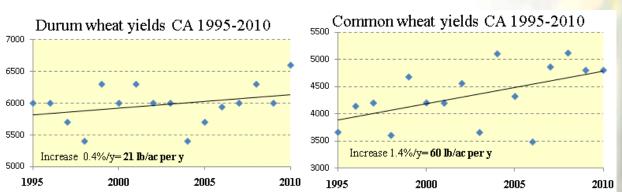
Progress in breeding for quality traits

CA Wheat Collaboration Meeting 10/20/10

J. Dubcovsky





Funded by:

CWC/UC Discovery & CCIA

UC Discovery–CWC grant

- 4 year grant awarded (2010-2014)
- CWC \$120,000 matched by UC-Discovery \$84,000 (\$204,000 per year)
- Will support the wheat breeding program and the regional testing program

Overall Objective

• Improve pasta and bread-making quality of California wheat varieties

Specific objectives

- Develop durum and common wheat varieties with increased resistant starch content, (higher amylose content) to increase dietary fiber.
- Develop durum wheat varieties with reduced Cadmium content.
- Improve gluten strength by incorporating the glutenin 7Bx-over-expressor allele (7BxOE) in bread wheat and the 1AL-1DL-1AL translocation carrying the Glu-D1a allele into pasta wheat .
- Increase grain protein content in durum and bread wheat varieties from California by incorporating the *Gpc-B1* high grain protein content locus from wild wheat.
- Discover new genes to improve grain protein concentration and improve nitrogen use efficiency

Increasing resistant starch in bread and pasta wheat

RESISTANT STARCH

The latest human research details ways in which less-digestible forms of starch may deliver important weight management benefits.

Suzanne Hendrich. Food Technology, March 2010 • **Definition**: **RS** is the sum of starch and products of starch degradation not absorbed in the small intestine of healthy individuals.

Consumption: Americans consume approximately **5** g of RS per day (range 3 to 8 g RS per day), considerably lower than intakes associated with health benefits.

• **Top sources**: wheat ~50% (bread 21%, cooked cereals/pastas 19%, cakes, muffins, waffles 7%, cookies 2%), vegetables and legumes (28%), bananas 14%. *J Am Diet Assoc. 2008;108:67-78.*

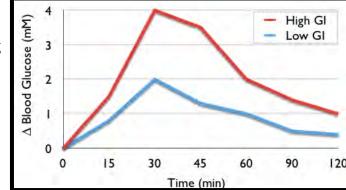
Classification:

- **RS1**: starch that is physically inaccessible to digestive enzymes (whole or **partly milled grains, whole grains**)
- **RS2**: starch that is resistant to digestion due to the nature of the starch granule (e.g. **high-amylose flour**).
- **RS3**: RS that forms from retrograded amylose and amylopectin during food processing. **Retrogradation of amylose is a major source of RS in cooked and cooled foods such as bread**.
- **RS4**: RS produced by chemical modification.

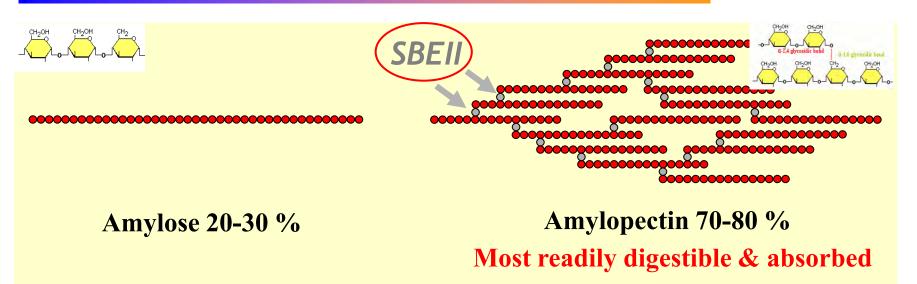
Beneficial health effects:

Large intestine: enhanced fermentation and laxation; increased uptake of minerals such as calcium; changes in the microflora composition, including increased *Bifidobacteria* and reduced pathogen levels; and reduced symptoms of diarrhea.

