

Use of Culture Filtrates of *Verticillium dahliae* as a Bioassay for Screening of Disease Tolerant Eggplant

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Introduction

Verticillium wilt, caused by *Verticillium dahliae* Klebahn, is a serious vascular wilt disease of eggplant (*Solanum melongena* L). The disease is worldwide in distribution and constitutes a major limiting factor to increasing eggplant culture^{1,2}. The most effective means for suppressing this disease are grafting on tolerant cultivars as rootstock and chemical control³. These practical methods, however, are not easily applicable and are limited by cost.

Recently, screening and selection of plant tissues *in vitro* for resistance to fungal toxins or culture filtrates has been successful for several species^{4,5}. In this approach, it is assumed that toxins secreted into the culture medium play an important role in pathogenesis, and that plant tissue culture systems express some selectable aspect of resistance at the cellular level *in vitro*⁶.

The objective of this study was to determine whether or not a reliable tissue culture screening system to detect *V. dahliae* resistance in eggplant could be established.

Materials and Methods

1. Plant materials

Three eggplant cultivars used in this experiment were "Mizunasu" and "Nagaokanagasu" susceptible to *V. dahliae*, and "Support No. 1" (*S. integrifolium* × *S. melongena*, a major rootstock cultivar) tolerant to it.

2. Initiation and maintenance of callus

Calli were initiated from hypocotyls on MS⁷ medium supplemented with 1.0 mg/l 2, 4-D, 1.0 mg/l benzylaminopurine and 0.8% agar in the dark at 25°C. Proliferating callus cultures were then routinely transferred to fresh medium every 4 weeks.

3. Culture of pathogen

A fungal isolate of *V. dahliae* isolated from a diseased eggplant was supplied by Miss Satoko Ohshima (Kimitsu Breeding Station, SAKATA Seed Coop.). The fungal isolate was maintained on Potato Sucrose Agar (PSA) medium at 20°C in the dark.

4. Preparation of culture filtrates of *V. dahliae*

One disc from 3-weeks culture of *V. dahliae* isolate on PSA was inoculated into 200 ml of liquid Czapek-Dox broth. The culture was shaken on an orbital shaker at 90 rev/min. at 20°C for 4 weeks. Culture filtrate was prepared from the liquid culture by filtration and centrifugation at $10,000 \times g$ for 20 min. to remove the mycelium and bud spores. Culture filtrate was sterilized by filtration through a 0.45 μm membrane and stored at -20°C until use.

5. Bioassay of culture filtrate

Culture filtrate was added to MS medium at final concentrations of 0.5, 1.0 and 5.0% (v/v) and pH of each medium was adjusted to 5.8. The test media were dispensed into 90(ϕ) \times 20 (height) mm sterile Petri dishes.

Calli (ca. 50 mg fresh weight) of each eggplant cultivar were placed on the surface of test media and incubated in the dark at 25°C for 2 weeks. Fresh weights of calli (10 pieces per treatment) were measured before and after the culture. All experiments were conducted in triplicate.

Results and Discussion

The differences in growth response between the susceptible and resistant eggplant calli exposed to culture filtrate of *V. dahliae* were quite apparent. Culture filtrate of *V. dahliae* suppressed callus growth of the two susceptible cultivars as the concentration of culture filtrate increased (Fig. 1, 2).

