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MAPPING POPULATION

Registration of the TCAP FAC-WIN6 Barley Panel for Genomewide Association Studies

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Abstract

Facultative/winter six-row malting barley is a distinct elite germplasm pool and a valuable resource that may prove useful in meeting the challenges of climate change. To preserve its diversity and make it accessible to the research and agricultural communities, the Oregon State University and University of Minnesota barley breeding programs are publicly releasing their winter/facultative six-row malt advanced lines named the TCAP FAC-WIN6 (MP-1, NSL 512632 MAP), which also function as a genomewide association studies (GWAS) panel. The FAC-WIN6 contains 296 lines—180 facultative and 116 winter—selected for disease resistance, malt quality, and general agronomic performance. To date, all lines have data for 6892 single nucleotide polymorphism (SNP) markers and phenotypic data from six experiments (representing 3 yr, eight locations), including traits such as malt quality, disease resistance, nitrogen use efficiency, and winter hardiness. The FAC-WIN6 is one of 24 barley and wheat mapping panels and populations from the USDA-ARS Triticeae Coordinated Agricultural Project (TCAP). As such, all of the TCAP FAC-WIN6 genotypic and phenotypic data can be freely downloaded from the TCAP's online database, T3 (http://triticeaetoolbox.org/barley/). Preliminary GWAS have identified novel loci for wort β-glucan, low temperature tolerance, and disease resistance. Given these results, the FAC-WIN6 is a singular resource both for future winter six-row barley breeding and for identifying and deploying genes for key barley traits in all backgrounds.

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The Triticeae Coordinated Agricultural Project (TCAP) facultative and winter six-row malt barley genomewide association studies (GWAS) panel (hereafter referred to as the TCAP FAC-WIN6 or FAC-WIN6) (MP-1, NSL 512632 MAP) is a set of elite inbred lines created for the dual purposes of variety development and gene discovery and validation. A major advantage of releasing these lines is to proactively secure the genetic diversity present in facultative/winter six-row malting barleys, which represent a discrete minority germplasm pool relative to the commonly grown two-row spring, two-row winter, and six-row spring varieties. Elite GWAS panels fill the gap between cultivar releases, GWAS diversity panels, and biparental quantitative trait loci (QTL) mapping populations. Cultivar releases are elite lines useful as parents but not as useful for mapping. Generally, GWAS diversity panels are composed of landraces or other nonelite material. Diversity panels are useful for mapping but not for identifying alleles that specifically contribute to differences between elite lines, and not useful as parents, without time-consuming prebreeding. Biparental QTL mapping populations, because they only represent two parents, lack genetic diversity and preclude estimation of background effects beyond the two parents.

The adapted lines of the FAC-WIN6 complement those in the unadapted GWAS panel of publicly released accessions and landraces used in the TCAP. That panel is referred to as the National Small Grains Center (NSGC) Core. The NSGC Core is a set of 2446 barley accessions and landraces selected to represent the global diversity in winter, spring, two-row, and six-row gene pools. However, as landraces, those lines are not likely to represent the genetics of modern high-performing malt varieties, especially malt varieties adapted for North America. The

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Abbreviations: BSR, barley stripe rust; Barley CAP, Barley Coordinated Agricultural Project; GWAS, genomewide association studies; LTT, low temperature tolerance; NSGC, National Small Grains Center; NUE, nitrogen use efficiency; QTL, quantitative trait loci; PCA, principal components analysis; PYT, preliminary yield trial; SNP, single nucleotide polymorphism; SSD, single seed descent; TCAP, Triticeae Coordinated Agricultural Project; THT, The Hordeum Toolbox; WUE, water use efficiency.

FAC-WIN6 is adapted to North America, and its 180 facultative lines may be grown as winter or spring types. Moreover, for GWAS analyses, the FAC-WIN6 has a much simpler population structure than in the NSGC Core.

The final advantage of both the FAC-WIN6 and the NSGC Core is data. All TCAP data are publicly available at the TCAP's online database, T3 (http://triticeaetoolbox.org/barley/; Blake et al., 2015). T3 is the successor of The Hordeum Toolbox (THT) (http://hordeumtoolbox.org/; Blake et al., 2012). T3 is rigorously curated, and its data download options include formatting for popular GWAS and BLUP-generating software. For the FAC-WIN6 alone, T3 holds genotypic data for 6892 single nucleotide polymorphism (SNP) markers, along with phenotypic data from 3 yr of field and greenhouse trials encompassing six experiments, eight locations, and 129 trial-trait combinations. Additionally, T3 has SNP and phenotypic data from early generations of the FAC-WIN6 (from the Barley Coordinated Agricultural Project [Barley CAP]), including malt quality data. Finally, our preliminary GWAS results detected novel FAC-WIN6 QTLs for key traits (e.g., winter survival and wort β-glucan), some of which colocalize with QTLs mapped in TCAP experiments of two-row and spring lines. Thus, we expect QTLs mapped in the FAC-WIN6 to be valuable both for six-row winter barley genetics and for QTL investigations in two-row and spring barleys.

