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ORIGINAL ARTICLE

QTLs for uniform grain dimensions and germination selected during wheat domestication are co-located on chromosome 4B

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Abstract
Key message **A major locus on the long arm of wheat chromosome 4B controls within-spikelet variation in both grain size and seed dormancy, the latter an important survival mechanism likely eliminated from wild wheat during domestication.**
Abstract Seed dormancy can increase the probability of survival of at least some progeny under unstable environmental conditions. In wild emmer wheat, only one of the two grains in a spikelet germinates during the first rainy season following maturation; and this within-plant variation in seed dormancy is associated with both grain dimension differences and position within the spikelet. Here, in addition to characterizing these associations, we elucidate the genetic mechanism controlling differential grain dimensions and dormancy within wild tetraploid wheat spikelets using phenotypic data from a wild emmer × durum wheat population and a high-density genetic map. We show that in wild emmer, the lower grain within the spikelet is about 30 % smaller and more dormant than the larger, upper grain that germinates usually within 3 days. We identify a major locus on the long arm of chromosome 4B that explains >40 % of the observed variation in grain dimensions and seed dormancy within spikelets. This locus, designated *QGD-4BL*, is validated using an independent set of wild emmer × durum wheat genetic stocks. The domesticated variant of this novel locus on chromosome 4B, likely fixed during the process of wheat domestication, favors spikelets with seeds of uniform size and synchronous germination. The identification of locus *QGD-4BL* enhances our knowledge of the genetic basis of the domestication syndrome of one of our most important crops.

Introduction

Wild plants possess various survival mechanisms that enable them to cope with unpredictable environmental conditions. One such mechanism is innate seed dormancy, which can enhance a plant's reproductive fitness under fluctuating environmental conditions by staggering the germination of its progeny through the current season and even into subsequent growing seasons (Finch-Savage and Leubner-Metzger 2006; Harel et al. 2011). Seed dormancy is a highly variable trait both within and among species (Simpson 1990); and its magnitude is determined by a dynamic interaction among environmental conditions (e.g. temperature, soil moisture, light, and nutrients), seed characteristics (e.g. age, size, and maturity), and genetic factors (Dyer 2004; Barrero et al. 2010). Many wild relatives of domesticated cereal crops possess dormancy mechanisms that prevent the germination of a proportion of their mature and viable grains (Simpson 1990; Hilhorst 1995). Wild emmer wheat (*Triticum turgidum* ssp. *dicoccoides*; $2n = 4x = 28$; BBAA genomes), a direct progenitor of domesticated wheats, exhibits such non-uniform germination; in fact,

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only one of the two seeds of its dispersal unit (spikelet) readily germinates (Cook 1913), a characteristic we refer to as 'differential dormancy'. In contrast, physiologically mature seeds of domesticated cereal crops exhibit uniform germination upon water imbibition and the detection of appropriate environmental cues, a condition defined as uniform germination (Abbo et al. 2014).

Generally, mechanisms of seed dormancy can be classified into five categories: physiological, morphological, morpho-physiological, physical, and combinational (physiological + physical) (reviewed in Finch-Savage and Leubner-Metzger 2006). Of these five, physiological dormancy is the most prevalent form, found in all major angiosperm clades (Baskin and Baskin 2004), and is subdivided into three levels: deep, intermediate, and non-deep. Most seeds have non-deep physiological dormancy which, depending on the species, can be broken by relatively short (<2 months) cold (0–10 °C) or warm (≥15 °C) stratification, though seeds may after-ripen in dry storage, resulting in a decrease in dormancy (Baskin and Baskin 2004).

The inability of an embryo to germinate can be related to its associated maternal tissues, such as the seed coat and endosperm (coat-based dormancy), or to the embryo itself (embryo-based dormancy). Both the seed coat and, to some extent, the glumes in cereals can physically separate the embryo from its surroundings and thereby prevent the uptake of water, oxygen, and nutrients needed for germination as well as the removal of inhibitory factors that may delay germination (Barrero et al. 2010). As a result, such grains can remain dormant for longer periods relative to grains with more permeable seed coats (Hilhorst 1995). Embryo-based dormancy, in contrast, is genetically determined as the grain develops within the spikelet; and it is this form of dormancy that has been implicated in the differential dormancy observed in several wild *Triticeae* species (Simpson 1990).

It is worth noting that wheat spikes flower gradually, starting with the middle third of the spike and progressing upwards and downwards such that the last spikelets to flower are the lower and upper ones. Spikelet position along the spike also affects grain dimension, with the largest grains generally developing in the central part of the spike (the first flowering spikelets) and grain size decreasing progressively upwards and downwards to the basal and terminal spikelets (Evers and Millar 2002). In some wild cereals, grain dimensions within a spikelet are dimorphic, with the first grain, positioned lower within the spikelet, usually smaller and less developed relative to the second grain, positioned slightly higher (Fig. 1) (Wurzburger and Koller 1973; Dyer 2004). Moreover, it has been reported in *Aegilops kotschyi*, *A. triuncialis*, and wild emmer wheat that the lower grains are inhibited and germinate poorly under conditions in which the upper grains in the spikelet

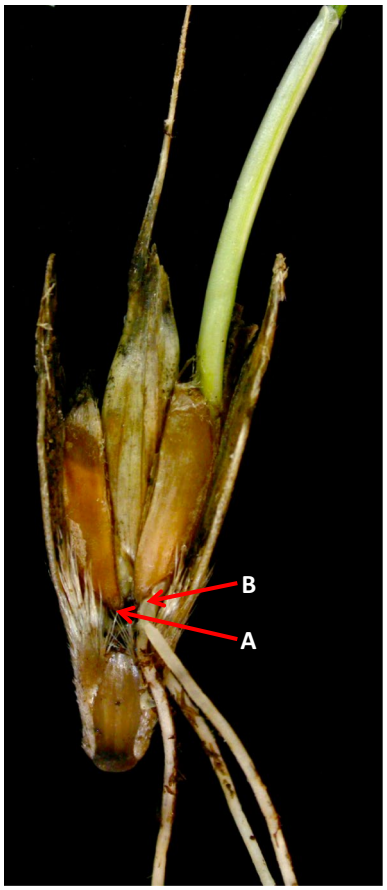

Fig. 1 Within-spikelet variation in grain dimensions between lower (*a*) and upper (*b*) grains of wild emmer wheat accession 'Zavitan'. The lower grain is smaller than the upper one and remains dormant while the upper grain germinates readily

