

Summary of Genotyping Data from the 2007 Regional Performance Nurseries

Hard winter wheat breeding lines from the 2007 Northern and Southern Regional Performance Nurseries were analyzed for 22 traits using 41 markers. The complete data set is included in the attached spreadsheet. The expected size (in base pairs) of each target band is included in the data set. Sizes preceded with the letter "T" are based on tailed primers and should be 18 base pairs longer than published reports. In the spreadsheet, a "+" indicates that the target band was positively identified, a "-" indicates that the target band was not present, and a "?" indicates that it was not possible to clearly determine the presence or absence of the band. The "NR" indicates that the assay was not run and is only used for excess control lines.

Except where noted, protocols used for all assays are listed on the MASWheat website (<http://maswheat.ucdavis.edu/protocols/index.htm>).

Fungal Resistance Traits

1. Wheat Scab (3BS QTL)

Three SSR markers (GWM493 and GWM533, TAG 2003 107:503-508; STS-3B-256, M. Pumphrey and Jim Anderson, personal communication) were used to detect the presence of a QTL on chromosome 3BS that confers resistance to wheat scab. No line contained the expected banding patterns for all three marker found in the controls Sumai 3. No line contained any two of the three markers. Two lines, 98x0435-15 and NW03681, did have the GWM493 band (211 bp) and GWM533 band (159 bp) respectively. The data suggests that none of the lines have the 3BS QTL.

2. Lr21

Newly designed primers were used for detecting the Lr21 resistance gene. This set of primers was based on the gene sequence provided by Li Huang. Known positive (WGRC07, WGRC27) and negative (WGRC02, Wichita) control lines were tested and found to be genotyped as expected using the new primers. Three entries, NE04490, 98x0338-13, and HV9W02-271W, had the band (214 bp) found in the positive controls and may have the Lr21 resistance gene. Other entries had the bands seen in the susceptible check line and may not have the Lr21 resistance gene.

3. Lr24/Sr24

Two STS markers were used to screen for Lr24/Sr24. Sr24#12 and Sr24#50 (Theor Appl Genet (2005) 111: 496–504) are both STS markers closely linked to Sr24 and typically amplify only one band. Resistant germplasm LcSr24Ag was positive for

both markers. Twenty-four lines were positive for marker Sr24#50. Sixteen lines were positive for marker Sr24#12 (512 bp). The following 16 lines were positive for both markers: CO03W054, CO03443, Jerry, NE03458, NE04537, NH03614, Nuplains, NW03681, OK03522, SD96240-3-1, SD98W175-1, TAM-107, TX02A0252, TX03A0563, TX99A0153-1, and Wesley. These 16 lines more likely have Lr24/Sr24 resistance gene.

4. Lr34/Yr18

The slow leaf rusting gene Lr34 and yellow rust resistance gene Yr18 are flanked by two markers (SSR marker SWM10, TAG 2006 113:1049–1062; STS marker csLV34-LR34, TAG 2006 114:21–30). Chinese Spring and Thatcher-Lr34 have the Lr34 resistance gene. CS7DS-4 (a deletion line of Chinese Spring) and Thatcher are both susceptible to Lr34. Chinese Spring and 20 lines have the “resistant” SWM10 206 bp band. Chinese Spring and 17 lines have the “resistant” csLV34-LR34 171 bp band. Fifteen lines were positive for both markers: 98x0338-13, CO03443, HV9W02-846R, KS980512-11-22, KS990498-3-&~2, NE04490, NWX03Y2459, NX03Y2489, OK05737W, OKBullet06ERU, TX01A7340, TX02A0252, TX03A0148, TX03A0563, and TX03M1096. These 15 entries more likely have Lr34/Yr18 resistance gene. However, Jagger also has the two resistance marker alleles and most likely does not carry the Lr34, therefore, caution needs to be taken for these materials have Jagger in their pedigrees.

