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EFFECT OF DROUGHT STRESS AND *GLOMUS* INOCULATION ON SELECTED PHYSIOLOGICAL PROCESSES OF SWEET PEPPER (*CAPSICUM ANNUUM* L. CV. 'SLÁVY')

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Abstract

JEZDINSKÝ, A., VOJTÍŠKOVÁ, J., SLEZÁK, K., PETŘÍKOVÁ, K., POKLUDA, R.: *Effect of drought stress and Glomus inoculation on selected physiological processes of sweet pepper* (*Capsicum annuum L. cv. 'Slávy'*). Acta univ. agric. et silvic. Mendel. Brun., 2012, LX, No. 3, pp. 69–76

Sweet pepper (*Capsicum annuum* L. 'Slávy' F1) plants were colonized by the vesicular-arbuscular mycorrhizal fungus *Glomus* and grown in two irrigational levels: 1. optimal water supply (the irrigation activation by available water capacity (AWC) < 65 %); 2. drought stress (irrigation activation by AWC < 45 %). In the field experiment selected physiological parameters, such as photosynthetic rate, transpiration rate, stomatal conductance and water use efficiency were observed. The highest photosynthetic activity was observed in the first measuring date in both experimental years (2010: 7.5–8.1 µmol . m^{-2} . s^{-1} 2011: 6.1–8.6 µmol . m^{-2} . s^{-1}). In the next measuring dates, when the temperature decreased and the plants were older stage, the activity of leaves decreased. The inoculation by *Glomus* species had not clear effect. The inoculation did not increased the activity of plants (photosynthesis, transpiration) under optimal water supply conditions. However, based on results from 2011, under drought stress the inoculation had slightly positive effect on the photosynthetic rate. Under drought stress conditions, the water use efficiency (WUE) of plants increased slightly by the *Glomus* inoculation (from 4.7 to 5.1 µmol CO_2 . mmol H_2O^{-1}).

Capsicum annuum, photosynthetic rate, transpiration rate, stomatal conductance, water use efficiency (WUE), *Glomus*

As a climate change sign the increase of frequency of extreme weather years can be considered. It can be observed based on the alternation of wet and extreme dry years, and also in the unexpected formation of extreme weather situations (storms, hail, strong wind) (Reddy, Hodges, 2000). The most important from the view of vegetable growing is the increasing frequency of the rainless years.

The change of photosynthetic activity and transpiration under abiotic stress are proved in case of more plants species (Sanchez *et al.*, 2001; Blum, 2005). The ratio of this two process – expressed by WUE (Water Use Efficiency) – is also changing under stress conditions (Martín-Closas, Recasens, 2001; Thiagarajan *et al.*, 2008).

Pepper is an important crop in most countries. Sweet pepper demands for the optimum temperature of 25–27 °C (Peet, Wolfe, 2000). The lowest threshold of growing is 10 °C (Maynard, Hochmuth, 2007), while the fruit set is inhibiting if the temperature is higher than 35 °C (Gyúrós, 2004). The total heat unit demand of the sweet pepper in the vegetation season is 3000 °C (Zatykó, 2006). Based on Maynard, Hochmuth (2007) it can be confirmed, that for the favorable yield the optimum of the average monthly temperature is 21–24 °C, the minimum is 18 °C, the maximum is 27 °C.

The light demand of sweet pepper is also high, the minimum of the fruit set is 12–14 hours light per day by 5000 lux light intensity required (Gyúrós, 2004).

Water demand is high also, the transpiration coefficient is 300 (Zatykó, 2006)–330 (Gyúrós, 2004). The evapotranspiration loss of the field for developing of 1 kg fresh fruit is 100 l of water.

After each 6 °C heat unit it can be observed 1 mm evapotranspiration loss on the pepper field. Pepper, under optimal climate conditions, needs 500 mm water amount in the season, and it can result 5 kg . m^{-2} yield. The optimum saturation of the field capacity for the sweet pepper is between 60–70%, the optimal air humidity is about 80 % (Zatykó, 2006).