Materials and Methods

General Selection Methods

The Oregon lines were selected from a variety of breeding projects, but with a shared general selection scheme. Full pedigree and selection scheme information are shown in Supplemental Data S1. Parents were all elite experimental lines (except Maja), chosen for some combination of malt quality, agronomic quality, cold tolerance, local adaptation, and disease resistance. For example, the Stab parental lines have high disease resistance and agronomic performance, along with moderate malt quality, and the Stab BC parental lines were Stab lines backcrossed to 88Ab536, then selected for good malt quality. NB3437f is a facultative cold-tolerant line from Nebraska. Maja, which was released by the Oregon Agricultural Experiment Station and is licensed to AgriSource of Burley, ID, advanced to commercial malting and brewing trials but was ultimately not recommended as a malting variety by the American Malting Barley Association (AMBA). This variety is resistant to barley stripe rust (incited by *Puccinia striiformis* f. sp. *hordei*) but is susceptible to barley scald (incited by *Rhynchosporium commune*).

The Oregon general selection scheme was as follows (summarized in Table 1). After a single cross, the F_1 plants were grown in the field and selfed. The F_2 seed was bulked and planted in the field in Pendleton, OR (moderately cold winters and dry spring and summer), from which $F_{2:3}$ heads were visually selected based primarily on winter survival, general plant quality, and large and uniform heads. $F_{2:3}$ heads were selected from bulk-planted populations of approximately 6500 F_2 plants and at a selection intensity of approximately 1.5%. With the exception of head rows, all plots were planted at a seeding rate of approximately 200 seeds/m^2. $F_{2:3}$ head row families were planted in Corvallis, OR (mild temperate climate with high rainfall), from which families and then heads within families were selected on the basis of the same criteria as in the previous generation, with the addition of selection for foliar disease resistance. All head rows used in breeding of the FAC-WIN6 were 0.465 m^2. Selections from head row nurseries and preliminary yield trials were determined with a selection intensity of approximately 10 to 15%. $F_{2:3}$ head rows had populations of approximately 9300 plants. $F_{3:4}$ head row families were planted and selected on the same criteria as the $F_{2:3}$ generation, with the addition of selection for family uniformity. $F_{3:4}$ head rows had populations of approximately 70 families. From the $F_{3:4}$ generation on, families were strictly rogued for off-types and then bulk harvested. Finally, the $F_{3:5}$ families were planted in preliminary yield trials (PYTs) in two or three locations between Oregon and Idaho, one location always in Corvallis. $F_{3:5}$ PYTs had populations of approximately

Table 1. Generalized breeding history for Oregon lines. A generalized selection scheme for the TCAP FAC-WIN6 lines contributed by the Oregon State University breeding program. The 148 lines from crosses made in winter 2005–2006 were used as primary examples for this table. The complete breeding history of all lines in this release is included as Supplemental Data S1. Here, "Year" begins in fall and ends at harvest in the subsequent summer.

Generation	Year	Environment	Selection unit†	Population size (*i*‡)	Key selection criteria
F_1	1	Field	N/A§	N/A	N/A
F_2	2	Field (Pendleton, OR)	F_2 headrow families, then heads within families	260 (9%)	Larger heads and grains, general plant quality, disease resistance, low temperature tolerance
$F_{2:3}$	3	Field	$F_{2:3}$ families, then heads within families	108 (21%)	Larger heads and grains, general plant quality, disease resistance
$F_{3:4}$	4	Field	$F_{3:4}$ families, then heads within families	248¶ (20%)	Larger heads and grains, general plant quality, disease resistance, uniformity
$F_{3:5}$	5	Field	$F_{3:5}$ families	137 (55%)	Preliminary yield trial. Strictly rogued.
$F_{3:6}$	6	TCAP NUE#	N/A	Fixed at 146	2012 NUE. Selection complete; lines fixed as families and strictly rogued.

† In units of selection unit from same generation. If multiple units are given, then refers to the first unit– e.g., for "F_2 headrow families, then heads within families," population size selection unit is F_2 headrow families.

‡ Selection intensity applied to same generation to obtain seed for next generation.

§ N/A, not applicable.

¶ Population size of current generation may be larger than that selected in prior generation, as multiple $F_{2:3}$ families may be obtained from the same F_2 headrow family.

NUE, nitrogen use efficiency.

