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Original article

# Investigation of *Ustilago maydis* Infection on Some Physiological Parameters and Phenotypic Traits of Maize

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**Abstract**

One of the most dangerous diseases of corn is *Ustilago maydis* DC. Corda, which damages the maize yield every year. It is difficult to protect against the corn smut infection because the efficiency of fungicide treatment is inadequate. The purpose of this research was to examine the effects of corn smut infection on some physiological and morphological parameters of maize (*Zea mays* L. cv. Armagnac). Furthermore, the aim of this research was to examine the possible compensation effects of gibberellin ($GA_3$) and ethylene (as ethrel) separately, and in combination on the corn smut infection. The plants were grown in a greenhouse, they were infected and treated in the five leaf phenological phase with two ml of sporidium suspension (10,000 sporidium/ml), injected into the stem. At the same time, one ml of gibberellin, ethylene and the combination of the two hormones were injected. The relative chlorophyll content, stem diameter and plant height were measured in the fourth and fifth leaves of plants, 7, 11 and 14 days after the infection (DAI). The photosynthetic pigments were determined from the fourth leaf, and the rate of lipid peroxidation was measured from the fifth leaves, 14 DAI.

The corn smut infection significantly reduced the relative-chlorophyll content 14 DAI compared to the control. The gibberellin reduced the harmful effects of the pathogen. No significant difference was recorded in case of stem diameter. The infected plants were shorter than the control plants when treated with ethylene.

The effect of corn smut infection was more pronounced in the terms of photosynthetic pigments and the rate of lipid peroxidation. Ethylene treatment increased while gibberellin treatment reduced the impact of the infection.

In this research, the corn smut infection and hormone treatments had larger impact on measured physiological parameters than on morphological parameters.

**Keywords:** Ustilago maydis, corn, lipid-peroxidation, chlorophyll content, plant height, stem diameter.

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## INTRODUCTION

The total cultivated area of maize was 187.95 million ha in 2018 woldwide (FAOSTAT 2018). Maize (*Zea mays* L.) is one of the most important crops in Hungary. Maize is mainly used in the feed industry, and sweet corn is grown for direct human consumption and used in the canning industry (Sots and Bnyiak, 2019). To achieve an adequate surplus of crops, satisfactory pest control is required (Tóth et al., 2018). Weed control in maize is successful due to the wide spectrum of herbicides permitted to be used (Janak and Grichar, 2016). Crop protection against insects is forecast by observing sticky and pheromone traps, which can be used to track the swarming dynamics of insects and butterflies (Tóth et al., 2017). The biggest problem is caused by fungal diseases in the production of maize (Frommer et al., 2018). In Hungary, seed coating is used to protect against fungal diseases. The effect of fungicides, which is permitted in maize, is unsatisfactory. Corn smut (*Ustilago maydis*) is one of the most important pathogens of maize in Hungary.

The origin of corn smut infection is the soil. Diploid teliospores are germinated and promicelium (basidium) is developed, which is followed by a nucleus merger (2n), a meiosis (2 piece n) and a mitosis (4-piece n) and haploid (single-nucleus) basidiospores are created (Olicón-Hernández et al., 2017). Basidiospores are placed on plants (with the help of wind or animal vectors), where the somatogamia occurs, the cell plasma of the hyphae is combined and dikaryotic (two opposite sexual cell nuclei) is formed (Kahmann - Kamper, 2004). The dikaryotic floccus penetrates the cell passages of the plant (mechanical injuries and insect bites open the door for the infection), it begins to withdraw the nutrients, and the tumour formation process begins. During tumour formation, karyogamy (nucleus fusion) occurs and diploid teliospores are created (Kahmann, 2017).

The pathogen can infect maize at both of the vegetative and generative stages. In the case of infection of a young plant, the following symptoms appear: chlorosis, necrosis, leaf deformation, and inhibition of shoot growth (Martinez - Soto and Ruiz - Herrera, 2016). Economic damage is caused by cob infestation. The resulting tumours initially have green colour, they turn brown as the maize ripens, and a mass of black spores forms.