The symbiosis of plant and microorganism is well-know natural process. Mycorrhizal symbiosis is means of mutual co-existence of higher plants roots with a specific group of soil fungi. Mycorrhiza allows the chemical compounds exchange between the fungus and the plant, which brings the advantages of allowing both organisms in situ thrive, which previously featured Pirozynski (1981). Mycorrhizal fungus gets energy from the host plants, reciprocally it support the nutrient uptake, especially phosphorus or nitrogen (Gianinazzi-Pearson, 1996). Further application of mycorrhiza leads to increased plant growth and yield by improving nutrient uptake (Smith, Read, 2008). Wang *et al.* (2008) also writes about the positive effects of inoculation in the initial stage of development to increase and improve plant growth. AM fungal mycelium significantly expands the volume of soil from which plants can draw mineral nourishment, but a vital physiological functions of the mycelium is the transport of many mineral nutrients from the soil into the root tissues (Gryndler *et al.*, 2005). The fungus is able to enhance plant defences against various abiotic and biotic stresses (Gianinazzi-Pearson, 1996).

More literature sources report the effect of colonization on the plant physiological process (water use efficiency, photosynthetic rates, stomatal conductance) (Davies *et al.*, 2002; Nowak, 2004). Ruiz-Lozano and Gomez (1995) found the positive effect of mycorrhiza colonization to the photosynthetic rate under drought stress.

An important genus of the vesicular-arbuscular mycorrhizal fungus is *Glomus*. Many positive experiences is known about using different *Glomus* species (*G. intraradices*, *G. fasciculatum, G. deserticola, G. mosseae*) in plant growing systems (Raju *et al.*, 1990; Aguilera-Gomez *et al.*, 1999; Kim *et al.*, 2002; Garmendia *et al.*, 2004; Nowak, 2004). The positive effect of sweet pepper inoculation was confirmed for example by increasing leaf number, leaf area, shoot, root and fruit mass – Aguilera-Gomez *et al.*, 1999; significant increase in plant dry weight and chlorophyll concentration – Kim *et al.*, 2002, improving plant growth and the early beginning of the reproductive stage, or higher yield – Garmendia *et al.*, 2004). Sensoy *et al.* (2007) confirmed colonization influence on sweet pepper cultivars. Based on they experiences, in general, inoculated plants were characterized by greater dry weights compared to control plants.

The aim of this work was to evaluate the effect of *Glomus* spp. on photosynthetic parameters in the sweet pepper grown at two water supply levels.

MATERIALS AND METHODS

The experiment was conducted in 2010 and 2011 in Lednice at the Faculty of Horticulture of Mendel University in Brno. The evaluation was conducted in four replications, at two different irrigation levels and under the inoculation treatment. As a model plant was selected *Capsicum annuum* L. 'Slávy F1'. Inoculation was done by mycorrhizal product Symbivit® (Symbiom Ltd., CZ). The product contains natural clay carriers and six mycorrhizal fungi (*G. intraradices* BEG140, *G. mosseae* 95, *G. etunicatum* BEG92, *G. claroideum* BEG96, *G. microaggregatum* BEG56, *G. geosporum* BEG199). This product was applied at 100 ml per 1 L of substrate Klassman TS 3. The variants consist of control and by plants treated with the product Symbivit® (referred to *Glomus* in the experiment). The size of one plot was 12 m^2.

In 2010, pepper was sown into the trays on February 26th. Transplants cultivation conditions were as follows: seed emergence 20–25 °C, after germination 17–20 °C by day, 12–14 °C by night. The transplants were fertilized with the 0.1% SAMPPI fertilizer every two weeks, by spraying. Planting took place on June 8th. In 2011, pepper was sown on March 3rd, under the identical growing conditions; plants were planted on May 17th. The spacing was 0.6 × 0.3 m and the planting on site took place by two pieces of plants. The experiment was based on two different levels of irrigation being activated automatically by moisture sensors VIRRIB, which triggered the irrigation when it goes below the set value. The two water supply level was:

- optimal water supply: the irrigation was activated when available water capacity (AWC) decreased below 65 %;
- drought stress: the irrigation was activated when AWC decreased below 45 %.

