Effects of *Torreya grandis* Root Rot on Seedling Growth and Screening of Its Chemical Control Agents

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Abstract [**Objectives**] This study was conducted to prevent the occurrence of root rot disease in *Torreya grandis* and improve the yield and quality of *T. grandis*. [**Methods**] One-year-old and two-year-old seedlings of *Torreya grandis* 'Xifei' and 'Cufei' were inoculated with the root rot pathogen *Fusarium fujikuroi*, and the changes in photosynthesis, chlorophyll content, malondialdehyde, and defense enzyme system (superoxide dismutase, peroxidase, and catalase) activity in leaves of *T. grandis* seedlings were investigated using water as a control. Meanwhile, the control effects of 80% carbendazim wettable powder, 64% metalaxyl · mancozeb wettable powder, 430 g/L tebuconazole suspension and 30% difenoconazole · cyproconazole EC on root rot in *T. grandis* were investigated. [**Results**] After inoculation with the pathogen *F. fujikuroi*, the net photosynthetic rates and transpiration rates in leaves of *T. grandis* 'Xifei' and 'Cufei' decreased, and the contents of chlorophyll decreased, while the contents of malondialdehyde increased, and the contents of superoxide dismutase and catalase increased with time. However, peroxidase showed a high activity in *T. grandis* 'Cufei' only, but a trend of "increasing-decreasing-increasing" in *T. grandis* 'Xifei'. Among the four chemical control agents, 64% metalaxyl · mancozeb wettable powder had the best control effect on root rot of *T. grandis* caused by the pathogenic fungus *F. fujikuroi*. [**Conclusions**] The net photosynthetic rate, transpiration rate, chlorophyll content, malondialdehyde content, superoxide dismutase activity and catalase activity could all be used as screening indicators for *T. grandis* varieties resistant to root rot. Meanwhile, 64% metalaxyl · mancozeb wettable powder could be used as a control agent for root rot on *T. grandis*.

Key words *Torreya grandis*; Root rot disease; Physiological and biochemical metabolism; Chemical control

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Torreya grandis, a species of *Torreya* in Taxaceae, is a rare economic dry fruit tree species unique to China, which is widely distributed in humid areas in southern China, including Anhui, Zhejiang, Fujian and Hunan[1-2]. *T. grandis* fruit is rich in unsaturated fatty acids such as oleic acid and linolenic acid, and the contents of fat and protein are high. After frying, it tastes crisp, making it a nutritious first-class dried fruit[3-4]. The aril of *T. grandis* contains many aromatic components such as alcohols, ketones, aldehydes and alkenes, and is thus a natural high-quality raw material for extracting high-grade aromatic oil and extract[5]. In addition, *T. grandis* can also reduce the content of cholesterol and blood lipid in serum, soften blood vessels, promote blood circulation and participate in the treatment of endocrine system diseases[1]. In recent years, with people's attention to the nutritional value and medicinal value of *T. grandis*, the area of artificially-cultivated *T. grandis* is increasing. Centralized planting will inevitably lead to the occurrence of *T. grandis* diseases and pests, which seriously affects the quality and yield of *T. grandis* fruit.

T. grandis root rot is a common disease in *T. grandis* diseases, which has the characteristics of great harm, high mortality, rapid spread and difficult early detection. Root rot is a soil-borne disease, which infects the roots of plants by microbes in the soil, causing the disease in plants, affecting the normal physiological metabolism of the roots, and finally leading to the death of plants. After *T. grandis* is infected with root rot disease, the symptoms are not obvious in the early stage, and in the later stage, the leaves turn completely green and yellow-brown, and the roots rot and show milky yellow, and could not absorb nutrients and moisture in the soil, leading to the death of *T. grandis*[6-7]. If we do not pay enough attention to root rot on *T. grandis*, it will easily lead to the death of *T. grandis* plants, which will have a huge impact on economy and ecology. Our previous study identified *Fusarium fujikuroi* as the pathogen causing root rot on *T. grandis* in Huangshan *T. grandis* plantations in Anhui Province, and analyzed its incidence regularity[8]. So far, the prevention and control of root rot are mainly crop rotation, chemical control and disease resistance breeding. Due to limited land resources in China, it is difficult to implement the rotation method in practical application, and it is difficult to fundamentally solve the disease. Chemical control has always been the main means to control soil-borne diseases, but so far no chemical pesticide can effectively control the occurrence of root rot on *T. grandis*. Screening of disease-resistant varieties is an effective method to control root rot. After the pathogen infects plant hosts, it can change the physiological and biochemical metabolism process of the hosts, and finally make the hosts show symptoms of disease. Therefore, comparing and analyzing the changes of physiological and biochemical metabolism of different *T. grandis* seedlings infected with root rot disease is of great significance to screening *T. grandis* varieties resistant to root rot.

