Dormancy in white-grained wheat: Mechanisms and genetic control

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INTRODUCTION

Although dormancy in white-grained wheats is relatively rare, genotypes with dormancy ranging from intermediate to dormant have been reported [1]. Despite this, dormant red-grained wheat is observed to be almost always more dormant than dormant white-grained wheat [1, 2]. A QTL located on chromosome 4A [3] was associated with dormancy in white-grained wheats of diverse origin whilst chromosome 4A has also been reported to contain the gene controlling embryo sensitivity to abscisic acid (ABA) [4]. In red-grained wheats, the red colour (*R*) genes, located on the distal end of the long arm of the homoeologous group three chromosomes, contribute to dormancy [5], possibly through an interaction between the *R* gene(s) and gene(s) on these chromosomes or chromosome 4A [6]. It is possible that the dormancy mechanism in white-grained wheat may have similarities to red-grained wheat.

This study examines a double-haploid population derived from white-grained dormant (SUN325B) x intermediate-dormant (QT7475) genotypes. These genotypes both contain the 4A QTL (derived from the same source), however QT7475 appears to lack the other dormancy gene(s) required to give the dormancy phenotype. The aim of this investigation was to locate QTLs other than 4A, in particular QTL associated with the putative seed coat factor, and to determine if there is any relationship between the chromosomal location of the seed coat factor in white-grained wheats and the *R* genes in red-grained wheats.

The *R* gene has been shown to be a transcription factor of the flavonoid biosynthesis pathway [7]. Based on these similarities, it seems possible that the mechanism of the seed coat factor in dormant white-grained wheat genotypes might involve an accumulation, but not polymerisation, of the flavonoid precursors of the red pigment. Himi *et al*. [8] have already shown that the genes controlling key enzymes required for synthesis of the red pigment are not expressed in the seed coat of non-dormant white wheats.

A comparison was undertaken of the expression of key genes in the flavonoid biosynthesis pathway in the seed coat of dormant and non-dormant genotypes. Dihydroflavonol 4-reductase (*DFR)* and chalcone synthase (*CHS)* are genes encoding major enzymes in the flavonoid biosynthesis pathway and the expression patterns of these genes were studied in the developing seed coats of four genotypes of differing dormancy. Developing grains at 10 and 15 days post anthesis (dpa) were selected as the time points for this analysis, based on the report by Himi and Noda [7].

In addition to gene expression analysis, direct evidence for the synthesis of flavonoids was examined using HPLC in mature and developing seed coat material. HPLC analysis was conducted in both red and white-grained wheat, with the red-grained wheat genotype serving as a positive control.

MATERIALS AND METHODS

The wheat genotypes, Hartog (white-grained, non-dormant), QT7475 (white-grained, intermediate-dormant), SUN325 (white-grained, dormant) and R/W635 (red-grained, dormant) were used in this study. For the reciprocal crosses, with all possible combinations, involving Hartog, QT7475 and SUN325B were prepared in three successive years under glasshouse conditions. F1 grain was harvested when all green colour had just disappeared from the spike, leaves and stems of the maternal plants, dried for 5 days then stored at –20°C until required for germination tests. Dormancy assays were conducted according to Mares and Mrva [9]. Dormancy was expressed by a germination index (GI), weighted according to grains which germinate rapidly, and is calculated from a standard formula [10]. For the QTL study, 96 doubled haploids derived from the population SUN325/QT7475, both of which contained alleles from the same dormant parent at the chromosome 4A dormancy QTL, were sown in four separate experiments (3 field and 1 glasshouse) over two years.

DNA was extracted from the parents and each of the DH lines using a DNA mini-prep method [11]. SSR markers close to the QTL located on chromosomes 3A, 3B and 3D were selected and tested for the possible linkage. Molecular and dormancy data for each of the lines were entered into Map Manager QTXb19 [12] for linkage and interval analyses.

The genotypes Hartog, QT7475, SUN325 and R/W635 were grown in a glasshouse, at anthesis spikes were tagged and single grains harvested at 10 and 15 dpa. Grains were extracted, and the embryo removed and discarded. The seed coat was then sliced through the dorsal side, exposing the developing endosperm which was subsequently scraped away with a spatula. For detection of flavonoid compounds, grains were harvested at 10, 15, 20, 25, 30 dpa. Primers were designed to conserved regions of published sequences for *CHS* in grass species. *GAPDH* was used as control gene.

