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Associations of sugar beet and nitrogen-fixing bacteria *in vitro*

N. MRKOVAČKI, S. MEZEI, I. VEREŠBARANJI, M. POPOVIĆ*, Z. SARIĆ and L. KOVAČEV

Scientific Institute of Field and Vegetable Crops, 21000 Novi Sad, M. Gorkog 30, Yugoslavia
*Faculty of Natural Sciences, 21000 Novi Sad, Dositeja Obradovića 3, Yugoslavia**

Abstract

The associations of four *Azotobacter chroococcum* strains (2, 5, 8 14) isolated from the rhizosphere with two sugar beet hybrids (Hy-11 and Dana) grown *in vitro* have been investigated. All tested strains caused an increase in dry mass of both hybrids almost proportionally to the nitrogen content in the medium. Plant nitrogen content was also higher in inoculated variants. The highest nitrogenase and glutamate dehydrogenase activities were in Hy-11 associated with strain 5 and in hybrid Dana associated with strain 8. These associations were the most effective in N_2-fixation 2 weeks after inoculation. The results showed specificity of individual strains with respect to individual hybrids.

Additional key words: Azotobacter strains, *Beta vulgaris*, glutamate dehydrogenase, nitrogenase.

Introduction

Numerous papers showed a positive plant response to inoculation with associative N_2-fixing bacteria - *Azotobacter, Azospirillum, Enterobacter, Klebsiella.* The average yield increase was 20 - 31 % with *Azospirillum*, and 11 % to 22 % with *Azotobacter* (Jagnow 1987). However, there are some papers where positive responses to inoculation were lacking.

The effect of *Azotobacter* on yield of bengal gram, wheat, rice, soybean, vegetables and *Setaria* has been described (Allison 1947, Cooper 1959, Maskey 1977, Khullar *et al.* 1978, Yahalom *et al.* 1984, Iswaran *et al.* 1987). Investigations of N_2-fixation with *Azotobacter* were related to wheat, maize and sunflower. It has been found that specific relation exists between *Azotobacter* strains and plants and that N_2-fixation varies due to strain and plant genotype (Sarić *et al.* 1991, Krstić *et al.* 1991 and Mrkovački *et al.* 1995).

We selected strains from natural conditions, *i.e.* from rhizosphere of sugar beet

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Abbreviations: NG - nitrogenase, GDH - glutamate dehydrogenase.

and tested association of 2 sugar beet hybrids and 4 *Azotobacter chroococcum* strains bred *in vitro*, according to their N_2-fixing activity. The aim of this paper was to determine whether the strains of *A. chroococcum* have the capacity to fix nitrogen in the association with sugar beet hybrids and whether some strain exhibit specificity with respect to some hybrid.

Materials and methods

The N_2-fixing activity of 4 *Azotobacter chroococcum* strains (2, 5, 8, 14) isolated from sugar beet rhizosphere and associated with sugar beet (*Beta vulgaris* L. ssp. *vulgaris* var. *altissima* Döll) hybrids Hy-11 and Dana (created in the Institute of Field and Vegetable Crops in Novi Sad) have been investigated. The experimental plants were grown in vials (volume of 100 cm^3, 1 plant per vial) on MS medium (Murashige and Skoog 1962) solidified with agar in a growth chamber (temperature 22 °C, photoperiod 16 h, relative humidity of air 90 %). Beside the treatment with the full nitrogen dosage (1 N - 20.6 mmol NH_4NO_3 + 18.8 mmol KNO_3) in the medium, we have also used treatments 1/4 N, 1/8 N and without nitrogen (0 N).

