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Nitrogen Metabolism of Groundnut Plants Inoculated with VAM Fungus and Rhizobium

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ABSTRACT

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The inoculation of groundnut plants with VAM fungus of *Glomus mossae* and *Rhizobium* sp. alone and also in combination greatly influenced the levels of total proteins, amino acids and nitrogen fractions. Both qualitative and quantitative changes in free and protein bound amino acids were studied. Accumulation of nitrogen fractions, total proteins, amino acids and the activity of glutamate dehydrogenase was more pronounced in the dual inoculated plants.

Key words : Amino acids, glutamate dehydrogenase, groundnut plant, nitrogen fractions, *Rhizobium*, vesicular - arbuscular mycorrhiza

INTRODUCTION

Vesicular arbuscular mycorrhizal (VAM) colonization stimulate significant increase in phosphate (P) uptake, resulting in dramatic increase in host growth[7,15]. In addition, increased P uptake in mycorrhizal legumes stimulates nitrogen (N) fixation by *Rhizobium*, thus indirectly causing an increase of N concentrations in the host[3,19]. VAM infection is known to cause changes in the biochemical constitution of the host plant[17,21].

The nitrogen nutrition of plants infected with VAM fungi has received very little attention compared with the number of studies on the interaction between mycorrhizal colonization and phosphate nutrition[2, 8]. The present investigation reports the effect of VAM fungus and *Rhizobium* infection on nitrogen metabolism of groundnut plants.

MATERIALS AND METHODS

Preparation of inoculum

Seeds of pure line of groundnut (*Arachis hypogaea* L.) var. TMV2 were employed throughout the study. The surface sterilized groundnut seeds were inoculated with 0.5 ml of rhizobial isolate (10^6 cells). The *Rhizobium* sp. was isolated from groundnut root nodules and maintained on yeast extract mannitol agar, as described by Ahmad et al. (1981) [1]. Mycorrhizal plants are produced by placing 500 mg of mycorrhizal inoculum (approximately 250 spores) 2 cm below seed and also around the seed. Soil : Sand (1:1) mixture containing spores and root segments of maize infected with *Glomus mossae* (Nicol & Gerd) Gerd & Trappe grown for 90 days was used as the mycorrhizal inoculum.

Method of sampling

The treatments T1 and T4 involved in the study and sampling of the material used for analyses at the three stages of infection were the same as described elsewhere[5].

The four treatments involved in the study are : 1. Control (uninoculated plants) : T1; 2. Inoculation with *G. mossae* : T2; 3. Inoculation with *Rhizobium* : T3 ; 4. Inoculation with *G. mossae* & *Rhizobium*: T4; i.e. dual inoculation.

The sampling was done at the stages outlined below :

Stage 1, 15 days after inoculation: characterized by the formation of hyphal threads on the roots. Infection started at this stage. **Stage 2**, 25 days after inoculation: characterized by the formation of arbuscules and vesicles. **Stage 3**, 45 days after inoculation : characterized by the presence of arbuscules, vesicles and spores.

Estimation of nitrogen

About one gram of fresh plant material was chopped into small pieces, extracted with 80% boiling ethanol, ground in a porcelain mortar and reextracted. The extracts were pooled, centrifuged and the supernatant was used for the estimation of nitrogen.

Total nitrogen was estimated according to the method of Markham (1942) [12] and protein nitrogen by that of Thimann and Loos (1957) [20]. Soluble nitrogen fraction was then calculated by subtracting protein nitrogen from total nitrogen. Amino nitrogen in the ethanol extract was determined by the ninhydrin method of Moore and Stein (1948) [14].

The proteins were extracted and estimated according to the method of Lowry *et al*. (1951)[11].

Extraction and assay of glutamate dehydrogenase activity was made by following the method of Triphenyl Tetrazolium Chloride (TTC)[4]. The activity was measured by spectrophotometer based on the formation of formazone as a reduced product of TTC at 495 nm.

