The effect and mechanism of Dufulin in controlling tomato yellow leaf curl virus on tomato plants

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Research

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Abstract

Background: Tomato yellow leaf curl virus (TYLCV) causes critical production loss in tomato cultivation. The control of TYLCV in tomato is done mainly by using pesticide which is difficult and expensive, making it essential to find an environmentally friendly chemical agent to control TYLCV. Dufulin has been widely used to prevent and control viral diseases in tobacco and rice in recent years. In this study, we investigated the effect and mechanism of Dufulin on TYLCV on tomato plants.

Methods: The control effect of Dufulin on TYLCV was evaluated by field experiments. The expression level of *PI II* and *NPR1* in healthy and TYLCV-infected tomato after treatments were determined by Real-time fluorescent quantitative PCR (qRT-PCR). Handheld chlorophyll meter was applied to compare the content of chlorophyll and nitrogen in healthy and TYLCV-infected tomato after treatments.

Results: It showed that the relative control effect of 20% Dufulin on TYLCV reached above 68% in 2018 to 2020. Jasmonic acid (JA) level was higher on healthy tomato, but lower on TYLCV-infected tomato plants treated with Dufulin compared to control. Salicylic acid (SA) level was higher on healthy and TYLCV-infected tomato plants treated with Dufulin compared to control. Chlorophyll content on healthy and TYLCV-infected tomato plants was higher after treatment with Dufulin compared to control. Nitrogen content on tomato plants showed no significant difference after spraying Dufulin compared to control.

Conclusions: We found the first evidence of control effects TYLCV using Dufulin. It induced plant defense and increased plant chlorophyll content to help plants resist infection which is helpful for future control of TYLCV in tomato.

Background

Tomato yellow leaf curl virus (TYLCV) belongs to the genus *Begomovirus* of the family Geminiviridae. It is transmitted by *Bemisia tabaci* in a persistent, circulative manner and is a plant virus that causes important disease in tomato [1-3]. TYLCV-infected tomato plants show various symptoms, for instance, leaf yellowing, curling and stunting, which result in a severe reduction in tomato cultivation production around the world [4, 5]. In addition to tomato, other cultivated plants including pepper, common bean, cucurbit and eustoma are also infected by TYLCV [6-9]. No matter in greenhouse or in open field production, the TYLCV management is difficult and expensive [10].

There are many different methods to reduce crop losses resulted from TYLCV, such as removing vectors, killing weeds, and changing planting season what have been applied in many crop areas [1]. The development of TYLCV-resistant commercial tomato cultivars is the best approach to control TYLCV so far [11]. However, virus may exist strong genetic variability, in addition, TYLCV-resistant tomato cultivars are easy to vary [12]. Also chemicals or pesticides are used for plant virus diseases and pest control, but they have negative effects on humans, wildlife and environment [13]. Therefore, it is necessary to find an environmentally friendly chemical agent to control TYLCV.

Dufulin, an amino phosphonate compound with a novel molecular structure ((2-fluorophenyl)-(((4-methylbenzothiazol-2-yl)-amino) methyl) phosphonic acid diethyl ester, Figure 1) [14-16]. It is a new antiviral agent and has highly effect on plant viruses. There are studies have shown that Dufulin inhibit tobacco mosaic virus (TMV) through inducing systemic acquired resistance (SAR) in plants [17]. It is highly effective against TMV, cucumber mosaic virus (CMV), potato virus Y (PVY) and southern rice black-streaked dwarf virus (SRBSDV) [18, 19]. Dufulin has been widely used to prevent and control tobacco and rice viral diseases in recent years. It is the first antiviral agent to meet environmental standards in China [20, 21]. In 2007, it was identified as a new chemical with high anti-TMV activity by the Ministry of Agriculture of China (LS 20071280 and 20071282). Subsequently, Dufulin was produced on an industrial scale and applied for field application [22].

The effect of Dufulin in controlling TYLCV is still poorly reported. In this study, we first investigated the effect of Dufulin in controlling TYLCV, and then we determined the plant defense and plant nutritional properties following treatment with Dufulin in tomato, and uncover the mechanisms of controlling TYLCV by Dufulin in tomato.

Methods

Field control effect of Dufulin on TYLCV

The field experiment was conducted in Chunhua vegetable-growing areas, Changsha City, Hunan Province, where TYLCV diseases occur every year. Tomato (*Solanum lycopersicum*, "Zuanhongmeina", Hunan Academy of Agricultural Sciences) was planted on April from 2018 to 2020. After planting for 3 days, the dead tomato plants were replaced. In the experiments, there were 2 treatments (Dufulin and water control). There were 3 replicates for each treatment, with 6 plots in total. Plots were arranged in random groups and 60 tomato plants were planted in each plot. Protective rows were set between plots. The reagent was 20% Dufulin suspension (Guangxi Garden Biochemical Joint Stock Company, Guangxi, China), water was used as control. Prior to Dufulin and water treatment, no other antiviral reagents were applied at the experimental field.