There is no direct protection against corn smut (Chavan and Shmith, 2014). However, plant hormones can reduce the effects of the corn smut infection. Resistance to the corn smut infection could be increased by treating them with salicylic acid (SA) (Djamei et al., 2011). According to Gurevich et al., (1975) the gibberellin hormone inhibited the germination of *Ustilago maydis* spores.

The aim of this research was to examine the effects of the corn smut infection on some physiological and morphological parameters of maize (*Zea mays* L. cv. Armagnac) such as plant height, stem diameter, chlorophyll contents, and the rate of lipid peroxidation. Furthermore, this study investigated the possible compensation effects of gibberellin and ethylene separately and in combination on corn smut infection.

## MATERIALS and METHODS

### Plant materials, pathogen inoculation, and hormone treatments

The test plants (*Zea mays* cv. Armagnac L.) were grown in a greenhouse, the irrigation was carried out daily, with a volume of 0.5-1 liters of water per plant. The plants were planted in peat, with the following composition: 70% brown, 30% medium ripe Sphagnum moss peat with the addition of 1.5 kg/m³ Multi mix fertilizer (14-16-18 MeOH) and water-binding additive. A total of 75 plants have been used for this research (15 plants / treatment). In each treatment five plants were used for morphological and physiological examination per sampling time.

The inoculum was created under laboratory conditions. The infected corn cobs were collected from fields, and the teliospores sprayed on the corn smut specific substrate. Then the pure culture was created. Next the pathogen was replicated in the liquid corn smut specific substrate and the dilution series (one -, ten -, one hundred -, one thousand -, ten thousand -, one hundred thousand- and one million fold) was made and the one hundred thousand and one million fold series were resulted the separated colonies (Figure 1). The colonies were grafted from the petri dish into slant agar of culture medium, and the strains were created.

