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Effect of Stem Polyphenol Content on the Susceptibility to Foot Rot Disease in Sweetpotato [*Ipomoea batatas* (L.) Lam.]

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Abstract. Foot rot (*Diaporthe destruens*) of sweetpotato generally infects the stem of the plant and then spreads throughout the plant. In Japan, foot rot is prevalent in the main area of sweetpotato production, and there is a pressing need for the development of resistant cultivars. It is generally believed that polyphenols are involved in defense against stress. However, little is known about the relationship between polyphenols and the susceptibility to foot rot in sweetpotato. We thus analyzed the polyphenol of the disease-free healthy stems and investigated their relationship between susceptibility to the disease to establish basic techniques for selecting cultivars resistant to foot rot. Polyphenol was analyzed by the Folin–Ciocalteu method and high-performance liquid chromatography. Resistance tests were conducted using the direct inoculation method. We observed a correlation between the total polyphenol content and the degree of foot rot disease severity. Surprisingly, cultivars with a low stem polyphenol content ('Tamaakane') had lower susceptibility to foot rot, whereas the cultivars with high stem polyphenol content ('Kokei No. 14') had higher susceptibility to foot rot. In particular, high positive correlations were shown with several polyphenols, including 3,4-dicaffeoylquinic acid and chlorogenic acid. Furthermore, the results of in vitro experiments also suggested that foot rot may metabolize trace amounts of chlorogenic acid. This discovery has potential applications in the selection of resistant cultivars, contributing to the streamlining of disease resistance tests in breeding programs. It could ultimately shorten the breeding period and help overcome foot rot disease in sweetpotato.

Sweetpotato [*Ipomoea batatas* (L.) Lam.] is one of the most important food crops in the world and is treated as a vegetatively propagated crop (Behera et al. 2022). It has traditionally been propagated by taking cuttings of stems and vines from storage roots (Sakaigaichi et al. 2022). In Japan, there are edible and industrial cultivars of sweetpotato. Industrial cultivars are used as a raw material for starch and liquor. Sweetpotato with yellow flesh and purple skin are popular as vegetable crops (Tanaka et al. 2017).

Foot rot is caused by a filamentous fungus (*Diaporthe destruens*) and is transmitted primarily from the stem of the sweetpotato (Nomiyama et al. 2022). Infected plants cause rotting of aboveground parts and storage roots of most cultivars, such as the popular yellow-fleshed cultivar Beniharuka, ultimately resulting in a drastic reduction in the yield of storage roots. Once

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contaminated in vegetatively propagated crops, the infection spreads and is easily passed on to subsequent generations (Clark et al. 2012). Since the identification of foot rot in Japan's Okinawa Prefecture in 2011 and Kagoshima and Miyazaki Prefectures in 2018 (Kobayashi 2019; Maeda et al. 2022). The southern regions of Japan including these three prefectures has been caused significant damage by foot rot, and we need to create resistant cultivars.

It has been reported that the sweetpotato cultivars Konaishin used for starch and Tamaakane used for liquor are resistant to foot rot, but yellow-flesh edible cultivars that are resistant to foot rot are not yet widely available [Bio-oriented Technology Research Advancement Institution (BRAIN) 2021; Tabuchi et al. 2024]. Evaluations of disease resistance are particularly labor-intensive. Having a field contaminated with foot rot disease is a risk for sweetpotato production areas and small research institutions, and the hurdle to resistance testing is even higher. If resistance can be determined by measuring genes and components in sweetpotato without the use of bacteria, these advances would contribute to risk management and labor savings in testing.

We have focused on changes in the polyphenol content of sweetpotato at different growth stages (Nakagawa et al. 2021; Setoguchi et al. 2023, 2024). Sweetpotato contain high amounts of chlorogenic acid and dicaffeoylquinic acids, and the relationship between these and biotic or abiotic stress during growth has been discussed (Setoguchi et al. 2023, 2024). Caffeoylquinic acid is a type of naturally occurring phenolic compound formed by the esterification of transcinnamic acid with quinic acid, which is present in almost all higher plants (Clifford et al. 2017). Although these compounds are sometimes referred to as chlorogenic acids, in the present report, we refer to 5-caffeoylquinic acid as chlorogenic acid. We also use dicaffeoylquinic acids as a generic term for 3,4-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid, and 4,5-dicaffeoylquinic acid.

Polyphenols are generally believed to have antifungal properties. Antimicrobial activity has been observed in extracts from polyphenol-rich plants (El-Shahir et al. 2022; Sangta et al. 2021). It has also been reported that chlorogenic acid > caffeic acid >3,5 dicaffeoylquinic acid >4,5 dicaffeoylquinic acid each inhibit bacterial growth, in that order, whereas 3,4 dicaffeoylquinic acid does not inhibit bacterial growth (Harrison et al. 2008). The available information on polyphenols in sweetpotatoes and foot rot infection is insufficient.

In this study, we investigated the differences in susceptibility to foot rot fungi among cultivars collected from the southern Kyushu region, which is the main sweetpotato production area in Japan. We also analyzed the polyphenol content and composition of the disease-free healthy stems (the main infection site of foot rot). The effects of polyphenols on the in vitro growth of foot rot fungi were evaluated.

