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Application of Population Sequencing (POPSEQ) for Ordering and Imputing Genotyping-by-Sequencing Markers in Hexaploid Wheat

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POPSEQ for Ordering GBS markers

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Abstract

The advancement of next-generation sequencing technologies in conjunction with new bioinformatics tools enabled fine-tuning of sequence-based high resolution mapping strategies for complex genomes. Although genotyping-by-sequencing (GBS) provides a large number of markers, its application for association mapping and genomics-assisted breeding is limited by a large proportion of missing data per marker. For species with a reference genomic sequence, markers can be ordered on the physical map. However, in the absence of reference marker order, the use and imputation of GBS markers is challenging. Here, we demonstrate how the population sequencing (POPSEQ) approach can be used to provide marker context for GBS in wheat. The utility of a POPSEQ-based genetic map as a reference map to create genetically ordered markers on a chromosome for hexaploid wheat was validated by constructing an independent *de novo* linkage map of GBS markers from a Synthetic W7984 x Opata M85 recombinant inbred line (SynOpRIL) population. The results indicated that there is strong agreement between the independent *de novo* linkage map and the POPSEQ mapping approach in mapping and ordering GBS markers for hexaploid wheat. Following ordering, a large number of GBS markers were imputed, thus providing a high-quality reference map that can be used for QTL mapping for different traits. The POPSEQ-based reference map and whole genome sequence assemblies are valuable resources that can be used to order GBS markers and enable the application of highly accurate imputation methods to leverage the application GBS markers in wheat.

Introduction

Bread wheat (*Triticum aestivum* L.) is one of the world's most important cereal crops that provides approximately 20% of all calories consumed by humans and is a staple food crop for 30% of the human population. Increasing wheat production at the global scale is key to the efforts of filling the anticipated future food shortage gap due to global population increase and adverse effects of climate change on crop production. Application of advanced and precise molecular tools is needed to speed the development of new wheat varieties.

The advent of next-generation sequencing technologies led to the emergence of high throughput sequence-based genotyping (Baird et al. 2008) approaches such as genotyping-by-sequencing (Poland and Rife 2012; Mascher et al. 2013). It also enabled the use of whole-genome shotgun sequencing (WGS) to generate genome assemblies of large and complex genomes. Although WGS assemblies of these large and complex genomes often contain many small, unordered contigs, assemblies can be made rapidly at low cost when compared with approaches based on physical maps (Mascher et al. 2013; Mascher and Stein 2014).

The 'gold-standard' method of genome sequencing is characterized by developing a physical map followed by sequencing of the minimum tiling path. Although the former needs coordinated efforts of several research laboratories, and requires considerable investment in terms of time and money especially for large genome species like wheat, it is still considered as the indispensable method that leads toward a reference genome of a crop species without a reference genome (Feuillet et al. 2011, 2012). However, as a more tractable working assembly, the low-copy gene-space from the whole-genome shotgun sequencing approach is becoming an important genomic resource for gene discovery and functional genomics in species without a reference genome. This application has been proved on large genome crop species such as

barley (*Hordeum vulgare*) and wheat by combining *de novo* WGS with classical genetic analysis (Mascher et al. 2013; Han et al. 2014; Chapman et al. 2015).

Population sequencing methodology, known as POPSEQ, was proposed as an integrated method to order and link contigs in WGS genome assemblies for gene isolation, genomics-assisted breeding, and genetic diversity assessment (Mascher et al. 2013; Ariyadasa et al. 2014). The method relies on the genetic segregation in bi-parental populations to create a linear order of contigs on an individual chromosome. The potential of POPSEQ to bring contigs into a linkage map was demonstrated on barley (Mascher et al. 2013) and then on hexaploid wheat (Chapman et al. 2015). A similar approach which was referred to as recombinant population genome construction (RPGC) was used to produce a high-quality genome assembly using a segregating population of *Caenorhabditis elegans* (Hahn et al. 2014).

Next-generation sequencing-based genotyping approaches have been applied to understand the biological basis of agronomic traits in several plant species and for making selections via whole genome prediction (Berkman et al. 2012). Studies on major crop species such as *Zea mays* (Bernardo and Yu 2007; Bernardo 2009; Crossa et al. 2013), *T. aestivum* (Heffner et al. 2010; Poland et al. 2012b; Daetwyler et al. 2014), *Oryza sativa* (Xu et al. 2014) and *H. vulgare* (Iwata and Jannink 2011) have indicated that genomic selection has the advantage of reducing the time needed to release new cultivars for production in plant breeding. Since genome-wide prediction needs a large number of evenly distributed markers per chromosome, GBS markers are suitable for accurately predicting breeding values of candidate individuals in plant breeding programs. The GBS platform provides tens of thousands of markers with relatively low investment (Elshire et al. 2011; Poland et al. 2012a) and allows marker discovery and genotyping to be conducted simultaneously. GBS has been shown as an effective marker platform for whole-genome

profiling and subsequent trait prediction (Poland et al. 2012b; Crossa et al. 2013; Zhang et al. 2014). Another promising area of application of GBS markers in plant breeding is for quantitative trait locus (QTL) mapping either by narrowing previously detected gene/QTL candidate regions or for the identification of novel gene/QTL regions that underlay economically important traits through saturating chromosomes with high density GBS markers.

The recent availability of a draft genome sequence and gene space assemblies for hexaploid and diploid wheat also makes the GBS platform an attractive approach for gene identification, and precisely mapping QTLs through anchoring trait-associated GBS single nucleotide polymorphism (SNP) tags on draft genome sequence and/or gene space assemblies. From recent QTL studies with GBS markers, saturating chromosome regions with markers enabled detection of previously detected and novel QTLs for aluminum tolerance and leaf width in rice (Spindel et al. 2013), for drought tolerance in chickpea (Jaganathan et al. 2014) and for precise identification of the location of a dwarfing gene called *Breviaristatum-e* in barley (Liu et al. 2014).

