Whealbi

Wheat and barley Legacy for Breeding Improvement

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Collaborative Project
SEVENTH FRAMEWORK PROGRAMME

Deliverable D6.4

Wheat lines with introgressed QTLs for yield and yield related traits originating from wild emmer wheat, accompanied with associated molecular markers for further use in breeding

Due date: M60

Actual submission date: M60

Project start date: January 1st, 2014 **Duration:** 60 months

Workpackage concerned: 6

Concerned workpackage leader: UH

Dissemination level: PU

Glossary and Definitions

CWR	Crop wild relatives
GPC	Grain protein content
GPD	Grain protein deviation
GY	Grain protein content
KASP	Kompetitive allele specific PCR
LG	linkage groups
MAS	Marker assisted selection
MEA	Multi-environment analysis
PEV	Percent of Explained Variation
QTL	Quantitative Trait Loci
RIL	Recombinant Inbred Line
WEW	Wild emmer wheat

Summary

Objectives
The overall goal on this work package is to improve the utilization of genetic resources in pre-breeding of wheat and barley.
Specific objective of WP 6.2 is to provide wheat lines with introgressed QTLs for yield and yield related traits originating from wild emmer wheat, accompanied with associated molecular markers for further use in breeding.

Rationale: Wheat is an important source for protein and micronutrients, however, grain protein content (GPC) of modern wheat cultivars is relatively low (10-14.2%). Consequently, improved GPC is one of major goal for plant breeding. Earlier studies indicated that wild emmer wheat (WEW), *Triticum dicoccoides,* collections have high diversity of GPC and grain mineral content (Chazav et al., 2010). *Gpc-B1* that was cloned from WEW in 2006 is currently the only gene in wheat with a characterized role in protein and nutrient remobilization (Uauy et al., 2006). It is an excellent example of successful introgression of functional allele of the gene from CWRs for improvement of grain quality in elite domesticated cultivars, however, to improve grain quality in diverse environments and in different genetic backgrounds, it is important to identify additional QTLs/genes for increasing GPC. Within the WHEALBI project, novel QTLs that were identified on chromosomes of WEW 4B, 5B, and 7A will be transferred into elite wheat cultivars by the marker assisted selection (MAS) approach.

Teams involved: UH

1. Marker assisted breeding of QTLs for high grain protein content (GPC) from wild emmer wheat (WEW)

1.1. Material and Methods

1.1.1. Plant Material

The genetic material used as a basis of this task is a F6 150 RILs mapping population derived from the cross *Triticum durum* cv. Langdon X *T. dicoccoides* acc. G18-16. This population was described in various papers including QTL mapping of drought resistance and QTLs for grain protein and minerals content (Peleg et al., 2009a and b). In the current project, specific RILs carrying GPC QTL alleles from WEW on chr. 4B, 5B and 7A were selected as potential donors for introgressions into Israeli elite cultivars that have high yield but low GPC: Ruta, Gedera, Galil, Barnir, Yuval and Zahir. The RILs were originally selected for introgression based on a previous genetic map, which included 600 SSR and DArT markers. However, due to the lack of markers in the QTL target regions, the mapping population was re-genotyped with single nucleotide polymorphism (SNP) array for the construction of a high density genetic map.

1.1.2. Construction of high-density genetic map and QTL analysis

1.1.2.1. Genotyping

The 150 RILs were genotyped using a 15K Infinium SNP array, which is an optimized and reduced version of the 90K iSELECT SNP-chip described by Wang et al. (2014). The development of the 15K SNP-chip and genotyping was performed by TraitGenetics GmbH (http://www.traitgenetics.com) (Muqaddasi et al., 2017).

1.1.2.2 Genetic mapping

The map was constructed using MultiPoint software, version «UltraDense» (http://www.multiqtl.com). After filtering for missing data (removing markers with more than 10% missing data points) and large segregation distortion ($\chi 2 > 35$), the function "bound together" was applied to select the best candidate skeleton markers representing groups of co-segregating markers with size of ≥2) (Ronin et al., 2017). Clustering of candidate markers into linkage groups (LG) was performed at the threshold of recombination fraction RF=0.2. The next step included marker ordering and testing of the local map stability and monotonicity for each LG (Mester et al., 2003; Korol et al., 2009). Reducing of the final number of LGs to 14, corresponding to haploid number of tetraploid wheat chromosomes, was performed by merging the LGs with minimum pairwise RF values expressed by their end markers (end-to-end association). Orientation of each LG in relation to the short (S) and long (L) chromosome arms was performed according to the correspondence of the mapped markers with those on the consensus maps of hexaploid (Wang et al., 2014) and tetraploid wheat (Maccafferi et al., 2015).