Materials and Methods

Plant materials. Five sweetpotato cultivars were used: Tamaakane, Konaishin, Benimasari, Kokei No. 14, and Beniharuka. These cultivars were selected based on known information on foot rot ecology and control measures (BRAIN 2023). Cultivars Tamaakane, Konaishin, and Benimasari are resistant to foot rot, whereas cultivars Kokei No.14 and Beniharuka are susceptible. There is no cultivar grown in Japan that is completely free from foot rot. Therefore, cultivars such as Tamaakane are considered relatively resistant to foot rot. These cultivars were grown with a normal cropping pattern and black mulch on two occasions in 2022 and 2023 in a field at Kushima city, Miyazaki Prefecture (31°33′30.6″N, 131°14′45.7″E). Stem cuttings of five cultivars were planted in late May, and stems were collected in early July. Stems were collected up to ∼40 cm from the base. These samples were frozen at −30 °C immediately after collection, lyophilized (FUD-1100, Tokyo Rikakikai, Tokyo, Japan), ground, and stored at −30 °C until polyphenol analysis.

Foot rot inoculation test. To investigate the resistance of the five sweetpotato cultivars to foot rot, we conducted an inoculation test using potted cuttings of each cultivar, following the method of Nomiyama et al. (2022). Stems collected from the field during Jul and Aug 2022 were planted in 10.5 cm black plastic pots, and seedlings that grew to ∼30 cm were used.

Inoculation was done by wounding the base of the stem (∼2 cm above the ground surface) with a toothpick. The trial began on 23 Aug 2022, and the seedlings were grown in an unheated glasshouse for 5 weeks. The temperature in the greenhouses varied between 10 and 40 °C. We used six strains named A through F, collected from stems or storage roots of diseased plants from Miyazaki and Kagoshima Prefectures, as foot rot pathogens, following a partial modification of the method described by Nomiyama et al. (2022) (Table 1). Five weeks after inoculation, the disease index was determined on a scale of 0 to 5 (Fig. 1). The disease severity was then calculated from the disease index, using the following formula:

$$\text{Disease severity} = \text{Mean of the disease index of strains A–F}/5 \times 100$$

Three biological replicates were performed for each treatment.

Determination of the total polyphenol content. The total polyphenol content of stems of the different cultivars was determined using the Folin–Ciocalteu method. Stems that were not inoculated with foot rot fungi were used for this experiment. First, 0.02 g of lyophilized powder was weighed for the two-season sample, 5 mL of 80% methanol was added, and the powder was extracted for 15 min in an ultrasonic generator. The extraction was then filtered through a 0.20-µm syringe filter. Phenol and saturated sodium carbonate reagents were added to the extract and allowed to stand at room temperature for 30 min, after which the absorbance was measured at 760 nm using a spectrophotometer. Standard solutions of 20, 50, 100, 150, and 200 $mg \cdot L^{-1}$ were prepared using gallic acid as a sample. The results are expressed as the equivalent of gallic acid per 100 g of fresh weight (FW). Three biological replicates were performed for each treatment.

Polyphenol composition analysis. To investigate the polyphenols in the stems further, we used high-performance liquid

Table 1. Detailed information on foot rot fungi (*Diaporthe destruens*) isolated from sweetpotato in the southern Kyushu region.

Strain	Sampling location	Cultivar	Organ
A	Kanoya-shi, Kagoshima	Koganesengan	Stem
B	Kanoya-shi, Kagoshima	Shiroyutaka	Stem
C	Kanoya-shi, Kagoshima	Koganesengan	Stem
D	Miyazaki-shi, Miyazaki	Unclear	Strage root
E	Miyakonojo-shi, Miyazaki	Unclear	Strage root
F	Miyazaki-shi, Miyazaki	Unclear	Stem

These pathogens were isolated and maintained by a partially modified method of Nomiyama et al. (2022).

Evaluation criteria					
0	1	2	3	4	5
No symptoms	Browning only at the site of inoculation	Progression of disease within 3 stem nodes	Progression of disease beyond 3 stem nodes	Disease symptoms spread throughout the plant body	Death
					

Fig. 1. Six evaluation criteria expressed after inoculation of foot rot (*Diaporthe destruens*) in potted cuttings of sweetpotato. Observations were made every week by examining disease symptoms appearing on stems and leaves, and a final decision was made after 5 weeks.

chromatography (HPLC) to determine the polyphenol composition. Polyphenols were extracted from the two-season sample in the same way as for the content measurement described previously. The extracts were analyzed by reversed-phase HPLC using a Prominence LC solution system and an ODS-3 column (particle size 5.0 μm, inner diameter 4.6 mm × length 250 mm; Shimadzu, Kyoto, Japan). The mobile phases were A = 100% ethanol, B = 20 mM KH_2PO_4 (pH 2.4). The binary gradient was as follows: 85% to 68%B (0 to 12 min), 68%B (12 to 15 min), 50% to 55%B (15 to 20 min), and 85%B (20 to 29 min). The column temperature was maintained at 40 °C, the detection wavelength was 320 nm, and the flow rate was 1.0 mL·min^{-1}. Chlorogenic acid, caffeic acid, 3,4-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid, and 4,5-dicaffeoylquinic acid were identified by comparing the retention times and spectra with pure standards. The results are expressed in mg/100 g FW, and the respective percentages were calculated. Three biological replicates were performed for each treatment.