RESULTS

Dormancy of grains derived from reciprocal crosses

Reciprocal crosses were performed using parents with non-dormant (Hartog), intermediate-dormant (QT7475) and dormant (SUN325) phenotypes. The dormancy phenotype of F_1 grains was influenced by both maternal and paternal genotype but in all cases where one parent was the non-dormant cultivar, Hartog, the resultant F_1 grains were also non-dormant (Fig. 1). In summary, Hartog x QT7475 or Hartog x SUN325B = Hartog self (non-dormant) = QT7475 x Hartog and SUN325B x Hartog, where the first named parent was the female. By contrast, QT7475 x SUN325B = or < QT7475 self whilst SUN325B x QT7475 was not significantly different from SUN325B self (Fig. 1).

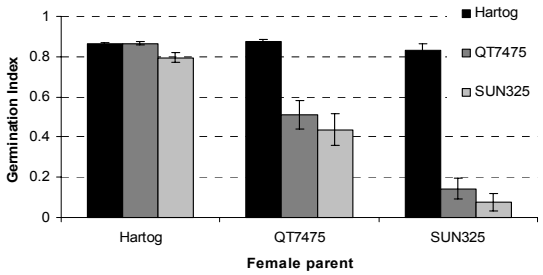

Figure 1. Germination Index (GI) of F1 progeny.

QTL Analysis

Microsatellite markers targeting chromosomal regions such as the long arms of group 3, 5BL and 2A, previously reported to be associated with dormancy, were examined in detail for linkage to dormancy phenotype. Only the 3BL markers gave a significant LOD score of 3.2, averaged across all environments. Marker analysis of individual genotypes detected six polymorphic SSR markers, wmc418, wmc527, gwm77, gwm285, wmc307 and gwm66 located in a region of 78.6cM on the long arm of chromosome 3B.

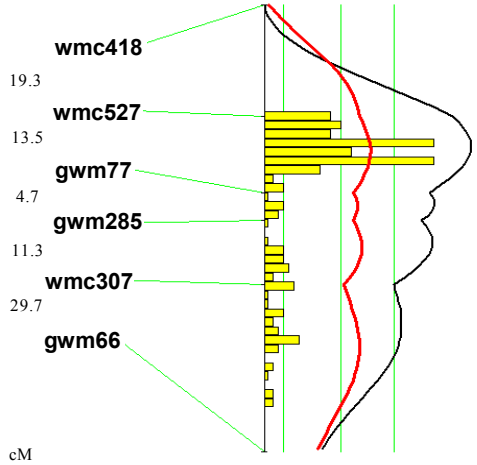

Figure 2. Partial interval map of QTL associated with grain dormancy on chromosome 3BL. The vertical lines indicate suggestive (P=0.63), significant (P=0.05) and highly significant (P=0.001) thresholds for LRS. Average LRS was 14.7, cM=centimorgan.

The QTL was consistently significant across all of the environments with maximum likelihood ratio statistic (LRS, [12]) values ranging from 11.7 (glasshouse 2003) to 17.7 (field 2003) and the averaged phenotypic data from the field experiments was used to generate the interval map (Fig. 2). The SSR markers wmc527 and gwm77 flanked the highly significant QTL (P<0.05) and the marker gwm77 explained 17.2% of the trait variation. There was also some evidence of another weakly linked QTL on chromosome 3A, with a LOD score of 1.7, but this was probably due to the lack of polymorphisms in the SSR markers available for this region in this population.

Expression of chalcone synthase (*CHS*)

All genotypes expressed *CHS* at 10 dpa. However, by 15 dpa, *CHS* expression was only detected in the seed coat of dormant red-grained and dormant white-grained genotypes (Fig. 3). No expression was detected in the seed coat of the non- and intermediate-dormant genotypes.

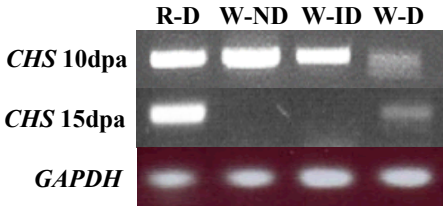

Figure 3. PCR amplified cDNA fragment of *CHS* in genotypes at 10 and 15 dpa. R-D (R/W635), W-ND (Hartog), W-ID (QT7475), W-D (SUN325).

