



SUPPLEMENTARY MATERIAL TO

Isoflavone content and antioxidant activity of soybean inoculated with plant-growth promoting rhizobacteria

BILJANA KIPROVSKI^{1*}, ĐORĐE MALENČIĆ², SIMONIDA ĐURIĆ², MIRA BURSAĆ³, JELENA CVEJIĆ³ and VLADIMIR SIKORA¹

¹*Institute of Field and Vegetable Crops, Maksima Gorkog 30, 21000 Novi Sad, Serbia,*

²*Faculty of Agriculture, University of Novi Sad, Trg Dositeja Obradovića 8,*

³*Faculty of Medicine, Department of Pharmacy,*

University of Novi Sad, Hajduk Veljkova 3, 21000 Novi Sad, Serbia

J. Serb. Chem. Soc. 81 (11) (2016) 1239–1249

MATERIAL AND EXPERIMENTAL DESIGN

Seeds of soybean (*Glycine max* (L.) Merr.) cultivar Bečejka were obtained from the Institute of Field and Vegetable Crops, Novi Sad, Serbia. A three-year experiment was conducted at the Rimski Šančevi (coordinates: 45°20'N, 19°51'E) experimental field located on calcareous chernozem on a loess terrace. The chemical properties of the soil were: pH 8.11 (in H₂O) and 7.43 (in KCl); N content 0.14 %; 1.56 % CaCO₃; 3.62 % humus content and available P and K contents of 31.14 and 31.10 mg 100 g⁻¹ soil, respectively. The seeds were inoculated before sowing by submersion in inoculums (700 mL water and 300 mL inoculum) of *Azotobacter chroococcum* (AB), *Streptomyces* sp. (S) and mixture of these bacteria (MIX, 700 mL water and 150 mL of each inoculum, AB and S) for 1 h. Control seeds were submersed in water. Seeds were sown at a depth of 10 cm. Sowing was performed between 1st and 10th April 2009, 2010 and 2011 in the early morning. The experimental design was a randomized block with four replications. Sowing was performed in an area of 34.5 m×16.5 m. The length of the experimental unit was 8 m and the width was 3.5 m. Plant spacing between rows was 0.5 m and within row, 3–5 cm. Weather conditions at the experimental field are given in Fig. S-1.

Plant material (leaves and roots) for biochemical analyses were sampled at four stages of development. The first period (I) was at the stage of the second trifoliolate (V2), the second period (II) was at the stage of the full bloom (R2), the third (III) was at the seed beginning stage (R5) and the fourth (IV) was the stage after the harvest, when only mature seeds were sampled. For biochemical analyses, 40 plants (*N* = 40) were sampled by hand from each replication, but the final harvest for the yield assessment was performed by sickle. Border plants were not harvested.

A part of fresh collected leaves and roots were immediately frozen in liquid nitrogen and the other parts were dried in a shaded and well-ventilated location. The fresh extracts were prepared by grounding 1 g of fresh leaves in liquid nitrogen with cooled mortar and pestle and

*Corresponding author. E-mail: bkiprovski@gmail.com

then homogenized with 10 mL of phosphate buffer solution (0.1 M K_2HPO_4 , pH 7.0). After centrifugation at 15000g for 10 min at 4 °C, aliquots of the supernatant were used for enzyme activity measurements. The dry extracts contained 10 mL MeOH:H₂O:CH₃COOH (140:50:10) and 0.2 g of dry leaves or roots powder. The extracts were rapidly vacuum-filtered through a sintered glass funnel and kept refrigerated until assayed.

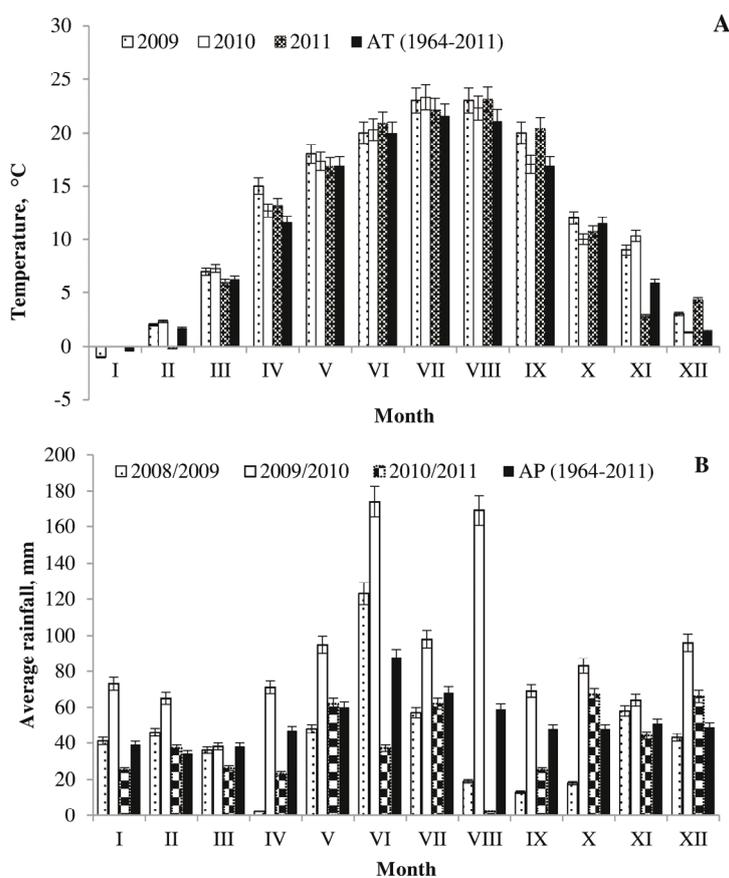


Fig. S-1. Average monthly air temperatures (AT) and precipitation (AP) (A and B, respectively) at the experimental field at the Rimski Šančevi site (45°20'N, 19°51'E, 86 m below sea level) in three-years of the experiment and in the period from 1964 to 2011.

TABLE S-I. Number of nodules per root (sampling period II), number of pods per plant (sampling period III), mass of 1000 seeds and seed yield per ha (end of the experiment). C - control, AB - *A. chroococcum* inoculum, S - *Streptomyces* sp. inoculum, MIX - mixture of AB and S. I, II and III - sampling periods. The results represent the mean of 3 years of research \pm standard error. Results marked with different letters differ significantly at $P < 0.05$ (Duncan's test).

Treatment	Number of nodules per plant	Number of pods per plant	Mass of 1000 seed, g	Seed yield t ha ⁻¹
C	25.2 \pm 1.0 ^b	31.3 \pm 1.2 ^{ab}	139.9 \pm 0.1 ^c	4.0 \pm 0.9 ^b
AB	33.3 \pm 1.9 ^{ab}	30.0 \pm 1.3 ^b	129.4 \pm 0.2 ^d	4.0 \pm 0.9 ^b
MIX	36.5 \pm 1.5 ^a	39.5 \pm 1.7 ^a	146.7 \pm 0.1 ^b	5.0 \pm 0.2 ^a
S	37.2 \pm 2.4 ^a	34.0 \pm 1.1 ^{ab}	150.5 \pm 0.4 ^a	4.1 \pm 0.2 ^b