



**Characterisation of morphological parameters, abscisic acid sensitivity and oxidative stressmarkers of cucumber (*Cucumis sativus* L.) cultivars**

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## **Introduction**

The climate change profoundly affect the annual crop production through that the plants are frequently challenged by abiotic stresses which cause impaired their growth and development. Thus, it is important to examine how cucumber hybrids adapted to either production system may respond to stresses. For this reason the aim of researchers is to design the more resistant genotypes against stresses.

The cucumber (*Cucumis sativus* L.) are an economically and nutritionally important non climacteric vegetable crop cultivated worldwide usually harvested in greenhouse or openfield either (Jasso-Chaverria 2005; Wang et al, 2013). The method is cucumber cultivation is wellknown there are a lot of information about appropriate soilless media and fertilizers (Kappel, 2011). Several bred variants are known (Wang et al, 2010). Growth and environmental conditions differ widely between these two production systems. Modern hybrids have been bred to be adapted to either production system may respond to different levels of mineral nutrients.

The terrestrial vegetation usually are exposed to the harmful effects of their environment. The environment parameter may be considered as stress effect if its intensity and its extent became more unfavorable condition that affects plant metabolism, growth or development (Taiz et al, 2015). For survival against adverse stress conditions, plants require an alteration in their architecture including plant physiology and metabolism (Maryam et al, 2019). The metabolism and the growing of plants decrease due to the effect of stresses, the reproduction may happen earlier than expected. The crop production of harvested cultivars may be damaged significantly.

The unfavorable alteration of condition results the acclimation of plants. The processes of acclimation include the plants respond to through signal transduction to cellular components, gene regulation of transcription factors, and eventually, metabolic changes that induce stress tolerance. The antioxidants and plant hormones are involved in defense against stresses (Kellös et al, 2008). The drought induces secondary stresses such as oxidative and osmotic stresses (Yu et al, 2018) thus these factors are frequently studied nowadays (Abedini et al, 2017).

The drought, cold, water, salinity and light stress can affect unfavorably the growth and development of plants in a poorly chosen production area. The adaptation to abiotic stresses is accompanied by the damaging of life processes which may lead to ruin of the crop production. For this reason, the examination of breeding cultivars is significantly important due to the elaboration of optimal growing conditions (Ördög és Molnár, 2011).

The inadequate fertigation may cause the excessive intake of nutrients to lead the stress development. The overfertilization is the dangerous problem for cultivated plants and the environment recently.

The abscisic acid is the tiny plant hormone that profoundly affects the growth and development of plants. It promotes the acclimation of in the stressed plants. Its effect is dependent on the hormone sensitivity of tissues (Lorrai 2018). The water deficit and seed maturation in plants are accompanied by endogenous production of ABA (Fujita et al, 2011). The relation between plant tolerance and sensitivity of ABA are highly emphasized (Deák et al, 2017). Either stress-induced or exogenous ABA may provoke the production of dehydrins. The dehydrins (DHN) mainly are examined on those plants that are exposed to dehydration and cold (Kosová et al, 2014; Greather & Boddington, 2014; Azarkovich et al, 2016). The biotic and abiotic stresses may be indirectly accompanied by oxidative damage of stressed tissues, the reactive oxygen species can spread within the cells (Janda, 2004).

### **The aims of our work**

1. To determine whether there is a difference in growth parameters (growth, hypocotyl and root system length growth, leaf surface area expansion, dry matter mass growth) between cucumber varieties bred for outdoor production ('Szatmár', 'Senzáció', 'Joker') and greenhouse ('Americana', 'Prior', 'Oitol').
2. To examine whether there is a difference in the sensitivity of the selected cucumber varieties to abscisic acid.
3. To find a difference between the molecular responses of the two groups of varieties following a single treatment of the ABA applied externally. Therefore, we planned to measure the expression level of dehydrin genes.
4. To determine the effect of different levels of nutrient supply on the selected cucumber varieties.
5. To study the growth rate and phenological behavior of two different cucumber hybrids (greenhouse 'Oitol and open field 'Joker') under elevated fertigation concentration. Investigate the expression of DHN3, APX1, GPX1, GR genes under applied treatments. Application of standard plant physiology assays such as FRAP antioxidant assay, Malondialdehyde assay and leaf osmotic potential to investigate the oxidative stress level in treated plants. Application of biophotonic imaging to study the level of biophoton emission as results of lipid peroxidation and cellular oxidative damage in samples of differentially treated plants at different fertigation level.

## MATERIALS AND METHODS

### Plants material and their seedling

For the experiments we worked with three varieties of cucumbers from the open field ('Joker', 'Szatmár', 'Szenzáció') and three greenhouse ('Prior', 'Oitol', 'Americana'). Four seeds per variety were planted in pots with rock wool cubes and perlite.

For the in vitro experiment, 15 seeds per variety were exposed into Petri dish and treated by 20 ml of mock solution for control and 20 ml of 5  $\mu$ M ABA solution every two days. For irrigation of cucumber plants, modified Hoagland medium was used (, Matsumoto & Tamura, 1981; Alan, 1989; Kappel, 2011).

### Examination of morphological parameters

The shoot length, hypocotyl length and root length values of cucumber plants have been determined. We measured the dry matter mass of the shoots and root system of the seedlings grown in perlite, recorded the fresh mass of the shoots for the ABA experiments.

Leave surface area values were calculated using ImageJ (ImageJ, Image Processing and Analysis in Java, USA). The surface of the cotyledons were determined by  $LA=k(s \cdot h)$  (s:width, h:longitude,  $k=0,857$ ) formula.

### RNA isolation and RT-PCR

RNA extraction was carried out by TRI reagent (Molecular Research Center, USA) and CTAB method (Jaakola et al, 2001) according manufacturers instructions. In experiments with ABA, cDNS synthesis was investigated by using the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific Inc., USA). qRT-PCR reactions ran on StepOnePlus Real-Time PCR System (Applied Biosystems, USA). The degree of gene induction were determined by the  $\Delta\Delta C_t$  method (Bookout & Mangelsdorf, 2003). Reverse Transcriptase Kit (Thermo Scientific) was used for cDNS synthesis in experiments on plants grown with HG solutions of varying concentrations. The primers are designed for *CSDHN1*, *CSDHN2*, AND *CSDHN3* *CSAPX*, *CSGR*, *CSGPX*, cucumber genes and the CsAct-3 gene used as control.