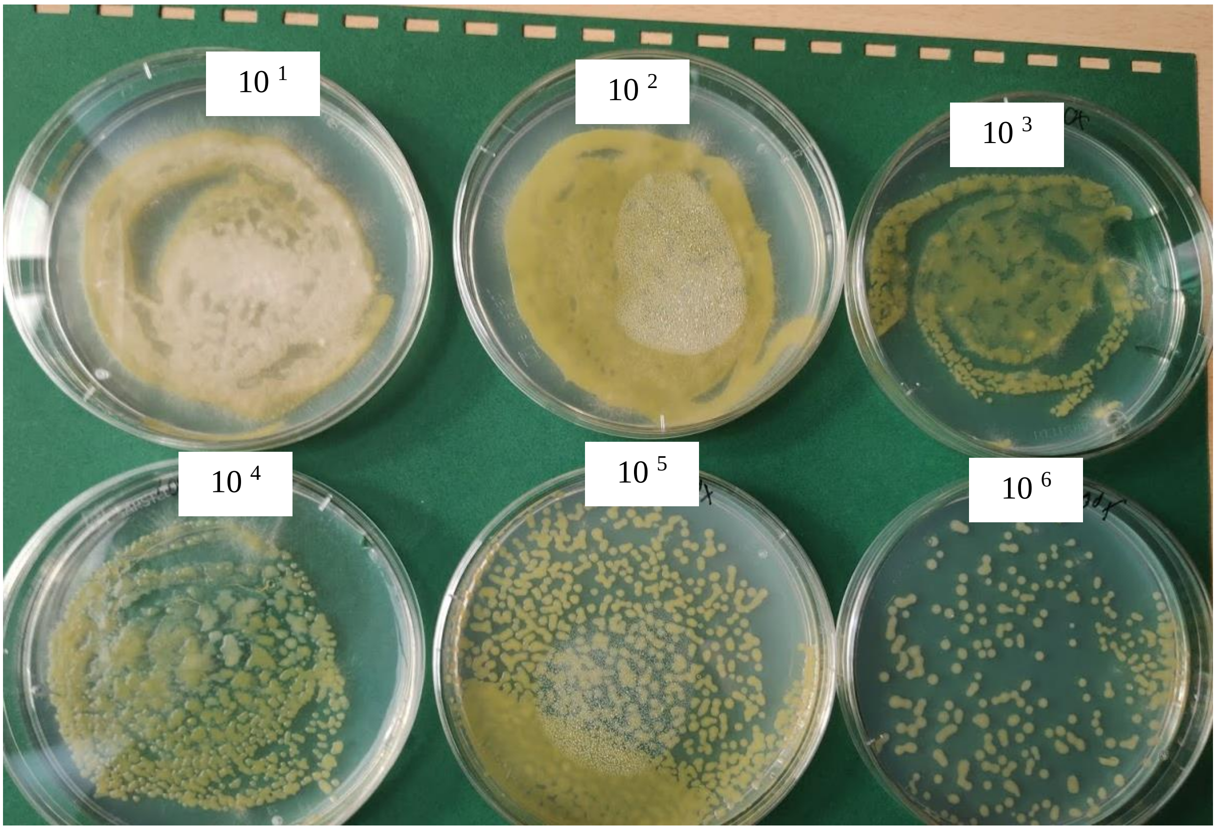


**Figure 1.** The colonies formed after the dilution

A compatibility test was performed with the newly created strains. The appearance of air mycelium indicated compatibility between the strains (the first, seventh and twelve strains) (Figure 2).

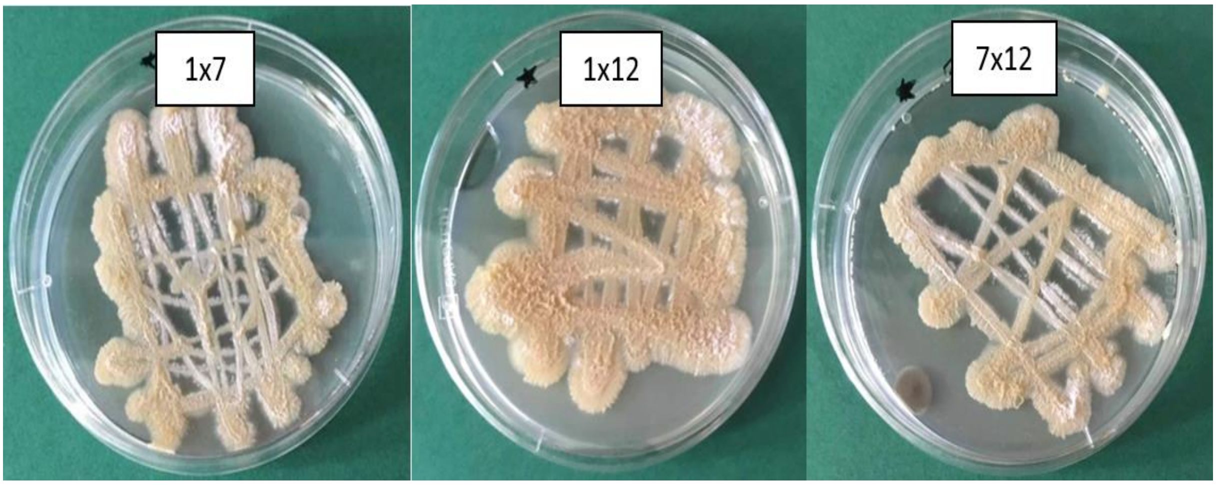


**Figure 2.** The compatibility strains

For the infection, the monosporidial strains were propagated on a liquid medium, grown for 48 hours and then mixed into a ratio of 1:1 before inoculation. The amount of cells was set to 10.000 sporidium number/ml in the Burker chamber.

The infection and the hormone treatments were carried out at the same time in the four-five leaf phenological stage of the plants. Two millilitres of sporidium suspension were injected into the stem section between the second and third nodes, and 1 ml of plant hormone was injected. In hormone treatments, gibberellin and ethylene were used separately and in combination. The hormones' forms were: $GA_3$ (gibberellin), and ethrel (ethylene). The infectious substance and hormone concentrations used are presented in Table 1.

**Table 1.** The applied treatments in the experiment

| Treatment | Number of sporidia (sporidia number/ml) | Concentrations of hormones |
|---|---|---|
| Control | - | - |
| Infected plants (I) | 10.000 | - |
| I + gibberellin | 10.000 | $10^{-3}$ M |
| I + ethylene | 10.000 | 1% |
| I + gibberellin + ethylene | 10.000 | $10^{-3}$ M + 1% |

For the statistical analysis of the results the IBM SPSS Statistics 25 program was used. The data normality was determined by Shapiro-Wilk and Kolmogorov-Smirnov tests. Then the Tukey HSD test was practiced to investigate the significant ($p \leq 5\%$) differences.

