

Wheat Research Progress Report

Project #: 3019-3450 AND 3019-5449

Title: Transferring 'CLEARFIELD' Herbicide Resistance and Stripe Rust Resistance into Washington Wheat Cultivars

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Progress Report Year: 2008

Accomplishments:

The objective of this project is to transfer resistance to imidazolinone herbicides such as 'Beyond'TM and stripe rust resistance to Washington winter, club and spring wheat to improve cultivars using marker-assisted background selection.

- We have developed an efficient, economical and reliable method of selecting plants carrying two-gene resistance against imidazolinone herbicides. The procedure involves first spraying plants with 20 oz/A or 5X rate of herbicide 'Beyond'TM to eliminate plants that carry wild type AHASL gene at all the three homeoloci (one each at chromosomes 6A, 6B and 6D) or carrying a single mutant gene at one of three homeoloci. The selected plants are then subjected to marker-assisted foreground selection with allele-specific DNA markers specifically designed for two homeoloci (chromosomes 6B and 6D) of the AHASL genes. Using this approach, we were able to select plants carrying the two mutant genes by screening only ~25% of the plants with markers. This is significant as the marker analysis is the most expensive part of the selection procedure. This procedure reliably differentiates plants carrying different allelic combinations of the AHASL genes, including differentiating plants carrying heterozygous alleles (Aa or Bb) for one and two genes (AaBb). To further reduce the expense associated with marker analysis for the AHASL gene, a new approach for marker analysis was optimized where the selection was performed for the flanking markers instead of gene specific markers. This simple approach should save us significant amount of money thereby allowing us to accommodate additional targets such as Xerpha without increasing the cost of the project.
- All of our field evaluations are performed using 5X rate of imidazolinone herbicides. At that high rate, our two gene lines perform really well compared to the single gene cultivars that show severe stunting and in general poor performance. This test not only helps us identify two gene lines without extensive marker analysis, but also shows the damage that excess herbicide can cause to the single gene varieties. Excess herbicide is a common occurrence in wheat fields at the field turns and as a residual herbicide from the previous crop rotation.

- Two-gene 'Clearfield' resistant lines (KCF08001 and KCF08002) are currently being tested in WSU variety testing program. Initial testing suggests quality of these lines to be marginal so we don't anticipate any of these lines to be released as a variety but we expect these to be very high yielding thus should serve as germplasm for our future introgressions. These lines were produced by an ultra-fast approach taking only three years from a cross to variety testing.
- For transferring two-gene Clearfield into Eltan and Madsen cultivars, by screening ~3000 BC₂F₃ plants for each cross, we have identified 153 BC₂F₃ plants carrying the two gene mutations in the Eltan background and 135 in the Madsen background. In close collaboration with Dr. Craig Morris, Solvent Retention Capacity (SRC) test was applied to evaluate quality at a single plant basis. This test is slightly modified version of Solvent Retention Capacity (SRC) test of AACCC (American Association of Cereal Chemist) Approved Method (56-11) for evaluating soft wheat flour quality. The test measures the ability of flour to retain a set of two solvents (50% sucrose and 5% sodium carbonate) after centrifugation. From each cross, ~50 BC₂F₃ plants from Madsen and Eltan with acceptable quality were selected and were used for marker assisted background selection using whole genome SSR markers. These plants were also evaluated for single plant yield and 1000-kernal weight. A line carrying maximum recurrent parent genome will be selected for each cross. Furthermore, a plant with the best yield, 1000-kernal weight, and with acceptable quality will also be selected for each cross. Therefore, two plants each from the Eltan and the Madsen crosses will be selected, and amplified to place in WSU variety testing program during the 2009-2010 growing season.
- With the objective to combine stripe rust resistance with the Clearfield resistance, backcross progeny of the Madsen, Eltan and Rod crosses were field tested at the Spillman farm. After screening for Clearfield two genes by spraying 5X rate of the herbicide, the selected plants were evaluated for stripe rust resistance, plant type, single plant yield and 1000-kernal weight. The best 20 plants were selected and analyzed for quality characteristics. Six plants with the best quality numbers were selected and were used as donor parents for transferring Clearfield resistance to cultivars Xerpha, Chukar and Louise.
- In response to the popular demand, two-gene Clearfield resistance is being transferred to Xerpha on priority. Overcoming the difficulties of obtaining Xerpha seed, now we have made crosses between two-gene Clearfield Madsen with Xerpha. The F1 plants will be used for making BC1 with Xerpha as well as will be used to produce 100-500 doubled haploid progeny. This progeny will be field tested in head rows possibly in the growing year of 2009-2010. This cross is also being used for the forward breeding approach (see progress report for project 3019-7450).
- Second year variety testing results for the wheat lines (WA8058 and WA8059) developed by transferring *Yr15* gene into cultivar Zak by marker assisted background selection, showed again that in the medium to high rainfall areas that are suited for growing Zak, the derived lines were either equal or better than cultivar Zak. We will propose to release one of these lines as a variety as there are very few if any, varieties carrying seedling resistance against stripe rust. Along with many other popular spring varieties, Louise is susceptible to stripe rust at the seedling stage. Therefore, we feel that it would be

valuable to have a variety carrying seedling resistance to stripe rust in case of a major stripe rust epidemic.

