XLIX. THE UREASE CONTENT OF CERTAIN INDIAN SEEDS.

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During preliminary work on the nitrogenous constituents of certain Indian seeds a number were tested for urease.

Owing to the fact that they contain urease, soy beans have been utilised by Marshall [1913, 1, 2), and by Plimmer and Skelton [1914] for the estimation of urea in urine. In the present work it was thought worth while to test certain well defined varieties of soy-beans in order to see if there were any large variation in their urease activity. During the course of the work a seed was found whose urease activity was many times greater than that of soy-bean and this short paper has chiefly been written to bring this fact to notice, since it should be very useful in the estimation of urea.

Zemplén [1912] has tested the seeds of many plants for urease. He found the enzyme in most papilionaceous but not in graminaceous ones.

In the work here described the following preliminary test for urease was applied to the seeds. They were finely powdered in a Maercker mill and about 0.5 g. was then placed in a test-tube containing 10 cc. of 1 per cent. urea solution with 3 drops of phenolphthalein and a few drops of toluene. Each tube was left 24 hours. If urease be present in the seed, ammonium carbonate should be formed and shew its presence by the reddening of the liquid.

By this method the following seeds appeared to contain urease :

Soy bean yellow, Soy bean spotted, Soy bean chocolate, Soy bean common, Soy bean black, *Sida spinosa*, Gram (*Cicer arietinum*), Rice (*Oryza sativa*), Cowa (*Setaria italica*), Sword bean (*Canavalia ensiformis*), (both a sample from U.S.A. and a sample grown at Dacca), *Urana lobata*, Khulti kalai (*Dolichos biflora*), Castor bean (*Ricinus communis*).

The method indicated the absence of urease in the following seeds : French beans (*Phaseolus vulgaris*), Velvet beans (*Mucuna pruriens*), Sona mung (*Phaseolus radiatus*), *Sida* sp. (from Burmah), *Sida cordifolia*, White til (*Sesamum indicum*), Black til (*Sesamum indicum*), Mati kalai (*Phaseolus mungo*), Cow pea (*Vigna catiang*), White mustard (*Brassica campestris* var. *sarson*), Red mustard (*Brassica campestris* var. *dichotoma*), Niger (*Guizotia abyssinica*), Taramira (*Eruca sativa*), Sweet pea (*Lathyrus sativus*).

The above test is by no means a reliable one, however, as it was found later (see below) that certain seeds which by the above test appeared to contain urease actually contained none at all, e.g., cowa and rice. The shell of castor bean is strongly alkaline and hence itself colours the test solution red.

A number of the above seeds were next taken for determination of their comparative urease activity.

The following method was used :

10 g. of the powdered seed were treated with 100 cc. of distilled water and allowed to stand with occasional shaking for one hour at room temperature in presence of toluene. 2 cc. of this extract were then added to 50 cc. of a 1 per cent. urea solution together with 0.5 cc. of toluene. 5 cc. of the liquid were then immediately removed and titrated, using methylorange as indicator, with N/10 HCl. Thence onward, generally at half hour intervals, successive portions of 5 cc. were titrated. The tests were carried on at room temperature, about 27°.

We thus have a small amount of enzyme acting on a large amount of substrate and therefore the method is a fairly accurate comparative test of the urease activity. The tables shew that there are considerable differences in the urease activity of different seeds.

The following were in this way found to contain no trace of urease : *Sida spinosa*, Rice (*Oryza sativa*), Cowa (*Setaria italica*), Peas (*Pisum arvense*), Mati kalai (*Phaseolus mungo*), Gram (*Cicer arietinum*), Sona mung (*Phaseolus radiatus*), Cow pea (*Vigna catiang*), Velvet beans (*Mucuna pruriens*).

A number of pure line types of Mati kalai (*Phaseolus mungo*) grown by the Economic Botanist to the Government of Bengal, besides certain well-defined local varieties, were tested. Not one was found to contain a trace of urease.

It might be here mentioned that during the course of this work a ground sample of Khulti kalai (*Dolichos biflora*) which is seen in the table to contain urease was tested two weeks later and gave no indication whatever of its

cc. N/10 HCl required.

Time in hours	Soya beans				Sword beans			Khuti kalai (<i>Dolichos</i> <i>biflora</i>)	Urena Lobata	Urena, Lobata, another sample
	Yellow	Common	Chocolate	Spotted	Black	Rymbasa Ktang	U.S.A. Dacca			
initial	0.4	0.5	0.5	0.8	0.5	0.5	1.2	0.2	0.2	0.4
0.5	1.8	2.2	2.0	2.9	1.5	1.4	8.7	2.7	1.9	1.7
1	3.6	3.5	3.0	4.2	3.0	2.7	13.0	3.2	4.0	3.9
1.5	4.6	4.4	4.6	5.6	4.4	3.7	15.7	3.7	5.4	5.2
2	5.4	5.4	5.0	6.7	5.2	4.1	15.9	4.2	—	6.8
2.5	—	—	—	—	—	—	—	—	—	7.9
3.5	—	—	—	—	—	—	—	—	9.1	—
4	—	8.5	7.7	10.3	7.9	7.7	16.0	—	—	—
5	—	9.7	8.9	12.2	9.4	8.5	—	6.2	—	—
6	—	11.5	10.0	13.6	9.4	9.2	16.0	7.2	—	—
7.5	—	—	—	—	—	—	—	—	—	—
14	15.9	16.5	16.7	16.5	16.4	16.1	—	—	10.4	—
15	—	—	—	—	—	—	—	—	15.8	—
24	—	—	—	—	—	—	—	—	15.8	—
26	—	—	—	—	—	—	—	—	—	13.3
50	—	—	—	—	—	—	—	—	—	14.3
	—	—	—	—	—	—	—	—	—	15.6

presence. Zemplèn [1912, p. 233] on the contrary found that powdered seed kept in a vacuum desiccator over sulphuric acid for six weeks was just as active as the fresh material. The powdered seed kept by me, however, was simply corked up in a test tube.

SUMMARY.

(1) The urease activity of Sword bean (*Canavalia ensiformis*) is many times greater than that of any of the Soy bean varieties which were tested. This is the case with both American and Indian seed. The table shows that an extremely rapid production of ammonium carbonate takes place immediately on adding sword bean extract to urea solution.

(2) The urease activity of five of the six varieties of soya bean examined is practically identical. The sixth variety is distinctly more active.

REFERENCES.

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