I: *Main climate characteristic of the vegetation period in the experimental years*

Parameter	Year	May	June	July	August	September	Veget. period
Average temperature [°C]	2010	14.4	18.4	21.7	19.2	14.3	17.6
	2011	14.6	19.6	19.2	20.7	17.4	18.3
Rainfall [mm]	2010	122.2	82.3	102.5	98.6	75.7	481.3
	2011	47.7	80.6	79.1	27.2	4.3	238.9
Sunny hours [hours]	2010	105.8	230.3	280.4	229.5	156.4	1002.4
	2011	296.9	242.1	203.0	255.4	239.9	1237.3

II: *Main climate, soil temperature and soil moisture conditions of the measuring dates*

Measu-ring period	Date	Air temperature			RH [%]	Number of sunny hours [h]	Soil temp. [°C]	Soil moisture AWC [%]
		Max. [°C]	Min. [°C]	Mean [°C]				
Experimental year: 2010								
1st	4. 8.	25.4	13.2	19.3	77,7	12,3	19.9	100.0
2nd	11.8.	28.0	12.8	20.4	77,0	9,1	22.7	61.9
3rd	24. 9.	23.1	9.2	16.2	79,0	9,5	12.0	100.0
Experimental year: 2011								
Optimal irrigation								
1st	23. 8.	34.3	19.8	27.1	60.3	10.0	24.8	100.0
2nd	6. 9.	22.9	12.3	17.6	70.3	10.7	21.8	100.0
3rd	29. 9.	24.1	8.1	16.1	62.7	7.6	17.5	100.0
Drought stress								
1st	22. 8.	33.7	16.3	25.0	69.3	10.9	21.9	52.2
2nd	5. 9.	31.1	18.5	24.8	65.0	7.5	20.5	25.9
3rd	30. 9.	24.1	8.8	16.5	69.7	9.9	15.2	30.1

The climate conditions of the experimental years are presented in the Tab. I.

In the first year it was not possible to observe the drought stress, because of unexpected frequency of natural precipitation.

The particular climate data and soil temperature and moisture data in the measuring days are presented in the Tab. II.

Methods of photosynthesis measurement: The portable photosynthetic system LCpro+ with an infrared gas analyzer and leaf chamber was used to measure the rate of photosynthesis. The conditions of chamber: 25 °C, light irradiance 650 μmol $m^{-2}s^{-1}$. The plants were measured three times in leaves of 80% of their final development. Values were measured in the upper leaf surface – determined from the tip of sheet. The 3plants were measured at each repetition.

The results were processed in "Statistica 9.0" (StatSoft Inc. 1984–2009) using method Anova and Tukey-Kramer pairwise comparison or Games-Howell pairwise comparison of means, at level of 95 % probability. 95% confidence intervals are presented in all figures.

RESULTS

The **rate of photosynthesis** (A) is showed on Fig. 1–2. In both years, the **photosynthetic rates** were decreasing during season.

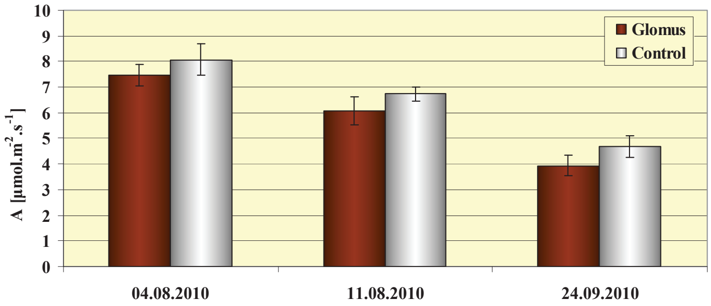
In the first year was not found the positive effect of *Glomus* on the photosynthetic rate. The highest average values (Control: 8.1; *Glomus*: 7.5 μmol.m^{-2}.s^{-1}) in both treatments were in the 1st measuring date, and the lowest average values (Control: 4.7; *Glomus*: 3.9 μmol.m^{-2}.s^{-1}) were observed in the 3rd measuring date. In comparison the measured values of all measuring dates, the photosynthetic rate of control plants were significantly higher than in the *Glomus* treatment (Control: 6.5; *Glomus*: 5.9 μmol.m^{-2}.s^{-1}).

