PHYSIOLOGICAL RESPONSE OF HOP (*Humulus lupulus* L.) PLANTS TO DROUGHT STRESS

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Abstract

The response of plants to water stress is well known in some agricultural crop species while in hop very little is known about the topics. In the research presented the response of hop plants vars. Savinjski golding and Aurora to drought stress was investigated. In a pot experiment the physiological response of hop plants was measured using total radical trapping potential (TRAP), chlorophyll *a* fluorescence and reflectometry. Based on the results obtained the hypothesis was confirmed – the variety Aurora is more drought tolerant compared to the variety Savinjski golding and has a higher regenerative capability. The results form a good basis for further investigations.

Keywords: hop, *Humulus lupulus* L., drought, physiological response, variety

FIZIOLOŠKI ODZIV RASTLIN HMELJA (*Humulus lupulus* L.) NA SUŠNI STRES

Izvleček

Odziv rastlin na vodni stres je dobro raziskan pri nekaterih kmetijskih rastlinah, medtem ko je pri hmelju o tem le malo znanega. V raziskavi smo proučevali odziv rastlin hmelja sort Savinjski golding in Aurora na sušni stres. V lončnem poskusu je bil merjen fiziološki odziv rastlin z uporabo TRAP testa (total radical trapping potential), meritve fluorescence klorofila *a* in reflektometrije. Rezultati potrjujejo hipotezo – sorta Aurora je bolj tolerantna na sušo v primerjavi s Savinjskim goldingom in ima višjo regenerativno sposobnost. Rezultati raziskave so dobra osnova za nadaljnja proučevanja.

Ključne besede: hmelj, *Humulus lupulus* L., suša, fiziološki odziv, sorta

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1 INTRODUCTION

Hop (*Humulus lupulus* L.) is a dioecious cone-bearing plant cultivated for commercial use, predominantly beer brewing [5]. In the cones of female plant the lupulin glands contain bitter acids and essential oils which impart bitterness, flavour and preservation to beer. Commercial hop cultivation occurs in many parts of the world, including Europe, North America, South Africa, Australia and New Zealand. Breeding programs are focused to develop new and improved cultivars, with quantity and quality of yield and disease resistance as main goals. Resistance to abiotic stress is not well known in hops, compared to pest and disease resistance. Much less is also known about genetics of resistance to abiotic constraints or physiological stress. Abiotic stress resistance is typically governed by polygenic inheritance and may be conditioned by multiple, interacting mechanisms. These and other factors make abiotic stress resistance especially difficult to study, both physiologically and genetically. In other agricultural species varieties with enhanced resistance to drought already exist on the market.

The effect of stress can be measured by determining specific or unspecific stress symptoms. Stress can be measured as the change of enzymatic activity, accumulation of osmotics, presence of stress hormones, inhibition of photosynthesis, etc. Drought stress in plants leads to gradually failing of cell antioxidative defense mechanism and subsequently accumulation of reactive oxygen species (ROS). The main consequences of ROS are oxidation of membrane lipids, proteins, nucleic acids and changed redox condition in the cell [6]. ROS are present in all aerobic cells, in equilibrium with antioxidants. When this balance is disturbed oxidative stress occurs. Aerobic organisms respond to oxidative stress by either non-enzymatic or enzymatic defense responses. Non-enzymatic defense involves glutathione, ascorbic acid, α-tocopherol, β-carotene and other compounds capable of quenching ROS. Enzymes involved in defense include superoxide dismutases, catalases, peroxidases, glutathione reductase and $NADP^+$ reducing enzymes [4, 8].

One of the methods for detecting ROS, particularly the formation of hydrogen peroxide, is luminol assisted chemiluminescence [1]. With this method it is also possible to determine ROS quenching activity indirectly in a biological sample, if an exogenous source of ROS is added. The resulting parameter is the total radical trapping potential (TRAP), which represents the cumulative action of intracellular and intercellular enzymatic and non-enzymatic antioxidants, active under experimental conditions.

The TRAP assay is based on a chemiluminescence signal, which occurs when enzyme horseradish peroxidase is oxidized by hydrogen peroxide and loses two electrons. When the horseradish peroxidase oxidizes luminol to recover the missing electrons, the latter becomes unstable and emits chemiluminescence [1].

