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EFFECT OF WATER STRESS ON SELECTED PHYSIOLOGICAL CHARACTERISTICS OF TOMATOES

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Abstract

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This work presents the results of a field experiment with tomato cv. Proton grown under water stress conditions and under well irrigated conditions. At the same time, the effects of Pentakeep supporting agent (consisting of aminolevulinic acid) was studied. The following characteristics of plant physiology were studied – specific leaf area (SLA), leaf water content (LWC) and leaf proline concentration.
The obtained results show a significant decrease in SLA during plant vegetation (from 190 to 165 $cm^{-2}.g^{-1}$). A decrease was also found under water stress treatment (163 $cm^{-2}.g^{-1}$), in contrast to the well watered control (184 $cm^{-2}.g$). LWC values ranged from 81 to 87% in both seasons. A significantly important decrease of LWC was found in the stressed variant. Pentakeep application did not affect either SLA or LWC characteristics.
The level of proline in tomato leaves varied from 0.6 to 1.5 $\mu mol.g^{-1}$. The highly significant effect of water conditions resulted in the increase of proline content under water stress treatment. Although Pentakeep increased the proline content by 9%, its effect was not of significant importance. The proline content was also influenced by the date of sampling.
The results indicate that physiological characteristics, such as SLA, LWC and proline content, are good indicators of water stress in tomato, proline content being a particularly reliable parameter corresponding to the actual water stress of plants.

water stress, tomato, specific leaf area, leaf water content, proline

Droughts in connection with high temperatures have become the most common abiotic factors influencing physiological processes in plants, and consequently the yield and its quality. Under water stress, plants naturally try to prevent loss of water by means of various mechanisms, such as pore closing (CHAVES et al., 2003), synthesis of osmoprotectants –sugars, aminoacid proline and other substances (RAMANJULU et al., 2002). It has been found out (CLAUSSEN, 2005) that proline content grows significantly as early as in 14 hours after water stress. When assessing stress in hydroponically grown tomatoes, it was found out that proline content in tomato leaves under water stress was higher in summer than at the end of the vegetation. The level of proline also depends on light intensity. A negative correlation of proline and leaf water content (LWC) was proved. The relative LWC decrease by 5% doubled the increase of proline content. SHTEREVA et al. (2008) mention the possibility to use a 'proline' test as an instrument for finding tomato genotypes tolerant to water stress.

Apart from the natural mechanisms reducing the stress, new possible uses of complementary substances leading to greater stress endurance of plants are being searched for. One such substance is aminolevulinic acid (ALA) which has a number of positive effects on plant physiology.

According to KORMAZ et al. (2009), spraying or dipping pepper seedlings in ALA solution clearly reduced the signs of cold damage. At the same time, an exogenous application of ALA increased the content of the monitored constituents, including proline. ALA also increased the relative leaf water content. The aim of this paper is to characterize water stress in tomatoes using some physiological characteristics such as specific leaf area (SLA), leaf water content (LWC) and proline content.

MATERIALS AND METHODS

The experiment took place in 2008 and 2009 in the field of the Faculty of Horticulture of Mendel University of Agriculture and Forestry Brno in Lednice (GSP location: 48°47'36.858" N, 16°47'49.526" E). The bushy mid-early tomatoes cv. Proton were planted in the field on 15th May 2008 and 17th May 2009 in 1.1 + 0.4 × 0.3 m spacing. The tomatoes were grown under two different watering conditions. The stressed plants (S) were watered when the available water capacity (AWC) dropped under 40 to 45 %, the optimally irrigated plants (O) when the AWC dropped under 65 to 70 %. Drip irrigation with automatic regulator, which turned the irrigation on with AWC decrease under the set level, was used. Pentakeep super (the main ingredient of which is 5-aminolevulinic acid, ALA) plant supporting agent which, according to its manufacturers' description enhances chlorophyll photosynthesis, photosynthetic capacity and regulates respiration, was applied to both the stressed and optimally irrigated treatments. The agent also contains NPK and Mg. It is manufactured by COSMO OIL CO. (JP). To check the results, an untreated variant was used. The experiment took place in 2008 in three repetitions and in 2009 in four repetitions.

