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Drought-driven respond of fast and slow growing components of an adapted clover *Rhizobium* strain

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ABSTRACT Drought tolerance of a selected *Rhizobium leguminosarum* bv. *trifolii* strain was tested in different, representative Hungarian soils *in vitro.* The stress-adapted strain (Zh-15) was isolated from the active root-nodules of the white clover (*Trifolium repens* L) grown in the highly salinic soil at Zám natural reserved area, Hungary. The water holding capacities (VK) of two sandy, calcareous and two, clayey, acidic soils was estimated and different water levels 30% (drought) and 60% ratio (optimum) of the total VK values was maintained for three weeks. The sterilised soils were inoculated preliminary with rhizobium suspension at a level of 10^6 cfu. g^{-1} soils. A selective plate counting method, modified in the lab. (Angerer et al. 1998) was used to assess the bacterium abundance in the soil. The survival of the drought-tolerant Zh-15 strain was highly depended on the soil characteristics on a certain water level. Clayey soils were found to protect the inoculums more efficiently, in comparison with the sandy soils. Such a modified cell-count, however, revealed that there are slow- and fast-growing components of the used, homogenous *Rhizobium* population. The fast growing, large colonies ("r" strategists) and the slow growing, tiny colonies ("k" strategists) had distinct survival capacities in the soils. The "k"-type cells were found to be more dependent on the soil characteristics, with an opposite survival tendency in the sandy or the clayey soils. This finding may outline the consideration of the strain- and soil characteristics, when designing microbial inoculums. The enumeration of the "r" and "k" strategist microbes, as introduced or indigenous in the soils, can be a new alternative in the environmental monitoring and eco-toxicology.

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KEY WORDS

microbial inoculums
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Nitrogen-fixing rhizobium inoculations are sustainable biotechnological treatments in the agriculture. The possible and efficient use of those inoculums is well known since 100 years (Biró 2002). A higher inoculums-dependency was found in those soils, where the macro- and micro-symbionts are not endemic, like the soybean (*Glycine max* L) - *Bradyrhizobium* symbiosis (Botha et al. 2004). Intensive agricultural practice with frequent use of the different agrochemicals (pesticides and fertilizers) or other pollutants may reduce the abundance of those beneficial microbes (Biró et al. 1993; Mikanova et al. 2001) which also highlights the importance of additional soil-plant-inoculations. Due to the deleterious effect of the abiotic environmental stress-conditions (drought, acidity, salinity…etc.), the free-living (*Azotobacter* sp.), the associative (*Azospirillum* sp.) and the symbiotic (*Rhizobium, Bradyrhizobium, Synorhizobium*…etc. sp.) nitrogen-fixers (Zahran 1999) and also the algae and cyanobacteria (Sprent and Sprent 1990) are known, as the most sensitive microbes in the soils. Adapted micro-symbionts, therefore can be used efficiently to survive and protect their hosts from those stressors (Vivas et al. 2003). Such beneficial microbes in the rhizosphere, as the first protecting level of the soil-plant-animal-human food chain (Kádár 1995) can increase the food production and can be used as possible biosensor of the soil fertility or functioning. More frequent studies are necessary, however, to learn how and which extent can the introduced microbial components survive and function in the soil-plant systems, as a function of the various environmental stress conditions *in situ* (Köves-Péchy et al. 2003).

Model experiment was designed with 4 Hungarian representative soil types, and with a drought- (and salt-tolerant) *Rhizobium* strain to detect the survival capacity and growth characteristics at different water levels.

Materials and Methods

Representative soils

Four Hungarian characteristic soils were selected for the study, such as the calcareous- sandy and chernoziem soils of Őrbottyán (Őb) and Nagyhörcsök (Nh), or the acidic sandy- and brown clayey forest soils of Nyírlúgos (Nyl) and Gyöngyös (Gy), respectively. The main soil characteristics are shown in the Table 1.

Test organism

One drought- and salt-tolerant strain (Zh-15) of the *Rhizobium leguminosarum* bv. *trifolii* species, which is the

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Table 1. Main soil physical and chemical characteristics of four representative Hungarian soils*

Soil and origin	pH H_2O	KCl	Plasticity (A_f)	Total salt (%)	$-CO_3$ (%)	Loamy part (%)	Humus (H %)
Chernoziem soil Nagyhörcsök (Nh)	8.1	7.6	40	0.02	10	36	2.55
Calcareous sand Őrbottyán (Őb)	8.3	7.3	22	0	15	6	0.69
Brown forest soil Gyöngyös (Gy)	6.8	5.8	44	0.04	0	57	3.05
Acidic sand Nyírlugos (Nyl)	5.4	3.9	25	0	0	5	0.71

*According to Buzás (1998)

microsymbiont of the white clover (*Trifolium repens* L.) was used in the study. The strain was isolated from the effective root nodules of the clover, grown in the highly saline circumstances at the Hortobágy National Park (Zám), Hungary (Biró et al. 2002).

