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Evaluation of tomato resistance to *Verticillium dahliae* culture filtrate in tissue culture

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Summary

Tomato (*Lycopersicon esculentum* Mill.) explants (hypocotyl fragments) and calli derived from susceptible and resistant cultivars to *Verticillium dahliae* Kleb. were exposed to fungal culture filtrates. Callus induction and growth rates in media containing the culture filtrates were investigated. Explants and calli from susceptible and resistant cultivars were sensitive to the culture filtrate of *V. dahliae*. In our experimental conditions, we failed to observe a correlation between the resistant gene to *V. dahliae* and the susceptibility of explants and calli to culture filtrates. These results suggested that *in vitro* bioassays using the fungal culture filtrates couldn't be used for evaluating sources of resistance in tomato to *V. dahliae*.

Key Words : Culture filtrate, Tomato, *Verticillium dahliae*

Introduction

Verticillium wilt is a vascular disease in tomato (*Lycopersicon esculentum* Mill.) caused by a soil fungus, *Verticillium dahliae* Kleb[1]. This pathogen persists in the soil for extended periods of time, the only effective control being the use of resistant cultivars[2]. Resistance in tomato to this wilt disease was reported to be controlled by one dominant gene (*Ve*)[3]. This monogenic specific resistance can be identified under greenhouse conditions when plants are inoculated with the fungus[2].

Plant tissue cultures could be used to either select disease resistant genotypes by *in vitro* selection or to evaluate disease resistance. A protocol that has been widely used for the selection of disease resistant lines is to grow calli in the presence of culture filtrates or toxin[4, 5].

Our objective was to develop an *in vitro* method for evaluating resistance to *V. dahliae*. This paper describes the effects of culture filtrates of *V. dahliae* on the induction and growth of calli derived from susceptible and resistant tomato cultivars.

Materials and Methods

Plant Material

Five tomato cultivars were used for this experiment on their differential responses to *V. dahliae*. 'Toyomasa' (genotype : *VeVe*), 'Momotarou' (*Veve*) and 'Hausu - Odoriko' (*Veve*) were resistant to Verticillium wilt. 'Kyouryokubeiju' (*Veve*) and 'Hikari' (*Veve*) were susceptible to it.

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Plant inoculation

A fungal isolate of *V. dahliae* isolated from a diseased tomato plant was supplied by Miss Sat oko Ohshima (Kimitsu Breeding Station, SAKATA Seed Coop.). To estimate virulence of the fungal isolate and resistance of the plants, plants were inoculated with it. To produce *V. dahliae* conidia, the pathogen was inoculated into Potato Sucrose Broth (PSB) in 200 ml Sakaguchi flasks for one week at 25℃. Fungal conidia were collected by washing mycelium growing in a PSB with autoclaved distilled water. The number of conidia/ml in the water was counted using a haemacytometer and the density was adjusted to 1×10^7 conidia/ml.

Twenty-five seedlings of each cultivar were gently uprooted and washed free of soil, and the roots were dipped in the inoculum suspension for 30 min. After inoculation seedlings were replanted in pots (five seedlings per pot) in the greenhouse.

Plants were scored individually for disease symptoms at 10 day intervals over 30 days following inoculation. The seedlings were scored on a scale of 0 − 5 (for wilting), where : 0 =green leaf without symptoms, 5 =whole leaf wilting.

Preparation of the fungal culture filtrates and toxic media

The fungal isolate was maintained on a Potato Sucrose Agar (PSA) medium at 20℃ in the dark. One disc from a 3 −week culture of *V. dahliae* isolate on PSA was inoculated into 100 mg of liquid Czapek-Dox broth. The culture was shaken on an orbital shaker at 90 rev/min at 20 ℃ for 4 weeks. Culture filtrate was prepared from the liquid culture by filtration and centrifugation at 10,000 × g for 20 min to remove the mycelium and bud spores. Culture filtrate was sterilized by filtration through a 0.45 μm membrane. The pH of the crude filtrate was adjusted to pH 5.7 with 1 N HCl. To avoid thermal degradation of toxic compounds in the fungal culture filtrate, it was added to the autoclaved basal medium. The different proportions used in this study were 1.0, 5.0 and 10% (v/v).

Effects of culture filtrates on callus induction

Seeds of the five cultivars were surface sterilized, rinsed and sown on Linsmaier and Skoog[6] (LS) medium, and seedlings were grown for 7 days at 25± 1 ℃ under continuous light. Hypocotyls (5 mm) were aseptically excised and transferred to an LS agar medium (0.4ppm 2, 4 − D, 1.0ppm. kinetin and 0.8% agar) containing different concentrations (1.0, 5.0 and 10%, 0 % : Control) of fungal culture filtrates. These explants were incubated for 14 days at 25± 1 ℃ in the dark. Fresh weights of induced calli (10 pieces per treatment) were measured after being cultured. This experiment was conducted in triplicate.

