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Interaction of *Rhizobium* and Urd Bean Mosaic Virus Infection on the Nitrogen Metabolism of Urd Bean

By

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With 2 Figures

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Summary

SINGH A. K. & SRIVASTAVA S. K. 1988. Interaction of *Rhizobium* and urd bean mosaic virus infection on the nitrogen metabolism of urd bean.–Phyton (Austria) 28 (2): 161–169, with 2 figures. – English with German summary.

The present study deals with the interaction of the virus and *Rhizobium* on the nitrogen metabolism of urd bean (*Vigna mungo* [L.] HEPPER) in soil and sand potting media. The total nitrogen, the nitrate-N- and nitrite-N-fraction, protein and free amino acids were higher in infected plants than in their healthy counterparts grown in sand and soil potting media and *Rhizobium* treated and untreated plants while NH_4-N was lowered under similar treatments. The contents of total-N, protein and total free amino acids were maximum in leaf followed by root and stem, NO_3-N and NO_2-N content followed the sequence roots > leaf > stem while the sequence of NH_4-N content was leaf > stem > root. The levels of nitrogen fractions were maximum on 50^{th} day (plants grown in soil media) or on 40^{th} day (in sand media) after inoculation. The increase/decrease of nitrogen fractions was more pronounced in untreated diseased plants than in *Rhizobium* treated ones. The virus infection increased the nitrate reductase activity, the maximum activity was observed in leaves followed by roots and stems. Highest enzyme activity was observed at 10^{th} and 20^{th} day after inoculation grown in soil and sand respectively and thereafter it decreased with increasing age of plant.

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Zusammenfassung

SINGH A. K. & SRIVASTAVA S. K. 1988. Wirkung von *Rhizobium* und Infektion mit Urd bean mosaic-Virus auf den Stickstoffwechsel der Kuhbohne. – Phyton (Austria) 28 (2): 161–169, mit 2 Abbildungen. – Englisch mit deutscher Zusammenfassung.

Die vorliegende Studie befaßt sich mit der Wechselwirkung von Virusbefall und *Rhizobium* auf den Stickstoffwechsel der Kuhbohne (urd bean, *Vigna mungo* [L.] HEPPER) bei Kultur in Erde und in Sand-Gesamtstickstoff, Nitrat und Nitrit-N, Protein und freie Aminosäuren waren in Erd- wie in Sandkulturen sowie mit und ohne *Rhizobium*befall in infizierten Pflanzen höher als in den gesunden Kontrollen, während der Gehalt an NH_4-N erniedrigt war. Der Gehalt an Gesamt-N, an Protein und freien Aminosäuren war in den Blättern am höchsten, gefolgt von Wurzeln und Sproß, Nitrat- und Nitrit-N folgten der Reihe Wurzel > Blatt > Sproß, der NH_4-N der Reihe Blatt > Sproß > Wurzel. Die N-Fraktionen erreichten am 50. Tag nach Inokulation (Erdkultur) bzw. am 40. Tag (Sandkultur) ihre höchsten Werte, Anstieg und Abfall war in den *Rhizobium*-freien virusinfizierten Pflanzen ausgeprägter als in den mit *Rhizobium* behandelten. Durch die Virusinfektion wurde die Aktivität der Nitratreduktase erhöht, maximale Aktivitäten wurden am 10. (Erdkultur) bzw. 20. Tag nach Impfung (Sandkultur) beobachtet, mit fortschreitendem Alter der Pflanze nahm die Aktivität ab.

Introduction

A large number of studies of the legumes have been made regarding the physiological disturbances of the host as influenced by virus infections but little attention have been paid on the physiological alterations produced due to interaction of *Rhizobium* and viruses. Urd bean (*Vigna mungo,* a pulse crop grown throughout in India, fixed nitrogen in the soil through their root nodules, sometimes is found infected by urd bean mosaic virus (SINGH & SINGH 1978). Therefore, in the present study, it was aimed to investigate the effects of *Rhizobium* and urd bean mosaic virus on the nitrogen metabolism of urd bean. In particular, very little information is available regarding the nitrate reductase activity of virus infected pulse crops (NARAYANASWAMY & RAMAKRISHNAN 1966, KHATRI & CHENULU 1973 and TRIPATHI & al. 1983). But an interaction between virus and *Rhizobium* had not been worked out so far. Therefore, the present study involves the effect of *Rhizobium* and urd bean mosaic virus interaction on nitrate assimilation by urd bean plants.

Materials and Methods

All the experiments were conducted in an insect proof chamber. Urd bean (*Vigna mungo* [L.] HEPPER) cv. Type-9 and urd bean mosaic virus (SINGH & SINGH 1978) maintained on urd bean were used as host and virus, respectively. Virus inoculations were made by usual leaf rubbing method using carborundum powder (600 mesh) as an abrasive at cotyledonary stage. The control plants were treated similarily by using neutral phosphate buffer solutions only.