Water and bacterial treatments

The maximum water holding capacity (VK) of the representative soils was estimated by Buzás, 1988. According to those data the optimal field-levels (calculated as the 60% of the total water holding capacity), VK-60, and the drought stressed conditions (at 30% level of the total), VK-30 was developed by additional watering after the bacterial treatment. 50-50 g of air-dried, homogenised soils was put into Petri-dishes in 3 replicates. *Rhizobium* strain culture was grown in microfermentor for 24 hours by putting one-loopful bacteria to 50 ml Nutrient broth. After 14-hours of incubation, the final cell counts of the broth was assessed in a Bürkher-chambre and a diluted 30 ml rhizobium suspension was mixed into the soil by developing a 10^6 cfu.g^{-1} soil starter cell counts.

Estimation of the bacterial survival

The treated soils were incubated at 28°C for three consecutive weeks. 1-1 g sub-samples were removed in each week, for making a dilution series and estimating the water content. A slightly modified plate-counting method (Angerer et al. 1998) was applied for counting the colony-forming units on Congo-red-supplemented yeast-extract-mannitol (YEM) agar plates.

Results and Discussion

According to the plasticity index and the humus content, the soils, examined were representing two main soil-types (Table 1), accomplished with distinct behaviours for the bacterial survival. Mean log-transformed data of the bacterium counts after a three-weeks of survival test can be seen on the Table 2. Data are converted to 1 g dry soil amount.

The survival of the rhizobia was less in the soils with low plasticity index and humus content, which represented the sandy soils (Őb, Nyl) examined. The number of countable rhizobia could be reduced by two orders of magnitude on those soils in general, which is considered as remarkable difference at the stress studies.

Although one single species was used, with “uniform” eco-physiological properties, still there were different sizes of colonies found on the selective plates. This show, that component of a certain population split to faster or slower growing cells, as a function of the environmental conditions. The soil characteristics were found less affecting the survival of the fast growing strains, which is called as “r” (rapid) strategists. The slow-grower component, however seem to be the stable (constant-“k”), slowly changeable fragment of the population, which was more efficiently depended on the main soil physical and chemical characteristics and the water content

Table 2. Abundance of fast- and slow- growing portion of Rhizobium leguminosarum bv. trifolii strain (ZH-15) at drought and optimal water levels after three weeks of incubation in four different Hungarian soils (LSD-5%= 0,11). More detailes about soils in Table 1.

Water level	Soils and abundance of slow- or fast-growing strategists Chernoziem r	k	Calcareous sand r	k	Brown forest r	k	Acidic sand r	k
Drought (VK 30%)* Log10cfu.g-1	5.33	5.87	4.21	5.23	5.28	5.79	3.99	5.16
Optimum (VK 100%) Log10cfu.g-1	5.36	5.72	4.42	5.18	5	6.07	4.47	5.06
Mean	5.34	5.79	4.31	5.205	5.14	5.93	4.23	5.11

*VK=30, VK=100, water content is the 30% or the 100% ratio of the water-holding capacity

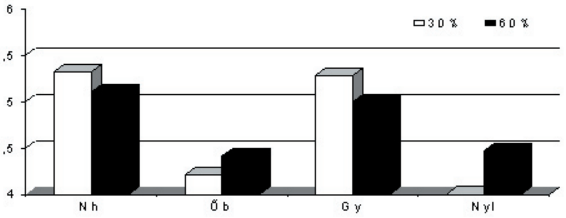

Figure 1. Abundance of k-strategist portion of *Rhizobium leguminosarum* bv. *trifolii* (Zh-15) strain at drought and optimum water levels (30% and 60% of the water-holding capacities) after three weeks of incubation in four different Hungarian soils (Nh, Őb, Gy, Nyl; $LSD_{5\%}$= 0,11). More detailes about soils in Table 1.

during this short examination period. Such behaviour of the particular k-type components can be seen on the Figure 1, where the abundance is increasing at the optimum conditions of the sandy soils or the drought stress of the clayey soils.

After this finding the consideration of such microbiological behaviour can be proposed at the evaluation of environmental stress in the various soil-plant ecosystems. It is assumed, that strains with less nutrient demand and slow-growing characteristics can survive longer to develop the effective nitrogen-fixing symbiosis, when they are used as microbial inoculums among the nutrient-poor and arid severe soil conditions. The "k" strategy can be an efficient tool for the nutrient competition in the soil, with potential for the soil-health and fertility.

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