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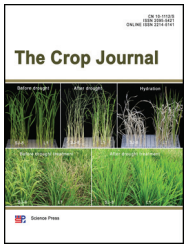

Identification of stably expressed QTL for resistance to black shank disease in tobacco (*Nicotiana tabacum* L.) line Beinhart 1000-1

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ABSTRACT

Cigar line Beinhart 1000-1 has effective durable resistance to black shank (BS) and is considered one of the most resistant sources in tobacco (*Nicotiana tabacum* L.). To investigate the inheritance and identification of stable quantitative trait loci (QTL) for BS response, F_2, BC_1F_2 individuals and $BC_1F_{2:3}$ lines were produced from a cross between Beinhart 1000-1 and Xiaohuangjin 1025. Two major quantitative trait loci (M-QTL) named *qBS7* and *qBS17* were repeatedly detected under different conditions. QTL *qBS7* was mapped to the region between PT30174 and PT60621 and explained 17.40%–25.60% of the phenotypic variance under different conditions. The other QTL *qBS17* in interval PT61564–PT61538 of linkage group 17 was detected in a BC_1F_2 population in the field and in $BC_1F_{2:3}$ in both the field and at the seedling stage, explaining 6.90% to 11.60% of the phenotypic variance. The results improve our understanding of the inheritance of resistance to BS and provide information that can be used in marker-assisted breeding.

1. Introduction

Black shank (BS) is a fungal disease caused by the soil borne oomycete *Phytophthora nicotianae* (van Breda de Haan) and is one of the most important diseases affecting tobacco (*Nicotiana tabacum* L.) production in many tobacco-producing areas [1]. *Phytophthora nicotianae* attacks tobacco roots, stems and leaves at all growth stages, leading to wilting, chlorosis, root and stem necrosis, stunting, and even plant death [2]. To date, strategies for plant disease prevention and control include genetic resistance, crop rotation and application of fungicides [3–5]. Resistance is an economically attractive approach when it can be incorporated into varieties without significantly affecting yield and quality characteristics.

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Physiological races 0 and 1 [6–9] are the predominant pathogenic races and are widely distributed throughout China and other major tobacco cultivation areas [10,11]. Shifts from race 0 to race 1 occurred when the *Php* or *Phl* gene was introgressed into *N. tabacum* [12–14]. Other described races such as race 2 and race 3 also cause BS [15–18]. The sources of resistance investigated to improve resistance to black shank (BS) exhibit either monogenic resistance or polygenic resistance in cultivated tobacco. Genes *Php* and *Phl* introgressed into *N. tabacum* from *Nicotiana plumbaginifolia* Viv. and *Nicotiana longiflora* Cav., respectively, confer immunity to *P. nicotianae* race 0. However, these resistance factors do not confer resistance to other pathogen races. Polygenic resistance most likely derived from the cigar variety Florida 301 [19] is partial in effect and non-race specific [20,21]. This type of resistance is currently present in commercial flue-cured and burley tobacco varieties [14,19].

Cigar tobacco line Beinhart 1000, derived from a selection of tobacco variety Quin Diaz reportedly has the highest reported level of BS resistance [1]. This line has been studied genetically [22–24].

Beinhart 1000-1 (hereafter referred to as BH) is a selection from Beinhart 1000 that has consistently shown black shank resistance equal to that of Beinhart 1000 [25,26], and is considered an important tobacco variety with high resistance to BS [27,28]. Heggestad and Lautz [29] reported that BH showed higher resistance than the cigar-wrapper variety Rg and Beltsville breeding lines with resistance derived from *Nicotiana plumbaginifolia*. Nielsen [30] evaluated BH response to black shank at eight locations in seven countries and it performed with the best resistance at all locations.

