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MAPPING POPULATION

Registration of the Triticeae-CAP Spring Wheat Nested Association Mapping Population

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Abstract

The Triticeae-CAP spring wheat nested association mapping population (Reg. No. MP-10, NSL 527060 MAP) consisting of recombinant inbred line (RIL) populations derived from 32 spring wheat (*Triticum aestivum* L.) accessions each crossed to a common spring wheat parent, 'Berkut', has been released. The spring wheat accessions consisted of 29 landraces and three cultivars. Each population consists of approximately 75 lines for a total of 2325 RILs (Reg. Nos. GSTR No. 14701–GSTR 17133). The RILs have all been genotyped with the Illumina wheat iSelect 90K single nucleotide polymorphism array using the Infinium assay method and through genotype-by-sequencing. This nested association mapping population provides a genotyped germplasm resource for the wheat community. A potential strategy for use of the material is to screen the parents for a trait of interest, followed by analysis of RIL of populations that are likely to be segregating for a target trait or sequence.

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Landrace accessions of wheat (*Triticum aestivum* L.) are a potentially important resource for superior genes to improve modern wheat cultivars. The landrace accessions themselves are typically inferior to modern cultivars for both agronomic and quality characteristics. This is particularly true when landraces from one region are grown in a different locale in that important alleles for adaptation to biotic and abiotic factors are lacking. A challenge for breeders is that superior alleles are difficult to detect in a background of undesirable alleles that typify the landraces. The increased efficiency of modern genotyping capabilities has provided an opportunity to identify superior alleles from nonadapted germplasm for incorporation into elite breeding material. The process of nested association mapping (NAM) was developed by the maize (*Zea mays* L.) community to allow identification of favorable alleles from a diverse germplasm set (Yu et al., 2008). The NAM population takes advantage of the power provided by traditional analysis of quantitative traits using bi-parental populations, as well as association mapping whereby a diverse set of lines are analyzed to identify favorable genes. Such NAM populations have been used to dissect genetic architecture in rice (*Oryza sativa* L.) (Fragoso et al., 2017), flowering time in barley (*Hordeum vulgare* L) (Maurer et al., 2015), and stem rust resistance in wheat (Bajgain et al., 2016).

The Triticeae-CAP spring wheat NAM population (Reg. No. MP-10, NSL 527060 MAP) was developed by crossing a genetically diverse set of 29 landraces and three cultivars to a common parent, named 'Berkut'. Berkut (pedigree: Irena/Babax//Pastor; released in 2002) was developed by the International Maize and Wheat Research Center (CIMMYT) in Mexico for drought-stricken areas around the world. Extreme height and late heading in the landraces make them difficult to phenotype in many North American environments used as

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Abbreviations: NAM, nested association mapping; RIL, recombinant inbred line; SNP, single nucleotide polymorphism.

part of the USDA Agriculture and Food Research Initiative Triticeae-CAP project "Improving barley and wheat germplasm for changing environments." Among the genes that differentiate Berkut from the landraces are ones for semidwarf growth habit and photoperiod insensitivity leading to earlier heading. As a result, recombinant inbred lines (RILs) derived from crosses of landraces with Berkut are expected to be heterogeneous for height and maturity. To allow more accurate field-based phenotyping, selection was applied during RIL derivation for semidwarf growth habit and photoperiod insensitivity.

A total of 2325 RILs were developed through this process, consisting of approximately 75 RILs for each of 32 crosses. The RILs were genotyped using both the Illumina 90,000 single nucleotide polymorphism (SNP) array and by genotype-by-sequencing (Jordan et al., 2018). The population will be useful for genetic studies aimed toward gene discovery in diverse wheat landraces.

