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The disease quantification analysis of cotton Verticillium wilt using the two methods of disease index and fungal biomass present high consistency

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Abstract: *Verticillium dahliae* is a broad host-range pathogen that causes vascular wilt in plants. The Verticillium wilt disease severity assay on plants caused by *V. dahliae* mainly includes two methods, one is a plant disease grade classification based on disease severity statistics [namely the disease index (DI)], and the other is the *V. dahliae* biomass quantification in plants (namely the fungal biomass). In this study, the relationships of pathogenicity with the DI, pathogenicity with the biomass, and the correlation analysis of the DI and relative fungal biomass were analysed. The results showed that pathogenicity assessment of *V. dahliae* strains using the DI method was able to give an intuitive reflection of the pathogenic ability for defoliating and non-defoliating strains; moreover, the method of quantitative PCR for fungal biomass also had high repeatability and stability. As a whole, the correlation coefficient between the DI and fungal biomass values of 28 strains was 0.728, indicating that the two data sets were highly correlated; however, the correlation coefficients of the defoliating strains and non-defoliating strains were only 0.5384 and 0.4547, respectively. In conclusion, the correlation coefficient between the DI and the fungal biomass presented high consistency, which could provide some meaningful exploration for the more accurate pathogenicity identification of *V. dahliae*.

Keywords: *Verticillium dahliae*; disease grade classification; qPCR; correlation coefficient

Verticillium dahliae is a soil-borne ascomycete fungi that causes Verticillium wilt in over 200 plant species, including many important crops such as cotton, potato, lettuce, and tomato, which causes

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billions of dollars in losses in agricultural annually (Klosterman et al. 2009). The list of hosts for *V. dahliae* is continually expanding, as new hosts in diverse ecological niches succumb to the pathogen (Bhat & Subbarao 1999). *V. dahliae* is an asexually reproducing, soil-borne, vascular wilt-causing phytopathogenic fungus (Klosterman et al. 2009). Extensive chromosomal reshuffling and horizontal gene transfer have been identified to drive the evolution of virulence and host adaption in this asexual pathogen (de Jonge et al. 2013; Chen et al. 2018). The survival structures of the microsclerotia produced by *V. dahliae* can keep it viable in the soil in the absence of a host for more than 14 years (Wilhelm 1955). As vascular pathogens, *V. dahliae* enters and colonises the plant vascular (xylem) system, disrupting water transport, and causes the characteristic symptoms of wilting, and often the vascular discoloration and death of aerial tissues (Pegg & Brady 2002; Fradin & Thomma 2006; Klosterman et al. 2009). These characteristics make *V. dahliae* a difficult pathogen to control, once it is introduced into the soil, and the spread of aggressive pathogen strains into previously non-endemic regions, which would be a major threat to plant health and food security.

Asexual organisms are often considered as evolutionary dead ends, however, *V. dahliae* has evolved abundant population variants (clonal lineages). At present, the divergence of clonal lineages of *V. dahliae* isolates from different hosts or geographical locations are well studied, including the six main vegetative compatibility groups (VCGs) identified by the compatibility of nitrate non-utilising (nit) mutants (Joaquim & Rowe 1991; Milgroom et al. 2014), race one and two that respond to host resistance differentials in tomato and lettuce (Vallad et al. 2006), two mating type idiomorphs, MAT1-1 and MAT1-2 (Usami et al. 2009), two pathotypes, defoliating (D) and non-defoliating (ND), identified based on the phenotype caused by Verticillium wilt (Schnathorst & Mathre 1966; Zhang et al. 2019). The symptoms (as leaf wilting, chlorosis, stunting, vascular discoloration, necrosis, and vein clearing) of Verticillium wilt caused by *V. dahliae* vary among different hosts, of which, in certain hosts, *V. dahliae* strains can cause the complete defoliation and, hence, are classified as D, and others that do not cause defoliation are classified as ND (Schnathorst et al. 1966; Carpita et al. 1993; Jiménez-Díaz et al. 2012; Chen et al. 2021). Based on the genetic variation analysis, researchers have also developed polymerase chain reaction (PCR)-based methods to detect races, pathotypes, mating-types, although the detection results have been negative in some cases (Pérez-Artés et al. 2000; Inderbitzin et al. 2011; de Jonge et al. 2012; Short et al. 2014).