germinate readily (Wurzburger and Leshem 1967; Maranon 1989; Dyer 2004). Heteromorphism in size and germination potential has also been reported in *Aegilops neglecta* and *Aegilops ovata* (Datta et al. 1970; Maranon 1987, 1989). In *A. ovata* the hulls of the dispersal units of seeds contain a germination inhibitor (Lavie et al. 1974), which may explain some extent of the dormancy of those seeds within intact hulls. However, a more recent study on wild emmer reported differential dormancy both for intact spikelets and for grains that were separated from one another and peeled (Horovitz et al. 2013), indicating that the phenomenon is neither the result of inhibitory factors extracted from the glumes nor the result of competition between the lower and upper grains under limited dispersal options (Cheplick 1992), but rather is attributable to internal factors within the grains themselves. Although the necessity of research on the physiological basis determining the somatic difference in the germination behavior of *Aegilops* and *Triticum* species was pointed out nearly three decades ago (Maranon 1989), the mechanisms and genetic bases of

these within-spikelet variations in both grain size and dormancy remain unknown.

In contrast to wild plants, the mature seeds of most domesticated crops germinate uniformly as soon as the appropriate conditions are met (Crocker 1916). Indeed, durum wheat (*T. turgidum* ssp. *durum* (Desf.) MacKey; $2n = 4x = 28$; BBAA genomes) and hexaploid bread wheat (*T. aestivum*; $2n = 6x = 42$; genomes BBAADD) both exhibit synchronized and ready grain germination (Fuller 2007). These are critical traits in the domestication syndrome of cereals, enabling the more uniform and complete germination of deliberately planted grains, thereby improving agronomic performance under cultivation (Barrero et al. 2010). There is a tradeoff, however, as crop plants subjected to selection pressure for more rapid and uniform germination can become prone to pre-harvest sprouting (PHS). In sprouting-prone plants, since mechanisms for delayed germination are absent, sprouting can occur while seeds are still on the mother plant, usually in response to rain. PHS causes substantial economic loss in cereals, especially in bread wheat (*Triticum aestivum* L.) (Barrero et al. 2015). One means of mitigating PHS losses is via genetic regulation of grain dormancy. In this context, the identification of genes controlling cereal grain dormancy is essential to breeding programs aiming to reduce PHS in commercial varieties. Dormancy-related and PHS QTLs, both of which may play a role in breeding for reduced PHS, have been previously identified in wheat, barley, rice, and the model plant species *Arabidopsis thaliana* (reviewed by Li et al. 2004; Kulwal et al. 2010; Mares and Mrva 2014). For example, the cloned *TaPHS1* gene on the short arm of wheat chromosome 3A is a wheat homolog of the *MOTHER OF FT AND TFL1* (*TaMFT*) gene, with two mutations in *TaPHS1* jointly altering PHS resistance (Nakamura et al. 2011; Liu et al. 2013b).

The long-term goal of the current research is to further elucidate the genetic mechanism(s) controlling grain dormancy in wheat through the use of quantitative genetics, as suggested by Koornneef et al. (2002). The specific objectives of this study were to (1) characterize the dimensional and dormancy differences between the lower and upper grains within spikelets of a Recombinant Inbred Line (RIL) population derived from a cross between durum wheat and its direct progenitor, wild emmer wheat; (2) confirm the association between dimorphism and dormancy differences within spikelets; and (3) identify and begin to characterize the genetic mechanism(s) underlying these important domestication traits. Our results not only shed new light on the process of crop domestication vis-à-vis uniform grain dimensions and ready, synchronous germination but also contribute to our understanding of the genetic mechanism responsible for within-spikelet variation in grain dimensions and dormancy observed in crop wild relatives.

Materials and methods

Plant materials

A population of 137 F_{7-8} RILs derived from a cross between wild emmer wheat (accession 'Zavitan', Zv hereafter) collected from the Zavitan nature reserve, Israel, and elite durum wheat cv. 'Svevo' (Sv hereafter) (Avni et al. 2014) was used for the current study. The RILs and the two parental lines were grown during two consecutive winter seasons, with a different location each year: (1) From November 2012–May 2013 in the botanical gardens of Tel Aviv University, Israel (32°6′N, 34°48′E; 30 MASL); and (2) From November 2013–May 2014 at the experimental farm of the Hebrew University in Rehovot, Israel (31°54′E, 34°47′N; 54 MASL).

A set of genetic stocks, consisting of 14 chromosome substitution lines (Joppa and Williams 1988), developed by LR Joppa and kindly provided by Drs. Xu and Faris (USDA-ARS, Fargo, ND USA), was used to validate the genetic mapping results. Each of these lines contains one substituted chromosome from wild emmer accession PI481521 within the genetic background of durum wheat cultivar 'Langdon' (LDN) (Table S2) and was grown from November 2014–May 2015 in the botanical gardens of Tel Aviv University, Israel.

In addition, we analyzed 51 accessions of domesticated emmer (*T. turgidum* ssp. *dicoccum*, BBAA genomes) for intra-spikelet grain size and dormancy differences. Four accessions (Leonessa 5, Agnone primaverile, Agnone invernale, and Potenza) were taken from the Salamini collection (Ozkan et al. 2005); two accessions (Khapli [CI 4013] and RTI-3424/75) were obtained from the collection of genetic stocks at the Institute for Cereal Crops Improvement (ICCI), Tel Aviv University; and 45 accessions originating from various regions were kindly provided by Tuberosa, University of Bologna (Table S2).

Phenotypic assessment

After harvest, spikes were left to dry in a ventilated oven at 37 °C for 1 month and then stored under low humidity (15–25 %) and low temperature (13–15 °C) until analyzed, 1–2 months after harvest. The plant material from the 2014 and 2015 growing seasons was used for all the phenotypic assessments described below. Plant material from the 2013 growing season was used only for grain dimension characterization.

Measuring dimensions of the lower and upper grains within spikelets

Twenty spikelets from each of the 137 RILs, the two parental lines, and the 51 *T. turgidum* ssp. *dicoccum* accessions were randomly selected from spikes. The lower (i.e.

first) and upper (i.e. second) grains were separated from each spikelet and placed in two separate petri dishes (PD) (90 × 15 mm, 10 cm diameter), such that each PD eventually contained 20 lower or 20 upper grains. Within each PD, the grains were arranged on top of two filter papers wetted with 6 ml of distilled water. After imbibition for 24 h at 4 °C in the dark, the grains from each PD were arranged crease-side down on a Qualmaster Computer Vision device (VIBE Technologies, Tel Aviv, Israel), which performs shape and size measurement for seeds. Its operation principle is as follows: after an image of the seed sample is captured by a camera with a geometry-calibrated lens, the background is removed using a color segmentation technique. As a result, each seed is represented as a shape on a binary image, which is then perspective-corrected for greater accuracy. Finally, a minimal bounding rectangle is extracted from each shape, from which the area, length, and width dimensions of each seed are acquired. For each genotype, ratios (upper grain/lower grain) were calculated based on the averages of each parameter (length, width, and area).