Results

Two-gene Clearfield Madsen, Eltan and Rod

With the objective to transfer two-gene Clearfield technology into winter wheat cultivars Eltan, Madsen and Rod ~1700 BC₁F₁ plants for the Eltan cross and ~1600 BC₁F₁ plants from the Madsen cross, along with their donor (CL-0618) and the recipient parents were grown in cell trays in the Green House. After 3-4 weeks (at 4-6 leaf stage), plants were sprayed using 'Beyond'TM in Dr. J. Yenish's spraying chamber facility. Herbicide was applied at the rates of 10, 15 and 20 oz/A along with 0.25% non-ionic surfactant and 2.5% liquid Nitrogen fertilizer. Symptoms were observed 2-3 weeks after spraying. On the basis of phenotypic selection (see progress report 2007), 436 resistant plants from the Madsen cross and 384 resistant plants from the Eltan cross were selected, that were then subjected to marker-assisted selection.

The above 436 BC₁F₁ plants from Madsen/CL618/Madsen and 384 BC₁F₁ plants from Eltan/CL0618/Eltan selected after spraying (previous section) were screened with DNA markers in order to identify plants carrying two genes for herbicide resistance, but carrying least amount of the donor parent background (DPB). The marker analysis for the gene of interest allowed selection of 86 plants from Madsen cross and 72 plants from Eltan cross to carry the mutant alleles for two AHASL genes. These selected plants were further analyzed using SSR markers for chromosome 6B and 6D that carry the two AHASL genes. Finally, the above analysis identified 6 plants from the Eltan cross and 8 from the Madsen cross that carry the least amount of the carrier chromosome from the donor line. These selected plants were again backcrossed to their respective recurrent parent to generate ~1200 BC₂F₁ progeny for each of the two crosses. These plants were again selected first by spraying and then using markers, as described above. This analysis identified 17 BC₂F₂ plants from the Madsen cross and 15 BC₂F₂ plants from the Eltan cross that not only carried the mutant alleles for both of the AHASL genes but also had the least amount of the donor type carrier chromosome.

About 1700 BC₂F₃ (100 seeds each from 17 selected BC₂F₂s) plants from the Madsen cross and 1500 (100 seeds each from 15 selected BC₂F₂s) plants from the Eltan cross were planted in cell trays in the green house. These plants were sprayed with the herbicide 'Beyond' (20 oz/A). Approximately 531 resistant BC₂F₃ plants from Madsen/CL-0618/Madsen and 417 BC₂F₃ plants from Eltan/CL-0618/Eltan were selected, and subjected to marker analyses, first using gene-specific markers followed in order by the SSR markers for the carrier chromosome (6B and 6D; see Figure 1). Available seed from the selected plants (out of 135 plants, 109 from Madsen and out 153 plants 142 plants from Eltan) with their control were used for the single plant quality analysis using SRC (Solvent Retention Capacity, as mentioned earlier). Results from single plant quality analysis using SRC are given in Table 1 and 2. On the basis of quality results, we made binary plots using % carbonate with break flour yield, % carbonate with predicted flour yield, % sucrose with break flour yield and % sucrose with predicted flour yield, estimated on the samples derived from each crosses (Figure 2). About 96 selected plants from each cross are currently being used for the whole genome background selection using about 200 SSR markers. From each cross two lines one carrying highest proportion of the recurrent parent genome and the other with the best phenotypic and quality values (total 4 lines) will be selected, grown in the greenhouse

for seed increase and will be placed in the WSU variety testing program during the growing season of 2009-2010.

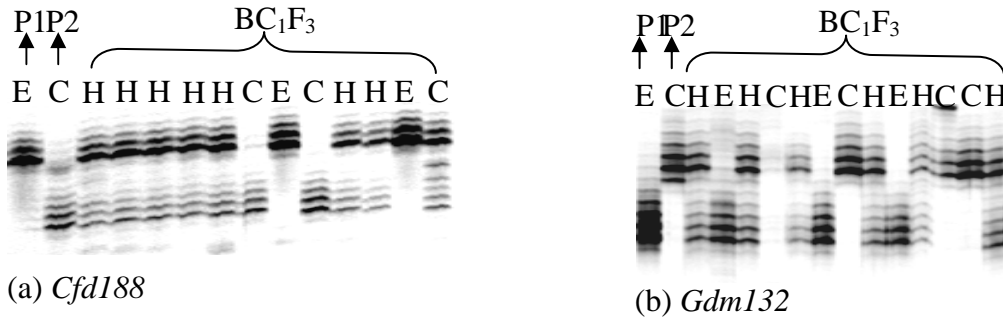


Figure 1: Amplification pattern of two SSRs markers belonging to the chromosome carrying AHASL gene. E = Eltan, C = CL618 (Clearfield donor parent), H = Heterozygous.