The Verticillium wilt disease severity assay on different plants caused by *V. dahliae* mainly includes two methods, one with a long history is the plant disease grade classification based on disease severity statistics, and the other is *V. dahliae* biomass quantification in plants after root-dip inoculation. Usually, different methods and standards of plant disease grade classification are used in different research groups when analysing the different host plants or inoculating different *V. dahliae* pathotypes. Plant disease grade classification mainly includes different methods, among which, the area under disease progress curves (AUDPC) method is mainly used in the potato Verticillium wilt survey (Rowe 1995; Derksen et al. 2013; Li et al. 2019). The percentage evaluation of wilting leaves in a certain number of investigated plants is also usually used to detect the Verticillium wilt degree of the *Arabidopsis*, where the symptoms are evaluated and the disease grade is classified as follows: 0 (no symptoms), 1 (0–25% wilted leaves), 2 (25–50%), 3 (50–75%) and 4 (75–100%) (Liu et al. 2014). The disease index (DI) method, has been widely used to evaluate the disease symptoms caused by many soil-borne pathogens, especially cotton Verticillium wilt (Powell et al. 1971; Liu et al. 2013). The DI method uses four grades for cotton wilt disease grade classification, grade 1: the cotyledon beginning to yellow; grade 2: the cotyledon and one euphylla wilting; grade 3: all the leaves wilting and chlorosis; and grade 4: plant death, with the formula of the DI value = [$\sum$ (the seedling of every grade × relative grade) / (total seedlings × 4)] × 100 (Powell et al. 1971). Similar to the above DI method during an investigation into cotton Verticillium wilt, the cotton foliar damage was also evaluated by the following disease grades: 0: health plant, 1: yellowing or necrosis of 1–2 cotyledons, 2: yellowing or necrosis of 1 true leaf, 3: more than 2 wilted or necrotic leaves, 4: no leaves left or dead plant, according to the research from Zhang et al. (2012). Moreover, when the Verticillium wilt caused by the defoliating *V. dahliae* strain was evaluated, the disease grade was also classified as 0 (0–25% defoli-

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ated leaves), 1 (25–50% defoliated leaves), 2 (50–75% defoliated leaves) and 3 (75–100% defoliated leaves) (Zhang et al. 2017). Therefore, the plant disease grade classification will be different using the above different standards in the same experiment. In addition, the disease grade division depends on individual observation differences to a great extent, which can cause controversy when conducting the same experiment.

With the development of molecular biology technologies, researchers prefer to explore whether they can quantify Verticillium wilt using the molecular quantification method based on the quantitative polymerase chain reaction (PCR) method. Based on the above consideration, the Santhanam et al. (2013) put forward one fungal biomass method to present the wilt disease degree according with the plant phenotype. This method was conducted as follows: the roots of the inoculated plants were harvested after inoculation. The samples were ground to a powder and the genomic DNA was isolated, and a quantitative PCR analysis on the genomic DNA was carried out. *V. dahliae* elongation factor 1-α (*VdEF1-α*) was used to quantify the fungal colonisation, and different host plant housekeeping genes (such as the tomato, *Arabidopsis*, and *N. benthamiana actin* gene, and the cotton *18S* gene) were used as endogenous plant controls (Santhanam et al. 2013; Gui et al. 2017; Chen et al. 2018). However, regarding this method, many researchers have also proposed that the fungal biomass could not reflect the host plant disease degree, as although many *V. dahliae* strains could infect different host plants, they had low virulence or no virulence to some host plants which resulted in even if the high *V. dahliae* fungal biomass does not correspond with the disease degree of the infected plants and could not represent the real pathogenicity of many *V. dahliae* strains.

In order to detect the real relationship between the two disease grade classification and fungal biomass methods in the cotton Verticillium wilt disease degree identification, we randomly selected 28 *V. dahliae* strains (including defoliating and non-defoliating strains with different pathogenicity) isolated from cotton, and conducted the disease grade classification using the DI method and with fungal biomass quantification. Moreover, the genome of these strains was also re-sequenced in our previous research (unpublished data). We hope to find the real relationship of the two methods in surveying the disease ability or virulence of different *V. dahliae* strains on cotton.

MATERIAL AND METHODS

Fungal culture and plant material cultivation. Twenty-eight strains of *V. dahliae* were used in this study, and the detailed source information was listed in Table 1. The 28 strains of *V. dahliae* were cultured on potato dextrose agar (PDA: potato, 200 g/L; glucose, 20 g/L; agar,

Table 1. Strains of cotton used in this study and the disease index (DI) statistics

Strains	Country/region	Defoliating (D)/ non-defoliating (ND)	Disease index (DI)
209-2	China/Xinjiang	ND	8.75
XJ14	China/Xinjiang	ND	17.50
D08165	China/Shanxi	ND	20.00
206-5-6	China/Xinjiang	ND	21.25
XJ63	China/Xinjiang	ND	22.50
XJ02	China/Xinjiang	ND	23.75
22-2-9	China/Xinjiang	ND	23.75
209-4	China/Xinjiang	ND	27.50
2-4	China/Xinjiang	ND	28.75
XJ45	China/Xinjiang	ND	28.75
XJ65	China/Xinjiang	ND	32.50
XJ36	China/Xinjiang	D	23.75
Vd991	China/Jiangsu	D	66.25
XJ28	China/Xinjiang	D	68.75
XJ592	China/Xinjiang	D	72.50
HB19	China/Jiangxi	D	72.50
XJ52	China/Xinjiang	D	73.75
D08142	China/Shanxi	D	75.00
HB42	China/Hunan	D	78.75
VD8	China/Jiangsu	D	78.75
HB40	China/ Hunan	D	80.00
3-4-6	China/ Xinjiang	D	82.50
3-4-3	China/ Xinjiang	D	86.25
HB39	China/ Hunan	D	86.25
206-5-7	China/Xinjiang	D	87.50
HB10	China/Shanxi	D	88.75
HB17	China/Jiangxi	D	92.50
D08107	China/Henan	D	95.00

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15 g/L) plates for five days at 25 °C in the dark. Then, these strains were transferred to a completed medium (CM) liquid for four days. The susceptible cultivar cotton *Gossypium hirsutum* cv. Junmian 1 was cultured in a 25 °C greenhouse with a 16 h light and 8 h darkness photoperiod. Throughout the growing period, these cotton plants were cultivated in a fixed position in the greenhouse, while different batches of cotton seedlings might be grown in different parts of the same greenhouse.

