Application of Barley Tweaky Spike Mutants

Subjects: Biology

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Barley developmental mutants tweaky spike (tw) with disturbed auxin pathways possess a unique feature of an increased level of mouldy germinating grains (MGG), which serves as a convenient model to investigate the effects of plant immunity-related substances. The effects of the auxin 2,4-dichlorophenoxyacetic acid (2,4-D), auxin inhibitors, salicylic acid (SA), and trans-cinnamic acid (TCA) were studied using the tw-WT system in surface-sterilized and unsterilized germinating grains under high rates of natural infection. Significant differences among the allelic tw mutants were revealed at the natural MGG level and in response to 2,4-D, SA, and TCA. The most effective means against MGG were sterilization and TCA. 2,4-D inhibited root growth in tw and tw2 mutants, occurring only in unsterilized and not sterilized germinating grains, while the opposite was observed for TCA and SA. The tw mutations influenced variations in the seed-borne fungal spectra, decreasing the frequency of Bipolaris sorokiniana and increasing Fusarium spp. Hypochlorite-based surface sterilization methods should be used with caution in studies where the action of exogenous 2,4-D will be analysed in germinating grains. Auxin pathway disturbances specific for pleiotropic tw mutants are generally restricted to organogenesis but not to germination events.

barley MGG assay salicylic acid trans-cinnamic acid tweaky mutants 2 4-D

1. Introduction

Grain contamination with fungi and their produced mycotoxins is not only a problem for organic producers but also for conventional agriculture [1]. For barley, special attention is required for malt and beer contamination with mycotoxins and their proper control and avoidance [2].

2. Analysis on Results

2.1. The Action of 2,4-D, SA and TCA on MGG and Root Growth of Allelic Tweaky Spike Mutants

Naturally, grain sterilization before germination is an effective treatment against MGG. In most cases studied, the MGG level was significantly lower in surface-sterilized grains than in unsterilized grains under the same experimental conditions, and independent of the plant genotype and the studied 2,4-D concentrations (**Figure 1**a and **Figure 2**a, Tables S1a and S2a).

