

LVII.—*Presence of Invertase in some Plants of the Gramineæ. Part I.*

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IN 1890) *Trans. Laboratory Club*, 3, 5), I drew attention to the presence of invertase in the rootlets and plumules of germinated barley; the object of the present paper is to prove that this enzyme is common to other and more mature plants of the *Gramineæ*.

The difficulty of extracting enzymes from the living cells of plants is now well known: even healthy yeast, as I have shown (*Trans.*, 1892, 61, 593), does not give up its invertase to water. The extraction of invertase from the plants under consideration is not necessary, however, if it is proved satisfactorily that there must be a substance within the cells of these plants which has the power of hydrolysing cane-sugar.

Extraction of Invertase from Maize and Oat Plants.

I have succeeded in preparing from maize and oats a small quantity of a substance which had an undoubted, although slight, hydrolytic action on cane-sugar. The method employed was as follows. Three healthy maize plants, grown in the open and about 12 inches high, were carefully uprooted and the roots cut off. The leaves and culms were cut up into small pieces, which were then well washed with recently distilled water containing chloroform. The pieces were ground with silver sand, left in contact with chloroform water for 24 hours, and then filtered. Alcohol was added to the filtrate until no further precipitation occurred, the precipitate being then collected on a filter and dried over sulphuric acid in a vacuum; only 0.135 gram of substance was isolated. In like manner, from eight strong oat plants, I obtained only 0.04 gram of substance.

Two small test-tubes, each containing 10 c.c. of a 5 per cent. cane-sugar solution, were plugged with cotton wool, sterilised, and cooled. A drop of chloroform and the whole of the substance from maize was put into one of the tubes, *a*, and into the other, *b*, the same amount of chloroform and the substance from oats. After remaining for 4 days in the cold, the solutions were carefully neutralised with potassium hydroxide and boiled for some time. The cupric oxide reducing power, *K*, of the solutions was determined gravimetrically, and the amount of cane-sugar hydrolysed calculated on the weight of substance used,* was, for *a*, 44 per cent., and *b*, 50 per cent.

Existence of Invertase in Roots and Leaves of Oats, Maize, and Wheat.

Oats.—Some oat grains were steeped in water for 48 hours, and then grown in moist sand. On the seventh day of growth, the roots and plumules were removed from 20 of the germinated grains. The roots weighed 0.25 gram, and the plumules 0.47 gram. These were washed with chloroform water, dried between filter paper, and added each to 10 c.c. of a 5 per cent. solution of cane-sugar saturated with chloroform.† After remaining for 18 hours at 15–18°, they were then removed by filtration, and washed until the filtrate and washings amounted to about 50 c.c. After neutralising, as above mentioned, the solutions were briskly boiled for some time, and the value of *K* for each determined, with the following results, expressed as before:

Roots = 33.1 per cent.; plumules = 14.7 per cent.

* All the expressions for the invertase are calculated throughout this paper in this manner.

† The cane-sugar solution throughout this paper means a 5 per cent. solution saturated with chloroform.

The residual roots and plumules were immediately immersed in the same quantity of cane-sugar solution, and treated in every way as just described. The results obtained in this second treatment were :

Roots = 10·7 per cent. ; plumules = 9·3 per cent.

When the oat seedlings had grown for some time, the leaves being about 5 inches long and the endosperm consumed, some of the leaves were cut off at their junction with the leaf-sheath. These weighed 0·831 gram ; they were divided equally by weight, and the two portions washed several times with chloroform water, and finally dried by pressure between filter paper. One part, *a*, was added to 10 c.c. of a water solution of chloroform, and the other part, *b*, to 10 c.c. of the sugar solution. After remaining for 18 hours at 15—18°, these were treated as already described, and the results obtained, expressed as before, were :

a = none ; *b* = 24 per cent.

The leaves used in *a* and in *b* were then immersed separately in 10 c.c. of the sugar solution, and, after remaining for 18 hours at 15—18° were treated as before, with the results :

a = 34 per cent. ; *b* = 16 per cent.

Maize.—Two maize plants, with the endosperm still attached, which were growing in the open, were carefully uprooted. The plumules and roots were cut off at their junction with the seed. The former weighed 0·43 gram, and the latter 0·24 gram. These were treated in every particular as in the case of the oat plant, and the results were :

Plumules = 16·7 per cent. ; roots = 14·9 per cent.

They were immediately immersed in the same quantity of the cane-sugar solution, digested under the same conditions, and treated in every way as in the last instance. The results obtained in this second treatment were :

Plumules = 10·0 per cent. ; roots = 22·0 per cent.

Two maize plants like those just described were similarly divided at the time. The plumules weighed 0·486 gram, and the roots 0·096 gram. These were each immersed in 10 c.c. of chloroform water, and at the end of 18 hours the extracts had no cupric reducing power. The plumules and roots from these experiments were each added to 10 c.c. of the sugar solution and digested as before at 15—18° for 18 hours, with the following results :

Plumules = 13·0 per cent. ; roots = 20·0 per cent.

Wheat.—Two quantities, each weighing 0.38 gram, of the seedling leaves of wheat were taken. To one, *a*, 10 c.c. of chloroform water were added, and to the other, *b*, 10 c.c. of the sugar solution. After 18 hours at 15–18°, the results obtained were :

$$a = \text{none} ; b = 52 \text{ per cent.}$$

The leaves from *b* were immediately immersed in the same quantity of the sugar solution, and the result obtained, expressed as before, was 54 per cent.

