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Validation of SNP chromosome locations via diverse molecular markers in three wheat mapping populations

S. Liu[1], S. Assanga[2], S. Dhakal[1], D.B. Hays[2], J.C. Rudd[1], A.M.H. Ibrahim[2], Q. Xue[1], S. Chao[3], R. Devkota[1], P. Sengodon[2], T. Huggins[2] and S Mohammed[2]

[1]Texas A&M AgriLife Research and Extension Center, Amarillo, TX 79106, USA; [2]Deptartment of Soil and Crop Sciences, Texas A&M University, College Station, TX 77843, USA; [3]USDA-ARS Biosciences Research Laboratory, Fargo, ND 58102, USA

E-mail: SLiu@ag.tamu.edu

Recombinant inbred lines (RILs) were developed from three wheat crosses, namely CO 960293-2/'TAM 111' (CT, 217 lines), 'Halberd'/'Len' (HL, 180 lines), and 'TAM 112'/TAM 111 (TT, 124 lines). The RILs and parents of each population were screened using the 90K Infinium iSelect array of single nucleotide polymorphic (SNP) markers. About 54,000 SNPs had valid scores in at least one population, but 35,500 SNPs (65%) were monomorphic across the three populations. About 9,100, 10,000, and 7,500 were polymorphic on CT, HL, and TT, respectively, with a total of 18,500 SNPs being polymorphic for three populations. However, only 1,437 SNPs (8%) were polymorphic across all three populations and 5,270 (28%) were polymorphic across two of the three populations. The numbers of polymorphic SNPs specific to CT, HL and TT were 2,947 (16%), 2,398 (13%), and 6,412 (35%), respectively. A subset of polymorphic SNP markers was used to construct genetic linkage groups following the confirmation of each RIL genotype. However, the chromosomal locations of most SNPs were still unknown. With known chromosomes of Diversity Array Technology (DArT), microsatellite, and sequence tagged site (STS) markers mapped in the TT population, these linkage groups were assigned to chromosomes. By aligning some published SNP markers with known chromosome locations, all the linkage groups were validated for chromosomal location. Saturated chromosome maps will be crucial for target gene/QTL identification.

Application of the Ug99 SNP assay on herbarium stem rust specimens

B. Visser[1], L.J. Szabo[2], L. Herselman[1] and Z.A. Pretorius[1]

[1]Department of Plant Sciences, University of the Free State, P.O. Box 339, Bloemfontein 9300, South Africa; [2]USDA-ARS, Cereal Disease Laboratory, 1551 Lindig Street, St Paul, MN 55108, USA

E-mail: visserb@ufs.ac.za

A recent genetic analysis using SSR markers of 24 herbarium specimens of *Puccinia graminis* f. sp. *tritici* collected between 1906 and 1945 in South Africa and other African countries, suggested that some of these may have contributed to the Ug99 lineage. This included a single specimen collected in Uganda in 1914. The two-stage SNP assay was used to determine whether any of these herbarium specimens belonged to the Ug99 lineage. Preliminary results were inconclusive and an extended set of SNP markers is being tested to speculate on the origin and historical presence of the Ug99 lineage in Africa.

Identification of markers linked to stem rust resistance in wheat landraces by bulked segregant analysis

E.M. Babiker[1], T.C. Gordon[1], S. Chao[2], M. Newcomb[3], S. Stoxen[3], M.N. Rouse[3] and J.M. Bonman[1]

[1]USDA-ARS, Small Grains and Potato Germplasm Research Unit, 1691 S 2700 W, Aberdeen, ID 83210, USA; [2]USDA-ARS, Cereal Crops Research, 1605 Albrecht Blvd., Fargo, ND 58102, USA; [3]USDA-ARS, Cereal Disease Laboratory; 1551 Lindig Avenue, St. Paul, MN 55108, USA

E-mail: Mike.Bonman@ARS.USDA.GOV

Stem rust is a destructive disease affecting wheat worldwide. *Pgt* race TTKSK (Ug99) is a threat to global wheat production. In previous efforts to identify resistance to Ug99 in the National Small Grains Collection, 34 wheat landrace accessions showed seedling resistance to race TTKSK. To improve the efficiency of breeding for resistance to stem rust, molecular markers tightly linked to novel resistance genes are needed to enable marker assisted selection. Bulked segregant analysis (BSA) is a rapid, efficient and less expensive strategy to map genes of interest to specific regions of the genome. The objectives of this study were to (i) identify and map intervals within the wheat genome containing stem rust resistance genes in these wheat landraces, and (ii) validate the detected markers in Recombinant Inbred Line (RIL) populations. The 34 resistant wheat accessions were crossed to a susceptible wheat genotype, LMPG-6. Parents, F_1 seedlings, and about 100 F_2 seedlings from each cross were screened against race TTKSK at the USDA-ARS Cereal Disease Laboratory. DNA was extracted from parents, F_1 seedlings, and two susceptible bulks each comprised of 10 F_2 susceptible seedlings and sent for 90K SNP analysis. Chi squared analyses revealed that segregation of reaction in 28 populations did not deviate from 3:1 resistant:susceptible ratios following inoculation with race TTKSK, suggesting that resistance in each of these populations is controlled by a single dominant gene. Four populations segregated 13:3 resistant:susceptible and one population, 9:7, indicating that two genes controlled resistance in these populations. Use of BSA and the 90K SNP chip may rapidly identify multiple new resistance genes from Ug99-resistant wheat landraces that could be used in wheat breeding.