Measuring differential dormancy

Immediately after scanning, the grains were transferred to a dark chamber held at 20 °C and the number of germinated grains was recorded every 24 h. Preliminary experiments showed that most of the lower grains from Zv germinate gradually in a timeframe of 21 days or less; therefore, this was determined as the maximum duration of the germination experiment. For this part of the study, a grain was considered germinated when it showed development of coleorhiza and coleoptile beyond the seed coat. As an internal control for the general viability of the grains for each genotype, we adopted a minimum acceptable threshold of 19 out of 20 germinated upper grains. This threshold was based on the observed distribution of upper grain germination among the RILs, namely that only 5 % of the RILs exhibited germination of less than 19 upper grains after 3 days. Therefore, if less than 19 upper grains germinated after 3 days, we repeated the experiment with that specific genotype. If it failed to reach the threshold again, the genotype was excluded from the experiment. In total, nine of the 137 RILs were excluded on this basis.

To quantify the extent of differential dormancy for each RIL, the number of days required to meet the threshold for full germination (i.e. 19 out of 20 grains) was recorded for both the lower and upper grains. Since the experiment was terminated at 21 days after imbibition, those genotypes that did not reach the full germination threshold were assigned a value of 21. The level of differential dormancy was then calculated as the difference to full germination (in days) between the lower and upper grains (ΔG hereafter). This point of this calculation is to isolate differential dormancy from other factors that might influence the germination differences between Sv and Zv unrelated to the position of the grains within the spikelet. The minimum ΔG value equals 0 and represents spikelets with uniformly germinating seeds, while the maximum ΔG equals 20 and represents spikelets with seeds exhibiting extreme differential dormancy. Grains from the 2013 growing season were not used for measuring differential dormancy due to long storage, which can affect seed dormancy (Finch-Savage and Leubner-Metzger 2006).

Effect of seed coat on differential dormancy

To test the potential effect of the seed coat on germination, three replications consisting of twenty punctured and unpunctured lower and upper grains of Zv were used. Specifically, a single hole of 2–3 mm diameter and 1–2 mm deep was made in the pericarp surface of each seed using a sterile 27 gauge stainless steel needle. After puncturing, the grains were placed crease-side down in petri dishes as described above and germination was recorded every 24 h.

Effect of gibberellic acid on differential dormancy

Three replications of GA (Gibberellin A_3, G7645 SIGMA) and control (mock) treatments of Zv lower and upper grains, each consisting of twenty seeds, were used to test the effect of GA on the differential dormancy trait. Each GA-treated replication received 6 ml of 10^{-5} M GA solution dissolved in distilled water and was subjected to the germination procedure described above. Germinated grains were counted daily and removed from the filter paper, and each PD received an additional 1 ml of the GA solution every 4 days until termination of the study at Day 21.

Effect of spikelet position on differential dormancy

The effect of spikelet position along the spike on differential dormancy was tested using two RILs exhibiting significant differential dormancy ($\Delta G > 12$ days) and two RILs exhibiting uniform germination ($\Delta G = 0$ days). Spikelets from three spikes of each genotype were separated according to their positions in the spike, namely three lower and three middle spikelets. Because the uppermost spikelets exhibited brittleness (i.e. spontaneous detachment from the rachis) in a large proportion of the RILs, only grains from the middle and lower spikelets were characterized for their dimensions and dormancy, as described above.

Statistical analysis

The JMP 7.0 software package (SAS Institute, Cary, NC, USA) was used for statistical analyses. Student's *t* tests

were used to test for significant differences in dimension parameters between the parental lines and also to test for differences between the two groups of RILs, namely those exhibiting differential dormancy and those exhibiting uniform germination.

Quantitative trait locus (QTL) analysis

In this study, we used a 2110 cM genetic map of Sv × Zv based on 14,088 polymorphic markers from the 90K iSelect SNP genotyping assay (Avni et al. 2014). Since the density of loci was relatively high for a QTL analysis, we developed a Perl script (ril_marker_parser.pl; available with documentation at https://github.com/halelab/Miscellaneous-Scripts.git) to systematically reduce the number of loci in evenly distributed genetic distances while taking into account marker quality. First, low quality loci were removed from the map based on both a maximum threshold of missing data (12 RILs) and whether the inclusion of a locus introduced a double crossover (DCO) within an interval of less than 2 cM. To prevent the introduction of large gaps in the map, such low quality loci were removed only if a sufficiently high-quality locus was present within 1.5 cM. In the next stage of parsing, the remaining set of 2247 high-quality "skeleton loci" [i.e. representatives of the unique loci in the original map; see Avni et al. (2014)] was further reduced using a 3 cM sliding window. This parsing step resulted in a final set of 472 high-quality loci, evenly distributed across the 14 chromosomes of tetraploid wheat, with an average of 33.71 markers per chromosome and an average distance of 4.47 cM between adjacent loci. It is worth mentioning that the MultiQTL software performs independent calculations for marker distances; therefore, minor differences exist between the full map distances (Avni et al. 2014) and the reduced markers map distances used in QTL analysis (Table S1).

Quantitative trait locus analyses of ΔG and grain dimension ratios were performed using the general interval mapping (IM) procedure for a RIL population in the MultiQTL software package (http://www.multiqtl.com). First, a 'single QTL, one trait' model was used to assess the likelihood of a single QTL at each position in the genome (Jansen 1993). To test whether an interval carries a QTL affecting the target trait, the H_1 hypothesis (the locus has a significant effect) was compared with the H_0 hypothesis (no significant effect) using permutation tests (n = 10,000). Next, a 'two linked QTLs' model was applied. For this, the H_2 hypothesis (two linked QTLs significantly affect one trait) was compared with two alternative hypotheses (H_1: only one QTL affects the trait; H_0: no effect) and significance was again estimated based on permutation tests (n = 10,000). Epistatic (e) interactions were then tested by comparing the H_0 hypothesis ($e = 0$) to the H_1 hypothesis ($e \neq 0$). If multiple, unlinked QTLs were identified that influence the trait, such QTLs were further analyzed using multiple interval mapping (MIM), a procedure that enables the reduction of residual variation caused by other QTLs located elsewhere in the genome, thus providing a more precise and powerful identification of real QTLs, separation of linked QTLs, and elimination of "ghost QTLs" (Kao et al. 1999). Finally, after choosing the correct model for each trait, a permutation test (n = 10,000) was applied to assess the significance of detected QTLs, followed by a bootstrap test (n = 10,000) to evaluate the accuracy of QTL positions, effects, and detection power. QTL boundaries were determined based on a 2 × drop in LOD score from the respective peaks.