Chlorophyll fluorescence is a widely used method for accessing the physiological status of the photosynthetic apparatus. PAM (pulse amplitude modulated) fluorometry has become a common tool in plant physiology and ecophysiology to access photosynthetic performance of plants non-invasively. Monitoring of primary photochemistry is extremely useful in the cases of plant stress caused by high temperature, chilling, high light and drought [3]. The photosynthetic parameters potential photochemical efficiency (Fv/Fm), photochemical

quenching (qp) and non-photochemical quenching (qn) are well described in several articles including [9].

Vegetation indexes are robust, empirical measures of vegetation activity at the land surface. They are designed to enhance the vegetation signal from measured spectral responses by combining two (or more) different wavebands, often in the red (600-700 nm) and Near-IR wavelengths (700-1100 nm). Stress is indicated by progressive decrease in Near-IR reflectance due to water loss and increase in red reflectance due to lower rates of photosynthesis.

In the research, the results of hop plant response to drought stress on physiological level using TRAP, PAM fluorometry and reflectometry (normalized difference vegetation index - NDVI) were obtained for the very first time. The good indication for higher tolerance to drought in the variety Aurora was obtained as it was expected.

2 MATERIAL AND METHODS

2.1 Material

In the experiment two well known Slovenian hop varieties were included which differ in drought tolerance in field conditions, Aurora as a more tolerant variety and Savinjski golding as a less tolerant or susceptible variety to drought.

2.2 Methods

2.2.1 The experiment in growth chamber

The experiment was conducted in the year 2009. Plants were grown in pots (substrate Gramoflor, Germany; volume 4 L); every plant was grown in a separate pot at the beginning of the experiment we provided the same volume density of substrate. Four different treatments were undertaken, with 20 plants in each of them:

1. Aurora – drought
2. Aurora – control
3. Savinjski golding – drought
4. Savinjski golding – control

The plants were grown in controled conditions (growth chamber RK - 13300CH, Kambič Laboratory equipment, Slovenia), different regimes were applied for each treatment:

- Control treatment: Normal lightness (15000 lux); relative air humidity 70 %; the day length 15 h; day temperature: 26 °C, night temperature: 20 °C.
- Drought treatment: Normal lightness (15000 lux); relative air humidity 70 % in the beginning of the trial and 55 % under drought conditions.

The plants were optimally irrigated based on tensiometer measurements and with the pot weighting every 3 days as well. At each sampling the soil humidity was determined gravimetrically in each treatment.

All plants were irrigated for the last time on 33rd day of the trial. The control plants were optimally watered every 3 days, while the plants in drought treatment were not watered till regeneration phase started (58th day). The sampling was performed on:

1. 39th day of the trial
2. 51st day of the trial
3. 58th day of the trial
4. 78th day of the trial (regeneration)

After the 3rd sampling (58th day, 25 days after the last watering of plants in drought treatment) all plants (control and drought treatment) were optimally watered every 3 days (310 ml/pot) till the end of the trial.

2.2.3 Physiological measurements

2.2.3.1 Total radical trapping potential (TRAP)

The TRAP assay is based on a chemiluminescence signal, which is produced when horseradish peroxidase is oxidized by H_2O_2 and loses two electrons. When the horseradish peroxidase oxidizes luminol to recover the missing electrons, the latter becomes unstable and emits chemiluminescence [1]. The samples for TRAP measurement were prepared in the following manner: Approximately 100 mg of fresh leaf tissue per sample was frozen in liquid nitrogen, homogenized in 1 ml of potassium phosphate buffer (50 mM, pH 7.0) and centrifuged (10 min, 10000 RPM, 4 °C). The supernatant was frozen and kept at -80°C until the measurement of luminescence. The luminescence reaction mixture contained horseradish peroxidase (0.13 units ml^{-1}) and 350 µM luminol in 0.1 M potassium phosphate buffer, pH 8.5. Into the reaction mixture first the sample and then hydrogen peroxide (final concentration of 870 mM) was added to start the reaction. The mixture was shaken for 15 s followed by 5 s of incubation at 20°C. Chemiluminescence was then recorded as relative light units every 20 s for 25 min with a Victor X5 Multilabel reader 2030 (Perkin Elmer). The TRAP value of plant tissue homogenates was determined as the quotient of blanks to treatments according to

$$TRAP = \frac{\sum I_0}{\sum I},$$

where I_0 represents the measured chemiluminescence of the blank sample (luminol, buffer, horseradish peroxidase and H_2O_2) and I represents the chemiluminescence of the tested sample (supernatant of homogenized plant tissue, luminol, buffer, horseradish peroxidase and H_2O_2).