Applying genomic selection to CIMMYT spring wheat for end-use quality

S.D. Battenfield[1], J.A. Poland[1,3], R.P. Singh[2], R.J. Peña[2], R.A. Miller[4] and A.K. Fritz[1]

[1]Kansas State University, Department of Agronomy, Throckmorton Hall, Manhattan, KS 66506, USA; [2]International Maize and Wheat Improvement Center, Apdo. Postal 6-641, 06600 Mexico D.F., Mexico; [3]USDA-ARS, Throckmorton Hall, Manhattan, KS 66506, USA; [4]Kansas State University, Department of Grain Science and Industry, Throckmorton Hall, Manhattan, KS 66506 USA

E-mail: sbatten@k-state.edu

Wheat cultivars must possess suitable end-use quality for release and acceptability; however, breeding for quality traits is often considered a secondary goal compared to enhancing yield, disease resistance and stress tolerance. Breeding programs evaluate end-use quality in advanced stages in the breeding process, thus allowing many undesirable materials to be advanced. Our aim is to develop and test genomic selection prediction models using the end-use quality phenotypes routinely generated by the CIMMYT wheat breeding program on advanced wheat materials. Once model accuracy is determined, genomic selection can be applied at earlier breeding stages and undesirable materials culled before implementing expensive replicated yield and quality tests. To test genomic selection model accuracy, we used data from CIMMYT wheat lines identified as candidates for inclusion in the 45th and 46th International Bread Wheat Screening Nurseries (n = 1,707). Each set was grown for one year at Ciudad Obregon, Mexico, under high yielding environments and evaluated for grain yield and quality parameters, viz. test weight, grain hardness, grain protein, flour protein, mixograph dough mixing time, alveograph dough strength (W) and tenacity/extensibility ratio (P/L), and loaf volume. High-density markers were generated with genotyping-by-sequencing, and SNPs were imputed. To test prediction accuracy, two validation methods using ridge regression were used: a year-to-year approach using one year as the prediction set and the other as the testing set, and a cross-validation scheme where training and testing sets were selected randomly and iterated 100 times. Prediction accuracies (r) for all quality parameters using cross-validation ranged from 0.476 (alveograph P/L) to 0.725 (test weight), while accuracies for year-to-year models were low (0.005 for grain hardness) to intermediate (0.535 for mixing time). Based on these prediction accuracies, we conclude that GS can be a useful tool to facilitate early generation selection for end-use quality.

Optimization of the barley stripe mosaic virus system for virus induced gene silencing in wheat

S.M. Clark, E. Liu and J.E. Page

National Research Council Canada, Saskatoon, Saskatchewan S7N 0W9, Canada

E-mail: Shawn.Clark@nrc.ca

Virus induced gene silencing (VIGS) is an effective tool for the rapid silencing of plant genes to study plant pathogen interactions. Barley stripe mosaic virus (BSMV) has been widely used for this purpose in wheat and other monocots. We are currently looking at ways to optimize this system to facilitate rapid high-throughput screening of gene function. A version of BSMV developed for *Agrobacterium*-mediated delivery via passage through *Nicotiana benthamiana* was previously developed by Yuan et al. (2011, PLoS One, 6, e26468). We found that passage through *N. benthamiana* led to a variable silencing phenotype in wheat. The variation was reduced with co-infiltration of the viral RNA silencing suppressor protein p19. The optimal insert size in this BSMV vector was validated using qPCR. A range of insert sizes was tested with the wheat phytoene desaturase gene with optimal insert size assessed with transcript abundance using qPCR and chlorophyll abundance using UV absorption at 645 and 665 nm. An insert size of 200 to 400 bp gave strong and consistent silencing, whereas larger inserts led to decreased silencing, which may be due to the removal of these inserts from the viral genome. Direct infection of wheat without passage through *N. benthamiana* was also investigated. Infiltration of the virus into wheat leaves proved to be inconsistent and ineffective; however, vacuum infiltration of wheat seedlings is quite promising. Sonication of seedlings followed by incubation with *Agrobacterium* carrying the virus under vacuum pressure led to a 15-20% infection rate in young plants. The streamlining of BSMV VIGS will provide a low cost rapid system for determining gene function and for investigating plant pathogen interactions in wheat.