Detached Leaf Test for Evaluation of Resistance to Powdery Mildew in Pepper

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Abstract

Powdery mildew caused by 

Leveillula taurica (Lév.) G. Arn. is a quite serious disease of pepper varieties (Capsicum spp.) worldwide. The use of resistant genotypes can control the disease. Tests of resistance against powdery mildew during pepper breeding studies have been carried out by directly brushing the conidia or spraying a conidia suspension on whole potted plants grown in the greenhouse. High amounts of inoculum, labour and large experimental areas are needed for these tests. The aim of this study was to investigate the possibility of using detached leaves for resistance evaluation. Detached leaves of a sensitive cultivar (Moonset F1) were placed on two different media (water agar and moistened filter paper) in petri dishes, were inoculated using three different methods (direct brushing conidia, spraying conidia suspension in distilled water, and in sterile distilled water). Inoculated leaves were incubated under two different conditions (16 °C for 24h in dark, then 22 °C for 14 h light/10 h dark, and 21 °C for 12 h light/12 h dark) in a climatically controlled room. The methods were combined with each other. The combination of water agar and moistened filter paper with inoculation by brushing conidia and spraying conidia suspension in distilled water at the second incubation condition gave infected leaf areas of 50% or more with powdery mildew at 25 day after inoculation. The authors suggest that detached leaves might be very practical to assess the resistance of pepper lines against to powdery mildew disease for breeding programmes.

Keywords: Detached leaves; Pepper (Capsicum annuum); Powdery mildew; Resistance test

Introduction

Powdery mildew caused by 

Leveillula taurica (Lev.) G. Arn. [anamorph: Oidiopsis taurica (Lev.) Salmon] causes economic yield losses in several groups of plants in addition to pepper varieties (Capsicum spp.) [1]. The fungus is an obligate biotrophic pathogen and needs living plants to develop and reproduce. In contrast to other powdery mildew genera, the germinated conidia of 

Leveillula and Phyllactinia develop adhesion bodies instead of appresoria to attach to the leaf surface. Infection hyphae penetrate host plants through stomata on the abaxial leaf surface, produce haustoria in the mesophyll cell and grow as endophyte [1]. Mildew colonies develop on the abaxial surface of infected leaves. Endophytic growth of the pathogen hinders the efficacy of chemical control [2, 3]. Additionally, indiscriminate use of fungicides has a negative impact on the environment and allows emergence of fungicide-resistant strains [4,5]. Breeding of resistant varieties appears to be the best control practice. Tests for resistance of pepper genotypes against powdery mildew disease are one of the main objectives for a breeding program. Assessments of powdery mildew resistance are performed on whole potted plants in a greenhouse. The abaxial surface of healthy plants with 45-60 days growth are inoculated by pressing gently sporulated areas of old infected leaves [6-7], by transferring the spores with a small brush [7-9]), and by spraying a conidia suspension (10^4-10^6 conidia/ml) in sterile distilled water or tap water [3,5,7,10-16]. In some studies, inoculation of plants was carried out by placing infected plants in the vicinity of healthy plants [17-22]. The plants were maintained at 16 °C for 24h in dark, then at 22 °C for 14h light and at 16 °C for 10h dark (3) or at day temperature (21 °C, 25 °C) and night temperature (19 °C, 22 °C) after inoculation [5,9,14-15]. Some authors kept pepper plants for 24h in plastic cages after inoculation [5,13,15-16]. Tests for resistance of pepper genotypes to powdery mildew throughout a breeding program under greenhouse conditions need high amounts of inoculum, labour, time and large experimental area. This study aims to examine the use of detached leaves placed on petri dishes by combining different media, inoculation methods and temperature conditions.