### Measurement of photosynthetic activity

The photosynthetic activity (A value) was measured using an IRGA photosynthesis meter (ADC Bioscientific Limited, LCi Console, serial number 31926, Herts, UK), taking the values of every second leaf of the all plants.

#### Determination of antioxidant capacity by FRAP method

The antioxidant capacity of the extract of the leaves of cucumber plants was determined by the Ferric Reducing Ability of Plasma method (BENZIE & Strain, 1999).

#### Measurement of gvaicol peroxidase activity by POD method

The activity of gvaicol peroxidase, was measured by the instruction which written by Chandrakar et al in 2016 with some modifications.

#### Measurement of lipid peroxidation by determination of TBARS content

The MDA content in leaf tissues during lipid peroxidation was determined and its concentration was expressed in nmol g<sup>-1</sup> (fresh weight) (Hodges et al, 1999).

#### Measurement of lipid peroxidation by biofoton emission imaging

The measurements were carried out on cucumber plants using the NightShade LB 985 Plant Imaging System (Berthold Technologies, Bad Wildbad, Germany). Images of intact leaves of similar size picked out from plants were taken in a darkroom with a sensitive thermoelectric cooling (-70 °C) CCD camera (NightOWLcam, Berthold Technologies).

#### Osmotic stress measurment

The method of measurement is based on a decrease in freezing points of the dilute solutions is proportional to the ozmolite content. Micro osmometer (Osmomat 030-D; Gonotec, Berlin, Germany) was used for the expreiments. (Bajji et al, 2001).

#### Statistical analysis

The evaluation of the resulting data was carried out with using the Microsoft Excel 2013 (Microsoft Corporation, 2013) spreadsheet program, for the correlation and one-way ANOVA analysis and homogeneity test, IBM SPSS Statistics and R statistical programs were used.

## RESULTS

### Morphological parameters of cucumber varieties grown in peat mixture

We used three varieties of openfield cucumbers ('Szatmár', 'Szenzáció', 'Joker') and three glasshouse ('Americana', 'Prior', 'Oitol') in peat mixtures and then irrigated the seedlings with tap water every two days. The average height of the 'Oitol' variety was significantly higher than the others, with the lowest growth of the 'Szenzáció' variety.

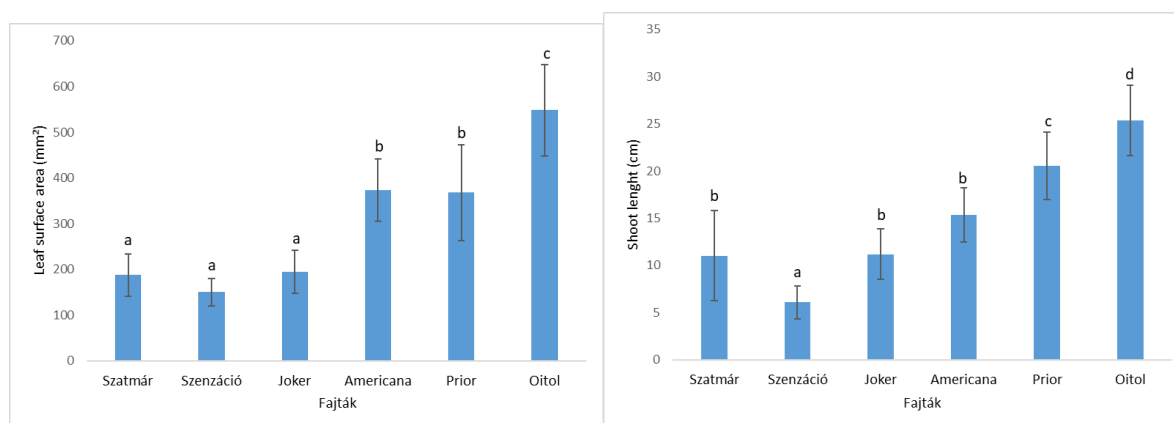


Figure 1. Cucumber varieties grown in peat mixtures are the values of shoot length and photosynthetic activity.

According the Figure 1, there was not significant difference between the varieties on photosynthetic activity, with only the 'Prior' variety being significantly different from the others. The plant size and the total leaf surface area were higher on the greenhouse varieties.

### Morphological parameters of cucumber varieties grown in perlite

Three openfield ('Szatmár', 'Szenzáció', 'Joker') and three greenhouse ('Americana', 'Prior', 'Oitol') cucumber varieties were grown in a perlite medium. The length of the root system, the length of hypocotyl, the length of the shoot, then the total leaf surface area per plant at least the dry weight of the seedling shoot and root system.were measured on 21-days plants. The root length of openfield varieties was significantly higher than greenhouse ones. As for the hypocotyl lengths, the surface area of cotyledon and the dry weight of the shoot, the greenhouse varieties had significantly higher values (Fig. 2) There were a positive correlation between the hypocotyl length and the surface area of cotyledons ( $r=0.86$ ,  $p<0.01$ ) and the dry mass and the surface area of cotyledon ( $r=0.739$ ,  $p<0.01$ ).

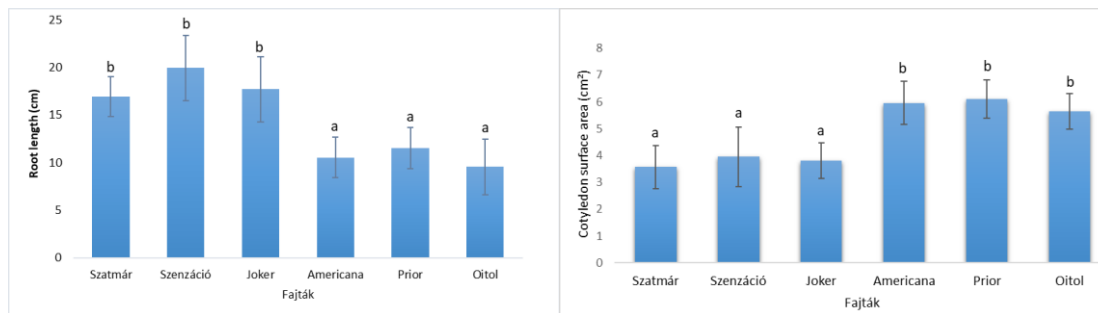


Figure 2. Growth parameters of cucumber varieties grown in perlite.