5. Lr37/SR38/Yr17

These three rust resistance genes are on a chromosome segment that does not appear to recombine with bread wheat chromosomes. The STS marker (VENTRIUP-LN2) is therefore completely linked with the resistance genes. The following 62 lines were positive for the marker and likely have the alien segment: 98x0338-13, 98x0435-15, 99x0212-2, BC98331-03\$-2W, BC98334-04\$-02\$, BC98334-10W-8W, BZ9W02-2051, CO01385-A1, CO03443, CO03W239, CO03W269, HV9W02-112W, HV9W02-271W, HV9W98A-1002R, Jerry, KS04HW47-3-4, KS970093-8-9-#1, KS980512-11-22, KS980512-2-2, KS990498-3-&~2, MT0419, MT0495, N98L20040-44, NE03458, NE04490, NE04537, NH03614, NI04420, NI05711, NI05714, NI05720W, Nuplains, NW03681, NWX03Y2459, OK02125, OK02522W, OK03305, OK05737W, OKBullet06ERU, SD02804-1, SD05004, SD05118, SD05179, SD05210, SD05W018, SD05W030, SD05W138, SD05W140, SD96240-3-1, SD98W175-1, T153, T159, TAM-107, Trego, TX01A7340, TX01V5136RC, TX02A0252, TX03A0148, TX03A0563, TX03M1096, TX04M410068, and Wesley.

6. Lr39/Lr41

These two resistance genes appear to be the same gene and are linked with SSR marker GDM35. No line had the expected 183 bp band clearly found in the positive controls WGRC02 and WGRC10. The data suggests that none of the lines

have Lr39/Lr41. The marker was run twice with DNA isolated from different plants.

7. Lr50

Lr50 is flanked by microsatellite markers GWM382 (6.7 cM) and GDM87 (9.4 cM) on wheat chromosome arm 2BL. In the resistant line WGRC36, marker GDM87 produces one distinct band of 124 bp. The following 19 lines have the GDM87 124 bp band as found in the positive control WGRC36: BZ9W02-2051, CO03W054, CO03W239, CO02W280, MT0419, MT0495, MTCL0477, NE03458, NI05711, NI05714, Nuplains, NX03Y2489, OK05737W, OKBullet06ERU, SD05118, SD05W018, SD96240-3-1, SD98W175-1, and Trego. None of the lines have the 156 bp band found in WGRC36 for marker GWM382. It is likely that none of the tested lines have the Lr50 gene. The 19 lines with the GDM87 124 bp band may have the Lr50 gene if they are from a pedigree with Lr50 gene.

8. Sr2

The Sr2 resistance gene has been effective worldwide for more than 50 years. It has recessive inheritance and is expressed primarily during the adult-plant stage. It is located on 3BS in the same region as the FHB QTL. The SSR marker, GWM533, produces a 133 bp band in resistant lines (Spielmeyer, 2003. *Crop Sci.* 43:333–336) and is only 1 to 2 cM away from the gene. The 133 bp band was present in all 15 Sr2 resistant lines tested from the US, Mexico, Canada, Kenya, and India and was present in all 12 Sr2 resistant lines from Australia; but was also present in 4 susceptible Australian lines (Spielmeyer, 2003. *Crop Sci.* 43:333–336). The 133 bp band was present in our positive controls (Eagle(USA), Sonalika) and was in the following 49 lines: 99x0212-2, BC98334-04\$-02\$, BC98334-10W-8W, BZ9W02-2051, CO02W280, CO03443, CO03W054, CO03W239, CO03W269, Harding, HV9W02-112W, HV9W02-267W, HV9W02-271W, HV9W96-1271R-1, HV9W98A-1002R, Jerry, KS980512-11-22, KS990498-3-&~2, MTCL0477, NE04424, NI04420, NI04428, NI05711, NI05714, Nuplains, NW03681, NWX03Y2459, OKBullet06ERU, Scout66, SD02804-1, SD05004, SD05210, SD05W012, SD05W018, SD05W030, SD05W140, SD96240-3-1, SD98W175-1, T153, T154, T158, T159, Trego, TX01A7340, TX01V5136RC, TX02A0252, TX03A0148, TX03A0563, and TX03M1096.