2.2.3.2 Chlorophyll fluorescence

Chlorophyll fluorescence was measured using a pulse amplitude modulated fluorometer from Opti Sciences (USA) OS-5 using a kinetic test protocol. Before measurement a dark clip was utilized for 10 minutes, then the F_0 of dark adapted leaves was obtained. The saturation pulse of the intensity 8000 µmol $(m^2s)^{-1}$ was switched on for 0,8 s and Fm was obtained. The steady state parameters Fs and Fm were measured after 3 minutes of actinic illumination of 160 µmol $(m^2s)^{-1}$.

From the measured fluorescence several physiological parameters were obtained: maximum quantum efficiency of PSII photochemistry $F_v/F_m=(F_m-F_0)/F_m$, photochemical quenching $qP=(F_m'-F')/(F_m'-F_0)$ and nonphotochemical fluorescence quenching $qN=(F_m-F_m')/(F_m-F_0)$ [9].

2.2.3.3 Reflectometry

Reflectance spectra was measured using an Ocean Optics HL-2000 Tungsten Halogen Light Source, the reflectance probe Ocean optics QR400-7-UV/BX and Ocean optics USB2000 spectrometer with the wavelength range from 400 – 1200 nm. Reflection probe was mounted 20 mm from the leaf at the 45 degrees incidence angle.

Reflection spectra was obtained using a SpectraSuit application software. First the dark spectra was subtracted. Light spectra was measured when 1 mm thick teflon sheet was inserted instead of the leaf. Reflection spectra was calculated as follows:

$R=(I_{leaf}-I_{noise})/(I_{teflon}-I_{noise})$

Every leaf was illuminated for 30 s before measurement to allow for the stabilization of reflectance in the red part of the spectrum.

NDVI was calculated as

$$NDVI = \frac{NIR - R}{NIR + R},$$

where NIR was taken at 800 ± 5 and R at 580 ± 5 nm.

The NDVI values were averaged between 5 plants with the same treatment.

2.2.3.4 Statistical analysis

The TRAP, Fv/Fm, qP, qN and NDVI values were averaged between 5 plants within the same treatment. The statistical significance was evaluated using a student t-test with a=0.05. All data groups were tested against their initial value at the start of the experiment. Additionally, averaged control and drought groups were tested for the significance of difference of their mean values.

3 RESULTS

3.1 Total radical trapping potential (TRAP)

As can be observed from the Figure 1, in the variety Savinjski golding, the TRAP values in control plants decrease in the third week as a consequence of non-optimal conditions in the growth chamber (too strong air ventilation), and again increase during the regeneration phase. The TRAP values in plants under drought conditions increase in the second week (as an active defense against ROS), while during the regeneration they are returned to the control level.

In the Aurora variety, the TRAP values decrease during the trial (as a consequence of the non-optimal conditions in the growth chamber) while in drought plants during the regeneration phase they reach the control level.

