DOLICHOS ENATION MOSAIC VIRUS (DEMV) AND SYMBIOTIC NITROGEN FIXATION IN FIELD BEAN (*DOLICHOS LABLAB* LINN.)*

by P. N. RAJU, *University Botany Laboratory, Madras*

Dolichos Enation Mosaic Virus (DEMV) infection decreased the levels of total carbohydrates and reducing sugars in roots and nodules of *D. lablab* after 24 days of growth. However, the total nitrogen content in these nodulated virus infected plants was more than that in the healthy plants. Again, nodules of virus infected plants contained higher amounts of total nitrogen after 24 days. There was a higher rate of transfer of fixed nitrogen from nodules to the tops and roots on virus infection of the host. Soluble proteins, total soluble nitrogen constituents and the total insoluble nitrogen increased in nodules following virus infection of the legume. Nitrogen fixation in infected plants increased during 24 to 31 days to levels higher than that in healthy plants and this high level was maintained throughout the growth period. An increased nitrogen fixing activity by symbiotic rhizobia in nodules of *D. lablab* following DEMV infection is indicated.

INTRODUCTION

There is little mention in literature on rhizobial symbiosis under abnormal conditions, especially those situations resulting in nitrogen stress in host metabolism. Disease, in general, alters the nitrogen metabolism of plants (Burris 1959) and this is particularly true of sap transmissible virus diseases (Bawden 1964).

Vanderveken (1964) and Joshi and Carre (1967) reported that the effectiveness of root nodules in nitrogen fixation was reduced on infection of white clover by clover phyllody virus. Nodulation in soybean was similarly affected by soybean mosaic virus and bean pod mottle virus (Tu, Ford and Quiniones 1970). In field bean infected with DEMV, Rajagopalan and Raju (1972) reported a reduction in nodule numbers in natural soil and an increase in nitrogen-free sand cultures. Pronounced increases in tissue nitrogen and altered C/N ratios were also shown to occur under pathogenesis.

Rhizobial symbiosis in virus infected legumes results in a triple interaction involving virus infection, host response and nodule bacterial activity. This paper describes certain changes in the nitrogen fixing ability of symbiotic rhizobia in the nodules of *D. lablab* infected with DEMV.

MATERIALS AND METHODS

Procedures for sand cultures, *Rhizobium* inoculation, virus inoculation and plant assemblage have been described earlier (Rajagopalan and Raju 1972).

*Memoir No. 158 from the Centre of Advanced Study in Botany, University of Madras, Madras-600005 (Tamil Nadu)

Reducing sugars in nodules were extracted in absolute ethanol. The alcohol soluble sugars and total carbohydrates were estimated by the method of Somogyi (1952).

Total nitrogen was determined by the Kjeldahl procedure (Humphries 1956). Total soluble nitrogen was estimated following the procedure outlined by Samborski, Forsth and Person (1958). The residue left over after extraction of soluble nitrogen was dried and used for the estimation of insoluble nitrogen by Kjeldahl method. Biuret method (Dawson *et al* 1959) was used for the determination of proteins.

RESULTS

Total carbohydrates (Table I)

Although the carbohydrate content in shoots was lowered initially on infection, differences thereafter were not marked between healthy and infected plants. In roots, however, carbohydrates accumulated upto 24 days and thereafter decreased to levels lower than that in healthy roots. There was a general reduction in carbohydrate levels in nodules during 24 to 45 days, but this was more pronounced in the infected plants.

TABLE I

Changes in total carbohydrates (mg/g dry matter) in shoots, roots and nodules of healthy and infected D. labalab *grown in nitrogen free sand cultures*
(mean of five observations)

Days after inoculation	Shoot		Root		Nodule	
	Healthy	Infected	Healthy	Infected	Healthy	Infected
17	143	92	100	126	—	—
24	138	147	92	119	224	266
31	132	130	96	92	193	129
38	124	133	146	133	100	73
45	134	147	142	138	81	78
52	148	128	158	128	119	108

Reducing sugars (Fig. 1)

In soil grown healthy plants the sugar level in nodules increased from 31 days, reached a maximum at 45 days and declined thereafter. In infected plants, however, the reducing sugar level was considerably lower than that in the corresponding healthy plants.

The levels of reducing suars in nodules remained comparable between healthy and infected plants in sand cultures at 31 days. These dropped subsequently in nodules of infected plants and remained so throughout the growth period.