The Pentakeep super agent was used in the concentration of 0.02 % (0.5 L.ha^{-1} in 2000 L water) in five applications. The first treatment took place one week after planting, the other four following in two weeks intervals. The last application in 2008 took place on 18th July and on 11th July in 2009.

Specific leaf area (SLA) was defined as the ratio of leaf area and dry matter (KVĚT et al., 1971). The measurement took place in 2008 and 2009 at the age of 47–91 days, or to put it differently, 32–103 days after planting, on the second top leaf of the length of at least 150 mm from. In the same period, the leaf water content (LWC) was determined. The dates of measurement were set in order to cover a longer action period of time (5 days) of either stressed or optimally irrigated conditions. The effects of water stress were monitored continually using the VIRRIB soil sensor (Amet, s.r.o., CZ). The proline content analysis was carried out on 19th and 25th August 2009, which corresponded to the plant age of 95 and 101 days after planting. The tomato leaves used for sampling were fully expanded without obvious stress damage. The determination of proline content was carried out by colorimetry (BATES et al., 1973) in the green matter in four repetitions with each variant.

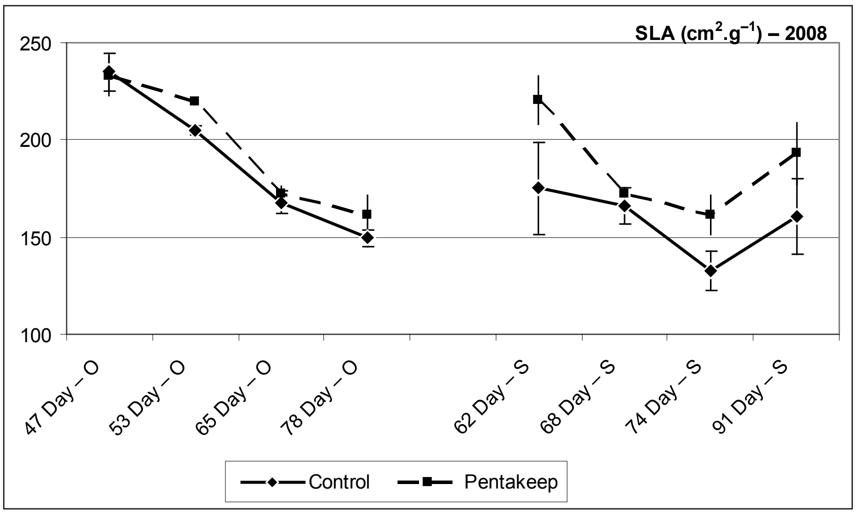
The statistical analysis was carried out in Unistat 5.1 programme using the ANOVA and LSD methods with a 95% level of probability.

RESULTS AND DISCUSSION

Graphs 1 and 2 indicate the average level of specific leaf area (SLA), in which there is a decreasing tendency with the growing age of plants in both levels of irrigation. The values refer to the age of the cultivation (date after planting) as well as with regard to the level of irrigation. The results in the two seasons are similar, with the exception of the last stressed variant sampling in 2008.

The average SLA decrease between the first and last analysis decreased from 190 to 165 cm^{-2}.g^{-1}. The observed fall in SLA with the growing age of plants corresponds to the findings observed in pepper (ABDELAZIZ et al., 2008). Similarly, ŠTAMBERA, PETŘÍKOVÁ (1970) report that levels of SLA in tomatoes are higher at the beginning of vegetation and the leaves being thinner. They also mention that the specific leaf weight of tomato varies according to the plant age, plant variety and the weather.

Pentakeep application led to a significant rise in SLA under stress conditions only during the first season when the level of soil moisture was consi-



1: *Specific leaf area in 2008*

derably lower. In 2009 the values in the treated and control variants were virtually comparable. The average SLA of the treated plants was 175 $cm^{-2}.g^{-1}$ and that of the untreated variety was 173 $cm^{-2}.g^{-1}$.

Compared to the stressed variant, higher values were observed under ideal irrigation conditions. Similarly, PAGANOVÁ et al. (2009) found out that there were significantly lower levels of SLA in stressed *Sorbus domestica* plants grown in 40% water saturated substrate than in the plants grown in 60% water saturated substrate.