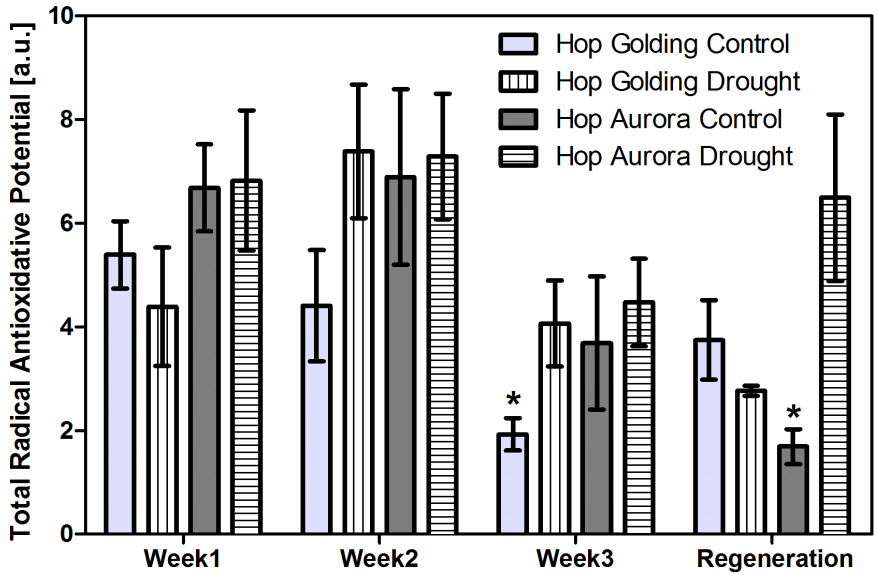

Figure 1: Total radical antioxidative potential (TRAP) of varieties Savinjski golding and Aurora in relation to drought stress duration. The watering was discontinued six days prior to 'Week1' time point. After 25 days of drought, the plants were watered to test their regeneration capability (after 20 days of re-watering). Data shown are mean values ± standard error of a single experiment performed in 5 replicates (n=5). Asterisk (*) represents statistically significant difference between control and drought exposed plants of the same variety at the same time point.

Slika 1: Rezultati TRAP testa sort hmelja Savinjski golding in Aurora, v odvisnosti od trajanja sušnega stresa. Zalivati smo prenehali šest dni pred "Week1" časovno točko. Po 25. dnevih suše smo rastline zalili, da smo preizkusili njihovo sposobnost regeneracije (po 20. dnevih ponovnega zalivanja). Prikazani podatki so povprečne vrednosti ± standardna napaka enega eksperimenta izvedenega v petih ponovitvah (n = 5). Zvezdica (*) predstavlja statistično značilne razlike v TRAP vrednostih, pri rastlinah, izpostavljenih suši in kontrolnimi rastlinami, iste sorte ob istem času.

3.2 Chlorophyll fluorescence

At the start of the experiment we obtained the theoretical optimum value of 0.83 for the parameter Fv/Fm (Figure 2a). In the second week the value dropped for drought exposed Aurora plants, but their state improved during the regeneration. The Fv/Fm value of control plants of Aurora variety dropped during regeneration showing non-optimal environmental conditions in the growth chamber.