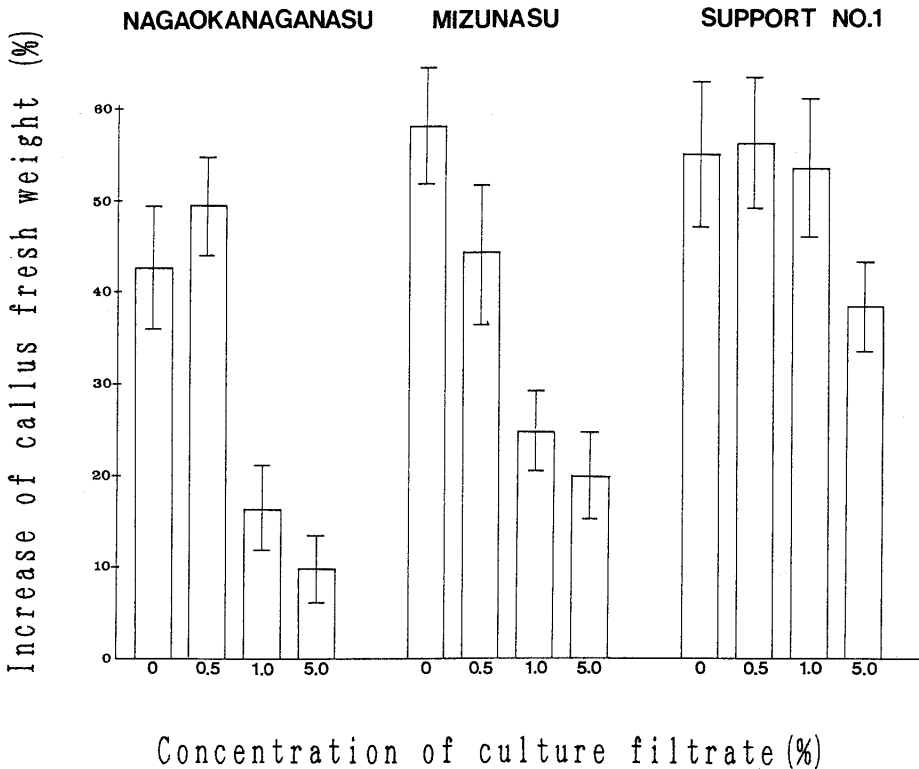


Fig. 1 Effects of *Verticillium dahliae* culture filtrate on increase of callus fresh weight (% increase of initial weight).

Calli of three eggplant cultivars (Nagaokanaganasu, Mizunasu and Support No. 1) were cultured for 2 weeks on MS media containing *V. dahliae* culture filtrate at different final concentrations.

Vertical bars indicate standard errors in three separate experiments.

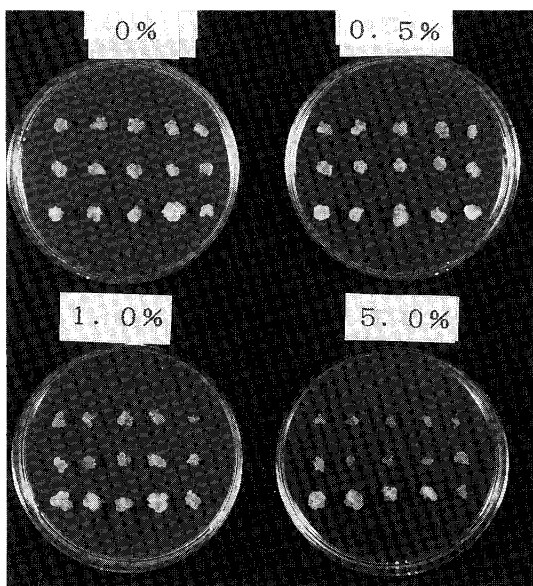


Fig. 2 Effect of *Verticillium dahliae* culture filtrate on callus growth in three eggplant cultivars.

Culture filtrates were added into MS medium at final concentrations of 0, 0.5, 1.0 and 5.0% (v/v). Calli were cultured on the MS medium for 2 weeks. In each dish, upper, middle, and lower lanes are Mizunasu, Nagaokanaganasu, and Support No. 1, respectively.

Callus growth of “Mizunasu” and “Nagaokanaganasu” were reduced significantly when culture filtrate was added at 1.0 and 5.0% (**Fig. 1**). The callus fresh weight of the tolerant eggplant (Support No. 1) did not decrease significantly at 1.0% of culture filtrate. When filtrate was added at 5.0%, the fresh weight of the calli decreased, but the decreasing rate was not higher than that of susceptible cultivars (**Fig. 1**).

The result presented in this report demonstrated that a strong inhibition in callus growth of the two susceptible eggplants was observed by the addition of a cell free culture filtrate of an isolate of *V. dahliae* into the culture medium. This decrease in callus fresh weight shows the possibility of the presence of toxic metabolites in the culture filtrate. It is known that *V. dahliae* produces wilting and cytotoxic toxin^{8,9}. Nachmias *et al.* isolated a phytotoxic protein-lipopolysaccharide (PLPS) complex from culture filtrate of *V. dahliae* and purified a toxic 3kDa 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 growth of callus cells may be affected by these toxins.

Tolerant and susceptible cultivars could be successfully distinguished using the *in vitro* callus screening system used in this study. This result suggests that this screening procedure is useful in performing selection for tolerance to the *Verticillium* wilt at the cellular level.

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《和文要約》

ナス半身萎ちよう病抵抗性スクリーニングのための *Verticillium dahliae* 培養濾液の利用

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半身萎ちよう病に感受性の栽培ナス2品種(みず茄, 長岡長茄)と耐病性の台木1品種(サポート1号)からカルスを誘導し, *Verticillium dahliae* の培養濾液に対する生育反応を調べた。感受性2品種のカルスは濾液1.0, 5.0%の含有培地で生育が抑制されたが, 耐病性台木品種のカルスは濾液1.0, 5.0%含有培地において, 感受性品種ほどの生育の抑制は認められなかった。