HPLC analysis of seed coat extracts

Fig. 4 shows the amount of flavone-*C*-glycosides (FCG) in the extract from the seed coat of developing grains. Statistical analysis of these peak area values from the three replications showed that there was a significant difference between the amount of the major flavone-*C*-glycoside in SUN325 and the other genotypes at each time point.

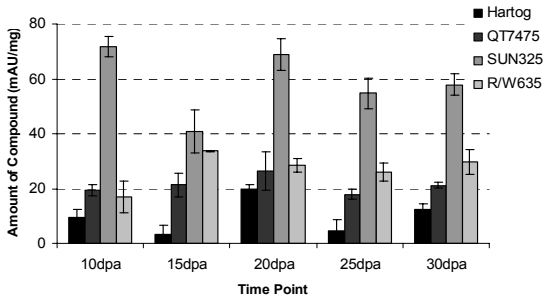

Figure 4. Flavone-*C*-glycosides content (mAU/mg) of the seed coat from developing grains

DISCUSSION

A model involving interaction between embryo sensitivity to ABA and a seed coat factor has been proposed to explain dormancy in white-grained wheat [13]. According to this model, complete dormancy only occurs with the expression of both of these factors, while

the embryo factor on its own confers only an intermediate level of dormancy. The presence of dormancy genes expressed in the seed coat which interact with the embryo sensitivity factor has also been reported in red-grained wheats, but in this instance the seed coat effect is thought to be associated with the *R* genes controlling red seed coat colour [6, 14]. The F1 progeny derived from the reciprocal crosses showed dormancy phenotypes that were in agreement with the proposed model that there are both embryo and seed coat factors involved in grain dormancy expression (Fig. 1).

The double haploid population, SUN325/QT7475 is homozygous for dormancy alleles at the 4A QTL that has previously been associated with embryo sensitivity to ABA [4] and grain dormancy in a number of mapping populations [3, 4]. The additional QTL on the long arm of chromosome 3B identified in this population (Fig. 3) was consistent across all of the environments and explains a significant proportion (LRS of 17.7) of the trait variation in the population. The 3BL region is therefore a likely candidate for the dormant seed coat factor. QTL regions relating to dormancy have also been found on group three chromosomes in other studies, for example Groos et al., [15] who detected a region on 3BL, Mori et al., [16] on 3AS and Kulwal et al., [17] on 3AL. Mares et al., [3] also suggested that there are possible QTL regions on the group three chromosomes. Despite this, there are other genes on the group three chromosomes which are related to dormancy and may be interfering factors with these studies.

In this study there was an increased quantity of FCG, compounds derived from the flavonoid pathway, which were most evident in the dormant white-grained genotype, SUN325. These compounds are not intermediates of the pathway leading to phlobaphene but rather are the products of an alternate branch in the pathway initiated with the common precursor, naringenin. CHS plays an integral role as the first step in the flavonoid biosynthetic pathway and generates important precursors and substrates for proceeding enzymes [18]. *CHS* was expressed in all genotypes at 10 dpa, but at 15 dpa was only expressed in the dormant genotypes, SUN325 and R/W635. The expression pattern in the red-grained control genotype was consistent with a previous study [19] and the deposition of red pigment. Whilst these observations do not prove that the biosynthetic pathway is fully functional, except in R/W635 where the accumulation of red pigment is an indicator of enzyme activity, they are nevertheless consistent with the possibility that specific compounds could accumulate in the seed coat of the white-grained genotypes and play a role in dormancy.

The observation that FCG compounds are present at a higher concentration in the seed coat of the dormant genotype SUN325, suggests that these compounds may have a function in dormancy. It is possible that FCG are a component of the dormant seed coat factor, as they are highly water-soluble compounds and may be dissolved in the seed coat and transferred to the embryo during imbibition. Despite this, there was not a paralleled increase in FCG in the seed coat dormant red-grained genotype, which would be expected if the dormancy mechanism was similar. An interaction between a factor produced by the dormant seed coat and embryo sensitivity to ABA is consistent with the dormancy model. The role of FCG compounds as the dormancy factor is unclear, but it is possible that there is a distinct FCG compound unique to both R/W635 and SUN325, which may interact with the dormant embryo during early imbibition.

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