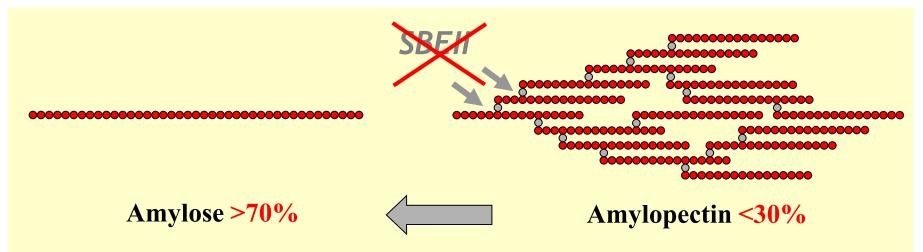
Systemic benefits: extended satiety (helps reduce weight), reduces glycemic index and demand for insulin, increases short-chain fatty acid production in the large intestine (RS fermentation)



Reduction of SBEII transcripts improved RS

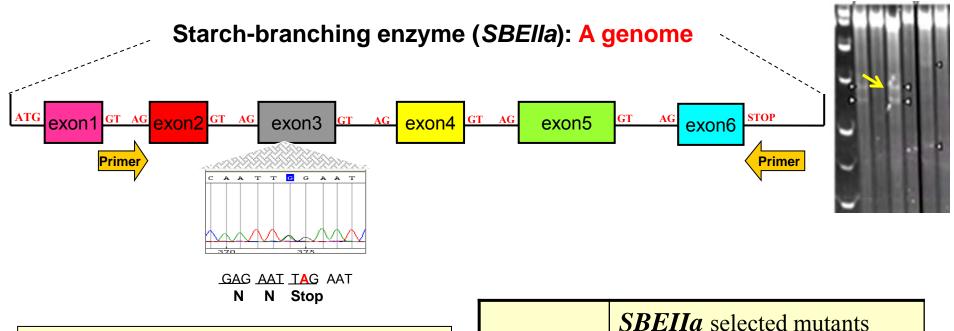


In 2006 Australian researchers showed that <u>inactivation of the *SBEIIa* gene</u> using a transgenic approach increased amylose content to >70%.



Mostly RS, escapes digestion & mimics fiber

TILLING: knock out of SBEII genes with no-transgenic approaches



- •165 mutations identified bread & pasta
- Selected best 1-2 for each copy in the different wheat genomes.
- Backcrossed two generations to reduce background mutations.
- We are now combining the different mutations in a single background to test effect on amylose content.

	SBEIIa selected mutants
Pasta A	Truncation (premature stop)
Pasta B	Truncation (splice site)
Bread A	2 amino acid mutations
Bread B	Truncation (splice site)
Bread D	Truncation (splice site)

Effect of high amylose content on pasta quality

Soh et al. Cereal Chemistry 2006 83: 513-519

EFFECT OF AMYLOSE ON PASTA QUALITY

Decreasing amylose below normal (waxy mutants):

Previous results

- decrease in pasta firmness
- increases stickiness
- pasta with inferior quality than normal durum

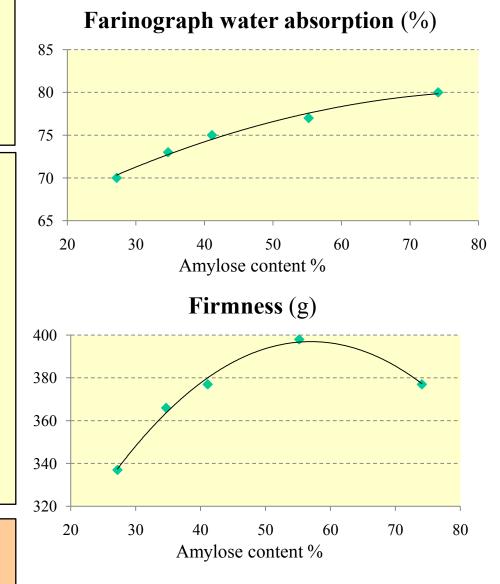
Increased amylose: reconstitution experiment

- semolina starch (28% amylose)
- replaced with high-amylose maize starch flour 27-74%
- constant gluten from the same durum wheat

Results

- An increase amylose content is correlated with:
- decrease in RVA parameters (>55% amylose no gelatinization)
- decrease in extensibility (from 33 to 22 mm)
- increased cooking loss (from 5.1 to 5.7)
- increased farinograph water absorption
- increased spaghetti firmness
- no significant changes in pasta stickiness

Conclusion: optimum quality at amylose content **32-44%**.



