Genome based QTL and meta-QTL analyses of thousand-kernel weight in tetraploid wheat

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Abstract

Wheat domestication and subsequent improvement formed a wide phenotypic variation in Grain Weight (GW) between the domesticated wheat species and their wild progenitors. GW continues to be an important goal of many wheat-breeding programs and yet, although studies found many quantitative trait loci (QTLs) for GW, not many genes that underlay these loci were identified. Here we performed QTL analysis for GW using a Recombinant Inbred Line (RIL) population based of a cross between wild emmer wheat accession 'Zavitan' and durum wheat variety 'Svevo'. Using the recent Zavitan genome assembly, we anchored the identified QTLs to the reference sequence and added the positions of previously published QTLs for GW in tetraploid wheat. This genome based meta-QTL analysis enabled us to identify a locus on chromosome 6A with a positive effect on GW that was contributed by wild wheat in a few studies. This locus was validated using an introgression line that contains the 6A GW QTL from Zavitan in the background of Svevo with higher grain weight. Using the reference sequence and genes associated with GW from rice, we were able to identify a wheat ortholog in the 6A QTL region to rice gene, *OsGRF4*. The coding sequence of this gene, *TtGRF4-A*, showed four SNPs between Zavitan and Svevo. Molecular marker developed for the first SNP showed that the Zavitan allele of *TtGRF4-A* is rare in a core collection of wild emmer and absent in domesticated emmer genepool. We suggest that *TtGRF4-A* is a candidate underlying the 6A GW QTL and breeding with its natural Zavitan allele may have the potential to increase wheat yields.

Keywords: Wheat, wild emmer, domestication, genome assembly, QTL, meta-QTL, grain weight, *GRF4*.

Introduction

Grain weight (GW) in wheat, typically expressed by Thousand Kernel Weight (TKW), is one of the most important determinants of yield together with grain number per unit area (e.g number of grains per spike and number of spikes per plant) (Campbell et al. 1999). GW factors grain size (length, width and area), grain shape and grain density (Gegas et al. 2010; Distelfeld et al. 2014) and considered as a very stable yield component, with relatively high heritability. Domesticated wheat (*T. aestivum* and *T. durum*) has little variation in grain size compared to wild wheat, as the result of a genetic bottleneck due to artificial selection during domestication (Dubcovsky and Dvorak 2007; Gegas et al. 2010). Wild emmer wheat (WEW: *T. turgidum* ssp. *dicoccoides*, genome BBAA) the progenitor species of all wheats grown commercially today, was domesticated about 10,000 years ago, most likely in southeastern Turkey (eastern population), with a major contribution from wild emmer populations in southern Levant (western population, Ozkan et al. 2005). The western population is further subdivided into two subpopulations, designated *Horanum* and *Judaicum*, which greatly differ in their morphological characters (Poyarkova et al. 1991). *Judaicum* is characterized with a tall, upright phenotype, wide spikes, large grains, and is more fertile than the *Horanum* subpopulation that exhibit a smaller stature, and a more slender spike. The large phenotypic variation in the wild present a potential allelic diversity for wheat improvement. Yet, considering that WEW is advocated as an important resource for wheat improvement (Aaronsohn 1910 and others), very few studies have looked into the yield potential of WEW or domesticated emmer (DEW). Transgressive segregation (TS) might be a good indication for the improvement potential of WEW and several studies have showed increase in TKW in genetic populations based on WEW x durum cross (Peleg et al. 2011; Eloufi and Nachit 2004; Nave et al. 2016) while Peng et al. (2003) did not find any TS for TKW. In addition, Thanh et al. (2013) showed TS in a biparental population of wild emmer and domesticated emmer.

Many QTL studies have been done in wheat (PubMed results for 'wheat' AND 'QTL' yields ~1200 results) yet these data seem to have no continuity, which begs the question what is the overlap among these studies? Furthermore, although many QTLs have been located for grain size in wheat, very few genes have been characterized (Hu et al. 2016, Nadolska-Orczyk et al. 2017). The better situation in cereals is in rice where there are close to 20 known genes involved in grain size and yield regulation (Huang et al. 2013; Zheng et al. 2015; Xu et al. 2016). These genes can affect rice yield in several ways including number of panicles per plant (similar to number of spikes in wheat), number of grains per panicle (similar to number of grains per spike in wheat) and GW. One example is the *GIF1* gene that encodes a cell wall invertase, *GIF1* mutants have lower GW due to loosely packed starch granules that

reduce the grain density and weight (Wang et al. 2008, Control of rice grain-filling and yield by a gene with a potential signature of domestication). Duan et al. (2016) have shown that *GIF1* interacts with *GRF4* and that overexpression of *GIF1* increases grain size and weight. Rice genotypes with a 2 bp mutation in the *GRF4* target site of *mir396* had larger grains in rice (Che et al. 2015; Duan et al. 2015; Tsukaya et al. 2016).

In the current study, we aim to identify the genetic elements controlling GW phenotypic variation in wheat and present our results from QTL analyses. We will associate these results with previously published GW QTL data and demonstrate a reference-based meta-QTL analysis. The genome data will also be used to associate rice GW genes and our meta-QTL data, allowing us to focus on a candidate gene underlying a major QTL on chromosome 6A with a positive effect on GW, contributed by wild emmer wheat.

Materials and Methods

Plant material and growth conditions

A segregating population consisted of 137 F_7 Recombinant Inbreed Lines (RILs) was developed by single-seed decent from from a cross between an elite durum wheat (cv. Svevo; Sv, hereafter) and a wild emmer wheat (accession Zavitan; Zv, hereafter). This population was previously genotyped (Avni et al. 2014) and used for various genetic studies (Avni et al. 2017; Hen-Avivi et al. 2016; Nave et al. 2016; Golan et al. 2018).