At present, there are few *T. grandis* varieties resistant to root

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rot, and the chemical control scheme of the disease is not clear. Therefore, in this study, the changes of physiological and biochemical indexes such as superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), malondialdehyde (MDA) and soluble sugar in different *T. grandis* varieties after inoculation with *F. fujikuroi* were compared, laying a foundation for further screening *T. grandis* varieties resistant to root rot. Meanwhile, the control effects of carbendazim, metalaxyl · mancozeb, tebuconazole and difenoconazole · cyproconazole EC on *T. grandis* root rot were compared, aiming to provide a scientific and high-quality control reference scheme for effectively controlling the disease and solving practical problems in production.

Materials and Methods

Experimental materials

T. grandis seedlings used in this study were all taken from the *T. grandis* cultivation base in Shexian County, Anhui Province. In specific, 200 seedlings of *Torreya grandis* 'Xifei' and 'Cufei', which were healthy, disease-free and consistent in growth, were selected and planted in plastic pots with a diameter of 40 cm, and after growing for 4 weeks, experiments were carried out, respectively.

The pathogenic fungus used was *F. fujikuroi* isolated and preserved in our laboratory in the early stage.

Pathogen inoculation

The preserved *F. fujikuroi* strain was taken out of a refrigerator at −80 ℃, inoculated on PDA plates after activation, and cultured at 25 ℃ for 5 − 7 d. Next, conidia were collected, and diluted with sterile water into a 1×10^6 cfu/ml spore suspension. Next, 110 plants of *T. grandis* 'Xifei' and 110 plants of 'Cufei' were selected, and the prepared pathogen spore suspension was used to infect the roots of the plants by combining the root trauma method and root irrigation method, and the same amount of sterile water was irrigated as the control. Next, a 5 g of leaf sample was collected from the plants at the 0, 10th, 20th, 30th, 40th and 50th days after inoculation in each group, respectively. After quick freezing in liquid nitrogen, the leaves were stored in the refrigerator at −80 ℃.

Determination of physiological and biochemical indexes

Determination of photosynthesis and chlorophyll contents

The net photosynthetic rates and transpiration rates of diseased plants of *T. grandis* 'Xifei' and 'Cufei' were measured by a portable photosynthetic apparatus (LI6400), and healthy *T. grandis* seedlings were used as control.

Chlorophyll content was determined by the acetone extraction method[9]. The contents of chlorophyll were calculated according to chlorophyll B concentration (mg/L): $Cb = 22.9A_{645} - 4.68A_{663}$, and total chlorophyll concentration (mg/L): $C(a+b) = Ca + Cb = 8.02\ A663 + 20.21\ A645$. Chlorophyll concentrations in the extract were converted into chlorophyll contents per gram of fresh leaves [mg/g (FW)].

Determination of malondialdehyde content The content of malondialdehyde (MDA) was determined by the thiobarbituric acid (TBA) method[10]. In specific, a 0.1 g of leaf sample was added with 10 ml of 10% (*W/V*) trichloroacetic acid (TCA), and ground in a mortar into paste, which was centrifuged at 7 000 r/min for 15 min to obtain a supernatant as the sample extract. Next, 2 ml of sample extract (the same volume of distilled water as control) was added with 0.6% thiobarbituric acid (TBA, prepared with 10% TCA), and the obtained solution was mixed and heated in a boiling water bath for 15 min, and then cooled quickly. The absorbance values of the supernatant at 450, 532 and 600 nm were determined, respectively.