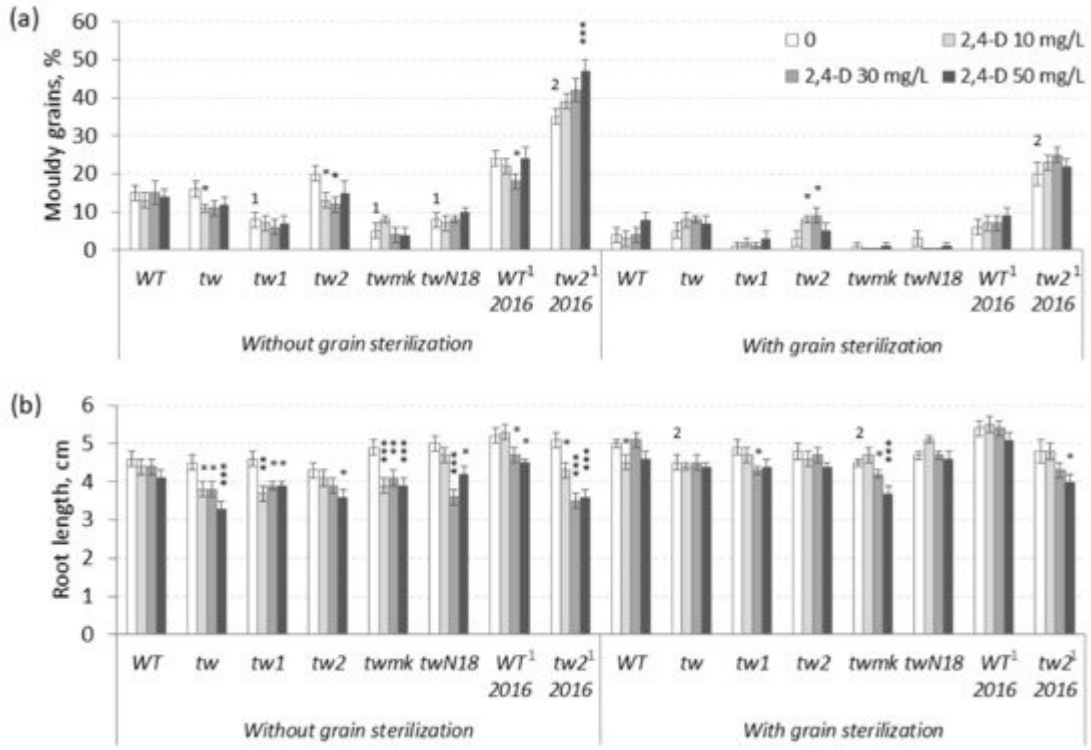

Figure 1. Effects of 2,4-D over a 10–50 mg L^{-1} range on barley *tweaky*-type mutants. (**a**) The frequency of mouldy grains (%) and (**b**) the root lengths (cm) of unsterilized and sterilized germinating grains measured after 5 days. For mouldy grains, n = 10 *Petri* dishes (100 grains); for root length n = 30; for tw_2, n = 15 *Petri* dishes (150 grains) and n = 60. *WT2016* and $tw_2 2016$, grain reproduction in 2016; other material, grain reproduction in 2013; the MGG assay was performed in 2017. The asterisks represent significant differences (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$) between the control and the 2,4-D treatment. The numbers denote significant differences (^{1} $p < 0.05$; ^{2} $p < 0.01$) between the controls of the *tw*-type mutant and the *WT*.

