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Cost-Effective Markers and Candidate Genes Analysis at Wheat MQTL Loci †

Rosa Mérida-García [1,*], Sergio Galvez [2], Etienne Paux [3], Gabriel Dorado [4], Laura Pascual [5], Patricia Giraldo [5] and Pilar Hernandez [1,*]

[1] Institute for Sustainable Agriculture (IAS-CSIC), Consejo Superior de Investigaciones Científicas (CSIC), Alameda del Obispo s/n, 14004 Córdoba, Spain
[2] Department of Languages and Computer Science, ETSI Informática, Campus de Teatinos, Universidad de Málaga, Andalucía Tech, 29071 Málaga, Spain; galvez@uma.es
[3] Université Clermont Auvergne, INRAE, GDÈC, 5 Chemin de Beaulieu, 63000 Clermont-Ferrand, France; etienne.paux@inrae.fr
[4] Departamento de Bioquímica y Biología Molecular, Campus de Rabanales C6-E17, Campus de Excelencia Internacional Agroalimentario (ceiA3), Universidad de Córdoba, 14071 Córdoba, Spain; bb1dopeg@uco.es
[5] Department of Biotechnology-Plant Biology, School of Agricultural, Food and Biosystems Engineering, Universidad Politécnica de Madrid, 28040 Madrid, Spain; laura.pascual@upm.es (L.P.); patricia.giraldo@upm.es (P.G.)
* Correspondence: rmerida@ias.csic.es (R.M.-G.); phernandez@ias.csic.es (P.H.)
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Abstract: High-resolution melting analysis (HRM) is a resolutive technique, using PCR amplification and in-tube detection, which is based on the PCR product's melting analysis. It is a promising technique for breeding analysis, as it does not require dedicated sequencing equipment. It can be performed using QRT-PCR equipment that can be available in small-medium molecular biology laboratories or locally by the breeders, and it does not require an electrophoretic step to analyze the amplified DNA fragments. To develop effective HRM assays, the search for highly polymorphic sites amenable to PCR amplification is a prerequisite, which is not an easy task in wheat due to its genome complexity. The insertion site-based polymorphism markers (ISBP) are PCR markers designed based on the knowledge of the sequence flanking transposable element (TE) sequences. The two PCR primers are designed one in the transposable element and the other in the flanking DNA sequence. TEs are very abundant and nested in the wheat genome, with unique (genome-specific) insertion sites that are highly polymorphic. In this work, we analyze the available HRM-ISBP assays for wheat 3B and 4A chromosomes, and update their applications in wheat diversity at drought and heat MQTL Loci.

Keywords: ISBP markers; high-resolution melting; drought; candidate genes; MQTL.

1. Introduction

Wheat is considered one of the most important crops worldwide [1]. Its development and final yield are affected by abiotic stresses as drought [2,3] and heat [4,5], whose effects are being increased as consequence of predicted climate change [6,7]. As result, tolerance to these abiotic stresses are important aims in plant breeding to increase crops production [8]. In this way, molecular markers as ISBP, can be developed and applied to identify genomic regions and genes of interest closely related to interesting traits as drought and heat tolerance [9]. ISBP are PCR markers designed based on the information of sequence flanking TE sequences [10]. They appeared as powerful and interesting tools to apply in genomic and genetic studies in wheat [10–12], and have been also used in marker-assisted selection (MAS) and as selecting tools in plant breeding programs [10]. These markers have been designed for several wheat chromosomes [9,11,13–16] and applied with different aims [17–21]. Mérida-García et al. [9] developed HRM assays based on ISBP markers for wheat chromosomes 4A

and 3B. Both chromosomes contain interesting QTLs related to biotic and abiotic stresses tolerance, and important agronomic traits, and some of the ISBP markers located within or in proximity to previously described MQTLs [22]. The meta-QTL analysis was developed to integrate results from individual QTL studies into a common dataset to identify and corroborate the interval of QTL regions [23]. MQTL analysis has been performed in wheat in several studies for root-related traits [24], grain weight [25,26] or heat and drought conditions [22,27]. In this presentation, we have assessed and updated the polymorphic HRM-ISBP assays developed for wheat chromosomes 4A and 3B, regarding their applications in wheat at drought and heat stresses MQTL loci.

2. Experiments

2.1. Insertion Site-Based Polymorphism Markers

ISBP markers were developed by Mérida-García et al. [9], using the IsbpFinder programme [10] to locate the ISBPs and Primer3 (http://primer3.sourceforge.net) for primer design. ISBP markers for wheat chromosome 3B were designed using BAC-end sequences as described in Paux et al. [10]. PCR setup and HRM analysis is described in Mérida-García et al. [9].

