methods of peroxydase determination described. An important fact is that his method of enzyme preparation includes a purification as regards to the reducing substances in the plant extract. The enzyme is precipitated by aethyl alcohol (96 %) and the residue is dissolved in a buffer solution.

Derx took 10 to 20 gram of plant material for his enzyme preparation. For our own use we often took no more than half a gram of plant material. The quantitative preparation of the residue thus demands more care, but it can very well be done with the use of small filter cups (Schott 63 G 3) 2 centimetres in diameter. There is no reason why in this way the determination should be less accurate.

In all cases the enzyme content was calculated per gram fresh weight of the plant material. Young leaves were always used for the enzyme preparations.

3. Results

Tropaeolum majus L.

Of this species there exist varieties which remain low near the ground and which do not climb. Comparing them with the normal climbing type their habitus strikes us as being stunted in growth and their leaves remain noticeably smaller. The small type being traded under the name of "nana compactum", "Tom Pouce", etc. Though the differences between these varieties are small the plants were used to test the methodology of the peroxydase determination. The results were the following.

variety	U.N.P./gram fresh weight
climbing type	0.015 ± 0.0006 (n = 12)
stunted type	0.023 ± 0.002 (n = 6)

Thus the peroxydase content of the stunted type was greater.

Phaseolus crosses

Geerts (1949), in his thesis, describes *Phaseolus* crosses in which in the F_1-generation giant types occurred besides dwarfs. The dwarf types were exceedingly small. Their habitus was badly stunted, their leaves were small and in many cases the growth of leaf-vein and -tissue was irregular. In the giant type the leaf was extraordinarily large. The phenomenon is not yet completely genetically explained, but possibly a plasmatic heredity plays a role here.

Table 1 gives the peroxydase contents found by the present author in the leaves of various types that occur in these cross-breedings. First of all the enzyme content of the two parents is given. Next that of types that have grown bigger than the parents and lastly of dwarf

types in the F_1- and F_2-generation. The F_2-generation also contained semi-dwarfs.

Table 1 clearly shows the extraordinarily high peroxydase content in the leaves of the dwarf types. The enzyme content of the semi dwarfs lies, in spite of their climbing type, in between normals and dwarfs, whereas in giant types (except for number 3192) the content

TABLE 1

Peroxydase content of *Phaseolus* plants; explanation: see text

		mean value U.N.P./gram $\times 10^{-3}$	stem length cm	number extractions
Phaseolus vulgaris P_1	302	70	40	5
Phaseolus multiflorus P_2	308	113	50	3
F_1 giant type	3191	36	65	9
F_1 giant type	3192	128	65	12
F_1 giant type	316	33	60	1
F_1 dwarf	3195	2400	25	3
F_1 dwarf	3199	3200	25	3
F_2 dwarf	32867	630	30	1
F_2 semi-dwarf	32607	130	200*	2
F_2 semi-dwarf	32926	260	170*	3

* Semi-dwarfs stand quite apart as to type. They are thinly built, climbing plants, but their leafs show the habitus of the dwarf type.

is below the normal. It looks as if the peroxydase content might be taken as a measure for the degree of dwarfing of the plant.

With such enormous differences occuring in the enzyme activity one wonders whether it is always the same enzyme one is dealing with. Therefore it was investigated whether in extracts of peroxydase from cripples the optimum of the Derx reaction occurred with the same hydrogen peroxide concentration and the same acidity as in extracts of peroxydase from normal plants. With the o-tolidine concentration of 8.3×10^{-3} per cent in the reaction mixture, in both cases the optimum hydrogen peroxide concentration was about 2.5×10^{-3} mol. (Compare with Derx's data). To determine the optimum acidity of the enzyme reaction a series of buffer solutions according to Mc Ilvaine was used. In both cases the optimum lay at pH 5.2 and 5.3. So it seems likely that in these cases the enzymes are the same.

Crosses of Nicotiana Tabacum L.

In 1944 I received seeds of tobacco crosses from the late professor J. A. Honing from Wageningen. For the genetical background of this material the reader is referred to Honing (1939). Some peroxydase extracts have been made of leaves from two types of the *Kloempang* dwarf race, one type being greater than the other in height as well as in size of the leaves. These two types of *Kloempang* dwarf race grow by heterozygosity from one and the same sowing. Apart from these, some determinations were carried out in extracts from the leaves of a necrotic tobacco dwarf, the plants of which remained exceedingly small. Table 2 gives a survey of the results.