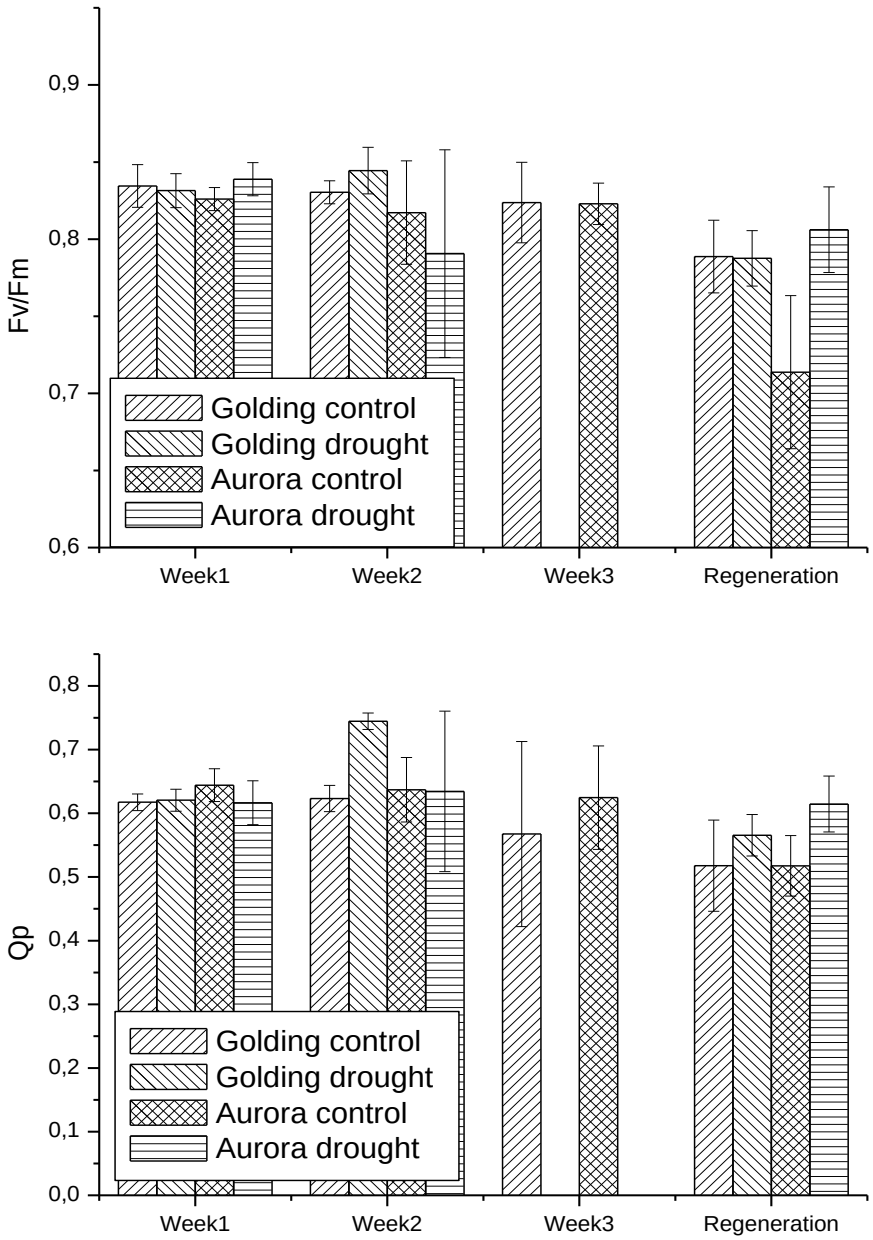

Figure 2a: Photochemical parameters Fv/Fm and qP of hop varieties Savinjski golding and Aurora after different time of drought exposure. Data shown are mean values ± standard deviation of a single experiment performed in 5 replicates (n=5).

Slika 2a: Fotokemična parametra Fv/Fm in qP pri sortah hmelja Savinjski golding in Aurora, po različnem času trajanja suše. Prikazani podatki so povprečne vrednosti ± standardni odklon enega poskusa, izvedenega v petih ponovitvah (n = 5).

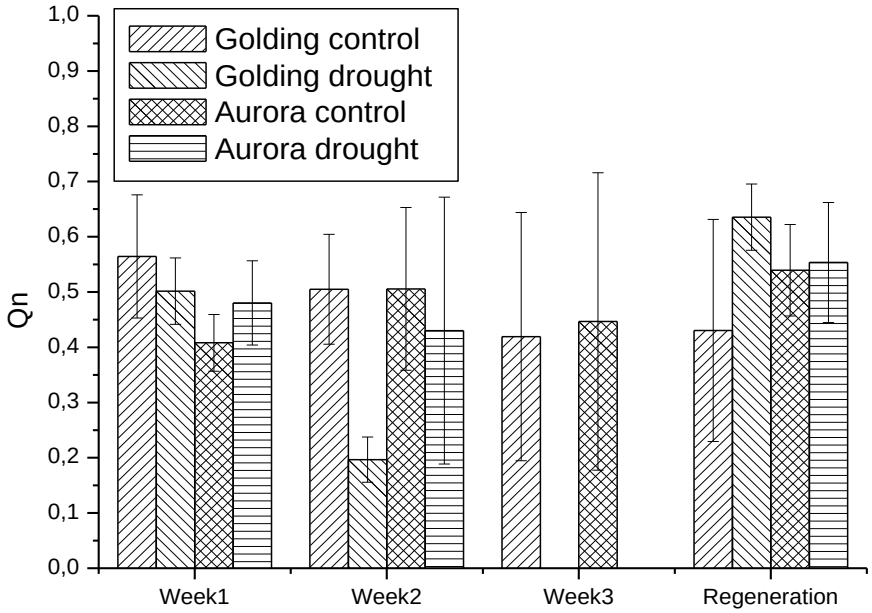

Figure 2b: Photochemical parameter qN of hop varieties Savinjski golding and Aurora after different time of drought exposure. Data shown are mean values ± standard deviation of a single experiment performed in 5 replicates (n=5).

Slika 2b: Fotokemični parameter qN pri sortah hmelja Savinjski golding in Aurora, po različnem času trajanja suše. Prikazani podatki so povprečne vrednosti ± standardni odklon enega poskusa, izvedenega v petih ponovitvah (n = 5).

We concentrated on the data from the regeneration stage which can give us some indication of the damage caused by drought. The increase of the photochemical quenching qP of the drought exposed plants during the regeneration show that the fitness of these plants increased after the stress has been removed. Although the high qN value of the Savinjski golding variety show some damage of the photosynthetic apparatus.