The first reagent application was made in accordance with the manufacturer's instructions on April from 2018 to 2020. The Singapore AGROLEX SPRAYER JACTO HD400 16-litre knapsack sprayer (Linon Private Limited Company, Singapore) was used to spray the leaf surface, with a spray pressure of 1-2 kgf/cm^2 and a flow rate of 255-950 g/min. The relatively steady rate of spraying was maintained and the weather was sunny. The reagent of 5 liters was sprayed on each plot, and was applied 3 times in a plot. Each interval is 7 days. Any phytotoxicity in tomato was observed after each application of 2 days. The morbidity was investigated by 5 point diagonal sampling method following the last application of 14 days, and 6 plants were investigated at each point. A total of 60 plants were investigated. The total number of investigated plants and the number of diseased plants at all levels were recorded. The disease index and control effect were calculated by the following formulate:

Disease index = Σ (Number of diseased plants at every levels × Level value) / (Total survey plants × the highest value);

Control effect = [(Disease index of control group - Disease index of treatment group) / Disease index of control group] × 100%.

Each plant was assigned a disease level according to the following scale [23]:

0 = Asymptomatic;

1 = Slight symptoms (apical leaves slightly yellowing and margin slightly curling);

2 = Moderate symptoms (apical leaves moderate yellowing, marginal moderate curling and wrinkling)

3 = Severe symptoms (severe marginal / interveinal yellowing, wrinkling and curling);

4 = Very severe symptoms (foliar symptoms similar to 3 accompanied by reduction in leaf size, branching, and severe stunting).

Effects of Dufulin on jasmonic acid (JA) and salicylic acid (SA) in tomato

This experiment was conducted in the laboratory. Tomato (*Solanum lycopersicum*, "Zuanhongmeina") plants were grown in pots (d = 12.5 cm, h = 15 cm) kept in whitefly-proof screen-cages (60 × 40 × 60 cm) in a greenhouse under photoperiod L16: D8, temperature 25 ± 1°C, and relative humidity 60 ± 10%. Healthy and TYLCV-infected tomato plants were used. TYLCV-infected tomato plants were obtained according to Ning et al. [24].

TYLCV-infected tomatoes and nonviruliferous tomatoes were sprayed with Dufulin purification after transplanting 35 days, water was used as control. Each tomato plant was sprayed with about 15 mL of the reagent. There were nine tomato plants for each treatment. About 0.1 g tomato leaf was collected into RNA free sampling tubes after spraying of 1, 2, 3, 4, 5 and 6 days, respectively. They were stored at -80°C for further detection. All tomato leaves RNA were extracted with TRIzol (TransGen Biotech, Beijing, China). The RNA concentration and purity were measured by NanoDrop 2000 (Thermo Fisher Scientific, Beijing, China). qRT-PCR (real-time fluorescent quantitative reverse transcriptase polymerase chain reaction) was used to measure the expression level of JA and SA associated genes in tomato after treatments. The first strand of cDNA for qRT-PCR was synthesized by using cDNA synthesis kit (TransGen Biotech, Beijing, China) in accordance with the manufacturer's instructions. qRT-PCR was carried out on qTOWER3G qPCR system (Analytik Jena, Jena, Germany) by using qPCR kit (TransGen Biotech, Beijing, China) in accordance with the manufacturer's instructions. We took *PI II* and *NPR1* as target gene. *PI II*, a downstream gene associated with JA pathway in tomato [25] and *NPR1*, a downstream gene associated with SA pathway in tomato [26]. Corresponding qPCR primers were listed in Table 1. Normalized gene expression was calculated using the $2^{-\Delta\Delta CT}$ method [27] with *ACT* and *UBI* as reference gene [28].

Effects of Dufulin on chlorophyll and nitrogen content in tomato

Handheld chlorophyll meter (Okechi Instrument Company, Henan, China) was used to measure the chlorophyll and nitrogen content in tomato leaves. The treatments were the same as above. The same leaves were measured after spraying of 1, 2, 3, 4, 5 and 6 days, respectively. The third leaf from the top of the plant was marked and measured in accordance with the manufacturer's instructions.

Data analysis

Data were analyzed using SPSS version 20.0 (SPSS Inc., Chicago, IL, USA). Independent sample *t*-test was used to compare the field control effect of Dufulin. The general linear model (GLM) repeated-measure was used to compare the effects of Dufulin on plant defense and nutrition in tomato. Differences between groups were considered statistically significant when the p value was less than 0.05.