Infection assays. The pathogenicity of the 28 strains of *V. dahliae* to the cotton seedlings (susceptible cultivar *G. hirsutum* cv. Junmian 1) was determined by the root-dipping inoculation method (Gui et al. 2017). The 1×10^7 conidia/mL suspension of each strain was obtained by filtration, harvested by centrifugation, washed, and diluted after shaking in the CM liquid for five days. Three-week-old cotton seedlings (reaching about two euphylla) were used for the subsequent inoculation experiments. The cotton seedlings were removed from the soil matrix, the soil was washed off by water, and these seedlings were dipped in the conidial suspension of different strains for 30 min. At least 20 cotton seedlings were inoculated for each strain, and five seedlings were replanted into a single cup. This assay was repeated three times. The symptoms of Verticillium wilt were photographed three weeks later. Then cotton stems were cut longitudinally to observe the vascular bundle browning. The vascular bundle browning in the stems of at least ten cotton plants was observed.

The disease index (DI) statistics. The disease severity of each seedling was recorded at three weeks post-inoculation (wpi) on the following five grades: 0 (no symptoms), 1 (0–25% wilted leaves), 2 (25–50%), 3 (50–75%), and 4 (75–100%). An overall DI was calculated as follows:

$$\mathrm{DI} = \frac{\sum_i^N \times i}{M \sum_i^N} \times 100 \quad (1)$$

where: N_i – number of plants with a disease score of i; M (=4) – the maximum scale level value.

The detailed criteria and calculation methods are described in previous studies (Powell et al. 1971; Liu et al. 2013, 2014) and are also presented in the introduction.

Quantification PCR (qPCR) for detection of the fungal biomass. For the analysis of the fungal biomass by qPCR, the root-stem junction tissue of the cotton seedlings was collected, the genomic DNA (gDNA) was extracted according to the DNAsecure plant kit (Tiangen, Beijing, China) for the detection of the relative fungal biomass. The amplification reaction mix was carried out using a 2 × Taq Pro Universal SYBR qPCR Master Mix (Vazyme). SYBR green-based qPCR was used to detect the relative biomass of *V. dahliae* with an initial 95 °C denaturation step for 3 min, followed by denaturation for 15 s at 95 °C, annealing for 30 s at 60 °C, and extension for 30 s at 72 °C for 40 cycles. The quantification of the *V. dahliae* DNA was carried out using the *V. dahliae* elongation factor 1α (*VdEF-1α*, F: 5' TGAGTTCGAGGCTGGTATCT 3'; R: 5' CACTTGGTGGTGTCCATCTT 3') with the cotton 18S rRNA (*18S*, F: 5' CGGCTACCACATCCAAGGAA 3'; R: 5' TGTCACTACCTCCCCGTGTCA 3') used in normalisation using the $2^{-\Delta\Delta Ct}$ method (Livak & Schmittgen 2001).

Statistical analysis. The standard errors were calculated for each treatment with three replicates. The unpaired Student's *t* test was performed to determine the statistical significance at $P < 0.01$. A one-way analysis of variance (ANOVA) and the least significant difference (LSD) were used to analyse the statistical significance of multiple groups using SPSS (version 23).

RESULTS

Genetic basis identification of the analysed *Verticillium dahliae* strains. Prior to conducting the pathogenicity evaluation, the genetic background of all the selected *V. dahliae* isolates were first analysed, except for confirming the defoliating (D) and non-defoliating (ND) characteristic of 28 strains using the N/ND specific primers. The characteristics identification of the races (responding to host resistance differentials) and mating type idiomorphs were also conducted using PCR for all the selected isolates. In this experiment, three strains (Vd991 strain with D, Race 2 and MAT1-2 marker; JR2 strain with ND, Race 1 and MAT1-2 marker; P48 strain with ND, Race 2 and MAT1-1 marker) were used as the controls. The results indicated that 11 strains (including 209-2, XJ14, D08165, 206-5-6, XJ63, XJ02, 22-2-9, 209-4, 2-4, XJ45, XJ65) were identified as a defoliating type, and 17 strains (including XJ36, Vd991, XJ28, XJ592, HB19, XJ52, D08142, HB42, VD8, HB40, 3-4-6, 3-4-3, HB39, 206-5-7, HB10,