A drawback of genotypic data from the GBS platform is often a large proportion of missing data points across samples as genomic DNA fragments are sequenced at low depth (Elshire et al. 2011). For GBS marker application in genomic selection, methods of imputing missing data points for unordered markers have been developed (Rutkoski et al. 2013). However, many other imputation approaches that have been developed first require markers to be linearly ordered on a chromosome. Here we demonstrate the utility of the POPSEQ methodology for mapping, ordering and imputing marker data from GBS.

Materials and Methods

Germplasm and genotyping

We used recombinant inbred lines (RILs) from a cross between Synthetic W7984 and Opata M85, ("SynOpRIL"; Sorrells et al. 2011) for this study. The total number of inbred lines used in this study was 183 lines, a sub-set of the larger population of ~ 2000 lines. Genomic DNA was extracted from seedlings of each individual line grown in a greenhouse. The GBS libraries were constructed in 96-plex following the two-enzyme system GBS protocol with restriction enzymes *PstI* (CTGCAG) and *MspI* (CCGG). (Poland et al. 2012a). Each library was sequenced on the Illumina HiSeq 2000 platform.

SNP calling and sequence data processing

Raw sequence data were processed with a custom java script in TASSEL 4, and a population-based SNP calling approach was used (Poland et al. 2012a). Unique sequence tags of 64 bp were aligned internally allowing mismatches of up to 3 bp to identify SNPs within the tags. Fisher's exact test was applied to determine independence of SNP alleles and then filter the SNPs. SNPs with up to 80% missing data points were retained for subsequent data analysis.

Linkage map construction and comparison with POPSEQ data

Construction of a linkage map for Synthetic W7984 x Opata M85 RIL population was done with MSTMap software (Wu et al. 2008) to group the markers into linkage groups. A total of 6,362 polymorphic markers with up to 20% missing data, minor allele frequency of greater than 30% and heterozygosity of less than 2% were considered for linkage map construction. Logarithm of odds (LOD) score of 10 was used to cluster the markers into linkage groups. Linkage groups from the same chromosome were merged together and markers of the same chromosome were reordered with MSTmap.

We used the synthetic wheat W7984 and Chinese Spring shotgun assemblies that were ordered with the POPSEQ approach using Synthetic W7984 x Opata M85 doubled haploid population (SynOpDH) to anchor SNP tags on genetically ordered WGS contigs (IWGSC 2014; Chapman et al. 2015). In brief the SynOpDH population, consisting of 90 individuals, was shotgun-sequenced and SNPs were identified to construct a high density linkage map that has all 21 wheat chromosomes. Then this highly dense genetic map was used to linearly order SNP-associated contigs in the WGS assembly of W7984 (Chapman et al. 2015). The contigs of Chinese Spring assembly were also integrated into the same map of SynOpDH population (IWGSC 2014).

Tags from which we detected SNPs were first aligned against both W7984 and Chinese Spring assemblies. To assemble the SNP tags, we used bwa software with "aln" method with default set up. Samtools "view" method was used to further process sequence output. The SNP tags were filtered using a minimum alignment quality score of 37, and then markers that passed the quality requirements were merged with the high-density genetic map from SynOpDH population. Both W7984 and Chinese Spring assemblies and the high density genetic map were linked by scaffold name in the former and by contig name in the latter which facilitated navigation from assembly to genetic map or vice versa.

Independently constructed *de novo* linkage maps were compared with the POPSEQ-based map for the number of markers correctly assigned to their respective linkage groups, and the linear relationship of marker order between *de novo* and POPSEQ maps. The step-by-step procedure of integrating GBS tags into the POPSEQ assembly is indicated in Supplemental Fig. 1.

Missing data imputation

Genotypic data imputation was done after anchoring SNP tags to the POPSEQ assembly. We aligned sequences of SNP tags for 33,664 SNPs that were obtained from the SynOpRIL population to W7984 and Chinese Spring assemblies to impute missing data points. For W7984 assembly, a total of 16,591 markers, and for Chinese Spring 9,709 markers were imputed using FSFHap (Full-Sib Haplotype imputation) method implemented in TASSEL 5 (Swarts et al. 2014). The algorithm first detects two parental haplotypes and recombination break points using a Hidden Markov model and Viterbi algorithm. A missing data point is imputed to the matching genotype of flanking markers. If genotypes of markers flanking a missing data point do not match, the missing data point is left missing.

Imputation accuracy of FSFHap method was also assessed using squared correlation coefficient (R^2). To calculate this parameter, five percent of the total genotypes were randomly masked for each marker in the dataset at genotypes where the SNP call was present. Squared correlation coefficient (R^2) was calculated by comparing the original data where SNP calls were masked with corresponding imputed data for each marker. The R^2 calculation was also done for the markers common between Chinese Spring and W7984 assemblies.

Results

Validating POPSEQ with de novo linkage map

The use of an ultra-dense genetic linkage map constructed from the 90-individual doubled haploid population (SynOpDH) with the POPSEQ approach was validated through integrating SNP tags of GBS markers used to construct *de novo* linkage maps. We used an independent

recombinant inbred line mapping population made from the same synthetic W7984 and Opata M85 parents for this validation work. A total of 23 linkage groups with greater than two markers were obtained with an LOD score of 10. There was a total of 6,360 markers in the linkage groups. Aligning sequences of SNP tags of these markers with sequences of linearly ordered gene space assembly of W7984 and Chinese Spring resulted in 3,364 markers and 2,049 markers that passed minimum quality alignment score of 37, respectively. A total of 3,357 (99.8%) for W7984 and a total of 2,037 markers (99.4%) for Chinese Spring were correctly assigned to their respective linkage group based on the sequence identity search in the assemblies. After removing incorrectly assigned markers and markers reported suspicious by the mapping algorithm in MSTmap, 21 linkage groups that correspond to the total number of hexaploid wheat chromosomes were obtained. Comparison of genetic map positions of the GBS markers in each of the 21 linkage groups based on the *de novo* map with that of positions of each marker based on the high density genetic map of POPSEQ also showed that there was a linear relationship between the two genetic maps (Fig. 1 and 2).