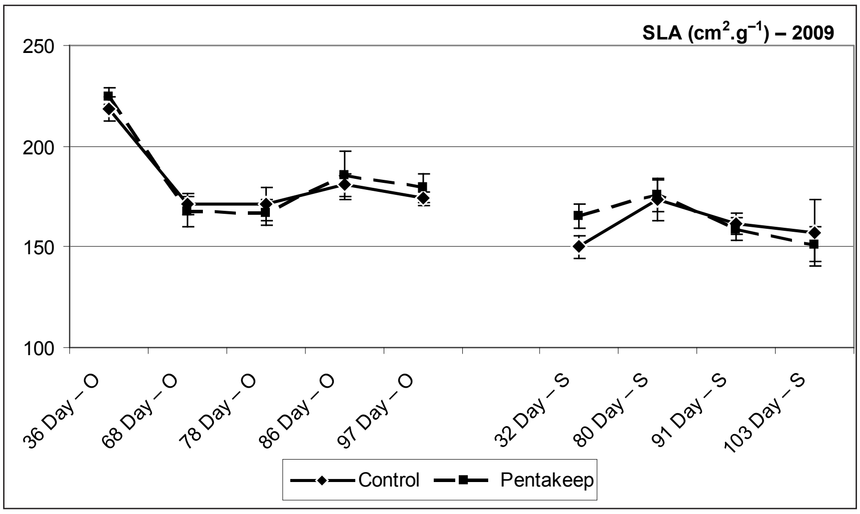
Comparing the two years, the course of SLA differed. While in 2008 the SLA decrease in the stressed variant was striking (with the exception of the day 91), it was less remarkable in 2009. This might have been related to a higher level of precipitations in the given period, which influenced the course of stress in 2009.

LEAF WATER CONTENT

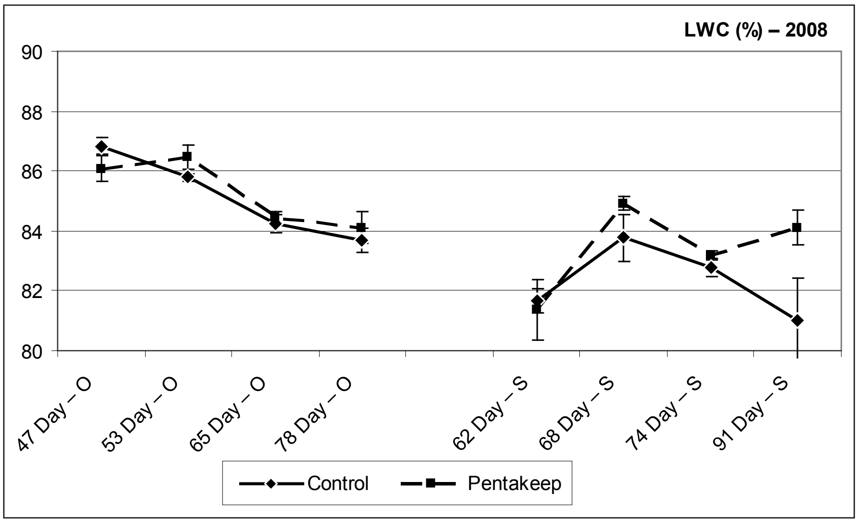
The results shown in graphs 3 and 4 indicate that the leaf water content (LWC) was not influenced by Pentakeep treatment in either of the variants or seasons except for the last sampling of the stressed variant in 2008. A significantly higher leaf water content was found in the optimally irrigated variant at the beginning of the experiment (87% in both years) in comparison to the stressed variant (81 and 82%).

In the optimally irrigated variant, with the growing age of the plants, the LWC decreased from 87% to as little as 84 or 85% in 2008 and 2009. It was most likely due to the fact that before flowering, plants require more water and nutrients which would not be available in stressed conditions (HANAFY, 1986). The results shown in graphs 5 and 6 confirm this conclusion in relation to the correlation (r = 0.99 and 0.86) between SLA and LWC in both irrigation variants in 2008 and 2009.

