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Selecting basil genotypes with resistance against downy mildew

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ABSTRACT

Downy mildew on sweet basil (*Ocimum basilicum*), caused by *Peronospora belbahrii*, has become a serious problem worldwide. In Germany, approximately 50 million pots with basil are produced annually on more than 25 ha of greenhouses. There are a few registered fungicides for the control of basil downy mildew. Increasing concern about the health and environmental hazards associated with the use of fungicides has intensified the need for mildew-resistant plants. A resistance screening method was established that allows a rapid selection of *P. belbahrii* resistant *Ocimum* genotypes under reproducible and favorable conditions for disease development. First, experiments were carried out to determine the optimal conditions for infection and further disease progress. Sporangia germination was favored between 5 °C and 15 °C *in vitro*. Freshly harvested sporangia germinated at nearly 90% in contrast to a germination rate of up to 25% observed for frozen sporangia. However, inoculation of basil plants with 3×10^4 fresh or frozen sporangia mL^{-1} resulted in high disease severity 14 days post-inoculation (dpi). A temperature of 20 °C was revealed as optimal for both infection of basil with *P. belbahrii* and sporulation of the pathogen on basil leaves at high relative humidity. For evaluating resistance of basil genotypes against downy mildew, basil plants at the 4-leaf-stage were inoculated with sporangia suspension (3×10^5 frozen sporangia mL^{-1}) and incubated for 24 h at 20 °C and 100% humidity in the dark. Afterward the plants were cultivated at 23/18 °C and 60/80% relative humidity with a 12 h/12 h day/night light-cycle. Before disease severity was assessed 5 to 14 dpi, plants were incubated for 18 h at 20 °C in the dark at nearly 100% relative humidity. Using these conditions, we assessed the susceptibility of 236 *Ocimum* genotypes against downy mildew. The genotypes *O. americanum* var. *americanum*/*O. canum*, *O. americanum* × *basilicum* 'Blue Spice', *O. americanum* var. *pilosum*, *O. campechianum*/*O. micranthum* 'Peruvian basil', *O. gratissimum* and, *O. tenuiflorum* 'Tulsi' showed resistance to downy mildew. However, they represented exotic basils, differing greatly in plant morphology, aromas and taste. These resistant genotypes could become potential sources for further breeding of basil cultivars resistant to *P. belbahrii*.

1. Introduction

The genus *Ocimum* (Lamiaceae) comprises annual and perennial herbs native to the tropical and subtropical regions of Asia, Africa and America (Paton et al., 1999). Sweet basil, *Ocimum basilicum* L., is one of the most popular culinary herbs and is considered to be of the highest quality and greatest economic importance among basil species in Europe and the United States (Simon et al., 1999). The recent infra-genetic classification of *Ocimum* by Paton et al. (1999) divides this genus into three subgenera, *Nautochilus*, *Gymnocimum* and *Ocimum*. The sections *Ocimum*, *Gratissima* and *Hiantia* belong to the subgenus *Ocimum*, which comprises the most widely used species *O. basilicum*, *O. americanum* and their hybrid *O.* × *citriodorum*. *Ocimum* species are cultivated globally as culinary herbs. Furthermore, some species are essential for oil production and medicinal use (Paton et al., 1999; Simon et al., 1999), such as *O. gratissimum* belonging to the section *Gratissima* and *O. selloi* belonging to the section *Hiantia* (Paton et al., 1999). Within the subgenera *Nautochilus* and *Gymnocimum*, *O. lamiifolium*, *O. tenuiflorum* (synonym *O. sanctum)* and *O. campechianum* are the most important species that serve as ornamental herbs, and medicinal/oil producing plants (Paton et al., 1999). Based on their classification method, Paton et al. (1999) assigned a total of 64 recognized species to the genus *Ocimum*.