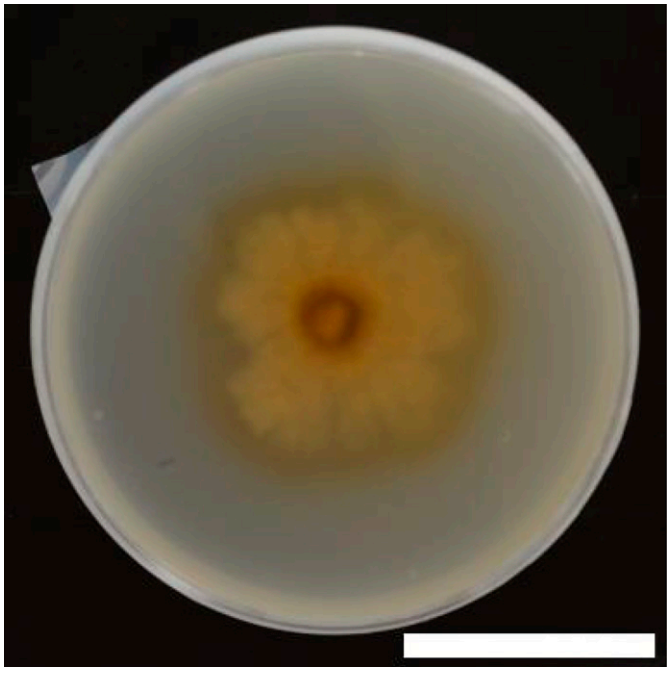

Fig. 2. Strain E of foot rot (*Diaporthe destruens*). Petri dish shows strain E after a week of incubation used for in vitro proliferation test. Bar = 5 cm.

In vitro proliferation test of foot rot fungi against polyphenols. We conducted an in vitro proliferation test to investigate the effects of polyphenols on the growth of foot rot fungi. Chlorogenic acid was used as a polyphenol contained in sweetpotato, and tannic acid, which is rarely found in sweetpotato and has high antibacterial properties (Fraga-Corral et al. 2021), was used as a control. For foot rot, a 7-d passaging culture of strain E (Fig. 2), which was used in the inoculation test, was performed at 30 °C. The basic medium was sweetpotato broth medium [sucrose-pepton-dichloran agar (SPDA); Dhingra and Sinclair 1985] supplemented with 5% glucose, 30 mg/100 g streptomycin sulfate, and 3% gellan gum. For the sweetpotato broth, 200 g of a 1 cm^3 piece of sweetpotato was boiled in distilled water for 30 min, filtered through gauze, and adjusted to l000 mL with distilled water. This sweetpotato broth was sterilized at 121 °C for 20 min after the addition of 5% glucose and 3% gellan gum. After the temperature was lowered, an antibiotic (streptomycin hydrochloride) was added at a concentration of 30 mg·L^{-1} to form the basic medium.

Polyphenols (chlorogenic acid or tannic acid) were added to each medium at seven concentrations (0, 20, 40, 80, 120, 160, and 200 mg per 100 mL). The polyphenols were injected using a syringe fitted with a filter. For the investigation of the effect of sweetpotato broth on the growth of foot rot fungi, a medium without sweetpotato broth was used as a control (gellan gum medium). Thus, four types of media were prepared at seven concentrations: gellan gum medium with polyphenols (chlorogenic acid medium), tannic acid medium, SPDA medium with polyphenols (SPDA + chlorogenic acid medium and SPDA + tannic acid medium), and three replicates of each medium.

The foot rot fungus was cut into 5-mm-diameter pieces and placed in the center of the medium used for the test. The medium was incubated in an incubator at 30 °C, and the progress was observed at 3, 5, and 7 d after the start of the incubation. Photographs were taken with a digital camera (D5100; Nikon, Tokyo, Japan) with the top part of the petri dish shaded from light, and the area of mycelium was measured using ImageJ software. Measurements were taken from the back of the petri dish.

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Statistical analysis. All experimental data were obtained from triplicate measurements (three extracts from three independent plants, three measurements per extract), and the data in the figures are the means ± standard deviation (n = 3). Multiple comparisons were performed with Tukey's test using Excel statistics. The coefficient of determination R^2 was calculated in Office365 Excel to determine the correlations among disease severity, total polyphenol content, and individual polyphenol content.

Results

Foot rot inoculation test. Inoculation tests were conducted using six strains of foot rot fungi, and differences in the disease index were observed after several weeks of inoculation. The foot rot index for most of the plants in all the treatments was 1 after 7 d of inoculation (only the inoculated areas turned black). The differences in disease index began to be observed 2 weeks after the inoculation, and some inoculated plants were dead after 5 weeks. Therefore, we used the disease index at 5 weeks post-inoculation as the final result of the resistance test. Differences in disease indices were also observed between strains A and F. The F strain of foot rot was the most severely affected, with dead plants of all five cultivars observed at 5 weeks post-inoculation. The other five strains also showed differences in the disease index, but the trends were similar among the sweetpotato cultivars tested (Supplemental Table 1). We calculated the disease severity of the A through F plants from the average of their disease index values at 5 weeks post-inoculation, and these severity values were used as the final measure of resistance to foot rot in the five sweetpotato cultivars.

Foot rot inoculation tests were conducted on the five cultivars, and the results demonstrated that the resistance to foot rot varied among the cultivars (Fig. 3). The disease severity values of 'Tamaakane' (50.0) and 'Benimasari' (52.2) were low. Most of the plants were infected at 5 weeks after inoculation, but only about 20% were dead. The cultivar with the highest disease severity was Kokei No. 14 (82.2). Seventy-two percent of the plants of this cultivar were dead at 5 weeks post-inoculation. The disease severity of 'Tamaakane' and 'Benimasari' was significantly lower than that of the weakly or slightly weakly susceptible 'Kokei No. 14'. The disease severity values of the cultivars Konaishin and Beniharuka were 61.1 and 73.3, respectively, which were not significantly different from those of the other cultivars.