Results

Field control effect of Dufulin on TYLCV

Three days after the application of 20% Dufulin, field observation showed that the tomato grew normally. The leaf color and plant height showed no obvious negative effects, indicating that Dufulin treatment was safe for tomato. There was a significant reduction in the incidence of TYLCV on tomato. The TYLCV morbidity of tomato was only 55.94% in 2018 (Fig. 2A), 53.97% in 2019 (Fig. 2B), 43.36% in 2020 (Fig. 2C) after spraying 20% Dufulin, which was significantly lower than control (2018, $F = 13.791$, $p = 0.037$; 2019, $F = 4.482$, $p < 0.001$; 2020, $F = 9.108$, $p = 0.006$). The incidence of tomato reached up to 98.72% in 2018, 99.62% in 2019, 99.58% in 2020 after control treatment. Also, the disease index of tomato after applying 20% Dufulin was significant less than water (2018, $F = 0.14$, $p = 0.004$; 2019, $F = 0.829$, $p < 0.001$; 2020, $F = 0.605$, $p = 0.001$), it reduced by 32.66% in 2018 (Fig. 3A), 32.95% in 2019 (Fig. 3B), 41.55% in 2020 (Fig. 3C). The average relative control effect of 20% Dufulin reached 68.29%.

Effects of Dufulin on JA and SA in tomato

After spraying Dufulin, the relative expression level of *PI II* gene showed no significant difference on healthy tomato compared with control on the whole ($F = 3.305$, p □ 0.05) (Fig. 4A). The relative expression level of *PI II* on healthy tomato was increased from 1 to 6 days. But the relative expression level of *PI II* gene showed significant lower on TYLCV-infected tomato compared with control ($F = 37.813$, $p = 0.001$) (Fig. 4B). The relative expression level of *PI II* gene on TYLCV-infected tomato was increased firstly, then declined from 2 to 6 days. The relative gene expression was lower after spraying Dufulin than control from 1 to 6 days.

The relative expression level of *NPR1* gene showed significant higher on healthy tomato and TYLCV-infected tomato compared with control (Healthy plant: $F = 434.469$; p □ 0.001; TYLCV-infected plant: $F = 179.074$; p □ 0.001). The relative expression level of *NPR1* gene on healthy tomato plants increased from 1 to 6 days (Fig. 5A). But the relative expression level of *NPR1* on TYLCV-infected tomato plants first declined and then increased from 2 to 6 days (Fig. 5B). And the relative gene expression was higher on healthy and TYLCV-infected tomato plants after spraying Dufulin compared to control.

Effects of Dufulin on chlorophyll and nitrogen content in tomato

After spraying Dufulin, the content of chlorophyll was significant higher on healthy tomato and TYLCV-infected tomato compared with control (Healthy plant: $F = 184.902$; p □ 0.001; TYLCV-infected plant: $F = 104.757$; $p = 0.001$). The chlorophyll content on healthy tomato plants was increased from 1 to 6 days (Fig. 6A). But the chlorophyll content on TYLCV-infected tomato plants was decreased from 1 to 4 days, then rosed (Fig. 6B). The content of chlorophyll on healthy and TYLCV-infected tomato plants was higher than control from 1 to 6 days.

The content of nitrogen on healthy and TYLCV-infected tomato were not significant difference compared with control (Healthy plant: $F = 1.174$; p □ 0.05; TYLCV-infected plant: $F = 0.023$; p □ 0.05;) The nitrogen content on healthy tomato plants decreased from 1 to 6 days (Fig. 7A). However the nitrogen content on TYLCV-infected tomato plants was first decreased, then rose from 3 to 6 days after spraying with Dufulin (Fig. 7B). The nitrogen content of nitrogen remained relatively stable on healthy and TYLCV-infected tomato plants in these two treatments.

Discussion

Dufulin has been widely used to prevent and control many tobacco, rice and other viral diseases. As a kind of highly effect on resist plant viral diseases, its effectiveness, active mechanism, environmental biotoxicity, and safety evaluation have been widely researched [29, 30]. Currently, TYLCV is a disease that seriously damages tomato production. In this study, Dufulin was applied to control TYLCV for the first time. There was a significant reduction in the incidence of TYLCV on tomato, and the average relative control effect reached more than 68% from the year of 2018 to 2020. It indicates that Dufulin has potential application value in the prevention and control of TYLCV.