According to Maass (1986) the best known species *O. basilicum* is divided into two subspecies, ssp. *minimum* L., which is cultivated in India and Greece as an ornamental plant, and *O.* ssp. *basilicum*, to which the European sweet basil belongs. *O. basilicum* varieties cultivated in Europe have arisen from long-term intentional and natural selections out of specific territories. *O. basilicum* features

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many varieties and forms characterized by high intraspecific variations, such as 'Neapolitan', 'Provence', 'Greek', 'Turkish', 'Thai', 'Osmin', 'Dark Opal', and 'Genovese' known as sweet basil. The 'Genovese' type is the variety mainly used in Central Europe, especially in Mediterranean cuisine (Garibaldi et al., 2004b).

Downy mildew, caused by the biotrophic oomycete *Peronospora belbahrii* Thines (Belbahri et al., 2005; Thines et al., 2009) has become a major disease in both field-grown and greenhouse-grown basil (McGrath et al., 2010). Disease occurrence has been reported in North and South America, Africa, Europe and Asia. It was observed in countries such as the United States (Roberts et al., 2009; McGrath et al., 2010), Cuba (Martinez de la Parte et al., 2010), Argentina (Ronco et al., 2009), South Africa (McLeod et al., 2006), Cameroon (Voglmayr and Piatek, 2009), Switzerland (Heller and Baroffio, 2001), Germany (Schmidt, 2004), Italy and France (Garibaldi et al., 2004a, 2005), Hungary (Nagy and Horvath, 2011), Iran (Khateri et al., 2007), Taiwan (Chen et al., 2010) and Israel (Cohen et al., 2013). *P. belbahrii* has a devastating effect on sweet basil production and can cause up to 100% losses (Wyenandt et al., 2010). Contamination of seeds appears to be responsible for the rapid distribution of the pathogen in various production areas worldwide (Garibaldi et al., 2004b; Belbahri et al., 2005). The epidemiological propagation of the downy mildew pathogen in basil stocks can arise from single infected plants originating from infested seeds (Djalali Farahani-Kofoet et al., 2012). The outbreak, rapid spread and development of the disease within basil crops are favored by high humidity and moderate temperature conditions, which coincide with cultivation conditions in most commercial basil production (Garibaldi et al., 2007). Using seeds not infested with the downy mildew pathogen and applying fungicides before disease onset are considered necessary steps to control downy mildew on basil (McGrath, 2013). Results of an evaluation program in the United States showed that many labeled as well as not registered fungicides have provided limited suppression, demonstrating the difficulty in controlling downy mildew in basil (McGrath, 2013). In Germany, the use of fungicides in basil is limited: the number of applications of chemicals on herbs is restricted and growers have to comply with long waiting periods (6 weeks) when using systemic fungicides. To avoid initial disease outbreaks, seed treatment with the active ingredients fludioxonil and metalaxyl-m has become more common in Germany.

An effective and consumer friendly method of controlling downy mildew disease is the cultivation of resistant cultivars. Currently, there are no sweet basil cultivars available that are resistant to basil downy mildew, although other species within the genus *Ocimum*, with its several related species belonging to spice and medicinal plants, can be a source for resistance breeding (Wyenandt et al., 2010). These species vary considerably in morphology, such as growth habit, leaf color, stem texture, as well as aroma and flavor (Simon et al., 1999). As a first step, mildew-resistant basil genotypes that can be found within the genus *Ocimum* must be identified. In field evaluations in the United States (New Jersey), Wyenandt et al. (2010) determined that *O. basilicum* was the most susceptible species among all *Ocimum* spp. evaluated. Importantly, they detected least susceptibility in *O. citriodorum* cv. Lemon and resistance in *O. americanum* × *O. basilicum* cv. Blue Spice, *O.* sp. cv. Spice and *O. basilicum* cv. Blue Spice F1. Disease expression under field and in greenhouse conditions can vary significantly, because both frequency of infection and inoculum density cannot be determined accurately. Importantly, weather conditions can also influence disease incidence and development of basil downy mildew, especially through key factors such as leaf wetness intensity and duration. The impact of these factors on disease development can be excluded when susceptibility of genotypes against the pathogen is assessed under controlled conditions using an aggressive downy mildew isolate at distinct inoculum densitiy. The selection of resistant varieties is a precondition for the development of improved resistant basil varieties in future breeding programs. Hence, the objective of our study was to evaluate the resistance/susceptibility of basil genotypes against the downy mildew pathogen *P. belbahrii* under reproducible controlled conditions. For this purpose, we established a method that allows the rapid screening of a large number of genotypes and progenies under favorable conditions for disease development. Detailed knowledge about the impact of environmental factors on pathogenesis of *P. belbahrii* on basil is still lacking. Therefore, we first focused our study on the impact of temperature on sporangia germination *in vitro*, of post-inoculation temperature and of inoculum density on disease progress.