In 2011, in the case of variant stressed by drought, the positive effect of *Glomus* was observed in each measuring date, in the 3rd measuring date significantly. Under optimal irrigation, this effect was observed only in the 2nd measuring date, but not significantly. In the 1st measuring date the control treatment at optimal irrigation resulted by statistically higher photosynthetic activity compared to the inoculated treatment. Within the first two measuring dates the plants from optimal irrigation were characterized by higher photosynthetic activity than the stressed plants. In the 3rd measuring date it was found the opposite. The differences between the stress and optimal conditions were significant in each measuring date. Comparison of the measured values of whole measuring period, the *Glomus* and control treatments from optimal condition resulted by statistically similar activity (Control: 5.8, *Glomus*: 5.4 μmol.m^{-2}.s^{-1}), and the lowest activity was observed under drought stress, in control (4.2 μmol.m^{-2}.s^{-1}). The average value in this treatment was significantly lower than the control treatment on the optimal water supply field, and than the inoculated treatment from the stressed area (4.9 μmol.m^{-2}.s^{-1}).

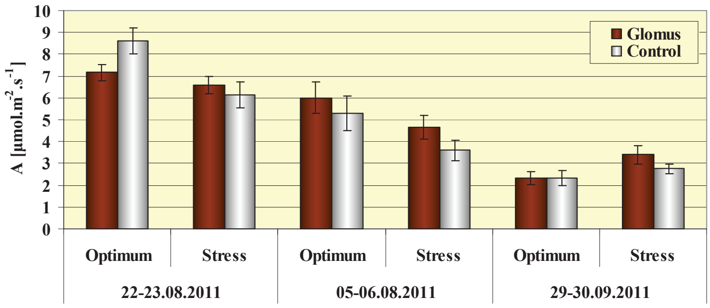
The two-factor analysis of variance confirmed, that the effect of water supply was stronger than the effect of inoculation by *Glomus* (Tab. III).

The **activity of transpiration** in the first year was observed of the same tendency like in the rate of photosynthesis (Fig. 3). The highest values were observed in the first measuring date (Control and *Glomus*: 2.0 mmol.m^{-2}.s^{-1}), and the lowest values in the 3rd measuring date (Control: 1.1, *Glomus*: 1.0 mmol.m^{-2}.s^{-1}). Statistical differences of treatments were found at the 2nd and 3rd measuring date.

In 2011 the highest average values were observed in the 2nd measuring date in case of plants growth under optimal irrigation (*Glomus*: 1.4 mmol.m^{-2}.s^{-1}) (Fig. 4). The higher average value was observed in the first measuring date in the control plots (stress:



1: *Photosynthetic rate (A) of sweet pepper leaves in 2010*



2: *Photosynthetic rate (A) of sweet pepper leaves in 2011*

III: *Effect of different conditions on the examined physiological parameters in 2011*

Conditions	Photosynthetic rate (A) [µmol.m^{-2}.s^{-1}]	Transpiration rate (E) [mmol.m^{-2}.s^{-1}]	Stomatal conductance of CO_2 (gs) [mol.m^{-2}.s^{-1}]	Water use efficiency (WUE) [µmol CO_2.mmol H_2O^{-1}]
Water supply *(Glomus and Control treatments together)*				
Optimal	5.58	1.03	0.073	5.83
Stress	4.52	0.93	0.057	4.87
Treatments *(Optimal water supply and stress conditions together)*				
Glomus	5.13	0.98	0.064	5.31
Control	4.97	0.98	0.066	5.39
Statistical effect of conditions (p-values and level of significance)				
Water supply	0.000*	0.006*	0.000*	0.003*
Treatment	0.399	0.986	0.519	0.805
Water supply x Treatment	0.007*	0.175	0.095	0.155

