Changes of relative chlorophyll content in sweet corn leaves of different ages infected by corn smut

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Summary

The smut fungus (*Ustilago maydis*) is a ubiquitous pathogen of corn. One of the symptoms of infected sweet corn by corn common smut is chlorosis on the leaves, so it is especially important to examine how the infection influence the leave chlorophyll content of different hybrids. Five sweet corn hybrids were investigated in the randomized small block field experiments. Hybrids were inoculated by injecting sporidial suspension in the stem, so to induce a high incidence of common smut of corn caused by *Ustilago maydis*. Two weeks after the inoculation the relative chlorophyll content was measured on seven leaves of different ages per plant by SPAD-502 chlorophyll meter. Because of the infection the relative chlorophyll content of younger leaves was decreased in comparison to the leaves of untreated control plants. The disease systematically spread within the plant causing chlorosis mainly on younger leaves.

Key words

biotic stress, maize, infection, *Ustilago maydis*

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Introduction

Among the most common symptoms of a plant diseases are the occurrence of various chlorosis. There are different causes for development of chlorosis, such as reduction of chlorophyll content in leaves, occurrence of functional disorders or ultimate destruction of chloroplasts. Chlorosis already means significant losses due to less effective photosynthesis based on a complex process. Generally the nutrient deficiencies, viz. magnesium (Jezek et al., 2014) and iron (Abadía et al., 1999), are the main causes of chlorosis, but biotic factors can also contribute to this symptom.

Corn smut (*Ustilago maydis*) is one of the most common maize diseases. The biotrophic fungus *U. maydis*, the causal agent of corn smut disease, uses numerous small secret effector proteins to suppress plant defence responses and transforms the host metabolism (Stirnberg and Djamei, 2016). However, the role of specific effectors remains poorly understood. Infection with the pathogen causes galls on all aerial parts of its host plant *Zea mays* (Christensen, 1963; Brefort et al., 2009). Large 2-10 cm galls (swollen, distended growths) are formed on stalks, tassels, ears and leaves. For example tumours that developed in infected leaves often span the entire leaf blade. Such a leaf infections first hamper the establishment of C_4 photosynthesis, which results in reduced CO_2 assimilation upon tumour initiation (Horst et al., 2008). During tumour development, a further reduction in overall photosynthetic capacity of infected leaves is observed. Consequently, soluble carbohydrate accumulation in tumours is similar to that in young sink leaves (Doehlemann et al., 2008). Plants with large galls on the lower stalk may be stunted, barren and produce small ears. The greatest damage results from corn ear or the stalk above the ear (William et al., 1991). When airborne sporidia from germinated teliospores or germinating teliospores contact susceptible tissue, form dikaryotic mycelia and penetrate directly or through wounds (Christensen, 1963). Therefore seed treatments with fungicides are not effective in control to this disease. Localized galls are the most conspicuous on the ears. Thus, yields of infected plants can be reduced by 40-100% (Shurtleff, 1980; Chavan and Shavannor, 2014). Losses vary with the year, location and cultivar grown, and the host resistance offers the most economical means of disease control (Smith and White, 1988; Aydogdu et al., 2015).

Chlorosis of the leaves is an observable common symptom of sweet corn infected by the *U. maydis*. Cytological analyses of chlorotic areas indicate that *U. maydis* dikaryotic hyphae can grow inter- as well as intracellularly within host tissue (Snetselaar and Mims, 1994). It seems to be no preference for hyphal invasion of particular cell types as hyphae grow within epidermal cells, parenchyma cells and vascular bundle cells in the first four days after inoculation. The deformed rupture site between host cell walls suggests that *U. maydis* may rely on, at least partially, a mechanical means to break cell walls and proliferate from cell to cell. Infected cells appear normal with the exception that chloroplasts are sometimes enlarged, containing many starch granules (Snetselaar and Mims, 1994). Branch primordial that resemble the clamp connections of other basidiomycetes are observed in *U. maydis* hyphae during colonization (Christensen, 1963; Snetselaar and Mims, 1994; Banuett and Herskowitz, 1996). As the most frequent symptom is choloris, it is especially important to examine how the infection influences the chlorophyll content of leaves in various hybrids. The chlorophyll content of leaves generally decrease in diseased plants (Hawkins et al., 2009) and in dependence of *in situ* nitrogen (N) status (Arregui et al., 2006; Ziadi et al., 2008; Yuan et al., 2016).