Plants cannot move to elude environmental threats. As a result, plants have evolved complex mechanisms for sensing aggression, such as viruses, fungi, bacteria, insects and so on, and then translate that perception into an adaptive response [31]. Plant defense plays an important role in its virus resistance. JA plays important roles in plant defense against insects. Whitefly nymphs are influenced by JA treatments, the development become slower and have a lower survival rate [32]. In this study, *PI II* relative expression level increased after spraying Dufulin on healthy tomato. However, *PI II* relative expression level rose first and then fell after spraying Dufulin on TYLCV-infected tomato. This phenomenon may be caused by TYLCV infection. Previous research showed that TYLCV infection of host plants are beneficial to *Bemisia tabaci* vectors by inhibiting JA signaling pathway [33]. Our results also illustrate this hypothesis. SA mediates plant defense against pathogens through accumulating in both infected and distal leaves, which can response to pathogen attack [34-38]. It was reported that Dufulin is an activator of systemic acquired resistance (SAR) by regulating the SA signaling pathway in tobacco, then the plant cells are given antiviral activity [29]. In our study, *NPR1* relative expression level was higher on healthy and TYLCV-infected tomato plant after spraying Dufulin compared to control. It is consistent with previous studies, suggesting Dufulin can resist TYLCV by regulating the SA signaling pathway in tomato. Treatment with Dufulin can control TYLCV by strengthening plant defenses, such as JA and SA. However, what specific regulatory mechanisms in the process need further verification.

Chlorophyll is the main pigment in photosynthesis and plays a central role in light absorption. Nitrogen is an essential macronutrient which is needed for plant growth and development. Chlorophyll and nitrogen concentration in leaves were also measured because they can be affected by the plant nutritional status [39]. There are studies showed that plant chlorophyll content was positively correlated with nitrogen content [40]. Therefore, the value of chlorophyll content in leaves can help understand the nutritional status of plants, guide fertilization management scientifically, and ensure the quality and yield of crops [41, 42]. Chlorophyll content on healthy and TYLCV-infected tomato plants was higher after treatment with Dufulin compared to control. This indicated that the effect of Dufulin in controlling TYLCV was related to the increase of chlorophyll content on tomato. Nevertheless, nitrogen content on healthy and TYLCV-infected tomato plants were not significant difference compared with control. This may indicate that Dufulin does not directly affect the nitrogen content of tomato plants. Further research is still needed to confirm the mechanism of the chlorophyll increase with Dufulin treatment.

Conclusions

In conclusion, we found the effects of Dufulin on controlling TYLCV for the first time, by inducing plant defense and plant nutrition. Our study uncovered the mechanisms of TYLCV controlling by Dufulin in tomato. Dufulin will be used more widely on other viral diseases in plants.

Abbreviations

TYLCV: Tomato yellow leaf curl virus; TMV: Tobacco mosaic virus; SAR: Systemic acquired resistance; CMV: Cucumber mosaic virus; PVY: Potato virus Y; SRBSDV: Southern rice black-streaked dwarf virus; JA: Jasmonic acid; SA: Salicylic acid; qRT-PCR: Real-time fluorescent quantitative PCR.

Declarations

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Authors’ contributions

YL and XBS performed the methodology and project application; ZZ, XGZ, ZHZ, LMZ, YG, FH, DYZ and XQT designed and coordinated the research; LPH, SXW and XBS performed the majority of the experiments and analyzed the data; LPH and XBS wrote the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

Not applicable.

Ethics approval and consent to participate

Because no animals and patients were involved in this study, the Ethics approval not applicable in this section.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1. Primers used in this study

Gene	GeneBank accession no.	Primer sequence [a]
ACT	BT013707	F: 5'-AGGCAGGATTTGCTGGTGATGATGCT-3'
		R: 5'-ATACGCATCCTTCTGTCCCATTCCGA-3'
UBI	X58253	F: 5'-TCGTAAGGAGTGCCCTAATGCTGA-3'
		R: 5'-CAATCGCCTCCAGCCTTGTTGTAA-3'
PI II	K03291.1	F: 5'-CCTATTCAAGATGTCCCCGTTC-3'
		R: 5'-GGGCAATCCAGAAGATGG-3'
NPR1	AY640378.1	F: 5'-ATATAGAATTCCTGCTCCAAAGGATCGGTTA-3'
		R: 5'-ATATACTCGAGCAGACAAGTCATCAGCATCCA-3'

[a] F denotes forward qPCR primer; R denotes reverse qPCR primer.