Originally, a total of 33,664 GBS markers with up to 80% missing data were obtained for the synthetic W7984 x Opata M85 RIL population. All marker tags, without considering the level of missing data, were integrated into POPSEQ data. After discarding markers with low alignment quality score (<37), map positions were found for 16,591 markers (49.3%) for W7984 assembly and 9,709 (28.8 %) for Chinese Spring assembly. The number of markers per chromosome was higher for W7984 assembly for all chromosomes than that of Chinese Spring (Fig. 3 and Supplemental Table 1). Similarly, the average gap size per chromosome was low for all chromosomes in the case of W7984 assembly (Fig. 4). Maximum gap size for markers anchored to W7984 assembly was lower than 20 cM for all chromosomes. All chromosomes had less than

30 cM maximum gap size for markers anchored to the Chinese Spring assembly (Supplemental Table 2). The two assemblies had a total of 7,040 markers in common, and there was a strong positive relationship between marker and map positions between the two maps (Supplemental Fig. 2).

Data imputation

Two datasets comprised a total of 16,591 markers (for W7984 assembly) and 9,709 markers (for Chinese Spring) were submitted to FSFHap for imputation. After imputation, 95% of the markers for W7984 assembly and 94% of the markers for Chinese Spring had less than 10% missing data points (Supplemental Table 3 and 4). Furthermore, 92.4% and 89.8% of the markers had less than 5% of missing data for W7984 assembly and Chinese Spring assembly, respectively. Only 2.7% of the markers for W7984 assembly and 3.2% of the markers for Chinese Spring assembly had over 20% remaining missing data points per marker. The highest average missing data points per chromosome in the imputed data set was recorded for chromosome 2D (Supplemental Fig. 3, and there was no clear relationship between the amount of missing data per chromosome in the original and imputed data sets (Supplemental Fig. 4 and 5). The number of un-imputed missing data points was higher for Chinese Spring than that of W7984 assembly in 15 chromosomes. The average proportion of heterozygous genotypes per marker was comparable between the two assemblies except for chromosomes 2A, 3D and 4A where differences between the two assemblies were large (Supplemental Fig. 6). The exceptionally high average proportion of heterozygous genotypes for chromosome 2A for Chinese Spring was due to high heterozygous genotypes per marker (28-37%) for markers within the interval 58.7-67.4 cM (around the centromeric region) (Supplemental Table 4). Markers on

chromosome 4D had high heterozygote genotypes for both assemblies. However, except for these two chromosomes (2A and 4D), the average proportion of heterozygote genotypes per marker was less than 10% for all chromosomes (Supplemental Fig. 6). There was no relationship between the amount of heterozygote genotypes before imputation and the amount remaining after imputation for both assemblies (Supplemental Fig. 7 and 8). Average proportion of heterozygote genotypes per markers in imputed data set was higher than that of before imputation for both assemblies for all chromosomes. The algorithm implemented in FSFHap is taking into account heterozygote under-calling for GBS platform SNP calling. However, taking into account the low proportion of heterozygote genotypes in the original data and the expected residual heterozygosity for recombinant inbred line populations, FSFHap may be overestimating the amount of heterozygote genotypes per marker in the imputed data.

Imputation accuracy of FSFHap was evaluated using squared correlation coefficient (R^2). The average R^2 for the markers anchored to Chinese Spring was 0.94, and 90% of the markers had R^2 greater than or equal to 0.8 (Supplemental Fig. 9). Similar average R^2 of 0.94 was obtained for the markers common between Chinese Spring and W7984 assemblies.

Discussion

We validated the utility of POPSEQ, a reference map-based marker ordering method, using a new recombinant inbred line population. We first constructed a *de novo* genetic linkage map using filtered high-quality GBS markers from the Synthetic W7984 X Opata M85 RIL population. For the POPSEQ map to be used as a reference map to order the GBS markers from different populations, first GBS markers within a *de novo* linkage group should be assigned to

253 the same chromosome with the chromosome assignment of the markers based on aligning SNP
254 tags sequences with the POPSEQ genome assembly. Secondly, marker order of the *de novo*
255 linkage map should also agree with the order of the same markers on the POPSEQ-based
256 reference genetic map. Using a high alignment stringency level, both chromosome assignment
257 and marker order indicated that there is a good agreement between the *de novo* linkage map and
258 POPSEQ-based high-density genetic map. This implies that the POPSEQ approach can be used
259 as an alternative method to assign and order GBS markers on wheat chromosomes.

260 The advantage of using the POPSEQ approach for mapping and ordering GBS markers is
261 twofold. In classical linkage mapping approach, chromosome assignment information is
262 required for all constructed linkage groups. With the absence of this information, anchor
263 markers are needed to guide linkage map construction and for downstream interpretation of QTL
264 analysis. In the case of GBS markers, for species without a reference genome sequence, this
265 information is presumably lacking and consequently construction of a *de novo* linkage map is a
266 tedious job. Moreover, genetic map construction is limited by the large number of markers with
267 a high proportion missing data. However, the POPSEQ anchored reference can give *a priori*
268 marker positions as with any reference genome and avoids the need of both anchor markers and
269 *de novo* map construction.

270 By using the POPSEQ approach we were able to genetically map and order 16,591 and 9,711
271 GBS markers anchored to W7984 and Chinese Spring assemblies, respectively. The higher
272 number of markers for W7984 assembly compared to Chinese Spring assembly may be due to
273 W7984 being one of the parents of the bi-parental population from which SNP tags were
274 detected. Chromosome 4D had the least number of markers while chromosome 3B the highest
275 number of markers for both assemblies (Fig. 3 and Supplemental Table 1). This agrees with the

observation that the larger the chromosome size, the more number of markers it contains (Poland et al. 2012a; Saintenac et al. 2013). Similarly, as expected the total number of markers mapped to the D genome (25% for both assemblies) was lower than that of A (31% for W7984 assembly and 28% for Chinese Spring assemblies) and B (44% for W7984 assembly and 47% for Chinese Spring assembly) genomes as the D genome of hexaploid wheat is smaller and less diverse than A and B genomes (Chao et al. 2010; Wang et al. 2013; Edae et al. 2014; Iehisa et al. 2014). However, the difference between the number of markers mapped to D and A genomes was lower than reported in the literature, which was about five-fold higher for both A and B genomes (Allen et al. 2011, 2013; Cavanagh et al. 2013). One of the probable explanations for this small difference between A and D genomes is due to re-introduction of D genome diversity through synthetic W7984 to the current hexaploid wheat mapping population.

