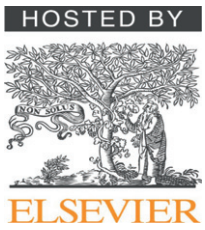
THE CROP JOURNAL 4 (2016) 344–352



Available online at www.sciencedirect.com

ScienceDirect

QTL and candidate genes associated with common bacterial blight resistance in the common bean cultivar Longyundou 5 from China

Jifeng Zhu[a], *Jing Wu*[a], *Lanfen Wang*[a], *Matthew W. Blair*[b], *Zhendong Zhu*[a], *Shumin Wang*[a,*]

[a]*Institute of Crop Science, Chinese Academy of Agricultural Sciences, Beijing 100081, China*
[b]*Department of Agriculture & Environment, Tennessee State University, Nashville, TN, USA*

ARTICLE INFO

Article history:
Received 3 May 2016
Received in revised form
28 June 2016
Accepted 7 July 2016
Available online 22 July 2016

Keywords:
Common bean (*Phaseolus vulgaris* L.)
Common bacterial blight
Quantitative trait locus
Days after inoculation

ABSTRACT

Common bacterial blight (CBB), caused by *Xanthomonas axonopodis* pv. *phaseoli* and *Xanthomonas fuscans* subsp. *fuscans* (*Xff*), is a worldwide disease of common bean (*Phaseolus vulgaris* L.). Longyundou 5, a Chinese cultivar in the Mesoamerican gene pool of common bean, displays resistance to the *Xff* strain XSC3-1. To identify the genetic mechanisms behind this resistance, we crossed Long 5 with a susceptible genotype to develop a mapping population of F_2 plants. Plant resistance to CBB was identified at 14 and 21 days after inoculation with *Xff* strain XSC3-1. A major QTL at 14 and 21 days after inoculation was mapped on chromosome Pv10 with LOD scores of 6.41 and 5.35, respectively. This locus was associated with *SAP6*, a previously-identified and much-used dominant marker, but in a 4.2 cM interval between new codominant markers *BMp10s174* and *BMp10s244*. Ten candidate genes were found between markers *BMp10s174* and *BMp10s244* on chromosome Pv10 and could encode defense response proteins responding to CBB pathogens. Four pairs each of epistatic QTL for CBB resistance were detected at 14 and 21 days after inoculation. Phenotypic variation explained by the epistatic QTL ranged from 7.19% to 12.15% and 7.72% to 8.80% at 14 and 21 days after inoculation, respectively. These results confirmed the importance of epistasis in CBB resistance in common bean. The adjacent markers found may be more efficient for marker assisted selection in common bean breeding for CBB resistance owing to their closer linkage to the target QTL.

Abbreviations: CBB, common bacterial blight; *Xap*, *Xanthomonas axonopodis* pv. *phaseoli*; *Xff*, *X. fuscans* subsp. *fuscans*; Long 5, Longyundou 5; Long 4, Longyundou 4; DAI, days after inoculation; QTL, quantitative trait loci (locus); SSR, simple sequence repeat; SCAR, sequence characterized amplified region; MAS, marker-assisted selection; RAPD, random amplified polymorphic DNA; PCR, polymerase chain reaction; ICIM, inclusive composite interval mapping; ICIM-ADD, inclusive composite interval mapping of additive QTL; ICIM-EPI, inclusive composite interval mapping of epistatic QTL; PVE, percentage of variance explained; GLP, germin like protein; NDPK, nucleoside diphosphate kinase; PK, protein kinase; LOX, lipoxygenase; CYP, cytochrome P450; PKc, protein kinase C; OxO, oxalate oxidase; SOD, superoxide dismutase; AGPPase, ADP glucose pyrophosphatase/phosphodiesterase; PPO, polyphenol oxidase; DA, dominant–additive; AD, additive–dominant; DD, dominant–dominant; SNP, single-nucleotide polymorphism.

* *Corresponding author.*
E-mail address: wangshumin@caas.cn (S. Wang).
Peer review under responsibility of Crop Science Society of China and Institute of Crop Science, CAAS.

http://dx.doi.org/10.1016/j.cj.2016.06.009

1. Introduction

Common bean (*Phaseolus vulgaris* L.) is one of the most important legumes on earth and provides many nutrients, high levels of proteins, unique carbohydrates, and essential vitamins for millions of people worldwide [1]. The annual production of dry beans is approximately 25 million tons, representing over half of the world's total food legume output in 2014 [51]. However, the production of common bean is limited by many plant diseases, such as halo blight, angular leaf spot, and CBB [2]. Of these, CBB is responsible for yield losses of 20%–60% in susceptible cultivars, especially under environmental conditions favoring disease [3].