9. Sr26

One STS marker (Sr26#43) was used to screen for Sr26 (Theor Appl Genet (2005) 111: 496–504). Three Sr26 resistant lines were positive for marker Sr26#43 (6AL-Ag-TA3933, Argus-Isoline-TA4025, Eagle-Aus). None of the tested entries appears to have the Sr26 gene.

Insect Resistance Traits

10. Hessian Fly (H9)

One STS marker was used to test lines for the presence of gene H9 which confers resistance to Hessian fly biotype L. The following 10 lines had the expected 909 bp band found in the positive control 'Iris' and likely have H9 gene: BC98331-03\$-2W, BC98334-04\$-02\$, BC98334-10W-8W, Kharkof, Millennium-27(ALS-1), MT0419, NH03614, NW03681, NWX03Y2459, and Trego.

11. Hessian Fly (H13)

Two SSR markers (GDM36 and CFD132) were used to test lines for the presence of gene H13 which also confers resistance to Hessian fly biotype L. No line contained both of the expected bands found in the positive control Molly. Only one line, HV9W96-1271R-1, has the CFD132 166 bp band. It is likely that none of the tested lines has H13 resistance gene.

12. Russian Wheat Aphid (Dn1, Dn2, Dn5, Dn6, Dnx)

Dn1, Dn2, Dn5, Dn6, Dnx, RWA genes....

We have data on GWM44 and GWM111, but since the controls for all of the RWA genes showed complex band patterns, we can not determine which band is corresponding to the resistance gene based on available information from the publication.

Viral Resistance Traits

13. Barley Yellow Dwarf Virus (Bydv2)

One SCAR marker (BYAgi) was used to detect the presence of the Bydv2 gene. No line had the expected 567 bp band clearly found in the positive control P961341. The data suggests that none of the lines have Bydv2.

14. Wheat Streak Mosaic Virus (Wsm1)

One STS marker (J15) was used to detect the chromosome segment containing the Wsm1 gene translocated from *Agropyron intermedium*. No line had the expected 431 bp band clearly found in the positive control KS93WGRC27. The data suggests that none of the lines may have Wsm1.

Quality Traits

15. 1RS Translocation

One rye SSR marker (SCM9, Euphytica 2003 132: 243–250, <http://maswheat.ucdavis.edu/protocols/drought/index.htm>) was used to detect the presence of the 1RS rye translocation. SCM9 amplified a 225 bp band

for the 1B/1R in check cultivar 'Aurora' and in 14 lines. SCM9 amplified a 242 bp band for the 1A/1R in check cultivar TAM107 and in 12 lines. The following 14 lines were positive for the SCM9 225bp band from rye only and likely have the 1B/1R translocation: HV9W02-846R, NW03681 SD05W012, SD05W018, SD05W030, SD05W138, SD05W140, SD05004, SD05118, OK02125, 98x0338-13, NI04420, NI04421, and NI04428. The following 12 lines were positive for the SCM9 242bp band and likely have the 1A/1R translocation: SD02804-1, SD05179, SD05210, T151, T153, T154, T158, BC98331-03S-2W, HV9W96-1271R-1, CO02W280, TX99A0153-1, and TX01V5136RC.

(Note: Five lines showed different results compared to the results from the USDA-ARS Grain, Forages and Bioenergy group at Lincoln, NE. One line, NI05714 showed 1R translocation in the Lincoln lab, but not in our report. Four lines showed 1RS translocation in our report, but not in the Lincoln lab, namely: HV9W02-846R, NWX03Y2459, BC98331-03S-2W and CO02W280. The marker was originally run in ABI3730. Because ABI sequencer is very sensitive and many samples showed false positive, the marker was rerun twice in an agarose gel using DNA isolated from different plants.)