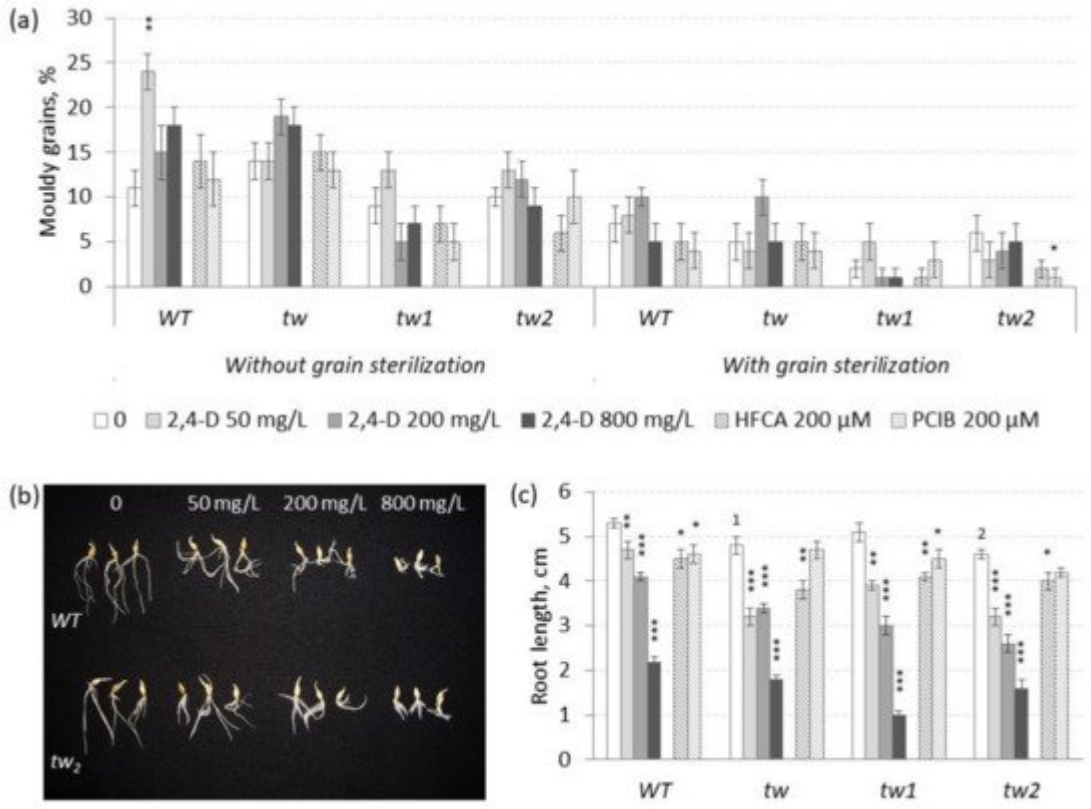

Figure 2. Effects of 2,4-D over a range of 50–800 mg L^{-1} and the auxin inhibitors HFCA and PCIB on (**a**) the frequency of mouldy grains (%) and (**b**,**c**) the root length (cm) of unsterilized germinating grains of *tw* mutants and the *WT*, as measured after 5 days. For mouldy grains, n = 10 *Petri* dishes (100 grains); for root length, n = 30. Grain was produced in 2013, and the MGG assay was performed in 2017. The asterisks represent significant differences (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$) between the control and the 2,4-D treatment. The numbers denote significant differences (^{1} $p < 0.05$; ^{2} $p < 0.01$) between the controls of the *tw*-type mutant and the *WT*.

In the range of 10–50 mg L^{-1}, an appreciable inhibitory effect of 2,4-D on the root growth of allelic *tw*-type mutants depended on (1) the plant genotype and (2) the sterilization status of grains (**Figure 1**b and Table S1b). In most cases, 2,4-D significantly inhibited root growth only in unsterilized germinating grains of all tested genotypes, including the nonallelic *twN18* and *twmk* mutants. The *twmk* mutant was the most sensitive to 2,4-D among all tested *tweaky*-type mutants. In contrast, 2,4-D-induced root growth inhibition in sterilized germinating grains mostly in a nonsignificant manner (**Figure 1**b and Table S1b).

The tested lower 2,4-D concentrations (10–50 mg L^{-1}) revealed an interesting dependence of 2,4-D-induced root growth inhibition on grain sterilization status; consequently, the effect of elevated 2,4-D concentrations was investigated in further experiments. In the range of 50–800 mg L^{-1} 2,4-D, a significant effect of 2,4-D on MGG was observed only in unsterilized grains of *WT* ($p = 0.0026$), in which 2,4-D increased the level of MGG (**Figure 2**a and Table S2a), while in the range of 10–50 mg L^{-1} 2,4-D, a significant increase in MGG was observed only in sterilized grains of the tw_2 genotype ($p = 0.024$; **Figure 1**a and Table S1a).

In contrast to the effect of the lower concentrations, the higher concentrations (50–800 mg L^{-1}) of 2,4-D induced a significant decrease in root length independent of the plant genotype and the grain sterilization conditions, and the inhibitory effect of 2,4-D on root growth was dose-dependent (**Figure 2**b,c and Table S2b). The root growth of the *tw* and tw_2 allelic mutants, but not the tw_1 allelic mutant, was weaker than that of *WT* germinating grains. An unsterilized grain background better revealed the inhibitory effect of 2,4-D on the germination rate, which was uniform independent of the plant genotype. In turn, grain sterilization revealed better differences among allelic mutants in the response to 2,4-D according to the germination rate (Table S2c). In general, 2,4-D in the range of 50–800 mg L^{-1} inhibited root length independent of the plant genotype (**Figure 2**c), while in the range of 10–50 mg L^{-1} 2,4-D, differences between *WT* and *tw*-type mutants and among allelic *tw* mutants themselves were observed (**Figure 1**).

Despite the proposed opposite effects to the action of 2,4-D, the auxin inhibitors HFCA and PCIB did not show a significant effect on MGG, except PCIB in sterilized grains of the tw_2 mutant, in which the MGG level decreased (**Figure 2**a and Table S2a). However, both auxin inhibitors suppressed root growth similarly to 2,4-D (**Figure 2**c and Table S2b).

Theoretically, the effects of TCA and SA are supposed to be opposite to those of 2,4-D, and TCA decreased the frequency of MGG in unsterilized germinating grains of the *WT* and all allelic *tw*-type mutants (**Figure 3** and Table S3).