The Sv × Zv population was characterized under field-conditions over multi-locations × years × treatments (Table 1). Three to six selected spikes from each experimental unit from the parental lines and their progenies were used for TKW measurements in all the experiments. In 2014R and 2015A the parental lines, Zavitan and Svevo, were evaluated for other grain characters (e.g. length, width and area) using a Qualmaster Computer Vision device (VIBE Technologies, Tel Aviv, Israel).

Development of Zv introgression line population and growth conditions

RILs from the Sv × Zv population were backcrossed three times to Sv, self-pollinated for 5 generations to create a set of BC_3F_5 lines or introgression lines. The introgression lines were genotyped with the wheat 90K iSelect SNP genotyping assay as previously described (Wang et al. 2014). ILs were grown in 2018 as described in table 1 and grains were evaluated for TKW.

Table 1. Location, year, experimental design and conditions used for growing the Sv × Zv segregating RIL population.

Experiment code	Population type	Location	Year	Experimental design and conditions *
2014R	RIL	Rehovot	2014	5 randomized complete blocks consisting of experimental unit with 10 plants per genotype in each block. Regular irrigation was applied unless rains were constant. Slow release fertilizer was applied upon sowing
2015A		Atlit	2015	
2016R (dry/wet)	IL	Rehovot	2016	Seedlings were transplanted into an insect-proof screenhouse protected by a polyethylene top. A split-plot factorial (RIL/IL × irrigation regime) block design with three replicates was employed; each block consisted of two main plots (for the two irrigation regimes), with main plots split into 137 sub-plots for RILs/ILs. Each sub-plot was planted as a single row, with five plants, 15 cm apart (75 cm long plots). The two plants at the edges of each plot served as borders, and the remaining three plants were harvested at the end of the experiment to estimate TKW.
2018R (dry/wet)		Rehovot	2018	

*__For all experiments:__ Seeds were disinfected (3.6% Sodium Hypochloric acid, for 10 minutes) and placed for vernalization in a moist germination paper for 3 weeks in a dark cold room (4ºC), followed by 3 days acclimation at room temperature (24ºC), then planted in the field. The two plants at the edges of each plot served as borders, and the remaining three plants were harvested at the end of the experiment to estimate TKW. The field were treated with fungicides and pesticides to avoid development of fungal pathogens or insect pests and was weeded manually once a week.

QTL analysis

QTL analysis followed the same procedure described by Nave et al. (2016), briefly, a reduced version of the Sv × Zv map, containing 472 markers, was used for QTL analysis with the MultiQTL software (http://www.multiqtl.com). Significance of each QTLs was calculated using a permutation test followed by a genotype × environment interaction analysis.

Meta-QTL analysis

The meta-QTL analysis included the TKW data collected in the current study and from previously published studies; five WEW × durum populations (Peng et al. 2003; Elouafi and Nachit 2004; Peleg et al. 2011; Tzarfati et al. 2014; Golan et al. 2015), two emmer (*T. turgidum* ssp. *dicoccum* Schrank) × durum populations (Faris et al. 2014; Russo et al. 2014), and one population of WEW × DEW (Thanh et al. 2013). To facilitate the identification of common QTLs, the peak marker for each QTL was positioned on the Zv reference genome (Avni et al. 2017) using blast alignment.

Wheat Rice colinearity analysis of yield related genes

We searched the literature for characterized yield-related-genes from rice (*Oryza* spp.) and aligned their sequences to the Zv genome using blast search. The best hits of this search were compared against the WEW annotation and ortholog WEW genes were identified including their genomic location on the

WEW genome.

***GRF4-A* SNP marker development and allelic variation study**

Sequences data with SNP information confined by [brackets] was uploaded to rhAmp® Genotyping Design Tool (IDT) resulting in the following assay: 'Allele Specific Primer 1-CTCCCCTTCTGCCG**T**', 'Allele Specific Primer 2-CTCCCCTTCTGCCG**C**' and 'Locus Specific Primer-GCACAAGAACACGCACCGAA'. The allele specific primers were labeled by different fluorophores. PCR and analysis were performed in PikoReal machine (Thermo). The *GRF4-A* marker was used to genotype our core collection of WEW and DEW genotypes (Avni et al. 2017, Table S10).

Results

Phenotyping

In 2014R and 2015A experiments, the parental lines Sv and Zv, differed in every measure of yield related parameters (Table 2.).

Table 2. Grain parameters of the parental lines Sv and Zv measured in field experiments in 2014 and 2015.

Trait	Location	S	Z
Yield	2014R	3.9±0.1	2.5±0.1
	2015A	4.0±0.1	1.6±0.8
TKW	2014R	61.9±0.2	45.8±0.07
	2015A	55.7±0.1	29.1±0.1
Area	2014R	21.6±0.2	23.3±0.3
	2015A	23.0±0.3	18.6±0.4
Width	2014R	3.2±0.03	2.8±0.03
	2015A	3.7±0.03	2.5±0.03
Length	2014R	8.1±0.08	10.2±0.1
	2015A	8.5±0.05	10.1±0.09

For example, Zv grains measured 10.2 mm and 9.8 mm in 2014R and 2015A, respectively, and were significantly longer (pv < 0.001) than those of Sv 8.1 mm and 8.6 mm for 2014R and 2015A, respectively. Contrary, Zv grain width was significantly shorter (pv < 0.001), 2.8 mm and 2.4 mm compared to Sv with 3.2 mm and 3.7 mm in 2014R and 2015A, respectively. Grain area was less consistent because in Zv grain area was larger than Sv in 2014R (23.3 mm^2 vs. 21.6 mm^2) but smaller in 2015A (17.8 mm^2 and 23.6 mm^2). Notably, TKW of Sv (61.8g and 56.5g in 2014R and 2015A, respectively) is much larger than that of Zv (45.8g and 29.7g in 2014R and 2015A, respectively).