Results

Intra-spikelet dimension and dormancy differences

The lower and upper grains within the spikelets of the wild parent (Zv) of the mapping population showed significant differences in area, length, and width (p = 0.0002, 0.0007, 0.0018, respectively), whereas no significance differences were found in the domesticated parent (Sv) (Table 1; Fig. 2a, b). The level of differential dormancy was then calculated as the difference to full germination (in days) between the lower and upper grains (ΔG). Germination of the lower and upper grains in Sv was found to be relatively uniform, ranging from 1 to 3 days across replications, with no difference between the lower and upper grains (ΔG = 0 days). In Zv, however, a significant difference was found in the number of days required for full germination of the lower and upper grains (p = 0.0004) (Table 1; Fig. 2c, d). While the upper grains of Zv germinated completely within 2 days, only 23 % of the lower grains germinated within 21 days after imbibition, when the experiment was terminated (ΔG = 19 days; "Materials and methods"). The 51 *T. dicoccum* accessions were also tested for intra-spikelet dimension and dormancy variation but, like Sv, no significant differences were found between the lower and upper grains in any of these emmer genotypes (Table S2). Differential dormancy values (ΔG) and germination percentages of upper and lower grains of the RILs population are presented in Table S3.

Effect of seed coat

To test the effect of the seed coat on differential dormancy in wild emmer, the germination pattern of punctured lower and upper grains of Zv was evaluated compared to control (un-punctured) grains (Table 1). The grains of both treatments showed the same pattern: the upper grains fully

Table 1 Grain dimensions and germination characterization (days to maximal germination) of lower and upper grains within the spikelets of wild emmer wheat accession 'Zavitan' (Zv) and durum wheat accession 'Svevo' (Sv)

Experiment type	Grain	Days	ΔG	Area (mm^2)	Length (mm)	Width (mm)
Zavitan vs. Svevo	Zv 1st	21 ± 0.33		24.3 ± 0.57	10.43 ± 0.12	2.96 ± 0.04
	Zv 2nd	2.3 ± 0.66	19	31.04 ± 0.48	11.76 ± 0.05	3.35 ± 0.04
	P	<0.001		<0.001	<0.001	<0.01
	Sv 1st	2 ± 0.57		26.35 ± 0.38	9.419 ± 0.09	3.56 ± 0.02
	Sv 2nd	2 ± 0.57	0	26.55 ± 0.36	9.397 ± 0.09	3.62 ± 0.02
	P	0.45 (NS)		0.35 (NS)	0.56 (NS)	0.089 (NS)

Experiment type	Grain	Days	ΔG	
GA treatment	Zv + GA 1st	1.6 ± 0.66		
	Zv-cont. 1st	14.3 ± 2.84		
	P	0.035		
	Zv + GA 2nd	1	0.6	GA (2nd)–(1st)
	Zv-cont. 2nd	1	13.3	Cont. (2nd)–(1st)
	P	>0.05 (NS)		
Punctured	Zv-1st punc. 1st punc. 1st	18.3 ± 0.33		
	Zv-1st cont.	19 ± 0.57		
	P	0.19 (NS)		
	Zv-2nd punc. 2nd	2 ± 0	17	Punc. (2nd)–(1st)
	Zv-2nd cont.	2 ± 0	16.3	Cont. (2nd)–(1st)
	P	>0.05 (NS)		

Data are means ($n = 3$) ± SD

1st lower grains, *2nd* upper grains, *GA* gibberellin treatment, *cont.* control (mock) treatment, *punc.* punctured grains, *ΔG* the difference to full germination (in days) between the lower and upper grains

germinated within two days and the lower grains germinated gradually (ΔG = 16.3 and 17 days for the control and punctured grains, respectively). These results indicate that perforation of the seed coat has no effect on the differential dormancy observed in Zv.

Effect of GA

The effect of exogenous GA on differential dormancy was measured on the grains of the wild parent (Zv). The upper grains germinated uniformly and completely in both the control and GA treatment within 24 h. For the lower grains, however, the GA treatment affected both germination pattern and percentage. The average ΔG was 0.6 days in the GA treatment compared to 13.3 days in the control treatment ($p = 0.017$) (Table 1). Over the course of the 21 day period, the average germination percentage was 68 % in the GA treatment, compared with only 13 % in the control treatment ($p = 0.004$).

Effect of spikelet position along the spike

To test if spikelet position along the spike affects ΔG, we evaluated four representative RILs from the genetic mapping population, two exhibiting differential dormancy and two not. No significant differences for ΔG values were found between the central and bottom spikelets for the two variably dormant RILs ($\Delta G_{RIL\#15}$ = 19.7 and 19.7 days, respectively; $\Delta G_{RIL\#146}$ = 19 and 19 days, respectively). Similarly, the average values of ΔG for the two uniformly germinating RILs showed non-significant differences for the central and bottom spikelets ($\Delta G_{RIL\#109}$ = 0.3 and 0.3 days, respectively; $\Delta G_{RIL\#124}$ = 0 and 0.3 days, respectively).

Differential grain dimensions and differential dormancy within the RIL spikelets

The upper grains of all RILs included in the analysis were found to germinate readily (within 1–3 days) and in high percentage (usually 100 %, Table S3). In contrast, the lower grains exhibited wide variation in dormancy response, with ΔG ranging between 0 and 20 days (Fig. 3). Based on a cutoff threshold of $\Delta G \leq 3$ days, we classified 48 % of the RILs as uniformly germinating (Group A; 0 days $\leq \Delta G \leq$ 3 days) and 52 % as exhibiting some degree of differential dormancy (Group B; 3 days $\leq \Delta G \leq$ 20 days). To test the correlation between differential dormancy and dimensional differences, we used *t* tests to compare the ratio of each dimension parameter (i.e. upper grain dimension: lower grain dimension) between the two germination groups.