The maximum gap size per chromosome for markers anchored to W7984 assembly (<20 cM) was lower than that of markers anchored to Chinese Spring assembly (<30 cM). The original reference map from POPSEQ had good marker coverage (maximum gap size less than 20 cM) for all chromosomes with the exception of 1A and 4D with maximum gap size of 26 cM and 28 cM, respectively (data not shown). Overall, the pattern of marker distribution on chromosomes observed for our population also has good agreement with that of reference map indicating the potential of POPSEQ approach of marker ordering and imputing. However, our final high quality SNP dataset represented only about 30% and 49% of the total original 33,664 SNP tags for Chinese Spring and W7984 assemblies, respectively. A majority of the markers did not pass the strict sequence alignment threshold we used to reduce the risk of assigning the tags to incorrect positions. Therefore, for full utilization of the POPSEQ-based gene space assemblies, consensus reference genetic maps of two or more populations are needed.

299 Imputing missing data is a necessary step particularly for the genotypic data sets with a large
300 proportion of missing data per marker (up to 80% in our case). The high-density genetic map
301 enabled us to use FSFHap as implemented in TASSEL 5 (Swarts et al. 2014). FSFHap generally
302 needs a large data set for best results. Imputation accuracy measurements (R^2) found here,
303 indicated that FSFHap is still accurate in imputing a relatively small dataset with a large
304 proportion of missing data. We found average imputation accuracy (R^2=0.94) roughly similar
305 with that of average imputation accuracy (R^2=0.97) obtained for a large genotypic data set of
306 maize nested association mapping panel (NAM RILs) used by Swarts et al. (2014). We observed
307 an increase in the number of heterozygous genotypes called in the imputed data Low coverage
308 sequencing with GBS is expected to under call true heterozygous genotypes. Therefore, an
309 increase in heterozygous calls is expected to some extent with the GBS imputation. Apart from
310 the effect of algorithms implemented in FSFHap, the higher levels of heterozygosity for some
311 markers near centromeric regions (e.g. 2A) in the imputed dataset may be due to deleterious
312 alleles in trans-linkage combined with low levels of recombination in these regions (McMullen
313 et al. 2009).

314 In conclusion, POPSEQ methodology can be used as an alternative method of mapping and
315 ordering GBS markers. For crop species lacking a high quality reference genome, like hexaploid
316 wheat, genetically ordered markers allow the implementation of marker-order-dependent but
317 highly accurate imputation algorithms (e.g. FSFHap) to impute genotypic data with a large
318 proportion of missing data points so that QTL/gene mapping can be done precisely. Since both
319 the Chinese Spring and W7984 POPSEQ results were based on the same SynOpDH genetic
320 mapping population, full integration of markers to the reference map needs POPSEQ-based high-

density genetic map and gene space assemblies that are developed from additional bi-parental populations.

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340 References

341 Allen, A.M., G.L.A Barker, S.T. Berry, J.A. Coghill, R. Gwilliam et al., 2011 Transcript-
342 specific, single-nucleotide polymorphism discovery and linkage analysis in
343 hexaploid bread wheat (*Triticum aestivum* L.). Plant Biotechnol J 9:1086–1099.

344 Allen, A.M., G.L.A Barker, P.Wilkinson, A. Burridge, M.Winfield et al., 2013 Discovery
345 and development of exome-based, codominant single nucleotide polymorphism
346 markers in hexaploid wheat (*Triticum aestivum* L.). Plant Biotechnol J 11:279–
347 295.

348 Ariyadasa, R., M. Mascher, T. Nussbaumer, D. Schulte, Z. Frenke et al., 2014 A
349 sequence-ready physical map of barley anchored genetically by two million
350 single-nucleotide polymorphisms. Plant physiology 164: 412-423.

351 Baird, N., P. Etter., T. Atwood, M. Currey, A. Shiver et al., 2008 Rapid SNP discovery
352 and genetic mapping sing sequenced RAD markers. PLoS ONE 3(10): e3376.
353 doi:10.1371/journal.pone.0003376.

354 Berkman, P., K. Lai, M. Lorenc, and D. Edwards, 2012 Next-generation sequencing
355 application for wheat crop improvement. American Journal of Botany 99(2): 365–
356 371.

357 Bernardo, R., 2009 Genomewide selection for rapid introgression of exotic germplasm in
358 maize. Crop cience 49:419–425.

359 Bernardo, R., and J.Yu, 2007 Prospects for Genomewide selection for quantitative traits
360 in maize. Crop Science 47:1082–1090.

361 Cavanagh, C.R., S. Chao, S. Wang, B.E. Huang, S. Stephen et al., 2013 Genome-wide
362 comparative diversity uncovers multiple targets of selection for improvement in

363 hexaploid wheat landraces and cultivars. Proc Natl Acad Sci USA 110:8057–
364 8062.
365 Chao, S.M., J. Dubcovsky, J. Dvorak, M.C. Luo, S.P. Baenziger et al., 2010 Population-
366 and genome-specific patterns of linkage disequilibrium and SNP variation in
367 spring and winter wheat (*Triticum aestivum* L.). BMC Genom 11:727 doi:
368 10.1186/1471-2164-11-727.
369 Chapman, J., M. Mascher, A. Buluç, K. Barry, E. Georganas et al., 2015 A whole-
370 genome shotgun approach for assembling and anchoring the hexaploid bread
371 wheat genome. Genome Biology 16:26. doi:10.1186/s13059-015-0582-8.
372 Crossa, J., Y. Beyene, S. Kassa, P. Pérez, J.M. Hickey et al., 2013 Genomic prediction in
373 maize breeding population with genotyping-by-sequencing
374 Genes|Genomes|Genetics 13: 1903-1926.
375 Daetwyler, H., U. Bansal, H. Bariana, M. Hayden, and B. Hayes, 2014 Genomic
376 prediction for rust resistance in diverse wheat landraces. Theor Appl Genet (2014)
377 127:1795–1803.
378 Edae, E., P. Byrne, S. Haley, M. Lopes, and M. Reynolds, 2014 Genome-wide
379 association mapping of yield and yield components of spring wheat under
380 contrasting moisture regimes. Theor Appl Genet 127:791-807.
381 Elshire, R.J, J.C. Glaubitz, Q. Sun, J.A. Poland, K. Kawamoto et al., 2011 A robust,
382 simple genotype-by-sequencing (GBS) approach for high diversity species. PLoS
383 one 6: e19379.
384 Feuillet, C., J.E. Leach, J. Rogers, P.S. Schnable and K. Eversole, 2011 Crop genome
385 sequencing: lessons and rationales. Trends Plant Sci 16: 77-88.