### Nutrient supply effect on cucumber varieties using of 0.125x and 0.5x Hoagland solutions

Three openfield ('Szatmár', 'Szenzáció', 'Joker') and three greenhouse ('Americana', 'Prior', 'Oitol') cucumber varieties were grown in a perlite and rockwool medium. The length of hypocotyl, the total leaf surface area per plant, the dry weight of the seedling shoot were measured on 21-days plants. The seedlings were treated with 250 ml of 0,125x and 0,5xHG solution per pots every two days. For plants irrigated with Hoagland solution with a concentration of 0,5x, hypocotyl length and shoot dry matter mass values were always given higher values than greenhouse varieties. The differences between the total leaf surfaces were less uniform, the lowest values were measured for outdoor varieties, while the largest for the greenhouse hybrid 'Oitol' was measured at the higher nutrient solution concentration (Fig 3).

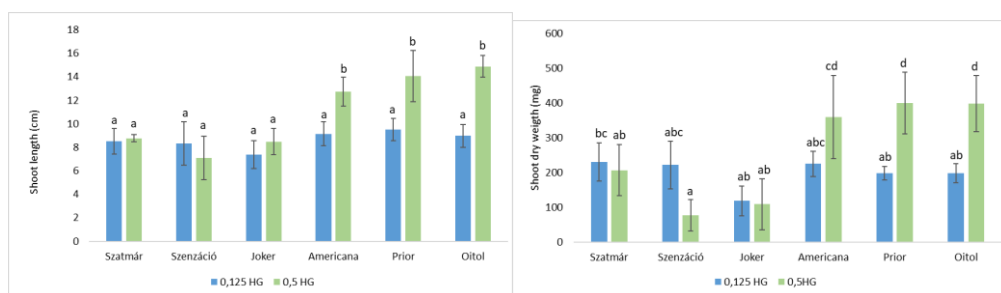


Figure 3. Growth parameters of cucumber cultivars treated with 0, 125x and 0.5 HG solution.

### Treatment with externally applied ABA hormone

#### *In vitro* experiments

The six cucumber varieties were treated with 5  $\mu$ M ABA and control mock solution in Petri dish. The 'Szenzáció' variety has been removed from our measurements due to its limited germination. As for the length of the root system, a significant difference in the effect of the hormone was detected for all varieties. As for shoot fresh mass, there was a significant



difference between the treated and control lines for the 'Prior' and 'Oitol' and 'Americana' varieties in the greenhouse (Fig. 4).

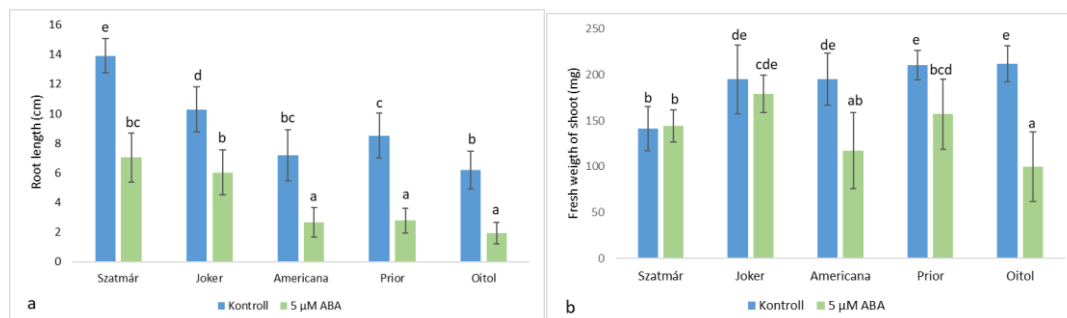
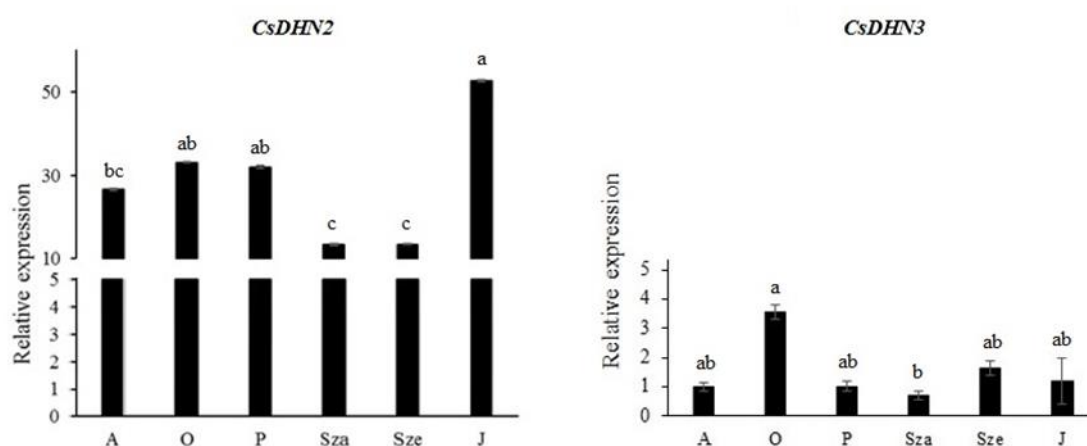


Figure 4. Growth parameters of ABA treated and control cucumber varieties

### Expression of CsDHN genes under the influence of ABA

In our studies, expression of *CsDHN2* and *CsDHN3* dehydrin genes were observed under the influence of externally applied ABA. Based on our results of RT-PCR and quantitative RT-PCR, the induction of the *CsDHN2* gene was stronger on those plants treated with ABA. *CsDHN3* expression in leaf tissue was also strong under control conditions, so the gene itself is likely to play a smaller role in the ABA-regulated stress response in the genotypings studied. The results of RT-qPCR showed a stronger expression of ABA treatment for all dehydrin genes tested compared to the *CsAct7* reference gene, with no significant differences between the varieties (Figure 5).