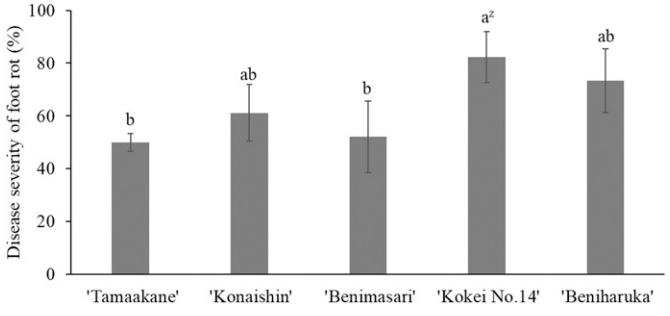

Fig. 3. Varietal differences of disease severity after inoculation with six strains of foot rot (*Diaporthe destruens*) in potted cuttings of sweetpotato. [z] Different letters beside each set of 5 cultivars represent significant differences at 5% level as determined by Tukey's multiple range test (n = 6).

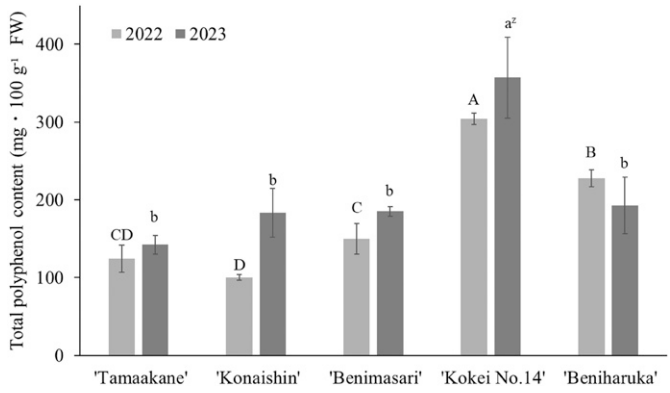

Fig. 4. Varietal differences in total polyphenol content of sweetpotato stem grown in 2022 and 2023. Stems of each cultivar were collected up to ~40 cm from the base in early July. [z] Different letters beside each set of five cultivars represent significant differences at 5% level as determined by Tukey's multiple range test (n = 3). Values for 2022 and 2023 were Tukey's multiple range test separately, and results were denoted by uppercase letters for 2022 and lowercase letters for 2023.

Total polyphenol content. The polyphenol content varied among the cultivars (Fig. 4). A similar trend was observed in the two-season samples. The cultivar with the highest value was Kokei No. 14 with 304.3 mg/100 g FW (2022) and 357.0 mg/100 g FW (2023). These values were significantly higher than those of the other four cultivars. The lowest values were obtained by 'Konaishin' (100.2 mg/100 g FW) in 2022 and 'Tamaakane' (142.2 mg/100 g FW) in 2023. However, both these cultivars had low values throughout the two seasons. The correlation between the total polyphenol content of the stems and the average disease severity was calculated, and high positive correlations were observed: R^2 = 0.7765 (2022) and R^2 = 0.7389 (2023). These results indicate that 1) sweetpotato cultivars with a low polyphenol content have a lower average disease severity for foot rot and 2) cultivars with a high polyphenol content have a higher average disease severity.

Polyphenol composition. For a more detailed analysis of the polyphenols in the stems, we used HPLC to determine the individual polyphenols. All five cultivars contained chlorogenic acid, 3,4-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid, and 4,5-dicaffeoylquinic acid (Fig. 5). The polyphenol composition differed in its occupancy when the two seasons were compared. In 2022, 'Konaishin' had high percentages of caffeoylquinic acids (chlorogenic acid, 3,5-dicaffeoylquinic acid, and 4,5-dicaffeoylquinic acid) at 38%, 38%, and 38%, respectively, and 'Konaishin' had the lowest percentage of caffeic acid at <1%.

Focusing only on chlorogenic acid, the results for 2022 show that 'Tamaakane' and 'Konaishin' had the highest percentages of chlorogenic acid at 38% and 35%, respectively. In the other three cultivars, 3,4-dicaffeoylquinic acid was the most abundant in 2022, accounting for 44% in 'Benimasari', 39% in 'Kokei No. 14', and 45% in 'Beniharuka'. The percentage of 3,5-dicaffeoylquinic acid was the highest in 'Benimasari' (44%), 'Kokei No. 14' (39%), and 'Beniharuka' (45%). The percentage of 3,5-dicaffeoylquinic acid was also relatively high, with chlorogenic acid, 3,4-dicaffeoylquinic acid, and 3,5-dicaffeoylquinic acid together accounting for >90% of the total (Fig. 5A). Throughout the two seasons, these three polyphenols accounted for >90% of the total polyphenol composition (Fig. 5B).