The combination of quantitative trait loci (QTL) mapping and molecular marker-assisted selection (MAS) is a good alternative strategy for improvement of complex traits [31]. Previous QTL mapping studies of resistance to BS in tobacco focused on the highly resistant resources Beinhart 1000 and Florida 301. Vontimitta and Lewis [23,24] detected two major QTL associated with resistance in a recombinant inbred line (RIL) population derived from a Beinhart 1000 × Hicks cross. The QTL located between markers PT61472 and PT30174 was repeatedly detected, explaining 24.7%–54.7% of the phenotypic variance in different environments. The second QTL associated with resistance to BS located between markers PT61373 and PT51164 explained 16.8%–20.4% of the phenotypic variance. Xiao et al. [19] conducted a QTL mapping study of resistance in line Florida 301. The major QTL between PT61472 and PT30174 derived from Beinhart 1000 was also found in Florida 301, suggesting that Beinhart 1000 and Florida 301 shared the same major locus.

Tobacco breeders have usually believed that the genetic bases of resistance to BS in Beinhart 1000 and BH were similar due to their shared origins and similar BS responses. The two sources are often confused and treated as the same material in tobacco breeding, particularly in China [32,33].

The primary objective of this study was to identify the genetic architecture and stable QTL of resistance to BS in a population derived from a Beinhart 1000-1 × Xiaohuangjin 1025 cross, and set a preliminary foundation for further study of the underlying mechanisms of BS resistance and for molecular-assisted selection (MAS) in breeding programs.

2. Materials and methods

2.1. Plant materials

Beinhart 1000-1 (hereafter referred to as BH, P_1), a selection of Beinhart 1000 expressed a high level of resistance to BS and was used as the female parent for this study. The flue-cured variety Xiaohuangjin 1025 (hereafter referred to as XHJ, P_2) developed by the Tobacco Research Institute, Chinese Academy of Agricultural Sciences, was used as the susceptible male parent and check (CK). An F_2 population was produced from a single BH × XHJ F_1 individual and the F_1 plant was also backcrossed to XHJ to develop a BC_1F_1 population with 100 plants. In 2012, 220 F_2 individuals were used in bulked segregant analysis (BSA) to detect markers associated with BS response. According to the results of QTL mapping, one BC_1F_1 plant was selected by MAS and sown to obtain a BC_1F_2 population with 120 plants. The corresponding BC_1F_2 plants were separately bagged to produce selfed $BC_1F_{2:3}$ lines (Fig. 1).

2.2. Pathogen isolation and inoculum preparation

The Plant Protection Laboratory of the Chinese Academy of Agricultural Sciences provided an isolate of race 0 of *Phytophthora nicotianae* for disease response tests. The isolate was maintained on oatmeal glucose agar medium at 28 °C in darkness for 7–10 days. Inoculum was prepared as described by Vontimitta and Lewis et al. [23] with minor modifications. A single agar plug (5 mm diameter) was removed from the actively growing edge of the oatmeal glucose agar culture and placed in a 500 mL glass flask containing sterilized millet (*Setaria italica*) grain. After 18 days, the millet seeds that were by then completely covered by hyphal growth were used for inoculation.

2.3. Inoculation and phenotyping

The F_2 population, BC_1F_1 populations and BC_1F_2 populations, along with the parental lines, were planted in BS nurseries at the Jimo Experimental Station, Tobacco Research Institute, Chinese Academy of Agricultural Sciences, Qingdao in Shandong province (35.4°N, 119.3°E, 15 m altitude), with a row length of 10 m, row spacing of 1.2 m and plant distance of 0.5 m. Forty days after transplanting, approximately 5 g millet grains colonized by *Phytophthora nicotianae* 0 race was added to the soil near each plant and then lightly covered with soil for inoculation.

The $BC_1F_{2:3}$ populations were tested in the field at Jimo Experimental Station and as seedlings in a growth chamber. The field evaluations were performed in a randomized complete block design with two replications. Each replication consisted of two 10-plant rows with a row length of 10 m, row spacing of 1.2 m and plant distance of 0.5 m. The inoculations and phenotypic evaluations of $BC_1F_{2:3}$ in the field were performed as described above.

The seedling evaluations were also performed in two replications. Experimental units consisted of 25 plants contained within a twenty-five-compartment segment of a 25 cm × 25 cm square plastic tray. The seeds were sown on Murashige and Skoog (1/2 MS) medium [34] under plastic domes with a 12 h/12 h light/darkness photoperiod at 25 °C

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