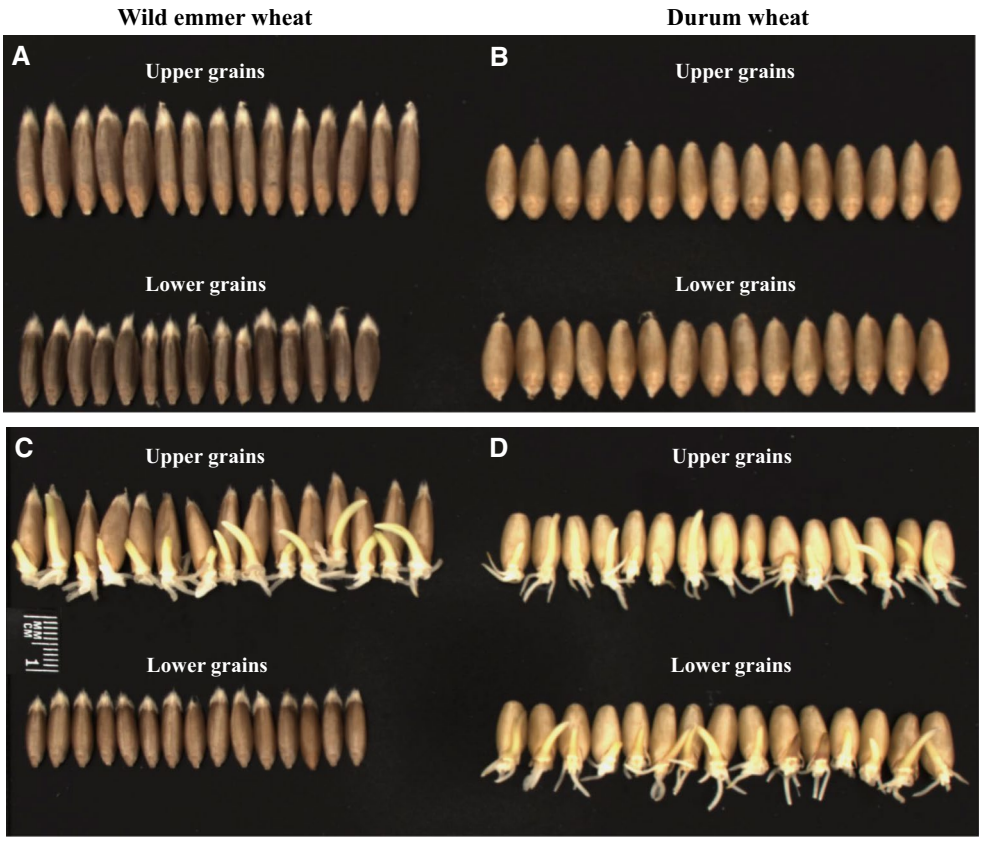

Fig. 2 Variation in grain dimension and germination pattern within spikelets. Dry grains separated according to their position within the spikelet (first/lower grain vs. second/upper grain) in wild emmer wheat accession 'Zavitan' (**a**) show size differences, whereas grain dimensions are uniform in durum wheat accession 'Svevo' (**b**). Upon imposing conditions conducive to germination for 2 days, the lower grains in wild emmer do not germinate, whereas the upper grains do (**c**). In contrast, rapid and uniform germination is observed in both the lower and upper grains of durum wheat (**d**). *Bar* 10 mm

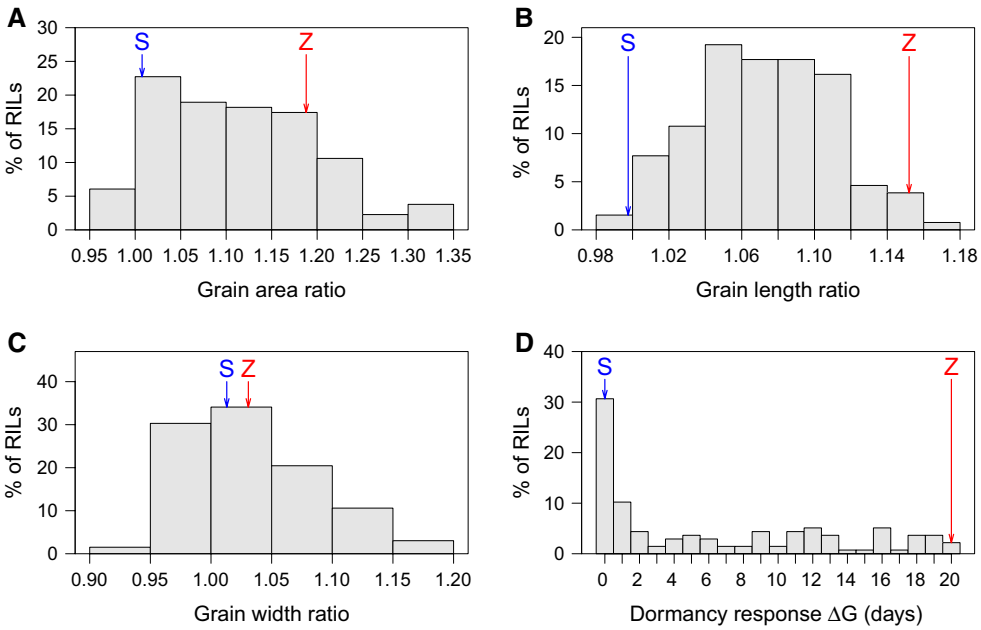

Fig. 3 Frequency distributions among the Svevo × Zavitan RIL population for dimension ratios between the upper and lower grains within spikelets [area (**a**), length (**b**), width (**c**)], as well as differential dormancy [ΔG (**d**)]