**Quantification of photosynthetic pigments**

The relative chlorophyll content was measured on the fourth and fifth leaves, seven, eleven, and fourteen DAI. The measurement was done with SPAD-502 Plus chlorophyll meter (Konica Minolta, Japan).

In addition, the absolute amount of the photosynthetic pigments (chlorophyll-*a*, -*b*, and carotenoids) was also measured with a Metertech SP 80 Spectrometer using the method of Moran and Porah (1980). Samples of fifty mg of fresh leaves were taken from the fourth leaf and dissolved in 5 ml N, N- dimethylformamide at 4 $^{0}$C for 72 hours. After 72 hours, the quantity of the chlorophyll-*a* was measured at 664nm, the chlorophyll-*b* at 647nm and the carotenoids at 480nm. The amount of photosynthetic pigments (mg $g^{-1}$) was calculated by using the method of Welburn (1994).

**Morphological parameters measurement**

The stem diameter was measured with slide calliper on the stem part between the second and third nodes. The plant height was measured from the peat surface to the base of the youngest leaf. The senescence of the leaf was determined by observing the yellowing of the cotyledon.

**The rate of lipid-peroxidation**

The rate of lipid peroxidation was determined with the quantity of malondialdehid (MDA) generated in the chemical assay by using the method of Heath and Packer (1968).

## RESULTS

**Effects of corn smut infection on chlorophyll content**

The changes in the relative chlorophyll content in the fourth and fifth leaves of maize were measured after 7, 11 and 14 DAI (Table 2 and Table 3).

**Table 2.** The effect of the corn smut infection (I) on the relative chlorophyll content (SPAD-unit) in the fourth leaf of maize treated with gibberellin and ethylene 7, 11 and 14 DAI

| Treatments | 7 DAI | 11 DAI | 14 DAI |
|---|---|---|---|
| Control | 33.98 ± 1.06 b | 36.50 ± 1.03 b | 33.06 ± 1.44 b |
| Infected plants (I) | 32.80 ± 0.74 b | 34.40 ± 0.90 b | 27.96 ± 0.93 a |
| I + ethylene | 25.88 ± 0.86 a | 23.26 ± 1.08 a | 21.42 ± 0.71 a |
| I + gibberellin | 35.30 ± 0.47 b | 35.60 ± 0.90 b | 33.78 ± 1.14 b |
| I+ ethylene and gibberellin | 29.60 ± 2.13 ab | 27.90 ± 1.27 a | 28.64 ± 0.44 a |

Values are means N ± SD (N= 25). The letters (a, b) denote the statistically similar groups per column.

When plants were infected, the relative chlorophyll content was significantly lower 14 DAI. The relative chlorophyll content significantly decreased (18.24%) when the infected plants were treated with ethylene compared to the control plants 7 DAI. Eleven (11) DAI, lower SPAD units were measured in the infected and ethylene treated, and in the infected and gibberellin and ethylene treated plants compared to control and the infected plants. The relative chlorophyll content was lower by 15.43% in the infected plants compared to the control plants 14 DAI. The infected plants showed 15.43 % lower SPAD units compared to the control plants. The intensity of the infection increased by 20% in the infected plants treated with ethylene.

**Table 3.** The effect of the corn smut infection (I) on the relative chlorophyll content (SPAD-unit) in the fifth leaf of maize treated with gibberellin and ethylene 7, 11 and 14 DAI

| Treatments | 7 DAI | 11 DAI | 14 DAI |
|---|---|---|---|
| Control | 35.06 ± 1.16 b | 39.20 ± 1.60 b | 37.16 ± 0.63 b |
| Infected plants (I) | 31.96 ± 0.78 b | 35.60 ± 1.21 b | 25.72 ± 1.04 a |
| I + ethylene | 24.88 ± 0.75 a | 28.62 ±2.82 a | 22.04 ± 1.38 a |
| I + gibberellin | 32.94 ± 1.28 b | 38.34 ±1.26 b | 35.56 ± 1.03 b |
| I + ethylene and gibberellin | 28.18 ± 2.05 a | 28.76 ± 2.20 a | 25.82 ± 0.67 a |

Values are means N ± SD (N= 25). The letters (a, b) denote the statistically similar groups per column

The results in Table 3 were similar to those measured in the fourth leaves. In the infected plants, the SPAD unit only decreased significantly 14 DAI. In the infected and ethylene treated plants, the levels of the relative chlorophyll content also decreased.