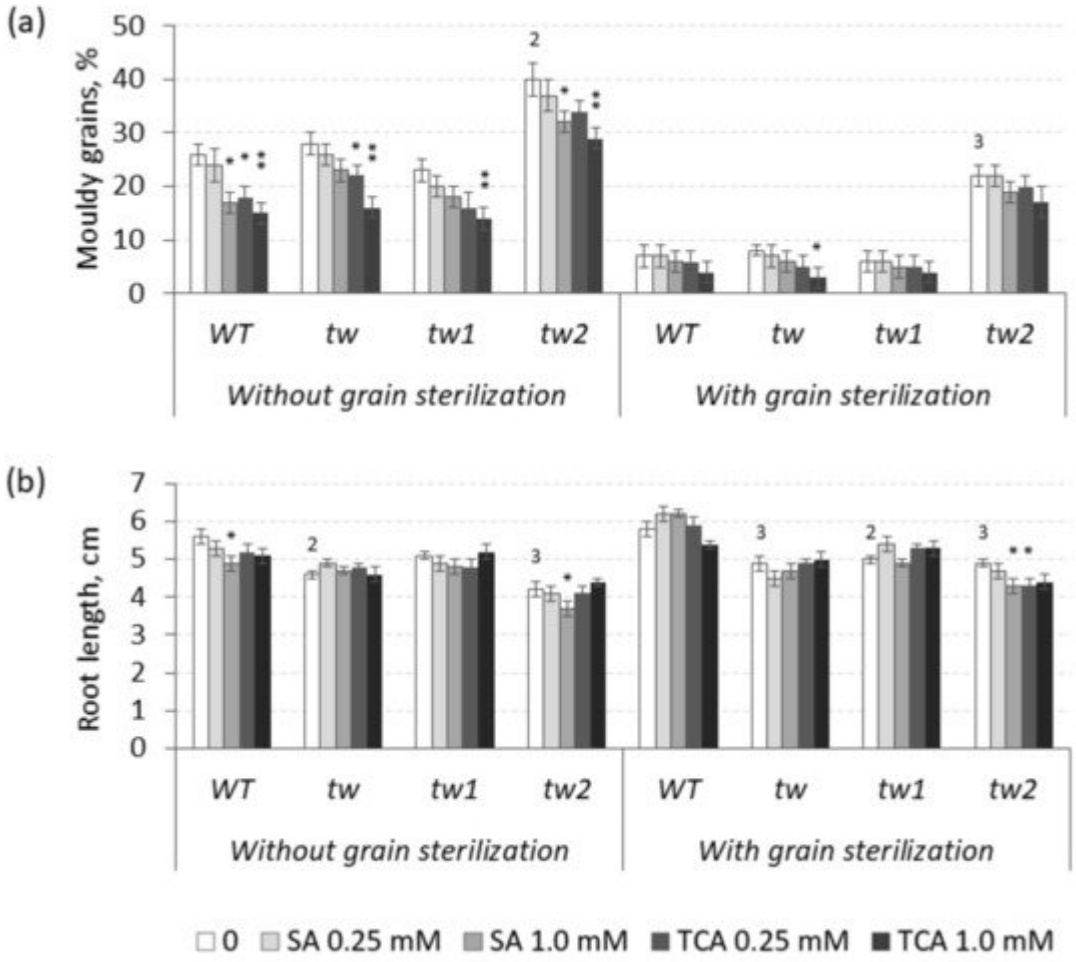

Figure 3. Effects of salicylic (SA) and *trans*-cinnamic (TCA) acids on (**a**) the frequency of mouldy grains (%) and (**b**) the root lengths (cm) of unsterilized and sterilized germinating grains of barley *tw* mutants and the *WT*, as measured after 5 days. For mouldy grains, n = 10 (100 grains); for root length, n = 30. Grain was produced in 2016, and the MGG test was performed in 2020. The asterisks represent significant differences (* $p < 0.05$; ** $p < 0.01$) between the control and the SA or TCA treatment. The numbers denote significant differences (^{2} $p < 0.01$; ^{3} $p < 0.001$) between the controls of the *tw*-type mutant and the *WT*.

However, in sterilized germinating grains, TCA decreased MGG at a significant level only in the allelic *tw* mutant, while SA decreased MGG at a significant level only in unsterilized grains of the tw_2 mutant (**Figure 3** and Table S3a). Similar to 2,4-D, SA and TCA also inhibited root growth, but only in the allelic mutant tw_2 (**Figure 3**b and Table S3b). Neither compound showed any effect on the grain germination rate (Table S3c).