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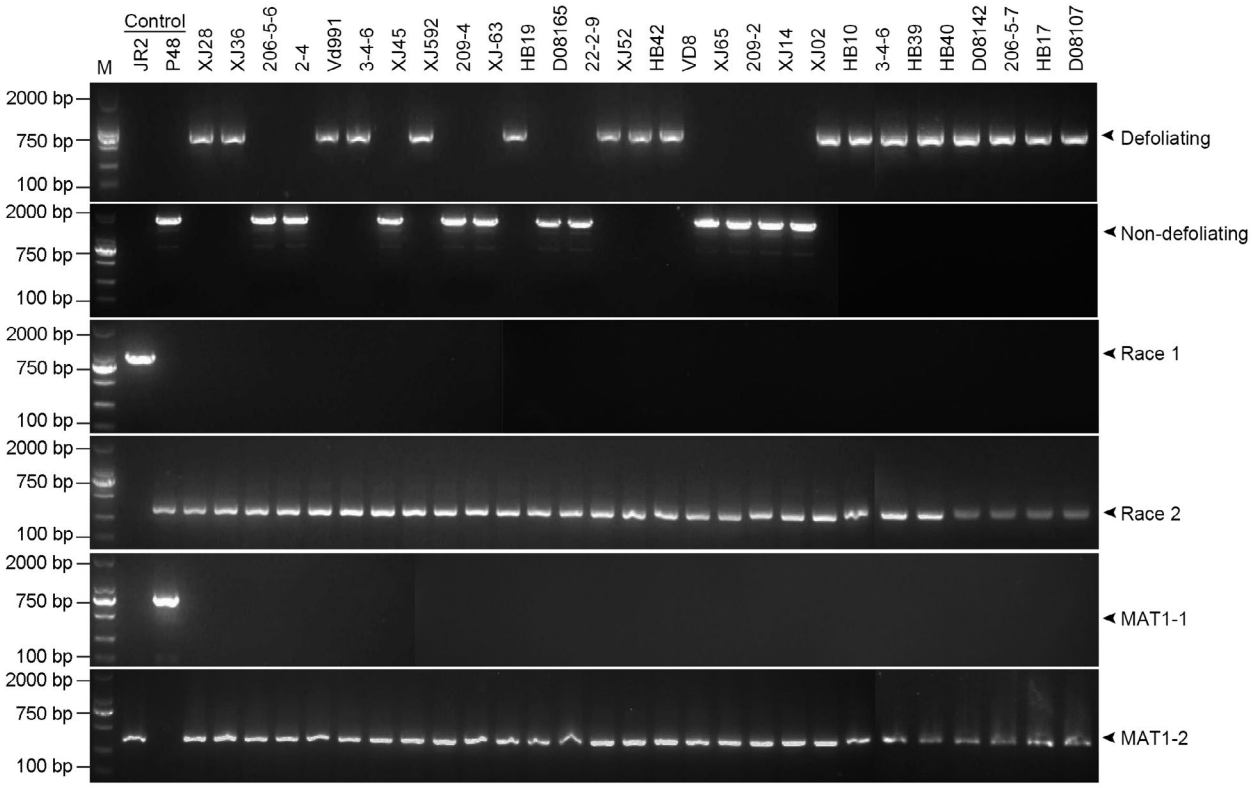

Figure 1. Genetic basis identification of the analysed *Verticillium dahliae* strains

The first line wild-type strains (Vd991 strain with D, Race 2 and MAT1-2 marker; JR2 strain with ND, Race 1 and MAT1-2 marker; P48 strain with ND, Race 2 and MAT1-1 marker) were used as the control; *M* = 2 000 bp DNA ladder used as a size marker

HB17, D08107) were identified as a non-defoliating type using the ND and D markers (Figure 1). All of the strains belong to MAT1-2 according to the mating type idiomorphs markers MAT1-1 and MAT1-2 (Figure 1). In the race identification, all the strains were amplified using the primers of the markers of race 1 and 2, and all the detected strains belong to race 2 (Figure 1), which was consistent with the previous finding that almost all *V. dahliae* strains were identified as race 2 in China. MAT1-1 *V. dahliae* isolates were only recently found in potatoes and sunflowers in the province of Inner Mongolia, most isolates in all other places own the MAT1-2 maker, and the number of *V. dahliae* strains with the D marker is increasing year by year in China due to their high pathogenicity.

The pathogenicity assessment of *Verticillium dahliae* strains using the disease index (DI) method. The severity of Verticillium wilt was firstly observed and calculated using the disease index (DI) method on cotton plants (Powell et al. 1971). In this study, the pathogenicity of 11 defoliating strains and 17 non-defoliating strains were identified by the method of DI statistics. The DI value of all the strains in at least three independent experiments is shown in Figure 2A. The results show that the DI value of the non-defoliating strains was below 35.00, of which the DI values of strain 209-2 and XJ65 were the highest (32.50) and lowest (8.75), respectively. In the defoliating strains group, except for XJ36 (23.75), the DI values of the other 16 strains were above 65.00, of which D08107 was up to 95.00. On the whole, the DI values of the defoliating group was significantly higher than that of non-defoliating group (Figure A), which suggested the pathogenicity of the defoliating strains was higher than the non-defoliating strains. From the pathogenetic phenotype, the cotton seedling leaves treated by defoliating strains showed yellow wilting or the leaves even fell off, such as with Vd991 and D08107, while only a few cotton seedling leaves treated with the non-defoliating strains showed slight yellowing and wilt (such as 209-2, XJ02, and XJ65) (Figure 2B).