6. Tests of cucumber varieties treated with Hoagland solution at different concentrations

7.

## Two varieties treated with five hoagland solutions of different strengths

Morphological parameters:

Two types of cucumbers (greenhouse 'Oitol', outdoor 'Joker') were examined after nutrient solution with five hoagland solutions of different concentrations ( $0 \times 5$ ,  $1 \times$ ,  $1,5 \times$ ,  $2 \times$ ,  $2,5 \times$  HG). According to shoot length, hypocotyl length and total leaf surface values, the 'Oitol' variety has significantly higher growth parameters than the 'Joker'. However, with different nutrient supply, there was no marked difference in plant growth within the varieties (Figure 6).

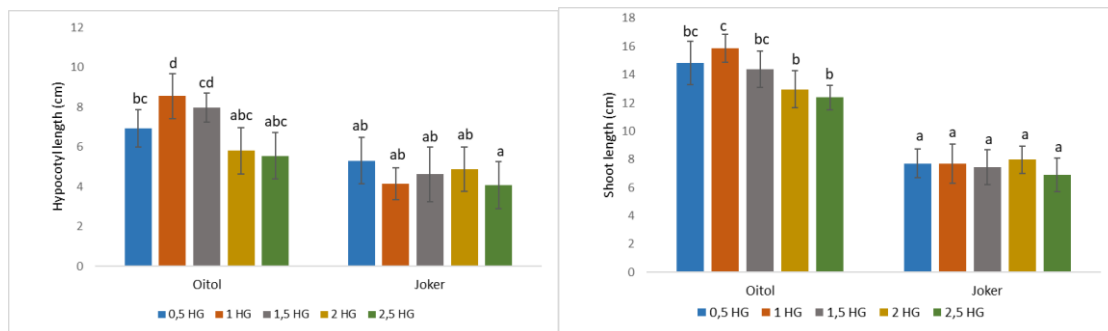


Figure 6. Morphological parameter values of cucumber varieties 'Oitol' and 'Joker' after treatment with 5 different concentrations of Hoagland solution.

Examinations of molecular markers of oxidative stress:

Gvajakol peroxidase activity was measured from the fresh leaves of 21-day plants according to the procedure described in the Material and Methods section. The enzyme activity raised after  $2,5 \times$  HG nutrient solution on both cultivars, but no significant differences can be seen between varieties. The 'Joker' variety has significantly lower FRAP values than 'Oitol' even with standard  $0.5 \times$  HG and higher nutrient solution. In both cases, the  $1.5 \times$ ,  $2 \times$  and  $2.5 \times$  treatments displayed significantly higher FRAP values than  $0.5 \times$  and  $1 \times$  HG. Measuring lipid peroxidation, significantly higher MDA values were given for the 'Joker' variety for all treatments. There was also a difference in results within varieties. In the 'Oitol' and 'Joker' varieties, malondialdehyde content increased significantly as the concentration of the HG solution increased (Fig.7). For both varieties, a positive correlation was found between the degree of lipid peroxidation in the leaves and the higher nutrient solution concentration used (for the Joker, the correlation was  $r=0,658$   $p<0,04$ ; Oitol  $r=0.543$ ,  $p<0.02$ ). There is a moderately negative correlation between the MDA content and the FRAP value of cultivars ( $r=-0.562$ ,  $p<0.01$ ).

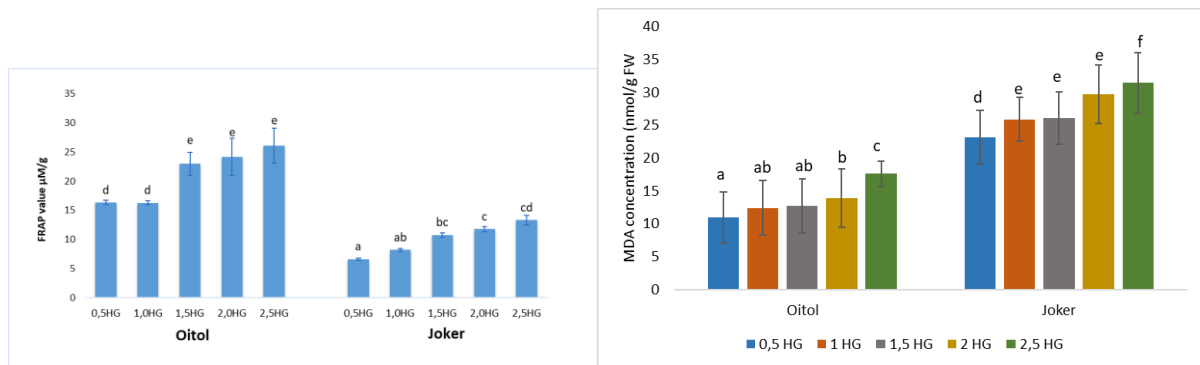


Figure 7. MDA content and FRAP value of cucumber varieties 'Oitol' and 'Joker' after treatment with 5 different concentrations of Hoagland solution.

In determining osmotic potential, there was a significant difference between the two varieties, with the 'Joker' variety possessing lower values (Fig. 8). There was also a significant difference within varieties, with treatment with more concentrated Hoagland solutions resulting in lower osmotic potential in both varieties. There was therefore a negative relation between the concentration of the nutrient solutions used and the osmotic potential values measured on the plants.