A quantity of the portion of wheat seedlings above ground was collected during a period of snow and severe frost, and divided into four parts of 1.3 grams each. After washing with chloroform water and drying as already mentioned, three of the parts, *a*, *b*, and *c*, were each added to 15 c.c. of the cane-sugar solution, and the fourth, *d*, to the same volume of chloroform water ; all were digested for 24 hours in the cold ; the results obtained were :

$$a = 8.9 ; b = 10.0 ; c = 10.9 ; d = 0.35 \text{ per cent.}$$

In order to compare with *a*, *b*, and *c*, the result obtained in *d* is expressed in terms of cane-sugar hydrolysed, as if cane-sugar had been present.

Four days after the last series of experiments were made, the weather having in no way improved, another quantity of the wheat plants was collected and divided into two parts of 0.354 gram each. One part, *a*, was treated as in the last series of experiments, and the second, *b*, as *a*, only it was digested at 49–50°. The results were :

a, 24 hours at laboratory temperature, 8.1 per cent.

b, 3 hours at 49–50° 6.3 „

The residue from *b* was again treated as before for 3 hours and gave 4.7 per cent.

This residue was treated in the same way for 16 hours, and gave 29.4 per cent.

In order to test how much of the cane-sugar, in the presence of the chloroform, would be hydrolysed at a temperature of 50°, the same quantity of the cane-sugar solution as used in the last series of experiments was digested for 24 hours at this temperature, and, calculated from the value of *K* obtained, the cane-sugar hydrolysed amounted only to 0.002 gram.

The total quantity of sugar, calculated as dextrose, in some of the same wheat leaves as used in the last series of experiments was determined, and amounted to 0.72 per cent.

Hydrolysis of Cane-sugar not due to Organisms attached to the portions of Plants employed.

In 1890, I concluded that organisms were not the cause of the inversion of the cane-sugar under the conditions of my experiments, and further proof of this view may now be given.

Some wheat plants were collected under the conditions already mentioned, and of these 4 grams were washed several times with small quantities of chloroform water until the total washings amounted to 250 c.c. To these washings, which were turbid, a little aluminium hydrate was added, and after well stirring, the whole was put aside in a tall cylinder for 24 hours; the supernatant liquid was then poured off and the deposit, *e*, retained. Microscopically, no organism could be detected in this deposit.

Seven of the same plants of equal size were chosen and the leaves cut from the culm of each were equally divided numerically into two portions, *a* and *b*, weighing respectively 0.29 and 0.301 gram. The seven culms, *c*, weighed 0.138 gram. The leaves, *a* and *b* separately, were washed with chloroform water, in small quantities at a time, until the washings in each case amounted to 500 c.c. To the 1000 c.c. of washings thus obtained, some Swedish filter-paper pulp was added, and after stirring well, the whole was filtered through a small filter paper, which, with its pulp contents, may be designated *d*.

The deposit, *e*, the leaves, *a* and *b*, the culms, *c*, and the filter paper, *d*, with its contents were each added to 15 c.c. of the sugar solution and digested for 6 hours at 49—50°. The results as expressed before were:

$$a = 16.5 ; b = 12.0 ; c = 13.1 ; d = 0.06 ; e = 0.05 \text{ per cent.}$$

The results obtained in *d* and *e* are given in terms of cane-sugar hydrolysed, calculated on the quantity of leaves which was washed.

Existence of Invertase in the Roots and Leaves of a nearly mature Maize Plant.

A maize plant about 16 inches in height and upon which the reproductive organs had not as yet appeared, was carefully uprooted in June, 1893. The weight of the leaves and culm was 8.26 grams, and that of the roots 0.841 gram.

The roots were divided into two equal parts, *a* and *b*, of 0.42 gram; each part was well washed with chloroform water, dried by pressure between filter paper, and added to 25 c.c. of cane-sugar solution. After remaining for 18 hours in the cold, the extent of hydrolysis was:

$$a = 26 \text{ per cent. ; } b = 24 \text{ per cent.}$$

These roots were then added each to 10 c.c. of cane-sugar solution, and after remaining for 18 hours the results were :

$$a = 38 \text{ per cent. ; } b = 35 \text{ per cent.}$$

The leaves of the plant were divided by cutting lengthways through the midrib, and from the halves thus made strips were cut at right angles to the midrib. Three equal portions of 2.02 grams were taken, and to one, *a*, 25 c.c. of a 5 per cent. cane-sugar solution and 0.1 c.c. of chloroform were added, to another, *b*, a similar quantity of sugar solution, and to the third, *c*, the same volume of chloroform water.

After remaining 18 hours in the cold, the numbers, calculated as before, were :

$$a = 10 \text{ per cent. ; } b = 5 \text{ per cent. ; } c = 0.94 \text{ per cent.}$$

These leaves were treated as before and the results obtained were :

$$a = 10.7 \text{ per cent. ; } b = 7.1 \text{ per cent. ; } c = \text{nil.}$$

A leaf was selected on an almost mature maize plant and a section of the midrib weighing 0.631 gram was cut out lengthways and divided into pieces which were washed with a small quantity of chloroform water and then dried as in the experiments mentioned. After remaining 48 hours in the cold with 10 c.c. of a 10 per cent. cane-sugar solution, the amount of sugar hydrolysed was 12.1 per cent.

A similar portion of the midrib of the same leaf, which was still attached to the growing plant, was cut out two days later. This weighed 0.295 gram, and, treated as in the last instance, the result indicated hydrolysis to the extent of 7.5 per cent.

To similar quantities of cane-sugar solution, the washings from the portions of midrib used in these experiments were separately added, and after digesting for the same time, the solutions were found to have no cupric oxide reducing power.
