Soybean field outlook: water use efficiency and *Bradyrhizobium japonicum*

**Abstract**

At the plant field level traditional definitions of water use efficiency (WUE) contain non-beneficial component of water consumed – evaporation. This is one of the reasons for the very big difference between WUE at the leaf and cenosis level (up to 30 times). That is why, without a doubt, the main way to increase WUE at the cenosis level must be due to the practices that reduce the soil water evaporation. Other component of WUE is yield and seed productivity per unit of plant leaf surface. For soybean local introducing of suspension of *Bradyrhizobium japonicum* with clay minerals in soil not only at sowing, but also at reproductive stages of ontogenesis, may be promising tool of yield increasing.

**Keywords:** soybean, water use efficiency, *Bradyrhizobium japonicum*

**Abbreviations:** B. japonicum, nitrogen fixing bacteria *Bradyrhizobium japonicum*; DAP, day after planting; E, soil evaporation; ET, evapotranspiration; ha, hectare; kg, kilogram; LA, leaf area per plant; mm, millimeter; T, transpiration; WUE, water use efficiency

**Introduction**

The efficient use of limited water resources is an urgent issue for the Republic of Moldova. In field experiments at the level of cenosis water-use efficiency (WUE) is defined as yield (Y) divided by applied water.¹ As the measure of applied water or water consumption of the crop usually used the evapotranspiration (ET)-the mass of water evaporated in the cenosis during the growing season by the soil (evaporation, E) together with plants (transpiration, T). E is included in water requirement since Briggs and Shantz (1913),² in view to practical difficulties to estimate separately beneficial (T) and non-beneficial (E) consumption of water. In connection with the use of portable gas exchange analyzers (simultaneously CO₂ and H₂O), it was proposed to evaluate WUE at the leaf level as a ratio of carbon dioxide assimilation (A) to water consumed, expressed as transpiration (T); the time-scale for defining WUE can be instantaneous (i).³

In addition to water consumption another component of WUE is the yield. The use of nitrogen-fixing bacteria *Bradyrhizobium japonicum* (B. japonicum) can be a means of increasing the soybean yield. Nitrate-N has disadvantages to increase yield. At first, nitrate reduces nodulation by 6 times.⁴ Secondly, when using nitrate, vigorous preanthesis growth can increase plant transpiration, at the expense of soil evaporation, and lead to a higher C fixation, but structural carbohydrate laid down earlier in the growing season is not readily mobilized to the grain, and receiving nitrate-N did not result in increased harvest index.⁵ In our study we set two objectives: 1) in one field experiment with soybean to determine WUE at the leaf level and at the level of cenosis; 2) in other field experiment to determine the effect of late (at the reproductive stages of plant ontogenesis) introduction into the soil of *B. japonicum* on some morphophysiological reactions of soybean plants. For these purposes, in 2019 two field experiments were carried out: 1) with the determination of yield and ET for calculating WUE at the level of cenosis, as well as determining instantaneous WUE at the leaf level with a portable gas analyzer; 2) microexperiment with late (70 DAP) local introduction of rhizobia into the soil under plants with the assessment of the stages of ontogenesis and seed productivity of the leaf area per plant with the observation of later nodulation.

The experimental site was located in Chisinau, Republic of Moldova, at the field of the Experimental Station of the Institute of Genetics, Physiology and Protection of Plants. The average (2001-2018) precipitation for period May-August is 221±4 mm. During the experiment, the site examined value was nearly to this average, 200 mm. The objects of the study were soybean plants Glycine max L. (Merr.) of variety Aura, the main variety in the Republic of Moldova. Seeds of the 2018 harvest were taken for sowing. A randomized experiment was in 4 replicates (plots of 3,6 m² with density 20 plants m⁻², distance between rows 0,50 m). Measurements of leaf gas exchange parameters was carried out with a portable gas analyzer ADC BioScientific LCpro-SD (Great Britain). Soil samples for determining the water content (by drying at 105°C to constant weight) were taken at sowing and harvesting (in a layer of 0-150 cm with an interval of 10 cm) to determine ET as the sum of soil water balance and precipitation for vegetation period in soybean cenosis). Several other determinations of soil water content in layer 0-150 cm were made at bare soil field for determining E (as the sum of soil water balance and precipitation). The microexperiment with late local introduction of rhizobia into the soil under plants was carried out in 4 different rows (repetitions) of 2 m, with 3 m long intermediate plant rows without introducing in soil *B. japonicum* and clay mineral between plots with introducing in soil or only bentonite or bentonite with *B. japonicum*. On 70 DAP (04.07.2019) the soil was artificially enriched by population of *B. japonicum* strain RD2. Pure culture of cells *B. japonicum* strain RD, was provided by Dr. L. Onofrash (Institute of Microbiology and Biotechnology, Republica Moldova, Chisinau). In the experiments, we used highly dispersed clay mineral bentonite, provided by Dr. O. Bolotin (Institute of Geology and Seismology, Republica Moldova, Chisinau). A suspension of *B. japonicum* with bentonite was prepared. During preparation, the ratio of bentonite with rhizobia was 10⁻⁴ mg of bentonite/bacterial cell. Inoculation rate...
was $2 \times 10^6$ B. japonicum strain RD$_2$ cells (in 100 ml of water) per plant. Distance in row between plants was 10 cm. In the control variant, the culture of rhizobacteria was not added to the suspension of clay mineral, which was introduced in soil as separate doses, each in 100 ml of water.