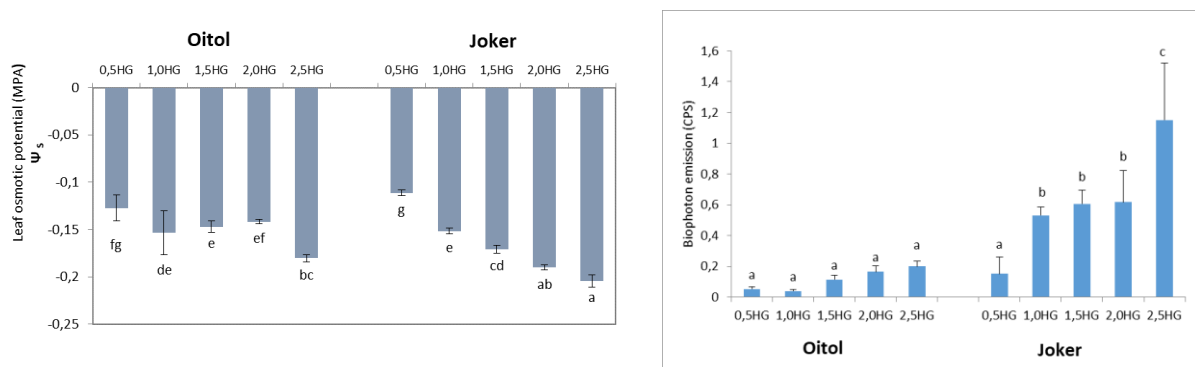
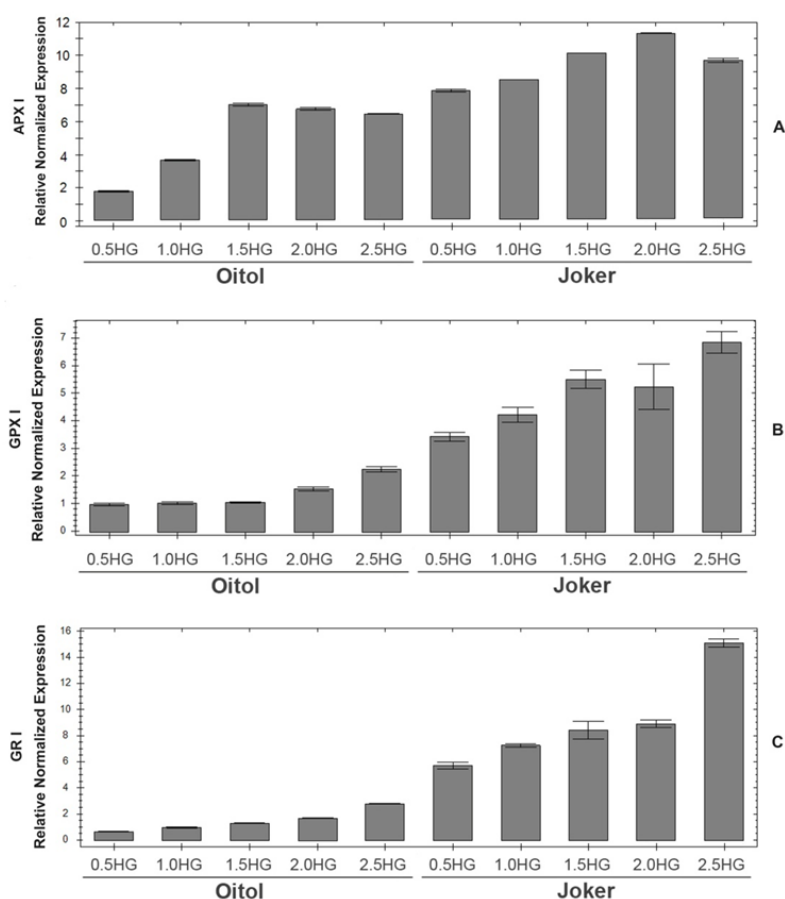


Figure 8. Osmotic potential and biofoton emission values of of cucumber varieties 'Oitol' and 'Joker' after treatment with 5 different concentrations of Hoagland solution.

Transcription of *DHN3* genes and some enzymes with antioxidant activity was carried out on cucumber 'Oitol' and 'Joker' plants with five different concentrations of Hoagland solution. In the qPCR analysis, a significant difference was detected among the expression of the *CsDHN3* gene in 'Oitol' plants: in higher concentration of HG accompanied by higher expression of *CsDHN3* (Figure 9). In addition, ultra-weak biofoton emission (UPE) imaging analysis was used to test lipid peroxidation processes, the number of biofotons leaving the leaves per second (CPS: count per second) was also determined (Fig. 8). For the 'Oitol' variety, there was no significant difference between the values obtained for the different treatments. For the 'Joker'

variety, the lowest value is 0,5 HG, the following set of values is given by plants treated with 1 x, 1,5 x and 2 x HG solutions, with the highest value being achieved during treatment with 2,5 x HG. The expression of the *APX1* gene in the 'Oitol' variety shows low expression in the 'normal' nutrient solution, while in the more concentrated Hoagland solutions the expression of the gene increases sharply in the 1.5 × HG and more concentrated Hoagland solutions, compared to 0.5× HG. In all cases, the 'Joker' has shown a high expression of this genes. For *GPX1* and *GR1* genes, there were also differences between the two varieties. No greater expression can be seen in the 'Oitol' variety after nutrient solution with the increasingly concentrated Hoagland solution. However, the 'Joker' variety has a stronger expression of genes, and the degree of expression increases with the concentration of the HG solution (Figure 9).



9. Expression patterns of APX1, GPX1, GR1 genes of two cultivar irrigated 5 different HG solution.

## **Conclusion**

### **Morphological parameters of cucumber varieties grown in peat mixture**

In terms of shoot length, greenhouse varieties generally had higher values, suggesting an increased elongated growth. Types of openfield and greenhouse varieties were also separated by leaf surface. This is explained by the different demands of the variety types. Each open-field and greenhouse variety must adapt to different environmental conditions due to their cultivation technology (Ackerl et al., 2004). For photosynthetic activity, no significant differences were found for either variety. With higher leaf area greenhouse varieties may be capable of producing larger crops, and more photo-synthylates may result in higher stress tolerances than openfield varieties.