Materials and Methods

A pepper cultivar (Moonset F1) very sensitive to powdery mildew was grown in a disease-free greenhouse for 80 days.
Detached leaves of the cultivar were used during the experiments. Leaves were surface-sterilized with 70% ethanol, then rinsed in sterile distilled water. After drying on sterile filter paper, leaves were placed bottom side up in petri dishes containing water agar (20g/l) supplemented with 30 mg/l benzimidazole (A) or three layers of moistened filter paper (B) (Table 1). Two sterile glass slides were put on the agar or filter paper to prevent contact of the leaves with media. Petioles were wrapped with sterilized wet filter paper to extend leaf viability. Powdery mildew inoculums were collected from infected pepper plants in a greenhouse in Antalya/Turkey. Leaves were inoculated with conidia using three different methods: slight brushing of conidia from sporulating zones onto the leaf (a), spraying a conidia suspension (1X10^5 conidia/ml) in distilled water (b) and in sterilized distilled water (c). Petri dishes sealed with parafilm were kept in two different conditions: 16 °C for 24h in dark, then at 22 °C for 14h light/10h dark (I) and 21 °C for 12h light/12h dark in a climatically controlled room (II). The methods were combined with each other and all combinations were repeated three times. Observations for sporulation were made at weekly intervals.

Table 1: Media, inoculation and incubation conditions used for method combinations

| Media       | Inoculation                     | Incubation Condition         |
|-------------|---------------------------------|------------------------------|
| A: Water Agar| a: Directly brushing conidia    | I: 16 °C for 24h in dark, then at 22 °C for 14h light/10h dark |
|             | b: Spraying conidia suspension in distilled water | II: 21 °C for 12h light/12h dark |
|             | c: Spraying conidia suspension in sterile distilled water |                           |
| B: Moistened filter paper | (a) as in A                        | (I) as in A                    |
|             | (b) as in A                        | (II) as in A                   |
|             | (c) as in A                        |                               |

Results and Discussion

First limited sporulation was observed on leaves in the method combination AbI at one week after inoculation, but symptoms then developed slowly (Table 2). Sporulation was detected on leaves of method combinations AaI, AbI, BbI and BbII at 15 days after inoculation. 40% and 25% of leaf area were covered with sporulation when leaves were inoculated by directly brushing conidia and spraying with conidia suspension in distilled water, respectively (Figure 1). The method combinations AcII and BcII gave less sporulation compared with AaII and BaII (Figure 2).

The method combinations AaI, AbI, BbI and BbII gave infected leaf areas of 50% or more with powdery mildew at 25 days after inoculation (Figure 3). There were no differences among the media used for sporulation. However, more brownish areas on leaves that were placed on moistened filter paper or inoculated by brushing conidia were observed at 25 days after inoculation as compared with other methods under incubation condition II. Infected leaf areas of 40% were observed for AcII and BcII at 25 days after inoculation (Figure 3). Disease evaluations on pepper plants were realized at 21-50 days after inoculation by spraying conidia [3,7,9,11,13-16] and at flowering time after inoculation by placing infected plants near to healthy plants [17-22] under greenhouse conditions. During these evaluations the genotypes, which had infected leaf areas of 40.1-51% or more, were accepted as highly susceptible [7, 18-21]. In the current study, infected leaf areas of 51% or more were as obtained with method combinations AaII, AbII, BbII at 25 days after inoculation. To our knowledge, this is the first time such a method is reported for evaluation of resistance in pepper against powdery mildew. Our method has some advantages: a- it is rapid, easy and requires little space, b- it permits testing a great number of genotypes at once, c- it is possible to organize the experiment with many replicates to justify the results, d- optimum conditions for pathogen growth can be developed, and thus, the experiment is not affected by other factors in greenhouse or field. However, moistened filter paper media and inoculation by brushing conidia had a disadvantage by causing browning on leaves.
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Conclusion

The detached leaf method reported here is suitable to evaluate resistance in pepper against powdery mildew. It has potential to be extended for other hosts of L. taurica. However, our studies have been continuing to ameliorate the moistened filter paper media and brushing conidia inoculation. In addition, further studies are needed to compare the results of detached leaves with greenhouse and field evaluations using different genotypes.

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