MDA concentration (μmol/L) = $6.45 \times (A_{532} - A_{600}) - 0.56 \times A_{450}$;

MDA content (μmol/g FW) = MDA concentration × VT × 2×10^{-3}/FW

Determination of defense enzyme activity

(1) Extraction of crude enzyme solution

T. grandis leaves were placed in a precooled mortar, and 0.05 mol/l phosphate buffer (pH7.2, containing 1% polyvinylpyrrolidone) was added according to the ratio of 1 ∶ 5 (*w/v*). The mixture was ground in an ice bath into a homogenate, which was centrifuged at 4 ℃ for 15 min at 10 000 r/min, and the obtained supernatant was the crude enzyme solution.

(2) Determination of superoxide dismutase (SOD) activity

The SOD activity was determined by the nitroblue tetrazolium photochemical reduction method[11]. The reaction system contained 3.9 ml of 0.05 mol/L PBS buffer (pH 7.8), 0.5 ml of 130 mmol/L methionine solution, 0.5 ml of 750 μmol/L nitroblue tetrazole solution (NBT), and 0.5 ml of 100 μmol/L EDTA-2Na solution, and finally, 0.5 ml of 20 μmol/L riboflavin solution and 0.1 ml of crude enzyme were added. After mixing, a group of control tubes were placed in the dark, and a group of control tubes were exposed to light without the addition of the enzyme solution, and other tubes were exposed to the sunlight of 4 000 lx for 20 min. The absorbance of each tube was measured at 560 nm, and the inhibition of 50% of NBT photochemical reduction was taken as one enzyme activity unit U/g.

(3) Determination of peroxidase (POD) activity

The POD activity was determined by the guaiacol method[12]. The reaction system contained 2.9 ml of 0.05 mol/L PBS buffer (pH 5.5), 1.0 ml of 2% H_2O_2, 1.0 ml of 0.05 mol/L guaiacol and 0.1 ml of enzyme solution. The system was heated in a water bath at 37 ℃ for 5 min, and PBS was used as a blank control instead of enzyme solution. Immediately after mixing, the absorbance change was measured at 470 nm within 3 min, and the change of 0.01 per minute was taken as one enzyme activity unit U/(g · min).

(4) Determination of catalase (CAT) activity

The CAT activity was determined by the ultraviolet absorption method[13]. The reaction system contained 1.5 ml of 0.2 mol/L phosphate buffer with pH 7.8, 0.1 ml of crude enzyme solution and 1 ml of distilled water. After being preheated to 25℃, the

system was added with 0.3 ml of 0.1 mol/L H_2O_2, and immediately measured for the absorbance at 240 nm, which was read once every 30 s for 2 min. The amount of enzyme with A_{240} reduced by 0.1 in 1 min was taken as one enzyme activity unit U/(g · min).

Determination of control effects of different chemicals *T. grandis* 'Xifei' and 'Cufei' inoculated with the spore suspension for 15 d were treated with 80% carbendazim wettable powder, 64% metalaxyl · mancozeb wettable powder, 430 g/L tebuconazole suspension and 30% difenoconazole · cyproconazole EC, respectively, according to 6 pots for each group, and 6 pots were treated with clean water as the control group. The experiment was done in three biological replicates. The symptoms of infection were regularly observed and the relative prevention effect was calculated:

Control effect of root rot = (Disease index of control − Disease index of treatment)/Disease index of control × 100%

Data analysis

SPSS20.0 and EXCEL 2021 were employed to sort out and analyze the data.

Results and Analysis

Changes in photosynthesis and chlorophyll content of *T. grandis* after infection with *F. fujikuroi*

After infecting different *T. grandis* varieties with the pathogenic fungus *F. fujikuroi*, the net photosynthetic rate and transpiration rate of leaves were significantly reduced compared with those without being inoculated with the pathogenic fungus ($P < 0.05$) (Table 1). After inoculation with the pathogenic fungus, the reduction rates in the net photosynthetic rate and transpiration rate of *T. grandis* 'Xifei' were greater than those of *T. grandis* 'Cufei'. The result indicated that compared with *T. grandis* 'Cufei', the inoculation of the pathogenic fungus *F. fujikuroi* had a greater impact on the photosynthesis of *T. grandis* 'Xifei'.