Materials and Methods

The relative chlorophyll content was investigated in five sweet corn (*Zea mays* L.) hybrids ('BOX-R', 'JUMBO', 'NOA', 'PRELUDE', and 'DESSZERT 73') after artificial inoculation made by common corn smut (*U. maydis*). The plots were designed in three repetitions within one experiment. The inoculation was made on three plants in each plot at the growth stage of 6-7 leaves (BBCH 31) (Hanway, 1963). The stem was inoculated at the second nodal height. Teliospores were collected and isolated from various types of infected maize by smut. Fungus was grown in both liquid and solid media as required by the strain. Monosporidial colonies required for the inoculation were obtained through dilution. Compatibility was specified based on the formation of aerial mycelium. The inoculation technique was injection. The inocula were provided by 1:1 mixture of the liquid culture grown for 24 hours of the two compatible monosporidial strains. The injection was performed using a special inoculation device. All the plants were injected with 2 ml of sporidial suspension to induce a high incidence of common smut. The sporidium concentration of the suspension was approximately 2000 ml^{-1}. Two weeks after the inoculation the relative chlorophyll content was measured from 1st leaf to 7th leaf in every hybrid by SPAD-502 chlorophyll meter (Minolta, Japan). During the measurement period the 7th leaf was the recently fully developed leaf, while the 1st leaf was the oldest. The effect of infection on elder leaves was compared to the infected younger ones. In order to obtain more accurate value, three measurements were performed and average value was calculated per leaf. Three-way analysis of variance (Tukey Test) using SigmaPlot 12 was performed and normality was tested using Shapiro -Wilk test.

Results

The relative chlorophyll content (SPAD value) of all infected leaves decreased for 14-45% in comparison to the healthy control leaves (Fig. 1). Significantly decreased SPAD value was observed in hybrid 'BOX-R' (by 45%) due to the infection. Reduction in relative chlorophyll content was also observed in other hybrids as well, however without statistical significance. The reduction of relative chlorophyll content was 14% in hybrid 'JUMBO', 23% in 'NOA', 26% in 'PRELUDE' and 28% in 'DESSZERT 73'

The disease systematically spread in the plants after stem inoculation. Younger leaves showed increased chlorosis and serious symptoms such as dwarfism and tumours. The relative chlorophyll content of younger leaves decreased to a greater extent in comparison to the elder ones for all hybrids (Table 1). The differences between infected differently aged leaves and control leaves are presented in Table 1. In the untreated healthy control, a smaller reduction was also observed in the elder leaves compared to younger ones, but the relative chlorophyll content in younger leaves did not decrease as much as in the infected ones. In 'JUMBO' hybrid the lowest differences were noticed in infected elder leaves (from 1st to 4th). The youngest leaf of the 'BOX-R' hybrid was the

Table 1. Changes of relative chlorophyll content (SPAD value) in infected sweet corn (BOX-R, JUMBO, NOA, PRELUDE, DESSERT 73) different age (1st to 7th) leaves presented as the percentage (%) compared to control

Leaf	BOX-R	JUMBO	NOA	PRELUDE	DESSERT 73
1st	82.5 ±1.9	108.3 ±8.5	75.1 ±4.7	73.8 ±8.1	80.2 ±8.3
2nd	76.3 ±5.9	89.1 ±5.1	79.9 ±1.7	80.2 ±3.8	81.9 ±8.4
3rd	63.8 ±4.1	91.2 ±6.8	87.2 ±7.2	69.5 ±10.4	71.3 ±5.4
4th	46.4 ±9.7	96.9 ±1.4	86.4 ±7.9	72.9 ±9.1	70.2 ±13.1
5th	42.5 ±8.5	70.9 ±5.1	85.9 ±10.2	73.2 ±8.4	67.3 ±16.5
6th	23.8 ±0.9	77.5 ±10.1	65.6 ±11.1	74.8 ±12.3	56.1 ±11.4
7th	22.4 ±5.9	63.9 ±15.6	60.2 ±14.2	74.2 ±12.5	60.4 ±13.3

n=3, ±s.e.

most sensitive for corn smut infection; the relative chlorophyll contents decreased by 70-80% compared to the control.

Results of statistical analyses are presented in Table 2. The hybrids, the treatment (infection) and the age of leaves showed statistically significant differences. Although the interaction between these parameters were not significant.

