CONTRIBUTIONS TO THE PHYSIOLOGY OF SANDAL

PART II. Influence of host on the nitrogen metabolism of sandal.

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The study of nitrogen metabolism of plants is of very great importance in view of its intimate relationship with the fundamental activities of life such as growth and reproduction. The metabolic changes proceed in the different parts of the plant but those occurring in the leaf are of special interest since it is the seat of protein synthesis and other related activities. Sandal being a root parasite, the changes taking place in its system would throw light on the nature of the nitrogenous constituents which the plant is capable of synthesising and the extent to which it depends on the host for elaborated food. Since the haustorial connections of sandal die out with the onset of spike-disease, it appeared probable that the conditions in diseased sandal would at least partly correspond to those in sandal when it is deprived of its host. In view of the above and the observation of Sreenivasaya that association with certain plants render sandal susceptible to spike while that with others imparts resistance to artificial infection of the disease, it was considered that the study of nitrogen metabolism of sandal, with and without hosts, would lead to findings of some practical importance.

EXPERIMENTAL.

The experimental plants were raised and the leaf materials prepared in the manner described in the previous part (*Jour. Indian Inst. Sci.*, 1933, **16A**).

Methods.—Total nitrogen was determined according to Gunning's modification of Kjeldahl's method to include nitrates. Protein nitrogen was estimated by the Stutzer's method (*J. Land.*, 1880, **28**, 103). Nitrates were estimated according to the method of Scales (*J. Biol. Chem.*, 1916, **27**, 327) and nitrite by colorimetric method using *Griess* Ilosway reagent for the purpose. Ammonia was estimated according to Grafe and Erich (*Zeit. Physiol. Chem.*, 1906, **48**, 300). Distribution of water-soluble nitrogen was determined by the methods of Jodidi, Kellog and True (*Jour. Agric. Res.*, 1918, **15**, 385) and amino nitrogen by the Van Slyke method (*Jour. Biol. Chem.*, 1911, **9**, 185; *ibid.*, 1912, **12**, 275). The details of the methods as adopted for different determinations are similar to those described by Narasimhamurthy and Sreenivasaya in their investigation on the distribution of nitrogen in healthy and spiked sandal (*Jour. Indian Inst. Sci.*, 1929, **12A**, 153).

As mentioned in a previous paper it was observed that sandal in association with the host (*Acacia farnesiana*) had a higher percentage of nitrogen (2.69 to 2.84) than the one without the host (2.03 to 2.19). Nitrite could not be detected in the extracts from sandal growing in association with the hosts while it was present only in traces in the control set of plants. Quantitative determination of nitrites was not therefore carried out.

The other results have been presented in the following tables :—

TABLE I

Distribution of nitrogen in the leaves of sandal with and without host plant.

Form of Nitrogen	PERCENTAGES ON MOISTURE-FREE BASIS			
	Set I		Set II	
	With host	Without host	With host	Without host
Total nitrogen ...	2·83	2·03	2·84	2·05
Protein ,, ...	2·07	0·86	2·08	0·86
Nitrate ,, ...	0·07	0·06	0·07	0·06
Total water-soluble N ...	0·68	1·19	0·77	1·20
Ammonia N ...	0·01	0·04	0·01	0·05
Amide ,, ...	0·08	0·01	0·06	0·01
Humin ,, ...	0·09	0·20	0·12	0·20
Basic ,, ...	0·14	0·46	0·19	0·44
Mono-basic ,, ...	0·19	0·21	0·19	0·21
Non-basic ,, ...	0·13	0·25	0·18	0·25
Peptide ,, ...	0·004	0·00	0·00	0·00

TABLE II

Distribution of the water-soluble nitrogen in the leaves of sandal with and without host plants

Material	Percentage of HCl used	Time of digestion in hours	PERCENTAGE OF THE TOTAL WATER-SOLUBLE NITROGEN						
			Ammonia	Amide	Humin	Basic	Mono-amino	Residual	Peptide
With host	4	½	1·89	11·76	13·60	20·29	28·11	18·94	...
Do.	20	8	1·89	11·76	13·60	...	30·01	17·04	0·63
Without host ...	4	½	3·75	0·50	13·49	38·87	17·72	21·16	...
Do.	20	8	3·75	0·50	13·49	...	21·30	17·58	...
With host	4	½	1·67	7·91	16·20	24·06	24·02	22·94	...
Do.	20	8	1·67	7·91	16·99	...	26·04	20·92	0·49
Without host ...	4	½	3·73	0·56	16·63	36·90	17·61	21·01	...
Do. ...	20	8	3·73	0·56	16·63	...	20·30	18·30	...

TABLE III

Percentages of nitrogen in the leaves of sandal occurring in association with different hosts

Host	Nitrogen	Host	Nitrogen
Lantana camara ...	1·29	*Murraya koenigii* ...	2·77
Divi Divi	2·17	*Dendrocalamus strictue* ...	1·58
Casuarina aquisetifolia ...	2·91	*Pongamia glabra* ...	3·23
Melia indica	2·78	*Acacia farnesiana* ...	4·22
Rutea graveolens	2·79		

TABLE IV

Form of Nitrogen	RESULTS EXPRESSED AS PER CENT. OF TOTAL N.			
	With host		Without host	
Total basic nitrogen ...	5·26		22·87	
Amino N.	2·45		8·64	
Non-amino N. ...	2·81		14·23	
Arginine N. ...		3·38		17·07
Histidine N.		0·49		2·14
Cystine N.		0·41		0·42
Lysine N.		1·01		3·23
Nitrogen in filtrate from bases	13·40		23·06	
Amino N.		8·88		12·50
Non-amino		4·52		10·56