Table 1 Effects of inoculation with pathogen *F. fujikuroi* on photosynthesis of *T. grandis* leaves

Variety	Net photosynthetic rate//m^2/s			Transpiration rate//m^2/s		
	Before inoculation	After inoculation	Reduction rate//%	Before inoculation	After inoculation	Reduction rate//%
T. grandis 'Xifei'	7.86 ± 0.26 A	2.73 ± 0.27 B	65.2	1.09 ± 0.15 A	0.38 ± 0.09 B	65.1
T. grandis 'Cufei'	8.17 ± 0.28 A	3.17 ± 0.31 B	61.2	2.74 ± 0.20 A	0.41 ± 0.10 B	64.0

The changes in chlorophyll contents in the leaves of different *T. grandis* varieties infected with the pathogen *F. fujikuroi* were shown in Fig. 1. After inoculation with the pathogen *F. fujikuroi*, the chlorophyll contents in the leaves of both *T. grandis* 'Xifei' and 'Cufei' continued to decrease with the extension of inoculation time, while the chlorophyll contents in the leaves of *T. grandis* 'Xifei' and 'Cufei' that were not inoculated with the pathogen remained basically unchanged. Compared with *T. grandis* 'Cufei', after inoculation with the pathogen *F. fujikuroi*, the chlorophyll content in the leaves of *T. grandis* 'Xifei' decreased more significantly, and especially at 10 and 50 d after inoculation, the chlorophyll content in the leaves decreased the fastest. The result indicated that after inoculation with *F. fujikuroi*, the response of *T. grandis* 'Xifei' to the pathogen was more sensitive than that of *T. grandis* 'Cufei'.

Changes in malondialdehyde (MDA) content of *T. grandis* after infection with *F. fujikuroi*

The changes in MDA content in leaves of different varieties of *T. grandis* seedlings inoculated with *F. fujikuroi* are shown in Fig. 2. After inoculation with the pathogen *F. fujikuroi*, the content of malondialdehyde in leaves of *T. grandis* 'Xifei' seedlings significantly increased over time. At 50 d after inoculation with the pathogen, the malondialdehyde content in the leaves reached its highest level, reaching 17.6 μmol/g. Different from the reaction in *T. grandis* 'Xifei' after inoculation with the pathogen, the content of malondialdehyde in leaves of *T. grandis* 'Cufei' after inoculation with *F. fujikuroi* showed almost no significant change until the 20th day of inoculation. Starting from the 30th day of inoculation with the pathogen, the content of malondialdehyde significantly increased and reached its highest value (23.5 μmol/g) on the 50th day. It indicated that the membrane lipid system of *T. grandis* 'Xifei' was damaged to varying degrees after early infection with the pathogen *F. fujikuroi*. Although *T. grandis* 'Cufei' was not severely damaged in the early stage, with the occurrence of root rot disease, both the membrane lipid systems of *T. grandis* 'Cufei' and 'Xifei' were damaged.

Changes in defense enzyme activity of *T. grandis* after infection with *F. fujikuroi*

In order to study the changes of defense enzyme activity in different varieties of *T. grandis* seedlings after inoculation with *F. fujikuroi*, the activity levels of SOD, POD and CAT enzymes in leaves of *T. grandis* 'Xifei' and 'Cufei' seedlings inoculated with the pathogen were determined. The results showed that the activity of SOD increased after inoculation with the pathogen *F. fujikuroi* in both *T. grandis* 'Xifei' and 'Cufei'. The SOD activity in *T. grandis* 'Xifei' reached its highest value on the 30th day after inoculation, while in *T. grandis* 'Cufei', it reached its highest value on the 20th day. However, with the prolongation of time, the changes in SOD activity in *T. grandis* 'Xifei' and 'Cufei' were different. The SOD activity in *T. grandis* 'Xifei' significantly decreased, while the SOD activity in 'Cufei' remained at a high level (Fig. 3a). After inoculation with the pathogen *F. fujikuroi*, the activity of POD increased with time in both *T. grandis* 'Xifei' and 'Cufei', and reached its maximum value on the 50th day of inoculation (Fig. 3b). After inoculation with the pathogen *F. fujikuroi*, the CAT activity in *T. grandis* 'Xifei' showed a trend of "increasing (10 d) − decreasing (30 d) − increasing (50 d)", while that in *T. grandis* 'Cufei' reached its highest value on the 20th day, followed by a slight decrease, but remained at a high level of activity (Fig. 3c). The