16. High Grain Protein Content, HGPC

One STS marker (UCW89) very closely (0.1 cM) linked with the Gpc-B1 gene was used to identify the gene for HGPC. The positive control 'Glupro' produces a band of 138 bp. No line had the distinct 138 bp band. It appears that no entry has the Gpc-B1 gene.

17. High Molecular Weight Glutenins

Three STS markers (Euphytica 2003 134:51-60) were used to determine some of the alleles at the 3 loci controlling high molecular weight glutenins. Marker HMWx2* produces one band of 1319 bp for Ax2* genotype, or no band for Ax1 genotype. HMWBx produces one band of 669 bp for Bx17 genotype, or 2 bands (630 and 766 bp) for all others (non-Bx17 genotypes). HMWDx5 will produce one 478 bp band for Dx5 genotype, or no band for all others (non-Dx5 genotypes). These three markers appear to be extremely sensitive to small changes in PCR conditions. Reproducibility of the data using these markers is low to moderate.

Twenty four lines without the HMWx2* band likely carry Ax1 allele (98x0435-15, 99x0212-2, BC98331-03S-2W, BC98334-04S-02S, BC98334-10W-8W, CO01385-A1, CO03W239, HV9W02-846R, Jerry, KS970093-8-9-#1, KS980512-2-2, MT0495, N98L20040-44, OK02125, OK02522W, OK03522, OK05737W, OKBullet06ERU, SD05118, SD05W012, SD96240-3-1, TX01A7340, TX03A0148, TX04M410068) and the remaining entries likely carry the Ax2* allele. Fifteen lines with the HMWBx 669 bp band likely carry Bx17 allele (CO03443, CO03W054, HV9W02-112W, HV9W02-267W, KS980512-11-22, KS980512-2-2, NE04490, NWX03Y2459, OK02522W, OK05737W, OKBullet06ERU, SD05210, T154, T158, T159) and the remaining entries likely carry non-Bx17 allele. Most of the lines tested with HMWDx5 produced a band of 478 bp and carry, therefore, Dx5 allele and only 7 lines (BC98334-10W-8W, Scout66, T151, T153, T154, T158, TAM-107) amplified no band and likely carry non- Dx5 allele.

18. Grain Texture (Pina-D1, Pinb-D1)

One dominant STS marker (Pina-D1) was used to screen for the presence of wild-type (Pina-D1a), soft alleles. The positive control, 'Newana' yielded the expected band size of 348 bp which is associated with soft texture. The following five lines were missing the 348 bp band and likely have the null allele (Pina-D1b) associated with hard texture: NE04490, NI05714, SD05118, SD05W018, and TX03M1096. The rest of entries have the 348 bp band, indicating the presence of the Pina-D1a (soft) allele.

A codominant PCR-CAPs marker (Pinb-D1) was used to screen for Pinb-D1 alleles. After PCR amplification and restriction using Bsr BI, a 320 bp band indicates the soft, wild allele (Pinb-D1a). A band of 200 bp indicates the hard, mutant allele (Pinb-D1b). Eighteen lines had the 320 bp band and therefore have the soft allele Pinb-D1a (Kharkof, NE04490, NI05714, SD98W175-1, SD05118, SD05210, NX03Y2489, KS04HW47-3-4, OK02125, 99x0212-2, CO01385-A1, CO02W280, CO03W054, TX01A7340, TX01V5136RC, TX03M1096, T159, TX04M410068). The remaining lines had the 200 bp band from the hard allele Pinb-D1b.

In summary, 4 lines have soft alleles of both markers Pina-D1a and Pinb-D1a: NE04490, NI05714, SD05118, and TX03M1096. Those lines are likely soft wheat.

19. Waxy Mutants

One STS marker (Waxy4) was used to detect null mutants at all three loci controlling granule-bound starch synthase (GBSS) or waxy protein. Almost all entries had all three bands and are non-mutants or non-waxy lines. No lines were missing more than one band. No line was missing either the Wx-D1 locus (314 bp) on 7DS or the Wx-A1 locus (273 bp) on 7AS. Three lines (HV9W02-112W, Trego, TX01A7340) were missing only the 243 bp band and are partially waxy null-mutants for the Wx-B1 locus on 4AL.