Figures

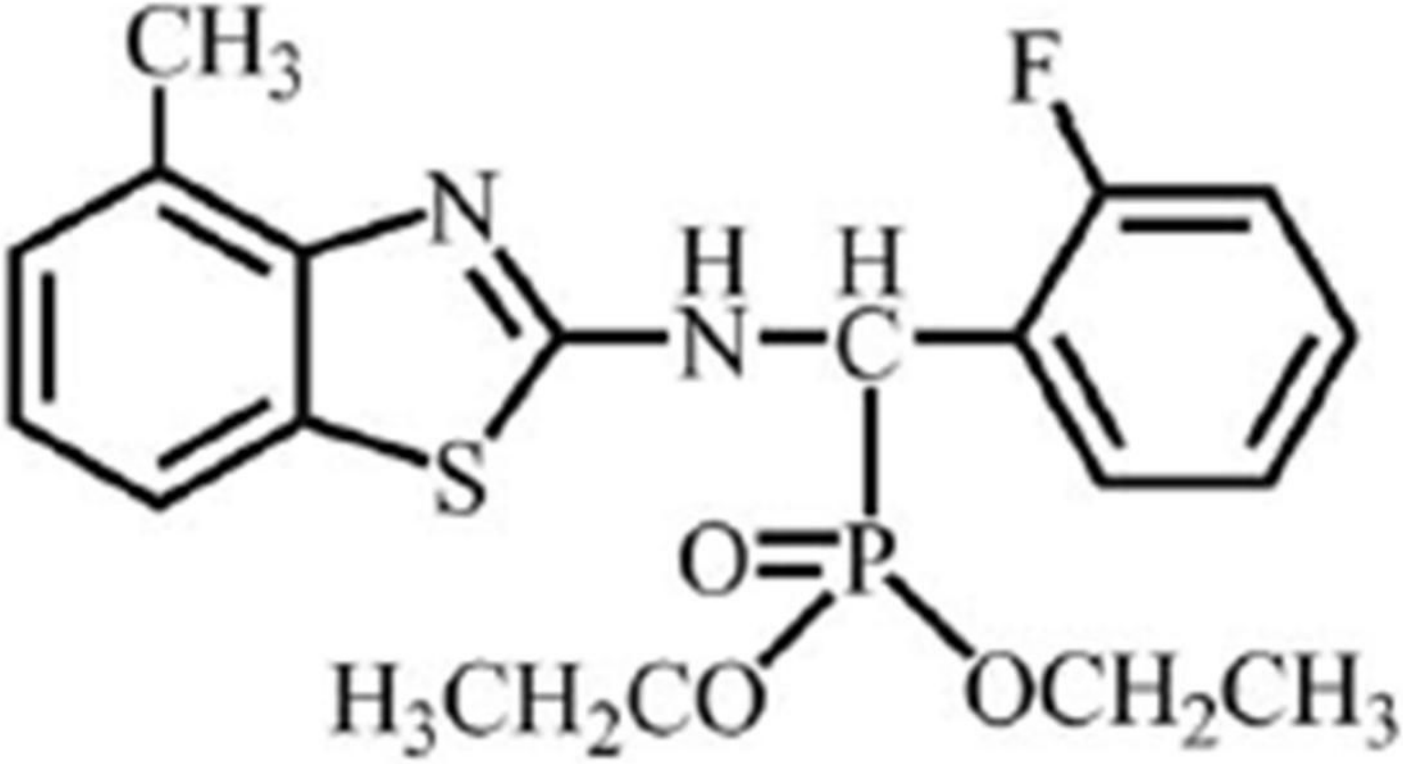

Figure 1

Structure of Dufulin.

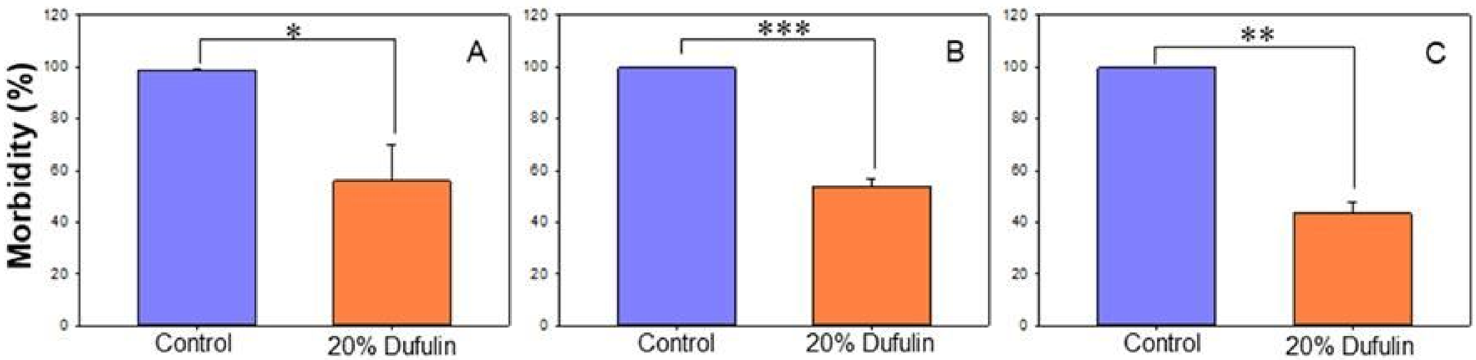

Figure 2

The morbidity of tomato in field experiments from 2018 to 2020. The morbidity in 2018 (A), 2019 (B) and 2020 (C) .Data are given as mean ± SD; Error bar: mean ± SEM; *$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$.