Methods

An initial set of 100 spring wheat landraces was selected on the basis of diversity of geographic origin for crossing to the common parent Berkut in 2009. Additional crosses were made to Berkut with local elite cultivars and international cultivars that showed evidence of drought or heat tolerance. The 100 lines used in initial crosses were included in a set of 2196 spring common wheat accessions obtained from the USDA-ARS National Small Grains Collection, Aberdeen, ID (Bonman et al., 2015), as part the Triticeae Coordinate Project. These accessions were genotyped with the Illumina wheat iSelect 9K SNP array using the Infinium assay method (Cavanagh et al., 2013). The SNP genotypes were clustered and scored using Illumina's GenomeStudio software v. 2011.0 followed by manual inspection of call accuracy. A total of 5634 SNPs were polymorphic among the spring accessions. Genetic structure present in this set of spring accessions was evaluated by principle component analysis using *cluster* function in R (Maechler et al., 2018) (Fig. 1).

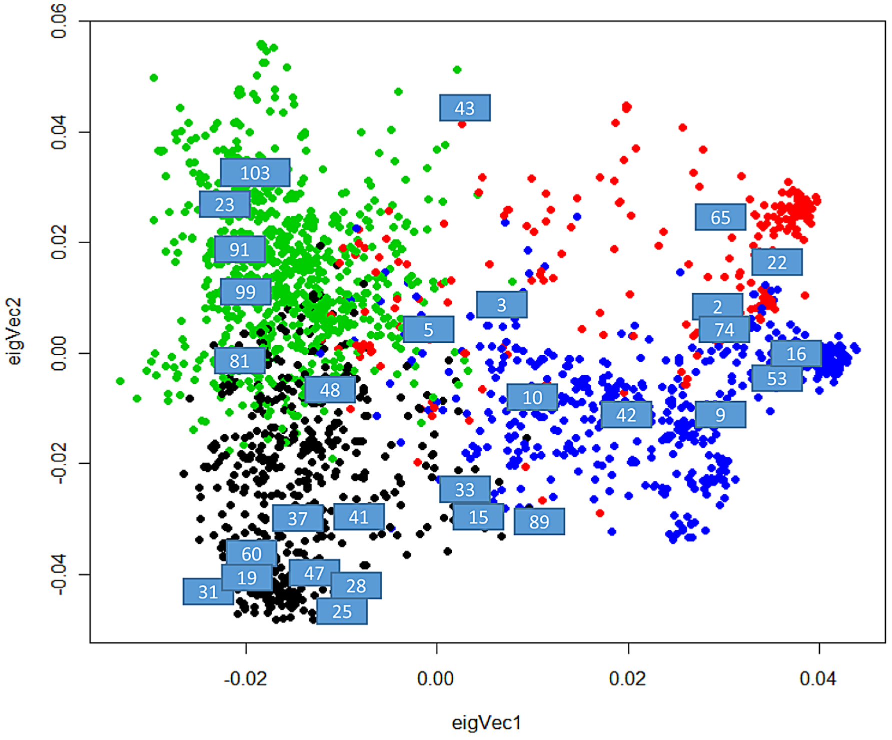

Fig. 1. Principle components analysis based on the iSelect 9K SNP array using Illumina's Infinium assay a set of 2194 spring wheat landrace accessions. The numbers correspond to the "Parent T-CAP No." in Table 1. As designated in Table 1, Cluster 1 is black, Cluster 2 is red, Cluster 3 is green, and Cluster 4 is blue.

Four genotype clusters were identified as shown in Fig. 1. Based on this data, a final set of 29 landrace accessions was selected for continuation of RIL development (Table 1). Population development from 11, 4, 6, and 8 accessions from clusters 1, 2, 3, and 4, respectively (Table 1), was continued to produce RIL families.

The parents for the populations were also genotyped for the *Rht* genes controlling plant height and the *Ppd* genes controlling photoperiod response, as described by Ellis et al. (2002) and Beales et al. (2007), respectively. Berkut contained the allele for semidwarf habit at *Rht-B1,* and the allele for photoperiod insensitivity at *Ppd-D1.* All of the landraces contained alleles for standard height at both *Rht-B1* and *Rht-D1*, and the allele *Ppd-D1b* for photoperiod sensitivity at *Ppd-D1.* Three cultivars were also included as parents in the NAM population due to their potential for drought tolerance. 'Vida' (Lanning et al., 2006) contained the *Rht-D1b* allele for semidwarf habit and the *Ppd-D1b* allele for photoperiod sensitivity. 'PBW 343' (Singh et al., 2017) was similar to Berkut with alleles for semidwarf habit at *Rht-B1* and photoperiod insensitivity allele *Ppd-D1a*. 'Dharwar Dry' (Kirigwi et al., 2007) also contained the semidwarf habit allele at *Rht-B1* but had the photoperiod sensitive allele *Ppd-D1b*.

