STUDIES ON SANDAL SPIKE

Part III. The Nitrate Reductase Activity in the Normal and Pathochemical States of Sandal (*Santalum album* Linn.)

By K. Parthasarathi, P. K. Ramaiah, T. R. Manjanatha and P. S. Rao, F.A.Sc.

(*Forest Research Laboratory, Bangalore*)

Received April 24, 1962

Nitrate reductase occupies a place of primary importance in the nitrogen metabolism of plant. Although this enzyme received some attention in the past, it is only in recent years that systematic studies have been made regarding its nature and its relation to nitrate reduction, photosynthesis and respiration in plants.[1] In continuation of the work in progress in this Laboratory on sandal spike, a study of the nitrate reductase activity of the leaves of the plant (*Santalum album* Linn.) has been taken up. The activity of the enzyme in the leaves from the spiked plant and from the healthy plant at different stages of development has been studied. For the sake of correlation the nitrate content of the healthy and diseased leaves as also the roots and stems has been determined.

Experimental

The sandal leaves were obtained from different representative localities, namely, Forest Research Laboratory Nursery, Kenchanahalli, Ragihalli, Dobbspet and Narasimha Devara Betta (N. D. Betta) as in the previous studies.[2, 3]

The nitrate reductase activity was determined following the method of Eckerson[4] as modified by Hibbard.[5] The fine pulp obtained by passing the leaf material (60–70 g.) through a meat juice extractor was squeezed through a lawn cloth to get the tissue fluid. To 4 ml. of this fluid taken in a conical flask, were added 4 ml. of KNO_3 solution (10%) containing 40 mg. of glucose and 16 ml. each of phosphate buffer (pH 7·3) and water. The pH of the contents was adjusted to 7·3 by adding NaOH (0·2 N) solution using phenol red as external indicator. After adding toluene (10–15 drops) to the contents, the flask was plugged with cotton and the contents were incubated at 37° C. for 17 hours. Activated carbon (7 g.) was then added to the contents which were filtered after 15 minutes, and, if the solution was still coloured, another treatment using 5 g. of activated carbon was given. The filtrate was made up to 100 ml. and the colour developed by 10 ml.

of the filtrate with 10 ml. of an acetic acid solution of α-naphthylamine and sulphanilic acid was compared with the colour developed using 10 ml. of a standard nitrite solution (0·00025 mg. nitrite N per 10 ml.). Nitrate reductase activity was expressed as mg. of nitrite N produced per 100 g. of tissue fluid.

For the nitrate estimation, the dried powdered sample (2 g.) was first extracted in soxhlet with acetone in order to remove the interfering colouring matters. The resulting powder was heated with water (50 ml.) over a water-bath for 45 minutes. The contents were filtered and thoroughly washed. To the filtrate was added sufficient amount of alumina cream so that the solution on filtration and washing gave an almost colourless, clear liquid which was evaporated to dryness in a china dish on a boiling water-bath. The resulting residue was used for developing colour with phenol-disulphonic acid reagent.[6] The contents were then made up to a known volume (250–1,000 ml.) so as to get a convenient degree of intensity of colour for comparison with an artificial standard of $K_2Cr_2O_7$ (0·001 N) which was previously standardised against the colour developed using a standard solution of KNO_3.

The results obtained are presented in Tables I, II and III. The first table gives the values of the nitrate reductase activity, while the other two present the figures for the nitrate content.

TABLE I

Nitrate reductase activity in healthy and spiked sandal leaves*

Place	Healthy Vegetative	Healthy Flower-bud	Healthy Flower	Spiked Vegetative
		July–August		
Forest Research Laboratory Nursery	0·0425	0·0595	0·0701	No spike disease in this area
Kenchanahalli ..	0·0486	0·0610	0·0726	0·1005
Dobbspet ..	0·0506	0·0683	0·0752	0·1155
Ragihalli ..	0·0469	0·0562	0·0811	0·1138
		October–November		
Forest Research Laboratory Nursery	0·0570	0·0550	..	No spike disease in this area
Kenchanahalli ..	0·0500	0·0530	..	0·0519

* Average of six values; expressed as mg. of nitrite N per 100 g. of the leaf tissue fluid.

TABLE II

Nitrate (expressed as KNO_3) content in healthy (vegetative) and spiked sandal leaves*

Month	Place	Healthy	Spiked
August 1961 ..	Kenchanahalli	0·142	0·273
	N. D. Betta	0·186	0·588
November 1961	Kenchanahalli	0·600	1·370
		0·375	1·500
February 1962	Kenchanahalli	0·294	0·400

* Percentage on zero moisture basis; healthy and spiked plants growing in the same locality were used for analysis.

TABLE III

Nitrate (expressed as KNO_3) content in the leaf, stem and root of the healthy (vegetative) and spiked plants at Kenchanahalli during November* 1961

Healthy			Spiked		
Leaf	Stem	Root	Leaf	Stem	Root
0·600	0·107	0·150	1·370	0·031	0·040
0·375	0·029	0·029	1·500	0·036	0·037

* Percentage on zero moisture basis; healthy and spiked plants growing in the same locality were used for analysis.