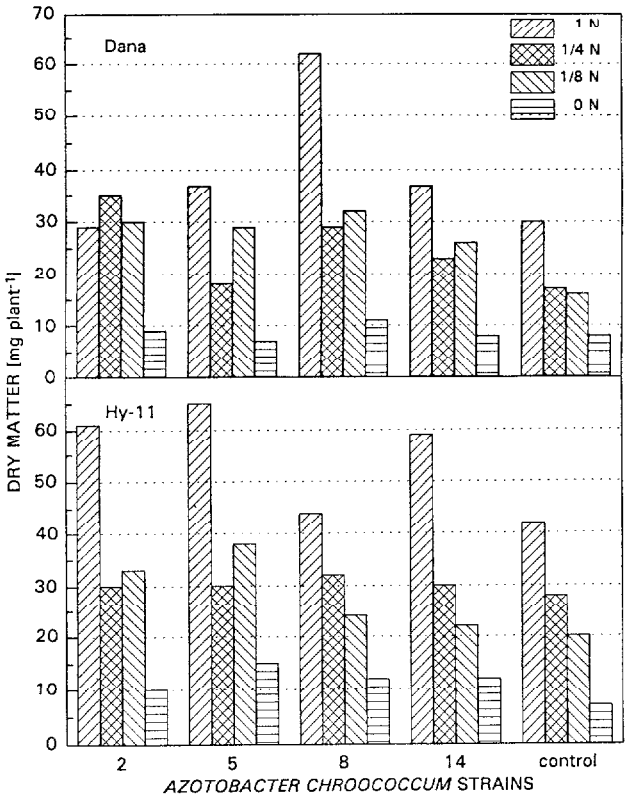

Fig. 1. Effect of inoculation with *Azotobacter chroococcum* strains and of medium nitrogen concentration on dry matter of two sugar beet hybrids.

Two weeks old seedlings were inoculated with liquid *Azotobacter* strain culture (0.1 cm^3 per plant; 10^9 cells per cm^3), grown in Fjodorov medium. Noninoculated plants served as control (C). The experiment was performed in 6 replications. Nitrogenase (NG) activity and nitrogen content was measured in plants 2 weeks after inoculation. NG activity was estimated by acetylene reduction assay of Hardy *et al.* (1968) using gas chromatograph *Hewlett Packard 5840* and nitrogen content by the Kjeldahl's method. Glutamate dehydrogenase (GDH) activity was estimated by the method of Farnden *et al.* (1980), in a reaction mixture: 1 cm^3 125 mM Tris - HCl (pH = 8.2), 0.2 cm^3 10 mM $CaCl_2$, 0.2 cm^3 155 mM α-ketoglutarate, 0.2 cm^3 enzyme extract, 0.2 cm^3 1.5 mM NADH and 0.2 cm^3 1850 mM CH_3COONH_4. The content of soluble proteins was determined in plant material extracted with phosphate buffer (pH = 7.5) by the method of Lowry *et al.* (1951).

The activity of enzymes (NG, GDH) and amount of soluble proteins in plants were statistically processed by the LSD method ($P = 0.05$).

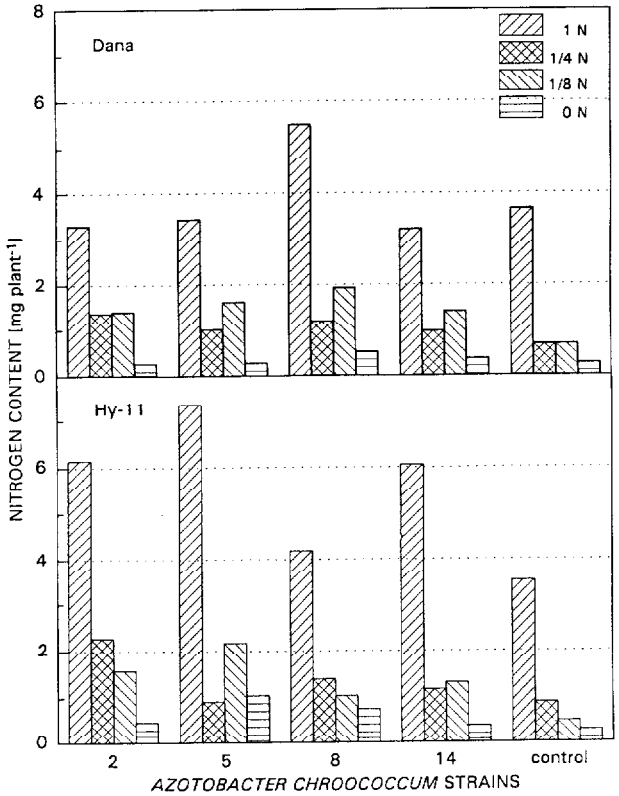

Fig. 2. Effect of inoculation with *Azotobacter chroococcum* strains and of medium nitrogen concentration on nitrogen content of two sugar beet hybrids.