A total of 864 F_2 seed was planted for each landrace and cultivar by Berkut combination in the greenhouse in Bozeman, MT, in 2010. Plants were grown under 16-h days. Approximately one-fourth of the F_2 plants were discarded based on plant height visually greater than the median height for the population. The expectation is that these plants were homozygous for the *Rht* alleles for standard height from the landrace accessions. This process was repeated in the F_3 generation. A group of approximately 430 F_4 individuals was planted in a growth chamber under 12-h days. Approximately one-half of the F_4 individuals were selected based on early flowering, indicating photoperiod insensitivity. Approximately one-fourth of the photoperiod insensitive individuals were discarded due to plant height greater than the median height. One F_5 seed from each F_4 plant was grown an additional generation in the greenhouse, and plants with excessive height were eliminated.

Seed from a single F_5 plant per RIL, comprising between 5 to 20 individuals, were planted as head rows in the field. Individual populations were distributed to Bozeman MT, Davis, CA, Pullman, WA, and Brookings, SD, for initial seed increase. Based on desirable height and maturity characteristics, each of the RIL families was reduced to a total of 75 lines. DNA was extracted

Table 1. Accessions used to generate the spring wheat nested association mapping (NAM) population. Approximately 75 recombinant inbred lines were derived per family after crossing to common parent Berkut. Eigen vector coordinates are given for the landrace accessions based on principal component analysis shown in Fig. 1. Cultivars Vida, PBW 343, and Dharwar Dry were not included in the principal component analysis.

PI no.	Parent T-CAP No.	NAM No.†	GSTR No. of RIL‡	EA No. of RIL§	No. of RILs¶	Cluster#	Eigen vector 1#	Eigen vector 2#	Origin
CItr 11223	LDRC 5	12	15538–15613	826–900	75	3	−3.21E-03	5.26E-03	Croatia
CItr 15134	LDRC 9	25	16374–16449	1801–1875	69	4	2.67E-02	−1.27E-02	Pakistan
CItr 15144	LDRC 10	18	15842–15917	1276–1350	74	4	8.14E-03	−1.48E-02	Saudi Arabia
CItr 4175	LDRC 2	11	15462–15537	751–825	72	4	3.28E-02	6.25E-03	Philippines
CItr 7635	LDRC 3	15	16982–17057	1051–1125	75	4	5.33E-03	4.99E-03	Russian Federation
PI 153785	LDRC 31		16170–16145	1501–1575	73	1	−2.34E-02	−4.35E-02	Brazil
PI 166333	LDRC 33		16146–16221	1576–1650	72	1	1.80E-03	−2.50E-02	Turkey
PI 185715	LDRC 37	26	16450–16525	1876–1950	75	1	−1.66E-02	−3.32E-02	Portugal
PI 192001	LDRC 41	23	16222–16297	1651–1725	71	1	−1.53E-02	−3.10E-02	Angola
PI 192147	LDRC 42	24	16298–16373	1726–1800	74	4	1.88E-02	−1.19E-02	Ethiopia
PI 192569	LDRC 43	27	16526–16601	1951–2025	72	2	2.64E-03	4.14E-02	Mozambique
PI 210945	LDRC 47	28	16602–16677	2026–2100	73	1	−1.50E-02	−4.08E-02	Cyprus
PI 220431	LDRC 48	29	16678–16753	2101–2175	73	1	−1.23E-02	−7.70E-03	Egypt
PI 262611	LDRC 53	13	15614–15689	901–975	73	4	3.66E-02	−4.15E-03	Turkmenistan
PI 278297	LDRC 60	30	16754–16829	2176–2250	75	1	−1.82E-02	−3.85E-02	Greece
PI 283147	LDRC 65	3	14854–14929	151–225	72	2	2.88E-02	2.64E-02	Jordan
PI 366716	LDRC 74	4	14930–15005	226–300	63	4	3.13E-02	1.24E-03	Afghanistan
PI 382150	LDRC 81	5	15006–15081	301–375	73	3	−2.23E-02	−4.44E-03	Japan
PI 43355	LDRC 19	19	15918–15993	1351–1425	75	1	−1.90E-02	−3.96E-02	Uruguay
PI 470817	LDRC 89	6	15082–15157	376–450	66	2	1.11E-02	−2.65E-02	Algeria
PI 477870	LDRC 91		16830–16905	2251–2325	73	3	−2.37E-02	1.96E-02	Peru
PI 565213	LDRC 99	7	15158–15233	451–525	75	3	−2.26E-02	1.06E-02	Bolivia
PI 572692	LDRC 103	2	14778–14853	76–150	75	3	−2.14E-02	3.39E-02	Georgia
PI 61693	LDRC 22		16906–16981	2326–2400	71	2	3.70E-02	1.76E-02	Malawi
PI 70613	LDRC 23	14	17058–17133	976–1050	75	3	−2.40E-02	2.64E-02	China
PI 82469	LDRC 25	17	15766–15841	1201–1275	73	1	−1.30E-02	−4.81E-02	Korea, North
PI 8813	LDRC 15	16	15690–15675	1126–1200	68	1	4.83E-03	−3.00E-02	Iraq
PI 94567	LDRC 28	20	15994–16070	1426–1500	75	1	−1.08E-02	−4.31E-02	Israel
PI 9791	LDRC 16	10	15386–15461	676–750	75	4	3.71E-02	1.13E-03	Uzbekistan
	Vida		15310–15385	601–675	75				
	PBW 343		15234–15309	526–600	71				
	Dharwar Dry		14702–14077	1–75	74				