Total nitrogen in plants (Fig. 2)

Analyses of total nitrogen content in plants showed that *Rhizobium* inoculation progressively increased the total nitrogen content in both healthy and virus infected plants compared to their respective controls. Nevertheless, in the *Rhizobium* inoculated virus infected plants, total nitrogen content was more than that in the healthy plants after 24 days. Again, nodules of virus infected plants contained higher amounts of total nitrogen after 24 days (Table II)

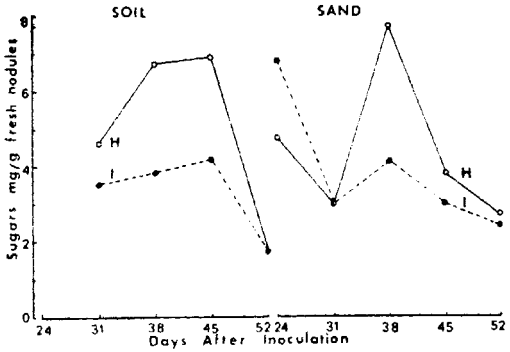

FIG. 1. Changes in reducing sugar levels in nodules. H=Healthy ; I=Virus infected.

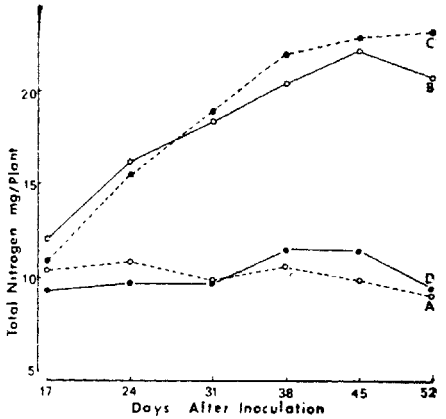

FIG. 2. Changes in total nitrogen content of *D. lablab*. A, Control ; B, Rhizobia inoculated, C, Rhizobia and virus inoculated ; D, Virus inoculated.

TABLE II

Nitrogen amounts, transferred nitrogen and fixation at different stages of growth of healthy and infected D. lablab *in sand cultures*
(Mean of five observations)

Days after inoculation	Nitrogen (mg/plant) in tops	in roots	Total transferred from nodules	in nodules	Total fixed	Ratio of N in nodules to transferred N	Transferred N as per cent of total N fixed
			Healthy				
17	0.83	0.82	1.65	—	1.65	—	—
24	2.97	0.85	4.82	1.58	6.40	1 : 3.1	75.3
31	5.96	1.08	7.04	1.80	8.84	1 : 3.9	79.6
38	6.88	0.89	7.77	2.02	9.79	1 : 3.8	79.4
45	8.75	1.31	10.06	2.23	12.29	1 : 4.5	81.8
52	9.98	1.10	11.08	1.60	12.68	1 : 6.9	87.4
			Infected				
17	1.52	0.02	1.54	—	1.54	—	—
24	4.08	0.85	4.93	0.92	5.85	1 : 5.4	84.2
31	6.60	0.72	7.32	1.90	9.22	1 : 3.8	79.3
38	7.98	0.98	8.96	2.52	11.48	1 : 3.6	78.0
45	8.30	1.26	9.56	2.90	12.46	1 : 3.3	76.7
52	9.55	2.28	11.83	1.75	13.51	1 : 6.7	87.1

Nitrogen (mg/plant) represents the amount in nodulated plants in excess of that of the respective controls.

Transfer of nitrogen from nodules to host (Table II).

A comparison of the tranfer of fixed nitrogen from nodules to host indicates a higher rate of transfer under virus infected conditions. During the period of active nitrogen fixation only 76 to 79 per cent of the fixed nitrogen are in the tops and roots of the virus infected plants. This is so because of the rapid fixation and accumulation of nitrogen in nodules.

Nitrogen fixation (Table II)

At 17 and 24 days after DEMV inoculation nitrogen fixation was lower in infected plants compared to healthy ones. However, during 24 to 31 days and up to 52 days the nitrogen fixed by infected plants increased to levels above those observed in the healthy plants.

Soluble and insoluble nitrogen in nodules (Fig. 3)

The soluble nitrogen content was higher in nodules of infected plants during 31 to 45 days. While the levels of insoluble nitrogen in nodules of healthy and infected plants did not show much change with age, nodules of infected plants had, in general, higher quantities at all times of plant growth.

Proteins in nodules (Fig. 4)

In both soil and sand cultures, nodules of infected plants showed higher quantities of soluble protein during 31 to 45 days following DEMV inoculation. The differences between healthy and virus infected plants were greater in soil cultures than in sand. While the soluble proteins decreased in nodules of infected plants after 45 days in sterile sand cultures, they remained higher throughout the growth period in natural soil.