2.2. Fungi Spectrum in the Internal Grain Tissues of Barley tw-Type Mutants and Revertants

To reveal the possible differences in the fungal diversity in surface-sterilized mouldy germinating grains of tested barley genotypes, the spectrum of fungi species was investigated. Among the fungi that frequently reside in the internal tissues of barley grains, *B. sorokiniana* prevailed in all the tested plant genotypes (**Figure 4** and Table S4). In addition to the *WT*, tw_1 and tw_2 mutants, the fungi spectra were also studied in several revertants that arose

during the phase of stabilization of tw_1 and tw_2 mutants. The revertant studies allowed for a broader understanding of the differences between *tw*-type mutants and the *WT*.

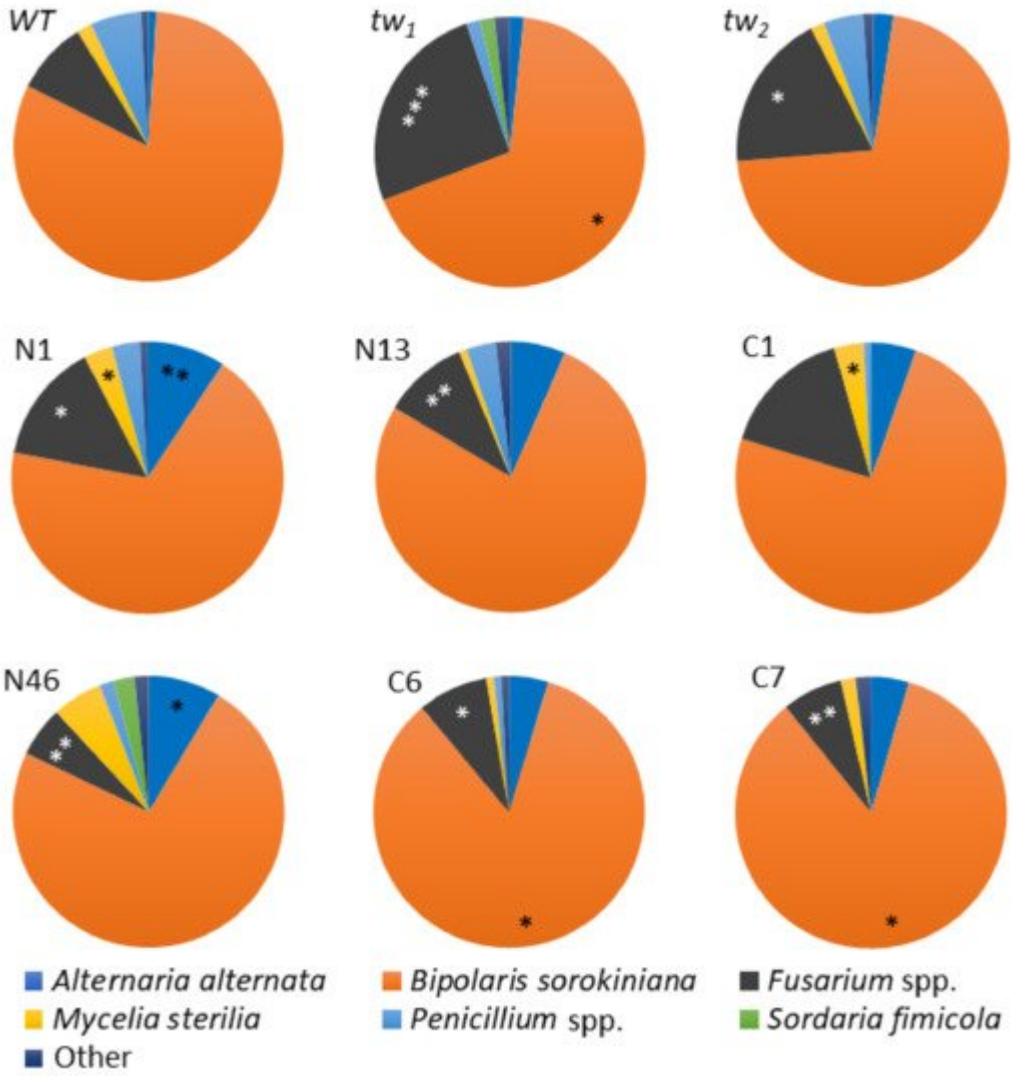

Figure 4. Spectra of fungi (%) in the internal grain tissues of barley *tw* mutants and the *WT*. The middle row—revertants from tw_1; the lower row—revertants from tw_2. N—revertants with normal spike and floral structure, C—revertants with normal floral structure but compactoid spikes. Grain was produced in 2013, and the analysis was performed in 2014. The asterisks represent significant differences (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$) between the *tw*-type mutant and the *WT* (in the upper row) or between the revertant and the respective initial *tw* mutant (N1, N13 and C1 derived from tw_1, N46, C6 and C7—from tw_2).

In the grains of the tw_1 and tw_2 mutants, the *B. sorokiniana* frequency was lower than that in the *WT* strain, but the difference was only statistically significant in tw_1. Interestingly, the level of *B. sorokiniana* was only significantly lower in grains of revertant N1 compared to *WT* ($p < 0.05$) and remained the same as in grains of its parental mutant tw_1. Such a tendency