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Research on TOBRFV Infection in Summer Tomato Seed Production in the Wafangdian Area

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Abstract. This study aimed to assess the infection status of Tomato brown rugose fruit virus (TOBRFV) in tomato seed breeding in Wafangdian area during early and late summer. By comparing infection rates and cycle threshold (Ct) values from early and late summer seed samples, we found a significant increase in infection rates and lower Ct values at the end of the growing season. The study revealed that seeds harvested at the end of summer are at a higher risk of carrying TOBRFV, which could impact seed quality and facilitate virus spread in subsequent plantings. The findings underscore the importance of monitoring and implementing control measures targeted at the end of the growing season to mitigate the risk of TOBRFV infection in tomato seeds. The results also highlight the need for further research into the factors contributing to the increased virus concentration and infection rates over the growing season.

Keywords: Tomato brown rugose fruit virus (TOBRFV); Seed infection; Wafangdian area; Summer seed breeding.

1. Introduction

Since its initial reporting by Brown and Jones in 2014 [1], Tomato brown rugose fruit virus (TOBRFV) has rapidly emerged as a significant threat to the global tomato industry. This virus has not only severely impacted the yield and quality of tomatoes but also spreads rapidly and is difficult to control, causing substantial economic losses to tomato growers. The transmission routes of TOBRFV are diverse, with seed-borne virus being the primary means of long-distance spread [2, 3]. Research indicates that the virus in seeds can be transmitted between different regions through the movement of seedlings, and this "relay-style" spread greatly increases the risk of epidemic expansion. The study by Zhang and Liu further emphasizes the impact of seed transmission on tomato production [2], noting that seed-borne virus not only affects the quality and safety of seeds but may also lead to the rapid establishment and spread of the virus in new planting areas.

Wafangdian City, located on the Liaodong Peninsula in China, has become an important core area for summer tomato seed multiplication in northern China due to its cool and dry climate conditions during the summer. Industry reports highlight the Liaodong Peninsula, including Wafangdian, as a crucial summer seed production base for solanaceous vegetables in China [4]. The annual seed production in this region accounts for a significant portion of the national supply, substantially impacting the tomato seed industry.

Considering the potential threat of TOBRFV to the tomato industry and the importance of Wafangdian in tomato seed breeding, this study aims to evaluate the infection status of TOBRFV in summer tomato seed breeding in the Wafangdian area. By analyzing the infection rates and viral spread dynamics across different samples collected at the beginning and end of the summer, this study seeks to provide a scientific foundation for developing effective prevention and control strategies to mitigate the impact of TOBRFV on the tomato seed industry.

2. Materials and Methods

2.1. Sample Collection

This study aims to assess the infection status of Tomato brown rugose fruit virus (TOBRFV) in tomato seed breeding in Wafangdian area during the early and late summer. Seed samples were collected from tomato seed breeding bases in the early and late summer, with 8 sets of seed samples collected at each time point, each set containing 10 replicates to ensure representativeness and reliability of the data.

2.2. Seed Treatment

The collected seeds were first dried at room temperature for 48 hours to reduce moisture impact on subsequent experiments. The dried seeds were stored in sealed sterile bags until RNA extraction.

2.3. RNA Extraction

Total RNA was extracted from tomato seeds using a commercial seed virus RNA extraction kit (e.g., DNase/RNase-Free Plant and Fungal DNA/RNA Purification Kit, Norgen Biotek Corp., Canada). The extraction process strictly followed the manufacturer's guidelines to ensure the quality and integrity of RNA. The extracted RNA was measured for concentration and purity using a spectrophotometer, and all RNA samples were stored at -80°C until further analysis.

2.4. Reverse Transcription PCR (RT-PCR)

RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, USA) was used to reverse transcribe the extracted RNA into cDNA. TOBRFV-specific primers were used to amplify the conserved region of TOBRFV.

2.5. Real-Time Quantitative PCR (RT-qPCR)

RT-qPCR analysis was performed using the 7500 Real-Time PCR System (Applied Biosystems, USA). Each reaction system contained 10μL PowerUp SYBR Green Master Mix (Applied Biosystems, USA), 0.5μM of forward and reverse primers, and 2μL cDNA template. The reaction conditions were: 95°C pre-denaturation for 10 minutes, followed by 40 cycles of 95°C denaturation for 15 seconds, and 60°C annealing and extension for 1 minute. Each sample was technically replicated three times.

2.6. Data Analysis

Data from RT-qPCR were analyzed using 7500 System Software v2.3 (Applied Biosystems, USA). The infection status of TOBRFV in the samples was assessed by comparing Ct values (cycle threshold). Lower Ct values indicate higher concentrations of TOBRFV in the samples. Ct value data from all samples were used to calculate infection rates and analyze infection differences among different samples.

The infection rate = (Number of positive samples / Total number of samples) × 100%.

3. Results and Data Analysis

3.1. TOBRFV Infection Status

Based on the data from Table 1 and Table 2, we analyzed the infection status of TOBRFV in tomato seed samples collected in Wafangdian area during the early and late summer. The following are the specific infection rates:

Table 1. Early Summer Seed Sample Infection Rates

Sample ID	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Infected Counts	Infection Rate
A	39.13	ND	ND	ND	37.55	38.76	ND	ND	2	25%
B	ND	ND	ND	ND	ND	ND	ND	ND	0	0%
C	ND	ND	ND	ND	ND	ND	ND	ND	0	0%
D	36.56	36.36	37.10	33.53	33.66	32.69	31.91	31.24	8	100%
E	31.82	38.68	37.04	39.50	36.07	35.27	ND	ND	6	75%
F	31.85	35.11	39.17	39.07	ND	ND	ND	ND	4	50%
G	20.33	ND	ND	ND	ND	ND	ND	ND	1	12.5%
H	ND	ND	ND	ND	ND	ND	ND	ND	0	0%