DISCUSSION

The nitrate reductase activity during July–August shows certain noteworthy features. The reductase activity shows a gradual increase in the healthy sandal from the vegetative stage through the flower-bud formation to the flower formation stage of the plant. However, in the leaves of spiked plant, which remains right through vegetative only, the reductase activity is higher than even that at the flower formation stage in the healthy sandal. The high levels of reductase activity at the reproductive stages of the healthy sandal, as compared to the activity at the vegetative stage, seems to be due

to a high metabolic activity involving the rapid utilization of nitrogen for incorporation in the floral organs. The high reductase activity in the leaves of the spiked plant also indicates a high rate of nitrogen utilization, which is possibly due to a rapid synthesis of the viral protein in the spiked leaves, since the spiked plant remains vegetative only right through.

The nitrate reductase activity during October–November shows yet different trends. While the activity in the leaves of healthy vegetative plant shows slight increase as compared to the activity of the earlier period, that at flower-bud stage appears to remain stationary as compared to the corresponding values obtained in July–August. It is interesting to note that the reductase activity in the spiked leaves falls to the level occurring in the leaves of healthy vegetative plant at that time. This leads us to suppose that the high rate of nitrogen utilization, existing earlier, is not there in October–November, possibly due to a considerable decrease in the rate of viral protein synthesis, which decrease, in its turn, may be due to the senescence of the leaf or the seasonal effect; the former appears to be more probable.

It is known that nitrate reductase shows an extreme degree of adaptive formation towards the supply of nitrate.[7–10] The fact that the spiked leaves show a high nitrate reductase activity indicates that the nitrate content of the spiked leaves should also be pretty high—higher than that of the normal healthy leaves. This has actually been found to be the case (*vide* results presented in Table II), although earlier workers reported otherwise.[11] This finding is also supported by the fact that spiked leaves suffer from iron deficiency and iron deficiency is recorded to lead to an increase in the nitrate level.[12] It is also of interest to note that the total nitrogen in spiked leaves is greater than in the healthy leaves.[11, 13] Hence the question naturally arises as to wherefrom the diseased plant is able to obtain the nitrates. It cannot possibly be from the host plant or from the soil, since in spike disease the haustorial connections and root ends cease to function; nor can it be from any already-stored material from other parts of the plant, since the stems and roots do not contain any abnormal quantities of nitrate (*vide* Table III). The high accumulation of the nitrates in the spiked sandal leaves may, therefore, possibly happen through the agency of the virus itself which may be a nitrogen fixer (*cf.* Narasimhamurthy and Sreenivasaya[11]).

It is known that molybdenum is the metal constituent of the nitrate reductase,[14, 15] and a deficiency of this element leads to a considerable decrease in the reductase level,[16–18] while its supply adds to its increase.[7] In the spiked leaves the nitrate reductase activity is never lower than in the

leaves of healthy vegetative plant at any period and hence it appears that the spike disease is not caused on account of any deficiency of this element.

REFERENCES

1. Kessler, E. .. *Symposia Soc. Exptl. Biol.*, 1959, No. 13, 87-105.
2. Parthasarathi, K. and Ramaswamy, M. N. *J. Sci. Industr. Res.*, 1961, **20 C**, 273-75.
3. ——— and Rao, P. S. .. *Proc. Ind. Acad. Sci.*, 1962, **55B**, 99–106.
4. Eckerson, S. H. .. *Contrib. Boyce Thompson Inst.*, 1931, **3**, 197-217.
5. Hibbard, A. D. .. *Plant Physiol.*, 1936, **11**, 657–58.
6. Snell, F. D. and Snell, C. T. *Colorimetric Methods of Analysis*, D. Van Nostrand Company, Inc., New York, 1959.
7. Candela, M. I., Fisher, E. G. and Hewitt, E. J. *Plant Physiol.*, 1957, **32**, 280–88.
8. Pei-Sung Tang and Hsiang Yu Wu *Sci. Record (Peking)*, 1957, **1**, 55–60; *vide Chem. Abstr.*, 1958, **52**, 9330.
9. Hsiang-Yu Wu and Pei-Sung Tang *Chih Wu Hsueh Pao*, 1958, **7**, 135–48; *vide Chem. Abstr.*, 1959, **53**, 15220.
10. Hageman, R. H. and Donna Flesher *Plant Physiol.*, 1960, **35**, 700-08.
11. Narasimhamurthy, N. and Sreenivasaya, M. *J. Indian Inst. Sci.*, 1929, **12 A** (10), 153–63.
12. Steinberg, R. A., Specht, A. W. and Roller, E. M. *Plant Physiol.*, 1955, **30**, 123-29.
13. Iyengar, A. V. V. .. *J. Indian Inst. Sci.*, 1928, **11 A** (9), 97-102.
14. Nicholas, D. J. D. and Nason, A. *J. Biol. Chem.*, 1954, **207**, 353-60.
15. Evans, H. J. and Hall, N. S. *Science*, 1955, **122**, 922-23.
16. Nicholas, D. J. D., Nason, A. and Mc Elroy, Wm. D. *J. Biol. Chem.*, 1954, **207**, 341–51.
17. Mulder, E. G., Boxma, R. and Van Veen, W. L. *Plant and Soil*, 1959, **10**, 335–55.
18. Cheniae, G. M. and Evans, H. J. *Plant Physiol.*, 1960, **35**, 454–62.