70 families. For the 146 lines from crosses made in the winter of 2005–2006 (the lines primarily used to create the example timeline in Table 1), the final selection was made post-harvest of the $F_{3:5}$ generation preliminary yield trials in the 2010–2011 season, and those lines were planted in the TCAP 2011–2012 nitrogen use efficiency (NUE) trials as $F_{3:6}$ families. All other Oregon lines were $F_{3:7}$ or later generation in the 2011–2012 TCAP NUE trials, and 83 of these lines have data from earlier generation experiments in the Barley CAP. For the remaining Oregon lines, in general, after selection in 1 yr of PYTs, they were selected in 1 to 2 yr of advanced or elite yield trials, where the population sizes ranged from 20 to 35 inbred lines, with a selection intensity of 15 to 50%.

The discrepancy between $F_{3:5}$ PYT population size and the product of $F_{3:4}$ population size and selection intensity (an $F_{3:4}$ population size of 70 combined with selection intensity of 10 to 15% should generate an $F_{3:5}$ PYT population size of 7 to 11 families, not 70) is due to the combining of projects across the Oregon breeding program at later generations. Thus, an $F_{3:5}$ PYT field would have 70 families, of which 7 to 11 would be from the previous year's $F_{3:4}$ facultative/winter six-row nursery. The selection intensity imposed on the $F_{3:5}$ families would be 10 to 15% across all PYT entries, but the selection intensity specifically on the $F_{3:5}$ facultative and winter families could range from 1.4% (one family) to 100%.

Each Minnesota line was selected by one of the following two general schemes (summarized in Table 2). (i) After a single cross, the F_1 plants were selfed in the greenhouse. The resulting F_2 plants were then backcrossed and the resulting BC_1F_1 plants planted in a spring field trial in St. Paul, MN, and selected for facultative growth. The selected plants were selfed to the $BC_1F_{2:3}$ generation by single seed descent (SSD) without selection. The $BC_1F_{3:4}$ families were grown in small plot spring trials in Crookston, MN, and selected for local adaptation and general agronomic quality. The selected $BC_1F_{3:5}$ families were then entered into the FAC-WIN6 TCAP NUE 2012 experiment. (ii) After a single cross, the F_1 families were selfed by SSD, no selection, until the $F_{2:3}$ generation. $F_{2:3}$ seed was fall-planted in St. Paul, and the plants selected for winter survival. Selected families were fall-planted as $F_{2:4}$ families in St. Paul and again selected for winter survival and general plant quality. Remnant seed from the $F_{2:3}$ plants was advanced by SSD to create $F_{4:5}$ seed. From the families selected in the $F_{2:4}$ fall-planted trial, $F_{4:5}$ families from the remnant seed SSD-advanced material were planted in a Christchurch, New Zealand, winter nursery for seed increase. The resulting $F_{4:6}$ families were planted in spring PYTs in three Minnesota locations and selected for yield and general agronomic quality. From selected families, $F_{4:7}$ seed was planted in the FAC-WIN6 TCAP NUE 2012 experiment.

FAC-WIN6 Oregon lines with early generation data from the Barley CAP are publicly available in two online databases: the Barley CAP THT and TCAP T3. Because early generations commonly segregate for key traits, the T3 database classifies the FAC-WIN6 panel as separate lines from the earlier generations (i.e., early generation data are stored under different line names than the FAC-WIN6). A simple naming convention was used for the FAC-WIN6 to facilitate finding early generation data. All FAC-WIN6 Oregon lines were given a synonym (T3 nomenclature for a supplementary name) with an "-FW6" suffix. If early generation data are available for a line, they will be stored under a name or synonym identified by removing the suffix "-FW6." For example, the FAC-WIN6 Oregon line TCFW6-200 has the T3 synonym 06OR-40-FW6. Barley CAP data for early generations of this same line (including five malt quality trials) are available in T3 and THT under the synonym 06OR-40.

Selection, Traits, and Experimental Design and Conditions

To date, the advanced generations of the FAC-WIN6 have been evaluated in six experiments: NUE (two locations, 2 yr), malt quality (MQ; part of standard N treatment in both years of NUE trials at one location), water use efficiency (WUE; one location, 1 yr), barley stripe rust (BSR; quantitative adult field resistance in one locations, 3 yr; race-specific resistance assessed against five races in one greenhouse trial), and low temperature tolerance (LTT; three locations, 2 yr). Experiments, years, locations, and traits are summarized in Table 3. All field experiments used a type-II modified augmented design, with one primary check and two secondary checks (Lin and Poushinsky. 1985), with the exception of the LTT trials grown outside of Oregon. The LTT trials in Mead, NE, and Aberdeen, ID, were unreplicated and augmented with repeated checks, with randomized incomplete blocks of 25 plots each, where the incomplete blocks were not arranged with regard to rows or ranges of the field. Each incomplete

Table 2. Generalized breeding history for Minnesota lines. The TCAP FAC-WIN6 lines contributed by the University of Minnesota breeding program were selected from two breeding projects (sets of crosses and selection trials). This table summarizes what is mostly shared by both projects. The complete breeding history of all lines in this released is included as Supplemental Data S1. Here, "Year" begins in fall and ends at harvest in the subsequent summer.