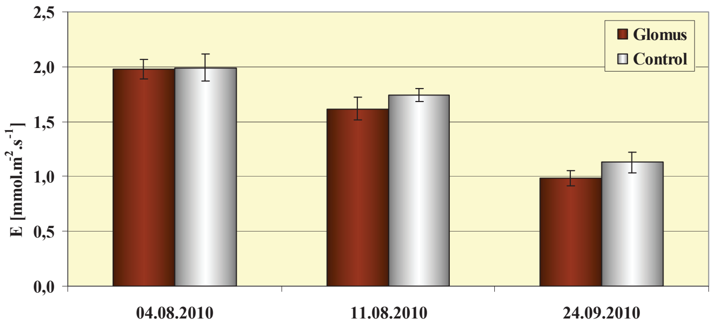
**: $p < 0.05$

1.4 mmol.m^{-2}.s^{-1}, optimal irrigation: 1.2 mmol.m^{-2}.s^{-1}), in the 2nd measuring date in the inoculated plots (stress: 1.4 mmol.m^{-2}.s^{-1}, optimal irrigation: 0.8 mmol.m^{-2}.s^{-1}). In the 3rd measuring date the effect of inoculation was not clear (it had positive effect under stress condition only).

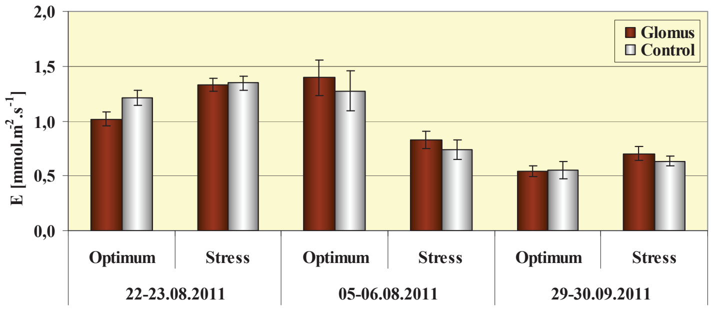
Comparison of the irrigation and inoculation treatment in all year, confirmed, that the irrigation has significant effect on the activity of transpiration, but the inoculation did not show clear effect (Tab. III). Under drought stress conditions the inoculation had slight improving effect on the activity of transpiration, but under optimal irrigation it was observed slight decreasing effect.

In case of **stomatal conductance** (Fig. 5–6) it was observed similar tendency like in activity of transpiration. Inoculation in 2010 decreased the stomatal conductance. In 2011, the increasing effect on the stomatal conductance was observed only in the second measuring date, but not significant. Examining the all year, based on the results of two-factor analysis of variance (Tab III.), the inoculation had no significant effect on the stomatal conductance, however the water supply had (stress: 0.06 mol.m^{-2}.s^{-1}, optimal irrigation: 0.07 mol.m^{-2}.s^{-1}), similarly to the activity of transpiration.

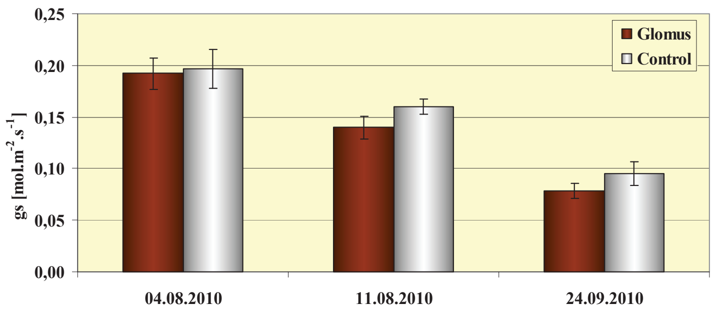
The highest **water use efficiency (WUE)** (Fig. 7–8.) was observed in the first measuring date of 2011, in the optimal irrigation treatments (7.2 µmol CO_2.