TABLE 2

Peroxydase content in several tobacco plants; explanation: see text

	mean value U.N.P./gram $\times 10^{-3}$	stem length cm	number extractions
Kloempang dwarf race 1561–120			
tall type	9	120	2
small type	12	40	2
necrotic dwarf	100	5	8

Though these observations were few, a connection between the peroxydase content and the degree of dwarfing is evident.

Zea Mays L.

In 1944 a number of dwarf maize cobs happened to be available in the Botanical Laboratory at Leiden, received by prof. L. G. M. Baas Becking in 1938 from J. van Overbeek. Due to the age of the material the maize grains had little germinating capacity. One cob was an exception to the rule however, and after sowing produced sound maize seedlings, 50 % normals and 50 % dwarfs. Table 3 gives the results of peroxydase determinations of the mesocotyl, the coleoptile and the primary leaf of these plants.

TABLE 3

Peroxydase content of the mesocotyl, coleoptile, and primary leaf of maize seedlings.

	U.N.P./gram fresh weight $\times 10^{-3}$ normals	U.N.P./gram fresh weight $\times 10^{-3}$ dwarfs	ratio U.N.P. dwarf/normal
mesocotyl	32	49	1.5/1
coleoptile	31	53	1.7/1
pr. leaf	27	28	1.0/1

For one peroxydase extract 12 to 14 coleoptile or mesocotyl pieces or primary leaves have been used. Though determinations could be made only with one plant series—which makes the conclusions somewhat doubtful—the result confirms van Overbeek's informations. VAN OVERBEEK (1935) found a proportion of the catalase content of the dwarf to that of the normal of 1.5 and 1.9 to 1. The proportion between the peroxydase contents in Table 3 is in accordance with it.

If we calculate the enzyme content on the basis of dry weight, the differences between dwarfs and normals become even greater. The differences remain, even when the enzyme content is calculated on the basis of the water content of the tissues.

4. DISCUSSION

As far as the small numbers of experiments allow a conclusion it has been shown that the peroxydase content of the leaves is in all cases correlated with the degree of dwarfing of the plant. The same

was the case with the tendency to form giant types. Determinations with coleoptile and mesocotyl pieces of dwarf maize show the same picture. In dwarfs of *Phaseolus* cross-breedings the peroxydase content was extremely high. It would seem useful to compare these figures with the content of this enzyme in other plants.

DERX (1942*a*) as well as WILLSTÄTTER (1928) give an account of the enzyme content in various plants. Among them *Cochlearia armoracia* L. has the highest content (U.N.P./gr 0.68 fresh and 2.63 dry). Though the methods of determination of the two authors differ, a comparision is very well possible because both state the enzyme content of *Cochlearia*. In both cases it can be expressed in millimol H_2O_2 converted per minute and per gram dry weight. Derx: 2.6 millimol H_2O_2/minute/gr. dry weight. Willstätter: 2.2 millimol H_2O_2/minute/gr. dry weight. These two figures are well in accordance with each other, considering that Derx worked with a reaction temperature of 25° C. and Willstätter at 20° C.

So, in comparison with the enzyme content in other plants the peroxydase content in *Phaseolus* dwarfs is shown to be extraordinarily high.

An open question is what significance should be attached to a higher or lower peroxydase content. According to Ross a high content is a symptom of an all-round increase of dissimilative metabolism of the plant. It should be examined whether and to what extent an increase or decrease of the enzyme content affects other enzymes than the peroxydase. Furthermore the question remains whether the peroxydase content is due to or is the cause of the dwarf growth. It is very well possible that the effect is a secundary one.

As remarked before, determinations of the pH-optimum and the optimum of the hydrogen peroxide concentration showed that in bean dwarfs very likely the same agent was isolated as in normal bean plants.

Because in some cases, as mentioned above, the giant growth may be considered as a heterosis phenomenon, there is a possibility that by means of the peroxydase test data will become available concerning the physiological aspect of the problem of heterosis.

SUMMARY

The information given in the literature concerning the peroxydase content of dwarf plants and giant plants has been quantitatively tested in various types of plants. The results were consistent with the conception that dwarf plants have a peroxydase content which is higher than normal and that giant types give exactly the reverse picture. In *Phaseolus* dwarfs an exceedingly high peroxydase content was found.

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