Generation	Year	Environment	Selection unit	Population size	Key selection criteria
F_1	Year 1	GH†	N/A‡	N/A	N/A
F_2	Year 1 (spring)	Field (Crookston, MN)	Heads		General plant quality, facultative habit
$F_{2:3}$	Year 2	Field	$F_{2:3}$ families, then heads within families		General quality, facultative habit, winter survival
$F_{3:4}$	Year 2	GH	N/A		N/A
$F_{4:5}$	Year 3 (winter)	GH	N/A		N/A
$F_{4:6}$	Year 3 (spring)	Field	$F_{4:6}$ families		Preliminary yield trial
$F_{4:7}$	Year 4	Field	N/A	Fixed at 35	2012 NUE. selection complete; lines fixed as families and stringently rogued

† GH, greenhouse.

‡ N/A, not applicable.

block contained a primary check in its "center" plot (the 13th of 25 plots, not the geometric center), and every other incomplete block (in total, six of 13 incomplete blocks) contained a randomly arranged plot of each of two secondary checks. The LTT trial in Orton, ON, was unreplicated without replicated checks. For these experiments not planted as type-II modified augmented designs, the data were not adjusted for field effects. This choice was made due to the geometrically unstructured arrangement of replicated checks (or lack of replicated checks in Ontario). All field trials were grown under standard recommended management for the region, with the exception of reduced N fertilizer in the low N treatment of the NUE trials, reduced irrigation in the low water treatment of the WUE trial, and exclusion of fungicide application in the disease resistance trials. All field trials were fall-planted, except the WUE trial (planted in late spring) and the 2013 disease trial (planted in early spring). The 2012–2013 NUE trial used yield trial plots (six rows, 8.9 m^2). The Utah NUE trials used small yield trial plots (six rows, 2.3 m^2). The Idaho and Nebraska LTT experiments were planted as single rows, 1.5 m in length. All remaining trials had two-row, 1.8-m^2 plots.

Quantitative disease data are from naturally inoculated field trials. The race-specific BSR disease response data are from greenhouse trials in Pullman, WA, inoculated and scored as described previously (Line et al., 1974; Chen and Penman, 2005). The races used in the greenhouse trials were PSH-33, PSH-46, PSH-51, PSH-71, and PSH-72 (Chen and Penman, 2005).

All phenotypic data collection methods were as defined by the TCAP, with descriptions at the T3 online database (http://triticeaetoolbox.org/barley/traits). Briefly, measurements of grain agronomic traits were taken post-harvest at the research farm at which they were harvested (Corvallis, OR, or Logan, UT). Malt quality was measured on samples from the Oregon 2011–2012 and 2012–2013 NUE trial standard N treatment, assayed by the USDA-ARS Cereal Crops Research Unit located in Madison, WI, following American Society of Brewing Chemists (ASBC, 1992) protocols (see Budde et al., 2008, for details of specific micromalting and malt quality methodologies). Plant height was equal to the distance from the soil surface to the top of the spike, excluding awns. Grain protein was measured with near-infrared spectrometry as the average of six subsamples. All spike traits used averages of three spikes per sample.

Marker Methods

TCAP FAC-WIN6 genotypic data were collected in 2013 from $F_{6:7}$ or later generation plants. Tissue from individual plants was taken from 2- to 3-wk-old seedlings grown in the greenhouse, immediately stored at −80, and lyophilized. DNA extraction and genotyping were performed at the USDA-ARS Regional Small Grains Genotyping Laboratory at Fargo, ND, following the methods of Sambrook et al. (1989). All markers were part of a 6892 SNP custom barley Infinium iSelect 9K Genotyping BeadChip (Illumina, Inc.; Close et al., 2009). Within those markers, 5198 had known locations on the latest barley consensus map (Muñoz-Amatriaín et al., 2014).

Statistical Analyses

Adjustments for field effects and calculations of means were made in Agrobase Generation II (Agronomix, Software, Inc.), except in the case of secondary check missing data. If any secondary check data were missing, then (i) for Method 1 adjustments, least squares means were used for row and column averages, produced by the GLM Procedure in SAS 9.3 (SAS Institute, Inc.), with the exception of the 2012 WUE low water trial grain protein data, where simple means were used for row and column averages (use of least squares or simple means was selected based on relative efficiency); and (ii) for Method 3 adjustments, any whole plots with missing data were removed from the estimation of field effect parameters and relative efficiency, after which all analyses and adjustments were calculated as in Agrobase (May et al., 1989) but performed in R version 2.14.2 (R Development Core Team, 2012). Analysis of variance of NUE experiments was calculated from unadjusted check data, using all check lines but only using whole plots with secondary checks, by the MIXED Procedure in SAS 9.3. Principle component analysis and preliminary GWAS were done in TASSEL v5.2.11 (Bradbury et al., 2007). Preliminary GWAS applied the mixed linear model approach, using a Q + K model (Q = first three PCA principal components), with the EMMA, P3D, and compression algorithms (Yu et al., 2005; Kang et al., 2008; Zhang et al., 2010). All line means presented here or used for GWAS were taken from those in the T3 online database at time of manuscript submission.