Feuillet, C., N. Stein, L. Rossini, S. Praud, K. Mayer et al., 2012 Integrating cereal genomics to support innovation in the Triticeae. Funct Integr Genomics 12: 573-583.

Hahn, M., S. Zhang, and L. Moyle, 2014 Sequencing, assembling, and correcting draft genomes assembly using recombinant populations. G3 Genes/Genomes/Genetics 4:669-679.

Heffner, E., A. Lorenz, JL. Jannink, and M. Sorrells, 2010 Plant breeding with genomic selection: gain per unit time and cost.

Iehisa, J.C.M., A. Shimizu, K. Sato, R. Nishijima, K. Sakaguchi et al., 2014 Genome-wide marker development for the wheat D genome based on single nucleotide polymorphisms identified from transcripts in the wild wheat progenitor *Aegilops tauschii*. Theor Appl Genet 127:261–271.

Iwata, H., and J.L. Jannink, 2011 Accuracy of genomic selection prediction in barley breeding programs: A Simulation study based on the real single nucleotide polymorphism data of barley breeding lines. Crop Science 51:1915-1927.

IWGSC, The International Wheat Genome Sequencing Consortium. 2014. A chromosome-based draft sequence of the hexaploid bread wheat (*Triticum aestivum*) genome. Science 345(6194):286.

Jaganathan, D., M. Thudi, S. Kale, S. Azam, M. Roorkiwal et al., 2014 Genotyping-by-sequencing based intra-specific genetic map refines a ‘‘QTL-hotspot” region for drought tolerance in chickpea. Mol Genet Genomics. DOI 10.1007/s00438-014-0932-3.

Liu, H., M. Bayer, A. Druka, J. Russell, C. Hackett et al., 2014 An evaluation of genotyping by sequencing (GBS) to map the *Breviaristatum-e* (*ari-e*) locus in cultivated barley. BMC Genomics 15:104.

McMullen, M. D., S. Kresovich, H. S. Villeda, P. Bradbury, H. Li et al., 2009 Genetic properties of the maize nested association mapping population. Science 325(7): 737-740.

Mascher, M., and N. Stein, 2014 Genetic anchoring of whole genome shotgun assemblies. Frontiers in Genetics 5(208). Dio:10.3389/fgene.2014.000208.

Mascher, M., G. Muehlbauer, D. Rokhsar, J. Chapman, J. Schmutz et al., 2013 Anchoring and ordering NGS contig assemblies by population sequencing (POPSEQ). The Plant Journal 76: 718–727.

Poland, J., P. Brown, M. Sorrells, and J.L. Jannink, 2012a Development of high-density genetic maps for barley and wheat using a novel two-enzyme genotyping-by-sequencing approach. PLoS ONE 7(2):e32253. doi:10.1371/journal.pone.0032253.

Poland, J., J. Endelman, J. Dawson, J. Rutkoski., S. Wu et al., 2012b Genomic selection in wheat breeding using genotyping-by-sequencing. The Plant Genome 5(3):92:102.

Poland, J., and T. Rife, 2012 Genotyping-by-sequencing for plant breeding and genetics. The Plant Genome 5(3):92-102.

Rutkoski, J., J. Poland, JL. Jannink, and M. Sorrells. 2013. Imputation of unordered markers and the impact on genomic selection accuracy. G3 (3):427-429.

Saintenac, C., D. Jiang, S. Wang, and E. Akhunov, 2013 Sequencing-based mapping of the polyploid wheat genome. G3 3:1105-1114.

Spindel, J., M. Wright, C. Chen, J. Cobb, J. Gage et al., 2013 Bridging the genotyping gap: using genotyping by sequencing (GBS) to add high‐density SNP markers and new value to traditional bi-parental mapping and breeding populations. Theor Appl Genet 126:2699-2716.

Sorrells, M., J. Gustafson, D. Somers, S. Chao, D. Benscher et al., 2011 Reconstruction of the Synthetic W7984 × Opata M85 wheat reference population. Genome 54:875-82.

Swarts, K., H. Li, A. Navarr, D. An, M. Romay et al., 2014 Novel methods to optimize genotypic imputation for low-coverage, next-generation sequence data in crop plants. The Plant Genome 7(3): doi: 10.3835/plantgenome2014.05.0023.

Truong, S., R. McCormick, D. Morishige, and J. Mullet, 2014 Resolution of genetic map expansion caused by excess heterozygosity in plant recombinant inbred populations. G3 (4):1963-1969.

Wang, J., M.C. Luo, Z. Chen, F. You, Y. Wei et al., 2013 *Aegilops tauschii* single nucleotide polymorphisms shed light on the origins of wheat D-genome genetic diversity and pinpoint the geographic origin of hexaploid wheat. New Phytol 198:925–937.

Wu, Y., P.R. Bhat, T.J. Close, and S. Lonardi, 2008 Efficient and accurate construction of genetic linkage maps from the minimum spanning tree of a graph. PLoS Genet, 4, e1000212.

Xu, S., D. Zhu, and O. Zhang, 2014 Predicting hybrid performance in rice using genomic best linear unbiased prediction. PNAS 111(34):12456–12461.