RESULTS

Various nitrogen fractions (Total, protein, soluble and amino nitrogen) in both inoculated and uninoculated plants were estimated at different stages and the results summarised in the Fig. 1. All the nitrogenous fractions (total protein, soluble and amino) showed a marked increase at various stages of growth of VAM fungus and *Rhizobium* inoculated groundnut plants. The increase was more pronounced in dual inoculated plants.

The present investigation also shows that inoculated and uninoculated groundnut plants vary with respect to the total protein content, whose concentration was more in dual inoculated plants as compared to the other treatments. In general, protein levels were higher in *Rhizobium* inoculated plants than in uninoculated plants (Fig. 2).

There was a considerable increase in the activity of glutamate dehydrogenase in the VAM and *Rhizobium* inoculated plant tissues, the increase being more pronounced in dual inoculated plants at 45 days (Fig. 2).

DISCUSSION

The greater benefits of dual inoculation for nitrogen and phosphorus uptake in peanut are in agreement with the reports of Moose (1977)[16] in *Stylosanthes* species in pot culture experiments. Increases in nitrogen content, either with individual or with dual inoculants, are in the confirmity with the results of Khan *et al*. (1995)[9]. Increase in protein nitrogen may be ascribed to increase protein synthesis and soluble nitrogen to increase synthesis or hydrolysis of proteins or both.

Increased levels of protein at 45th day in the dual inoculated plants could be attributed to either the presence of fungal proteins or post-infectional stimulation of protein synthesis in the host plant[10]. Mycorrhizal and non-mycorrhizal plants are known to differ in their biochemical constitution particularly in the amino acid and protein fractions[17]. According to Krishna *et al*. (1983)[10] the protein levels were higher in mycorrhizal roots of groundnut plants than in non-mycorrhizal roots.

Increase in the activity of glutamate dehydrogenase with dual inoculation is in confirmity with the results reported earlier by Dehne (1986)[6] who has observed increased activities of glycolytic enzymes and various dehydrogenases in VAM plants. Rosendahl (1992) reported only minor changes in the activity of GDH in the host plant[18]. The most important function of GDH in plants is the catalysis of amino acids and diminution of glutamate[13]. The presence of VAM in the roots may be influencing the amino acid metabolism of the host by increasing the specific activity of the enzymes concerned.