QTL analysis

Combining TKW data from the two experiments (2014R and 2015A) with three additional experiment (2017N, 2016R_wet and 2016R_dry) using the same mapping population, we could get much more statistical strength to our analysis. The TKW mean for each population 2015A, 2016R_wet, 2016R_dry and 2014R was 43.2, 45.0, 46.4 and 49.6, respectively (Figure. 1). The QTL analysis for TKW using these four environments showed 29 significant QTLs with a LOD score ≥ 3 on chromosomes 1A, 1B, 2A, 2B, 3A, 3B, 4B, 5A, 5B, 6A, 6B and 7A (Figure).

The largest QTL (LOD = 10.85) was contributed from WEW and was located on chr 1B, yet this QTL was specific for the 2015A experiment. The 6A QTL had a positive effect contributed by the Zv allele and was found in four environments (2014R, 2016R_dry and 2016R_wet) with LOD values ranging between 3.8 to 6.1. An additional QTL with a positive effect from Zv allele on chromosome 2A was common for 2014R and 2016R_wet (Figure. 1).

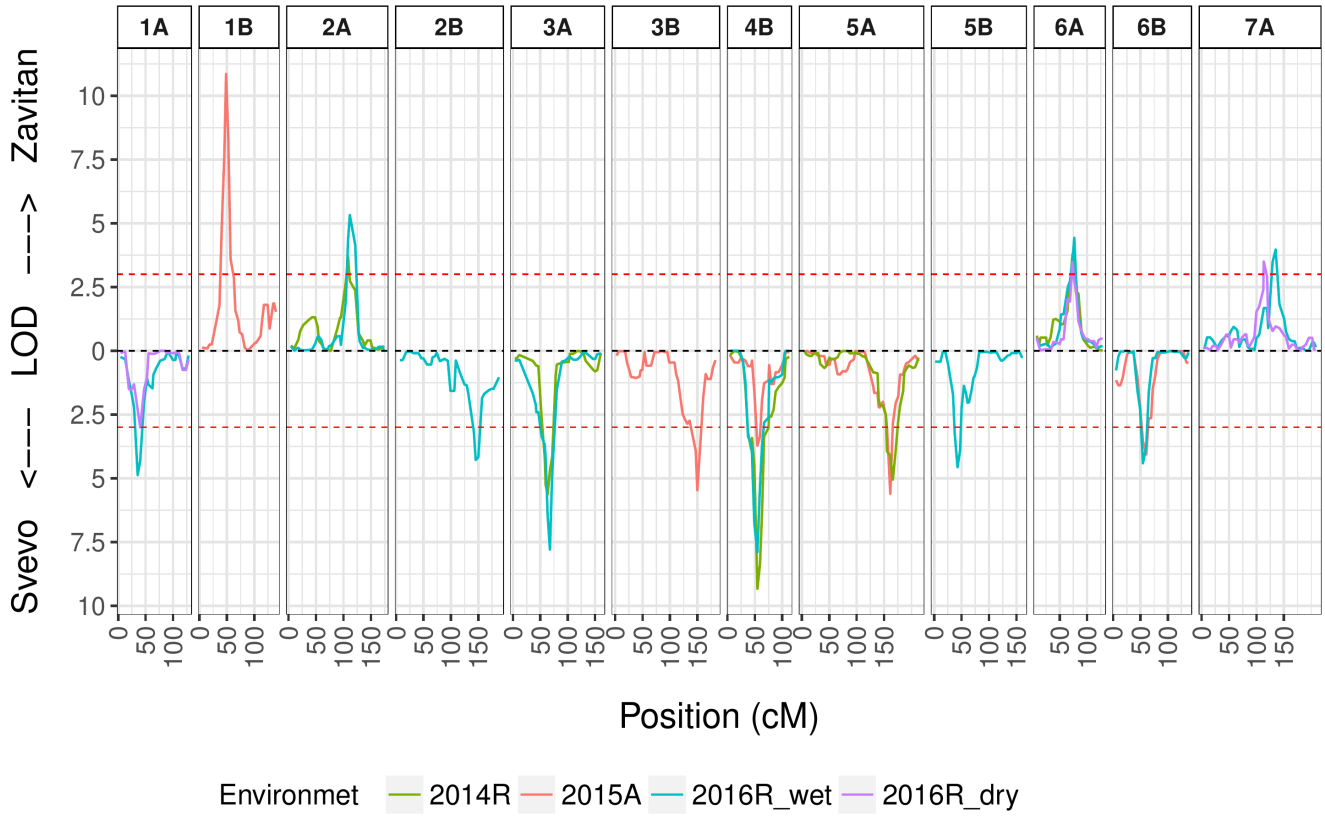

Figure. 1 QTL analysis for TKW in four experiments, the x-axis shows the genetic position (cM) and the y-axis shows the LOD score with the positive effect of each allele Zv (above x-axis) or Sv (below x-axis) contributing the high TKW value.