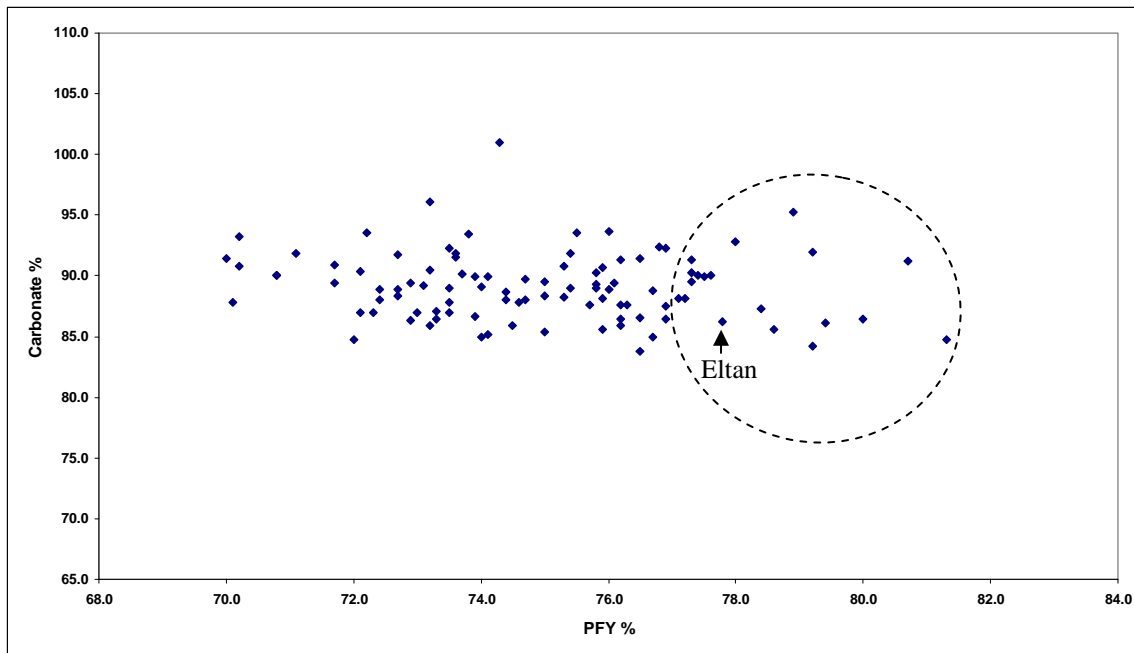


Figure 2: Binary plot representing correlation between % sodium carbonate and predicted flour yield (PFY) estimated from Clearfield Eltan BC2F3 progenies with Eltan without Clearfield. Selected plants for quality are in Circle.

Combining stripe rust resistance with Clearfield resistance and transferring these traits into Xerpha, Chukar, and Louise

About 6000 BC₁F₃ plant, 2000 from each of the Madsen, Eltan, and Rod crosses, were grown at the Spillman farm for phenotypic evaluation. These plants were sprayed with 20 oz/A rate of herbicide 'Beyond'. After the herbicide application, ~25% BC₁F₃ plants (including 565 for Eltan, 475 for Madsen and 420 for Rod) showed resistance that is typically seen for the two gene Clearfield resistance. These plants were analyzed using SSR markers specific for chromosome 6B and 6D that carry the two AHASL genes. Finally, the mutant alleles of the AHASL genes were successfully distinguished from the normal alleles and ~90 plants from Madsen, ~70 from Rod and ~60 plants from the Eltan cross were selected. These plants were used as a donor for Clearfield and stripe rust resistance to making crosses with Xerpha, Chukar and Louise (see Table 3).

Table 3: Crosses were made using Xerpha, Chukar and Louise as a recipient with three Clearfield donors Madsen, Eltan and Rod (Madsen, Eltan and Rod lines are the different selections from BC₁F₃ progenies carrying two genes for Clearfield with stripe rust resistance).

S. No.	Xerpha x Eltan	S. No.	Xerpha x Madsen
1	Xerpha x ECFBC1F3 1-12	22	Xerpha X MCFBC1F3 13-74
2	Xerpha x ECFBC1F3 1-17	23	Xerpha X MCFBC1F3 2-36
3	Xerpha x ECFBC1F3 1-29	24	Xerpha X MCFBC1F3 12-35
4	Xerpha x ECFBC1F3 1-40	25	Xerpha X MCFBC1F3 12-53
5	Xerpha x ECFBC1F3 1-58	26	Xerpha X MCFBC1F3 12-30
6	Xerpha x ECFBC1F3 1-61	27	Xerpha X MCFBC1F3 13-54
7	Xerpha x ECFBC1F3 1-62	28	Xerpha X MCFBC1F3 22-1
S. No.	Xerpha x Rod	29	Xerpha X MCFBC1F3 12-94
1	Xerpha x RCFBC1F3 1-4	30	Xerpha X MCFBC1F3 2-1
2	Xerpha x RCFBC1F3 2-79	31	Xerpha X MCFBC1F3 2-2
3	Xerpha x RCFBC1F3 1-12	32	Xerpha X MCFBC1F3 2-2
4	Xerpha x RCFBC1F3 1-20-1	S. No.	Chukar x Madsen
5	Xerpha x RCFBC1F3 1-20-2	1	Chukar X MCFBC1F3 2-1
6	Xerpha x RCFBC1F3 1-21	1	Chukar X MCFBC1F3 12-30
7	Xerpha x RCFBC1F3 1-28	2	Chukar X MCFBC1F3 12-44
8	Xerpha x RCFBC1F3 1-32	3	Chukar X MCFBC1F3 13-13
9	Xerpha x RCFBC1F3 1-36	4	Chukar X MCFBC1F3 13-67
10	Xerpha x RCFBC1F3 1-50	5	Chukar X MCFBC1F3 13-92
11	Xerpha x RCFBC1F3 1-83	6	Chukar X MCFBC1F3 22-65
12	Xerpha x RCFBC1F3 1-86	S. No.	Louise x Eltan or Madsen
S. No.	Xerpha x Madsen	1	Louise x ECFBC2F3 10-2
1	Xerpha X MCFBC1F3 13-12	2	Louise x ECFBC2F3 6-1
2	Xerpha X MCFBC1F3 12-58	3	Louise x ECFBC2F3 10-1
3	Xerpha X MCFBC1F3 13-45	4	Louise x ECFBC2F3 7-1
4	Xerpha X MCFBC1F3 13-48	5	Louise x MCFBC1F3 2 (3)
5	Xerpha X MCFBC1F3 2-38	5	Louise x MCFBC1F3 2 (2)
6	Xerpha X MCFBC1F3 13-67	6	Louise x MCFBC2F3 17-3
8	Xerpha X MCFBC1F3 13-42	7	Louise x MCFBC2F3 17-4

