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Real-time quantitative PCR based method for the quantification of fungal biomass to discriminate quantitative resistance in barley and wheat genotypes to fusarium head blight

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ABSTRACT

Fusarium head blight (FHB) caused by *Fusarium graminearum* is one of the devastating diseases of small grain crops, including barley and wheat. Breeding for resistance is one of the best and ecofriendly strategies to manage the FHB. However, the existing methods used for screening genotypes, both under field and greenhouse conditions, often resulted in high experimental error, leading to inconsistent ranking of genotypes over years. In the postgenomic era, precise assessment of resistance is crucial to identify candidate genes. Here, we report a pathogen inoculation procedure and a real-time quantitative polymerase chain reaction (qPCR) based protocol for the quantification and discrimination of quantitative resistance among barley and wheat genotypes to FHB. Using *Fusarium* specific primer pair Tri6_10, for the trichothecene biosynthetic cluster (*Tri6*) gene, we successfully quantified the relative fungal biomass in both spikelets and rachis. A qPCR of spikelets and rachis collected on 6 dpi, from inoculated three alternate spikelet regions, discriminated resistance with less experimental error than those based on the proportion of spikelets diseased (PSD) at 9 dpi. This method can be applied for medium to high-throughput barley and wheat breeding programmes to discriminate quantitative resistance among genotypes against FHB.

1. Introduction

Fusarium head blight (FHB), also known as head scab, is one of the most devastating and alarming fungal diseases of small grain crops including barley (*Hordeum vulgare* L.) and wheat (*Triticum aestivum* L.) (McMullen et al., 1997). Several species of *Fusarium* are associated with FHB in field, of which *Fusarium graminearum* Schwabe [teleomorph: *Gibberella zeae* Schw. (Petch)], is the most predominant causal agent leading to enormous yield losses (Trail, 2009). FHB infection most likely occurs in warm and humid conditions during flowering and early stages of kernel development (Gilbert and Tekauz, 2000). The fungus initially colonizes florets, spreads through the rachis to adjacent spikelets causing bleaching and shriveling “tombstone” kernels (Quirin, 2010). FHB causes severe losses in yield as infected florets and spikelets are often destroyed or, if not, the seed quality is deteriorated due to the accumulation of trichothecene mycotoxins, such as Deoxynivalenol (DON) and Nivalenol (NIV) (Bai and Shaner, 2004; Gilbert and Tekauz, 2000). DON has serious consequences on human and animal health, as the toxin causes food refusal, vomiting and acts as an immune modulator (Bai and Shaner, 2004). Enormous losses due to FHB epidemics have been reported worldwide, wherever barley and wheat are cultivated (McMullen et al., 2012). In one of the most notorious FHB epidemics in the United States during the 1990s, barley and wheat losses were estimated to be close to $3 billion (McMullen et al., 2012).

Several disease control strategies have been followed to manage FHB, such as application of fungicides, adoption of various

Abbreviations: DON, deoxynivalenol; dpi, days post inoculation; FHB, fusarium head blight; hpi, hours post inoculation; NIL, near isogenic line; NIV, nivalenol; PDA, potato dextrose agar; PSD, proportion of spikelets diseased; QTL, quantitative trait loci; qPCR, quantitative polymerase chain reaction; RIL, recombinant inbred line; *Tri6*, trichothecene biosynthetic cluster gene.

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biological and cultural practices involving crop rotation patterns, appropriate tillage practices, and collection and disposal of the infected waste residues from the initial harvest (Bai and Shaner, 2004). All these strategies however, were impractical, ineffective and contributed little to reduce FHB incidence. The plant resistance is considered to be the most efficient, cost effective and eco-friendly approach to manage FHB and the accumulation of mycotoxins (Bai and Shaner, 2004; Steffenson et al., 2003).

Resistance to FHB is highly complex and quantitative in nature. Evaluation of resistance under field conditions gets complicated due to interaction among genotypes, pathogen and the environment. Several methods such as disease incidence, disease severity, fusarium damaged kernels and DON accumulation in the grain have been routinely used to screen genotypes for resistance (Cuthbert et al., 2007). Fungal biomass has been quantified based on ergosterol, however, there was poor correlation between FHB severity and amount of fungal biomass, suggesting evaluations solely based on visual scoring of FHB severity can't be used to discriminate resistance under field conditions (Schlang and Duveiller, 2011). The correlation between the FHB severity and the amount of toxins in grains was also quite inconsistent (Bai and Shaner, 2004). Under more favorable environmental conditions for disease development, a high disease severity was associated with low FHB incidence and DON content in the grains (Ittu et al., 2008). Both wheat and barley plants can detoxify DON to DON-3-O-glucoside and thus the amount of DON remaining in the plant varies with DON detoxification rate and it is important to determine total DON produced (Bollina et al., 2011). The development of resistant cultivars is very challenging because of limited understanding of genetics of resistance and lack of cost effective means of phenotyping (Cuthbert et al., 2007; McMullen et al., 1997).