Meta-QTL analysis

In the meta-QTL analysis we included the above mentioned 5 field experiments with the Sv x Zv population and additional eight studies published previously (Peng et al. 2003; Elouafi and Nachit 2004; Peleg et al. 2011; Thanah et al. 2013; Faris et al. 2014; Russo et al. 2014; Tzarfati et al. 2014 ; Golan 2015). In these eight studies and our current study, the TKW ranged between 10g to 48g in the wild emmer parents and 30g to 74g in the domesticated parents (including emmer), while population means ranged from 29.9g to 58.9g (Table 3.).

To identify overlap between QTLs, we anchored the peak marker of every TKW QTL from all the studies to the WEW genome by a BLAST alignment. The best alignment was chosen by the highest percent of identity, e-value and agreement with the genetic maps. This process was successful in most cases, except when the marker sequence was absent from the public databases (*wPt-9555* and *gwm263* from Peleg et al. 2011; *MctcEagg84* and *gwm144* from Elouafi and Nachit 2004) or when multiple BLAST hits indicated that the peak marker contained a repetitive sequence (MctcEaag350 and *gwm582* from Elouafi and Nachit 2004; *gwm403* Peng et al. 2003). Altogether, we could not find a meta-QTL that was common to all studies, but there were meta-QTLs for two or more studies on all but chromosome 7B. Meta-QTLs in which the wild parent contributed to a higher TKW were found on chromosomes 1A, 1B, 2A, 2B, 3A, 3B, 4A, 4B, 5A, 6A, 6B and 7A while QTLs in which the domesticated parent was responsible for a higher TKW were found on chromosomes 1A, 1B, 2A, 2B, 3A, 3B, 4B, 5A, ,5B, 6A and 6B (Figure. 2).

Table 3. Mean TKW of parental lines and segregating populations in studies used for the current meta-qtl analysis.

Paper	TKW (g) wild/emmer	TKW (g) durum/emmer	TKW (g) mean of population
Elouafi and Nachit 2004	28.6	32.1	29.9
Peleg (well-watered) 2011	42	46	42
Peng 2003	10	30	-
Thanh 2013	19	52 (emmer)	-
Faris 2014	28.84 (emmer)	55.12	40.84
Russo (2011, 2015 exp.) 2014	58, 45.5 (emmer)	74, 54.5	58.9, 56.9
Golan (Wild and durum, mean of 12 acc. Each) 2015	37.7	55.7	-
Tzarfati (2011, 2012 exp.) 2014	48, 45	56, 51	-
Avni (2014R, 2015A exp.) 2018 (current)	45.8, 29.7	61.8, 56.5	49.6, 43.1

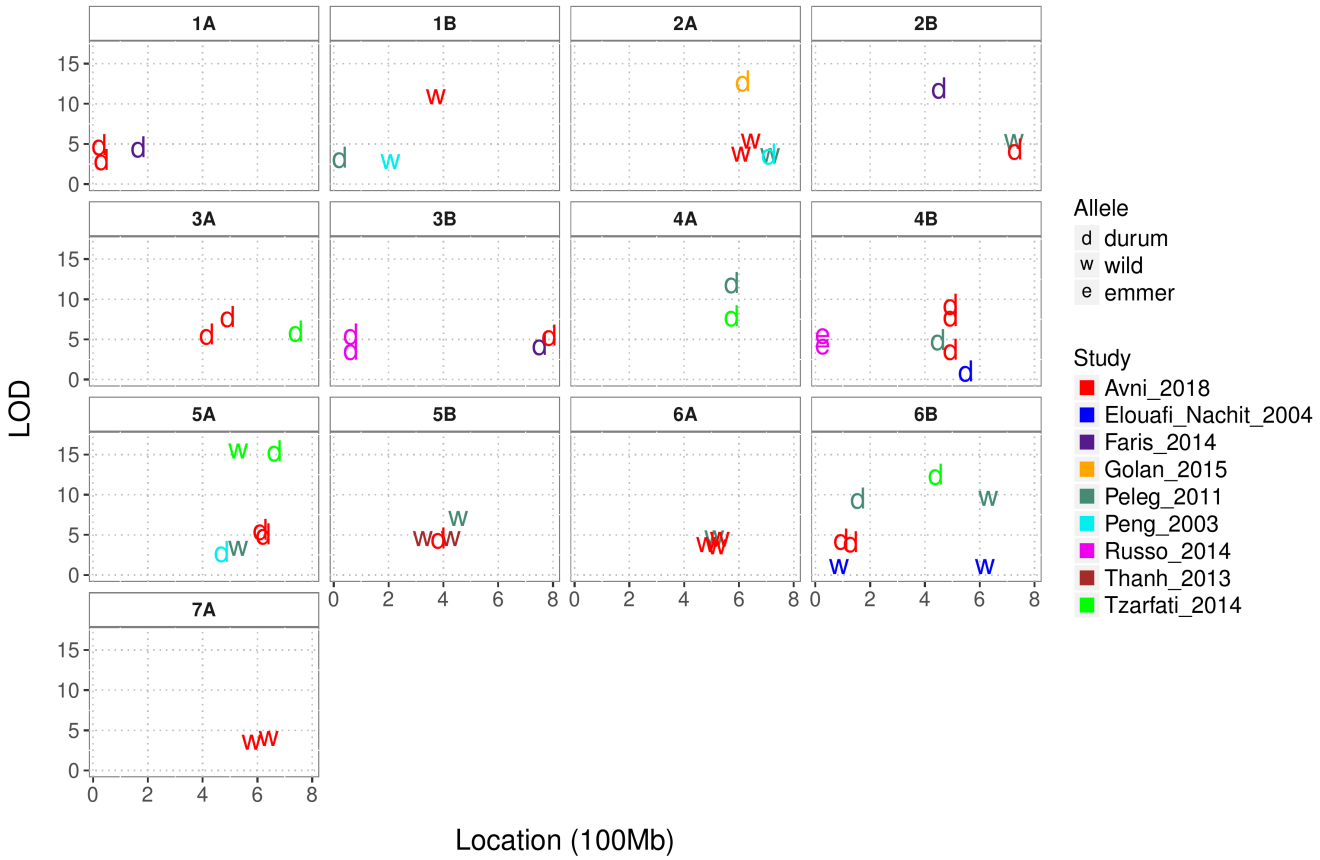

Figure. 2 Meta-QTL analysis of TKW QTLs using the WEW genome assembly. X-axis shows the position on the WEW genome (1 unit = 100Mb) and the y-axis shows the LOD score. Each study is represented in a different color (see legend) and the positive allele is indicated as (d) for durum, (w) for wild, or (e) for emmer.