9	Xerpha X MCFBC1F3 12-82		8	Louise x MCFBC1F3 2 (1)
11	Xerpha X MCFBC1F3 2-44	S. No.		Chukar x Rod
12	Xerpha X MCFBC1F3 12-4		1	Chukar X RCFBC1F3 1-21
13	Xerpha X MCFBC1F3 13-15		2	Chukar X RCFBC1F3 1-36
14	Xerpha X MCFBC1F3 1-53		3	Chukar X RCFBC1F3 1-52
15	Xerpha X MCFBC1F3 22-65		4	Chukar X RCFBC1F3 2-25
16	Xerpha X MCFBC1F3 12-96		5	Chukar X RCFBC1F3 2-79
17	Xerpha X MCFBC1F3 12-10	S. No.		Chukar x Eltan
18	Xerpha X MCFBC1F3 2-66		1	Chukar X ECFBC1F3 1-55
19	Xerpha X MCFBC1F3 13-13		2	Chukar X ECFBC1F3 1-58
20	Xerpha X MCFBC1F3 2-25		3	Chukar X ECFBC1F3 1-62
21	Xerpha X MCFBC1F3 13-92			

Table 1: Results are micro SRC (Solvent Retention Capacity) test conducted for quality analysis for Clearfield resistant Madsen BC2F3 plants with their recipient parent as a control.

Serial No.	VARIETY Name	% Break Flour yield	% Predicted Four yield	SRC (micro) % Sucrose	SRC (micro) % Sodium Carbonate
1	Madsen	19.8	78.0	99.0	92.8
2	MCFBC2F3-1-1	19.4	77.4	108.2	90.0
3	MCFBC2F3-1-15	16.7	72.7	103.0	88.9
4	MCFBC2F3-1-19	16.3	73.5	97.0	89.0
5	MCFBC2F3-1-23	14.6	75.0	102.0	89.5
6	MCFBC2F3-3-9	16.7	76.2	104.3	91.3
7	MCFBC2F3-3-10	18.6	74.4	98.1	88.7
8	MCFBC2F3-3-19	16.9	73.8	101.5	93.4
9	MCFBC2F3-3-21	–	–	98.1	91.4
10	MCFBC2F3-3-22	16.2	72.1	102.4	90.4
11	MCFBC2F3-3-28	16.7	72.9	95.6	86.3
12	MCFBC2F3-3-32	15.0	71.7	104.4	90.9
13	MCFBC2F3-4-13	14.6	73.2	101.5	96.1
14	MCFBC2F3-4-28	16.7	74.1	100.0	85.2
15	MCFBC2F3-5-2	16.1	72.3	101.5	87.0
16	MCFBC2F3-5-4	17.6	73.5	101.0	87.8
17	MCFBC2F3-5-5	14.7	76.5	98.6	91.4
18	MCFBC2F3-5-6	17.0	71.7	95.0	89.4
19	MCFBC2F3-5-7	17.9	74.6	101.0	87.8
20	MCFBC2F3-5-8	14.3	70.1	100.5	87.8
21	MCFBC2F3-5-9	13.3	73.3	99.0	87.1
22	MCFBC2F3-5-10	16.7	75.9	91.6	85.6
23	MCFBC2F3-5-14	16.9	70.8	98.5	90.1
24	MCFBC2F3-5-16	17.0	73.6	92.8	91.8
25	MCFBC2F3-5-17	13.8	72.4	96.1	88.9

26	MCFBC2F3-5-18	14.7	72.1	96.2	87.0
27	MCFBC2F3-5-22	17.2	74.7	101.9	89.7
28	MCFBC2F3-5-24	16.7	72.2	111.4	93.5
29	MCFBC2F3-5-26	14.3	76.8	106.5	92.4
30	MCFBC2F3-5-29	16.4	76.7	97.0	88.8
31	MCFBC2F3-5-30	17.1	77.1	104.8	88.1
32	MCFBC2F3-5-31	17.8	77.8	103.0	86.2
33	MCFBC2F3-5-32	16.7	75.0	103.0	88.3
34	MCFBC2F3-5-33	17.2	75.3	103.5	90.8
35	MCFBC2F3-5-34	16.2	73.5	103.0	92.3
36	MCFBC2F3-5-37	16.2	74.3	103.8	101.0
37	MCFBC2F3-5-38	14.3	76.2	106.4	87.6
38	MCFBC2F3-5-40	19.7	75.8	106.5	89.0
39	MCFBC2F3-5-42	18.1	74.7	113.2	88.0
40	MCFBC2F3-5-43	21.1	78.9	102.0	95.2
41	MCFBC2F3-5-44	14.5	75.4	100.0	91.9
42	MCFBC2F3-5-46	15.9	76.1	98.1	89.4
43	MCFBC2F3-6-4	18.5	74.1	103.5	89.9
44	MCFBC2F3-6-5	15.4	76.9	102.5	87.5
45	MCFBC2F3-6-8	12.0	76.0	100.5	88.9
46	MCFBC2F3-6-10	17.5	77.2	100.5	88.1
47	MCFBC2F3-6-11	16.9	74.0	103.4	84.9
48	MCFBC2F3-6-12	14.9	74.5	99.5	85.9
49	MCFBC2F3-6-14	13.6	72.7	98.5	88.4
50	MCFBC2F3-6-17	17.9	78.6	102.4	85.6
51	MCFBC2F3-6-18	15.3	70.8	102.4	90.0
52	MCFBC2F3-6-21	15.3	73.6	103.8	91.5
53	MCFBC2F3-6-22	14.7	76.0	100.5	93.7
54	MCFBC2F3-6-26	15.4	74.4	100.5	88.0
55	MCFBC2F3-6-27	15.4	73.1	100.0	89.2
56	MCFBC2F3-6-28	16.2	75.7	98.6	87.6
57	MCFBC2F3-6-29	18.2	81.8	100.5	–
58	MCFBC2F3-6-31	20.8	79.2	100.0	92.0
59	MCFBC2F3-6-32	16.3	76.3	94.3	87.6
60	MCFBC2F4-12-5	16.4	77.6	100.0	90.0
61	MCFBC2F4-12-6	14.0	80.0	96.6	86.4
62	MCFBC2F4-12-15	19.6	78.4	98.5	87.3
63	MCFBC2F4-12-16	15.8	78.9	109.8	–
64	MCFBC2F4-12-17	15.2	73.9	104.8	86.6
65	MCFBC2F4-12-19	14.7	76.5	98.1	86.5
66	MCFBC2F4-12-20	17.4	73.9	100.5	89.9
67	MCFBC2F4-12-22	17.2	75.9	98.5	88.1
68	MCFBC2F4-12-23	11.4	77.3	99.0	90.3
69	MCFBC2F4-12-25	14.7	79.4	96.7	86.1
70	MCFBC2F4-12-26	15.4	76.9	104.8	92.3
71	MCFBC2F4-12-29	17.7	75.8	98.6	89.3