† Designation in Jordan et al. (2018).

‡ RIL, recombinant inbred line. Registration number assigned by the USDA-ARS National Small Grains Collection. The first number in each population is assigned to the parent. The core parent Berkut is assigned GSTR No. 14701.

§ Local identifier used in population development.

¶ Numbers less than 75 indicate attrition during seed increase and missing RILs.

Based on Fig. 1.

from one plant per line for genotyping (Jordan et al., 2018). The F$_7$ RILs were grown in subsets of 25 lines per RIL in 2014 in Montana, Washington, and South Dakota. The seed deposited in the USDA National Small Grains Collection is from these trials.

A total of 28 RIL populations were genotyped to investigate the control of recombination in wheat (Jordan et al., 2018) using the Illumina wheat iSelect 90K SNP array (Wang et al., 2014) and genotyping-by-sequencing (Saintenac et al., 2013). A total of 50,143 markers were placed on the 28 family-specific genetic maps (Jordan et al., 2018).

Characteristics

Each RIL population consists of approximately 75 lines for a total of 2325 RILs (Reg. Nos. GSTR No. 14701–GSTR 17133). Genetic maps for 28 NAM families are available in Jordan et al. (2018, Supplemental Table S11) and on the Akhunov Wheat Genomics Laboratory website (http://wheatgenomics.plantpath.ksu.edu/nam). This NAM population provides a genotyped germplasm resource for the wheat community. A potential strategy for use of the material is to screen the parents for a trait of interest, followed by analyses of RIL of populations that are likely to be segregating for target trait or sequence. Genotype data from the NAM population has been used to determine the distribution and genetic control of recombination in the wheat genome (Jordan et al., 2018). Subsets of the NAM population were also used to identify novel alleles for resistance to the wheat stem sawfly (Varella et al., 2017), to identify a novel allele for resistance to wheat stem rust race Ug99 (Nirmala et al., 2016), and to validate a quantitative trait locus for grain yield (Zhang et al., 2018).

Availability

A total of 2325 RIL consisting of approximately 75 RIL for each of 32 crosses with Berkut as a common parent has been deposited for immediate distribution in the USDA National Small Grains Collection. Some RIL are missing due to poor seed increase. Seed for all of the parent landraces and cultivars has also been deposited in the USDA National Plant Germplasm System.

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