Next, we focused our efforts to study the meta-QTL on chromosome 6A (designated *mQTL-GW-6A*) because it showed consistent contribution of higher TKW from WEW, therefore may present genetic diversity with breeding potential that is currently absent from the domesticated genepool.

Validation of the *mQTL-GW-6A* using Sv × Zv introgression lines

To learn more about *mQTL-GW-6A*, we selected a BC3F5 introgression line designated NIL-21.1, based on the results of the 90K iSelect SNP genotyping assay. NIL-21.1 carry most of Zv chr. 6A (37-553 Mb) in the background of Sv and includes the *mQTL-GW-6A* region (480-540Mb). In addition, NIL-21.1 carry small introgressions (<40 Mb) on chromosomes 3B, 5B and 6B. In 2018R field experiment, NIL-21.1 showed higher TKW than Sv (average of 67.0g vs. 61.8g, *pv*=0.005, Figure 3A and B).

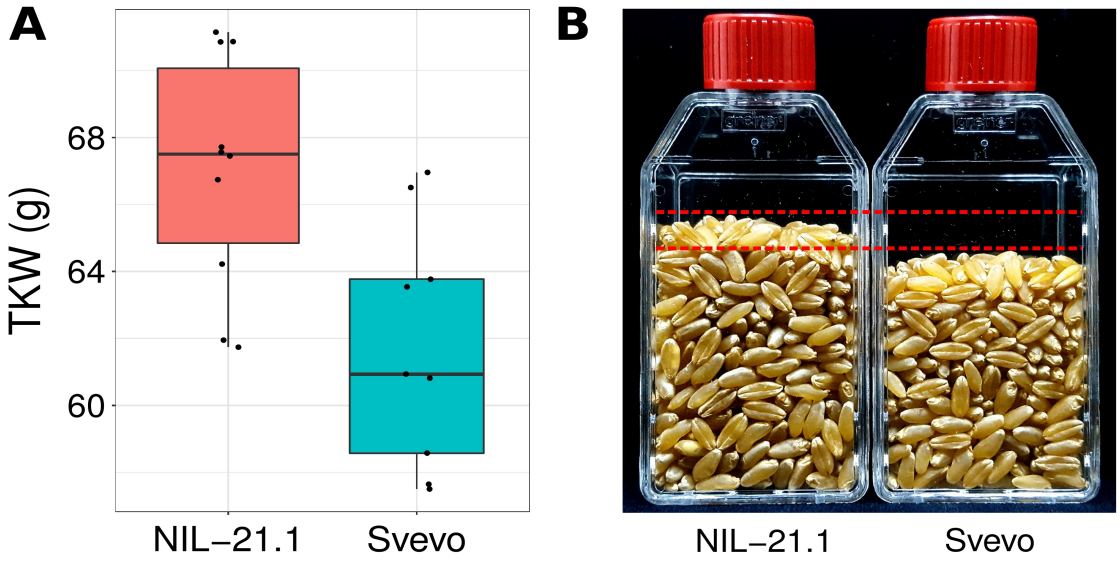

Figure. 3 Differences in TKW between Sv and NIL-21.1 carrying *GRF4-A*, (A) boxplot shows quantile statistics for TKW from the 2018R experiment (*pv*=0.005). (B) Samples from 2018R field experiment of 500 grains from NIL-21.1 and Sv showing the overall larger grains of the introgression line that carry *mQTL-GW-6A* compared to Sv.

Rice and wheat yield related genes

The extensive work that was done in rice to identify yield related genes may provide candidate wheat genes responsible to the TKW QTLs and meta-QTLs like *mQTL-GW-6A*. To explore this possibility, the sequences of yield related genes from rice were aligned to the WEW genome and, in most cases, we could find their orthologs on the A and B subgenomes of wheat. For some rice genes such as *OsGRF4*, *OsGW5* and *OsSRS3*, we detected wheat paralogous genes in addition to orthologs (Table 4.).

Table 4. Summary of association between yield-related genes from the literature and WEW genes using the WEW reference genome. The table shows the rice function, and reference paper, the wheat chromosome, start and end as found in a blast alignment and the WEW gene function and location (start and end) from the WEW annotation.