72	MCFBC2F4-12-30	15.1	75.5	100.5	93.6
73	MCFBC2F4-12-32	16.7	76.2	99.5	86.4
74	MCFBC2F4-12-33	15.9	77.3	97.1	91.3
75	MCFBC2F4-12-34	16.0	74.0	96.8	89.1
76	MCFBC2F4-12-35	13.2	71.1	101.0	91.9
77	MCFBC2F4-12-36	16.7	75.9	96.2	90.7
78	MCFBC2F4-12-38	18.2	77.3	100.0	89.5
79	MCFBC2F4-12-40	12.2	73.2	99.0	90.5
80	MCFBC2F4-12-41	17.5	77.5	97.5	89.9
81	MCFBC2F4-12-45	15.2	75.8	99.0	90.3
82	MCFBC2F3-16-2	14.9	70.2	102.0	93.2
83	MCFBC2F3-16-3	20.0	76.0	97.6	–
84	MCFBC2F3-16-4	20.0	75.0	101.6	–
85	MCFBC2F3-16-5	16.4	72.7	100.5	91.7
86	MCFBC2F3-16-6	19.3	73.7	95.5	90.2
87	MCFBC2F3-16-7	16.9	75.3	98.5	88.2
88	MCFBC2F3-16-8	17.5	70.2	100.5	90.8
89	MCFBC2F3-16-9	17.4	75.4	97.0	89.0
90	MCFBC2F3-16-10	18.8	75.0	100.6	–
91	MCFBC2F3-16-11	18.6	72.9	97.0	89.4
92	MCFBC2F3-16-12	18.2	77.3	97.5	–
93	MCFBC2F3-16-14	20.0	72.0	98.9	84.7
94	MCFBC2F3-16-19	17.6	73.5	97.6	87.0
95	MCFBC2F3-16-20	19.7	73.2	97.9	85.9
96	MCFBC2F3-16-23	17.5	73.0	97.5	87.0
97	MCFBC2F3-17-1	20.0	75.0	100.5	–
98	MCFBC2F3-17-4	19.0	76.2	100.5	–
99	MCFBC2F3-17-6	20.8	79.2	101.0	84.2
100	MCFBC2F3-17-7	20.6	76.5	99.5	83.8
101	MCFBC2F3-17-8	21.2	76.9	103.0	86.4
102	MCFBC2F3-17-9	16.7	76.2	104.4	85.9
103	MCFBC2F3-17-10	15.0	73.3	98.4	86.4
104	MCFBC2F3-17-11	9.1	72.7	105.8	–
105	MCFBC2F3-17-12	13.3	80.0	105.8	–
106	MCFBC2F3-17-15	17.2	72.4	106.7	88.0
107	MCFBC2F3-17-19	21.4	71.4	110.4	–
108	MCFBC2F3-17-20	21.1	78.9	106.2	–
109	MCFBC2F3-17-21	15.0	76.7	101.5	85.0
110	MCFBC2F3-17-23	18.8	75.0	104.5	85.4

(–) indicated the missing quality data due to insufficient quantity of flour sample.

Table: Results are micro SRC (Solvent Retention Capacity) test conducted for quality analysis for Clearfield resistant BC2F3 plants with their recipient parent as a control.