1.1.2.3 Phenotyping and QTL analysis

1.1.2.3.1 Phenotyping

Yield components were obtained from Peleg et al. (2009 a); GPC results used for QTL mapping were obtained from Peleg et al., (2009). Nitrogen in the grain was determined by using a C/N analyzer (TruSpec CN, Leco Co., USA). Grain nitrogen concentration was multiplied by 5.83 to obtain GPC values. Phenotypic measurements in introgression lines included the following traits: plant height, days from planting to

heading, chlorophyll content at the heading stage, number of productive spikes per plant, number kernels per spike, thousand kernel weight, total yield per plant, GPC and grain protein yield per plant. Grain Nitrogen was calculated based on Kjeldahl method, multiplied by 5.83 to obtain GPC values.

1.1.2.3.2 QTL analysis

QTL mapping was applied using the general interval mapping (IM) procedure of MultiQTL software package (http://www.multiqtl.com). First, single-QTL and two-linked-QTL models were used for screening of genetic linkage for each trait in each environment separately (Korol et al., 2009). Multi-environment analysis (MEA) was performed by joint analysis of trait values scored in two environments (WL and WW). After separate analysis for each chromosome, multiple interval mapping (MIM) was used for reducing the residual variation for each QTL under consideration, by taking into account QTLs that reside on other chromosomes. The significance of the detected QTL effects was tested using 5000 permutation runs. Significant models were further analyzed by 5000 bootstrap runs to estimate standard deviations of the chromosomal position and QTL effect. Overlapping QTL effects, when a detected QTL affects two or more separate traits, were referred to as multi-trait QTLs.

1.2. Results

1.2.1. High-density genetic map

Genotyping of the G×L RIL population, followed by quality control, resulted in 4,347 polymorphic SNP markers. Out of these, 4,015 SNPs representing 1,369 unique loci (skeleton markers) were clustered into 14 LGs. The genetic map covered 1835.7 cM (953.1 cM for the A genome and 882.6 cM for the B genome). The number of skeletal markers and length of individual chromosome maps ranged from 51 (84.6 cM) for chr. 4B to 146 (165.3 cM) for chr. 5B. The order of markers on the current genetic map showed highly similar positions on the WEW pseudomolecules (average rank correlation coefficient 0.999). The most significant result of the new genetic map is the exposure of chromosomal regions that were not present in the previous map, such as the complete 4BS arm that was missing in the previous map, as well as new regions in 3AS, 4A, 5A, 5B, 7A and 7B.

1.2.2. QTL analysis

Negative correlations were found between GY and most of the grain nutrient traits. Grain protein (GPD) and grain nutrient deviations, which are based on the residuals of linear regression between GY and nutrients content, were applied to obtain traits that are independent of productivity QT. The ultra-dense genetic map exposed new chromosomal regions carrying novel QTLs, which were not present in the previous map. Furthermore, the new map improved the resolution of QTLs, with increased LOD scores, and higher percent of explained variation (PEV), and reduced interval length. A total of 13 QTLs for GPC were identified, of which 11 were contributed by the WEW parent, with LOD score range of 2.0–19.6 and PEV range of 1.2–26.9%. The aim was to select QTLs for GPC while avoiding chromosome regions that negatively affect yield. Of the 11 GPC QTLs we selected the most promising ones for introgression into wheat cultivars: *QGpc.huj.uh-4B* on chromosome 4BS; *QGpc.huj.uh-7A* on 7AL and *QGpc.huj.uh-5B* on 5BS (Table 1). QTL mapping results indicated that QTLs affected GPD that excluded negative effect of those QTLs on grain yield at F6 RILs. The GPC QTLs (located on 4BS, 5BS, 6BS and 7AL) had also pleiotropic effects on other nutrients. The 4BS QTL had pleiotropic effects on GPC and chlorophyll content (Chl),

suggesting alterations in N-use or remobilization as an underlying nature of the QTL. The 5BS QTL had pleiotropic effects on GPC and Sulfur concentration in the grains, and the 7AL QTL had pleiotropic effects on GPC, copper, zinc and iron content. The QTL on 6BS was located in putative region for the known gene *Gpc-B1* affecting GPC and mineral content.