3.3 Reflectometry (NDVI)

The NDVI values of Savinjski golding variety are lower already at the beginning of the experiment. There was a drop in NDVI for the Aurora variety, which was not statistically significant. During the regeneration phase we obtained similar values for the control and drought exposed plants, but the difference between the varieties remained (Figure 3).

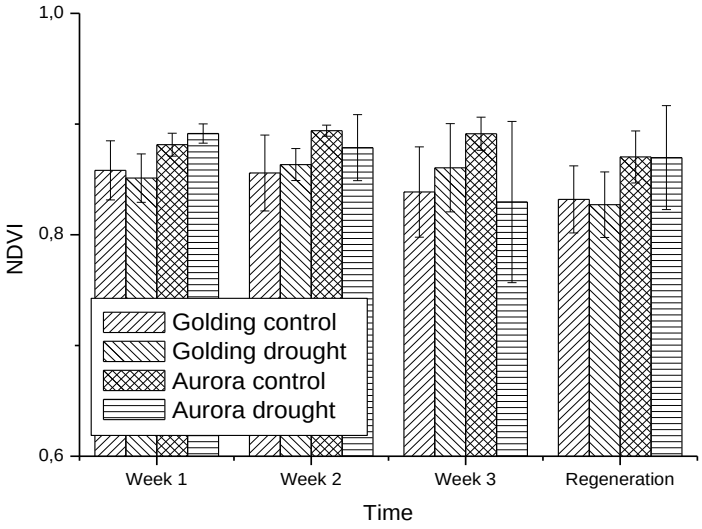

Figure 3: Measured values of the reflectometric index NDVI of hop varieties Savinjski golding and Aurora after different timef drought exposure. Data shown are mean values ± standard deviation of a single experiment performed in 5 replicates (n=5).

Slika 3: Izmerjene vrednosti reflektometričnega indeksa NDVI sort hmelja Savinjski golding in Aurora po različnem času trajanja suše. Prikazani podatki so povprečne vrednosti ± standardni odklon enega poskusa, izvedenega v petih ponovitvah (n = 5).

4 DISCUSSION

The results obtained from TRAP test revealed that the variety Savinjski golding exhibited a typical stress response, as the antioxidative network was transiently induced in the stressed plants. This suggests higher activity of antioxidative enzymes and/or increased concentrations of low molecular antioxidants, such as glutathione [7, 8]. The TRAP value later gradually decreased and reached the lowest point in the drought-stressed regenerated plants (Figure 1). This indicates that antioxidative network in the Savinjski golding plants was overburdened and that the plants could not undo the damage caused by the drought induced oxidative stress.

In contrast, the TRAP value of drought-stressed plants of the variety Aurora decreased progressively in three weeks of drought conditions, however the same was observed for the control plants (Figure 1). During regeneration the TRAP value in drought-stressed plants increased to the initial values and surpassed the control plants' TRAP value. This demonstrates that the antioxidative network of the Aurora plants was not irreversibly damaged during drought stress. Therefore the Aurora variety is more drought tolerant compared to Savinjski golding variety and has a higher regenerative capability.

The results of the regeneration phase can point out some insight into the physiology of the photosynthetic apparatus. The most robust parameter Fv/Fm representing maximum quantum efficiency of photosystem II shows that drought exposed plants managed to avoid permanent damage as expected in the case of mild water stress [2, 6]. The good regeneration capacity is

especially evident for the Aurora variety. During the regeneration phase we obtained the same values for non-photochemical quenching in control and drought exposed plants.

NDVI is a measure of the water content in leaf [10]. The difference between the two varieties at the beginning of the experiment shows higher water content in the Aurora variety. There was some drop of the NDVI during the drought for Aurora but the difference was not significant and quickly returned to the start values during the regeneration phase showing that there was no irreversible damage to the plants.

Based on the results obtained in this preliminary investigation we can conclude that variety Savinjski golding appeared to be more drought sensitive than the variety Aurora. The variety Aurora shows better regeneration capacity as well. The same indication was obtained from the results of TRAP and PAM fluorometry. In the further research the experiment will be conducted in optimal conditions. Finally, the presented results confirm the data obtained from the observations in field conditions as was expected. The use of optimized TRAP method for drought selection in hop breeding program will be studied in the future.

5 ACKNOWLDEGEMENT

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