Moreover, the phenotype of the euphylla yellowing and wilting was positively correlated with the DI val-

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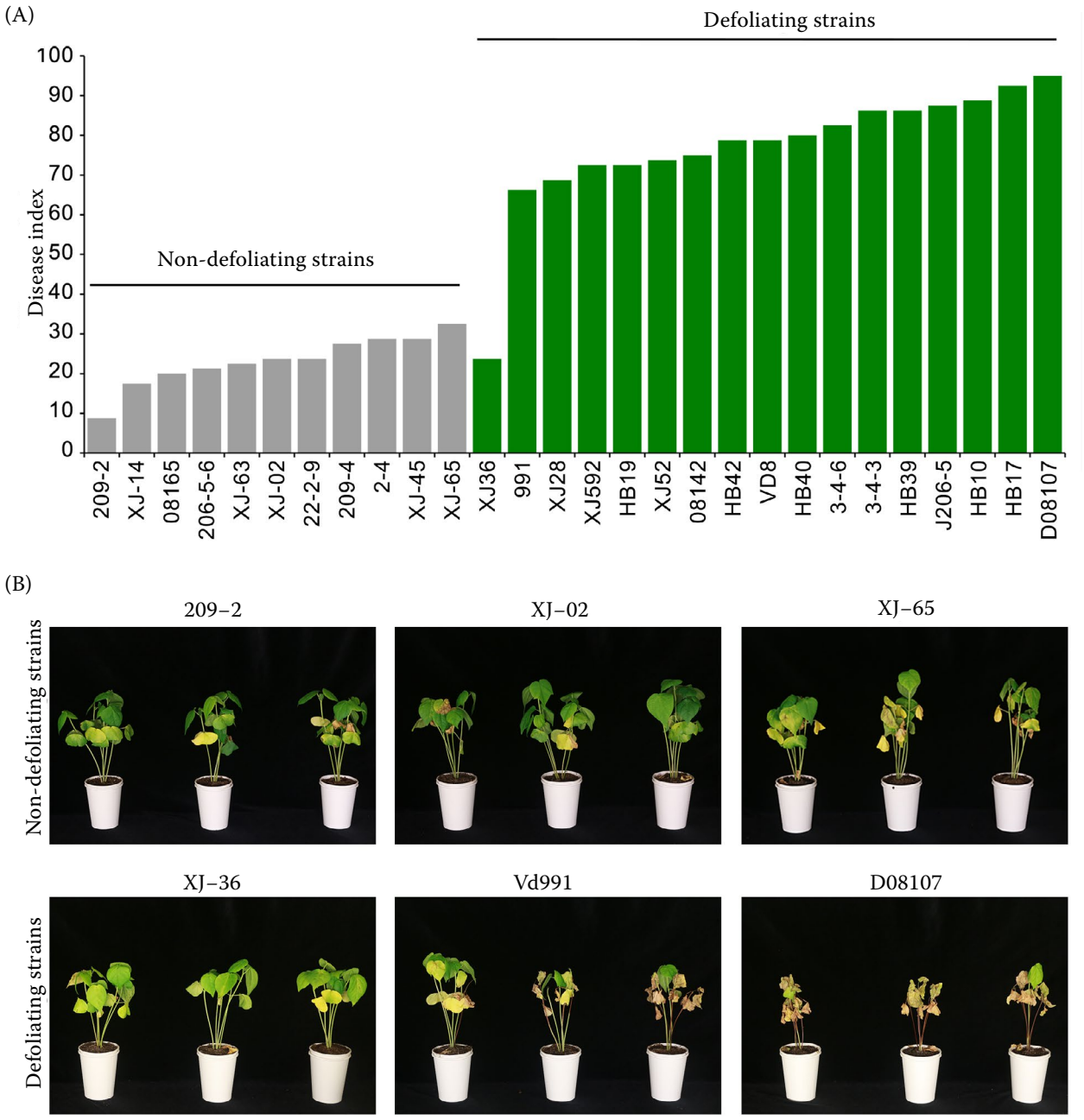

Figure 2. The pathogenicity assessment of the *Verticillium dahliae* strains using the disease index (DI) method
Disease index analysis of cotton plant infected with the 28 species of isolates. The cotton seedlings were photographed and subjected to disease index analyses at three weeks post inoculation (3 wpi). The disease indexes were evaluated with three replicates generated from 20 plants for each strain. (A) Disease index analysis of cotton seedlings infected with the 28 V. dahliae strains. (B) Disease symptoms of cotton seedlings inoculated with three defoliating and three non-defoliating strains were showed which were collected at 3 wpi

ue. For example, in the non-defoliating group, the strain XJ-65 with the highest DI value only caused one-third of the euphylla yellowing and wilting, correspondingly its DI value was only 32.50; strain XJ-02 could cause three or four euphylla yellowing and wilting, and its DI value was 23.75; strain 209-2 showed the weakest pathogenicity and could only cause the cotton seedling cotyledon and the wilting of a few euphylla 14 days after inoculation, and its DI value was only 8.75 (Figure 2). For the defoliating strains, excepting for XJ36, the DI values of other strains mainly focus on the range of 65.00 ~ 95.00,