CBB is a seedborne disease caused by *Xap* or *Xff* [4] and occurs at any developmental stage of bean [5]. The two variants have the same host range and epidemiological features and show similar biochemical phenotypes, except that *Xff* colonies produce a brown pigmentation in media [6]. However, studies of host–pathogen interactions have shown that these pathogens vary in their virulence and prevalence in the two gene pools of common bean [7–9]. Most commonly, *Xap* is associated with CBB in large-seeded bean cultivars of the Andean gene pool, whereas *Xff* is associated with CBB in both Andean and Mesoamerican bean cultivars [8]. In addition, *Xff* strains appear to be more pathogenic towards their hosts than *Xap* strains [7]. In China, common beans are cultivated mainly in the northern region of Heilongjiang province, where CBB is a severe and destructive disease. Two small-seeded bean cultivars (Long 5 and Long 4) of the Mesoamerican gene pool are the leading varieties in Heilongjiang. The black-seeded Long 4 is susceptible to CBB, whereas the white-seeded Long 5 is resistant. Pathogen strains isolated from diseased samples of Long 4 in this region were identified as *Xff* by our laboratory.

Genetic disease resistance is the most biologically safe, socially acceptable, effective, and environmentally friendly way to control bacterial, fungal, and viral plant pathogens [10]. Molecular markers for disease resistance are powerful tools for analyzing the genome and are comprehensively applied in mapping genes and MAS [11]. To date, 24 QTL conferring resistance to CBB have been identified, distributed across all 11 chromosomes of common bean [2,12–15]. Among these loci, most have been tagged with SCAR markers. Examples include *BC420* [13,16], *SU91* [17], and *SAP6* [5,18], which are linked with three major QTL of particular interest to researchers in CBB resistance [5,9–12,15,19,20].

BC420 is located on chromosome Pv06 [12] and is thought to be derived only from tepary bean (*Phaseolus acutifolius*) via the breeding line XAN159 [21]. This locus accounted for 62%–63% of phenotypic variation for CBB resistance [16,22]. Upon map-based characterization, 21 novel genes were predicted in this region, among which *BC420–CGs10* and *BC420–CGs14* showed strong associations with CBB resistance [20]. Another QTL of importance in conditioning resistance to CBB from XAN159 is the SCAR marker *SU91* [17], located on chromosome Pv08 in at least one study to date [12]. This locus explained 14%–17% of phenotypic variation for CBB resistance [10]. Sixteen genes have been identified as linked to this locus by a map-based cloning approach, among which *SU91–CGs10* and *SU91–CGs11* presented strong associations with CBB resistance [20]. A third QTL linked to *SAP6* has been found in the Mesoamerican common bean gene pool, derived from the great northern landrace cultivar Montana No. 5, and was located on chromosome Pv10 [5]. The presence of this locus accounted for 35% of the variation for resistance to CBB in an F_2 population derived from the cross of Montana No. 5 (resistant) with Othello (susceptible) [5].

In recent years, *BC420*, *SU91*, and *SAP6* have been widely used in MAS of common bean despite being dominant markers [11,12,16]. For example, the breeding line HR67 was selected for CBB resistance based on *BC420* [3], while ABCP-8 pyramided two QTL based on *SU91* and *SAP6* [23]. Researchers have developed codominant markers to replace *BC420* and *SU91* [20]; but no new codominant markers have been developed for *SAP6* and these codominant markers have not been tested for their efficiency in MAS for CBB resistance.

Considering the less codominant markers have been developed in MAS for CBB resistance, our objective in this study was to identify QTL controlling CBB resistance in a cross between the two modern cultivars Long 5 × Long 4. A detailed understanding of resistance in Long 5 will help breeders use this resistant parent to improve future cultivars. The new *Xff* strain from Heilongjiang was selected to investigate the phenotype of CBB resistance in this study because of its prevalence and because most previous studies have used the *Xap* strain to study the inheritance of the trait. Another novel aspect of this study was the use of many codominant markers rather than previously developed RAPD-derived markers for the analysis of CBB resistance. Careful physical mapping of multiple CBB resistance loci from Long 5 is useful for the dissection of new genes involved in pathogenicity and plant response. We also developed and genetically mapped new codominant SSR markers that could be used to replace older SCAR markers for MAS. The finding of multiple QTL in the Long 5 variety is useful for breeding within the white-seeded commercial class or across other Mesoamerican and possibly Andean seed types.

2. Materials and methods

2.1. Plant materials

The parents used for developing the population were Long 5 (resistant) and Long 4 (susceptible) (Fig. 1). Long 5, a dry bean with small white seeds, used as the male, was developed from a cross between Dabaidou and B-7150 and is widely cultivated in Heilongjiang Province, China. The Long 5 × Long 4 hybrids were advanced from the F_1 generation by selfing to yield an F_2 population comprising 803 individuals for fine mapping of CBB resistance. All of the plants were grown in plastic pots (23 cm × 18 cm × 18 cm) under a 14 h/10 h photoperiod at 25 °C (day) and 20 °C (night) in a greenhouse in Beijing, China.

2.2. Inoculation and phenotypic evaluation of CBB

The *Xff* strain identified as XSC3-1 was isolated from CBB-infected samples in the Heilongjiang Province of China and was used for phenotypic evaluation of the F_2 population and parental controls. Pathogenic bacteria were selected on Milk Tween agar medium for 4–5 days at 28 ± 2 °C [24], after which