Characteristics

Highlights of ranges, means, standard deviations, and narrow-sense heritability (h^2) estimates of key traits are listed

Table 3. Summary of TCAP FAC-WIN6 trials currently available at T3, the TCAP online database. TCAP FAC-WIN6 data from both the Barley CAP (early generations of a subset of TCAP FAC-WIN6 lines) and the TCAP are freely available for download, viewing, and analysis at T3 (http://triticeaetoolbox.org/barley/).

Experiment	Locations	No. of years	No. of data sets in T3†	Key traits
NUE	2	2	76	Malt, yield, CSR‡
WUE	1	1	18	Yield, WUE,§ leaf rust
Disease (field)	1	3	10	Stripe rust, scald, leaf rust
LTT	3	2	4	LTT¶
Disease (qualitative)	N/A	N/A	5	Barley stripe rust (5 races)

† A data set here is defined as a trial-trait combination.

‡ CSR, canopy spectral reflectance.

§ WUE, water use efficiency.

¶ LTT, low temperature tolerance; note that these trials specifically were only measured for winter survival.

Table 4. Summary of key trait and trial TCAP FAC-WIN6 data. In this table are malt quality, yield, nitrogen use efficiency, and disease resistance highlights from the TCAP FAC-WIN6. Full mean, range, and h^2 summaries from all TCAP FAC-WIN6 trials on T3 are available in Supplemental Data S2.

Experiment	NUE†								WUE†
Location	Oregon							Utah	Oregon
Year	2013							2013	2012
Trait	Yield HiN‡	Yield LoN§	Malt extract	DP¶	Grain protein	Plump grain	Malt β-glucan	Yield HiN	Leaf rust severity HiW#
	kg/ha	kg/ha	%	°ASBC	%	%	μg/mL	kg/ha	%
Min.	1,750	2,090	74.9	53.7	7.9	47.3	27.5	2,180	0
Mean	7,150	5,920	80.1	108.1	9.6	92.5	280.0	8,730	15.8
Max.	10,230	8,540	85.5	196.0	12.5	99.3	821.0	13,380	52.1
Std. error	410	460	0.1	1.8	0.3	1.2	19.6	1,230	8.4
h^2	0.93	0.79	0.94	0.95	0.84	0.75	0.81	0.43	0.82

† NUE, nitrogen use efficiency; WUE, water use efficiency.

‡ Standard N fertilizer rate.

§ 70% of standard N fertilizer rate, where the same rate was applied in fall, but a differential rate in spring.

¶ DP, diastatic power.

Standard irrigation regime.

in Table 4, and histograms of key traits are given in Fig. 1. Those same statistics are listed for all traits within each trial (or within treatments of each trial) in Supplemental Data S2. Heritability (h^2) estimates ranged from 0 to 0.99, although most estimates fell between 0.45 and 0.80 (Supplemental Data S2). Yield within trials ranged from approximately 600 to 13,400 kg/ha. The lowest-yielding trials were the 2012 WUE trials, presumably due to an especially late spring planting. The highest-yielding trials were the 2013 NUE Utah trials. Yields from barley and wheat experiments adjacent to the 2013 NUE Utah trials were also extraordinarily high (D. Hole, personal communication, 2013). The lower yields are mostly represented by Minnesota lines in the Corvallis environment. Lines with top malt quality are summarized in Table 5. The Minnesota lines produced high-quality grain for malting purposes in Oregon, but yields were low, likely due to susceptibility to barley stripe rust (not present in Minnesota) and to early heading in Corvallis. Notably, an apparent association between early heading date and lower yield was present in Corvallis but not in Logan, which is approximately equal in latitude but does not have the warm winter and spring of Corvallis. In the BSR experiment, barley stripe rust ranged from 0 to 100% severity in 2011–2012 and 0 to 90% severity in 2012–2013, with means of 7.1 and 4.8% in 2011–2012 and 2012–2013, respectively. Nitrogen use efficiency trial results are summarized in the ANOVA table of Table 6. Across all