It may be seen from the above that the nitrogen content of sandal is determined by the nature of the host with which it is associated. Thus, the plants growing in association with the two leguminous hosts, *Pongamia glabra* and *Acacia farnesiana*, have much higher nitrogen contents than those with other hosts. The results, together with pot culture observations, showed that the supply of suitable hosts particularly those of leguminous type is necessary to promote the rapid vegetative growth of sandal. Sandal without hosts leads a struggling existence and since the improved growth observation in association with a host is accompanied by increase in nitrogen content, it would appear that the supply of nitrogen by the host plant is one of the limiting factors in the growth of sandal, at any rate, in the early stages. The leaves of sandal without host turn yellow and the plants make no appreciable growth but if the host plant is provided, preferably one of leguminous type, the natural green colour is restored and the plant makes very rapid growth.

The nitrate content of sandal with host is slightly higher than that of the one without it. Sandal fed by host is richer in amide nitrogen than the one without it. Since amides play a very important part in transformations of nitrogen into proteins it would appear the conditions are more favourable in sandal with host than in the one without. This observation is supported by the protein contents which show that the host-fed sandal is richer than the one without (Table I). The observations relating to synthesis of proteins are further supported by the comparatively smaller amounts of water-soluble nitrogen in host-fed plants while the corresponding condition in hostless sandal would point to proteolytic degradation taking place therein.

The state of sandal that is deprived of its host corresponds to that of starvation observed by Chibnall in the case of the runner bean plant which showed decrease in protein nitrogen, followed by increase in water-soluble forms with the progress of starvation (*Biochem. J.*, 1912, **16**, 344). Chibnall also observed that the starved plants contained a greater quantity of what he called the 'other N', which corresponds to the 'residual N' in column 10 of Table II in the present paper. Drastic treatment (with concentrated HCl for 16 hours) to effect complete hydrolysis led to increase in ammonia and basic nitrogen followed by decrease in 'other N'. This suggested to Chibnall that 'other N' might consist of ureides like barbituric acid which resist mild hydrolysis, but which, on prolonged hydrolysis, yield bases giving a precipitate with phosphotungstic acid. Basing his conclusions on those of Fosse (*Compt. rend.*, 1913, **156**, 567, 1938; 1914, **158**, 1374) who demonstrated the presence of traces of urea in higher plants, Chibnall drew the conclusion that the catabolic process of the nitrogen metabolism in the leaves proceed along the line leading to increased production of 'other N'. The results presented in column 10 of Table II are similar to those obtained by Chibnall. Although drastic hydrolysis with concentrated acid was not conducted, yet prolonged treatment with 20 per cent. hydrochloric acid led to increase in the mono-amino nitrogen content of the hydrolysate, thereby suggesting that the latter was derived from the 'residual' nitrogen. It would follow therefore that the values for mono-amino nitrogen given in column 9 of Table II, did not represent the entire amount of mono-amino nitrogen present in the water extract. Another reason for concluding that column 9 does not represent the actual amount of mono-amino nitrogen is that according to Hart and Bently (*J. Biol. Chem.*, 1915, **22**, 477) and Roxas (*J. Biol. Chem.*, 1916, **23**, 71) at least a part of the humin nitrogen formed as a result of hydrolysis with HCl is formed out of the mono-amino nitrogen. For the same reason column 11 cannot represent the actual amount of peptide nitrogen present in the extract as part of the increase of amino-nitrogen due to hydrolysis of peptide by acid may be converted into humin nitrogen. In view of these defects in the method of acid hydrolysis, a development of the method of hydrolysing the peptides with a specific enzyme seems necessary for the estimation of the true peptide nitrogen.

The results given in Table III show that the nitrogen contents of sandal leaves in association with different host plants are dependent on the nature of the hosts with which it is associated.

Table IV gives the results of the distribution of the hexone bases in the leaves of sandal with and without host plant according to the method of Van Slyke as modified by Plimmer and his co-workers (*Biochem. J.*, 1925, **19**, 1004; *ibid.*, 1927, **21**, 247). The proportion of water-soluble nitrogen in the leaves of sandal with and without host is as 1 :3 and the amino nitrogen in the filtrate obtained after separation of bases is less in the case of sandal with host plant than in one without. The significance of these observations is still somewhat obscure.

When the hexone bases are expressed as percentages of the total basic nitrogen arginine and histidine are found to exist to the same extent in sandal raised with and without hosts. Lysine is present to a slightly greater extent and cystine very much more so in sandal nourished by hosts than in without. Since cystine is known to be essential for the growth and active functioning of plants, it is probable that the inadequacy of supply of that essential amino-acid may be partly responsible for the poor development of hostless sandal.

SUMMARY

A detailed investigation of the nitrogen metabolism in the leaves of sandal with and without hosts has been made.

Total nitrogen in the leaves of sandal is largely influenced by the nature of the host plant with which it is associated.

A great proportion of the nitrogen in host-fed sandal exists in the form of protein. Absence of host plant seems to bring about a

decrease in protein followed by an increase in the amount of water-soluble nitrogen.

Basic nitrogen content of leaves of hostless sandal is greater than that of the host-fed plant.

Sandal without host resembles the spiked plant in that its protein nitrogen is low and water-soluble nitrogen (made up chiefly of basic forms) high. On the other hand, the ammonia content of hostless sandal is higher and amide nitrogen lower than that of host-fed sandal. This observation differs from that in the case of spiked sandal, the ammonia and amide contents of which are not significantly different from those of the healthy one.

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