2. Material and methods

2.1. Pathogen inoculum production and inoculation

All experiments were carried out with the *P. belbahrii* isolate PA06 which was initially isolated from diseased sweet basil plants and kept in a freezer (see below). This isolate was found to be highly virulent on basil (*O. basilicum* var. *basilicum* cv. Bavires, GHG Saaten, Aschersleben, Germany) in previous tests comparing the virulence of 12 *P. belbahrii* isolates obtained from various basil production areas in Germany. No significant differences in aggressiveness between the 12 isolates were observed (data not shown). Due to the obligate biotrophic nature of *P. belbahrii*, the inoculum was routinely produced on living basil plants (*O. basilicum* var. *basilicum* cv. Bavires, GHG Saaten, Aschersleben, Germany) as follows: Basil seeds were sown in pots (four seeds per pot, Ø 9 cm, 180 mL) containing the substrate [Fruhstorfer Einheitserde Typ P, Germany; chemical analysis (mg per 100 g): N = 75, P = 75, K = 125; pH 5.9] and were initially cultivated under greenhouse conditions (min. temperature of 18 °C and max. temperature of 23 °C; 60–70% humidity) until the 4-leaf-stage and further under controlled conditions (23/18 °C and 60%/80% relative humidity with a 12 h day/night-cycle and a light intensity of 500 μmol m^{-2} s^{-1}) in growth chambers (York, Mannheim, Germany). Four plants grown in one pot were inoculated by spraying 5 mL of a sporangia suspension (3×10^5 sporangia mL^{-1}) on the leaf surface until run off at 4-leaf-stage using a plastic trigger sprayer. Inoculated plants were exposed to 20 °C and 100% humidity for 24 h in the dark. To ensure 100% humidity, crucial for both sporangia germination and infection, the plants were covered with plastic hoods, their inner surface being sprayed with water. Basil leaves with freshly developed sporangia were harvested from plants (6-leaf-stage) to obtain pathogen inoculum. Harvested leaves with sporangia were either used immediately or put in plastic bags (1 L) and frozen at −20 °C in a freezer (Liebherr Premium no-frost GSN 3336, Germany) for storage until use.

For producing sporangia suspensions fresh or frozen leaves were suspended in tap water. The number of sporangia was microscopically counted (Zeiss Axioskop 2, Germany) using a haemocytometer and adjusted to densities corresponding to 3×10^3, 3×10^4 and 3×10^5 sporangia mL^{-1} by dilution with tap water.

2.2. Assessment of downy mildew disease severity

In all experiments, DS was evaluated based on the percentage of sporulating leaf area according to Kofoet and Fischer (2007). The DS of four inoculated leaves per plant was assessed and the response of the four leaves from a single plant was averaged. DS was rated in eight categories: 1 = 0% (no symptoms, no sporulation); 2 = <0.1%; 3 = 0.2–5%; 4 = 5.1–10%; 5 = 10.1–30%; 6 = 30.1–60%; 7 = 60.1–90%