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and the cotton seedlings showed the typical leaf yellowing and wilting phenotype with almost all the plants being nearly dead three weeks after inoculation. Among them, the strain D08107 had the highest virulence on cotton, and all the inoculated cotton plants showed the typical leaf wilting phenotype and almost all the plants were nearly dead, its DI value was up to 95.00; strain Vd991, as in our previous research, could rapidly cause the most euphylla leaves to wilt 14 days after inoculation, and its DI value was 66.25; but here is the interesting thing: although strain XJ-36 was identified as a defoliating strain, its pathogenicity was very weak on cotton seedlings, which could only cause partial cotyledon yellowing 14 days after inoculation, and its DI value was only 23.7 (Figure 2). Taken together, according to the above pathogenic phenotypes and DI values of the defoliating and non-defoliating strains, we can roughly judge that the pathogenicity of the strains almost corresponded to the DI values.

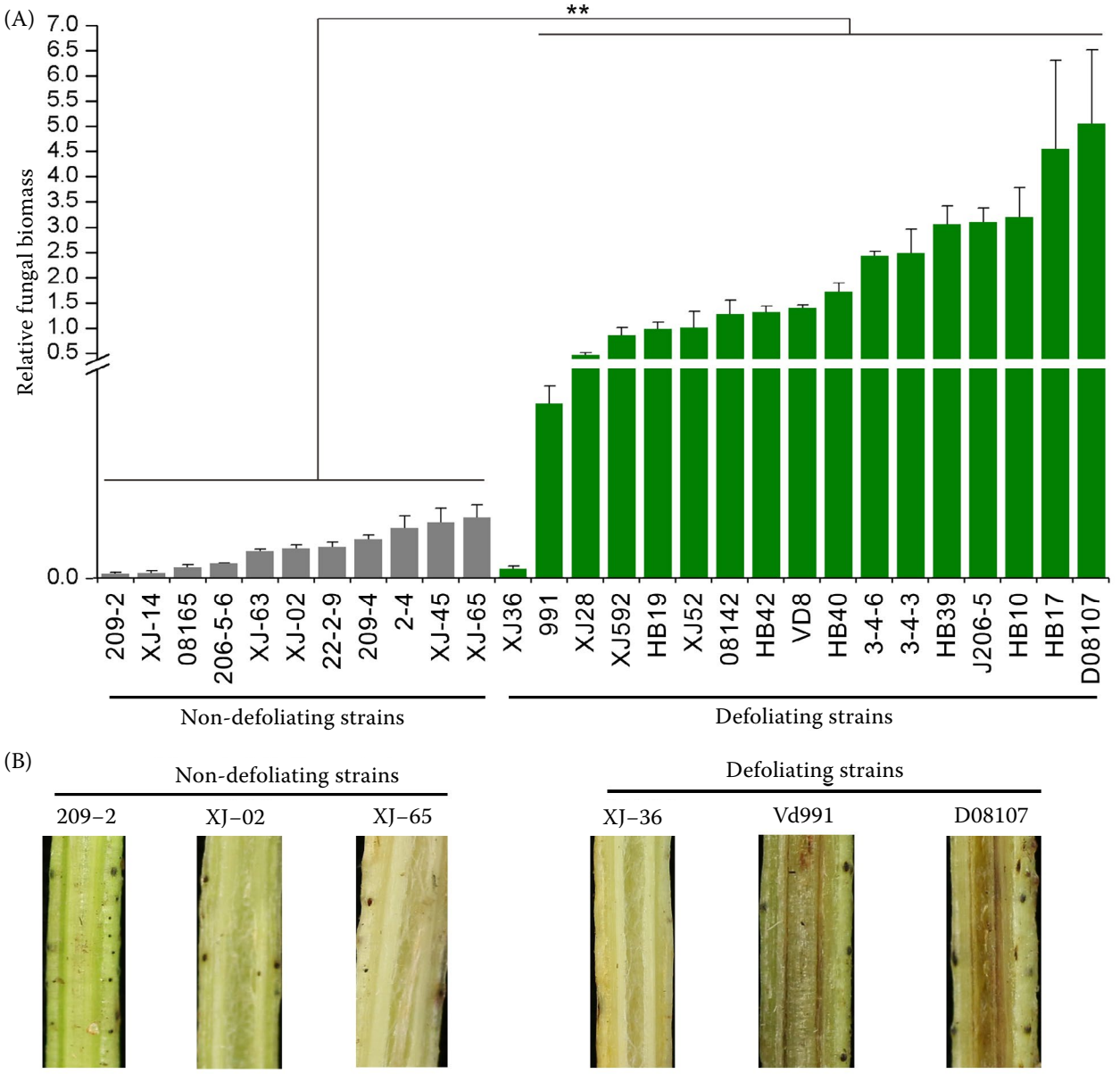

Figure 3. The pathogenicity assessment of the *Verticillium dahliae* strains using the quantitative PCR (qPCR) method for the fungal biomass