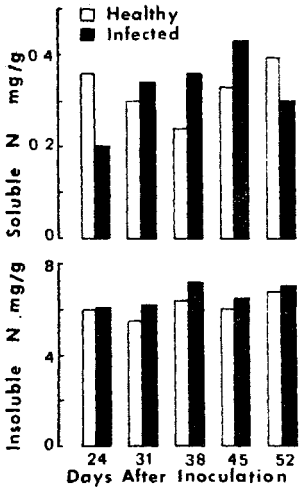

FIG. 3. Changes in soluble and insoluble nitrogen in nodules of healthy and virus infected *D. lablab*.

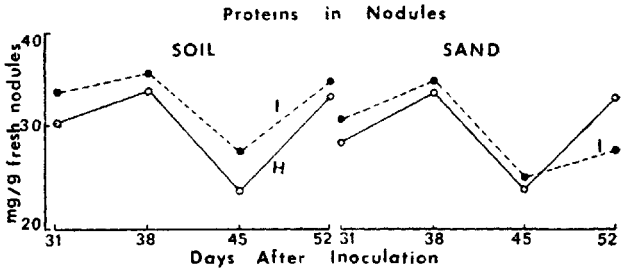

FIG. 4. Changes in soluble proteins in nodules of field bean. H=Healthy; I=Virus infected.

Discussion

Inoculation of *D. lablab* with an effective cowpea *Rhizobium* and DEMV increased carbohydrate levels in roots during the early growth period up to 25 days (Table I). In nodulating legumes, transport of carbohydrates from photosynthesizing leaves to roots and nodules is known (Pate 1966). In studies on the transport of labelled carbon from leaves fed with $^{14}CO_2$, Pate, Walker and Wallace (1965) showed that the carbohydrates produced in the leaves can contribute to the synthesis of a variety of amino compounds in roots and nodules. Viral mosaics generally retard rate of photosynthesis but augment respiration (Bawden 1959). Therefore, any consideration of *in vivo* changes in C/N ratios will have to examine *per se* the interaction of virus infection on nodulation and nodule activity as indicated by host tissue response at different age levels.

In DEMV infected *D. lablab* inoculation with an effective *Rhizobium* strain increased the total nitrogen content of infected plants over the healthy after 24 days (Fig. 1). This increase was especially pronounced in nodules (Table II) during the period of active nitrogen fixation (i.e. 31 to 45 days). The increased tempo of turn over of fixed nitrogen in infected plants with age was accompanied by a sizable reduction in carbohydrate content of roots and nodules after 24 days (Table I). In a nodulated legume the carbohydrate supply to the root nodule is the primary determinant of the extent and progress of symbiotic nitrogen fixation (Gibson 1966). The nitrogen fixing capacity of root nodules diminishes rapidly after removal of carbohydrate supply from the plant tops (Bach, Magee and Burris 1958). The concentration of reducing sugars also decreased in nodules of infected plants during 31 to 52 days in both soil and sand cultures (Fig. 1). The decline in carbohydrates and reducing sugars might be associated with their oxidation possibly supplying reducing power for the conversion of nitrogen into ammonia (Bergersen 1960). In fact, very high rates of endogenous respiration are reported in nodules of virus infected plants during 31 to 52 days (Raju 1968). The observed reduction in carbohydrates and reducing sugars and the concomitant increase in nitrogen with virus infection of the host can be considered to reflect a rapid utilization of carbohydrates in the synthesis of soluble nitrogen compounds in nodules following DEMV infection.

The amounts of nitrogen in tops and roots also increased during this period thus indicating a higher rate of transfer of fixed nitrogen from the nodules (Table II). Fixation in root nodules is always associated with a direct export of fixed nitrogen to the shoot (Pate 1958; Stewart 1962). The higher rate of transfer of fixed nitrogen from nodules is suggestive of a greater fixation activity in the nodules. The gradual decrease in the ratio of nitrogen in nodules to that in tops and roots (Table II) is, therefore, taken to be due to increased fixation and accumulation of nitrogen in nodules of infected plants during this time.

Nodules contain amino acid activating enzymes and can incorporate amino-acids into water soluble proteins including haemoglobin (Moustafa and Proctor 1962) and can, therefore, be regarded as a probable site for protein synthesis (Proctor and Moustafa 1963). The concentration of soluble proteins was higher in nodules of infected than in healthy plants (Fig. 4). Apart from this, there was a pronounced

increase in total soluble nitrogen constituents of nodules during 31 to 45 days (Fig. 3).

The amount of nitrogen fixed in infected plants increased during 24 to 31 days to levels higher than that in healthy plants (Table II) and this high level was maintained throughout the growth period.