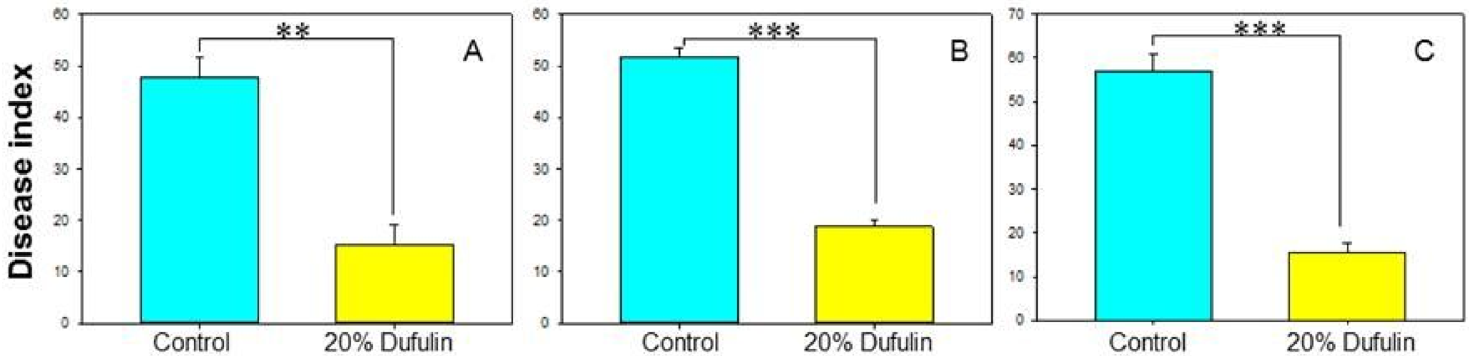

Figure 3

The disease index of tomato in field experiments from 2018 to 2020. The disease index in 2018 (A), 2019 (B) and 2020 (C). Data are given as mean ± SD; Error bar: mean ± SEM; $**p \leq 0.01$; $***p \leq 0.001$.

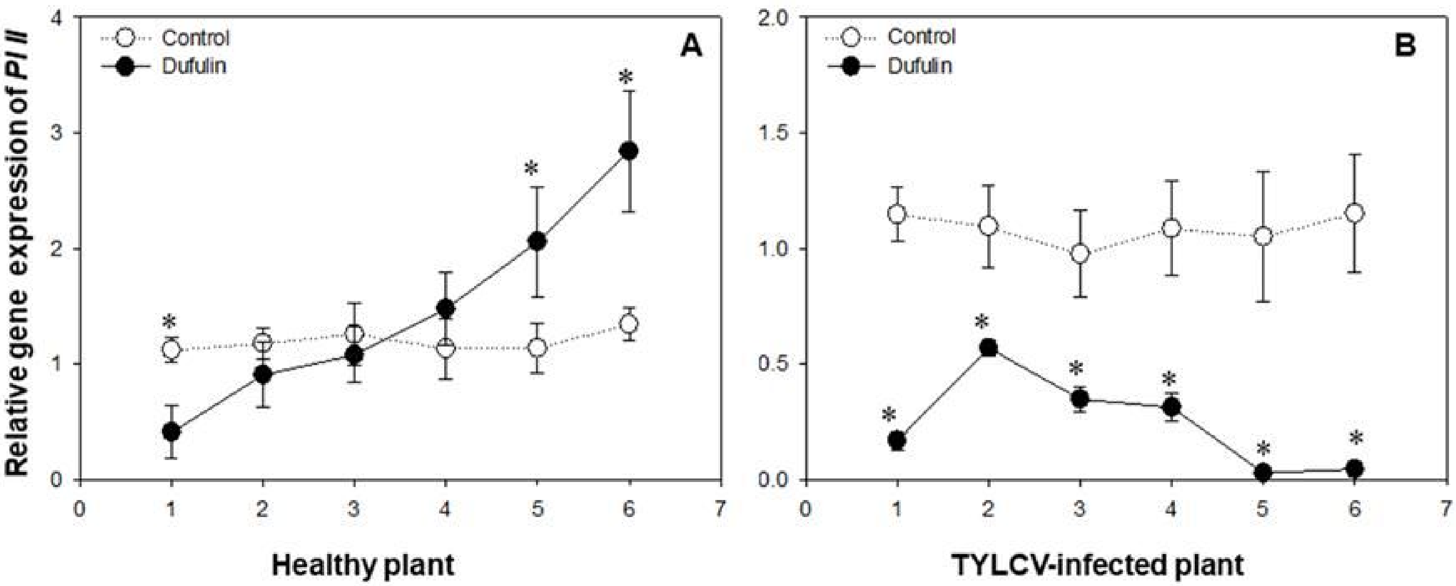

Figure 4

Relative expression level of PI II on healthy and TYLCV-infected tomato after spraying with Dufulin and water for 1, 2, 3, 4, 5, 6 days. (A) Healthy tomato. (B) TYLCV-infected tomato. Data are given as mean ± SD; Error bar: mean ± SEM; *$p \leq 0.05$.