As relative chlorophyll content is only a relative value, the amount of photosynthetic pigments (chlorophyll-*a*, chlorophyll-*b*, carotenoids) was also measured (Table 4).

**Table 4.** The effect of corn smut infection (I) on photosynthetic pigments (chlorophyll-*a*, chlorophyll-*b* and carotenoids) content (mg $g^{-1}$) of maize treated with gibberellin and ethylene 14 DAI

| Treatments | Chlorophyll-*a* | Chlorophyll-*b* | Carotenoids |
|---|---|---|---|
| Control | 14.72 ± 0.74 c | 10.32 ± 0.83 c | 6.76 ± 0.81 c |
| Infected plants (I) | 9.02 ± 0.30 b | 6.52 ± 1.04 b | 5.33 ± 1.29 b |
| I + ethylene | 6.76 ± 0.96 a | 3.22 ± 1.89 a | 2.91 ± 0.69 a |
| I + gibberellin | 15.58 ± 2.20 c | 12.18 ± 2.19 c | 5.94 ± 0.91 bc |
| I + ethylene and gibberellin | 12.75 ± 0.99 c | 12.26 ± 1.75 c | 3.26 ± 0.60 a |

Values are means N ± SD (N= 5). The letters (a, b, c) denote the statistically similar groups per column.

The chlorophyll-*a* and chlorophyll-*b* contents were significantly lower in the infected plants compared to the control. The chlorophyll-*a* and chlorophyll-*b* decreased by 38.77% and 36.81%, respectively. The amount of chlorophyll-*a* and chlorophyll- *b* increased by 73.72% and 88.04 % in the infected and gibberellin treated plants compared to the infected plants. The amount of these two pigments was significantly lower (50%) when infected plants were treated with ethylene, compared to the control plants. Control plants showed 21.18% more carotenoid content in comparison to the infected plants. No significant difference was observed between the infected and the infected and gibberellin treated plants. The carotenoid content was significantly lower (57%) when plants were infected and treated with ethylene compared to the control.

**Effect of corn smut infection on stem diameter**

Measuring the stem diameter of test plants, the control had thicker stem compared to the infected plants at all three sampling times, however, significant differences were not observed. Statistical significant difference (17.86%) was detected between the infected plants and the infected and gibberellin and ethylene treated plants, 11 DAI (Table 5).

**Table 5.** The effect of corn smut infection (I) on the stem diameter of maize (mm) treated with gibberellin and ethylene 7, 11 and 14 DAI

| Treatments | 7 DAI | 11 DAI | 14 DAI |
|---|---|---|---|
| Control | 7.44 ± 0.22 a | 8.32 ± 0.30 ab | 13.47 ± 0.95 a |
| Infected plants (I) | 7.19 ± 0.28 a | 7.95 ± 0.21 a | 11.65 ± 0.40 a |
| I + ethylene | 7.00 ± 0.31 a | 8.36 ± 0.20 ab | 11.61 ± 0.59 a |
| I + gibberellin | 7.25 ± 0.10 a | 8.37 ± 0.14 ab | 11.80 ± 0.32 a |
| I + ethylene and gibberellin | 7.24 ± 0.09 a | 9.37 ± 0.22 b | 11.86 ± 0.69 a |

Values are means N ± SD (N= 5). The letters (a, b) denote the statistically similar groups per column.

Results of the leaf senescence showed that there were no significant differences among the treatments at any sampling times. Yellowing was observed on all seed-leaf of plants (results are not shown).

**Effect of corn smut infection on plant height**

The infection did not have any considerable influence on plant height (Table 6). Plant height increased when infected plants were treated with gibberellin compared to the infected plants 11 and 14 DAI (7.31% and 9.84 %), but not significantly.