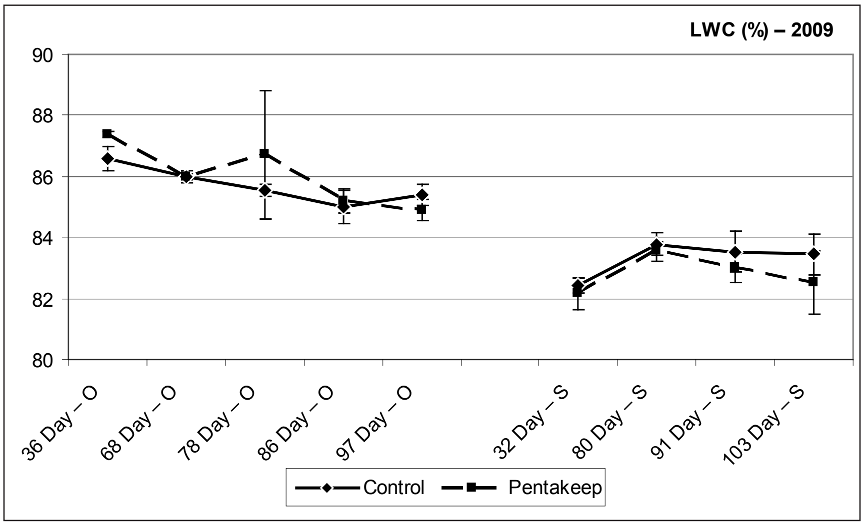
PAGANOVÁ et al. (2009) found out a positive linear relation between SLA and LWC, which is typi-



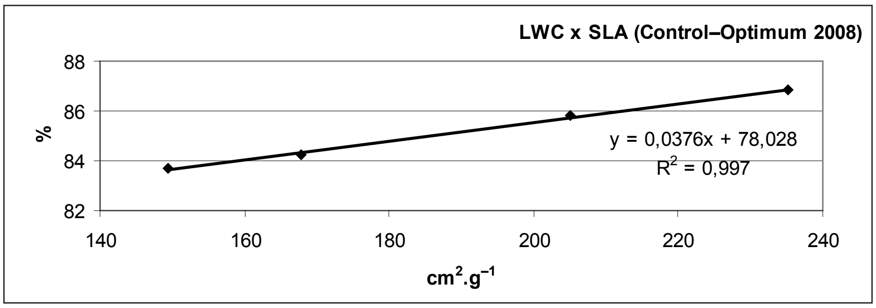
2: *Specific leaf area in 2009*



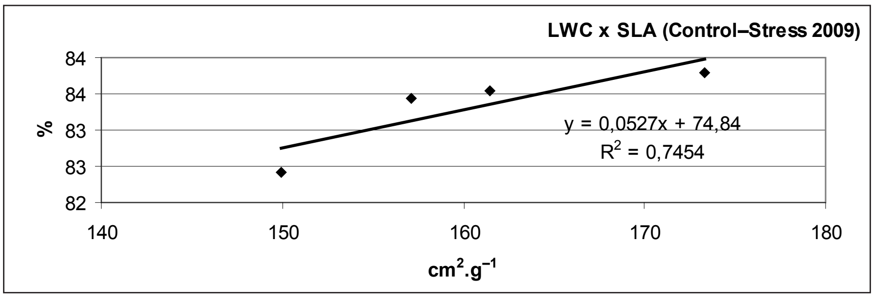
3: *Leaf water content in 2008*



4: *Leaf water content in 2009*



5: *LWC and SLA correlation in the control variant under optimal irrigation – 2008*



6: *LWC and SLA correlation in the control variant under water stress – 2009*

cal of plants with delicate leaves. SLA and LWC are regarded as suitable indicators of plant adaptability to water stress.

PROLINE CONTENT

The proline content results are summarized in table I. It indicates that the average proline content ranged from 0.63 to 1.51 μmol.g^{-1} f. w. Proline content was highly significantly ($p = 0.0001$) influenced by water stress, in the course of which its average level in the control and treated variants increased to 1.20 μmol.g^{-1} f. w. in contrast to the optimally irrigated variants (content of 0.75 μmol.g^{-1} f.w). A significant ($p = 0.0466$) effect of the sampling date was also found out. The effect of Pentakeep treatment was not significant despite the fact that proline content after Pentakeep treatment increased by 9%. The differences between its content in the different variants are presented in graph 7.