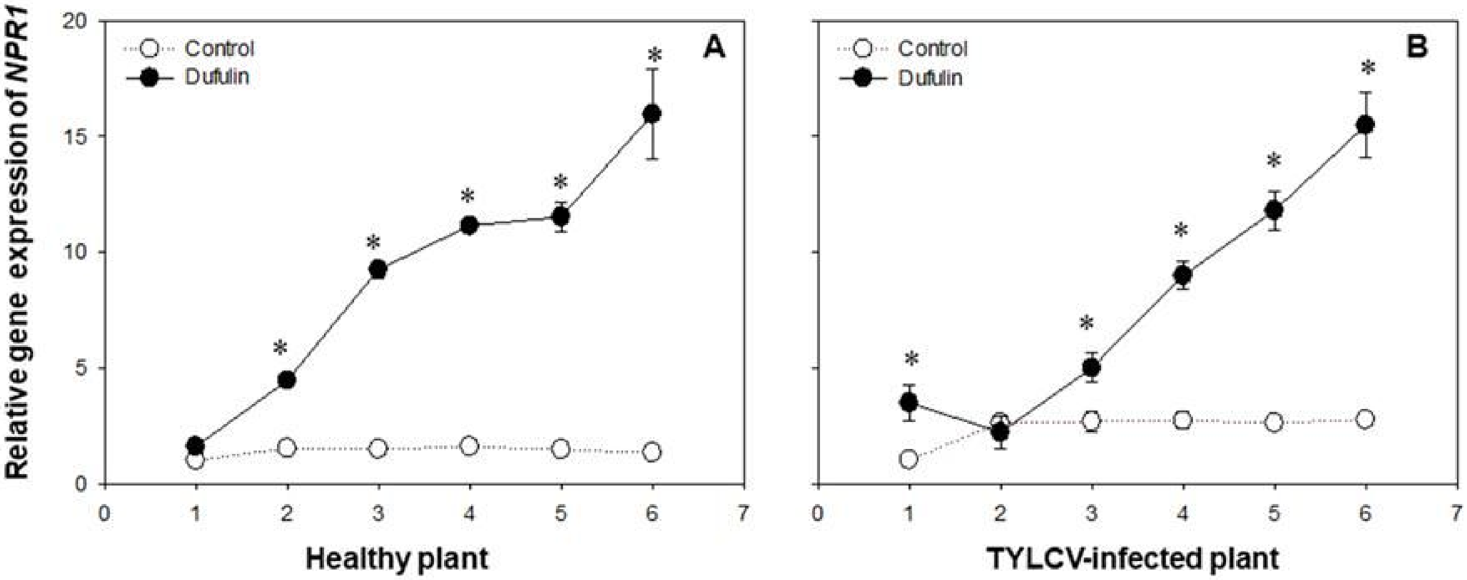

Figure 5

Relative expression level of NPR1 on healthy and TYLCV-infected tomato after spraying with Dufulin and water for 1, 2, 3, 4, 5, 6 days. (A) Healthy tomato. (B) TYLCV-infected tomato. Data are given as mean ± SD; Error bar: mean ± SEM; *p ≤ 0.05.