2.2. Candidate Genes

ISBP markers were mapped and blasted against the RefSeq v1 [15] as described in Mérida-García et al. [9]. Candidate genes for markers for wheat chromosomes 4A and 3B were assessed within a window of +/−20 kb and +/−300 kb (due to the reduced density of genes found for 3B), respectively, of marker's hit in the pseudomolecule [15] gene model annotation. ISBP marker positions were compared to the wheat MQTLs described in Kumar et al. [27] for drought tolerance. The position of MQTL was determined with flanking markers [27] and using BLAST against the RefSeq v1 [15].

2.3. Gene Expression Analysis

Gene expression analysis was performed using the information previously published by Liu et al. [29] [experimental seedling samples grown under controlled conditions (NCBI SRA ID SRP045409): control (IS), heat and PEG induced drought stress for 1 and 6 h (PEG1 and PEG6, respectively)], Ma et al. [30] [experimental samples grown in a shelter (NCBI SRA ID SRP102636): anther stage irrigated leaf phenotype (AD_C), anther stage drought-stressed leaf phenotype (AD_S), tetrad stage irrigated developing spike phenotype (T_C), and tetrad stage drought-stressed developing spike phenotype (T_S)], and Gálvez et al. [31] [flag leaf samples from field grown plants (NCBI SRA ID SRP119300): irrigated (IF), mild stress (MS), and severe stress (SS)]. This information was applied to construct gene expression heatmaps, using data retrieved from Wheat Expression (www.wheat-expression.csom/) and the R package 'NMF 0.21.0' [31]. Transcripts per kilobase millions (TPMs) of genes under each condition and differential gene expression analysis were performed on the raw data using the RefSeq v1 [15] gene models and two bioinformatic pipelines [9].

3. Results and Discussion

A recent wheat MQTL analysis [27] described a novel drought tolerance MQTL (MQTL3), which is mainly placed in the wheat chromosome 3B centromeric region (Figure 1). Akhunov et al. [32] and Munkvold et al. [33] highlighted a positive gradient of gene density from the centromere to the telomeres in wheat, which is consistent with the low presence of ISBP markers found in the MQTL3 [27] proximities. Two ISBP markers (HRM3B_331497483 and HRM3B_465802537 [9]) for the wheat chromosome 3B were found surrounding this MQTL (58 and 52Mb, respectively). Marker HRM3B_465802537 was previously located within MQTL26 (previously described by Acuña-Galindo et al. [22]) and mapped to two interesting genes (TraesCS3B01G290200 and TraesCS3B290300) by Mérida-García et al. [9], who highlighted they encode a glycosyltransferase and ABC transporter B family protein, respectively. Both are related to plant responses to abiotic stresses [34,35] and both genes were found up-regulated under different drought conditions [9].

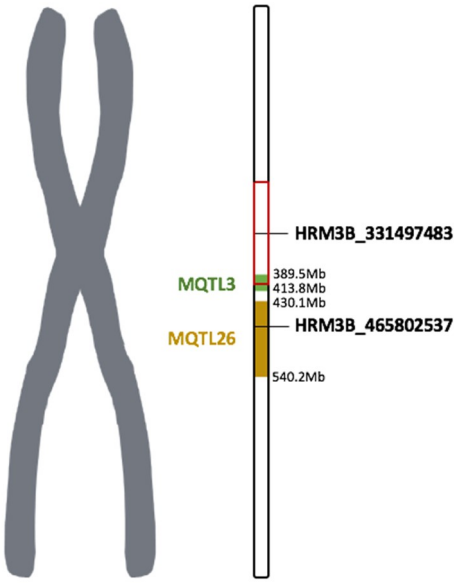

Figure 1. Location of MQTL3 [27]**, MQTL26** [22] **and the surrounding ISBP markers found in wheat chromosome 3B.** MQTLs flanking positions are indicated in Mb (megabase); Centromere region in red color, was delimited by the genome positions indicated in [15].

Within MQTL3 [27] we have found 269 HC and LC genes (Appendix A, Supplementary Table 1), of which 31 and 8 genes respectively, were found differentially expressed under different drought stress conditions (Figure 2). The gene TraesCS3B01G246000 encodes a MYB-related transcription factor, which plays a crucial role in the control of plant-specific processes as responses to abiotic and biotic stresses [36]. This gene was found down-regulated under PEG treatments (Supplementary Table 1 and Figure 2), which agrees with previous studies that indicated the expression of many MYB genes is regulated by drought [37]. The gene TraesCS3B01G246300 encodes an ATP-dependent zinc metalloprotease FtsH, some of which have been proposed as contributors to stress responses in plants [38] and also related to photosynthesis and protein stability [39]. This gene was found up-regulated under heat and PEG induced drought (PEG1 and PGE6) conditions (Supplementary Table 1 and Figure 2), which is in agreement with previous studies in wild barley in response to heat stress conditions [39]. The gene TraesCS3B01G251100 encoding a 3′-N-debenzoyl-2′-deoxytaxol N-benzoyltransferase, which has been related to cellular biogenesis and its overexpression promotes the increase of root growth in maize [40]. In this regard, this gene was found up-regulated under PEG treatments (Supplementary Table 1 and Figure 2).