Serial No.	VARIETY Name	% Break Flour yield	% Predicted Four yield	SRC (micro) % Sucrose	SRC (micro) % Sodium Carbonate
1	Eltan	18.0	81.3	96.4	84.7
2	ECFBC2F4-1-3	26.8	73.2	93.0	82.0
3	ECFBC2F4-1-13	25.6	74.4	93.2	81.2
4	ECFBC2F4-1-15	24.6	75.4	91.8	84.8
5	ECFBC2F4-1-16	23.7	71.1	96.0	86.3
6	ECFBC2F4-1-17	19.5	70.7	101.0	87.4
7	ECFBC2F4-1-23	20.0	73.3	96.5	90.2
8	ECFBC2F4-1-24	23.4	74.5	89.4	86.2
9	ECFBC2F4-2-5	20.0	68.0	101.5	89.2
10	ECFBC2F4-2-6	20.5	70.5	90.9	81.7
11	ECFBC2F4-2-13	20.9	72.1	94.4	84.3
12	ECFBC2F4-2-16	19.6	71.7	93.5	83.6
13	ECFBC2F4-2-29	24.4	73.3	92.1	84.9
14	ECFBC2F4-3-4	20.9	74.4	91.9	85.2
15	ECFBC2F4-3-5	20.0	75.0	91.0	85.4
16	ECFBC2F4-3-6	25.4	74.6	94.9	84.7
17	ECFBC2F4-3-7	21.9	71.9	91.1	82.8
18	ECFBC2F4-3-8	17.0	74.5	92.2	81.8
19	ECFBC2F4-3-10	19.2	69.2	91.3	83.8
20	ECFBC2F4-3-13	21.9	75.0	96.6	85.7
21	ECFBC2F4-3-16	22.6	71.0	95.8	88.0
22	ECFBC2F4-3-18	22.2	74.1	96.1	82.7
23	ECFBC2F4-4-2	23.3	76.7	94.7	83.5
24	ECFBC2F4-4-4	22.4	73.5	97.9	83.9
25	ECFBC2F4-4-7	23.5	73.5	92.5	84.0
26	ECFBC2F4-4-8	26.6	73.4	100.0	83.2
27	ECFBC2F4-4-10	25.5	74.5	95.2	83.1
28	ECFBC2F4-4-12	27.6	74.1	93.6	81.6
29	ECFBC2F4-4-13	22.0	70.7	95.2	84.7
30	ECFBC2F4-4-15	21.9	71.9	89.1	82.5
31	ECFBC2F4-4-16	20.9	74.4	90.6	84.7
32	ECFBC2F4-4-17	25.0	75.0	92.7	83.7
33	ECFBC2F4-4-18	20.0	73.3	100.0	88.1
34	ECFBC2F4-4-20	18.4	71.1	87.1	86.9
35	ECFBC2F4-4-21	23.7	73.7	91.2	82.0
36	ECFBC2F4-4-22	24.3	73.0	91.6	85.4
37	ECFBC2F4-4-23	18.9	70.3	90.6	83.1
38	ECFBC2F4-4-24	20.0	70.0	89.9	82.7

39	ECFBC2F4-4-25	16.7	70.0	91.8	85.5
40	ECFBC2F4-4-28	20.0	75.0	91.2	84.2
41	ECFBC2F4-4-29	24.4	73.3	87.6	84.3
42	ECFBC2F4-5-1	17.4	76.1	87.6	88.0
43	ECFBC2F4-5-2	19.0	76.2	94.8	90.0
44	ECFBC2F4-5-3	23.7	78.9	89.7	86.3
45	ECFBC2F4-5-4	13.2	76.3	94.3	91.2
46	ECFBC2F4-5-5	11.8	76.5	93.0	–
47	ECFBC2F4-5-6	14.7	70.6	91.0	91.3
48	ECFBC2F4-5-7	15.8	73.7	90.4	96.0
49	ECFBC2F4-5-9	12.5	80.0	93.2	94.3
50	ECFBC2F4-5-10	14.3	78.6	90.5	86.5
51	ECFBC2F4-6-5	21.1	77.2	91.9	87.1
52	ECFBC2F4-7-14	15.2	71.7	96.6	93.3
53	ECFBC2F4-7-21	19.6	71.7	93.8	85.4
54	ECFBC2F4-7-22	14.3	78.6	90.3	88.0
55	ECFBC2F4-7-23	17.9	79.8	89.4	89.4
56	ECFBC2F4-7-27	15.6	77.8	92.6	87.1
57	ECFBC2F4-7-28	16.1	77.4	93.7	88.5
58	ECFBC2F4-7-32	14.5	72.6	92.3	88.9
59	ECFBC2F4-7-33	17.6	71.6	87.0	82.2
60	ECFBC2F4-7-35	16.4	78.2	93.8	90.5
61	ECFBC2F4-8-1	22.6	74.2	94.8	87.9
62	ECFBC2F4-8-2	24.1	74.7	85.9	82.8
63	ECFBC2F4-8-5	20.6	73.5	98.0	84.3
64	ECFBC2F4-8-6	21.4	74.3	95.1	88.2
65	ECFBC2F4-8-12	23.9	73.9	93.2	84.9
66	ECFBC2F4-8-13	21.1	71.1	92.8	84.5
67	ECFBC2F4-8-14	22.1	73.7	93.4	86.5
68	ECFBC2F4-8-15	23.1	74.4	93.2	84.7
69	ECFBC2F4-8-16	18.8	68.8	91.5	87.6
70	ECFBC2F4-8-17	17.6	73.5	89.7	85.2
71	ECFBC2F4-8-19	16.0	72.0	90.0	82.8
72	ECFBC2F4-8-20	16.7	73.3	97.5	87.9
73	ECFBC2F4-8-21	22.1	73.5	90.8	81.2
74	ECFBC2F4-8-22	20.5	71.8	96.7	81.8
75	ECFBC2F4-8-24	21.5	73.4	87.1	82.4
76	ECFBC2F4-8-26	19.2	71.2	97.6	86.2
77	ECFBC2F4-8-27	18.6	72.1	93.7	82.0
78	ECFBC2F4-8-28	19.0	74.6	92.9	83.7
79	ECFBC2F4-8-29	18.9	75.5	96.2	84.8
80	ECFBC2F4-11-3	25.0	75.0	92.7	80.6
81	ECFBC2F4-11-4	20.9	74.4	91.4	82.7
82	ECFBC2F4-11-5	20.0	72.5	93.2	83.7
83	ECFBC2F4-11-6	20.0	72.9	91.6	78.9
84	ECFBC2F4-11-8	20.3	73.4	91.8	83.2