above results indicated that after inoculation with the pathogenic fungus *F. fujikuroi*, the activity of defense enzymes in leaves of both *T. grandis* 'Xifei' and 'Cufei' seedlings changed, especially the changes in SOD and POD, which could be used as important indicators for screening resistant varieties.

Differences in the control effect on *F. fujikuroi* among different chemical agents

After 15 d of inoculation with the pathogenic fungus *F. fujikuroi* into the roots of *T. grandis* 'Cufei' and 'Xifei', the control effects of four different pesticides (80% carbendazim wettable powder, 64% metalaxyl · mancozeb wettable powder, 430 g/L tebuconazole suspension, and 30% difenoconazole · cyproconazole EC) on root rot were observed and recorded regularly. The results showed that the same chemical control agents had similar control effects on root rot disease in different varieties of *T. grandis*, and there were significant differences in the control effect among the four chemical control agents mentioned above (Table 2). Compared with the clear water control group, the incidence rates of root rot disease caused by *F. fujikuroi* (20% for *T. grandis* 'Xifei' and 10% for 'Cufei') and the disease indexes (10 for *T. grandis* 'Xifei' and 6 for 'Cufei') were the lowest after the treatment with 64% metalaxyl · mancozeb wettable powder (88.64% for *T. grandis* 'Xifei' and 92.30% for 'Cufei'). The control effects of 30% difenoconazole · cyproconazole EC and tebuconazole suspension on the incidence rates and disease indexes of root rot in *T. grandis* 'Xifei' and 'Cufei' took the second place, and the relative control effects were slightly lower. However, the incidence rates of root rot in *T. grandis* 'Xifei' and 'Cufei' treated with 80% carbendazim wettable powder were the highest (40% for both *T. grandis* 'Xifei' and 'Cufei'), and the disease indexes were also high (35 and 32, respectively), and the control effects were the lowest (60.23% for *T. grandis* 'Xifei' and 58.97% for 'Cufei'), lower than those of other chemicals. In addition, the dosage of 64% metalaxyl · mancozeb wettable powder was also the lowest compared with other three drugs. Therefore, the use of 64% metalaxyl · mancozeb wettable powder could effectively prevent and control the occurrence of root rot on *T. grandis*.

Table 2 Comparison on control effects of four chemical treatments

Variety	Treatment	Dosage // g/kg	Incidence // %	Disease index	Relative control effect // %
T. grandis 'Xifei'	80% carbendazim wettable powder	2	40	35	60.23 d
	64% metalaxyl · mancozeb wettable powder	0.5	20	10	88.64 a
	430 g/L tebuconazole suspension	4	30	24	72.73 c
	30% difenoconazole · cyproconazole EC	1	20	16	81.82 b
	Clear water control	–	100	88	–
T. grandis 'Cufei'	80% carbendazim wettable powder	2	40	32	58.97 d
	64% metalaxyl · mancozeb wettable powder	0.5	10	6	92.30 a
	430 g/L tebuconazole suspension	4	35	28	64.14 c
	30% difenoconazole · cyproconazole EC	1	20	17	78.21 b
	Clear water control	–	85	78	–