Abiotic Stress and Agronomic Traits

20. Aluminum Tolerance

Two SSR markers WMC331 and ALMT1-SSR3A (Mol Breeding 2006 18:171–183) were used for screening of 4DL Al-resistance QTL. Twelve entries (98x0338-13, 99x0212-2, KS970093-8-9-#1, NE04490, NH03614, NI05720W, OK02125, SD05118, SD96240-3-1, TX01A7340, TX03M1096, and Wesley) were positive for both SSR markers are most likely have the Al tolerance QTL on chromosome 4D.

Additional eight lines that were positive for only the ALMT1-SSR3A SSR marker

(98x0435-15, KS04HW47-3-4, KS990498-3-&~2, NI05714, Nuplains, NW03681, SD05W018, and T159) may also carry the Al tolerance QTL on chromosome 4D.

21. Plant Height Genes (Rht1, Rht2, Rht8)

Two gene-specific STS markers were used to detect the Rht1 and Rht2 genes (TAG 2002 105:1038-1042). One linked SSR marker (GWM261, TAG 1998 96:1104-1109) was used to detect Rht8. All but seventeen lines (BC98331-03\$-2W, BZ9W02-2051, CO03W239, Harding, Jerry, Kharkof, KS970093-8-9-#1, NI05720W, Scout66, SD02804-1, SD05004, SD05118, SD05179, SD05W138, SD05W140, T159, and TX04M410068) had the 255 bp band indicating the presence of the Rht1 gene. Only three lines (BZ9W02-2051, KS970093-8-9-#1, and SD02804-1) had the 270 bp band diagnostic for the Rht2 gene. Four lines (CO01385-A1, OK03305, TX03A0148, Wesley) had the 212 bp band linked with Rht8 and may carry the Rht8 gene.

22. Vernalization (VRN-1)

Three STS primer sets (MGG 2005 273:54-65) were used to determine if deletions were present in the first intron of the VRN-1 gene in the A (Intr1/C/F & Intr1/AB/R), B (Intr1/B/F & Intr1/B/R4), and D (Intr1/D/F & Intr1/D/R4) genomes. One STS primer set (VRNA1F-VRNA1R, TAG 2004 109:1677-1686) was used to determine the presence of insertions or deletions (in/dels) in the VRN-A1 promoter. Winter genotypes have no intron deletions in the VRN-A1, VRN-B1, or VRN-D1 genes and no VRN-A1 promoter in/dels. Either an in/del in the VRN-A1 promoter or a deletion in the VRN-A1 gene itself is associated with a strong spring growth habit. A deletion in the intron of VRN-B1 or VRN-D1 indicates the dominant Vrn-B1 and Vrn-D1 alleles associated with spring growth habit. The deletions in Vrn-B1 and Vrn-D1 do not have as great an effect as the dominant Vrn-A1 alleles, and usually flower later than the Vrn-A1 spring types, but much earlier than winter types. There are other alleles associated with spring growth that are not detected by the primer sets used here, so it is possible to have no promoter mutations and no deletions in any of the VRN-1 genes yet still have a spring type.

One entry (BC98334-10W-8W) appears to have an indel in the VRN-A1 promoter and is likely strong spring types. Four entries (Harding, HV9W98A-1002R, SD05004, and Wesley) have deletions in the VRN-A1 gene contributing to spring growth habit. Seven entries (KS980512-2-2, NH03614, NI05720W, NW03681, SD05179, SD05W012, and SD05W138) appear to have indels in the VRN-B1 gene contributing to spring growth habit. Two entries, MTCL0477 and N98L20040-44, appear to have deletions in the VRN-B1 gene contributing to spring growth habit. One entry, OK02125, appear to have a deletion in the VRN-D1 gene contributing to spring growth habit.