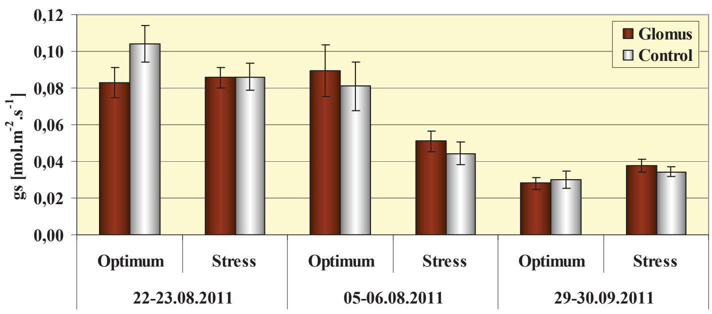
3: *Transpiration rate (E) of sweet pepper leaves in 2010*



4: *Transpiration rate (E) of sweet pepper leaves in 2011*



5: *Stomatal conductance (gs) of sweet pepper leaves in 2010*



6: *Stomatal conductance (gs) of sweet pepper leaves in 2011*

mmol H_2O^{-1}, in the Control and *Glomus* treatments identically.

In 2010 the inoculation led to the WUE decrease. The statistical analysis proved the differences between the treatments in the first measuring date. The highest values were observed in the 3rd measuring date (Control: 4.3 µmol CO_2.mmol H_2O^{-1}, *Glomus*: 4.1 µmol CO_2.mmol H_2O^{-1}).

In 2011, under drought stress, the not-significant positive effect of *Glomus* on WUE in each measuring dates was observed. Under optimal irrigation the effect of inoculation was not clear during the season.

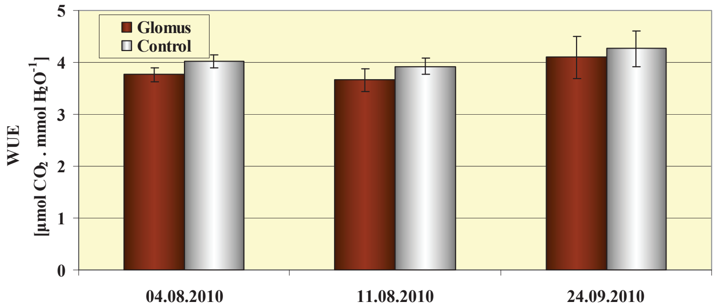
Comparison of the water supply conditions, in the first measuring date under stress conditions the plants were characterized by lower WUE value, while in the subsequent measuring dates the stressed plants had WUE value higher.

The two-factor analysis of variance presented, that the water supply condition has significant effect on the WUE, in contrary to the inoculation (Tab. III).

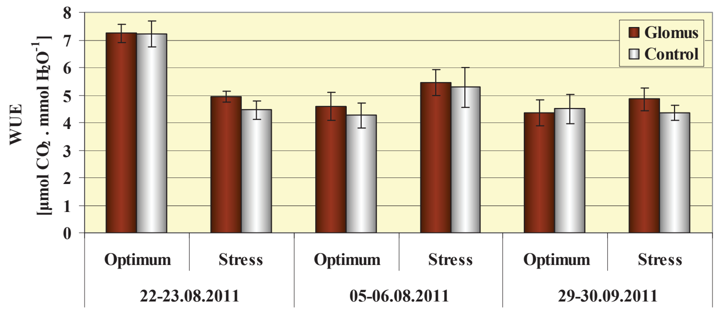
The **coefficient of transpiration** was calculated based on reciprocal value of WUE (Tab. IV). In the 2010 the values were between 234 and 273 mmol H_2O.mmol CO_2^{-1}. In the second year, in the first measuring date at the optimal irrigation, and in the second measuring date at the stress conditions the coefficient was lower than 200 mmol H_2O. mmol CO_2^{-1}.