Conclusions and Discussion

T. grandis, as a woody oil plant species with Chinese characteristics, not only has high economic and medicinal value, but also can be used as an ornamental and ecological tree species. Developing the *T. grandis* industry is an important part of China's woody oil plant industry strategy, which is conducive to developing the mountainous economy, increasing farmers' income, enriching natural landscapes, and improving the ecological environment[14-16]. In recent years, with the rapid expansion of the planting area of *T. grandis*, coupled with unreasonable afforestation, seedling cultivation and transitional management, its disease and pest problems have become increasingly apparent, which not only affects the yield and quality of *T. grandis* fruit, but also causes the death of *T. grandis* trees in serious cases, which seriously hinders the sustainable development of the *T. grandis* industry[17-18]. At present, there are more than 60 reported diseases and pests of *T. grandis*, and root rot on *T. grandis* seriously affects the growth of *T. grandis* plants because it is difficult to find it early and eradicate it completely[17-20]. In 2022, we conducted a study on the incidence and pathogens of root rot disease in *T. grandis* plantations in Shexian County, Anhui Province, and found that *F. fujikuroi* was the pathogen causing root rot on *T. grandis*. The prevention and control of root rot mainly rely on methods such as resistance breeding and chemical control. Therefore, this study aimed to establish the relation between physiological and biochemical indexes and disease resistance in *T. grandis* through changes in physiological and biochemical indexes of *T. grandis* after it was infected with pathogen *F. fujikuroi*. Meanwhile, the control effects of four chemical control agents on root rot in *T. grandis* were also studied.

The results of this study showed that the net photosynthetic rate, transpiration rate, chlorophyll content, MDA content, SOD activity, POD activity and CAT activity of *T. grandis* 'Xifei' and 'Cufei' were all changed after inoculation with the pathogen *F. fujikuro*. Photosynthesis of plant leaves is the basis of their growth and development, which provides necessary energy and organic matter for plants and can reflect the growth state of plants. When the pathogen *F. fujikuro* infected *T. grandis*, the net photosynthetic rate and transpiration rate of *T. grandis* 'Xifei' and 'Cufei' decreased significantly, and the chlorophyll content in leaves also decreased significantly, but the decline degrees of *T. grandis* 'Cufei' was less than those of *T. grandis* 'Xifei'. When plants suffer from adversity stress, they will produce a large

number of superoxide radicals, which can destroy membrane lipids and produce malondialdehyde. Excessive accumulation of malondialdehyde in cells will lead to changes in the structure and function of cell membrane, so its content can directly reflect the peroxide level of plasma membrane[21]. When the pathogen *F. fujikuro* infected the roots of *T. grandis*, the content of malondialdehyde in leaves increased obviously, indicating that the membrane lipid was destroyed. Defensive enzymes (SOD, POD, CAT) in plants can scavenge free radicals in the matrix, thus protecting cells to maintain normal physiological functions[22]. After *T. grandis* was infected with *F. fujikuro*, SOD and POD increased to different degrees, while CAT only maintained a high level in *T. grandis* 'Cufei'. It indicated that net photosynthetic rate, transpiration rate, chlorophyll and malondialdehyde content, SOD and POD activity could be used as evaluation indicators for *T. grandis* varieties resistant to the pathogen *F. fujikuro*. Further research is needed on CAT as a screening indicator for resistant varieties.

In this study, four common chemicals, 80% carbendazim wettable powder, 64% metalaxyl · mancozeb wettable powder, 430 g/L tebuconazole suspension and 30% difenoconazole · cyproconazole EC, were selected to determine the disease index and relative control effects of them on root rot in *T. grandis* caused by *F. fujikuro*. It was found that 64% metalaxyl · mancozeb wettable powder had the best control effect. Wang *et al.*[23] showed that tebuconazole had a strong inhibitory effect on three main pathogenic Fusarium species of soybean root rot. Li *et al.*[24] selected five chemicals, namely chlorothalonil, mancozeb, triadimefon, thiophanate-methyl and metalaxyl, which inhibited the growth of pathogen colonies and spore germination to varying degrees. There was a difference between the above research and our results, which shows that as 64% metalaxyl · mancozeb wettable powder has a good control effect on root rot in *T. grandis* caused by *F. fujikuro*, factors such as pathogens and hosts also need to be considered in the selection of control agents. In addition, we also found that the dosage of 64% metalaxyl · mancozeb wettable powder was the smallest when controlling root rot on *T. grandis*. Chemical control has always been the main means to control soil-borne diseases, but with the extensive use of chemical pesticides, environmental pollution and other worrying problems have arisen. Therefore, in the process of selecting chemicals, it is also necessary to consider factors such as less pollution to the environment and less interference to human and livestock activities. Reasonable selection of chemical control agents can reduce the occurrence and spread of soil-borne diseases, and we should not abuse or misuse them, and develop towards low toxicity, less residue and environmental friendliness.