85	ECFBC2F4-11-9	34.5	67.2	90.5	80.3
86	ECFBC2F4-11-14	26.1	73.9	90.1	80.3
87	ECFBC2F4-11-15	23.3	76.7	85.9	78.9
88	ECFBC2F4-11-17	24.6	75.4	88.0	80.8
89	ECFBC2F4-11-18	22.6	73.6	89.4	81.6
90	ECFBC2F4-11-19	22.9	72.9	90.5	86.0
91	ECFBC2F4-11-20	23.1	74.4	89.2	82.4
92	ECFBC2F4-11-21	25.6	74.4	89.9	85.0
93	ECFBC2F4-11-22	21.3	73.3	92.2	83.4
94	ECFBC2F4-11-27	19.4	72.2	91.4	82.9
95	ECFBC2F4-11-28	19.0	74.1	87.1	82.5
96	ECFBC2F4-11-31	22.8	73.7	91.6	83.6
97	ECFBC2F4-11-32	22.2	74.6	95.7	84.7
98	ECFBC2F4-12-1	25.0	73.3	89.0	83.1
99	ECFBC2F4-12-3	21.3	74.5	88.6	82.8
100	ECFBC2F4-12-5	27.7	76.6	92.5	78.9
101	ECFBC2F4-12-6	23.5	72.5	83.8	78.3
102	ECFBC2F4-12-7	21.7	71.0	86.3	80.2
103	ECFBC2F4-12-9	23.4	74.5	93.8	78.8
104	ECFBC2F4-12-10	25.0	75.0	89.8	79.9
105	ECFBC2F4-13-4	19.1	66.0	89.5	83.2
106	ECFBC2F4-13-5	20.5	71.2	92.9	83.8
107	ECFBC2F4-13-6	18.4	73.5	87.8	80.5
108	ECFBC2F4-13-7	23.1	73.8	89.3	81.9
109	ECFBC2F4-13-18	20.3	71.9	87.8	82.1
110	ECFBC2F4-13-19	15.0	70.0	92.0	83.0
111	ECFBC2F4-13-20	17.5	72.5	85.5	80.7
112	ECFBC2F4-14-1	25.0	80.0	85.7	79.6
113	ECFBC2F4-14-2	21.7	78.3	84.9	81.6
114	ECFBC2F4-14-5	20.4	71.4	88.5	79.5
115	ECFBC2F4-14-6	18.2	77.3	89.7	85.3
116	ECFBC2F4-14-7	22.9	77.1	86.2	82.6
117	ECFBC2F4-14-8	22.8	73.7	88.2	81.8
118	ECFBC2F4-14-9	18.0	72.0	86.6	83.2
119	ECFBC2F4-14-10	18.2	76.4	90.4	85.2
120	ECFBC2F4-14-11	14.3	73.2	91.7	90.7
121	ECFBC2F4-14-12	18.8	75.0	87.9	87.6
122	ECFBC2F4-14-15	20.8	70.8	94.4	81.9
123	ECFBC2F4-14-16	20.5	74.4	92.2	88.0
124	ECFBC2F4-14-17	21.3	78.7	90.5	86.3
125	ECFBC2F4-14-18	20.0	75.6	90.0	84.1
126	ECFBC2F4-14-19	17.8	75.6	85.2	87.5
127	ECFBC2F4-14-20	13.6	77.3	89.4	77.3
128	ECFBC2F4-14-21	19.5	73.2	86.0	74.6
129	ECFBC2F4-14-22	19.6	74.5	91.2	74.2
130	ECFBC2F4-14-23	22.4	73.5	91.6	74.2

131	ECFBC2F4-14-24	20.7	75.9	90.6	75.5
132	ECFBC2F4-14-25	25.6	76.7	90.4	78.8
133	ECFBC2F4-14-26	22.0	73.2	93.3	71.9
134	ECFBC2F4-14-28	19.6	74.5	89.7	73.6
135	ECFBC2F4-15-3	15.0	70.0	90.6	74.8
136	ECFBC2F4-15-5	16.9	74.6	94.9	77.1
137	ECFBC2F4-15-7	17.5	71.9	95.1	75.6
138	ECFBC2F4-15-10	17.6	72.5	89.6	72.4
139	ECFBC2F4-15-16	17.2	71.9	88.9	76.3
140	ECFBC2F4-15-19	19.5	75.6	93.1	76.8
141	ECFBC2F4-15-20	20.4	72.2	91.0	83.5
142	ECFBC2F4-15-22	18.0	72.1	93.2	66.0
143	ECFBC2F4-15-35	17.5	71.9	96.5	81.8

(-) indicated the missing quality data due to insufficient quantity of flour sample.

Field evaluation for Clearfield

Last year, the remnant seeds (~4000 BC₂F₂ plants) of the progeny of 17 BC₂F₂ plants from the Madsen cross and ~1500 BC₂F₂ plants from Eltan carrying two genes for Clearfield were space planted at Spillman Agronomy Farm. The above plants were sprayed in the spring season with 20 oz/A rate of herbicide 'Beyond'. After spraying, stunted plants possibly carrying single gene for herbicide resistance were rouged out. Only 21 BC₂F₃ plants from the Madsen/CL-618/Madsen and 25 BC₂F₂ plants from the Eltan/CL-618/Eltan carrying two genes for Clearfield were selected and used for DNA marker analysis. The selected few with good agronomic and quality characteristics were used to cross with Xerpha, Louise and Chukar cultivars. Plant carrying two gene Clearfield and agronomically superior were planted this year as head rows/plots at the Spillman Agronomy Farm.