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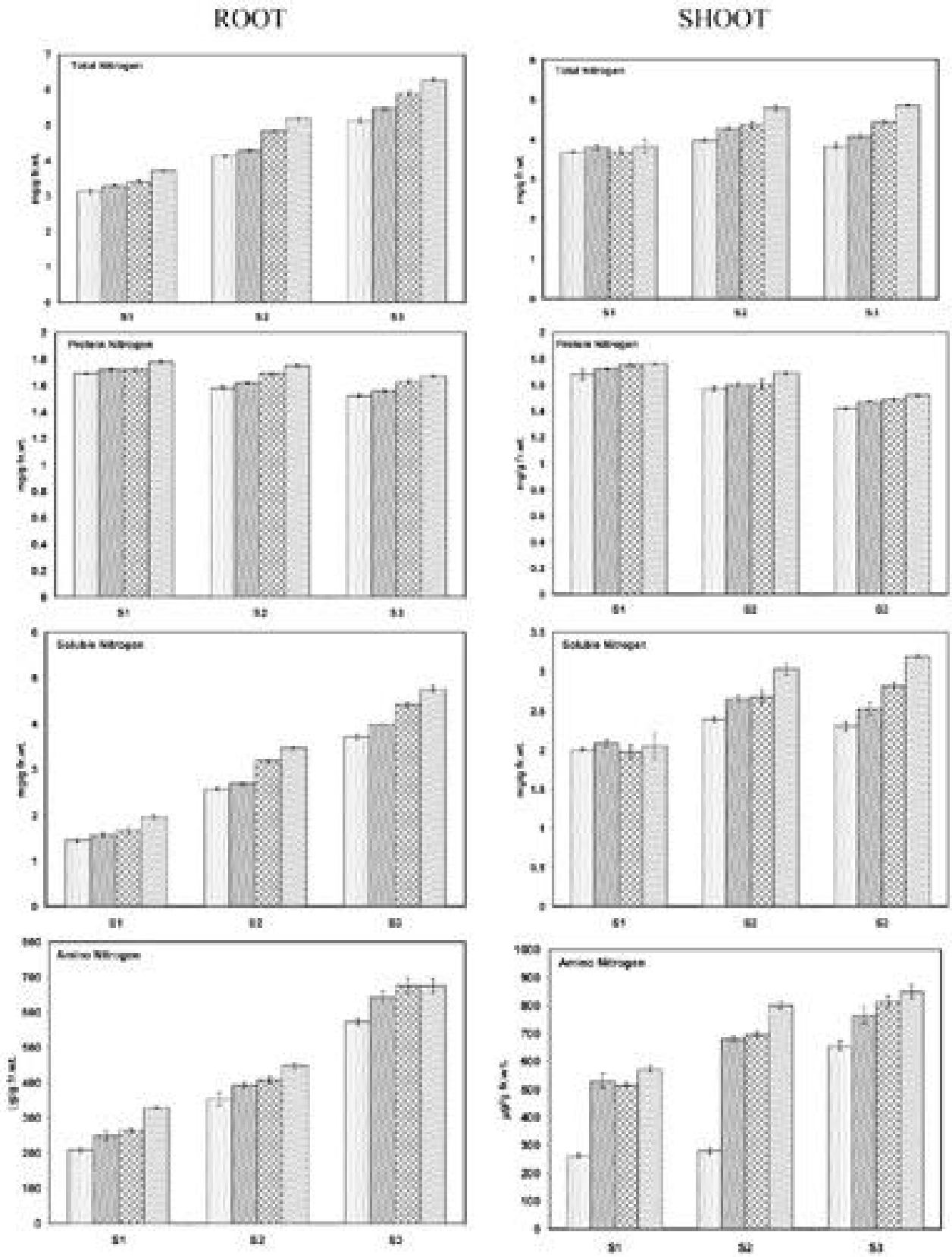

Fig. 1. Effect of VAM fungus and *Rhizobium* on nitrogen fractions of groundnut. (S1, S2, S3 representing 15, 25 and 45 days after inoculation; T1-check, T2-VAM, T3-*Rhizobium*, T4-VAM+*Rhizobium*, represent treatments involved (cf. text)).