The breeding programs used several types of resistance, such as resistance to initial infection of spikelets (type-I), resistance to spread within the spike or rachis resistance (type-II), and amount of mycotoxin accumulation in the grain (type-III) to screen wheat and barley populations to identify quantitative trait loci (QTLs) to FHB (Bai and Shaner, 2004; Miller et al., 1985). However, these field screenings were often associated with high experimental errors, especially for spikelet resistance (Collard et al., 2005). These errors were mainly due to variations in inoculum availability, amount of inoculum, spore deposition, and the environment (Buerstmayr et al., 2009; Cuthbert et al., 2007). The type of florets, open or closed, also significantly affect the pathogen inoculation, thus leading to disease avoidance (Yoshida et al., 2008). In the case of wheat, discriminating rachis resistance from spikelet resistance under field conditions was difficult due to multiple primary infections of spikelets. Under high disease pressure, multiple infection sites may lead to erroneous interpretation of rachis resistance (Bai and Shaner, 2004).

Screening for FHB resistance under controlled environmental conditions, such as greenhouse and growth chamber, has been associated with reduced levels of experimental error, unlike in the field. Still the spray inoculation resulted in high experimental error due to uneven distribution of inoculum deposited on or in spikelets. Spikelets are generally resistant to *F. graminearum* infection because of a thick wax layer, and the germtube enters spikelets between lemma and palea, eventually gaining access through stomata on the inner sides of lemma and palea. Single floret inoculation in a spike has been successfully used in greenhouse conditions to evaluate rachis resistance in wheat (Zhu et al., 1999). However, single floret inoculation to assess spikelet resistance in barley, which generally has a very high level of rachis resistance, under greenhouse conditions was unable to discriminate small phenotypic variations in FHB severity based on visual assessment (Smith et al., 2004; Yli-Mattila et al., 2008). Therefore, a method that can discriminate both spikelet and rachis resistance is crucial for high throughput screening of genotypes in barley and wheat breeding programs.

Technological advancements such as metabolo-genomics are newly emerging areas for candidate gene identification, however, these studies require accurate FHB resistance phenotyping (Kushalappa and Gunnaiah, 2013). Resistance to FHB is polygenically controlled and thus highly influenced by environment, and it is imperative these must be held constant to reveal true resistance in genotypes. Real-time qPCR based methods enable rapid and sensitive detection of pathogens in plant systems or other matrices (Horevaj et al., 2011; Yli-Mattila et al., 2008). Though qPCR based methods have been employed in recent years for fungal biomass quantification, none described a protocol applicable to screen wheat and barley genotypes varying in their level of resistance with high precision and accuracy. Considering the above experimental errors normally encountered in evaluating resistance under greenhouse and field conditions, due to high variability in inoculum and the environment, here we developed a method to screen wheat and barley genotype for resistance to FHB under greenhouse conditions, with potential to be used in field conditions. The objectives were: (i) to establish inoculation and sampling methods that can discriminate genotypes varying in resistance to FHB; (ii) to develop an associated method to quantify *F. graminearum* biomass based on qPCR for the discrimination of quantitative resistance in barley and wheat to FHB. Our study is the first to report a very reliable method, based on a combination of inoculation techniques, sample collection and fusarium biomass quantification protocol, to discriminate quantitative resistance in barley and wheat, for both spikelet and rachis resistance.

2. Materials and methods

2.1. Genetic background of plant materials used

2.1.1. Barley

Two two-row, yellow barley cultivars namely CI9831 (resistant) and H106-371 (susceptible) varying in resistance to FHB (seeds provided by Dr. T.M. Choo, AAFC) were selected. H106-371 was selected from a population of 190 doubled-haploid lines developed from a cross between Léger and CI9831 (Choo et al., 2004; Kumaraswamy et al., 2011).

2.1.2. Wheat

One pair of, resistant and susceptible, recombinant inbred lines (RILs) and two pairs of, resistant and susceptible, near isogenic lines (NILs) were used, in separate studies. All the three pairs of genotypes were reported to have mainly rachis resistance, and some spikelet resistance as the NILs had susceptible background (McCartney et al., 2007; Somers et al., 2003, 2005). The RILs were derived from a cross between BW-278 (AC Domain*2/Sumai 3, FHB resistant) and AC Foremost (HY320*5/BW553//HY320*6/7424-BW5B4, FHB susceptible) (Cuthbert et al., 2007). Sumai-3 was the source of resistance, from which the resistant parent BW-278 was derived. The RILs were designated as RIL-R (R = resistant) and RIL-S (S = susceptible). It contains QTL loci Fhb2, localized on the short arm of chromosome 6B, one of the major QTLs conferring rachis resistance to FHB.

The NIL-1, with FHB susceptible/resistant alleles (Somers et al., 2003), was derived from a cross between BW301 × HC374 (McCartney et al., 2007; Somers et al., 2005). It contains QTL 2DL on the chromosome 2DL and is one of the major QTLs, associated with rachis resistance to FHB. The BW301 is FHB susceptible hard red spring wheat line from western Canada and HC374 is resistant to FHB (derived from the cross Wuhan1 × Nyubai). FHB resistance in HC374 is coming from Wuhan1.