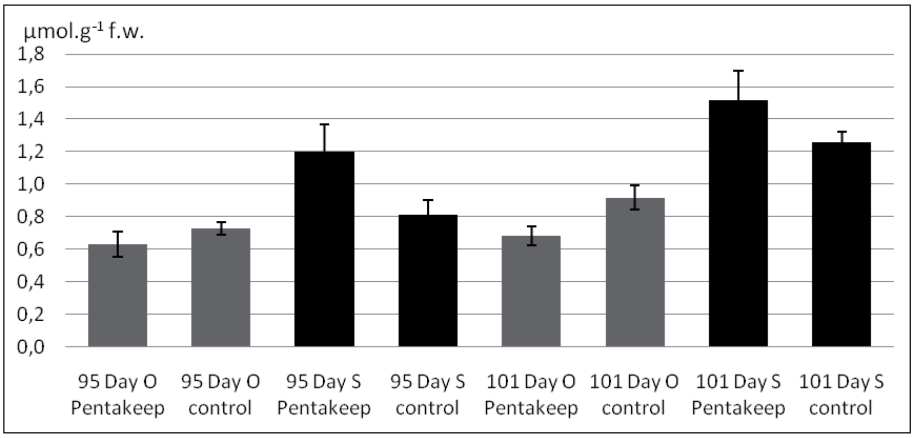
Within the framework of this research, it has been proved that proline plays an important role in assessing stress to which plants are exposed (CLAUSSEN, 2005). When the plants tested were exposed to water stress, the proline content, in comparison to the optimally irrigated control, increased by as much as 61%. In accordance with other sources a negative correlation between LWC and proline content has been found in our experiments.

Although KORKMAZ et al. (2009) report the positive effect of 5-aminolevulinic acid, the active ingredient of Pentakeep agent, on the increase in proline content, this finding has not been confirmed by the experiments, which might be related to the length of the period between the last Pentakeep application and proline content assessment.

I: *Average proline content*

Variant	Mean content µmol.g⁻¹ f.w.		Standard deviation	Standard error	Conf. interval lower 95%	Conf. interval upper 95%
95 Day O Pentakeep	0.6315	a	0.1583	0.0792	0.6551	0.8517
95 Day O control	0.7283	a	0.0794	0.0397	0.6551	0.8517
95 Day S Pentakeep	1.2011	bc	0.3277	0.1638	0.9036	1.3834
95 Day S control	0.8096	a	0.1854	0.0927	0.6551	0.8517
101 Day O Pentakeep	0.6807	a	0.1167	0.0583	0.6551	0.8517
101 Day O control	0.9171	ab	0.1454	0.0727	0.6551	1.2145
101 Day S Pentakeep	1.5143	d	0.3691	0.1846	1.2291	1.5400
101 Day S control	1.2548	cd	0.1374	0.0687	1.0725	1.5400

Rem.: The different letters show the significance of the difference in the average values



7: *Effect of irrigation variants and Pentakeep treatment on average proline content*

SOUHRN

Vliv vodního stresu na vybrané fyziologické charakteristiky rajčat

Byly zjištěny fyziologické charakteristiky – specifická listová plocha (SLA), obsah vody v listech (LWC) a obsah L – prolinu u keříčkových rajčat odrůdy Proton pěstovaných při optimální úrovni závlahy: závlaha při poklesu vodní využitelné kapacity (VVK) pod úroveň 65–70 % a stresovaných: závlaha při poklesu VVK pod úroveň 40–45 %. Závlaha byla kapková s automatickým řízením závlahovým regulátorem, který spouštěl závlahu při poklesu pod nastavenou hodnotou VVK. U stresované i optimálně zavlažované varianty byl aplikován podpůrný přípravek Pentakeep super (hlavní složkou je 5-aminolevulová kyselina (ALA). Aplikace v koncentraci 0,02 % (0,5 l.ha⁻¹ do 2000 l vody) byla provedena celkem pětkrát. První ošetření proběhlo týden po výsadbě, další čtyři po čtrnácti dnech. Hodnoty SLA a LWC byly zjišťovány na 2. listu od vegetačního vrcholu, který dosáhl délky cca 150 mm. Prolin byl stanoven kolorimetricky (BATES et al., 1973) z plně vyvinutého listu. Termíny měření byly určovány tak, aby vystihly působení delšího časového úseku (5 dnů), kdy byly rostliny stresované nebo naopak v podmínkách dostatečného zásobení vodou.