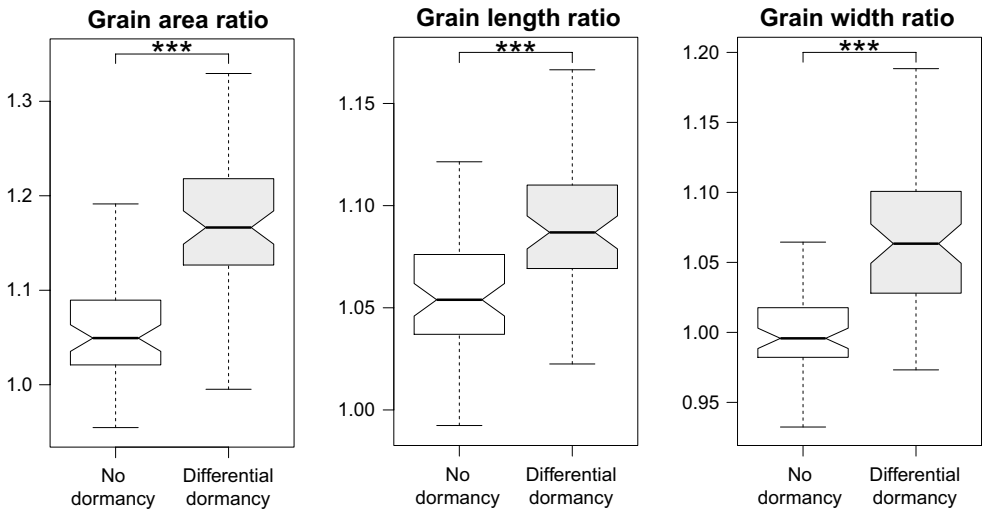

Fig. 4 Grain dimension ratios of differentially dormant RILs compared to non-dormant RILs. All ratios are significantly different between the two groups ($p < 0.05$). *Triple asterisk* indicates significance at $P \leq 0.01$

The differences between Group A (uniformly germinating) and Group B (variably dormant) were significant ($p < 0.001$) for all parameters (Fig. 4) and demonstrate a strong association between the germination pattern and grain dimension differences between the lower and upper grains.

QTL mapping

Uniform germination QTL

Primary analysis using the 'single QTL, one trait' model revealed three putative uniform germination QTLs, two on chromosome 1A and one on chromosome 4B. Subsequent analysis using the 'two linked QTLs' model refuted the likelihood of more than one significant QTL per chromosome; furthermore, no significant epistasis was found among the loci. Therefore, after eliminating what appeared to be a false positive effect of chromosome 1A, focus was turned to the major QTL on the long arm of chromosome 4B. This QTL, with peak LOD score 12.9, explains 43.3 % of the observed phenotypic variance in ΔG, with Zv contributing the allele associated with higher values of ΔG (Table S4).

Grain dimension ratio QTLs

Mapping to the same interval on chromosome 4BL as the uniform germination QTL described above were three major grain dimension QTLs. These QTLs for grain area, grain length, and grain width ratios, with peak LOD scores of 20.63, 12.06, and 21.66, explain 59.1, 40.1, and 58.3 % of the observed phenotypic variances, respectively (Table S4). The higher ratios between the upper and lower grains for all parameters were associated with the Zv alleles; and the grain dimension QTLs were consistent across the two growing environments in 2013 and 2014 (Fig. 5).

In summary, a major QTL was identified for each of the measured traits (three dimensional ratios and differential dormancy). These four QTLs co-localize to an interval of 4.97 cM, flanked by markers *IWB72369* and *IWB73477*, on the long arm of chromosome 4B (Fig. 5). We designate this interval the '*Chromosome 4BL Grain Domestication Locus*' (*QGD-4BL*).

QTL validation

To validate the *QGD-4BL* locus, we used an independent set of cytogenetic stocks comprises 14 chromosome substitution lines, namely the individual chromosomes of *Triticum turgidum* ssp. *dicoccoides* accession PI481521 substituted into durum wheat cv. 'Langdon' (LDN). Only the 4B-chromosome substitution line LDN (PI481521-4B) showed a significant difference in the number of days required for full germination of the lower and upper grains ($p = 0.0132$), as well as significant differences between lower and upper grain lengths, widths, and areas ($p = 0.0129$, 0.0042, and 0.0273, respectively). Like Sv and LDN, all other substitution lines were found to exhibit uniform grain dimensions and ready, synchronized germination ($p > 0.05$) (Table S2).

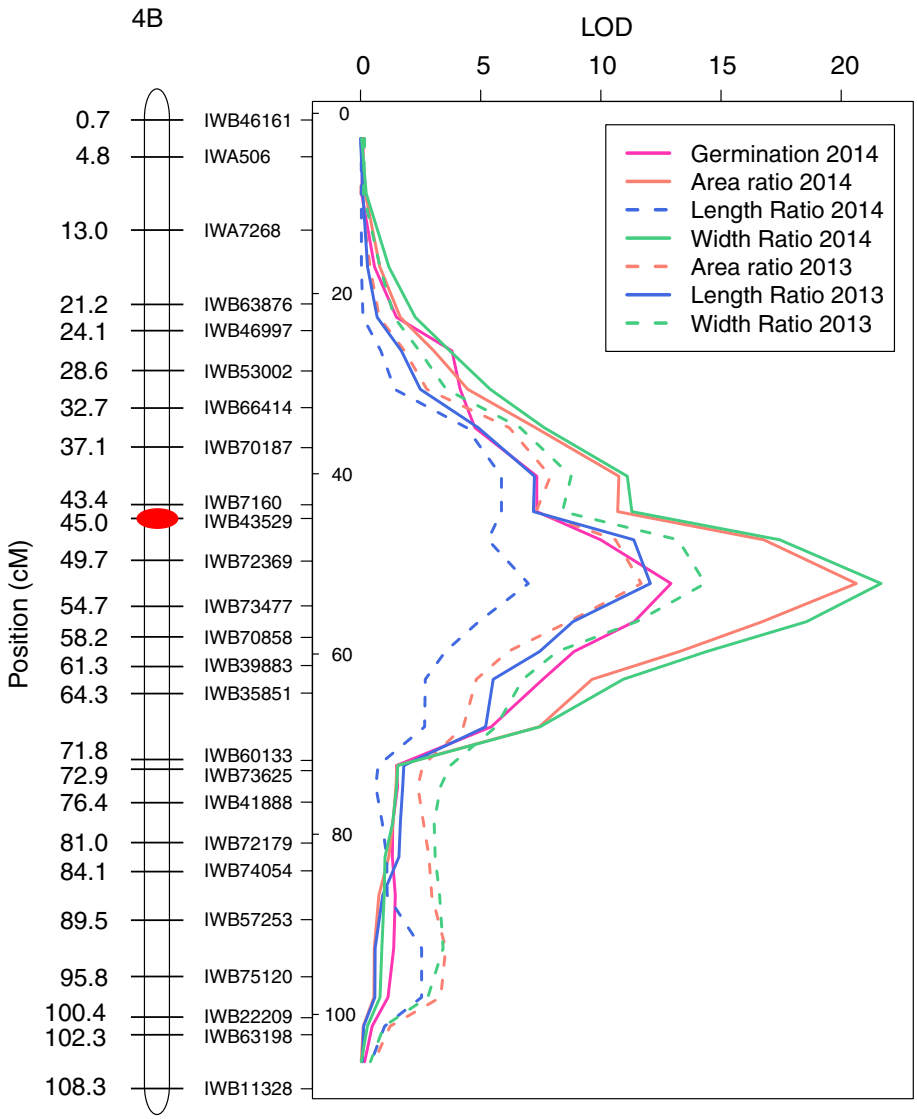

Fig. 5 Co-localization of grain dimension ratio QTLs (2013, 2014) and the differential germination QTL on chromosome arm 4BL. *Red mark* centromere