Results

Dry mass of inoculated plants of both hybrids of sugar beet was higher than that of noninoculated plants (Fig. 1). The lowest dry mass had the plants on medium without nitrogen both inoculated, and noninoculated and the highest dry mass had plants on medium with a full nitrogen concentration. All tested *Azotobacter* strains caused an increase in plant dry mass almost proportionally with the nitrogen content in the medium. The highest dry mass was obtained in Hy-11 inoculated with strain 5 and Dana with strain 8 at full nitrogen dose (Fig. 1).

Nitrogen contents in plants were higher in Hy-11 associated with all strains and in Dana only associated with strain 8 (Fig. 2).

Two weeks after inoculation, only plants grown on media with 1/8 N and 0 N showed NG activity (Fig. 3). The highest NG activity (significantly different from others) was in Hy-11 inoculated with strain 5 and in Dana with strain 8. Noninoculated plants did not show any nitrogenase activity.

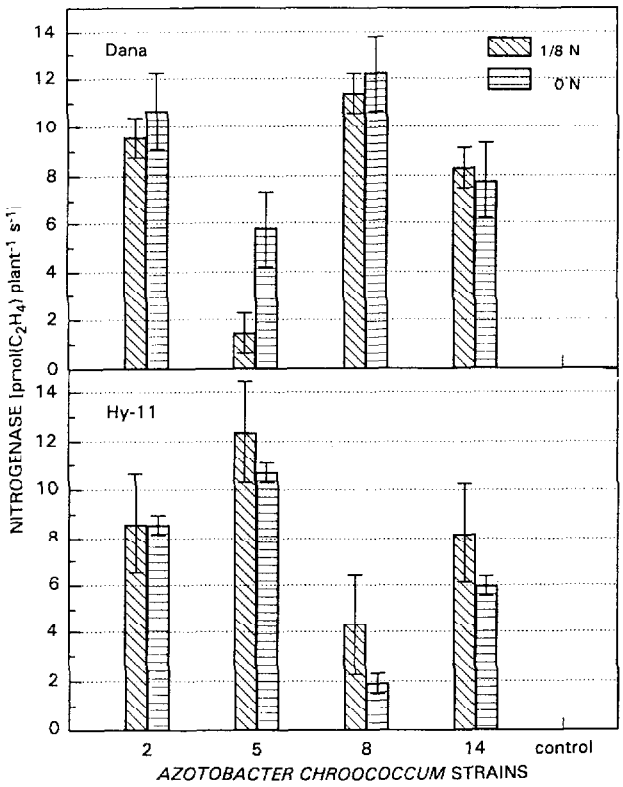

Fig. 3. Effect of inoculation with *Azotobacter chroococcum* strains and of medium nitrogen concentration on nitrogenase activity of two sugar beet hybrids.

GDH activity in inoculated plants was slightly (but significantly) higher than in noninoculated plants of both hybrids (Fig. 4). High GDH activity was noticed in Hy-11 in association with strain 5 grown on medium with 1/8 N and 0 N. High

nitrogen concentration in medium (1 N) suppressed GDH activity in Hy-11. The highest GDH activity was in Dana inoculated with strain 8 grown on medium with 1/4 N and 1/8 N.

The content of soluble proteins was significantly higher in inoculated plants of both hybrid (Fig. 5) grown at all nitrogen concentrations in the medium. Especially effective was strain 5 in association with the hybrid Hy-11 and strain 8 with the hybrid Dana.