Table 2. Late Summer Seed Sample Infection Rates

Sample ID	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Infected Counts	Infection Rate
A	27.80	28.22	20.04	31.30	31.11	31.74	37.01	35.19	8	100%
B	37.77	38.01	36.21	35.41	36.18	37.66	ND	ND	6	75%
C	39.08	36.80	38.33	38.06	ND	ND	ND	ND	4	50%
D	34.63	34.59	33.91	ND	39.15	39.73	ND	ND	5	62.5%
E	39.22	37.71	32.15	37.73	36.85	ND	ND	ND	5	62.5%
F	39.16	34.18	22.79	26.41	26.48	25.12	26.42	ND	7	87.5%
G	22.11	ND	ND	ND	ND	ND	ND	ND	1	12.5%
H	ND	ND	ND	ND	ND	ND	ND	ND	0	0%

3.2. Infection Rate Analysis

To provide a comprehensive analysis of the infection rates and to understand the dynamics of TOBRFV infection throughout the summer, we compared the Tomato brown rugose fruit virus (TOBRFV) infection rates and Ct values between early and late summer seed samples from the Wafangdian area.

Early Summer Infection Rates and Ct Values:

Sample A had an infection rate of 25% with Ct values ranging from 37.55 to 39.13, indicating a relatively low virus concentration.

Sample B and Sample C had no positive detections, suggesting either no infection or below detection limit virus concentrations.

Sample D showed a high infection rate of 100% with Ct values ranging from 31.24 to 37.10, indicating higher virus concentrations compared to Sample A.

Sample E had an infection rate of 75% with Ct values ranging from 35.27 to 38.68, suggesting moderate virus concentrations.

Sample F exhibited an infection rate of 50% with Ct values ranging from 31.85 to 39.17, indicating variable virus concentrations.

Sample G had a low infection rate of 12.5% with a single Ct value of 20.33, indicating a high virus concentration in the positive replicate.

Sample H had no positive detections, indicating no detectable virus.

Late Summer Infection Rates and Ct Values:

Sample A showed a complete infection rate of 100% with Ct values ranging from 27.80 to 37.01, indicating higher virus concentrations compared to early summer.

Sample B had an infection rate of 75% with Ct values ranging from 35.41 to 38.01, suggesting a significant increase in virus concentration from early to late summer.

Sample C exhibited an infection rate of 50% with Ct values ranging from 36.80 to 39.08, indicating a moderate increase in virus concentration.

Sample D had an infection rate of 62.5% with Ct values ranging from 33.91 to 39.73, showing a slight increase in virus concentration.

Sample E also had an infection rate of 62.5% with Ct values ranging from 32.15 to 39.22, indicating a similar trend to Sample D.

Sample F demonstrated a high infection rate of 87.5% with Ct values ranging from 22.79 to 39.16, indicating a substantial increase in virus concentration, especially with the lower Ct values suggesting higher concentrations.

Sample G had a low infection rate of 12.5% with a single Ct value of 22.11, indicating a high virus concentration in the positive replicate.

Sample H had no positive detections, indicating no detectable virus.

The comparison between early and late summer infection rates and Ct values reveals significant differences. The late summer samples generally exhibited higher infection rates and, in many cases, lower Ct values, indicating higher virus concentrations. This trend suggests that the virus accumulates over the growing season, leading to increased infection rates and higher virus concentrations by the end of the summer.

The increase in infection rates and lower Ct values in late summer samples could be due to several factors, including prolonged exposure to the virus, increased virus replication within the plants, and higher environmental conditions conducive to virus spread. This pattern indicates that seeds harvested at the end of the summer are more likely to carry the virus, which could impact seed quality and the spread of the virus in subsequent plantings.

This detailed analysis underscores the importance of monitoring and control measures targeted at the end of the growing season to mitigate the risk of TOBRFV infection in tomato seeds. It also highlights the need for further research into the factors contributing to the increase in virus concentration and infection rates over the growing season.

4. Conclusion

The study results indicate that as the growing season progresses, both the infection rate and viral concentration of TOBRFV increase. This may be due to the long-term accumulation of the virus in the field, increased viral replication within plants, and environmental conditions that are more conducive to virus transmission. Consequently, seeds harvested at the end of summer are more likely to carry a higher concentration of TOBRFV, posing a potential risk to seed quality and subsequent viral spread during planting.

Given that the TOBRFV infection risk for seeds at the end of summer is significantly higher than at the beginning, this could negatively impact the quality and safety of tomato seeds. A high infection rate may lead to reduced germination rates, stunted growth, and decreased yield and quality. Moreover, the virus carried by seeds could further spread through seedling transportation, increasing the risk of epidemic expansion.

5. Discussion

The study findings underscore a seasonal trend in TOBRFV infection rates and viral concentrations in tomato seeds from the Wafangdian area, with higher rates observed at the end of the growing season. This increase likely results from cumulative viral exposure and favorable environmental conditions for virus proliferation [5]. Consequently, seeds harvested at the end of summer are at a greater risk of carrying higher TOBRFV concentrations, which could compromise seed quality and facilitate virus spread in subsequent plantings [6, 7].

The potential negative impact on seed quality and the subsequent spread of the virus highlight the need for robust control measures. Implementing stringent seed testing and disinfection protocols, along with developing TOBRFV-resistant tomato varieties, are critical steps in mitigating the spread of the virus [8]. Future research should focus on understanding how environmental factors influence TOBRFV accumulation in seeds and exploring innovative seed treatment technologies to enhance seed safety and reduce transmission risks.

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