DISCUSSION AND CONCLUSION

The climate conditions in the two experimental years were different. The main difference was observed based on the rainfall and number of sunny hours. In 2010 the amount of rainfall was almost two times higher than in 2011, and this was accompanied also by the low value of sunny hours. Based on the air temperature, the differences between both years were recorded from August, and in this term 2011 was warmer. However, both years the total heat unit of vegetation period (in 2010: 2695 °C, in 2011: 2802 °C) was near to the 3000 °C, which value fulfil



7: *Water use efficiency (WUE) of sweet pepper leaves in 2010*



8: *Water use efficiency (WUE) of sweet pepper leaves in 2011*

IV: *Mean value and 95% confidence interval (C.I.) of the coefficient of transpiration [mmol H_2O.mmol CO_2^{-1}]*

Measuring date	Water supply	Glomus			Control		
		Mean	95% C.I.		Mean	95% C.I.	
Experimental year: 2010							
4. 8. 2010	optimal	266	256	276	249	241	257
11. 8. 2010	optimal	273	257	289	255	244	265
24. 9. 2010	optimal	244	220	268	234	216	253
Experimental year: 2011							
22.–23. 8. 2011.	optimal	138	132	144	139	130	147
	stress	202	194	210	225	208	241
5.–6. 8. 2011	optimal	218	193	242	234	210	258
	stress	184	168	199	189	163	215
29.–30. 9. 2011	optimal	230	206	255	223	197	248
	stress	206	189	223	229	214	243

the optimal demand of the sweet pepper Zatykó (2006). The average month temperature in both years was in the demanded interval of sweet pepper (18–27 °C – Maynard, Hochmuth, 2007) during 3 month (June, July, August). In 2010 the theoretical water demand of sweet pepper was almost satisfied by the natural rainfall (500 mm – Zatykó, 2006), while in 2011 the natural rainfall was only 239 mm from May to end of September. Thus the effect of drought stress was detectable only in 2011.)

According to the results of the experiment, it can be confirmed, that the highest photosynthetic activity was observed in the first measuring date in both experimental years. In the next measuring dates, when the temperature decreased and the plants were older, the activity of leaves decreased. The transpiration rate and the value of stomatal conductance changed almost simultaneously. Under optimum water supply the values were higher, than under drought stress.

The inoculation by *Glomus* species showed not clear effect. The inoculation not increased the activity of plants (photosynthesis, transpiration) under optimal water supply conditions. However, based on results from 2011, under drought stress conditions the inoculation had slight positive effect on the photosynthetic rate. The data of photosynthetic rate were lower than the most of published data (20–25 μmol.$m^{-2}.s^{-1}$), maybe because of the different genotype and experimental conditions. (Aguilera-Gomez *et al.*, 1999).

Under drought stress conditions, the water use efficiency (WUE) of plants increased slightly by the *Glomus* inoculation. In the 1st measuring date the optimal supply resulted by the higher WUE, while in 2nd and 3rd ones the stress condition resulted by WUE higher value. This is similar to the experiences of Thiagarajan *et al.* (2008), who examined the effect of mild water stress on water use efficiency in carrot.

The coefficient of transpiration was significantly lower than the values published by literature (300– Zatykó, 2006; 330 – Gyúrós, 2004). The cause of this can be the different experimental conditions and the properties of tested cultivar.

According to Raju *et al.* (1990) and the data of soil temperature sensors of this experiment, the experiences can show that it would be important to examine more particularly the different environmental demand of *Glomus* species and harmonised inoculation material for sweet pepper.

SUMMARY

This experiment was focused on evaluation of some physiological parameters and mycorrhizal inoculation of pepper with *Glomus* in water stress condition. The experiment was conducted in 2010 to 2011 in Lednice. Plants were grown under field conditions. In the experiment were observed selected physiological parameters of pepper: photosynthetic rate, transpiration rate, stomatal conductance and water use efficiency. The two water supply levels were: 1. optimal water supply (the irrigation activation by available water capacity (AWC) < 65 %); 2. drought stress (irrigation activation by AWC < 45 %).

The highest photosynthetic activity was observed in the first measuring date in both experimental years. In the next measuring dates, when the temperature decreased and the plants were older, the activity of leaves decreased. The inoculation by *Glomus* species had not clear effect. The inoculation did not increase the activity of plants (photosynthesis, transpiration) under optimal water supply conditions. However in 2011, under drought stress conditions the inoculation had slight positive effect on the photosynthetic rate. Under drought stress conditions, the water use efficiency (WUE) of plants increased slightly by the *Glomus* inoculation.

Acknowledgment

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