### **Morphological parameters of cucumber varieties grown in perlite**

Plants were grown and harvested under controlled conditions thus the differences between the types of varieties were clearly visible. In the case of hypocotyl length characteristic of elongated growth, greenhouse varieties and openfield varieties for root lengths gave higher values. It can be assumed that the increased elongated growth in the greenhouse varieties was made possible by controlled environmental conditions, while in the case of openfield varieties the longer root system can ensure adequate water supply. Interestingly, the cotyledon surface area was found as a sensitive parameter correlating with elongated growth. This easy-to-measure plant organ in the selection of breeding lines may provide an early indication of growth strength.

### **Nutrient supply effect on cucumber varieties using of 0.125x and 0.5x Hoagland solutions**

Soilless growth media and nutrient solutions were applied according to the needs of plants. For cucumbers grown on different concentration of HG solution, there were significant differences in morphological parameters (Li and Cheng, 2015) which supported by shoot length values that greenhouse cucumber varieties are capable of higher elongated growth (0.5x HG values). The hypocotyl lengths showed a similar trend, but were less sensitive. The shoot dry mass values were also higher than greenhouse hybrids for normal (0.5x HG) nutrient solution. Since differences in dry weight and seedling sizes between groups of varieties are more pronounced than leaf surface differences, it can be assumed that greenhouse varieties may show increased biomass growth in their strongly crescent stems. The sensitivity of greenhouse varieties and the insensitivity of the openfield ones to reduce the concentration of nutrient solution were demonstrated for all measured parameters.

## **Treatment with externally applied ABA hormone**

### *In vitro* experiments

Absciscic acid (ABA) is a phytohormone that has multiple roles in plant growth and development, it is involved in various biological processes. (Lorrai et al, 2018). Different ABA sensitivity may indicate different stress tolerances of the varieties. The ABA can promote or inhibit root development and decrease shoot growth, as part of the stress tolerance response. The greenhouse varieties usually raised under optimal conditions, so in their case the effects of stress hormones should be less emphasized. Based on our results, we found a significant difference between the elongation of the root system of the ABA treated and control cucumber varieties. However, there was no clear difference between the responses of varieties groups. However, for measuring the fresh weight of the shoot, the increased sensitivity of the greenhouse varieties to the ABA were found compared to openfield ones. The greenhouse hybrids are less exposed to environmental stress, thus the ABA may be able to produce a more significant response.

### Expression of CsDHN genes under the influence of ABA

Research has shown the significant role of the DHN gene family in the abiotic stress response. The inductability of dehydrin, ABA is a well-studied process at both protein and mRNA levels (Greather & Boddington, 2014). The experiments were completed with expression studies on putative dehydrin genes (CsDHNs), which was investigated after external ABA treatment. In our study, strong induction of the *CsDHN2* gene was observed in all of the varieties for externally used ABA treatment. At the same time, the expression of *CsDHN3* was also high in control plants. Presumably, the role of the *CsDHN3* gene product is different, not involved in the ABA's mediated stress responses. F1 hybrids of the same cultivation type did not form distinct groups according to their transcriptional responses to the ABA treatment applied.

## **Two varieties treated with five hoagland solutions of different strengths**

### Morphological parameters and nutrient supply

The growth responses are one of the best indicators of stress on plants (Zhang et al, 2017), which may also be a consequence of excessive nutrient intake. We examined the effect of nutrient solution with five different strengths (0.5x, 1x, 1.5x, 2x, 2.5x) Hoagland solution on cucumber varieties 'Oitol' and 'Joker'. Based on the measured data, we confirmed our previous results that of the two varieties, 'Oitol' had significantly higher leaf surface values. However,

the use of the more concentrated Hoagland solution did not result in a significantly more extensive leaf surface for either variety. The hypocotyl length and seedling size increased to 1x HG for both varieties, but this was significant only for the 'Oitol'. Excessive nutrient supply may be behind the growth. The seedling size values showed an increase in higher nutrient solution concentrations, which was also significant for the 'Oitol' variety. This may be explained by several stress physiological phenomena. Due to the osmotic effect of concentrated nutrient solution, the water potential may have been reduced between the cells of the soil and roots. Thus the water intake is inhibited, osmotic stress can develop in the plant's root cells (Zhang et al., 2017), which adversely affects growth. Excessive nutrient uptake can also disrupt homeostasis of ions. This may also explain why the 'Oitol' plants grown on the 2.5x HG solution were smaller in size than the 1x HG treated plants.

#### Relationship between oxidative stress and stress tolerance

The lipid peroxidation is one type of cellular damage. During the process malondialdehyde (MDA) is formed, which is an excellent marker of the indication of lipid peroxidation (Hegedűs and Stefanovics, 2012; Zhang et al., 2008; Zhu et al., 2008). The degree of oxidative damage may be related to the stress tolerance of a particular cultivar. Cucumber varieties grown openfield have a higher stress tolerance than greenhouse ones (Szegő et al., 2019) due to their growing technology. Based on our results, the use of higher concentrations of Hoagland solutions generated a more oxidative response than the openfield 'Joker' variety than in the greenhouse 'Oitol'. The greenhouse varieties are better adapted to excessive nutrient intake than those in the openfield, due to their harvesting technology. In the case of openfields, excessive nutrient intake can lead to a more stress (osmotic stress, oxidative stress). If mineral elements are more efficiently incorporated into the faster-growing tissues of the greenhouse variety ('Oitol'), this may result a higher osmotic potential. Excess salts may be stored in the tissues of the slower-growing 'Joker' variety, thus lowering the osmotic potential. Therefore, based on our results, there is a significant difference in the mineral nutrient management of the two varieties and in the adjustment of the osmotic balance formed by osmolites. Increased stress level may be accompanied by high level of ROS generation which can lead to tissue damages. The tolerance of a particular genotype may be manifested in its ability to cope with oxidative stress caused by overdosed fertigation. The mobilization of the enzymatic and non-enzymatic redox defense systems may be monitored by some molecular markers and gene expression assays in plants, giving a broader perspective to determine stress tolerance and dynamics in those cultivars. The results of the malondialdehyde (MDA) determination and the antioxidant

capacity (FRAP) measurement together indicate a different tolerance of the two varieties following increased nutrient solution. Osmotic stress, triggered by increased nutrient concentrations, was likely to have contributed to the development of oxidative stress, which was more stronger for the Joker. Lipid peroxidation processes are characterized by active biophoton emissions, from the level of emissions of biophotons, we can indirectly deduce the oxidative state of the studied plants (Birtic et al., 2011).. The use of Hoagland solution with increasing concentration in the 'Joker' variety triggered a strong bioluminescence, suggesting stronger oxidative stress than 'Oitol'.