Organism	Gene	Rice gene function	Reference	Wheat chr.	Wheat alignment start	Wheat alignment end	Wheat gene function	WEW gene ID
Rice	D11/ DWARF11	Cytochrome P450 (CYP724B1) enzyme	Reviewed by Huang 2013	2A	561795447	561798557	Cytochrome P450 superfamily protein	TRIDC2AG048380
				2B	496938005	496941148	Cytochrome P450 superfamily protein	TRIDC2BG050840
	D2	Cytochrome P450 (CYP90D) enzyme	Reviewed by Huang 2013	2A	4843464	5209969	Cytochrome P450 superfamily protein	TRIDC2AG001470
				2B	5686865	5987817	Cytochrome P450 superfamily protein	TRIDC2BG001370
	D61	BR insensitive (BRI)-like leucine-rich repeat (LRR) receptor kinase	Reviewed by Huang 2013	3A	465976238	465979780	Leucine-rich receptor-like protein kinase family protein	TRIDC3AG036670
				3B	453931439	453935096	receptor-like protein kinase 2	TRIDC3BG041310
	GIF1	Cell wall invertase	Reviewed by Huang 2013	2A	503854205	503855081	Beta-fructofuranosidase, insoluble isoenzyme 2 (Cell wall invertase 2)	TRIDC2AG042730
				2B	447195335	447196211	Beta-fructofuranosidase, insoluble isoenzyme 2 (Cell wall invertase 2)	TRIDC2BG045820
	GRF4/GS2	Growth-Regulating Factor 4 (OsGRF4)	Duan 2015, Sun 2016	2A	680343644	680346735	Growth-regulating factor 3	TRIDC2AG062550
				2B	649416512	649417723	Growth-regulating factor 3	TRIDC2BG066890
				6A	497985063	497985958	Growth-regulating factor 4	TRIDC6AG041360
				6B	517412993	517416246	Growth-regulating factor 4	TRIDC6BG048340
	GS3	Membrane protein with multiple domains	Reviewed by Huang 2013	4A	714924235	714925670	Grain length protein	TRIDC4AG069340
				7A	5283743	5283958	Grain length protein	TRIDC7AG001510
	GS5	Serine carboxypeptidase	Reviewed by Huang 2013	3A	182355936	182359086	serine carboxypeptidase-like 33	TRIDC3AG023140
				3B	212372375	212373474	Carboxypeptidase Y homolog A	TRIDC3BG026960
	GW2	RING-type E3 ubiquitin ligase	Reviewed by Huang 2013	6A	230789449	230809149	Protein SIP5 (*TaGW2)	TRIDC6AG027660
				6B	294434000	294448424	Protein SIP5	TRIDC6BG033820
	GW5	Arginine-rich protein of 144 amino acids	Reviewed by Huang 2013	1A	142379896	142381359	IQ-domain 26	TRIDC1AG017640
				1B	185320338	185321816	IQ-domain 26	TRIDC1BG021520
				3A	69160021	69161092	IQ-domain 26	TRIDC3AG013280
				3B	111226601	111227636	IQ-domain 26	TRIDC3BG017740
	GW8/SPL16	SQUAMOSA promoter-binding protein-like 16	Reviewed by Huang 2013	7A	251030195	251034936	undescribed protein	TRIDC7AG033770
				7B	230000953	230005263	Squamosa promoter-binding-like protein 16	TRIDC7BG025060
	qGL3	Ser/Thr phosphatase of the protein phosphatase kelch-like (PPKL) family	Reviewed by Zheng 2015	5A	683802818	683803388	Bifunctional inhibitor/lipid-transfer protein/seed storage 2S albumin superfamily protein	TRIDC5AG075900
	SRS3	Kinesin 13 protein	Reviewed by Huang 2013	1A	131830406	131835083	Kinesin-related protein 6	TRIDC1AG016970

				1B	142745967	142751957	Kinesin-related protein 6	TRIDC1BG017960
				3A	274848912	274857469	Kinesin-related protein 6	TRIDC3AG027550
				3B	295258442	295261011	Kinesin-related protein 6	TRIDC3BG032430
	DEP1	G protein γ subunit	Xu 2016	5A	422466437	422469555	Guanine nucleotide-binding protein subunit gamma 3	TRIDC5AG033880
				5B	391766206	391769237	undescribed protein	TRIDC5BG035790
Wheat	TaCKX6	Cytokinin oxidase/ dehydrogenase 6-like	Reviewed by Nadolska-Orczyk 2017	1A			Cytokinin dehydrogenase 3	TRIDC1AG024270
				1B			Cytokinin dehydrogenase 3	TRIDC1BG029500
	TaTGW6	IAA-glucose hydrolase	Reviewed by Nadolska-Orczyk 2017 /Hu 2016	Un			strictosidine synthase-like 3	TRIDC0UG011660

This analysis enabled us to identify a candidate gene located within the region of *mQTL-GW-6A* (480-540Mb); This gene is designated as *Growth-Regulating Factor 4* (*OsGRF4*; Duan et al. 2015; Sun et al. 2016) and is located on rice chromosome 2. The best two hits in the WEW genome for *OsGRF4* were on chromosomes 6A (positions 497980067-497986236, 73% identity) and 6B (positions 517,412,655-517,414,135, 73% identity). These regions correspond to two genes designated in the Zv gene annotation as *TRIDC6AG041360* (*GRF4-A*) and *TRIDC6BG048340* (*GRF4-B1*).

GRF4-A polymorphisms

Sequence comparison of *GRF4-A* 1227-bp coding sequence in Zv and Sv (Maccaferri et al, in publication) showed four SNPs in positions 93, 342, 5610 and 5661. The first SNP is synonymous but the other three SNPs translate into three AA changes between Zv and Sv; P83S, R319G and G336S.

Allelic diversity study using molecular marker for the *GRF4-A*

We have developed a molecular marker based on the SNP in position 93 of *GRF4-A*. This marker was used to genotype a core collection of wild and domesticated tetraploid genotypes. The results showed that only two additional WEW genotypes (WE-10 and WE-12, both from Israel, Table S1) carried the Zv allele while all the rest of the accessions (wild and domesticated) carried the Sv allele (Table S1).