In conclusion, it may be stated that enhanced nodule activity resulting in augmented nitrogen fixation is the sequela of the DEMV *Rhizobium* interaction in *D. lablab*. Under such stress conditions the activity of the symbiotic rhizobia as the primary nitrogen supplying mechanism is put to a serve test. In fact, *Rhizobium* is capable of a great deal of adaptive variation (Joshi, Carr and Jones 1967) and the increased effectiveness of symbiotic rhizobia following DEMV infection of the host reported here might well be an 'adaptation' to meet the greater metabolic demands.

ACKNOWLEDGEMENT

I am indebeted to Prof. T.S. Sadasivan, for valuable advice and continued interest; and to Dr. N. Rajagopalan for all help.

REFERENCES

Bach, M.K., Magee, W.E., and Burris, R.H. (1958). Translocation of photosynthetic products to soybean nodules and their role in nitrogen fixation. *Pl. Physiol.*, **33**, 118-123.

Bawden, F.C. (1959). Physiology of virus diseases. *Ann. Rev. Pl. Physiol.*, **10**, 239-256.

———(1964). Plant Viruses and Virus Diseases (4th ed.) The Ronald Press Company, New York, pp. 361

Bergersen, F.J. (1960). Biochemical pathways in legume root nodule nitrogen fixation. *Bact. Rev.*, **24**, 246.

Burris, R.H. (1959). Nitrogen nutrition. *Ann. Rev. Pl. Physiol.*, **10**, 301-328.

Dawson, R.M.C., Elliot, D.C., Elliot, W.H., and Jones, K.M. (1959). *In:* Data for Biochemical Research. p. 284. Oxford Clarendon Press.

Gibson, A.H. (1966). The carbohydrate requirements for symbiotic nitrogen fixation. A whole plant growth analysis approach. *Austr. J. biol. Sci.*, **19**, 499-515.

Humphries, E.C. (1956). Determination of nitrogen. *In:* Modern Methods of Plant Analysis. **1**, 479-481. Springer Verlag, Berlin.

Joshi, H.U., and Carr, A.J.H. (1967). Effect of clover phyllody virus on nodulation of white clover (*Trifolium repens*) by *Rhizobium trifolii*. *J. gen. Microbiol.*, **49**, 385-392.

Joshi, H.V., Carr, A.J.H., and Jones, D.G. (1967). Effect of clover phyllody virus on nodulation of white clover (*Trifolium repens*) by *Rhizobium trifolli*. *J. gen. Microbiol.*, **47**, 139-151.

Moustafa, E., and Proctor, M.H. (1962). Amino acid dependent exchange between pyrophosphate and adenosine triphosphate in root nodules. *Biochim. biophys. Acta.*, **63**, 93-97.

Pate, J.S. (1958). Nodulation studies in Legumes. I. Synchronisation of host and symbiotic development in the field pea, *Pisum arvens* L. *Austr. J. biol. Sci.*, **11**, 366-381.

Pate, J.S. (1966) Photosynthesizing leaves and nodulated roots as donors of carbon to protein of the shoot of the field pea (*Pisum arvense* L.). *Ann. Bot.*, **30**, 93-109.

Pate, J.S., Walker, J., and Wallace, W. (1965). Nitrogen containing compounds in the shoot system of *Pisum arvense* L. II. The significance of amino acids and amides released from nodulated roots. *Ann. Bot.*, 29, 475-493.

Proctor, M.H., and Moustafa, E. (1963). Incorporation of amino acid by root nodules of *Galega officinalis*. *Nature, Lond*, **197**, 262-263.

Rajagopalan, N., and Raju, P.N. (1972). The infiuence of infection by *Dolichos* enation mosaic virus on nodulation and nitrogen fixation by field bean (*Dolichos lablab* L.). *Phytopath. Z.*, **73**. 285-309.

Raju, P.N. (1968). Studies on legume nodulation. Doctoral Thesis. Univ. Madras.

Samborski, D.J., Forsth, F.R., and Person, C. (1958). Metabolic changes in detached wheat leaves floated on Benzimidazole and the effect of these changes on rust infection. *Can. J. Bot.*, **36**, 591-601.

Somogyi, M. (1952). Notes on sugar determination. *J. biol. Chem.*, **195**, 19-23.

Stewart, W.D.P. (1962). A quantitative study of fixation and transfer of nitrogen in *Alnus*. *J. exp. Bot.*, **13**, 250.

Tu, J.C., Ford, R.E., and Quiniones, S.S. (1970). Effects of soybean mosaic virus and/or bean pod mottle virus infection on soybean nodulation. *Phytopathology*, **60**, 518-523.

Vanderveken, J. (1964). Influence d'um virus sur la nodulation chez *Trifolium repens*. *Ann. Inst. Pasteur, Paris*, **107**, 143-48.