Dehydrins can greatly help tolerance to certain abiotic stress. The degree of their expression is tissue-specific and usually involves different levels of expression of different stress effects (Allagulova et al., 2007; Yu et al, 2018). Under the experimental conditions used, we were able to detect expression of the *CsDHN3* gene, the expression of which was low for the 'Joker' variety. For 'Oitol', the expression levels of the *CsDHN3* gene were higher at 1,5x HG nutrient concentration and above this. The DHN3 protein belongs to a class of dehydrine protein family that also has membrane protection function, which can inhibit lipid peroxidation processes. With higher *CsDHN3* expression, the lipid peroxidation processes in the leaves of the 'Oitol' variety due to increased nutrient concentration were not significant compared to the 'Joker' variety. The expression of antioxidant enzymes (APX1, GPX1, GR) was fundamentally higher in the 'Joker' variety, even in normal nutrient solution (0.5 ×HG, 1 ×HG) than in the case of 'Oitol'. This indicates the presence of increased oxidative stress in the 'Joker' variety (there may be an increased ascorbate glutathione cycle).

Overall, the open field 'Joker' exhibited slower growth, lower values for antioxidant/reducing power (FRAP) and higher level of lipid peroxidation (MDA) indicating more oxidative stress which was also generally associated with more concentrated Hoagland nutrition. Moreover, elevated fertigation caused deeper osmotic potential to 'Joker', promoting osmotic stress as a likely explanation for the more strained oxidative status of this hybrid. As far as dehydrins are concerned, *CsDHN3* displayed specific induction under high nutrition in 'Oitol' hybrid only. This suggests unique role of this protein in protecting plants from the nutrient induced oxidative damage. Biophoton emission imaging was successfully used to confirm biochemical data about oxidative damage in the leaves that showed especially higher value on 'Joker'. These data indicates differential activation of molecular tolerance mechanisms in the two cultivars in case of elevated nutrient provision (tathione).



## New scientific results

In the light of the results of our series of experiments with cucumber F1 hybrids, we can identify the following new scientific results:

1. Three openfield ('Szatmár', 'Senzáció', 'Joker') and three greenhouse cucumber varieties ('Americana', 'Prior', 'Oitol') have been examined in more detail in terms of their morphological parameters and there are significant differences between the varieties. We found that the roots of openfield hybrids are typically larger than greenhouses ones. The cotyledon surface was found to be a sensitive parameter correlating with elongated growth.
2. The ABA sensitivity of the studied varieties is different. The externally applied ABA has little effect on the shoot elongation of openfield hybrids, but it significantly inhibited for greenhouses. As for the expression of *CsDHN3* and *CsDHN2* genes to the plant hormone ABA, *CsDHN2* is most powerfully activated.
3. Our experiments revealed significant differences in the oxidative stress state of the two selected varieties, which maybe related to differences in tolerance of groups of varieties to stress. In particular, we examined the stress caused by excessive nutrient supply. In the light of the results, it can be concluded that increased nutrient solution had only a small impact on the growth of hybrids. For normal and elevated nutrient solution, the antioxidant capacity of 'Oitol' is typically higher and its lipid peroxidation was lower than that of the 'Joker'. The genes of antioxidant enzymes involved in the protection against oxidative stress were expressed higher in the 'Joker' hybrid. These results demonstrate a higher oxidative stress level of the 'Joker'. With increased nutrient solution, oxidative stress parameters (e.g. lipid peroxidation). Furthermore, elevated concentration nutrient solution in the 'Oitol' hybrid specifically increased the expression of the *CsDHN3* gene.

## REFERENCES

- ABEDINI R., GHANE GOLMOHAMMADI F., PISHKAM RAD R. (2017): Plant dehydrins: shedding light on structure and expression patterns of dehydrin gene family in barley. *Journal of Plant Research*. **130**:747–763p
- ALLAGULOVA C.R., MASLENNIKOVA D.R., AVALBAEV A.M., FEDOROVA K.A., YULDASHEV R.A, SHAKIROVA F.M.(2015): Influence of 24-epibrassinolide on growth of wheat plants and the content of dehydrins under cadmium stress. *Russian Journal of Plant Physiology*. **62**:465-571p.
- ALAN R. (1989): The effect of nitrogen nutrition on growth, chemical composition and response of cucumbers (*Cucumis sativus* L.) to nitrogen forms in solution culture. *Journal of Horticultural Science*. **64**(4): 467-474p.
- BENZIE I. F. F., STRAIN J. J. (1996): The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”:the FRAP assay. *Analytical Biochemistry*. **239**(1):70-76p.
- BIRTIC S., KSAS B., GENTY B., MUELLER M.J., TRIANTAPHYLIDÉS C., HAVAUX M. (2011): Using spontaneous photon emission to image lipid oxidation patterns in plant tissues. *The Plant Journal*. **67**(6):1103–1115p.
- DE BEVER A, NDAKIDEMI P. A., LAUBSCHER C. P (2012): Effects of different combinations of Hoagland’s solution and *Azolla filiculoides* on photosynthesis and chlorophyll content in *Beta vulgaris* subsp. *Cyca* ‘fordhook giant’ grown in hydroponic cultures. *African Journal of Biotechnology*. **12**(16):2006-2012p.
- GLITS M., GÓLYA E., GYÚRÓS J., GYÖRFI J., HODOSSY S., HOLB I., HRASKÓ I., KOVÁCS A., KOVÁCSNÉ GYENES M., NAGY GY., NAGY J., NÉMETHY Z., OMBÓDI A., PÉNZES B., SLEZÁK K., SZÖRINÉ Z. A., TERBE I., ZATYKÓ F. (2005): Zöldségtermesztés termesztőberendezésekben. *SZIE Kertészettudományi Kar Zöldség- és Gombatermesztési Tanszék. Budapest*. 32-40p.
- GRAETHER S. P., BODDINGTON K. F. (2014): Disorder and function: a review of the dehydrin protein family. *Frontiers in Plant Science*. **5**:576p.
- HEGEDŰS ATTILA, STEFANOVITSNÉ BÁNYAI ÉVA (2012): Természetes antioxidáns forrásunk: a gyümölcs. Debreceni Egyetem, AGTC, Kertészettudományi Intézet ISBN 978-615-5183-26-3, 26-31 p.