Discussion

It is well established that domesticated wheat has heavier grains than its wild progenitor, the grain of domesticated wheat is usually also wider and shorter while wild wheat has longer and narrower grains (Gegas et al., 2010; Golan et al. 2015). Contrary to domesticated wheat, in rice, grain length is much more variable with a range of 3 to 11 mm (Huang et al. 2013). Yet, the wild varieties of wheat have a large variation in grain length with a range of up to 5.6mm (Gegas et al. 2010; Nave et al. 2016). It appears as though in wheat the domestication process targeted grain width and this was explained by (1) selection due to pleiotropic effects during domestication (Gegas et al. 2010) or (2) by a relation between glume shape, softness and grain width in domesticated wheat (Xie et al. 2018; and in rice Shomura et al. 2008; Okamoto et al. 2013). The genetic mechanisms that are involved in this evolutionary process are not well understood and therefore we initiated a genetic dissection of GW using a biparental tetraploid mapping population. Reference genomes for the parental genotypes, wild emmer wheat (accession Zv) and durum wheat Sv were assembled and annotated (Avni et al. 2017; Maccaferri et al. 2018) making the Sv × Zv RIL population an excellent resource to study wheat domestication and to identify genetic factors responsible for the different grain characteristics. Interestingly, our genetic studies with the Sv × Zv population shows several RIL genotypes with higher TKW than the parental lines and this transgressive segregation points to the improvement potential of WEW.

Since we wanted to identify QTLs that possibly associated with wheat domestication, we used all the populations that used wild wheat (*T.dicoccoides* and *T.dicoccum*) as parental line and conducted a meta-QTL analysis. Until recent, meta-analysis studies usually relay on a consensus map constructed by either combining maps based on common markers by a homothetic projection process and solving conflicting markers locally (Goffinet and Gerber 2000; Arcade et al. 2004 'BioMercator') or by completely avoiding conflicting markers and instead analyzing all datasets as a single population (by reducing the consensus-mapping problem to single-population ordering via constructing a synthetic distance matrix from all datasets, hence avoiding situations with conflicting markers) (Mester et al. 2015; Maccaferri et al. 2014). Here, we used the WEW reference genome to anchor the QTL markers using their sequences alignment to the genome. This process was efficient as we were able to find the physical location of most QTL markers. This strategy allowed a straightforward comparison between the results from all the QTL studies, accurately locate similar overlapping QTLs, avoiding the need to have even one common marker between the populations. The current study focused on TKW but the general scheme is true for all QTL experiments in wheat, which can now be analyzed using a reference genome and without genetic distance estimations. Our meta-QTL analysis showed more than 10 loci

that are associated with higher TKW from wild wheat and we chose to focus on a 6A locus that showed increased TKW in most studies. This locus, *mQTL-GW-6A*, spans a 60 Mb region (480-540Mb) including 650 genes of which 411 are high-confidence genes and 239 are low-confidence genes as defined by Avni et al. (2017). To validate this result we selected an introgression line with a large region of chromosome 6A in the background of durum wheat and conducted field experiment to evaluate TKW. This introgression line, NIL-21.1, showed higher TKW, width, length and area compared with Sv, a result that may indicate a potential for wheat breeding programs aimed to increase yield. The introgression in NIL-21.1 includes *TaGW2-A* (Simmonds et al. 2016, located at ~230Mb on Zavitan genome) which may further contribute to the large grain size of NIL-21.1, yet since the meta-QTL on 6A did not include TaGW2 we think that the 6A QTL is independent of the *TaGW2* effect. Classically, the next step in genetic dissection of a QTL region would include saturation of the region with critical recombinant plants (Distelfeld et al. 2004). This is also valid in the case of *mQTL-GW-6A* where further validation using backcrossed NIL-21.1 progeny is needed in order to clean the background from other wild introgressions and to reduce the 6A introgression. This process is time consuming taking typically few years but it usually allows a thorough examination of the QTL effects, including the study of trade-off with other yield components, and genotype by environment interactions. Alternatively, we decided to proceed with a candidate gene approach using knowledge from the literature about yield related genes.

Studies in rice have found many genes related to grain size and yield (Huang et al. 2013) and a large natural variation (Lu et al. 2013). The comparison between our meta-QTLs and grain size genes from rice revealed that *GRF4-A* a homolog of *OsGRF4* (Duan et al. 2016) lies within the range of *mQTL-GW-6A*. *OsGRF4* is a transcription factor involved in chromatin-remodeling factors, with a higher expression in rice panicles. *OsGRF4* expression is negatively regulated by OsmiR396 which cleaves the transcript at a specific target site. In certain rice varieties there is a mutation TC to AA at the cleavage site, which results in lower expression of *OsGRF4*. Rice plants with the site resistant to miR396 cleavage had larger and especially longer hull and grain due to higher expression of *OsGRF4* (Duan et al. 2016). To further associate between *GRF4-A* and *mQTL-GW-6A* we examined the allelic differences between Sv and Zv, identified four SNPs in the coding sequence and developed a molecular marker for the first SNP. Allelic diversity analysis on a core collection using this *GRF4-A* marker revealed that the Zv allele is rare (Only two genotypes out of 64 wild and domesticated genotypes, table S10, Avni et al. 2017). In fact, the genotypes that carry the Zv *GRF4-A* allele cluster together (Fig. 4 in Avni et al. 2017) to a branch that is associated with the *Judaicum* subpopulation, consisting of Zv

and five WEW accessions from southern Levant. The *Judaicum* subpopulation have previously shown to possess a more robust grain phenotype than the more widespread *Huranum* subpopulations (Poyarkova et al. 1991; Ozkan et al. 2011; Sela et al. 2014). Therefore, we suggest that the polymorphisms in *GRF4-A* may be associated with those phenotypic differences between the two subpopulations.