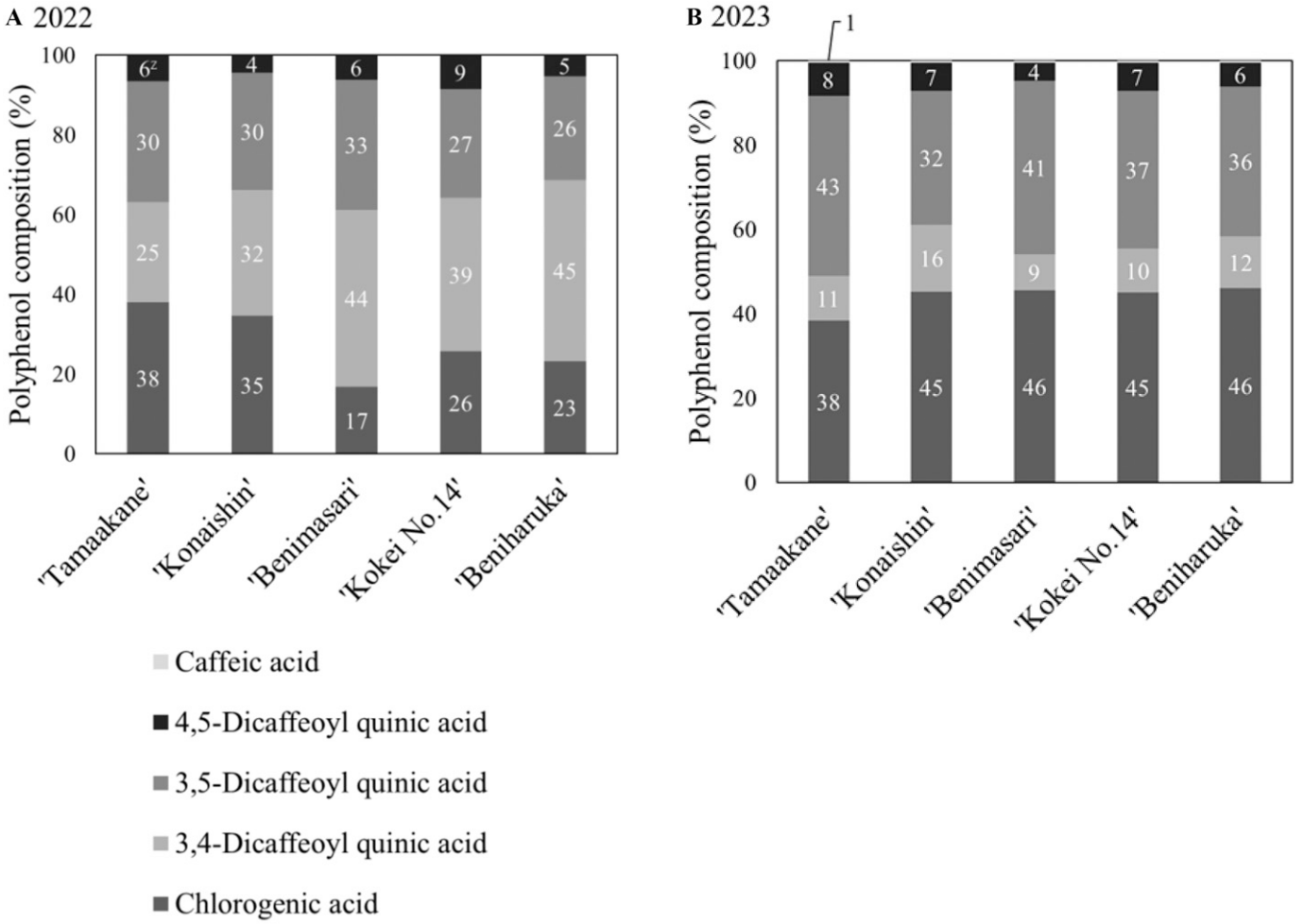

Fig. 5. Varietal differences in polyphenol composition of sweetpotato stem grown in (**A**) 2022 and (**B**) 2023. Stems of each cultivar were collected up to ~40 cm from the base in early July. [z] The numbers in the box indicate the percentage of each polyphenol composition. Numbers less than 1% are not shown.

Correlations between the individual polyphenol content and disease severity as well as the total polyphenol content were also observed: $R^2 = 0.7722$ for 3,4-dicaffeoylquinic acid, $R^2 = 0.7006$ for chlorogenic acid, and $R^2 = 0.6708$ for 3,5-dicaffeoylquinic acid in 2022. Similar correlations with disease severity were observed for the polyphenol content in 2023, with values of $R^2 = 0.7722$ for 3,4-dicaffeoylquinic acid and $R^2 = 0.7006$ for chlorogenic acid. The polyphenol contents of 3,4-dicaffeoylquinic acid, chlorogenic acid, and 3,5-dicaffeoylquinic acid in 2023 were also correlated with disease severity, with $R^2 = 0.9411$, 0.8297, and 0.6563, respectively. Among the polyphenols, 3,4-dicaffeoylquinic acid showed the highest correlation with disease severity.

In vitro proliferation test of foot rot fungi against polyphenols. The results of the in vitro proliferation test confirmed the effects of the polyphenols on the growth of the foot rot fungi. The appearance of the medium after 3, 5, and 7 d of incubation is shown in Fig. 6. The brown area of the medium expanded in concentric circles from the center as the fungus grew. A clear difference was observed between the medium with chlorogenic acid and the control medium with tannic acid, with the tannic acid inhibiting the growth of the bacteria to a greater degree. This brown zone was the area of mycelial growth, and its area was measured using ImageJ software and shown in Fig. 7.