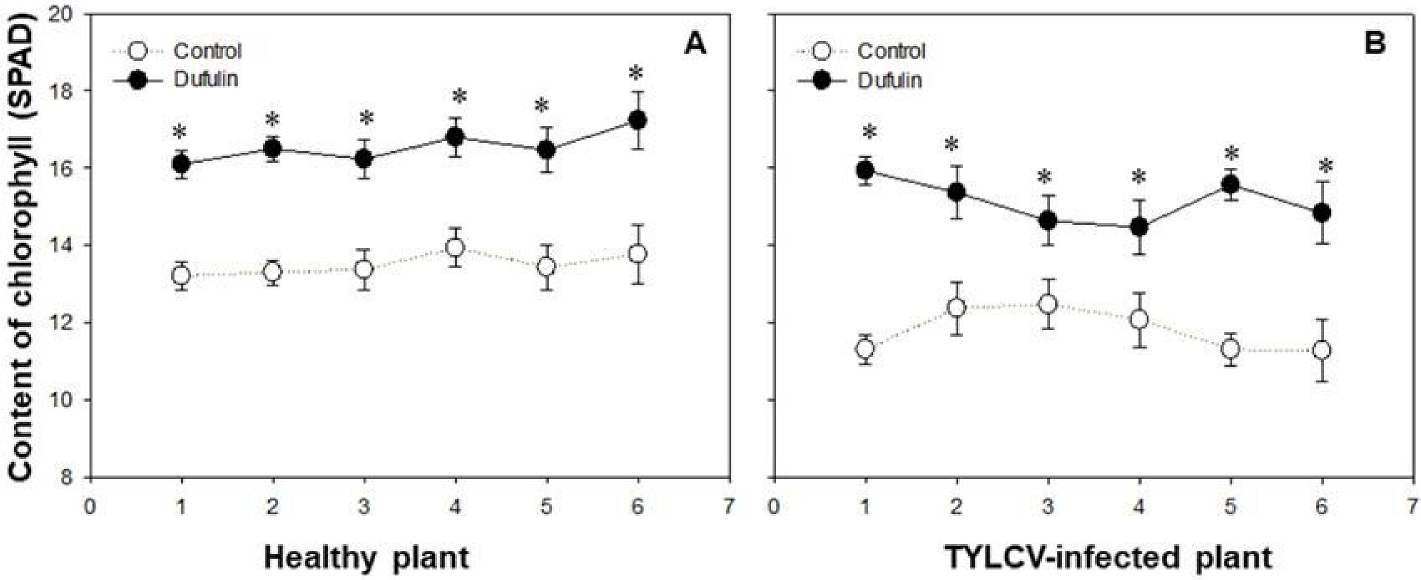

Figure 6

The content of chlorophyll (SPAD) on healthy and TYLCV-infected tomato after spraying Dufulin and water for 1, 2, 3, 4, 5, 6 days. (A) Healthy tomato. (B) TYLCV-infected tomato. Data are given as mean ± SD; Error bar: mean ± SEM; *p ≤ 0.05.

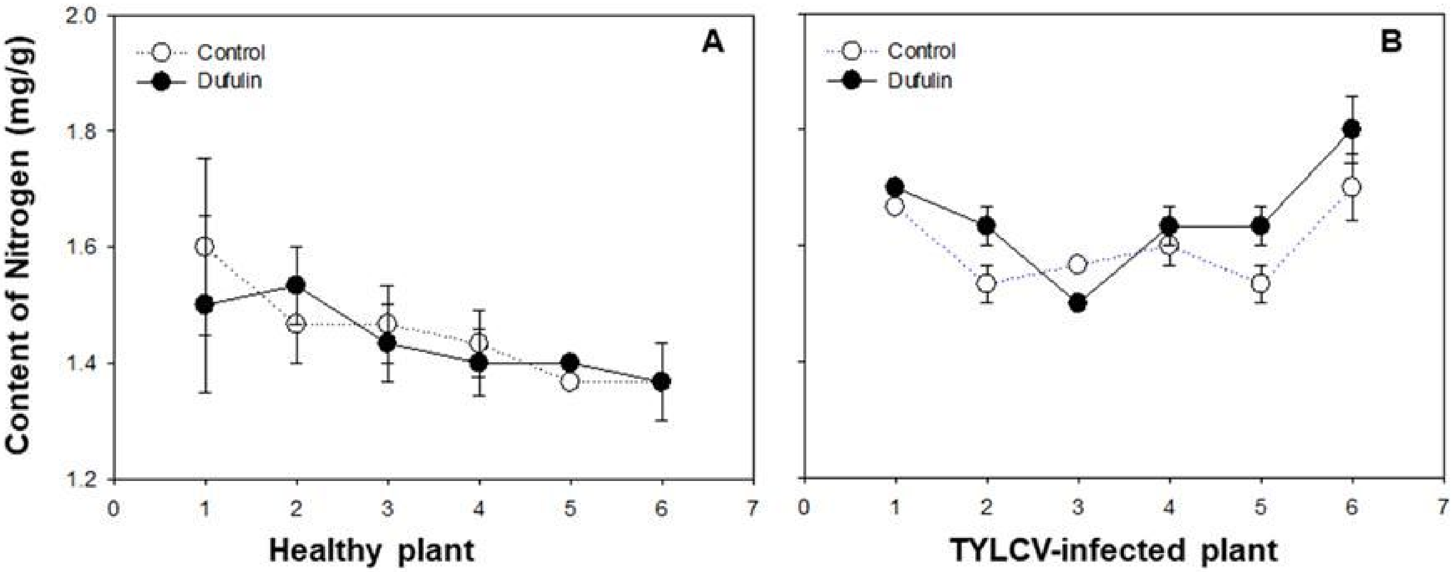

Figure 7

The nitrogen content (mg/g) on healthy (A) and TYLCV-infected (B) tomato after spraying with Dufulin and water for 1, 2, 3, 4, 5, 6 days. (A) Healthy tomato. (B) TYLCV-infected tomato. Data are given as mean ± SD; Error bar: mean ± SEM.