The first group of the plants were raised from *Rhizobium* treated and untreated urd bean seeds separately in clay pots (25 cm diameter) containing sterilized soil (sand, loam and compost in 1:1:2 ratio). The second group of the plants were raised from *Rhizobium* treated and untreated urd bean seeds in clay pots filled with purified sterilized sand. For *Rhizobium* treatment, before sowing the seeds were treated with 6 days old culture of *Rhizobium phaseoli* effective on urd bean. Sixty pots per treatment were taken containing 5 plants per pot. The detailed experimental plan for supply of nutrient solution and harvesting was the same as described by SINGH & SRIVASTAVA 1985.

Leaf, stem and root were dried in an electric oven at 80±5°C for 24h. Estimation of different nitrogen fractions of plant parts were determined from oven dried samples. The total nitrogen (DONEEN 1932), nitrate and nitrite nitrogen (HUMPHRIES 1956), ammoniacal nitrogen (STROGANOV 1964), total free amino acids (WIGGINS & WILLIAMS 1955) were estimated as

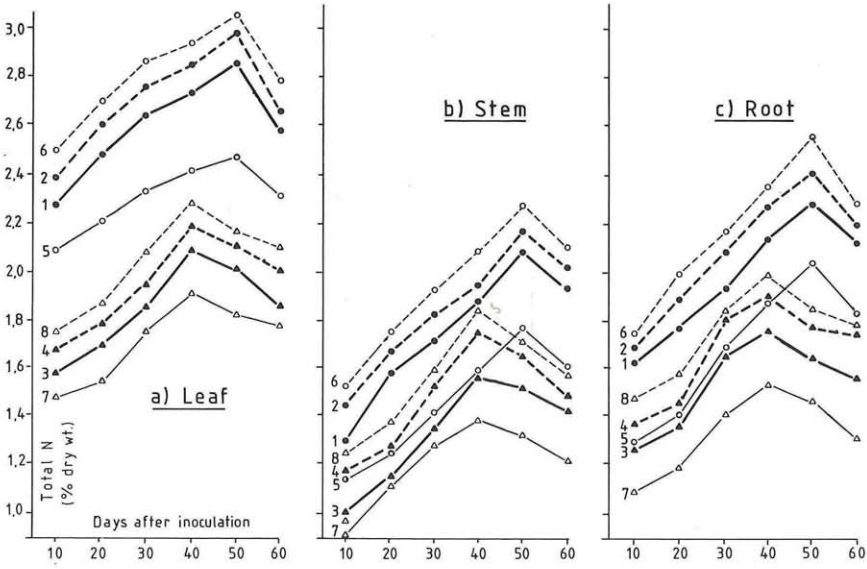

Fig. 1. Effect of urd bean mosaic virus (UBMV) and *Rhizobium* interaction on the total nitrogen content (mg/100 mg = percent dry weight) of leaf (a), stem (b) and root (c) of urd bean.

Symbols: Plants grown in soil; 1 ● ——— ● = *Rhizobium* treated healthy control plants; 2 ● – – – – – ● = *Rhizobium* treated and with UBMV infected plants; 3 ▲ ——— ▲ = untreated healthy control plants; 4 ▲ – – – – – ▲ = untreated with UBMV infected plants. Plants grown in sand: 5 ○ ——— ○ = *Rhizobium* treated healthy control plants; 6 ○ – – – – – ○ = *Rhizobium* treated with UBMV infected; 7 △ ——— △ = untreated healthy control plants; 8 △ – – – – – △ = untreated with UBMV infected plants.

described. For protein the samples were ground with 10% TCA, centrifuged and the residue was placed in an oven at 70°C for drying and nitrogen content was estimated as above and multiplied by 6.25 to get the value of protein. The nitrate reductase activity was measured as described by SRIVASTAVA 1974.

The experiments and the estimations were repeated thrice and their avarages are presented.

Results and Discussion

Fig. 1 shows the course of nitrate nitrogen in leaf, stem and root of virus infected urd bean plants treated with *Rhizobium* and untreated ones in comparison to their healthy counterparts. The level of nitrate nitrogen increases upto 50th day after inoculation (plants grown in soil media) and upto 40th day (grown in sand media) after which they showed a gradual decrease. Because the curves of the other nitrogenous compounds investigated are essentially similar as shown in Fig. 1, although with different amplitudes, its unnecessary to present them in particular. It is sufficient to compare the maximal values completed by indication of the point of time when the maxima appear (Table 1).