Ethylene treatment significantly decreased plant height compared to the control plants at all sampling times (39.30; 54.38 and 50.20 %). Plant height significantly decreased when infected plants were treated with ethylene at both sampling times. Both gibberellin and ethylene treated plants were significantly shorter than infected plants 11 and 14 DAI (Table 6).

**Table 6.** The effect of corn smut infection (I), gibberellin and ethylene treatments on the plant height (mm) 7, 11 and 14 DAI

| Treatments | 7 DAI | 11 DAI | 14 DAI |
|---|---|---|---|
| Control | 514 ± 33,41 a | 776 ± 23,15 c | 988 ± 32,77 c |
| Infected plants (I) | 476 ± 13,27 a | 786 ± 22,49 c | 916 ± 25,02 c |
| I + ethylene | 312 ± 17,72 b | 354 ± 12,88 a | 492 ± 15,30 a |
| I + gibberellin | 472 ± 23,75 a | 848 ± 5,83 c | 1016 ± 16,00 c |
| I+ ethylene and gibberellin | 480 ± 27,20 a | 630 ± 8,94 b | 706 ± 36,55 b |

Values are means N ± SD (N= 5). The letters (a, b, c) denote the statistically similar groups per column.

**Effect of corn smut infection on the rate of lipid peroxidation**

The rate of lipid peroxidation was measured with the quantity of produced malondialdehyde (MDA). The MDA contents significantly increased (37.90 %) in the infected plants in comparison to the control plants. The amount of MDA increased by 20% in the infected and ethylene treated plants. There were no significant differences among the infected plants, the infected and gibberellin treated, and infected and gibberellin and ethylene treated plants (Figure 3).

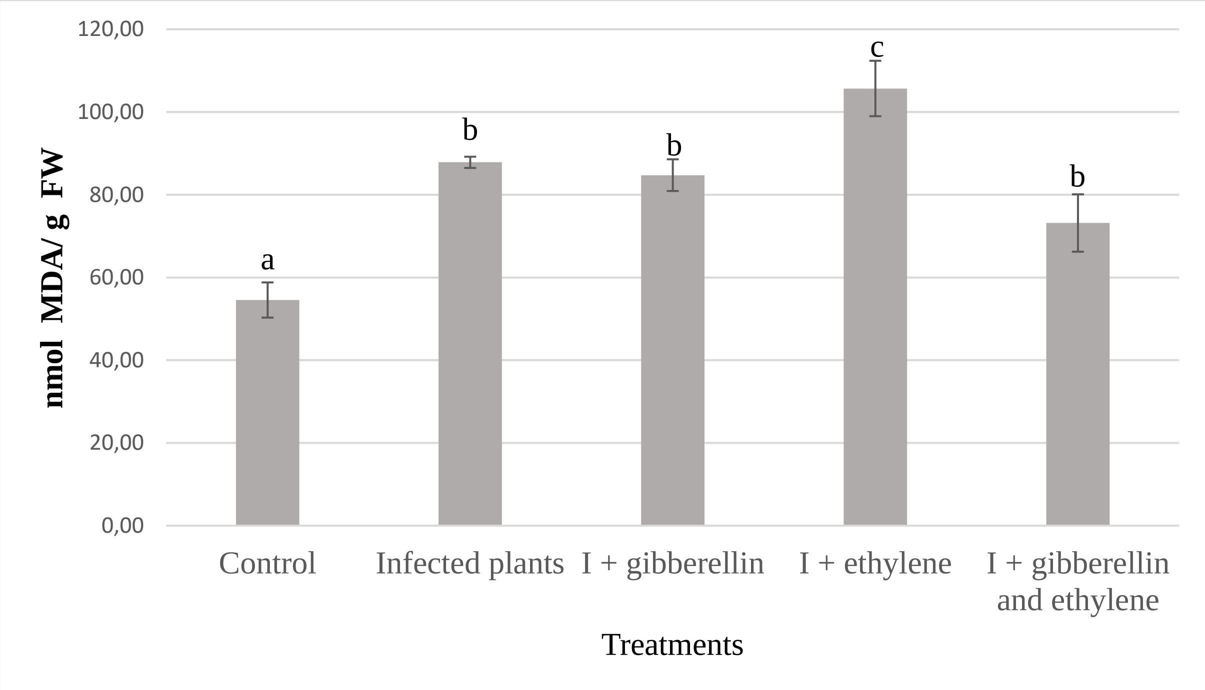


**Figure 3.** Effect of corn smut infection (I) on MDA content in the fifth leaves of maize treated with gibberellin and ethylene (nmol MDA/g FW)