The mycelial area tended to spread more on the medium with a small amount of chlorogenic acid than on the medium without it (0 mg) (Fig. 7A and C). In particular, after 7 d on the SPDA + chlorogenic acid medium, the mycelial area was 543.3 mm^2 at a concentration of 0 mg, whereas it was 835.2 mm^2 at 20 mg, a significant difference (Fig. 7C). The mycelial area peaked at chlorogenic acid concentrations between 20 and 80 mg, and it decreased at higher concentrations. This was more pronounced in the gellan gum medium with chlorogenic acid (Fig. 7A).

In contrast, the mycelial area decreased with increasing concentrations of tannic acid in the tannic acid–added medium. A significant difference was observed between 0 and 20 mg of tannic acid. After 7 d on tannic acid medium, the mycelial area was 588.8 mm^2 at 0 mg, whereas at 20 mg, it was 319.6 mm^2. After 7 d on the SPDA + tannic acid medium, the area was 1420.2 mm^2 at 0 mg and 883.7 mm^2 at 20 mg (Fig. 7B and D). In the 200-mg tannic acid medium, mycelium hardly grew at all; the area was still 67.3 mm^2 after 7 d (Fig. 7B).

Discussion

Foot rot is a disease that develops from the stem at the base of a plant. Once infected, the basal stems turn black and the leaves wilt, defoliate, and eventually die. Dark brown, somewhat

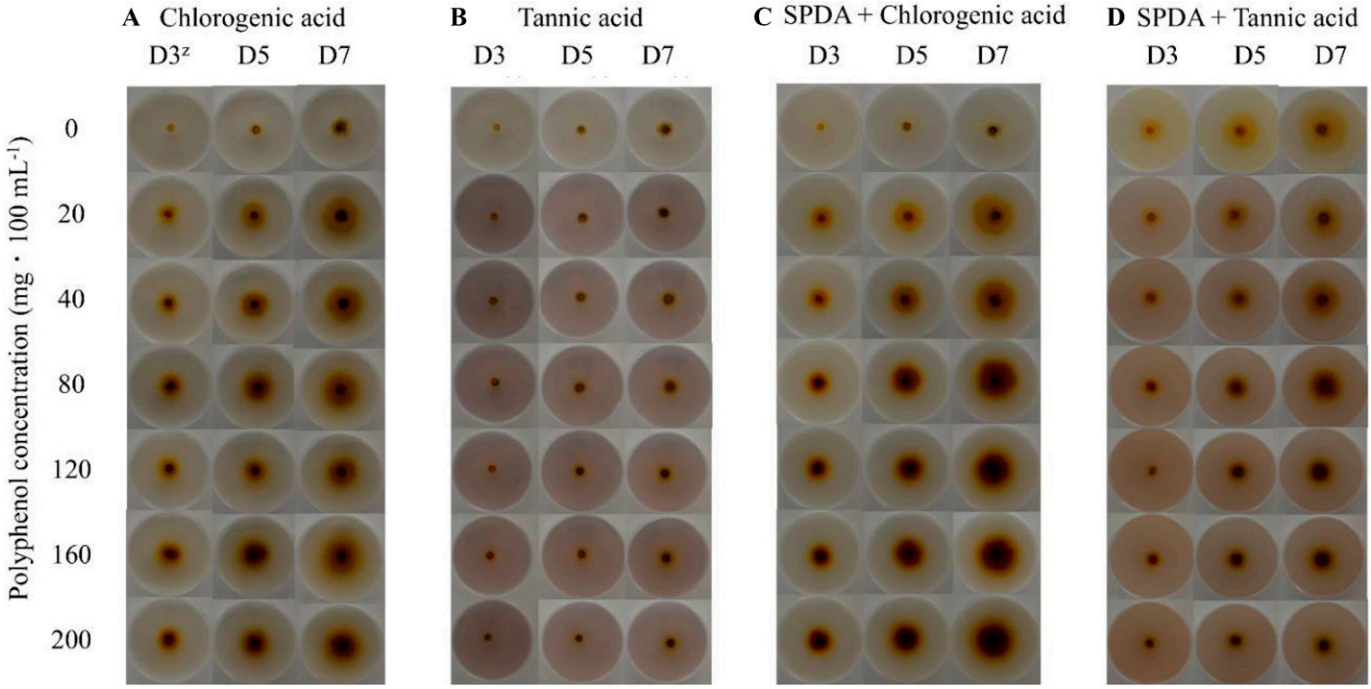

Fig. 6. Change in the proliferation of foot rot (*Diaporthe destruens*) by addition of polyphenols to the culture medium. (**A**) Chlorogenic acid medium. (**B**) Tannic acid medium. (**C**) SPDA + chlorogenic acid medium. (**D**) SPDA + tannic acid medium. [z] D3 = 3 d after inoculation. This marker indicates the number of days after inoculation.

hard rot slowly develops from the base of the stem in storage roots (Kobayashi 2019). Most of the research concerning sweetpotatoes has focused on the underground storage roots, but in recent years interest in the relationship with the aboveground parts has increased (Jin et al. 2024; Lin et al. 2021; Luo et al. 2024), and the importance of the aboveground parts in acquiring disease resistance has been proposed (Sakaigaichi et al. 2024). In the present study, we focused on the base of the stem (i.e., the main infection site of foot rot) to investigate the relationship between disease infection and polyphenols.