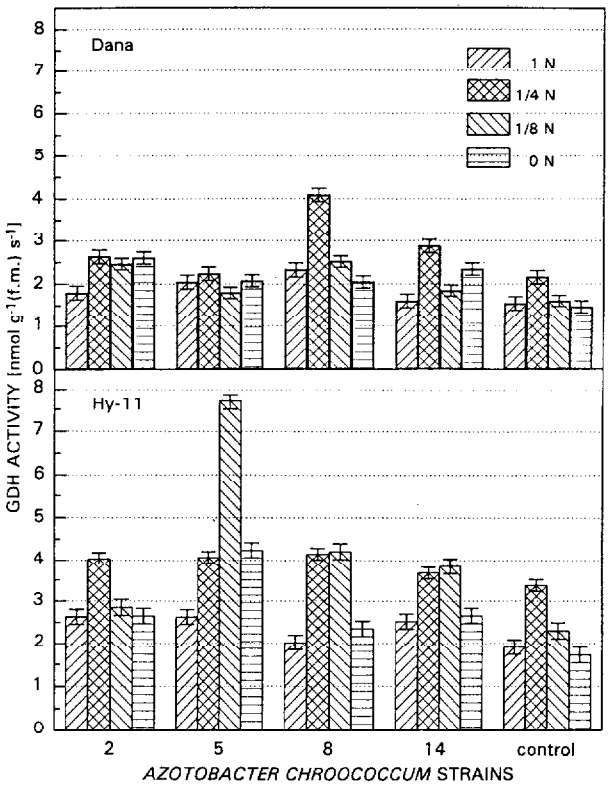

Fig. 4. Effect of inoculation with *Azotobacter chroococcum* strains and of medium nitrogen concentration on glutamate dehydrogenase activity of two sugar beet hybrids.

Discussion

Berkum and Sloger (1983) estimated a negative correlation between NG activity in roots of cultivated rice and wild rice and relative supply of nitrogen in the root environment. Similar inverse relationship between N_2-fixation and availability of nitrogen was estimated with *Azotobacter vinelandii* in pure culture and in soybean field. On the other hand, in our experiments nitrogen content in sugar beet plants increased with the increase of nitrogen concentration in the medium, which corresponds to the results of Sarić *et al.* (1987).

Although grass crops require nitrogen fertilizer for good growth (Scarsbrook 1965, Viets 1965), nitrogen in soil repressed NG activity of N_2-fixing bacteria (*e.g. Azotobacter* - Burns 1975) but increased nitrogen concentration of bacterial cells.

Therefore, we were able to determine nitrogenase activity in treatments 1/8 N and without N. Berkum (1980) concluded that also during longer period of root

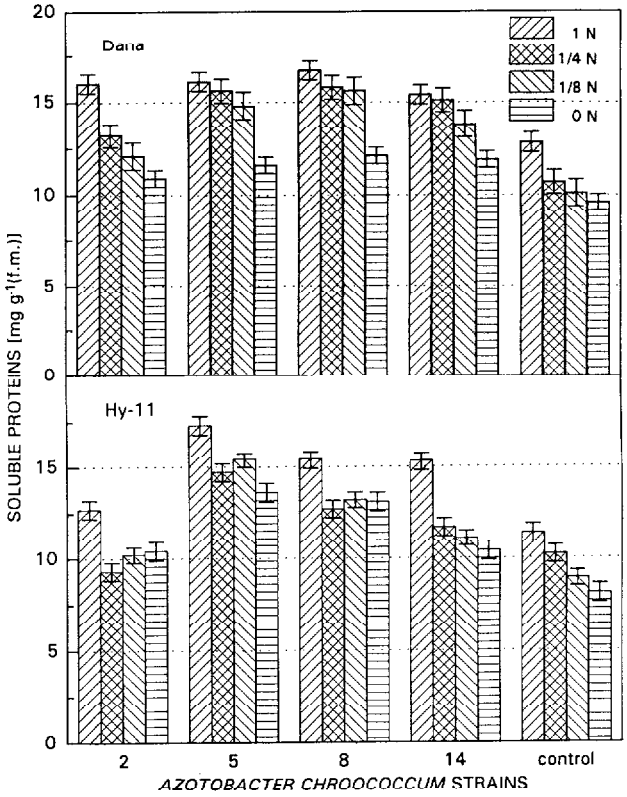

Fig. 5. Effect of inoculation with *Azotobacter chroococcum* strains and of medium nitrogen concentration on protein contents of two sugar beet hybrids.

incubation, NG activity was increased when the quantity of nitrogen in bacterial medium was sufficiently decreased. In our experiments the same effect could be seen on medium with 1/8 N. Gašić *et al.* (1990) also obtained dependence of NG activity on nitrogen content in the medium in association of *Azotobacter* and wheat, but the differences among strains were not significant.

The obtained results show that the investigated associations of sugar beet plants and *Azotobacter* strains are characterized by significant differences in plant growth and N_2 fixation. These differences improved selection of active strains. The most effective were associations of hybrid Dana with *Azotobacter* strain 8 and hybrid Hy-11 with *Azotobacter* strain 5.

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