was not observed. A similar tendency was also observed in other revertants, except for the compactoid (C)-type revertants from tw_2, but only in an insignificant manner. However, a decrease in the *Bipolaris* proportion occurred at the expense of the increasing *Fusarium* portion in the fungi spectra, and the

observed effect was statistically significant (**Figure 4**). Comparable results were also obtained after analysing the fungi spectra in the internal grain tissues in our previous studies [3]. This finding provided a pretext for studying the effects of grain meals made from the different *tw*-type allelic mutants and the *WT* on *B. sorokiniana* growth.

2.3. The Impact of Meals from Grains of tw-Type Mutants on the Colony Growth of Bipolaris sorokiniana

The growth of *B. sorokiniana* colonies on MEA media supplemented with meals prepared from the grounded dry grains of allelic mutants *tw*, tw_1, and tw_2 was compared with *B. sorokiniana* growth on MEA medium containing meals from the grains of *WT*. Additionally, the effects of the SA and TCA concentrations were investigated on such media (**Figure 5**). The meals from the allelic *tw* mutants significantly decreased the growth of *B. sorokiniana* colonies. In most cases, a further statistically significant decrease in *Bipolaris* colony growth occurred only after TCA but not SA addition. SA decreased the growth of *B. sorokiniana* in a concentration-dependent manner only in tw_2. TCA also showed a strong inhibitory effect on *B. sorokiniana* growth on MEA media with meals from the *WT*. Even the lowest concentration of TCA, 0.05 mM, significantly decreased the growth of *Bipolaris* colonies, and a further increase in TCA concentration did not enhance its inhibitory effect on *B. sorokiniana* growth in *WT* media (**Figure 5**).