Values are means N ± SD (N=5). The letters (a, b, c) denote the statistically similar groups per column

## DISCUSSON

This study examined the effects of corn smut infection on morphological and physiological parameters of maize. The pathogenicity depends on the susceptibility of the host plant and the plant pathogen fungi's infection intensity. Bojtor et al. (2019) studied the influence of bean rust (*Uromyces* *appendiculatus* PERS. STRAUSS) on the amount of photosynthetic pigments, and the rate of lipid peroxidation. The study found that at 14 DAI, the amount of photosynthetic pigments decreased, and the quantity of MDA increased. Shareef et al. (2020) observed the same tendency when they examined the effects of salt and heat stresses in date palm leaves. Tani and Yoshikawa-Naito (1973) published that oat crown rust (*Puccinia coronata*) decreased the chlorophyll content in oat.

Frommer et al. (2019) described that the relative chlorophyll content decreased in each leaf of sweet corn after the infection. In this study the corn smut infection reduced the amount of photosynthetic pigments in the leaves. The symptoms of the pathogen are leaf yellowing, necrosis, decreased number of chlorophylls in the infected plants. The appearance of leaf discoloration takes time, as proved by the measurements of photosynthetic pigments.

The corn smut infection did not reduce the plant height significantly. Frommer et al. (2015) carried out the infection in seedling phenological phase while the resistance of the plant is much weaker. As the plant grows, the resistance to pathogens increases. Plants that are more developed can better tolerate the harmful effects of diseases. Lévai (1975) aslo studied the effects of ethylene and gibberellin in the corn smut infected plant at the seedlings' phenological stage. He stated that the gibberellin treatment reduced, the ethylene treatment increased the effects of corn smut infection. In addition, it had been shown that the magnitude of the sporidia number determines the effect of gibberellin and ethylene.

## Conclusion

In this study the focus was on the corn smut infection effects on the morphological and physiological parameters. The fungal disease depends on the susceptibility corn plants, and the pathogenicity of the fungi. The infection did not cause any major changes in the morphological parameters in our experiment. There are several reasons for this. First of all, the resistance increases with the plant development. The five-leaf corn is better able to tolerate the disease than the younger corn. Secondly, the susceptibility of corn plants is also important. In this study, the Armagnac hybrid was used as a test plant. For this hybrid there are no reports yet for the corn smut infection susceptibility. According to our results, the corn smut infection was well tolerated by the Armagnac hybrid. Finally, the pathogen's virulence also affects the disease process. Infection can occur after the compatibility of the strains. The virulence of the compatible strains is different, which has been proved by previous researches. The corn smut infection also had negative effects on the chlorophylls content and the rate of lipid peroxidation.

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**Conflict of Interest**

The authors declare that they have no conflict of interest.

## REFERENCES

Bojtor, Cs., Győri, Z., Sipos, P., Radócz, L. & Tóth, B. (2019). Effect of bean rust [*Uromyces appendiculatus* (PERS.) STRAUSS] on photosynthetic charachteristics, superoxide-dismutase activity, and lipid peroxidation of common bean (*Phaseolus vulgaris* L.). Acta Alimentaria., 48 (2), 253–259.

Chavan, S. & Smith, S. M.: (2014). A rapid and efficient method for assessing pathogenicity of *Ustilago maydis* on maize and teosinte lines. Journal of Visualized Experiments., 83, 1-7.

Djamei, A., Schipper, K., Rabe, F., Ghosh, A., Vincon, V., Kahnt, J., Osorio, S., Tohge, T., Fernie, A. S., Feussner, I., Meinicke, P., Stierhof, Y. D., Schwarz, H., Macek, B., Mann, M. & Kahmann, R.: (2011) Metabolic priming by a secreted fungal effector. Nature., 478, 395–398.

FAO (2018). Food and Agriculture Organization of the United Nations. FAOSTAT Statistics Database.