Effect of culture filtrates on callus growth

Calli (20−40mg) induced from hypocotyls of each cultivar on LS agar medium were placed on the surface of the test media and incubated in the dark at 25± 1 ℃ for 14 days. Fresh weights of calli (10 pieces per treatment) were measured before and after the cultures. This experiment was conducted in triplicate.

Results

Fig. 1 shows the progress of the disease as mean wilting scores for the five cultivars over 30 days. Verticillium wilt of tomato seedlings starts as yellowing of lower leaves, then progresses acropetally with chlorosis and necrosis of older leaves, and culminates in dehydration, and with death of the plants in extreme cases. Significant differences between wilting scores of resistant ('Toyomasa', 'Momotarou' and 'Hausuodoriko') and those of susceptible cultivars ('Kyouryokubeiju' and 'Hikari') were apparent at all obsevation times.

The inhibition of callus induction (as percentage

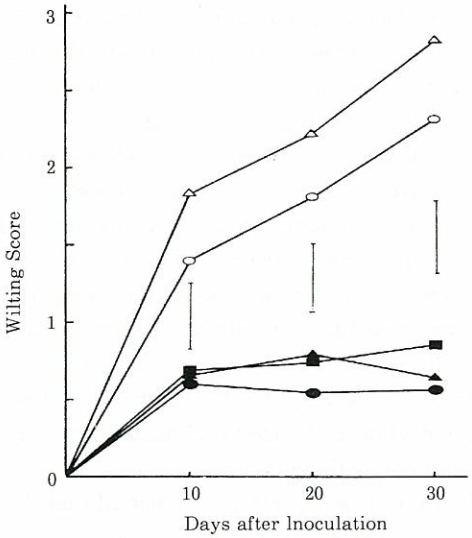

Fig. 1. Development of Verticillium with in five tomato cultivars. Each point is the mean value for 25 plants. Vertical lines denote the L. S. D. P=0.05
■, Toyomasa ; ●, Momotarou ; ▲, Hausu odoriko ; △, Kyouryokubeiju ; ○, Hikari

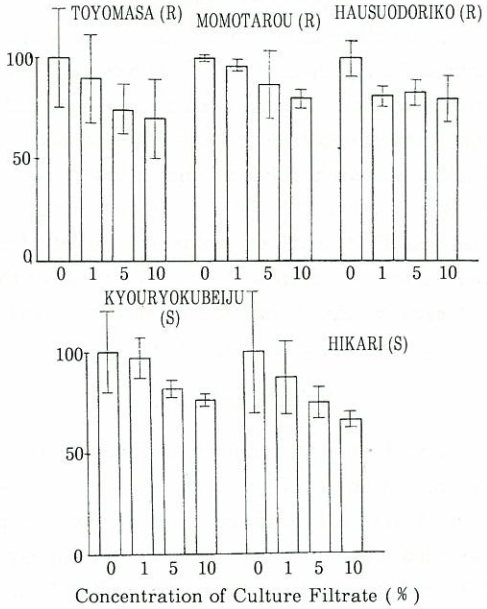

Fig. 2. Effect of culture filtrates on the induction of calli from hypocotyl explants of various tomato cultivars. Fresh weights were determined after 30 days. Callus fresh weight means % of control. Vertical bars indicate standard error.

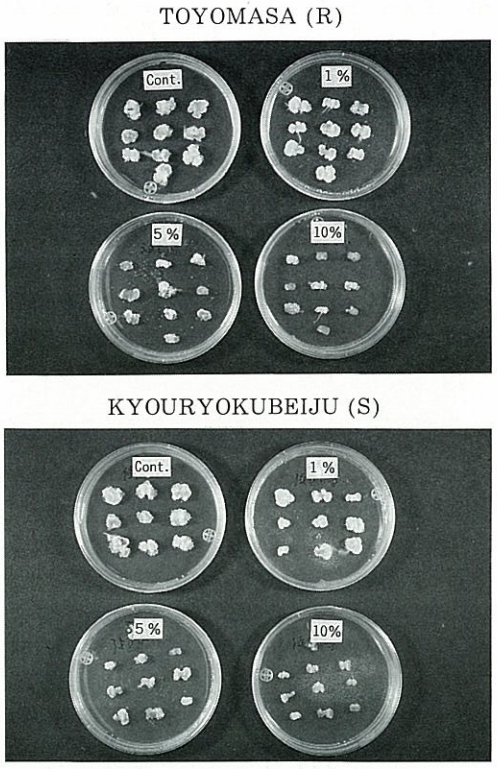

Fig. 3. Effects of *Verticillium dahliae* culture filtrates on the callus induction from hypocotyl tissues of resistant (R) and susceptible (s) tomato cultivars (30days after incubation).

of control weight) of susceptible and resistant cultivars grown on media at different concentrations of culture filtrate is shown in Figs. 2 and 3. Final fresh weights of induced calli decreased with increasing culture filtrate concentration for all five cultivars. The intensity of growth inhibition depended on the concentration of culture filtrates. Toxic effects caused by culture filtrates were obtained in all cultivars tested, independent of the resistance level expressed in the intact plants.