Conclusions

We showed here that the recent assembly of the wild emmer genome opened the way for genome based genetic dissection of phenotypic variation. The existence of a high quality ordered genome facilitate co-localization of QTLs from different studies and different organisms (e.g rice). Combining such meta-QTL study with a well annotated genome can point out to a potential gene underlying the studied trait. *GRF4-A*, the ortholog of yield related gene in rice, *OsGRF4*, was associated with *mQTL-GW-6A*, a meta-QTL with a positive effect on grain size originating from WEW. *GRF4-A* marker may be related to the differences between the *Huranum* and *Judaicum* subpopulations of WEW and the Zv allele of this gene is absent from domesticated wheat genepool. *GRF4-A* appears to be a valid target for genome editing and the integration of the Zv allele in different backgrounds is needed in order to assess its potential to regulate grain size and increase yields in wheat.

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Supplementary

Table S1.

Label	Location	Accession	Species	Improvement status	GRF4_marker
WE-1	Central Israel	PI 471021	dicoccoides	wild	-
WE-2	Northern Israel	PI 538673	dicoccoides	wild	-
WE-4	Central Israel	PI 471038	dicoccoides	wild	-
WE-6	Kazin, Syria	PI 466946	dicoccoides	wild	-
WE-7	Northern Israel	Qazerin (UH 5)	dicoccoides	wild	-
WE-8	Northern Israel	PI 466957	dicoccoides	wild	-
WE-9	Northern Israel	Nesher (UH 27)	dicoccoides	wild	-
WE-10	Central Israel	PI 471060	dicoccoides	wild	+
WE-11	**Northern Israel**	**Zavitan**	**dicoccoides**	**wild**	**+**
WE-12	Northern, Israel	PI 467008	dicoccoides	wild	+
WE-14	Central Israel	PI 470962	dicoccoides	wild	-
WE-15	Central Israel	PI 466950	dicoccoides	wild	-
WE-16	Central Lebanon	PI 428132	dicoccoides	wild	-
WE-17	Central Lebanon	PI 352322	dicoccoides	wild	-
WE-18	Northern Israel	Mt. Hermon (UH 1)	dicoccoides	wild	-
WE-19	Central Israel	Mt. Gerizim (UH 17)	dicoccoides	wild	-
WE-20	Halab, Syria	PI 487264	dicoccoides	wild	-
WE-21	Iraq	Iraq (UH 41)	dicoccoides	wild	-
WE-22	Central Lebanon	PI 428129	dicoccoides	wild	-
WE-23	Central Turkey	PI 428066	dicoccoides	wild	-
WE-24	Diyarbakir, Turkey	PI 428084	dicoccoides	wild	-
WE-25	Karacadag, Turkey	PI 538666	dicoccoides	wild	-
WE-26	Diyarbakir, Turkey	PI 428054	dicoccoides	wild	-
WE-29	Diyarbakir, Turkey	PI 538642	dicoccoides	wild	-
WE-30	Diyarbakir, Turkey	PI 428072	dicoccoides	wild	-
WE-31	Central Turkey	PI 428070	dicoccoides	wild	-
WE-32	Diyarbakir, Turkey	PI 428036	dicoccoides	wild	-
WE-33	Diyarbakir, Turkey	PI 428025	dicoccoides	wild	-
WE-34	Diyarbakir, Turkey	PI 538631	dicoccoides	wild	-
DE-1	Oman	PI 532302	dicoccum	domesticated	-
DE-2	India	PI 322232	dicoccum	domesticated	-
DE-3	Central Turkey	PI 319868	dicoccum	domesticated	-
DE-4	Central Turkey	PI 319869	dicoccum	domesticated	-
DE-5	Central Israel	PI 352347	dicoccum	domesticated	-
DE-6	Southern Turkey	PI 355454	dicoccum	domesticated	-
DE-7	Central Israel	PI 355496	dicoccum	domesticated	-
DE-8	Central Israel	PI 352357	dicoccum	domesticated	-
DE-10	Central Israel	PI 352367	dicoccum	domesticated	-
DE-11	Southern Turkey	PI 352352	dicoccum	domesticated	-
DE-12	Central Turkey	PI 182743	dicoccum	domesticated	-
DE-14	Italy	PI 352361	dicoccum	domesticated	-
DE-15	Spain	PI 191091	dicoccum	domesticated	-
DE-16	Spain	PI 276007	dicoccum	domesticated	-
DE-17	Central Turkey	PI 606325	dicoccum	domesticated	-
DE-18	Central Turkey	PI 352329	dicoccum	domesticated	-
DE-19	Ukraine	PI 94741	dicoccum	domesticated	-

DE-20	Slovenia	PI 377658	dicoccum	domesticated	-
DE-21	Croatia	PI 264964	dicoccum	domesticated	-
DE-22	Bosnia and Herzegovnia	PI 434995	dicoccum	domesticated	-
DE-23	Iran	PI 254158	dicoccum	domesticated	-
DE-24	Iran	PI 254169	dicoccum	domesticated	-
DE-26	Central Turkey	PI 470739	dicoccum	domesticated	-
DE-27	Central Turkey	PI 470738	dicoccum	domesticated	-
DE-28	Armenia	PI 94661	dicoccum	domesticated	-
DE-29	Central Turkey	PI 470737	dicoccum	domesticated	-
DE-30	Georgia	PI 326312	dicoccum	domesticated	-
DDW	**Italy**	**Svevo**	**Durum**	**domesticated**	**-**