Ze stanovených fyziologických charakteristik vyplývá, že rostliny rajčat reagovaly na vodní stres průkazným snížením specifické listové plochy (SLA) a průkazným snížením obsahu vody v listech (LWC). SLA dosahovala hodnot 184 cm⁻².g⁻¹ u optimálně zavlažované varianty a 163 cm⁻².g⁻¹ u stresované. Aplikace Pentakeep super vedla k průkaznému zvýšení SLA ve stresovaných podmínkách pouze v průběhu první sezony, protože byla úroveň půdní vláhy výrazně nižší. V roce 2009 byly hodnoty mezi ošetřenou variantou a kontrolou prakticky srovnatelné. Průměrná hodnota LWC byla u stresované varianty o 2,4 % nižší v roce 2008 a o 2,6 % v roce 2009.

Obsah prolinu v listech dosahoval hodnot u optimálně zavlažované varianty 0,6–0,9 µmol.g^{-1} a u stresované 0,8–1,5 µmol.g^{-1} v č. h. Bylo zjištěno, že obsah prolinu byl průkazně zvýšen u stresované varianty oproti optimálně zavlažované. Toto zvýšení dosáhlo až 60 % a bylo spojeno s poklesem LCW. Ošetření přípravkem Pentakeep nemělo průkazný vliv na obsah prolinu, i když u stresované varianty Pentakeep zvýšil obsah prolinu o 9 %.
Práce potvrdila, že sledované fyziologické charakteristiky SLA, LWC a obsah prolinu jsou vhodné indikátory pro stanovení reakce rostlin na vodní stres, přitom nejvýrazněji byl stav charakterizován koncentrací prolinu v listech.

vodní stres, rajče, specifická listová plocha, obsah vody v listech, prolin

SUMMARY

This work describes the following physiological characteristics – specific leaf area (SLA), leaf water content (LWC) and proline content in bushy tomato cv. Proton grown under optimal irrigation (minimal available water capacity AWC kept at 65–70 %) and under water stress (AWC kept at 40–45 %). The field was irrigated by drip irrigation with automatic regulation maintaining the above mentioned AWC levels. The plant supporting agent Pentakeep super, which contains 5-aminolevulinic acid, was applied as foliar spray to both irrigation variants. Its application in the concentration of 0,02 % (0.5 L.ha^{-1} in 2000 L water) took place 5 times. The 1st treatment was done 1 week after planting, the other four following in 2 week intervals. The levels of SLA and LWC were analysed on the 2nd leaf from the plant top having reached 150 mm of length. Proline content was analysed by colorimetric method (BATES et al., 1973) from the fully expanded leaves. The time of analyses was set in correspondence to the longer period (5 days) of water stress and saturated irrigation levels effects.
The results of physiological characteristics show that tomatoes respond to the water stress by a significant decrease in SLA and LWC. While under optimal irrigation treatment, the SLA was 184 cm^{-2}.g^{-1}, its level under water stress conditions was 163 cm^{-2}.g^{-1}. Pentakeep super application led to a significant increase in SLA under stress treatment in the first year only. The values in Pentakeep treated plants and the control in 2009 were comparable.
The content of proline in leaves ranged from 0.6 to 0.9 µmol.g^{-1} of fresh weight under the optimal treatment, and from 0.8 to 1.5 µmol.g^{-1} in the stressed variants. The proline content was significantly increased by stress conditions. This increase was represented by 60 % and was accompanied by the decrease of LWC. Pentakeep application did not affect proline content. However, under water stress, its application increased the proline level by 9 %.
The work confirmed that the selected physiological characteristics (SLA, LWC and proline) are good evaluating parameters for the determination of water stress in plant. The most sensitive parameter is probably proline concentration in leaves.

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