Zhang, X., Pérez-Rodríguez, K. Semagn, Y. Beyene, R. Babu et al. 2014 Genomic prediction in biparental tropical maize populations in water-stressed and well-watered environments using low-density and GBS SNPs. Heredity 1-9. doi:10.1038/hdy.2014.99.

Figure legends

Figure 1. Marker order relationship between de novo map and POPSEQ map for all 21 chromosomes of hexaploid wheat for markers anchored to W7984 assembly.

Figure 2. Marker order relationship between de novo map and POPSEQ map for all 21 chromosomes of hexaploid wheat for markers anchored to Chinese Spring.

Figure 3. Genome-wide distribution of GBS markers anchored to both W7984 and Chinese Spring assemblies.

Figure 4. Average gap size (cM) for the markers anchored to both W7984 and Chinese Spring assemblies.

Supplemental Figure 1. Schematic representation of GBS marker integration into POPSEQ data.

Supplemental Figure 2. Common markers between W7984 and Chinese Spring assemblies.

Supplemental Figure 3. Average proportion of missing data points remain after imputation for W7984 and Chinese Spring anchored markers.

Supplemental Figure 4. Average proportion of missing data points in imputed and unimputed markers anchored to W7984 assembly.

Supplemental Figure 5. Average proportion of missing data points in imputed and unimputed markers anchored to Chinese Spring assembly.

Supplemental Figure 6. Average proportion of heterozygote genotypes after imputation for W7984 and Chinese Spring.

Supplemental Figure 7. Average proportion of heterozygote genotypes in imputed and unimputed markers anchored to W7984 assembly.

Supplemental Figure 8. Average proportion of heterozygote genotypes in imputed and unimputed markers anchored to Chinese Spring assembly.

Supplemental Figure 9. Imputation accuracy of FSFHap imputed Chinese Spring anchored markers.

Figure 1

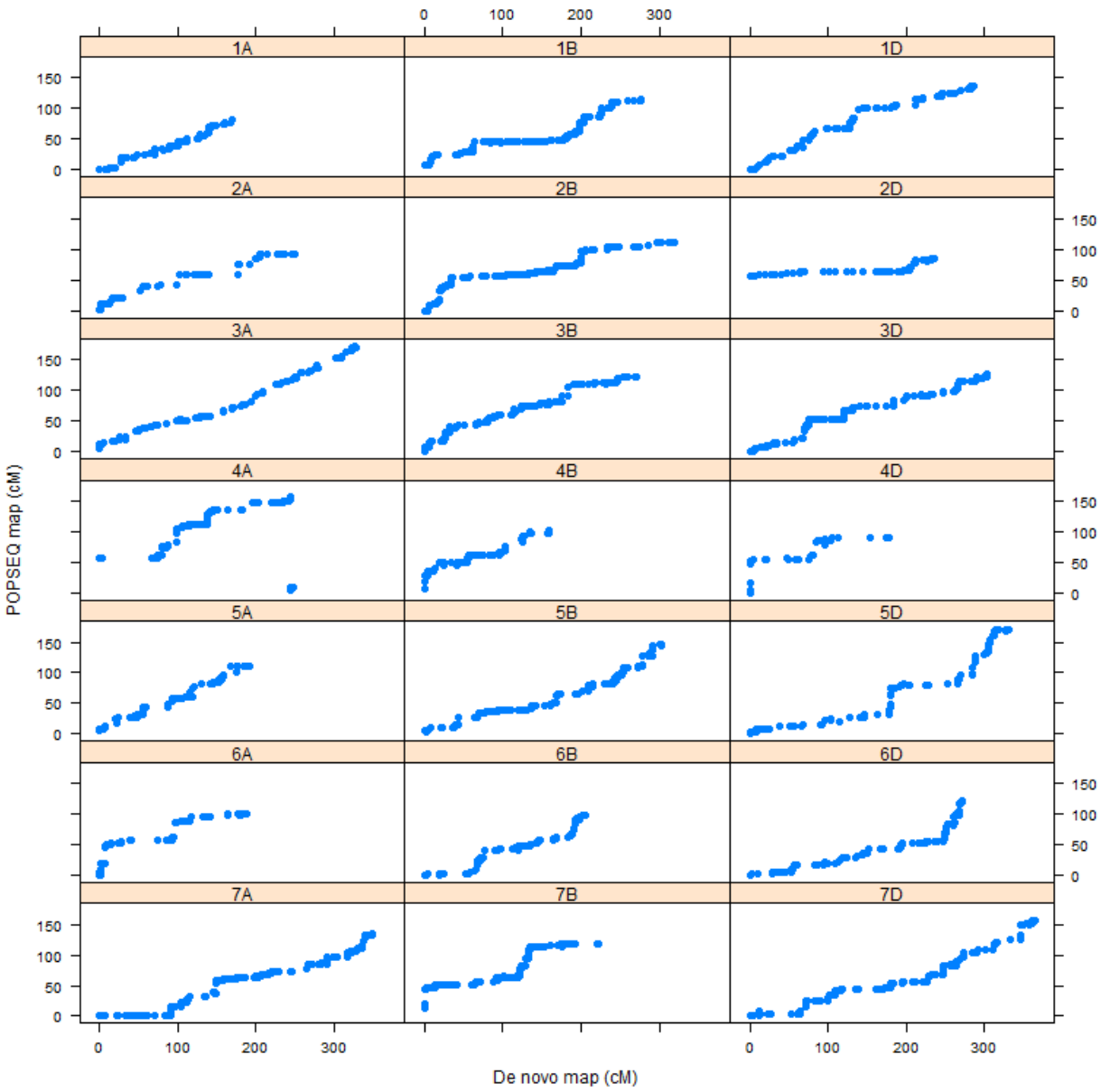

1A
1B
1D
2A
2B
2D
3A
3B
3D
4A
4B
4D
5A
5B
5D
6A
6B
6D
7A
7B
7D
0
100
200
300
0
50
100
150
POPSEQ map (cM)
De novo map (cM)