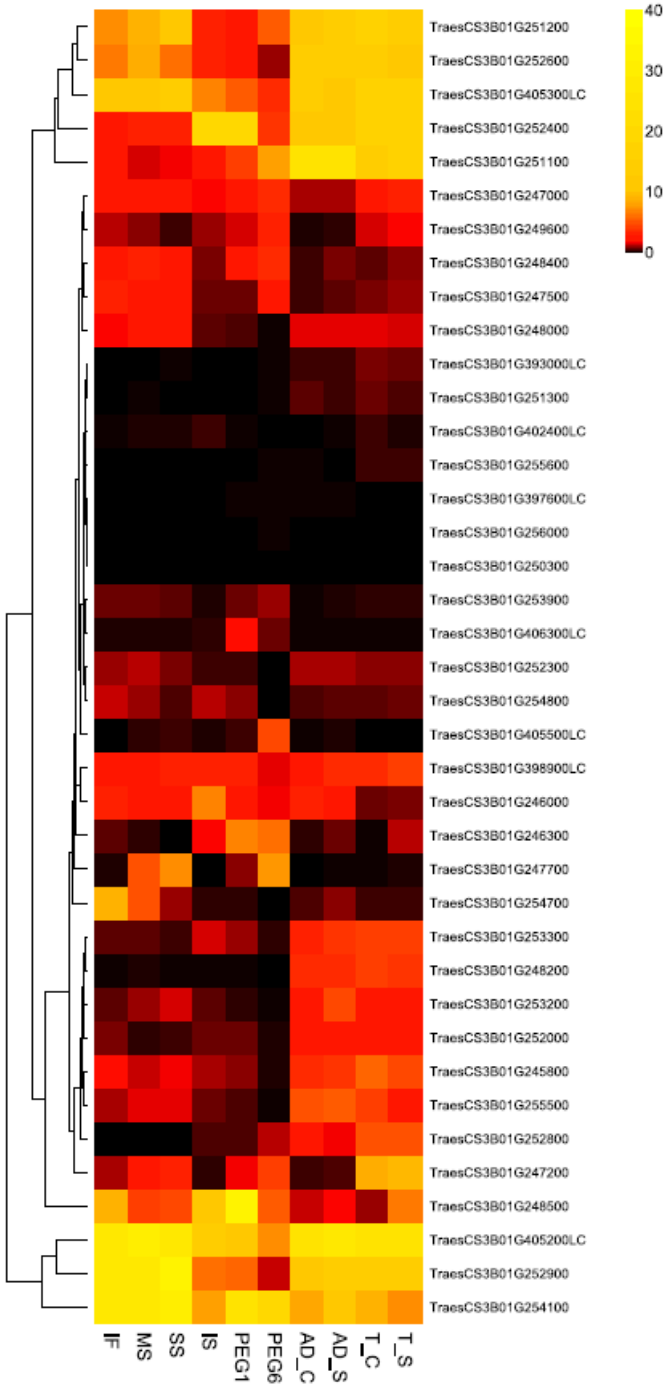

Figure 2. Heatmap for gene expression analysis under several stress conditions for the candidate genes found within wheat MQTL3 [27] that were differentially expressed under drought conditions. IF: irrigated field conditions; MS: mild stress conditions; SS: severe stress conditions [31]; IS: seedling PEG shock control; PEG1: seedling 1h PEG stress; PEG6: seedling 6h PEG stress [29]; AD_S: anther stage irrigated shelter phenotype; AD_S: anther stage drought stressed shelter phenotype; T_C: tetra stage irrigated shelter phenotype; and T_S: tetrad stage drought shelter phenotype [30].

Author Contributions:. S.G. performed bioinformatics analyses. R.M.-G., S.G., E.P., G.D., L.P., P.G., and P.H. analyzed the results. R.M.-G. and P.H. drafted the manuscript. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Abbreviations

The following abbreviations are used in this manuscript:

HRM	High Resolution Melting
MQTL	Meta-Quantitative Trait Loci
TE	Transposable Element
ISBP	Insertion Site-Based Polymorphism
bp	base pairs
Mb	megabase

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