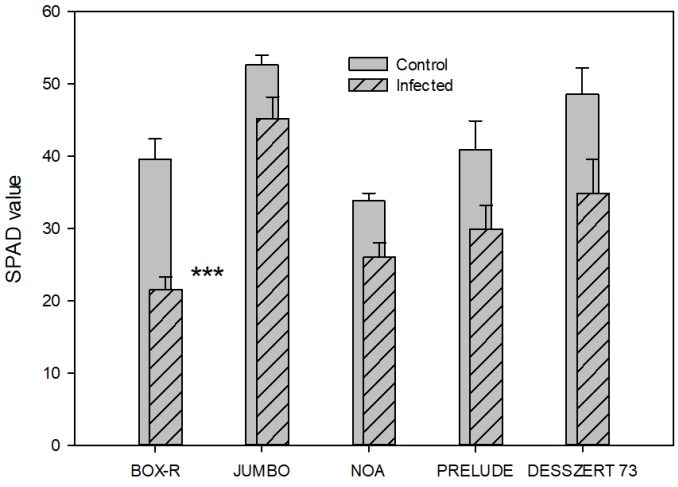

Figure 1. Relative chlorophyll content (average SPAD values of seven leaves per hybrid) changes due to corn smut infection.
***p <0.001 (n=9, ±s.e.)

Table 2. Results of Three way analyses of variance

Source of Variation	P
Hybrid	<0.001
Treatment (infection)	<0.001
Age of leaf	<0.001
Hybrid x Treatment	n.s. (0.052)
Hybrid x Age	n.s.
Treatment x Age	n.s.
Hybrid x Treatment x Age	n.s.

Discussion

Five different sweet corn hybrids were investigated after artificial inoculation by *Ustilago maydis*. The susceptibility of sweet corn hybrids to common smut (*U. maydis*) may vary (Frommer et al., 2018). The relative chlorophyll contents of all artificially inoculated hybrids were decreased in comparison to the control. In previous studies there was revealed that chlorotic spots can be observed in one day after inoculation (Banuett and Herskowitz, 1996) and chlorosis became more extensive in the second to third day following inoculation (Snetselaar and Mims, 1992, 1994). There was also described that anthocyanin production may occurre far away from infection site (Banuett and Herskowitz, 1996). In the leaves of infected plants by corn common smut the relative chlorophyll content decreased in all examined hybrids by 14-65% in comparison to the untreated healthy plants, depending on the age of leaf. A significant decrease was observed in the case of 'BOX-R' hybrid. However the reduced relative chlorophyll content in other hybrids was not statistically significant. Furthermore slow and stunted growth of shoots, dwarfism, deformity and slanting were experienced that considerably decreased the length of the shoots compared to healthy plants (Frommer et al., 2018).

The relative chlorophyll content was also decreased in the younger leaves compared to the elder ones. According to a recent publication senescence of elder leaves could delay as a consequence of pathogen infection (Robin et al., 2009). A delay senescence of elder leaves has been observed upon infection of *Ricinus communis* by the plant parasite *Cuscuta reflexa* (Jeschke and Hilpert, 1997), which taps directly into the phloem of the host, where it acquires carbohydrates and amino acids. In *U. maydis* infected maize plants, increased demand for nitrogen and carbon by the tumours provokes a stimulation of carbon and nitrogen export in elder leaves, which consequently necessitates increased carbon and nitrogen assimilation (Robin et al., 2009). As a result, photosynthetic performance in elder leaves of infected plants will remain above the critical threshold for longer time than that of corresponding leaves from uninfected plants, thereby delaying senescence induction (Smart, 1994). These observations could explain greater reduction of chlorophyll content of young leaves than that of older ones in infected plants when compared to the healthy (control) plants. The relative chlorophyll contents of elder leaves (leaf No. 1-3) were much higher in comparison to younger ones (4th-7th). The measured average reduction between the eldest

leaf (1st) and the youngest one (7th) was 41%. Relative chlorophyll content in the elder infected leaves (1st-3rd) decreased in a greater extent in comparison to the untreated healthy control. The average reduction between the eldest leaf (1st) and the youngest one (7th) was 26%.

The significant decrease of the relative chlorophyll content demonstrates that the appearances of the symptoms are much more intense in the case of the susceptible hybrids, which could have greater yield loss. The main trend is to grow hybrids that are resistant to common corn smut disease. The results of this study indicate differences in susceptibility among investigated hybrids.

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