Frommer, D., Veres, Sz., Radócz, L. & Lévai, L.: (2015). Investigation the resistance of maize hybrids against corn smut disease. In 57$^{th}$ Georgikon Scientific Conference., 117-121.

Frommer, D., Veres, Sz. & Radócz, L.: (2018). Susceptibility of stem infected sweet corn hybrids to common smut disease. Acta agraria Debreceniensis., 74, 55-57.

Frommer, D., Radócz, L. & Veres, Sz. (2019). Changes of relative chlorophyll content in sweet corn leaves of different ages infected by corn smut. Agriculture Conspectius Sciencetificus., 84 (2), 189-192.

Gurevich, L. S,. Krylenkova, N. L. & Slepyan, E. I.: (1975). Gibberellin-like substances and inhibitors of the spores of smut fungi in connection with their parasitic way of life. Soviet Plant Physiology., 22 (2), 321-325.

Heath, R. L. & Packer, L. (1968). Photo peroxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fattyacidperoxidation. Archives of Biochemistry and Biophysics., 125 (1), 189-198.

Janak, T. W. & Grichar, W. J. (2016). Weed control in corn (*Zea mays* L.) as influenced by preemergence herbicides. International Journal of Agronomy., 2016, 1-9.

Kahmann, R. & Kamper, J.: (2004). *Ustilago maydis* how its biology relates to pathogenic development. New Phytologist., 164 (30), 31–42.

Kahmann, R.: (2017). *Ustilago maydis* effectors and their impact on virulence. Nature Reviews Microbiology., 15, 409–421.

Lévai, L.: (1975). Hormonális változások hátterének vizsgálata golyvásüszöggel (*Ustilago maydis* Cda.) fertőzött kukorica növényekben, különös tekintettel az etilén és a gibberellin kölcsönhatására. University of Debrecen. Degree work.

Martinez-Soto, D. & Ruiz-Herrera, J.: (2016). Induced resistance to *Ustilago maydis* in *Zea mays* inoculated in nonsterile conditions. International Journal of Biotechnology for Wellness Industries., 5, 51-59.

Moran, R. & Porath, D.: (1980). Chlorophyll determination in intact tissues using N, N-Dimethylformamide. Plant Physiology., 65 (3), 478-479.

Olicón-Hernández, D. R., Araiza-Villanueva, M. G., Pardo, J. P., Aranda, E. & Guerra-Sánchez, G.: (2019). New insights of *Ustilago maydis* as yeast model for genetic and biotechnological research: A review. Current Microbiology., 76, 917–926.

Shareef, H., Abdi, G. & Fahad S.: (2020). Change in photosynthetic pigments of Date palm offshoots under abiotic stress factors. Folia Oecologica., 47 (1), 45-51.

Sots, S. M. & Bnyiak, O. V.: (2018). Use of corn grain in production of food products. Grain Products and Mixed Fodder's., 18 (2), 20-25.

Tani, T. M. & Yoshikawa-Naito. N.: (1973). Effect if rust infection of oat leaves on cytoplasmic and chloroplast ribosomal ribonucleic acid. Phytophatology., 63, 491-494.

Tóth, M., Szarukán, I., Nagy, A., Furlan, L., Benvegnù, I., Rak, C., Ábri, T., Kéki, T., Körösi, S., Pogonyi, A., Toshova, T., Velchev, D., Atanasova, D., Kurtulus, A., Kaydan, B. & Signori, A.: (2017). European corn borer (*Ostrinia nubilalis* Hbn., Lepidoptera: Crambidae): comparing the performance of a new bisexual lure with that of synthetic sex pheromone in five countries. Pest Management Science., 73 (12), 2504-2508.

Tóth, T., Szilágyi, A. & Kövics, Gy.: (2018). Preliminary estimation of the efficacy of Fusarium sporotrichioides Sherb. as biological control agent against common milkweed (*Asclepias syriaca* L.). Acta Agraria Debreceniensis., 74, 201-204.

Wellburn, R. A. (1994). The spectral determination of chlorophylls *a* and *b*, as well as total carotenoids, using various solvent with spectrophotometers of different resolution. Journal of Plant Physiology., 144, 307-313.