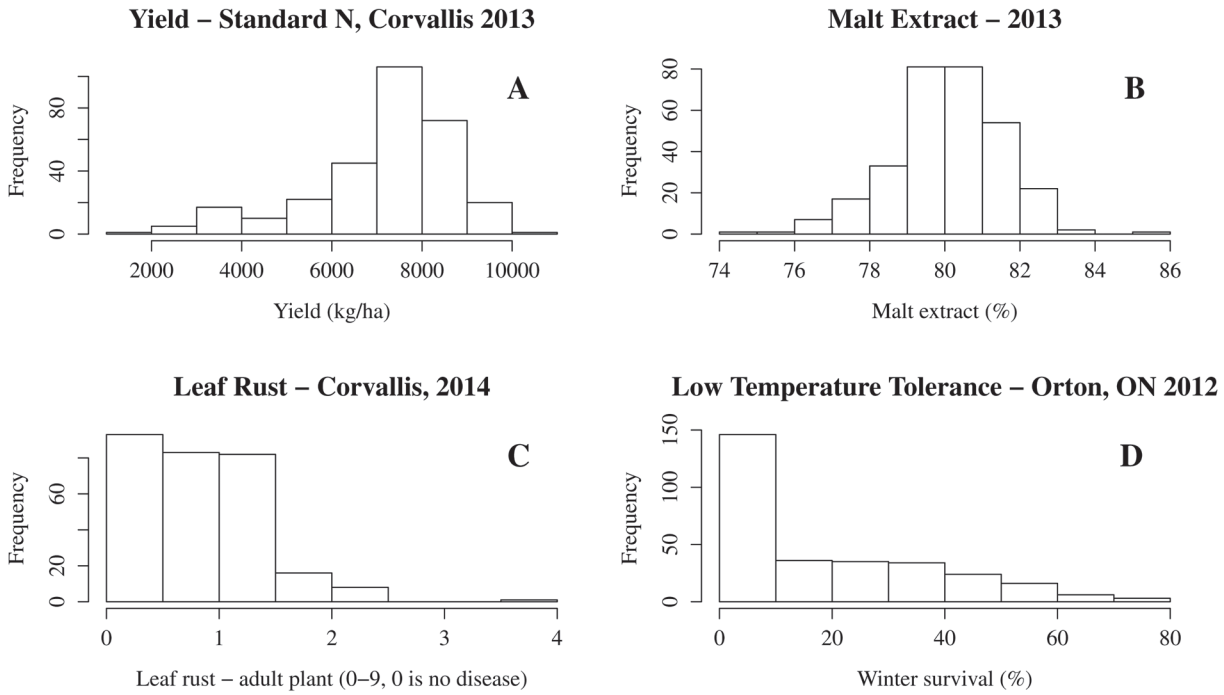

Fig. 1. Histograms of select phenotypic datasets. Histograms of T3 data from select FAC-WIN6 trials. Genotypic variance is significant ($P \leq 0.008$) for (A) yield from the HiN treatment (i.e., standard N application) in Corvallis, OR, 2013 nitrogen use efficiency trial, with full yield trial size plots and (B) malt extract, from same trial; nonsignificant ($P = 0.11$) for (C) adult plant leaf rust in Corvallis, OR, 2014 disease trial; and unavailable (no replicated checks) for (D) winter survival in Orton, ON, 2014 low temperature tolerance trial.

Table 5. Malting data highlights for select TCAP FAC-WIN6 lines. A sample of malt quality and yield data for the FAC-WIN6 lines with the best malting quality. Full malt quality data for the TCAP FAC-WIN6 can be found at the TCAP online database, T3.

T3 line name	Malt extract	DP†	FAN‡	β-glucan	α-amylase	Yield	Growth habit
	%	°ASBC	μg/mL	μg/mL	20°DU§	kg/ha	
TCFW6-235	81.8	165	191	61	117	8420	Facultative
TCFW6-244	81.1	122	160	145	68	8520	Facultative
TCFW6-193	80.9	107	184	154	64	9550	Winter
TCFW6-194	81.6	107	152	160	77	9240	Facultative
TCFW6-017	81.6	131	181	272	77	9650	Facultative

† Diastatic power.

‡ Free amino nitrogen.

§ DU, dextrinizing unit.

Table 6. ANOVA results from nitrogen use efficiency trials replicated check data, from Corvallis, OR, and Logan, UT, in the 2011–2012 and 2012–2013 field seasons.