Discussion

Differential grain dimensions and seed dormancy in wild plants of the tribe *Triticeae*

Intra-spikelet grain size and dormancy differences in wild emmer

More than a century ago, it was shown that approximately 50 % of wild emmer grains germinate during the first rainy season following maturation (Cook 1913). More recently, Horovitz et al. (2013) described how the upper grain in a spikelet of wild emmer is well developed and germinates readily in the first rainy season following its dispersal. In contrast, the lower grain is thinner and may germinate the following year or even later, whether the spikelet is left entire or the grains are separated from one another. In the current study, we have confirmed these observations and quantified them with precise morphometric characterizations (Figs. 1, 2; Table 1). In agreement with these previous studies, the dimensional and dormancy differences between the lower and upper grains within wild Zv spikelets were found to be highly significant. Delaying germination of up to 50 % of a plant's seeds reduces the risks associated with unfavorable environmental conditions (e.g. severe drought, grazing, fire, etc.) by increasing the chance of survival of at least some progeny and enhancing reproductive fitness. The dormant lower grains of Zv, genetically programmed to germinate in the next growing season (or beyond), provide a clear example of this common strategy.

The effects of intra-spikelet grain position on dimensional differences and dormancy were previously identified in close wild relatives of the genus *Aegilops* within the grass family, such as *Ae. geniculata* (Zhukovsky, 1928; Datta et al. 1970; Maranon 1989), *Ae. triuncialis* (Maranon 1989; Dyer 2004), and *Ae. kotschyi* (Wurzburg and Koller

1973). In these studies, the possible mechanisms responsible for smaller seeds of dimorphic pairs have included a water-soluble chemical on the dispersal unit induced by maternal tissue (Lavie et al. 1974; Dyer 2004), varying internal levels of Gibberellin, and sibling competition for essential metabolites (Wurzburger and Leshem 1967). In the present study, lower grain dormancy was observed in isolation from both maternal tissues and upper grains, indicating that the cause for the differential dormancy in wild emmer is most likely not attributable to a chemical factor in the glumes or to inter-grain competition. It is worth noting that such investigations of within-spikelet variation are not possible in *T. urartu*, a diploid ($2n = 2x = 14$) progenitor of wild emmer and donor of the A genome, due to the fact that each spikelet of that species contains only one grain.

Effects of seed coat and GA on differential germination

A thick seed coat can disrupt germination if the embryo is confined or if water uptake and gas diffusion are obstructed (Hilhorst 1995; Barrero et al. 2010). It has been shown that embryo growth is not inhibited and dormancy is reduced in *Arabidopsis thaliana* mutants with various defects in the testa or seed coat structural components (Debeaujon et al. 2000). To determine if the seed coat may be responsible for the differential dormancy observed in wild emmer, we examined the effect of puncturing the seed coat before imbibition ("Materials and methods"). Disrupting the integrity of the seed coat in this way did not eliminate the observed differential dormancy, nor did it change the germination percentage of the lower grains. We therefore conclude that the differential dormancy described in this work is not attributable to the physical barrier of the seed coat. It is worth mentioning that such puncturing not only disrupts the physical barrier of the seed coat but can also induce wound signals and secretion of hormones such as jasmonic acid (JA) (Creelman and Mullet 1995). It has been shown that JA is involved in the control of seed dormancy in wheat by reducing dormancy levels under certain conditions (Jacobsen et al. 2013). Nevertheless, the punctured grains in this study showed no reduction in seed dormancy levels; therefore, we posit that wound-induced JA also plays no significant role in the differential dormancy described in this work. To definitively prove this putative embryo-based dormancy of lower grains in wild emmer wheat, further investigations using excised embryos and crossed seeds are required.

Two main hormones have been shown to influence grain dormancy and germination (Hilhorst and Karssen 1992): abscisic acid (ABA) and gibberellin (GA). While ABA usually promotes dormancy (Frey et al. 2004), GA generally promotes germination by antagonizing ABA (Debeaujon and Koornneef 2000). Moreover, GA has been shown to play an active role in promoting germination in many plant species (De Vries 1971), specifically in seeds with intermediate and non-deep physiological dormancy (Baskin and Baskin 2004). In addition, the level of endogenous GA in the lower grains within spikelets of *Ae. koyschyi* has been shown to be lower than that in upper grains (Wurzburger and Koller 1973). Recently, Liu et al. (2013a) suggested that changes in the expression of GA biosynthesis genes in response to prolonged storage indicate that GA enhances dormancy release and germination. To assess the potential role of GA on the differential dormancy observed within spikelets of wild emmer, we treated Zv lower grains with exogenous GA and found that such treatment significantly enhanced both the germination rate and percentage (Table 1), indicating that the differential dormancy observed in Zv is somehow functionally related to the presence of GA. Taken all together, these results support the classification of the differential dormancy of wild emmer as embryo-based, physiological dormancy.

Effect of spikelet position on differential germination

Grain dimension is known to correlate with spikelet position along a spike (De Vries 1971). In this study, we investigated the relationships between spikelet position on both grain dimension ratios as well as differential dormancy between the lower and upper grains within a spikelet. Our results indicate that both the dimension ratios and the extent of differential dormancy are uniform along the spike (Table 1). Thus, we conclude that spikelet position does not affect size or dormancy differences between the upper and lower grains within a spikelet.