Field evaluation for Clearfield and stripe rust resistance

(1). During the 2006-07 growing season, a large BC₁F₂ population from the crosses involving Madsen/CL-618/Madsen, Eltan/CL618/Eltan and Rod/CL618/Rod were grown at the Spillman Agronomy Farm. After spraying with herbicide 'Beyond' 16 resistant plants for Rod/CL618/Rod, nine for Eltan/CL618/Eltan and seven plants for Madsen/CL-0618/Madsen crosses were selected. The seeds of these plants were evaluated for quality traits (1000-kernel weight, seed color, protein content, kernel hardness) and for physical appearance. Progeny of these plants was again space planted and sprayed with herbicide 'Beyond'. Only 44 plants from Rod, 40 from Eltan and 26 plants from Madsen cross were selected with superior agronomic traits and were grown as head rows this year. A progeny of the Madsen cross showing homozygosity for herbicide resistance was planted this year at the Spillman Agronomy Farm as a yield plot.

(2). About 5000 F₂ plants from the Eltan × CL0618 cross, ~3000 from the Madsen × CL0618 and ~4000 from the Rod × CL0618 cross were space planted during 2006. After screening for herbicide resistance, plants carrying stripe rust resistance and good phenotypic characteristics were selected. Only nine plants from the Rod cross, eight from Madsen and seven plants from the Eltan cross were selected. After single plant quality evaluation, only few plants were selected from each cross and the progeny was again grown in the field again as space planting. After

selection for herbicide resistance, further phenotypic selection reduced the number of plants to 39 plants from Rod, 31 from Madsen and 29 plants from the Eltan x CL0618 cross. Selected plants were grown as head rows this year.

(3). Clearfield KCF08001 and KCF08002 lines: Progeny of two of the eight plants from the Rod × CL0618 cross (see previous section) was very uniform for the herbicide resistance as well as for height and other phenotypic traits Figure 3. DNA analysis of few random plants from this progeny confirmed presence of two gene Clearfield in all tested plants. After removing the off type plants harvested from the progeny was tested for quality. These lines do not have acceptable level of quality but the yield and other agronomic traits are very good. These two lines are currently in the WSU variety testing program. We expect these two lines to serve as germplasm for our future Clearfield transfers and for forward breeding program (see progress report for project 3019-7450)



Figure 3: Field evaluation of Clearfield KCF08001 and KCF08002 lines with checks grown in the Spillman Farm after 5X spray with ‘Beyond’; (a) ‘KCF08001’ two gene Clearfield resistance line. (b) ‘KCF08002’ two gene Clearfield resistance line. (c) ‘Rod’ Clearfield susceptible variety used as a control. (d) ‘ORCF-101’ one gene Clearfield resistance line.

Variety testing results of the Zak derived stripe rust resistant lines ‘WA8058’ and WA8059
Stripe rust resistant ‘Zak’ lines (WA8058 and WA8059) carrying the *Yr15* gene were evaluated for stripe rust and for yield trial in WSU variety testing program and the data is given in (Table 4 and Table 5). Based on the yield performance, two plants from one line were selected for seed increase. Variety testing data on stripe rust shows, both lines are seems better than Zak (Table

4). This year both lines were also evaluated for yield and other traits. The yield performance of our both lines are either equal or better than Zak in high rainfall location but these do not perform good at medium or low rainfall locations (Table 5).

Table 4. The data from WA8059 line and Zak evaluated at different places for stripe rust resistance in 2008.

Line # Name	Spillman Farm (Pullman)		Plant Path Farm (Pullman)		Whitlow Farm (Pullman)		Mt. Vernon, WA		Walla Walla		Lind	
	IT	S %	IT	S %	IT	S %	IT	S %	IT	S %	IT	S %
WA8059	2	20	2	5	2	5	0	0	0	1	0	0
Zak	5	80	8	100	8	50	8	10	8	80	3	2

IT = Infection type; S % = Severity percent

Table 5. The data from Wa8058 and WA8059 with Zak evaluated at different locations as Variety Testing Nurseries 2008 Zak .

Location	Line	Yield (Bu/A)	Test Wt. (Lb/Bu)	Protein (%)
Pullman	WA8058	64.30	56.90	13.70
	WA8059	64.90	60.70	12.00
	Zak	62.80	60.00	11.50
Farmington	WA8058	60.30	60.80	11.90
	WA8059	62.10	60.30	11.70
	Zak	61.00	59.20	11.10
Mayview	WA8058	47.20	62.90	11.70
	WA8059	51.50	62.70	12.50
	Zak	51.50	61.00	11.10
Dayton	WA8058	53.70	57.50	12.70
	WA8059	54.90	57.20	12.90
	Zak	57.30	56.60	12.60
St. John	WA8058	44.40	58.10	13.10
	WA8059	57.20	57.70	13.10
	Zak	65.90	58.00	12.20
Walla Walla	WA8058	37.50	60.50	13.70
	WA8059	42.90	60.30	13.60
	Zak	51.90	60.50	12.60

Publications:

Randhawa H.S*, Mutti J.S.* and Gill K.S. 2008. Optimizing marker-assisted background selection for rapid introgression of desirable genes. Published in 11th International Wheat Genetics Symposium. *First two authors contributed equally to the work. ISBN: 9781920899141; <http://hdl.handle.net/2123/3324>.

Randhawa H. S.*, Mutti J.S.*, Kidwell, K, Morris, C. F, Chen, X. M, and Gill K.S. Rapid introgression of single genes into popular cultivars using marker-assisted background selection. PNAS (Submitted). *First two authors contributed equally to the work.

Presentations and Reports

An overview of this project was given by Kulvinder Gill at the Washington Grain Alliance annual meeting in Spokane on November 13, 2008. The meeting was attended by approximately 100 wheat growers and WSU / ARS scientists.