	Oregon and Utah				Oregon only						
Source of variation	df	Yield (kg/ha)	Protein (%)	Heading date (Julian days)	df	Height (cm)	Lodging (%)	Plump (%)	Test weight (g/L)	BSR† (%)	Scald (0–9)
		F value				*F* value					
Entry	2	6.86***	0.77	191.08***	2	55.99***	7.81***	44.56***	31.41***	1.00	57.58***
Location (loc)	1	8.92**	33.3***	4877.91***	–	–	–	–	–	–	–
Year	1	7.17**	52.73***	108.07***	1	16.09***	2.36	11.46**	255.84***	0.47	6.91*
Loc × year	1	50.39***	273.17***	26.26***	–	–	–	–	–	–	–
Treatment (trt)	1	5.73*	23.88***	0.16	1	28.57***	8.56**	4.54*	16.93***	0.49	3.65
Loc × trt	1	4.19*	0.39	0.16	–	–	–	–	–	–	–
Year × trt	1	0.78	22.40***	0.01	1	1.43	0.01	4.37*	21.91***	0.49	0.81
Loc × year × trt	1	0.23	13.66***	0.71	–	–	–	–	–	–	–
	df		Error		df			Error			
Whole plot error	76	490,001	0.2370	1.0749	32	12.7065	0.0001	0.0019	0.0001	0.0393	0.0001
CV (%)		0.15	0.07	0.02		0.04	0.44	0.03	0.02	10.44	0.77

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

† Barley stripe rust.

NUE trials, grain protein ranged from 5.5 to 16.7%, with an across-trials mean of 10.9%. As examples of malting quality parameters, the following ranges were observed: malt extract (73–85%), α-amylase (26–122 20° dextrinizing units), diastatic power (54–206°ASBC), and wort β-glucan (28–821 μg/mL).

Discussion

A GWAS panel's efficacy depends on the panel size, genetic structure, degree of relatedness between lines, genetic marker quality, and distribution of values across useful phenotypes (von Zitzewitz et al., 2011). The FAC-WIN6 panel's combined size and genotypic structure fit what is predicted to be effective for GWAS panels. Major elements of the population structure are summarized by the first three principal components from the principal components analysis (PCA; Fig. 2 and Supplemental Fig. S1). Using empirical data to perform GWAS with differently sized random subsets of a spring barley GWAS panel, Wang et al. (2012) determined a minimum panel size of 384 lines, 28% larger than the FAC-WIN6 full panel, to consistently detect QTLs for traits of high heritability. This is in contrast to the results of Bradbury et al. (2011), who, using simulated phenotypic data with a barley GWAS panel, determined a minimum panel size of 300 lines for adequate GWAS power with traits of moderate to high heritability (e.g., heading date). However, the FAC-WIN6 is better represented by the Bradbury et al. (2011) experiment. Not only did that experiment include early generations of FAC-WIN6 lines present in the Barley CAP, but it better represents the population structure and phenotypic range of the full FAC-WIN6. The Wang et al. (2012) panel subsets were equally divided between eight breeding programs, thus having substantially greater structure than the FAC-WIN6. Also, despite representing eight breeding programs, their panels' range for heading date (13 d) is approximately half that of the ranges from the FAC-WIN6 trials (Supplemental Data S2). Moreover, Type I and II errors may be satisfactorily reduced in small GWAS panels by use of known or candidate genes as anchoring loci, even in panels smaller than otherwise recommended (Cuesta-Marcos et al., 2010). Even without use of anchoring loci, Gutierrez et al. (2011) were able to detect novel and known malt quality QTLs in GWAS panels much smaller than the FAC-WIN6 ($71 \leq n \leq 96$), panels that included earlier generations of many lines from the FAC-WIN6.

The accessions within the FAC-WIN6 display a wide spectrum of interrelatedness, and based on preliminary results, the Q+K model seems to readily account for the population

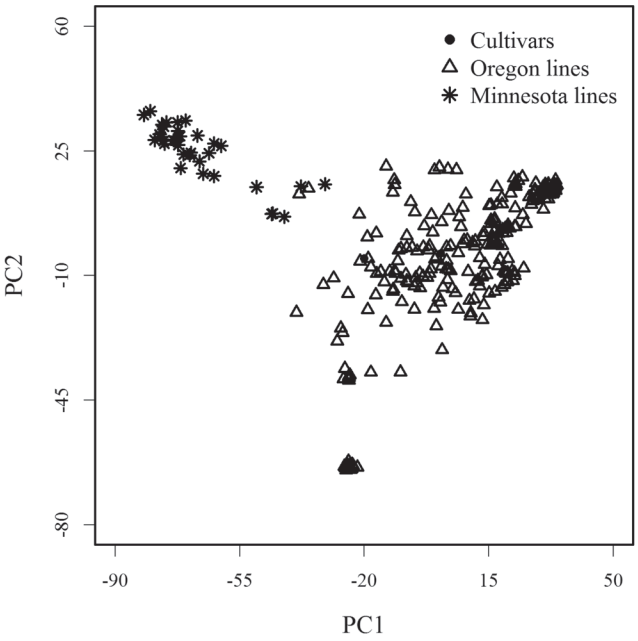

Fig. 2. Principal component analysis (PCA) of full FAC-WIN6 panel. Scatterplot of the second (PC2) versus the first principal components (PC1) from the PCA of the full 300-line FAC-WIN6 panel, based on single nucleotide polymorphism data. Triangles and asterisks represent the 296 lines in this germplasm release.