(A) Fungal biomass of the *Verticillium dahliae* strains on the cotton seedlings was determined by qPCR ** represents significant differences at $P < 0.01$ according to the unpaired Student's *t* test; (B) the stem longitudinal sections of the cotton plants that are shown on the bottom were collected 3 weeks post-inoculation

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Disease index analysis of the cotton plant infected with the 28 species of isolates. The cotton seedlings were photographed and subjected to disease index analyses at three weeks post-inoculation (3 wpi). The disease indices were evaluated with three replicates generated from 20 plants for each strain. (A) Disease index analysis of the cotton seedlings infected with the 28 *V. dahliae* strains. (B) Disease symptoms of the cotton seedlings inoculated with three defoliating and three non-defoliating strains were showed which were collected at 3 wpi.

The pathogenicity assessment of the *V. dahliae* strains using the quantitative PCR method for fungal biomass. To determine the colonisation ability of the pathogenic fungi in plants, a quantitative PCR (qPCR) analysis was used to detect the relative biomass of 28 strains in the cotton seedlings 14 days after inoculation. The tissue at the junction of the roots and stems was harvested 14 days after inoculation. The samples were ground to a powder and the genomic DNA was isolated. The relative biomass was determined using qPCR based on the genomic DNA, and the fungal biomass of strain XJ592 was set as 1.0. Vd991 was reported as a defoliating strain with strong virulence in a previous study (Zhang et al. 2019). For most defoliating strains (expect for XJ36), their fungal biomass was obviously higher than Vd991 (Figure 3A). For example, the relative biomass of strain VD8 and HB39 was 5.07 and 4.56-fold than Vd991, respectively. It is similar to the previous DI value results, the biomass of strain D08107 was also the higher than the other defoliating strains, and strain XJ36 was obviously lower than Vd991 even though it was identified as a defoliating strain (Figure 3A). On the contrary, the fungal biomass of all the non-defoliating strains was significantly lower than Vd991, of which, the biomass in the cotton roots and stem for strains XJ14 and XJ63 was extremely lower, and only about one percent of Vd991. The other non-defoliating strains were basically the same and only 10 ~ 50% of Vd991, such as strains XJ65 and 209-2 (Figure 3A). In addition, the longitudinal sections of the cotton stems were observed, and these results showed that all the non-defoliating strains could barely cause any vascular discoloration, but most defoliating strains could cause the cotton stem to turn brown and suffer necrosis (Figure 3B). Together, except for strain XJ36, the fungal biomass in most defoliating strains was obviously higher than the non-defoliating strains, which indicated the colonisation and reproduction ability of the non-defoliating strains in the cotton stem and root tissue was weaker than most of the defoliating strains.

Correlation analysis of the DI value and relative fungal biomass. In order to further clarify the relationship of the DI value and fungal biomass, a correlation analysis based on the above two sets of data from all the *V. dahliae* strains was conducted. The result showed that the correlation coefficient between the two sets of data of the 28 strains was 0.728, indicating that the DI value and fungal biomass value were highly correlated (Figure 4A). The correlation between the DI and the biomass of the defoliating and non-defoliating strains was also analysed, respectively. Moreover, the results indicated that the correlation coefficient between the DI value and the fungal biomass value of the non-defoliating strains and defoliating strains was 0.5384 and 0.4547, respectively (Figure 4B), which indicated that the two sets of data are moderately correlated in both the defoliating group and non-defoliating group.

DISCUSSION

The disease index (DI) method has been widely used to evaluate the disease symptoms caused by many soil-borne pathogens (Powell et al. 1971). In this research, the pathogenic phenotypes of the *V. dahliae* strains on cotton seedlings was nearly consistent with the DI value (Figure 2), which indicated the pathogenicity assessment of the *V. dahliae* strains using the DI statistics method was able to give an intuitive reflection of the pathogenic ability for the defoliating and non-defoliating strains. Although some strains had a slightly higher error value using this method in three dependent experiments, most strains showed a consistent and stable trend. In addition, regarding the DI method, the disease grade division depends on individual observation differences to a great extent which could cause controversy when conducting the same experiment.

For the cotton Verticillium wilt evaluation, the DI method has been used for many years, however, the fungal biomass detection method has only been used recently (Santhanam et al. 2013; Gui et al. 2017; Chen et al. 2018). For the relative