A similar result was obtained in the assay for callus growth. Fig. 4 shows the inhibition of callus growth (as percentage of control weight) of susuceptible and resistant cultivars grown on media at different concentrations of culture fil-

trate. Final fresh weights of growth calli decreased with increasing culture filtrate concentration for all five cultivars. There was no difference of toxic effect of culture filtrates on callus growth between resistant and susceptible cultivars.

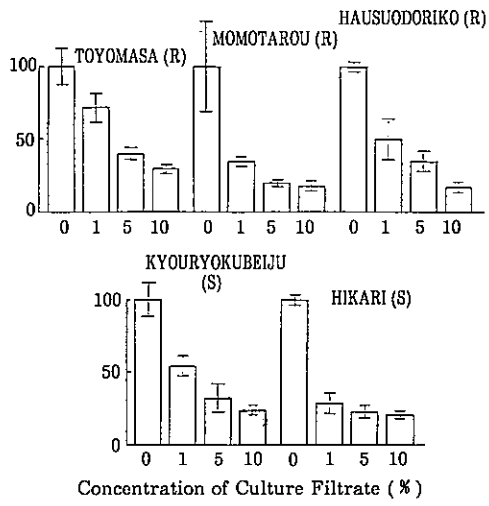

Fig. 4. Effect of culture filtrates on the growth of calli derived from various tomato cultivars. Fresh weights were determined after 2 weeks. Callus fresh weight means % of control. Vertical bars indicate standard error.

Discussion

The observations of the present study indicate that hypocotyl and calli derived from three resistant cultivars ('Toyomasa', 'Momotarou' and 'Hausuodoriko') exhibited sensitivity to the culture filtrates similar to the sensitivity of the two susceptible cultivars ('Kyouryokubeiju' and 'Hikari'). A good relation between the two parameters studied (induction and growth of calli) depending on filtrate concentrations was observed. Resistant cultivars were not resistant to the culture filtrate. The resistant gene *Ve* does not appear to be expressed against toxins secreted in filtrate, when the host is cultured *in vitro* as explants. It could be concluded, therefore, that some of the toxic components of the culture filtrates of the pathogen used in the selection medium have limited roles in pathogenesis and this toxic agents cannot be used to distinguish resistant from susceptible plant material *in vitro*.

It is known that *V. dahliae* produces wilting and cytotoxic toxin[7)·8)·9)] Nachmias *et al.* isolated a phytotoxic proteinlipopolysaccharide (PLPS) complex from culture filtrate of *V. dahliae* and purified a toxic 3 kDa glycopeptide derived from PLPS by extended dialysis as based on wilt symptom-producing activity by a detached potato leaf bioassay[10)·11)]. In this experiment, the induction and growth of callus tissue may be affected by these toxins.

In a previous paper[12)], we reported that there was a good correlation between *in vivo* resistance of eggplants and *in vitro* response to *V. dahliae* culture filtrate. From these results, the question is raised as to why culture filtrates of the same pathogen (different strain) showed different toxicity to their host plants. However, this phenomenon remains to be understood.

On the other hand, in alfalfa-*V. albo-atrum* interactions, Koike *et al.* [13)] showed there was no correlation between *in vivo* resistance and *in vitro* response to crude culture filtrates. But their further research indicated that a low molecular weight fraction (<3,500 Da) of culture filtrates has specific toxicity to protoplasts derived from susceptible genotypes[14)·15)]. We need further research in order to isolate fractions which induce cultivar-specific toxicity *in vitro*.

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組織培養によるトマト半身萎ちょう病抵抗性の評価

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和　文　摘　要

細胞レベルにおけるトマト半身萎ちょう病に対する

抵抗性を調べるために，感受性2品種（強力米寿，ヒカリ）と抵抗性3品種（豊将，モモタロウ，ハウスおどりこ）を用いて菌培養濾液に対する反応を調べた。菌培養濾液を含んだLS寒天培地上で，胚軸からのカルス誘導とカルスの生育を調査したところ，誘導されたカルスと生長したカルスの生重量は，濾液の濃度が高まるにつれて抑制される傾向にあり，抵抗性および感受性品種間に反応の差は認められなかった。