STUDIES ON SANDAL SPIKE

Part X.* Deoxyribonuclease and Ribonuclease Activities and Nucleic Acid Levels in Sandal (*Santalum album* Linn.) Affected by Spike Mycoplasma

By K. Parthasarathi, C. R. Rangaswamy, K. Jayaraman and P. S. Rao

(*Forest Research Laboratory, Bangalore-3*)

Received August 20, 1972

Abstract

A study of the deoxyribonuclease and ribonuclease activities and nucleic acid levels in sandal affected by spike mycoplasma was made. A serious disturbance in the nucleic acid metabolism noticed at the young leaf stage in the diseased plant suggested a rapid multiplication of the mycoplasma at that stage. The relative levels of nucleic acids and nuclease activities appear to indicate the migration of the infective organism from the older diseased leaf tissue to regions of fresh vegetative growth.

Observation of mycoplasma-like bodies as the causative agents in certain plant diseases is of recent origin.[1] These bodies contain both RNA and DNA.[2] Our studies on sandal spike disease have shown it to be mycoplasmal in nature.[3-6] The study of the nucleic acid balance, ribonuclease (RNase) and deoxyribonuclease (DNase) activities in sandal affected by this disease has been made now, since it throws light on the gross disturbances in nucleic acid metabolism concomitant with the development of the infective organism in the plant. In addition to the phosphorus fractions corresponding to RNA and DNA, those of acid-soluble phosphorus (which comprises to a large extent nucleotides and coenzymes, and hence hereinafter referred to as nucleotide-P), phospholipids and total-P were determined.

Experimental

Fresh leaf samples, at different stages of development, were taken separately from six healthy and six spiked sandal plants (10-15 years age) growing in the same area, collecting the samples always between 8 and 9 A.M. In the case of healthy plants, when flowering season started,

---

* Part IX of this series has appeared in *Current Science*, 1971, 40, 640.

samples were taken only from those branches where there was no flowering; spiked plants remained vegetative right through.

Total-P

Fresh leaves (2 g.) were dried (80°C) and digested with 10N H_2SO_4 (40 ml.) and anhydrous Na_2SO_4 (1 g.), adding 2 ml. of H_2O_2 after getting a uniform suspension of charred matter and then continuing the digestion until a clear solution resulted (about 4 hrs. time). The digest, after neutralising with 10N KOH and re-acidifying with 0·8 ml. 10N H_2SO_4, was made up to 500 ml. filtered, and used for total-P determination using chlorostannous acid reagent.[7]

P-fractions

The P-fractions representing phospholipids, nucleotides, RNA and DNA were separated according to the method of Volkin and Cohn,[8] using trichloro-acetic acid wherever needed. The extracts of these P-fractions were evaporated to dryness, transferred to a Kjeldahl flask using a minimum amount of water, and digested as indicated above. The digest, after neutralisation and re-acidification, was made up to 100 ml., filtered and used for P determination as indicated above. The data obtained are presented in Tabe I.

TABLE I

Phosphorus content of various fractions in healthy (H) and spiked (S) sandal leaves*

Time of collection		Growth condition	Total		Phos-pholipid		Nucleo-tide		RNA		DNA		RNA/DNA	
Month	Week		H	S	H	S	H	S	H	S	H	S	H	S
July	2	Young	201	190	32	22	30	48	98	70	27	45	3·6	1·6
September	2	Fairly grown-up	54	56	17	16	15	20	8	12	5	7	1·6	1·7
November	2	Mature	94	88	13	30	50	32	15	18	6	7	2·5	2·6

* μg. of phosphorus per 100 mg. of leaf tissue; average values obtained with six plants.

RNase Activity

*R*Nase activity of the leaf tissue was estimated by the amount of nucleo-tides liberated from yeast RNA substrate, according to the method of Kessler

and Engelberg[9]. Using 5 g. of fresh leaf, 50 ml. of enzyme extract was prepared. Yeast RNA substrate was prepared by dissolving RNA in water, made slightly alkaline.[10] Incubation period was 2 hours. Blanks were run using water in place of substrate. In both the cases, the filtrate obtained after seperating the acid-insoluble precipitate, was digested and estimated for P as before. The data obtained are given in Table II.