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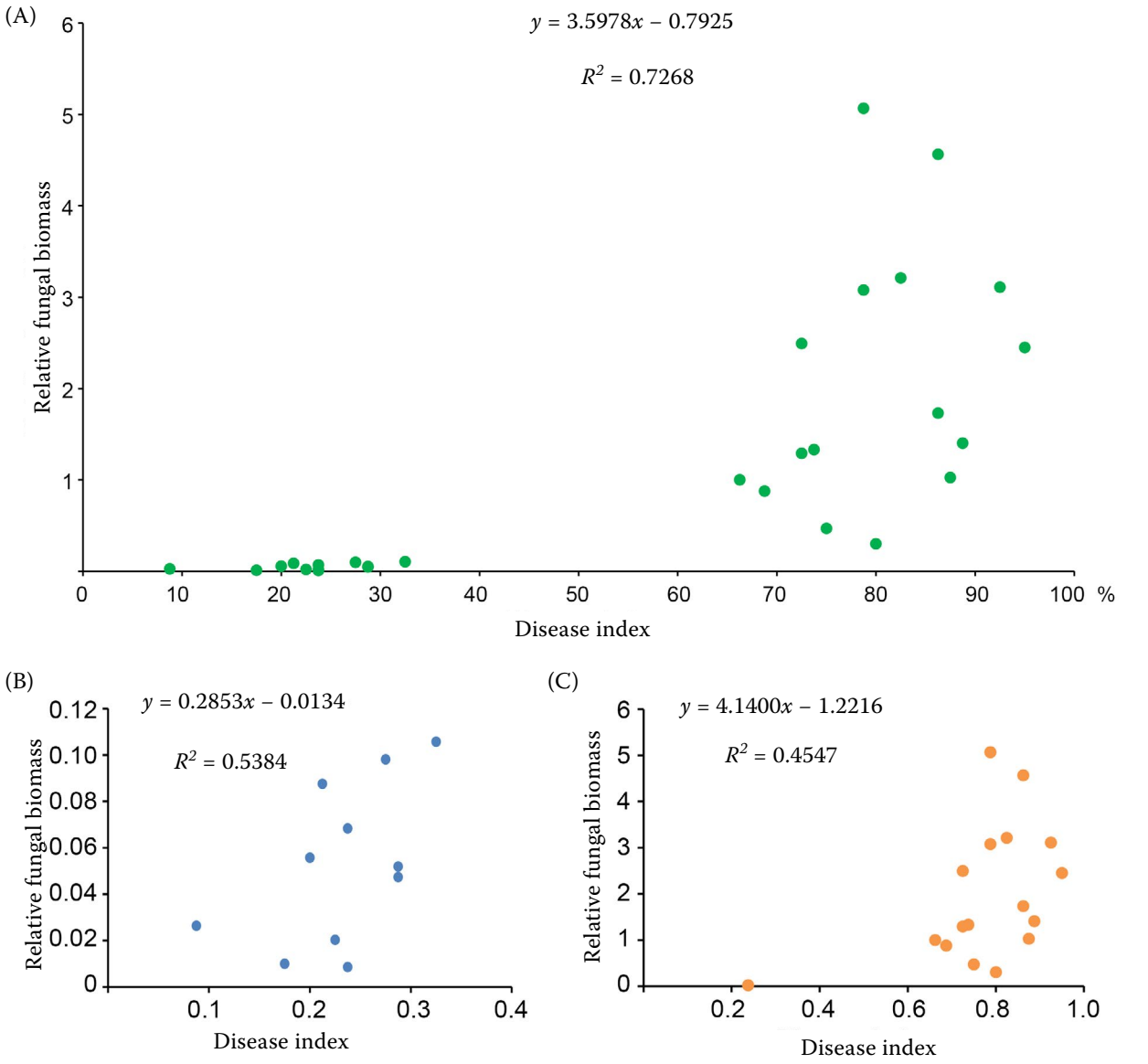

Figure 4. Correlation analysis of the results from the disease index (DI) and fungal biomass method
(A) the correlation coefficient between the disease index value and fungal biomass value; (B) the correlation coefficient between the disease index value and fungal biomass value of the non-defoliating strains; (C) the correlation coefficient between the disease index value and fungal biomass value of the defoliating strains

fungal biomass detection using the qPCR method, we found this method could correctly reflect the amount of *V. dahliae* in the cotton root and stem tissue, and the error value was relatively smaller than the DI method, indicating the high repeatability and stability with this method. However, there was another defect with this method, where the value of *V. dahliae* biomass of some strains did not correspond with the disease phenotype and the stem vascular discoloration degree, indicating that this method probably could not really respond the pathogenic ability of some strains on cotton.

In this research, the correlation coefficient between the DI values and the fungal biomass of 28 *V. dahliae* strains was 0.728, indicating that the DI value and fungal biomass value were highly correlated (Figure 4). However, when the correlation between the defoliating group and non-defoliating group was calculated separately, the correlation coefficient was found to be lower than the population value. The reason may be that although the two methods alone can reflect the strength of the pathogenicity to a certain extent, the correlation coefficient may be low due to the difference in the individual strains in the

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association analysis. Thus, there may be a respective error in the two methods, and neither of them could be perfectly accurate in reflecting the pathogenic ability of the different strains on cotton plants. Therefore, for one experiment, we suggest that one should only use one method to calculate the pathogenicity and not to simultaneously use two methods. In addition, regarding which method to choose, one should make the selection according to the actual experimental situation.

In conclusion, the pathogenicity of the defoliating and non-defoliating *V. dahliae* strains was measured by different methods including DI statistics and relative fungal biomass. The correlation coefficient between the DI and fungal biomass presented high consistency, which could provide some meaningful exploration for the more accurate pathogenicity identification of *V. dahliae.*

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