Figure 2

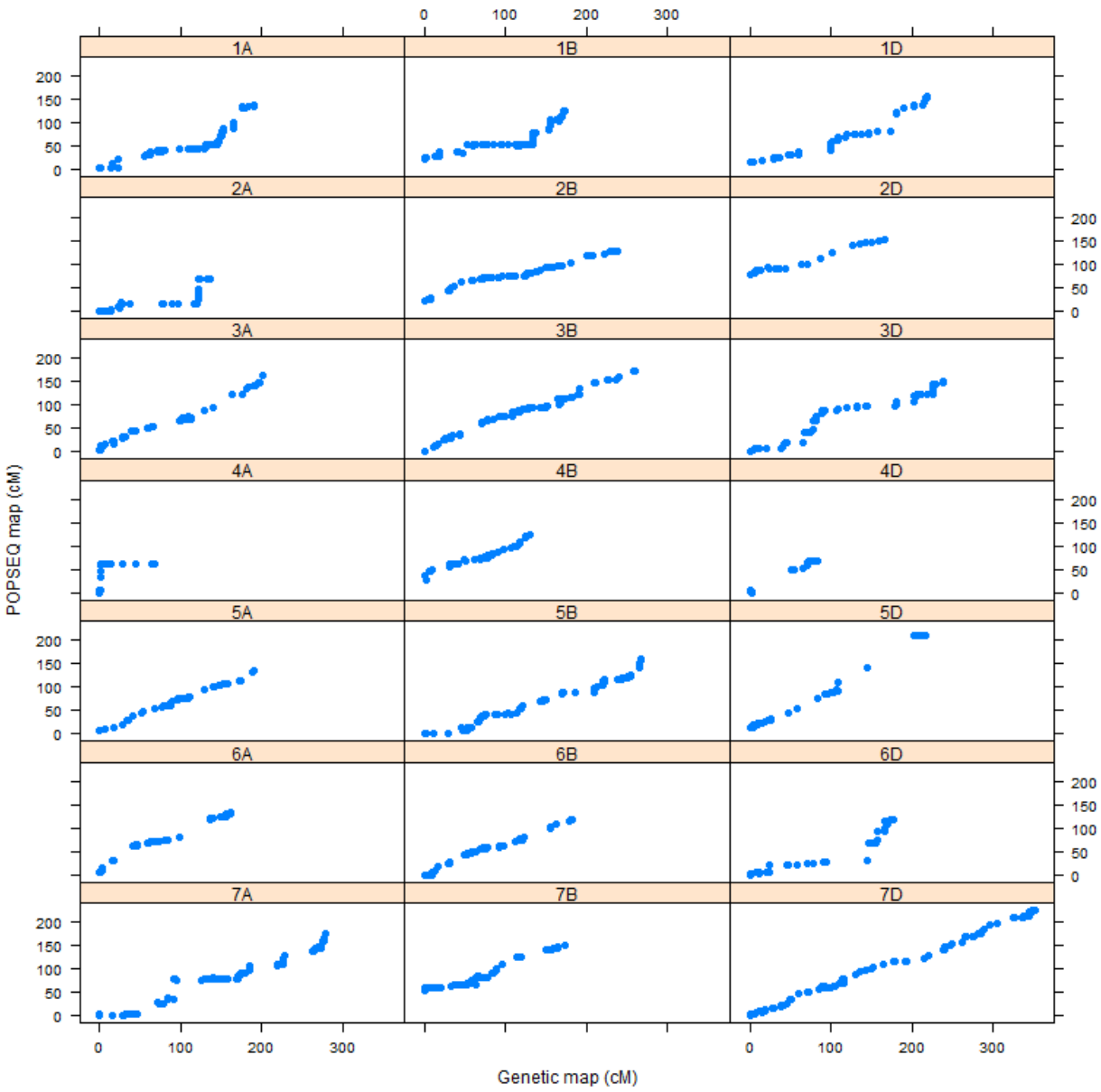

1A
1B
1D
2A
2B
2D
3A
3B
3D
4A
4B
4D
5A
5B
5D
6A
6B
6D
7A
7B
7D
0
100
200
300
0
50
100
150
200
POPSEQ map (cM)
Genetic map (cM)