HOAGLAND D.R. & ARNON D.I. (1950): The Water-Culture Method for Growing Plants without Soil. *California Agricultural Experiment Station*. Circular-347. 1-31p.

JANDA TIBOR (2007): Termesztett növények abiotikus stresszfolyamatai és egyes védekező mechanizmusai, különös tekintettel az antioxidáns rendszerekre. Akadémiai Doktori Értekezés, MTA Mezőgazdasági Kutatóintézete Martonvásár, 2007

JASSO-CHAVERRIA C., HOCHMUTH G. J., HOCHMUTH R. C., SARGENT S. A. (2005): Fruit Yield, Size, and Color Responses of Two Greenhouse Cucumber Types to Nitrogen Fertilization in Perlite Soilless Culture. *Horticultural Technology*. **15(3)**:565-571p.

KAPPEL N. (szerk.) (2011): Tökfélék termesztése. Mezőgazda Kiadó Kft. Budapest 55-58p

KELLŐS T., TÍMÁR I., SZILÁGYI V., SZALAI G., GALIBA G., KOCSY G. (2008): Stress hormones and abiotic stresses have different effects on antioxidants in maize lines with different sensitivity. *Plant Biology*. **10(5)**:563-72p

MARYAN E. K., LAHIJI S. H., FARROKHI N., KOMELEH H. H. (2019): Analysis of Brassica napus dehydrins and their co-expression regulatory networks in relation to cold stress. *Gene Expression Patterns*. **31**:7-17p.

MILLNER P.D., KITT D.G. (1992): The Beltsville method for soilless production of vesicular-arbuscular mycorrhizal fungi. *Mycorrhiza*. **2**:9-15p.

LORRAI R., BOCCACCINI A., RUTA V., POSSENTI M., COSTANTINO P., VITTORIOSO P. (2018): Absciscic acid inhibits hypocotyl elongation acting on gibberellins, DELLA proteins and auxin. *AoB Plants*. **10(5)**:061p.

ÖRDÖG V., MOLNÁR Z. (2011): Növényélettan. Debreceni Egyetem. Nyugat-Magyarországi Egyetem. Pannon Egyetem, Debrecen

TALANOVA V. V., TOPCHIEVA L. V., TITOV A. F. (2006): Effect of abscisic acid on the resistance of cucumber seedlings to combined exposure to high temperature and chloride. *Biology Bulletin*. **33(6)**:619–622p.

TAIZ L., ZEIGER E., MOLLER I. M., MURPHY A. (2015): 24 th Chapter: Abiotic stress In *Plant Physiology and Development*. 6th Edition. ISBN10 1605352551

SZEGŐ A., BADICS E., GUBALA D., OSZLÁNYI R., BAT-ERDENE O., KAPPEL N., PAPP I., KISS-BÁBA E. (2019): Diverse responsiveness of dehydrin genes to abscisic acid and water stress treatments in cucumber F1 cultivar hybrids. *The Journal of Horticultural Science & Biotechnology*. **94(6)**:726-734

YU Z., WANG X., ZHANG L. (2018): Structural and functional dynamics of dehydrins: A plant protector protein under abiotic stress. *International Journal of Molecular Sciences*. **19**:3420p.

WANG S., SIU X., HU L., SUN J., WEI Y., ZHANG Z. (2010): Effects of exogenous abscisic acid pre-treatment of cucumber (*Cucumis sativus*) seeds on seedling growth and water-stress tolerance. *New Zealand Journal of Crop Horticultural Science*. **38(1)**:7-18p.

WANG Y., JI K., DAI S., HU Y., SUN L., LI Q., CHEN P., SUN Y., DUAN C., WU Y., LUO H., ZHANG D., GUO Y., LENG P. (2013): The role of abscisic acid in regulating cucumber fruit development and ripening and its transcriptional regulation. *Plant Physiology and Biochemistry*. **64**:70-79p.

ZHANG G.W., LIU ZL., ZHOU JG., ZHU YL. (2008): Effects of Ca(NO<sub>3</sub>)<sub>2</sub> stress on oxidative damage, antioxidant enzymes activities and polyamine contents in roots of grafted and non-grafted tomato plants. *Plant Growth Regulation*. **56**:7–19p.

ZHU J., BIE Z.L., LI Y.N. (2008): Physiological and growth responses of two different salt-sensitive cucumber cultivars to NaCl stress. *Soil Science Plant Nutrition*. **5(3)**:400–407p.

## **Publications**

Szegő, A.; Badics E.; Gubala D.; Oszlányi R.; Bat-Erdene O.; Kappel N.; Papp I.; Kiss-Bába E. (2019): Diverse responsiveness of dehydrin genes to abscisic acid and water stress treatments in cucumber F1 cultivar hybrids. *The Journal of Horticultural Science & Biotechnology*. Vol 94, 6: 726-734 doi.org/10.1080/14620316.2019.1628665 IF: 0.64

Oszlányi, R.; Mirmazloun I.; Pónya Zs.; Szegő A; Bat-Erdene O.; Papp I.: Oxidative stress level and dehydrin gene expression pattern differentiate two contrasting cucumber F1 hybrids under high fertigation treatment. *International Journal of Biological Macromolecules* Volume:161, (15):864-874, <https://doi.org/10.1016/j.ijbiomac.2020.06.050> IF: 4,784

Oszlányi,R.; Papp I.,(2021): Uborka (*Cucumis sativus* L.) F1 hibridek növekedési paramétereit és abszcizinsav érzékenysége. *Kertgazdaság* 2021. évf. 1. szám