The observations of the Table 1 indicate that total nitrogen, nitrate nitrogen, nitrite nitrogen, protein and total free amino acids were higher in urd bean mosaic virus infected urd bean plant parts than their healthy counterparts grown in soil and sand potting media and *Rhizobium* treated and untreated plants while ammoniacal nitrogen was lowered under similar treatments. The contents of total nitrogen, protein and total free amino acids were maximum in leaf followed by root and stem. Nitrate and nitrite nitrogen were higher in root followed by leaf and stem while the ammoniacal nitrogen was higher in leaf followed by stem and root. The level of nitrogenous fractions were maximum on 50th day of inoculation in the plants grown in soil media and on 40th day of inoculation in the plants grown in sand media.

The increase/decrease of the nitrogenous fractions was more pronounced in the *Rhizobium* untreated diseased plants than the *Rhizobium* treated diseased plants.

Similar to the present findings, increase/decrease of nitrogenous fractions in virus infected leguminous plants have been reported in cowpea infected with cowpea mosaic virus (KHATRI & CHENULU 1973), pigeon pea infected with pigeon pea sterility mosaic virus (NARAYANASWAMY & RAMAKRISHNAN 1966, NAMBIAR & RAMAKRISHNAN 1969), *Dolichos lablab* infected with Dolichos mosaic virus (JOHN 1963, RAJAGOPALAN & RAJU 1972).

The increase in level of various nitrogenous fractions appear as a result of virus multiplication involving synthesis of virus specific proteins. It has been reported that in addition to virus protein itself, infected plants con-

Table 1

Effect of urd bean mosaic virus and *Rhizobium* infection on the total-, nitrate, nitrite- and ammoniacal-N, protein and free amino acids of roots, stems and leaves of urd bear *(Vigna mungo)*. The numbers indicate the maximal contents obtained 50 (plants grown in soil, A) and 40 days (plants grown in sand, B) after inoculation respectively. All values (nitrite excepted) as mg/100 mg (= percent) dry weight, nitrite-N as μg/100 mg dry weight.

	Total - N			Nitrate - N			Nitrite - N			Ammoniacal - N			Protein - N			Total free amino acids		
	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf
A. Plants grown in soil																		
Rhizobium - treated																		
Control	2.25	2.06	2.85	0.27	0.17	0.19	15.8	10.3	12.2	0.120	0.125	0.148	10.9	10.2	14.2	1.60	1.35	2.40
Infected	2.36	2.16	2.98	0.31	0.20	0.23	16.2	10.6	13.1	0.112	0.115	0.136	12.9	11.1	15.5	1.72	1.42	2.65
untreated																		
Control	2.00	1.77	2.48	0.25	0.15	o.17	15.6	10.0	11.9	0.114	0.118	0.138	6.5	5.4	9.0	1.49	1.28	2.22
Infected	2.5o	2.24	3.04	0.32	0.21	0.24	16.3	10.9	13.3	0.095	0.100	0.120	8.4	8.0	12.1	1.80	1.50	2.76
B. Plants grown in sand																		
Rhizobium - treated																		
Control	1.75	1.58	2.05	0.13	0.08	0.11	9.4	7.0	8.0	0.095	0.100	0.1o8	7.5	7.4	11.2	0.98	0.90	1.55
Infected	1.88	1.76	2.14	0.15	0.10	0.12	10.0	7.2	8.3	0.080	0.088	0.102	8.6	8.0	12.5	1.09	1.04	1.70
untreated																		
Control	1.52	1.40	1.90	0.12	0.08	0.09	9.1	6.7	7.8	0.090	0.092	0.105	6.9	6.0	9.4	0.86	0.81	1.45
Infected	1.95	1.82	2.25	0.17	0.11	0.14	10.2	7.4	8.5	0.065	0.070	0.096	9.0	8.4	12.8	1.15	1.10	1.74

tained various amounts of abnormal proteins (TAKAHASHI & ISHII 1953, COMMONER & al. 1953, BAWDEN & KLECZKOWSKI 1957, VAN RYSSELBERGE & JEENER 1957).

The accumulation of total free amino acids in virus infected plant parts seems to be due to synthesis of more amino acids to meet the demands of rapid protein synthesis during virus multiplication. An enhanced amine activating system in virus infected plants, reported by HAYASHI 1962, seems to be operative in the present case.

The increase in nitrate and nitrite nitrogen indicated that the virus infection increased the nitrogen demands of the host plant which resulted into enhanced absorption of nitrogen from soil and its speedy conversion into utilizable forms like nitrite nitrogen. Decrease in ammoniacal nitrogen strengthens this view.