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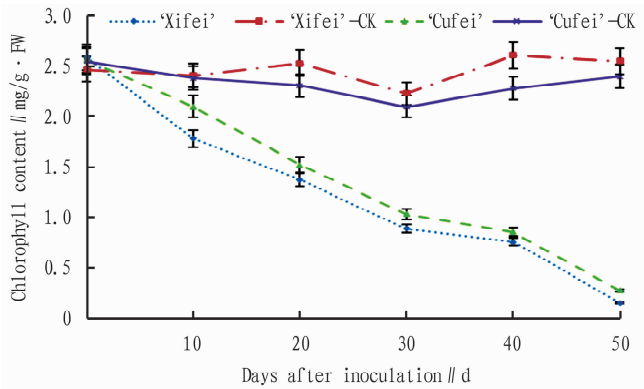

Fig. 1 Changes in chlorophyll content in leaves of different varieties of *T. grandis* seedlings after inoculation with the pathogenic fungus *F. fujikuroi*

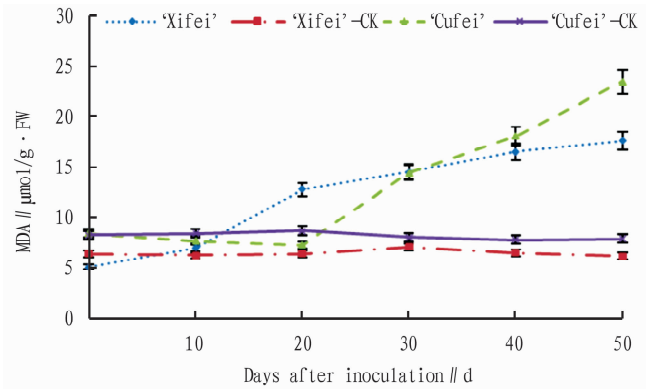

Fig. 2 Changes in MDA contents in leaves of different varieties of *T. grandis* seedlings after inoculation with the pathogenic fungus *F. fujikuroi*

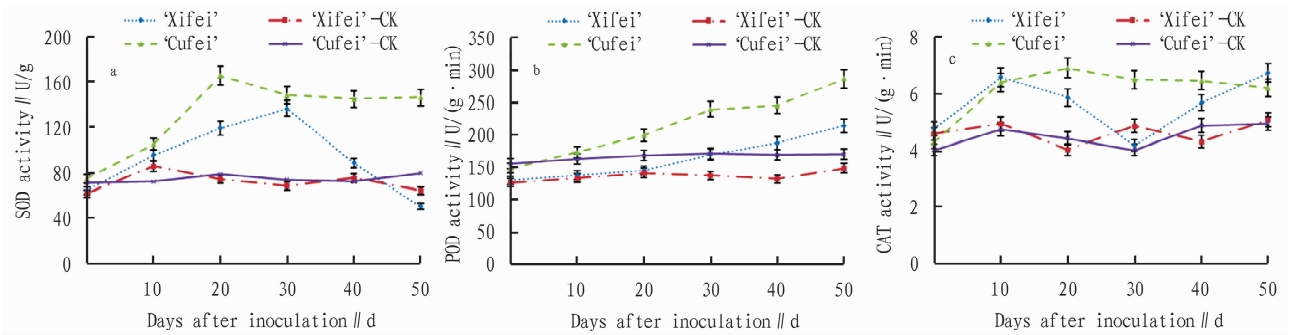

Fig. 3 Changes in defense enzyme activity in leaves of different varieties of *T. grandis* seedlings after inoculation with the pathogenic fungus *F. fujikuroi* (a: SOD; b: POD; c: CAT)

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(Continued from page 103)

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