TABLE II

RNase and DNase activity in healthy (H) and spiked (S) sandal leaves

Time of collection		Growth condition	RNase activity*		DNase activity †	
Month	Week		H	S	H	S
July	2	Young	478	524	50	40
September	2	Fairly grown-up	480	535	84	14
November	2	Mature	330	223	66	5

*μg. P liberated/250 mg. tissue/2 hours; average values obtained with six plants.

† μg. DNA hydrolysed/6·2 mg. tissue/30 minutes; average values obtained with six plants.

DNase Activity

DNase, unlike RNase,[11] sometimes leaves a resistant core[12,13] during hydrolysis of the DNA substrate. Hence for the present study the method of Kurnick and Sandeen[14] which was based on the determination of the substrate DNA left unhydrolysed, has been adopted. This method, which was originally developed for animal tissues, has now been altered suitably for the plant tissues, and the details are as follows:–

The substrate solution consisted of 40 ml. of calf thymus DNA (2 mg./ml.), 40 ml. methyl of green (0·04 %, extracted with chloroform until the chloroform layer becomes colorless), 20 ml. of $MgCl_2$ (0.3M), 40 ml. of acetate buffer (pH 5·2) and 1 ml. of merthiolate (1%). As a further precautionary measure against microbial contamination, 2 ml. of toluene was added to the substrate which was then preserved in a refrigerator. Fresh substrate solution was prepared once in 10 days. For preparing the enzyme extract, 2 g. of fresh clean leaves were ground using glass-distilled water (20 ml.) and reagent-grade sand (2 g.). The slurry was filtered through a muslin cloth into a 50 ml. volumetric flask, washing the residue with two 10 ml. aliquots of water. The filtrate was made up to 50 ml.

and centrifuged for 8 minutes. The supernatant was used as the enzyme source. The reaction mixture (12 ml. of substrate + 1 ml. of enzyme extract) was incubated for 30 mins. at 37°C, whereafter two 2 ml. aliquots of the reaction mixture were pipetted out into two test tubes each of which contained 1·5 ml. tris buffer (pH 7·5) and 0·5 ml. sodium citrate (0·33 M). After adding 3 drops of toluene to each of these tubes, they were closed with plastic caps and kept in dark for 4½ hrs., for the fading away of free methylgreen. The percentage transmission of the resulting solution was determined at 640 mμ. The control consisted of all the constituents of the test solution except methylgreen. By referring the % transmission of the test solution to a standard graph showing the relation between the amount of DNA present in the DNA-methylgreen complex and the % transmission, the amount of unhydrolysed DNA was found. The difference between the original and residual contents of DNA measured the DNase activity expressed as μ g. DNA hydrolysed/6·2 mg. tissue/30 minutes. Standards were prepared by adding 1 ml. of water to 12 ml. of substrate solution, pipetting out aliquots upto 2 ml. from this, adjusting the volume of each aliquot to 2 ml. with water and then subjecting it to the same sequence of treatments as in the case of the sample. The results are shown in Table II.

Discussion

From the results it is clear that both in the healthy and spiked leaves the total P-content tends to decrease with the growth, and again shows a slight rise at maturity. However, differences in the P-fractions exist between the healthy and the diseased tissues. A notable feature is that the RNA-P/DNA-P ratio differs considerably at the young stage between the healthy and diseased leaves indicating a derangement in the nucleic acid metabolism, probably due to multiplication of the infective principle at that stage.

RNase activity remains more or less at a high level in the young leaves of both spiked and healthy sandal. Thus the level of RNase has not affected the RNA content in the young leaves. RNase functions mainly as a degradative enzyme,[9,15] although some synthetic function under certain growth conditions is also attributed. In the healthy as well as spiked leaves, the low level of RNA during the fairly grown-up and mature stages, is due to the degradative action of RNase. Perhaps multiplication of the mycoplasma occurs at young leaf stage and thus appears to account for the high RNA level in the young spiked leaves despite the existence of a high RNase activity at that stage.