Significant reduction in ammoniacal nitrogen in diseased samples seems to be in agreement with the observations made by COMMONER & DIETZ 1952 in TMV infected tobacco. They proposed the theory of *de novo* synthesis of TMV protein from ammonia and nitrogen free carbon source.

The maximum accumulation of total nitrogen, protein and total free amino acids was observed in the infected leaves followed by root and stem. This indicated rapid translocation of these fractions to the foliage where the active virus synthesis was going on.

The results of the present investigation indicate that urd bean mosaic virus infection affected the nitrogenous fractions more in *Rhizobium* untreated plants than the *Rhizobium* treated plants. This may be due to the interaction of virus and *Rhizobium* in *Rhizobium* treated plants leading to lower the concentration of virus with the result accumulation of nitrogenous substances.

Conjointly with the alterations of the nitrogen compounds the nitrate reductase activities were investigated. A perusal of Fig. 2 a–c indicates that virus infection increased the activity of the enzyme in all experiments. The maximum enzymatic activity was observed in the leaf followed by root and stem. The plants grown in soil had more enzymatic activity than of those grown in sand and plants treated with *Rhizobium* than of those *Rhizobium* untreated plants. Highest enzymatic activity was observed at the 10^{th} day and 20^{th} day of inoculation in the plants grown in soil and sand, respectively and thereafter it decreased with increasing age of the plant.

Similar to the present findings, increase in the activity of nitrate reductase in the virus infected leguminous plant parts have been reported in pigeon pea affected with pigeon pea sterility mosaic virus (NARAYANASWAMY & RAMAKRISHNAN 1966), cowpea infected with cowpea mosaic virus (KHATRI & CHENULU 1973) and urd bean infected with broad bean mosaic virus (TRIPATHI & al. 1983).

The higher nitrate reductase activity in virus infected plants is indica-

tive of higher metabolic activity involving utilization of nitrogen which could result from accelerated protein synthesis. Due to alteration in nitrogen status of virosed plants, it seems that most of the nitrogen, after absorption is translocated to the leaves as such, where active virus synthesis is going on. This seems to be a possible reason for the highest activity of the

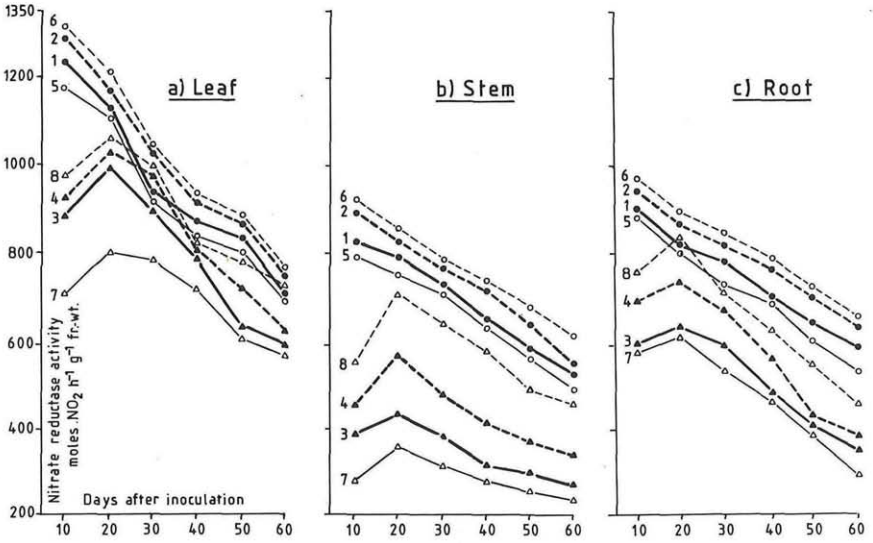

Fig. 2. Effect of *Rhizobium* and UBMV infected on nitrate reductase activity (as moles NO_2 h^{-1} g^{-1} fresh weight) in leaf (a), stem (b) and root (c) of urd bean *(Vigna mungo)* at differents periods of infection under soil and sand potting media. Symbols see Fig. 1.

enzyme in the leaves. The low nitrate reductase activity in the roots may due to the negative geotropic nature of the roots. A much more efficient synthesis of the enzyme takes place when the plant is exposed to light (BEEVERS & al. 1965, HAGEMANN & FLESHER 1960, KANNANGARA & WOOLHOUSE 1967 and ASLAM & al. 1976).

The *Rhizobium* untreated diseased plants have higher nitrate reductase activity than *Rhizobium* treated diseased plants. This is due to the rhizobia that reduces the effect of virus in *Rhizobium* treated diseased plants. It may also be due to the increased demand of nitrogen by *Rhizobium* untreated diseased plants to meet the higher rate of virus multiplication that the *Rhizobium* treated diseased plants.

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