1.2.3. Marker assisted breeding

Three parental RILs (RIL12; RIL55; RIL105) were selected for MAS backcross program for transferring three QTLs, based on the QTL analysis and graphical genotyping. These RILs were crossed with six Israeli bread wheat cultivars to produce F1, after which three rounds of backcrossing and three generations of selfing were conducted. Plants were grown in the greenhouse at the University of Haifa. It is important to note that a delay at the beginning of the project occurred due to large amount of mortality of F1 hybrids between the hexaploid cultivars and tetraploid RILs, which required additional crosses.

MAS was implemented using KASP markers, which were converted from the 15K SNP array (a subset of 90K array) using Polymarker (http://polymarker.tgac.ac.uk/). Three KASP markers were used to follow each QTL across backcross, generations: two flanking and one in the center of the QTL region to avoid influence of recombination. The heterozygote BC1F1, BC2F1 plants were crossed with recurrent parental lines and BC3F1 were selfed to produce BC3F2 and seeds. Altogether 344 BC2F1, 707 BC3F1 and 537 BC3F2 plants were screened. This procedure yielded the following: (i) 129 BC3F3 lines based on Ruta cultivar; (ii) 30 BC3F3 lines based on Gedera; (iii) 3 BC3F3 lines based on Galil, and (iv) 3 BC3F3 lines based on Barnir. Based on the results we selected seeds of BC3F3 for validation of the phenotypic effect of the introgressions. We selected homozygote introgression lines which had either a full target QTL region/s, recombinants between tested markers, and their controls (sister lines without introgressions). A total of 63 BC3F3 lines, from 33 BC families, were tested in field experiment in 2018 for phenotypic validation of introgressions in relation to GPC and GY. For the field validation we have selected introgression lines with RIL12 as the tetraploid parent since BC3F3 plants with RIL55 or RIL105 as a parents were less successful. Plants were grown in three randomized blocks, each BC3F3 was represents by six plants in each block (i.e. total of 600 plants were included in the experiment).

1.2.4. Phenotypic effects of WEW alleles of GPC loci in hexaploid wheat.

BC3F3 introgression lines with three major GPC QTLs on 4BS, 5BS and 7AL in the background of high-yielding with low-GPC Israeli hexaploid cultivars, were grown in the field in 2018 for phenotypic validation of GPC and GY. The preliminary results of GPC and GY are presented here for the introgressions in Ruta and Gedera (both with ~ 13% GPC, recorded by the Israeli Ministry of Agriculture as mean of 10 years) (Fig. 1). The results obtained for NILs with full homozygosity of the introgression regions (Fig. 1) showed an increase in GPC with an average of 10.0% for 4BS NILs, 11.8 % for 5BS NILs, 15.1% for 7AL NILs in Ruta background and 8.7% in average for all NILs in Gedera background. Samples obtained from the single high-yielding plants carrying WEW introgressions did not show a decrease in GPC, indicating that the increase in GPC is independent of GY in these NILs. The similar increase in GPC for introgression lines developed based on two cultivars (Ruta and Gedera) confirmed the independence of introgression regions effects in different genetic backgrounds.

Table 1. Biometrical parameters of effects for three QTL that affect GPC selected for introgression into bread wheat cultivars.

QTL effects	1.5 LOD support interval	DRY 2005		WET 2005	
	LOD	PEV	d	PEV	d
4BS					
QGpc.huj.uh-4B	8.7	0.17	1.44	0.1	1.13
QGpd.huj.uh-4B	6.8	0.15	0.77	0.03	0.33
5BS					
QGpc.huj.uh-5B.1	11.1	0.13	1.22	0.07	0.99
QGpd.huj.uh-5B.1	7.6	0.13	0.74	0.07	0.51
7AL					
QGpc.huj.uh-7A	8.5	0.02	0.4	0.06	0.84
QGpd.huj.uh-7A	5.5	0.02	0.26	0.1	0.61