### WUE on the leaf and cenosis levels

According to measurements of leaf transpiration and CO$_2$-assimilation on soybean plants in field, instantaneous WUE (A/T) at leaf level was 15.6 ± 0.3 g CO$_2$/kg H$_2$O (Table 1).

#### Table 1 Leaf transpiration (T), CO$_2$-assimilation (A) and instantaneous WUE (A/T) for soybean variety Aura at leaf level in field (2019, 97 DAP)

<table>
<thead>
<tr>
<th></th>
<th>T, mmol H$_2$O m$^{-2}$ s$^{-1}$</th>
<th>A, μmol CO$_2$ m$^{-2}$ s$^{-1}$</th>
<th>instantaneous WUE (A/T), g CO$_2$/kg H$_2$O</th>
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<tbody>
<tr>
<td></td>
<td>2.09 ± 0.12</td>
<td>13.33 ± 0.86</td>
<td>15.6 ± 0.3</td>
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</table>

The amount of precipitation for the 2019 vegetation season is approximately 200 mm. During this period, in soybean cenosis soil water reserves in the 0-150 cm layer decreased by 128 mm. The evapotranspiration value is 200+128=328 mm. The soybean yield was 164.5±10.0 g m$^{-2}$ or 0.50±0.03 g seed kg$^{-1}$ of water for evapotranspiration. This value corresponds to the literature WUE value for soybean fields, 0.6-1.0 kg grain m$^{-3}$ ET water$^6$ and 30 times less than instantaneous WUE (A/T) at leaf level. To assess the magnitude of evaporation, we determined the change in soil water reserves at the bare soil field. From the time of sowing (April 26, 2019) to July 22, 2019 (87 DAP), the soil water reserves of bare soil decreased by 35 mm. Rains (80% of which in 2019 were less than 20 mm) did not lead to an increase in water reserves of bare soil (these reserves even decreased). Accounting precipitations of 153 mm for May-July 2019, evaporation (bare soil) for 87 days of 2019 (counting from the sowing) was 188 mm (average ~2 mm day$^{-1}$). It is important that in the cenosis during the first half of the growing season, the leaf canopy cannot significantly reduce the evaporation losses of water. Non-beneficial losses of available water of 200 mm – this is very much, more than half of the precipitation amount (318 mm), needed for a maximum soybean WUE.$^7$ For example, for nearly 40x10$^6$ ha of soybean fields in USA 100 mm corresponds to 40 billions m$^3$ liters of potentially potable water. That is why, without a doubt, the main way to increase WUE at the cenosis level must be due to the practices that reduce the soil water evaporation.

### The late local introduction of B. japonicum into the soil near plants

1.5 months after the introducing of bentonite and B. japonicum in soil, the developmental stage was determined according to Fehr, Caviness (1977).$^8$ The stage of 10 plants in each of 4 repetitions was taken in account. 17.08.2019 (113 DAP) in both variants stage of ontogenesis was the same, 5.4±0.1. The leaf surface of reference plants was measured non-destructively several times per season (last time, August 23). Seed productivity of plant leaf surface was determined as seed weight per plant at harvest (16.09.2019) divided on plant leaf surface area (in dm$^2$). Including already fallen leaves: these values in both variants were the same, 0.57±0.07 g seeds dm$^{-2}$ for control plants and 0.59±0.07 g seeds dm$^{-2}$ for plants after introducing B. japonicum in soil. Thus, late addition of B. japonicum does not show (at least 43 days after inoculation) plant developmental shift. When harvesting, plants of two variants in study differed in nodulation: more nodules are on the plant roots with introducing B. japonicum in soil (Figure 1).

### References


### Acknowledgments

None.

### Conflicts of interest

Author declares no conflict of interest.