The results of our foot rot inoculation test showed a trend that is similar to those observed in previous studies (BRAIN 2023; Tabuchi et al. 2024). We conducted inoculation tests for five sweetpotato cultivars, and the test results confirmed that the

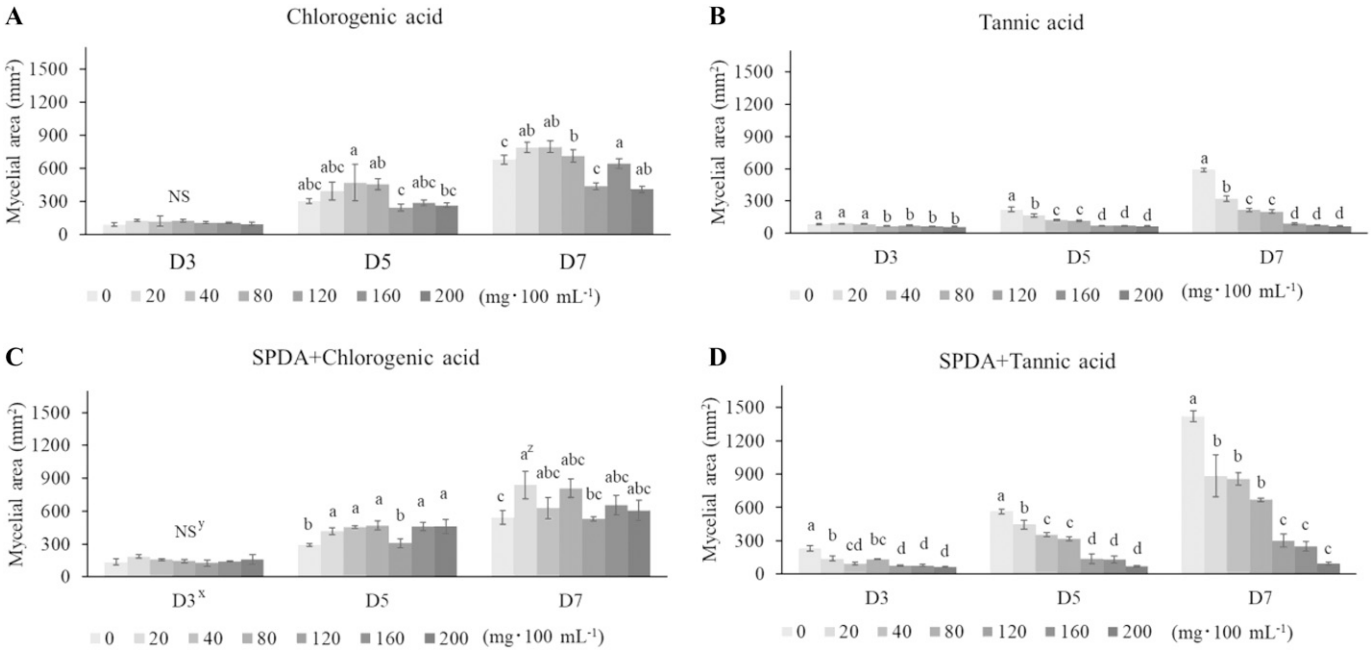

Fig. 7. Effect of type and concentration of polyphenols on the in vitro proliferation (mycelial area) of foot rot (*Diaporthe destruens*). (**A**) Chlorogenic acid medium. (**B**) Tannic acid medium. (**C**) SPDA + chlorogenic acid medium. (**D**) SPDA + tannic acid medium. [z] Different letters beside each set of four culture medium represent significant differences at 5% level as determined by Tukey's multiple range test (n = 3). [y] NS = not significant. [x] D3 = 3 d after inoculation. This marker indicates the number of days after inoculation.

resistance to foot rot varied among the cultivars. The disease severity of 'Kokei No. 14' (82.2), which is considered weak or slightly weak, was significantly higher than those of 'Tamaakane' (50.0) and 'Benimasari' (52.2), which are considered strong or slightly strong. The disease severity of 'Beniharuka' (73.3), which is also considered weak or slightly weak, was the second highest after 'Kokei No. 14', but it was not significantly different from those of the other cultivars. This result suggests that the cutting quality and/or other environmental factors may have affected the differences among individuals. As described earlier, the degree of resistance of the genotype was in agreement with the degree of resistance to foot rot for each cultivar already reported, thus reaffirming the degree of resistance of the genotypes as well as the appropriateness of the inoculation test method used in this study.