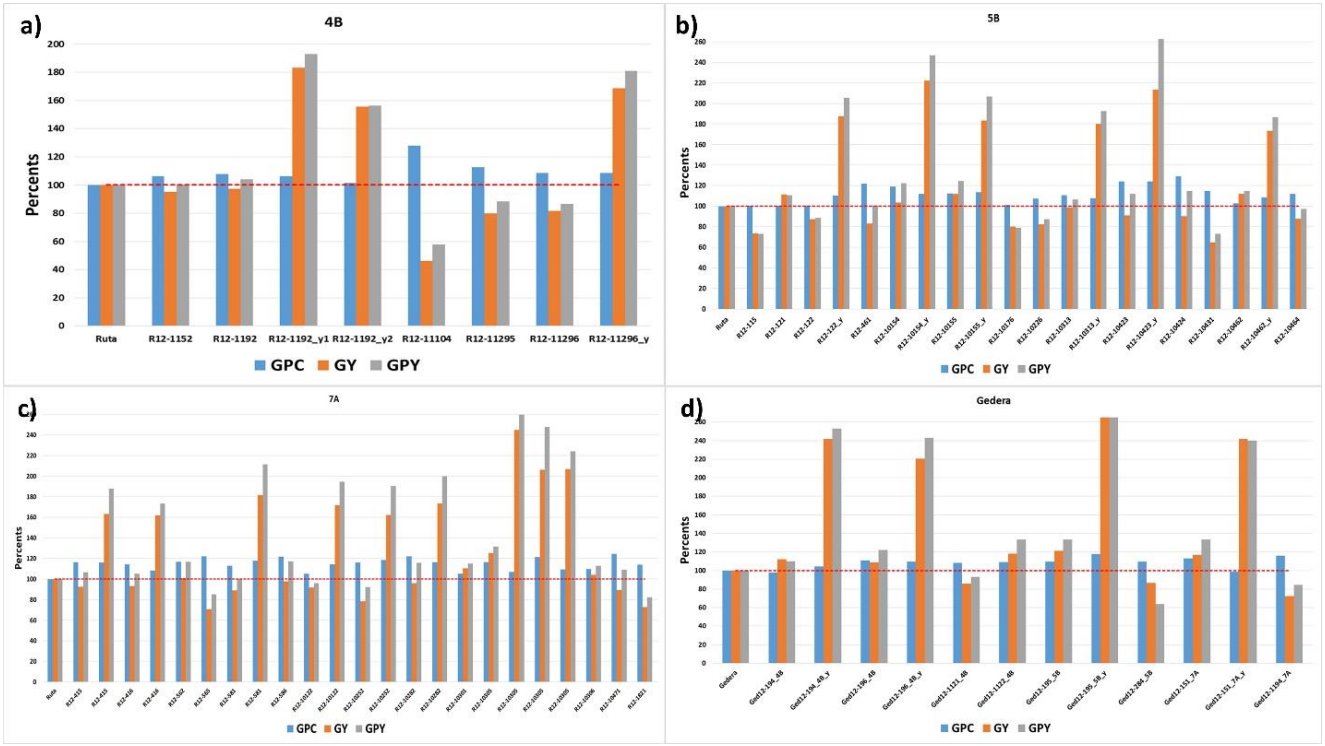

Figure 1. Comparison of GPC, GY and GPY for recurrent parental lines and homozygous NILs: a) comparison of 4BS NILs in Ruta background; b) comparison of 5BS NILs in Ruta background; c) comparison of 7AL NILs in Ruta background; d) comparison of NILs in Gedera background. Results obtained from the single high-yielding plants include prefix “y”.

CONCLUSION

Wild crop relatives are regarded as promising gene pools for crop improvement, which is of future cardinal importance in light of the increasing world population. The genepool of WEW, the progenitor of domesticated durum and bread wheat is a valuable source for improvement of resistance to abiotic and biotic stresses and for improvement of grain nutritional values. The current study demonstrates the introgression of GPC QTLs from WEW from Israel by MAS. We dissected the genetic basis of high GPC, and used the advances in wheat genomics, including the full genomic sequence of WEW to follow the precise introgressions and accelerate the process from trait discovery to gene cloning, validation, and utilization.

The BC3F4 introgression lines developed within the Whealbi project are regarded as pre-breeding material with high potential to improve GPC in bread wheat. However, further evaluation of their contribution to increased GPC is still needed. We will continue phenotyping high GPC lines in dense plots, and genotyping using 20K SNP array in order to select lines with shorter intervals of the introgressed QTLs. The resulted high GPC lines with shorter introgression segments will be subjected to additional backcrossing to reduce negative linkage drag. These introgression lines will be available for academic/private collaborative research under MTA and/or commercial agreements.

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