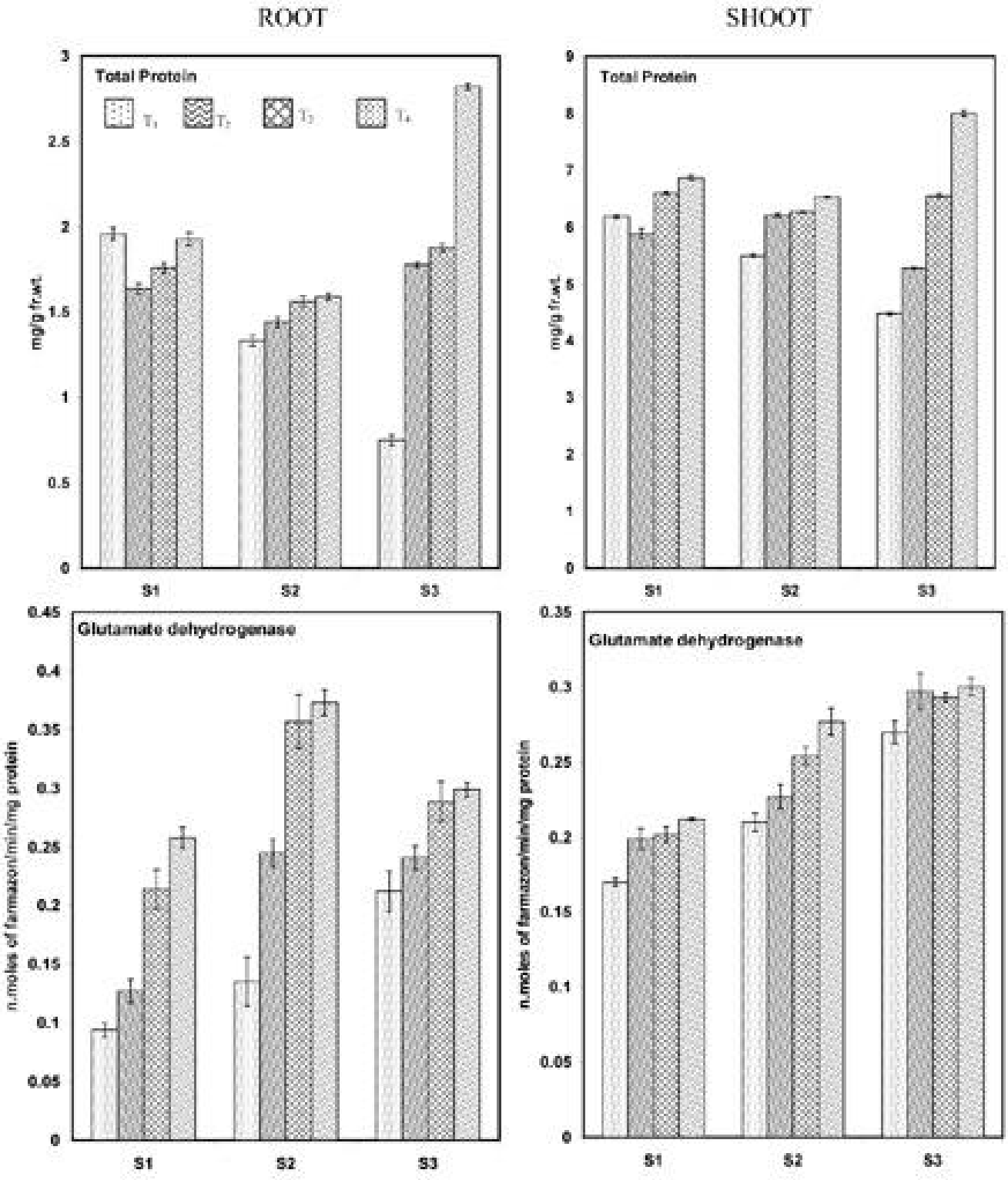

Fig. 2. Effect of VAM fungus and *Rhizobium* on total protein contents and glutamate dehydrogenase activity in groundnut. (S1, S2, S3 representing 15,25 and 45 days after inoculation; T1-check,T2-VAM,T3-*Rhizobium*,T4-VAM + *Rhizobium*, represent treatments involved (cf. text))

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摘 要

Charitha Devi, M.[1] and Reddy, M. N.[1,2] 2005. 內生菌根菌與根瘤菌對落花生氮素代謝之影響. 植病會刊14: 177-182. ([1] Department of Applied Microbiology, Sri Padmavti Mahila Visvavidyalayam, Tirupati - 517 502, India; [2] Corresponding author : E-mail : mopuri_nr@yahoo.com)

將內生菌根菌 (*Glomus mossae*) 與根瘤菌 (*Rhizobium* sp.) 共同或單獨接種落花生根部，均會嚴重影響其蛋白質總量、氨基酸及氮素部份的含量。本試驗探討蛋白質中氨基酸與游離氨基酸的量與質，發現共同接種內生菌根菌與根瘤菌者，其氮含量、蛋白質總量、氨基酸及 glutamate dehydrogenase 的累積均較單獨接種者為明顯。

關鍵詞：氨基酸、glutamate dehydrogenase，落花生、氮素代謝、內生菌根菌、根瘤菌