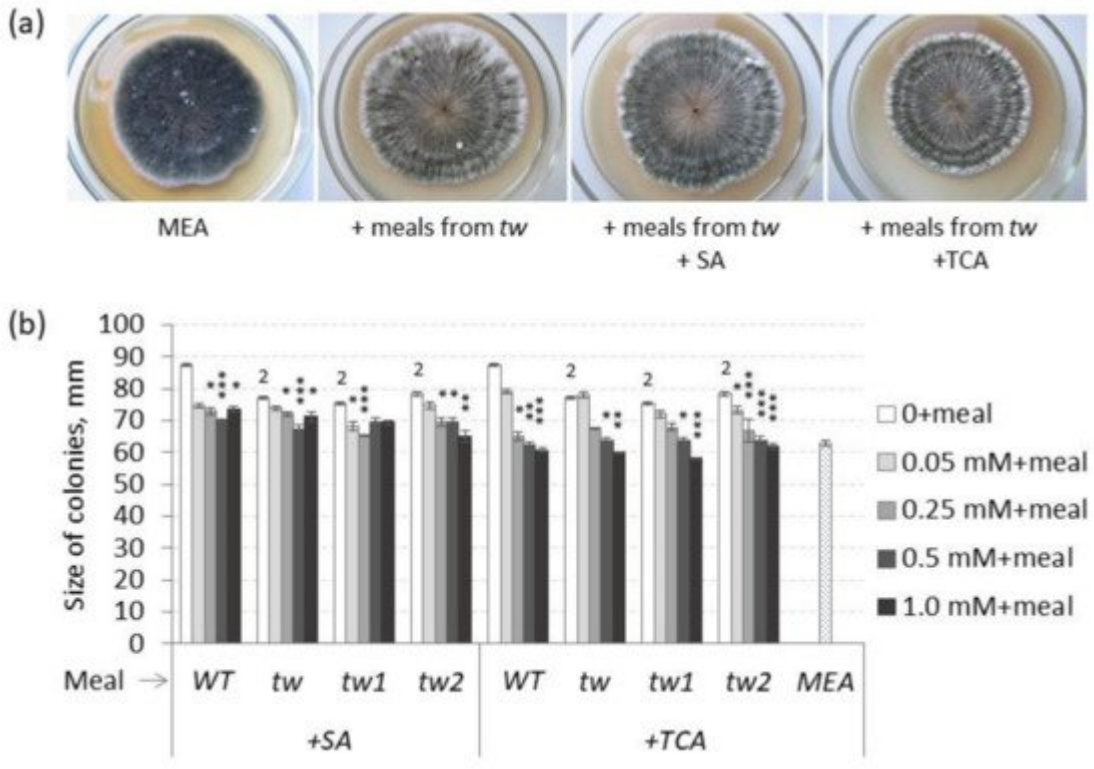

Figure 5. Effects of salicylic acid (SA) and *trans*-cinnamic acid (TCA) on the growth of *Bipolaris sorokiniana* after 7 days of growth on medium containing meal from *tw*. (**a**) The morphology of *B. sorokiniana* colonies grown on MEA media with different supplements. (**b**) Size of the colonies 7 days after inoculation. MEA, malt extract medium. Grain was produced in 2013, and the experiment was performed in 2014. The asterisks represent significant

differences (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$) between the control and the SA or TCA treatment. The numbers denote significant differences (^{2} $p < 0.01$) between the controls of the *tw*-type mutant and the *WT*.

3. Current Insights

The present study showed that more appreciable conclusions on the action of the modifying factors could be made when the MGG assay is performed in parallel with the root growth test. The inhibition of root elongation of germinating grains is one of the earliest and most distinct symptoms exhibited in response to auxins, especially 2,4-D [4], but auxin promotes lateral root formation [5], and this auxin feature is related to pathogen invasion [6]. In the present study, significant differences in 2,4-D-induced root growth inhibition among the different allelic *tw*-type mutants were revealed only in the lower range (10–50 mg L^{-1}) of 2,4-D concentrations (**Figure 1**b). Moreover, significant root length inhibition with 2,4-D in the allelic mutants *tw* and tw_2 and the nonallelic mutant *twN18* was only observed in unsterilized germinating grains, whereas in sterilized grains, root growth inhibition in these mutants was absent (**Figure 1**). While differences in MGG frequency between sterilized and unsterilized grains are naturally expected, the dependence of root growth after 2,4-D treatment on grain sterilization status was quite an unexpected phenomenon. Hypochlorite-based agents, including commercial bleach, are routinely used for surface sterilization of various plant materials [7][8]. Hypochlorite was proposed to react with the seed surface, forming a chlorine cover that is not completely removed by rinsing, and subsequently can be converted into highly toxic chloramines that easily penetrate plant tissues [7]. Furthermore, various salts are known to antagonize the phytotoxicity of several herbicides, including 2,4-D [9][10]. After surface sterilization with commercial bleach, nonremovable chlorine compounds can antagonise exogenously applied 2,4-D and subsequently diminish the effect of root growth inhibition in comparison to that of unsterilized 2,4-D-treated grains. This observation highlights the importance of comparing sterilized and unsterilized grain conditions in studies of plant-auxin–pathogen relationships where the action of exogenous auxin will be analysed in germinating grains since hypochlorite-based sterilization itself can lead to underestimation of the 2,4-D effect on root growth.

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