Surprisingly, a new finding was that the total polyphenol content of the sweetpotato stems differed among the cultivars. Edible parts of globe artichoke [*Cynara cardunculus* var. *scolymus* (L.) Fiori] accumulate more polyphenols in a nitrogen/phosphorus/potassium balanced environment than in an overfertilized environment reported (Heimler et al. 2017). The total polyphenol content in leaves and branches of rabbiteye blueberry (*Vaccinium virgatum* Aiton) has also been reported to vary with season (Koga et al. 2024). Thus, the total polyphenol content has also been reported to increase or decrease with the environment. Still, our result was confirmed to be reproducible in a similar study over a 2-year period. Luo et al. (2024) reported that total polyphenol content in sweetpotato leaves varies significantly by genotype. This suggests that, as with leaves, the total polyphenol content of sweetpotato stems is determined primarily by genetic factors. In addition, the present study calculated the correlation between the total polyphenol content of the stems and the average of the disease severity values, and we found a high positive correlation. Surprisingly, cultivars with a low stem polyphenol content had lower susceptibility to foot rot, whereas the cultivars with high stem polyphenol content had higher susceptibility to foot rot. It is generally believed that polyphenols are involved in defense against ultraviolet light and pathogens (Pinto et al. 2020). In rice (*Oryza sativa* L.), it is known that the polyphenol content of resistant cultivars is significantly higher than that of susceptible cultivars (Wakimoto and Yoshi 1958). It has also been reported that the application of Fe-polyphenol along with CaO_2 to the soil helps control soilborne diseases (Morikawa 2018). Furthermore, based on the relationship between the developmental stage of sweetpotato storage roots and the total polyphenol content, we have also considered that the accumulation of polyphenols with high antioxidant activity is a strategy used by a plant to protect itself from biotic and abiotic stresses (Setoguchi et al. 2023, 2024). However, contrary to our storage root speculation, the high polyphenol content of sweetpotato stems (the main infection site) observed herein may promote disease infection and growth in foot rot. In summary, because the polyphenol content of sweetpotato stems varies by genotype and there is a correlation between polyphenol content and susceptibility to foot rot, it is possible to select cultivars resistant to foot rot by measuring the total polyphenol contents in healthy stems that are not infected with the disease.

Our further analysis of the polyphenol composition of the stems showed that chlorogenic acid, 3,4-dicaffeoylquinic acid, and 3,5-dicaffeoylquinic acid accounted for >90% of the total polyphenol content. The correlation between the polyphenol content (mg/100 g FW) of each of these three polyphenols and the disease severity was examined and found to be as high as that for the total polyphenol content. These are all similar compounds of caffeic acid and quinic acid, but 3,4-dicaffeoylquinic acid showed a particularly high correlation. Harrison et al. (2008) tested the effects of dicaffeoylquinic acids isolated from sweetpotato tissue and caffeic acid and chlorogenic acid standards on the growth of *Fusarium solani* (Sacc.) Mart. They reported that chlorogenic acid > caffeic acid > 3,5-dicaffeoylquinic acid > 4,5-dicaffeoylquinic acid > 3,4-dicaffeoylquinic acid inhibits bacterial growth in that order, and 3,4-dicaffeoylquinic acid hardly inhibits the growth at all. It thus appears that 3,4-dicaffeoylquinic acid showed a higher correlation compared with the other polyphenols. It has also been reported that the higher the concentrations of chlorogenic acid and 3,5-dicaffeoylquinic acid, the stronger their inhibitory effects (Harrison et al. 2008). Polyphenols may have two opposing effects, that is, a favorable effect on foot rot fungi fungal infection and growth, as well as the inhibition of fungal growth. In the case of sweetpotato stems, we observed that the favorable effect of the polyphenol concentration was stronger, and the present study also confirmed a high correlation for individual polyphenols.

The results of our in vitro proliferation tests confirmed the effects of polyphenols on the growth of foot rot fungi. In our study, the common polyphenol tannic acid inhibited the growth of foot rot fungi, whereas moderate amounts of chlorogenic acid promoted their growth. It has been reported that microorganisms metabolize and use secondary metabolites for their growth (Piekarska-Radzik and Klewicka 2021). Furthermore, the presence of bacteria that use phenolic substances as a carbon source (Hopper and Mahadevan 1997) and bacteria that degrade cinnamic acid (Fritsch et al. 2017) and chlorogenic acid (Fritsch et al. 2016) has been reported. Our present study material, foot rot, may also metabolize chlorogenic acid as a nutrient source because the chlorogenic acid-supplemented medium promoted the growth of foot rot fungi. However, the growth of the bacteria peaked at concentrations of 20 to 80 mg/100 mL of chlorogenic acid and was inhibited at higher concentrations, suggesting that there may be an optimal concentration of the chlorogenic acid content that the bacteria can metabolize.

Xu et al. (2022) reported that sweetpotatoes differ in their polyphenol content and composition depending on their organs and tissues such as young leaves, young stems, mature leaves, mature stems and storage roots, and the growth stages. Although chlorogenic acid and other polyphenols in sweetpotatoes vary by organ and genotype, the action of infection may begin when the concentrations of chlorogenic acid and other polyphenols in the stem reach the optimal concentration for infection by foot rot fungi.

As described earlier, the relationship between sweetpotato foot rot disease and polyphenols was investigated over a 2-year period to establish basic techniques for selecting cultivars resistant to foot rot. We calculated the correlation between the total polyphenol content of stems and the average disease severity for each cultivar and obtained high positive correlations: $R^2 = 0.7765$ (2022) and $R^2 = 0.7389$ (2023). Surprisingly, the cultivars with a low polyphenol content had lower susceptibility to foot rot, and the cultivars with a high polyphenol content had higher susceptibility to foot rot. A causal relationship was also confirmed in a bioassay of base rot fungi using chlorogenic acid in the stems. The stem polyphenol content could become a marker for determining a

cultivar's resistance to foot rot, as confirmed in the present 2 years of trials. This finding is expected to be applied to the selection of foot rot-resistant sweetpotato cultivars and will lead to labor-saving disease resistance tests at breeding sites, shortened breeding times, and the amelioration of sweetpotato foot rot. Our research group is currently selecting sweetpotato lines that are resistant to foot rot by measuring the polyphenol content of the stems of crossbred sweetpotato seedlings. In the near future, we expect to produce new cultivars selected based on polyphenol markers of resistance to foot rot.

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