Considerations when analyzing grain size differences

In grain dimension studies, seed length and width are typically measured on bulk samples of grains, without regard for position along a spike or within a spikelet. Using such a strategy, several loci associated with grain dimension variation *among* genotypes of tetraploid wheat were recently identified (e.g. grain length QTLs on chromosomes 1B, 2B, 3A, 7A; and grain width and area QTLs on chromosome 4B) (Russo et al. 2014). In hexaploid wheat (*Triticum aestivum* L.), QTLs for several components of grain size and morphology have been detected on almost all 21 wheat chromosomes (Wu et al. 2015). On chr 4B, QTLs for grain length were identified on the long arm (Breseghello and Sorrells 2007; Wu et al. 2015) and for grain width on the short arm (Ramya et al. 2010). In contrast to this bulk sample approach, in this study we investigated differences in dimensions (width, length, and area) and dormancy specifically between the lower and upper grains *within* spikelets of tetraploid wheat. Using a bi-parental population of RILs

(Avni et al. 2014), we discovered a major QTL for each of these four traits, all co-located to the same ~5 cM interval on the long arm of chromosome 4B—the *QGD-4BL* locus. In light of this result, when phenotyping segregating populations derived from crosses between wild and domesticated wheats, we suggest that patterns of grain variability within genotypes should be taken into account when characterizing grain traits (morphology, weight, germination, etc.). This co-localization of the differential dormancy and grain dimension QTLs in the *QGD-4BL* region suggests that the observed pattern of size and dormancy differences between lower and upper grains may be controlled by a single gene with pleiotropic effects or a set of tightly linked genes.

The consistency of the identified QTLs across two separate environments and the relatively high percentage of explained variation attributable to the QTLs (40–59 %) suggest a limited environmental effect on these traits, under these conditions. Moreover, the presence of the *QGD-4BL* locus on chromosome 4B was validated using a set of independent chromosome substitution lines (Joppa and Williams 1988). The chromosome substitution line LDN(PI481521-4B) that carries chromosome 4B from wild wheat accession PI481521 in the background of the durum wheat cultivar 'Langdon' (LDN) showed clear differences in intra-spikelet grain dimensions and dormancy, similar to wild accessions PI481521 and Zavitan, while in the other chromosome substitution lines no significant differences were found in grain dimensions parameters and germination of upper and power grains (Table S2).

A domestication locus for uniform grain size and uniform germination

Plant domestication is a process whereby a wild plant population is systematically modified at the genetic level through selection of certain phenotypes to accentuate traits that benefit human-mediated cultivation. In grasses, mutations in genes controlling important spike-related traits such as rachis fragility (brittle or non-brittle rachis) were selected during domestication and subsequently became fixed in cultivated populations due to positive selection pressure. The earliest non-brittle domestic wheat has been dated to ~9250 years B.C. (Tanksley and McCouch 1997; Tanno and Willcox 2006; Feuillet et al. 2008; Gegas et al. 2010; Pourkheirandish et al. 2015). Such crucial domestication traits are often governed by one or two major loci (Abbo et al. 2012) exhibiting low levels of polymorphism within either wild or domesticated accessions, resulting in distinct monomorphic phenotypes within wild and domesticated gene pools.

Similar to non-brittle rachis, uniform grain dimensions and synchronized, uniform germination are key traits differentiating modern domesticated crop species from their wild ancestors (Abbo et al. 2014; Gao and Ayele 2014). Fuller and Allaby (2009) suggested that the selection toward rapid germination of crops following planting was encouraged simply by cultivation itself, since seeds that do not germinate freely will not be harvested. Therefore, it is plausible that reduction in dormancy occurred under very early cereal cultivation (Fuller 2007). Our results indicate that such reduction in dormancy is strongly affected by a single and major locus on the long arm of chromosome 4B; thus uniform germination controlled by the *QGD-4BL* locus qualifies as a trait within wheat's domestication syndrome. Similar to the *Q* loci that control spike characteristics in wheat (Simons et al. 2006), we posit that the *QGD-4BL* haplotype(s) of domesticated wheats may carry mutation(s) resulting in rapid and synchronous grain germination, simultaneous ripening, and ultimately improved performance under agricultural conditions. To test our hypothesis that the genetic modification in the wild emmer *QGD-4BL* locus occurred in the early stages of crop domestication and that the transition to uniform grain dimensions and germination was crucial for cultivation, we analyzed 51 accessions of domesticated emmer for intra-spikelet grain size and dormancy differences (Table S2). In all the tested accessions, no significant differences in grain dimensions or germination were found between the upper and lower grains, thus suggesting that the *QGD-4BL* locus may have been fixed in the transition from *Triticum turgidum* ssp. *dicoccoides* to *Triticum turgidum* ssp. *dicoccum* as part of the domestication syndrome of wheat.

Previously, several studies reported the detection of *among-genotype* dormancy-related QTLs on group 4 chromosomes (reviewed by Kulwal et al. 2010). The *QGD-4BL* locus, a *within-genotype* dormancy-related QTL, is located ~5 cM from the centromere on the long arm of chromosome 4B. While Kato et al. (2001), Mares et al. (2005), and Barrero et al. (2015) reported dormancy QTLs on chromosome 4AL, it is unlikely that the *QGD-4BL* locus is homoeologous to these QTLs given that the *QGD-4BL* is a QTL for a different trait (within-genotype differential dormancy) and, as such, is primarily related to wheat's domestication syndrome rather than to the post-domestication process of wheat improvement for pre-harvest sprouting (PHS) resistance. The *QGD-4BL* locus represents an essential difference between wild and domesticated wheat (such as durum), whereas the reported QTLs for PHS have been identified on the basis of variation within domesticated species (durum and bread wheat). During wheat domestication, the initial selection (most likely unconscious) was made for uniform germination, favoring the elimination of dormancy from the lower seed within each spikelet. Later, after domestication and the spread of wheat to environments more prone to PHS, selection was made

for seeds that did not germinate on the mother plant regardless of their position within the spikelet. Still, it is possible that the mechanisms controlling differential dormancy in wild wheat and PHS in domesticated wheat share the same genes; thus the identification of the genes that underlay these various QTLs is needed.

Conclusions

In this study, we identified a major domestication locus on the long arm of wheat chromosome 4B controlling uniform germination. This locus is also shown to regulate differences in grain dimensions within the spikelet. Likely contributed by the diploid donor of the B genome to wild emmer, the differential dormancy trait can be assumed to increase reproductive fitness in relatively unpredictable wild habitats. Under cultivation, however, such a trait is undesirable and we hypothesize that its elimination was one of the changes wheat underwent during domestication.

Currently, we are sequencing the genome of wild emmer wheat accession Zv that was used for the identification of *QGD-4BL* and expect that this effort will facilitate the identification of the gene(s) underlying this important domestication QTL.

Author contribution statement AD and MN designed the research. MN and BB conducted the experiments. MN, RA, and IH analyzed the data. MN, IH, and AD wrote the manuscript; and all authors reviewed the paper.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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