Likewise, the levels of DNase activity had no obvious relationship to the content of DNA in the spiked plant. The young spiked leaves had a high DNase activity, while they had a high DNA content as well. The normal function of DNase is degradative,[16] but it is reported to have some synthetic role also, because in certain tissues, where rapid cell division and DNA synthesis occur, a high DNase activity has also been noticed.[17–20] A plausible explanation for this is that the rapid multiplication of the mycoplasma at this stage has contributed to this increase.

A decrease in the DNase activity normally results in an accumulation of DNA, but in the case of spiked sandal the DNA content also decreases along with the decrease in the DNase activity as the leaf grows and matures. A plausible explanation for this is that the mycoplasmal DNA which probably constitutes a major part of total DNA at the young leaf stage, has gone down at these later stages due to migration of the mycoplasma to the regions of fresh vegetative activity. This means that the concentration of the infective principle gets much reduced in the older leaf tissue.

In animal cells [21,22] affected by mycoplasma, an increase in DNase activity was reported. It is of interest to note that in the spiked sandal leaves there is, in contrast, a decrease in DNase activity.

References

1. Maramorosch, K., Shikata, E. and Granados, R. R. *Trans. N.Y. Acad. Sci.*, 1968, **30**, 841.
2. Anonymous .. *Nature*, 1968, **219**, 438.
3. Rao, P. S. .. *Sci. and Cult.*, 1969, **35**, 585.
4. Varma, A., Chenulu, V. V., Raychaudhuri, S. P., Nam Prakash and Rao, P. S. *Ind. Phytopathol.*, 1969, **22**, 289.
5. Hull, R., Horne, R. W. and Nayar, R. M. *Nature*, 1969, **224**, 1121.
6. Dijkstra, Jeanne and Ie, T. S. *Neth. J. Pl. Path.*, 1969, **75**, 374.
7. Jackson, M. L. .. *Soil Chemical Analysis*, Prentice-Hall of India Private Ltd., New Delhi, 1967, p. 142.
8. Volkin, E. and Cohn, W. E. .. *Methods of Biochemical Analysis*, David Glick, Editor, Interscience Publishers, Inc., New York, 1954, **1**, p. 287.
9. Kessler, B. and Engelberg, N. *Biochim. et Biophys. Acta.*, 1962, **55**, 70.

10. Markham, R. .. *Modern Methods of Plant Analysis*, K. Peach and M. V. Tracey, Editors, Springer-Verlag, Berlin, 1955, **4**, p. 249.

11. Anfinsen, C. B. and White, F. H. *The Enzymes*, P. D. Boyer, H. Lardy and K. Myrback, Editors, Academic Press, New York, 1961, **5**, p. 95.

12. Brawerman, G. and Chargaff, E. *J. Biol. Chem.*, 1954, **210**, 445.

13. Laskowski, Sr., M. .. *The Enzymes*, P. D. Boyer, H. Lardy and K. Myrback, Editors, Academic Press, New York, 1961, **5**, p. 123.

14. Kurnick, N. B. and Georgianna Sandeen *Biochim. Biophys. Acta.*, 1960, **39**, 226.

15. Barnard, E. A. .. *Ann. Rev. Biochem.*, 1969, **38**, 677.

16. Lehman, I. R. .. *Ibid.*, 1967, **36**, Part II, 645.

17. Stern, H. .. *J. Biophys. Biochem. Cytol.*, 1961, **9**, 271.

18. Hotta, Y. and Stern, H. .. *Ibid.*, 1961, **9**, 279.

19. Alfrey, V. and Mirsky, A. E. *J. Gen. Physiol.*, 1953, **36**, 227.

20. Gautier, R. and Leonard, A. *Exptl. Cell Res.*, 1962, **28**, 335.

21. Russel, W. C. .. *Nature*, 1966, **212**, 1537

22. Stock, D. A. and Gentry, G. A. *J. Virology*, 1969, **3**, 313.

3993/73. Published by B. S. Venkatachar, Editor, 'Proceedings of the Indian Academy of Sciences", Bangalore and Printed by V. J. F. Jesudason at the Bangalore Press, Bangalore-560018