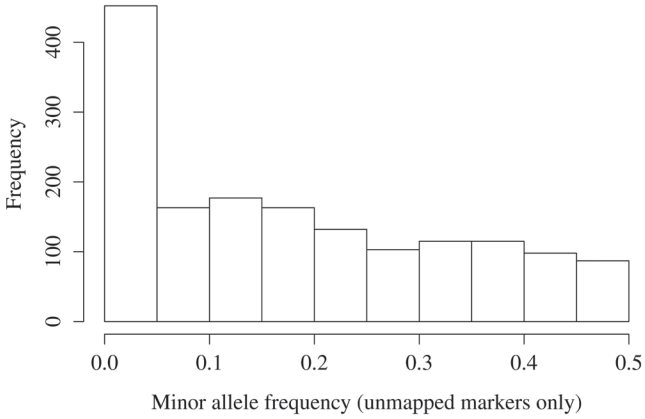

Fig. 3. Histogram of minor allele frequency across all unmapped polymorphic markers. From the publicly available single nucleotide polymorphism marker data of the full 300-line FAC-WIN6, for those 1605 polymorphic markers not yet placed on the consensus map (Muñoz-Amatriaín et al., 2014), a histogram of minor allele frequency.

division between the Oregon and Minnesota breeding material (Belcher, unpublished data, 2014). The 6892 SNP set has a high level of polymorphism and a low rate of missing data. Mean minor allele frequency is 0.19 (minor allele frequency of unmapped markers and mapped markers summarized in Fig. 3 and Supplemental Fig. S2, respectively). The mean rate of missing data per line is 0.6%. The proportion of markers that were polymorphic per chromosome ranged from 78.4% on 2H to 88.9% on 6H.

The panel displays a highly quantitative distribution of phenotypic data across key malt and agronomic traits, with elite performance and significant h^2 (note h^2 here calculated with only one generation). For example, diastatic power ranged from 58 to 206°ASBC in 2012 (Supplemental Data S2) and 54 to 196°ASBC in 2013 (Table 4), with significant estimated h^2 of 0.95 and 0.99, respectively ($P \leq 0.004$). Mean yields ranged from 1720 to 8730 kg/ha, with h^2 of 0.23 to 0.93 ($4.7 \times 10^{-5} \leq P \leq 0.30$). Moreover, all of these data are freely available on T3, where they can be readily downloaded pre-formatted for analysis in TASSEL (Bradbury et al., 2007), ASREML (VSN International), or the R packages rrBLUP (Endelman, 2011) and GAPIT (Lipka et al., 2012). Ranges, population structure PCA, and histograms can be generated and viewed at T3 in most Web browsers.

Finally, the best indicator of potential is repeated success. Within the TCAP FAC-WIN6 BSR experiments preliminary GWAS results, the FAC-WIN6 population detected 18 maturity-independent adult plant quantitative disease resistance QTLs across five trials and three diseases (Belcher et al., 2014). Of those, two QTLs were mapped in all trials for the corresponding disease (three trials for barley stripe rust, and two trials for leaf rust), and two stripe rust QTLs were mapped in two of three trials.

Availability

Seed is maintained by the Barley Project at Oregon State University, Corvallis, OR 97331. Seed for research purposes will be available on request from the corresponding author for at least 5 yr. It is requested that appropriate recognition of source be given when this cultivar contributes to development of new germplasm or cultivars. Upon acceptance of any FAC-WIN6 panel germplasm to the National Plant Germplasm System (NPGS), the NPGS identification numbers of those lines will be listed at the TCAP T3 online database. All FAC-WIN6 phenotypic data mentioned are also available for download at T3, including SNP data. The name(s) used for each line in T3 are provided in Supplemental Data S1. For those who wish to use these lines for research purposes, we ask that recognition be given by citing this article.

Conclusions

The wealth of available data, paired with the convenience of its access at T3, makes the FAC-WIN6 association studies panel a valuable resource. As the lines are fixed, all of the TCAP genotypic and phenotypic data will remain valid for future studies. Public release of this germplasm has vast potential for genetics and breeding in six-rowed facultative and winter barley.

Supplemental Material

Two supplemental figures and two supplemental data sets accompany this text: further representations of FAC-WIN6 population structure via principal components (Supplemental Fig. S1), FAC-WIN6 minor allele frequencies across all barley chromosomes (Supplemental Fig. S2), full breeding history of all FAC-WIN6 lines (Supplemental Data S1), and a short summary of data from all phenotypic datasets available on T3 (Supplemental Data S2).

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