Figure 3

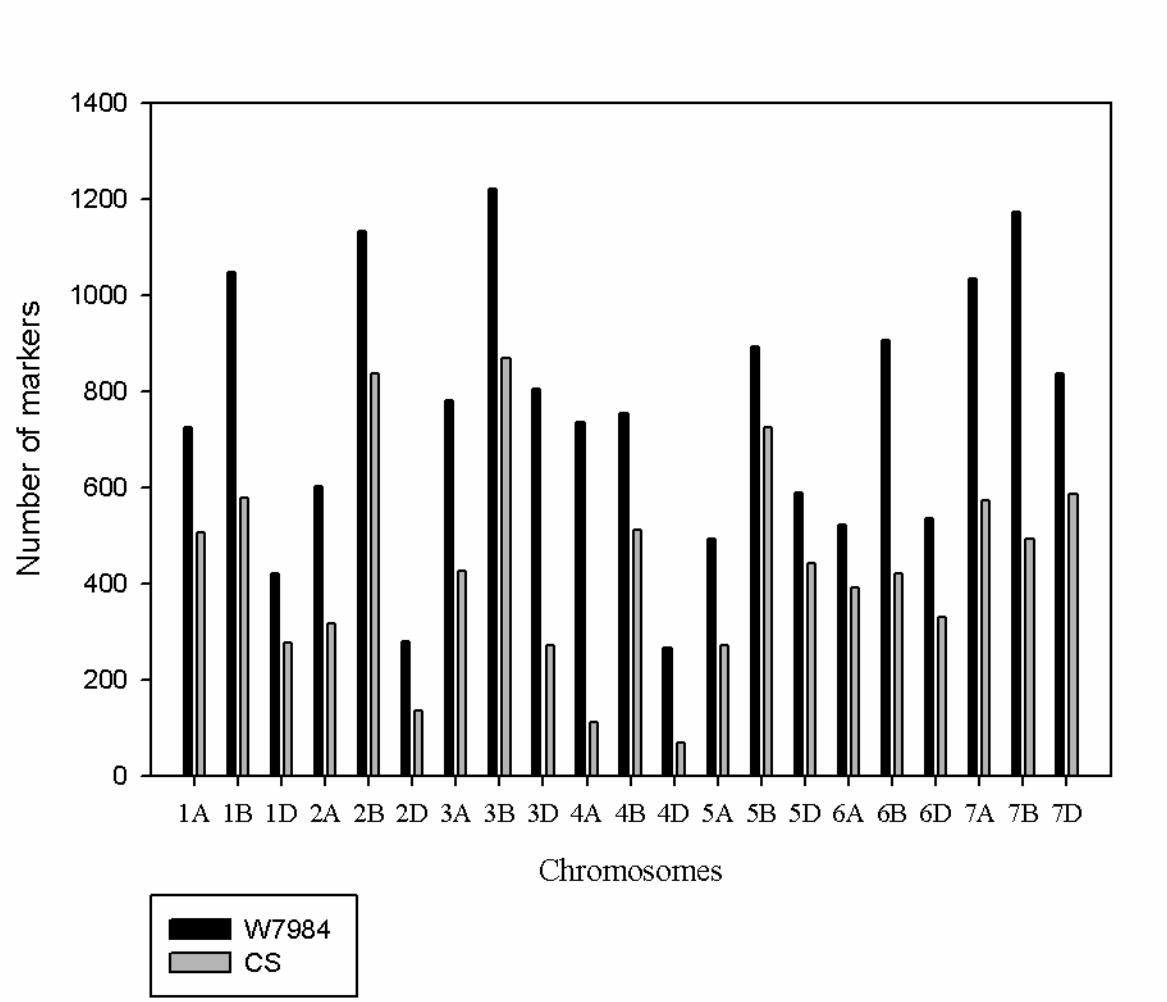
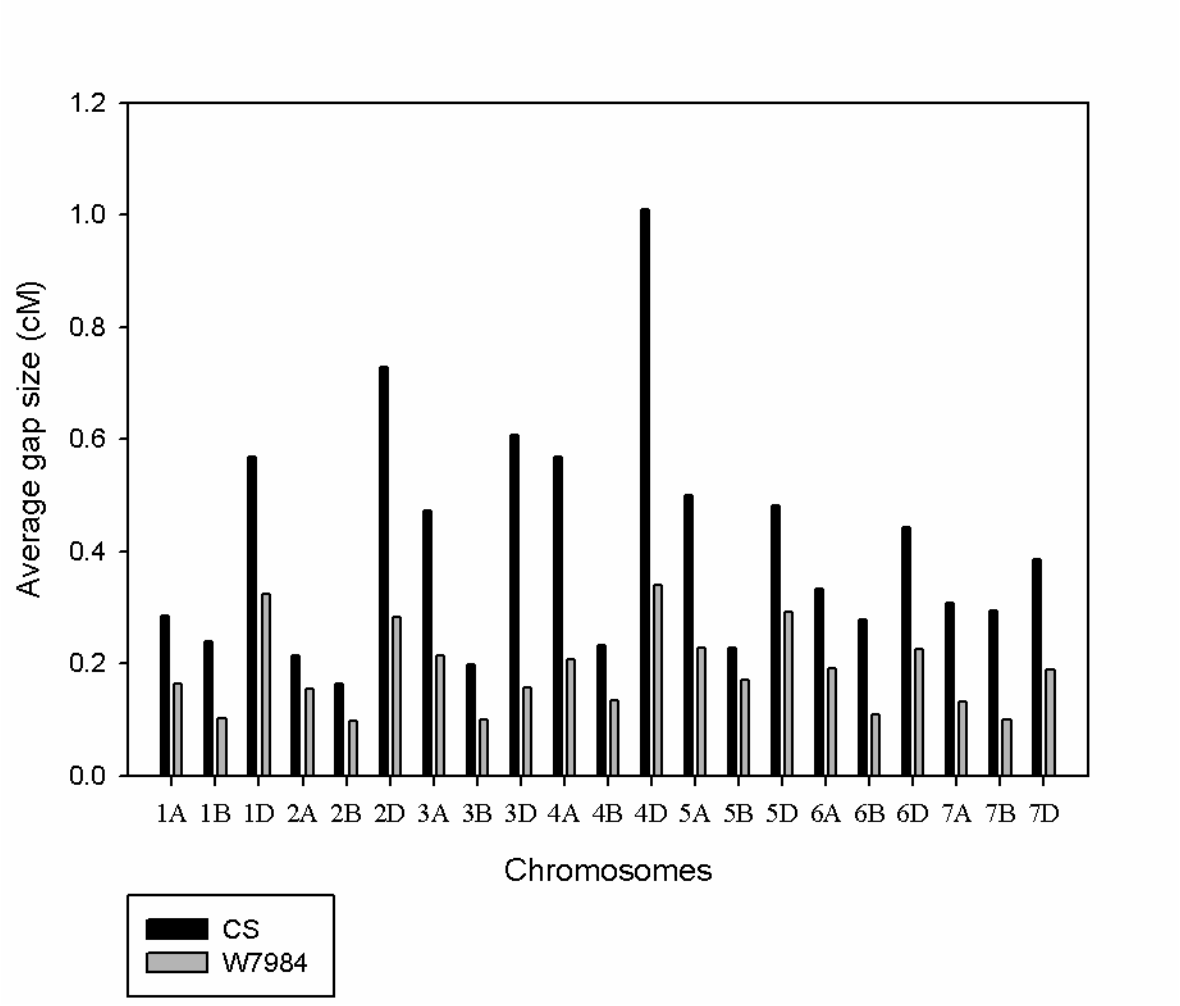

1400
1200
1000
800
600
400
200
0
Number of markers
1A 1B 1D 2A 2B 2D 3A 3B 3D 4A 4B 4D 5A 5B 5D 6A 6B 6D 7A 7B 7D
Chromosomes
W7984
CS

Figure 4


Average gap size (cM)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
1A 1B 1D 2A 2B 2D 3A 3B 3D 4A 4B 4D 5A 5B 5D 6A 6B 6D 7A 7B 7D
Chromosomes
CS
W7984