Effect of elevated amylose content on breadmaking quality

EFFECT ON BREAD QUALITY

Reconstitution experiments: Substitution of 20% of the wheat flour by 75%-amylose maize flour (final 10% increase in amylose). Gluten was added to similar levels.

Properties of dough from high amylose

- Same mixing time
- Higher water absorption (59.2% to 65.9%)

Properties of bread from high amylose

- same loaf volumes (663 ml to 656 ml in HA)
- same specific volume (4.7 to 4.5 in HA)
- same crumb color and structure
- same aroma, taste and mouth-feel)
- significant increased RS and dietary fiber (DF) (Table)
- reduced staling during storage (Figure)

Conclusion. 10% increase in amylose content:

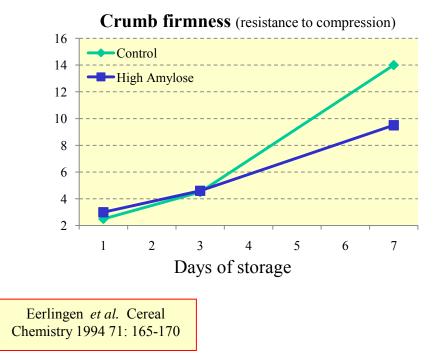
- produced breads with significant increases in RS.
- RS increased with storage (amylopectin retrogradation).
- increased water absorption and reduced staling
- no significant effect on bread volume or sensory charact.

		Flour			ead lay		ead ays
	Amy- lose	RS %	Di. Fib.	RS %	Di. Fib.	RS %	Di. Fib.
Normal	25%	14.5	1.0	0.0	2.4	4.0	2.4
High amylose	35%	27.7	4.2	7.7	6.9	10.2	9.1

• Most of the RS is lost during backing in normal amylose wheat

• Increase of RS and DF in breads from high amylose flour

• RS and DF increase with storage



Reduction of acceptable Cadmium levels in durum grain

Cd is relatively poorly absorbed into the body, but once absorbed is slowly excreted, like other metals, and **accumulates in the kidney** causing damage.

Europe 2006

Commission Regulations No 1881/2006: wheat limit 2.0 µg/kg of wet weight (200 ppb)

Europe March 2009

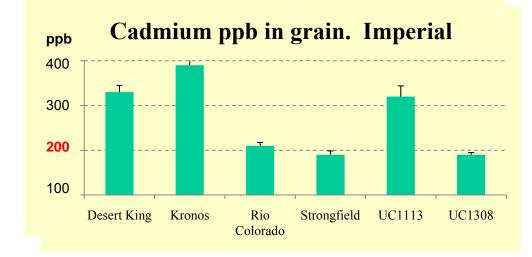
- The European Food Safety Authority (EFSA) established tolerable weekly intake (TWI) for cadmium of 2.5 μ g/kg bw (=2.5 ppb per week)
- The mean exposure for adults across Europe is ~2.5 ppb. Subgroups such as vegetarians and children may exceed the TWI by about 2-fold.

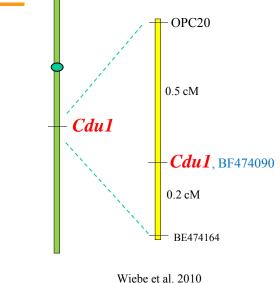
• They concluded that the Cd intake of the European population should be reduced, and are revising limits on foods.

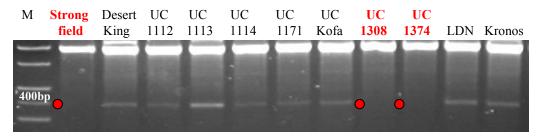
June 2010

• The FAO/ WHO JECFA recently adopted a provisional tolerable <u>monthly</u> intake (PTMI) for cadmium of 25ug/kg bw (=25 ppb per month, 2.5-fold higher than EFSA).

Low Grain Cadmium uptake: Cdu1 (chromosome 5BL)

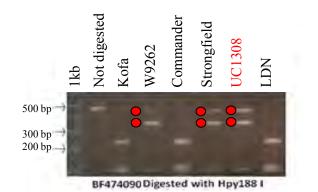








Problem: dominant marker in repulsion

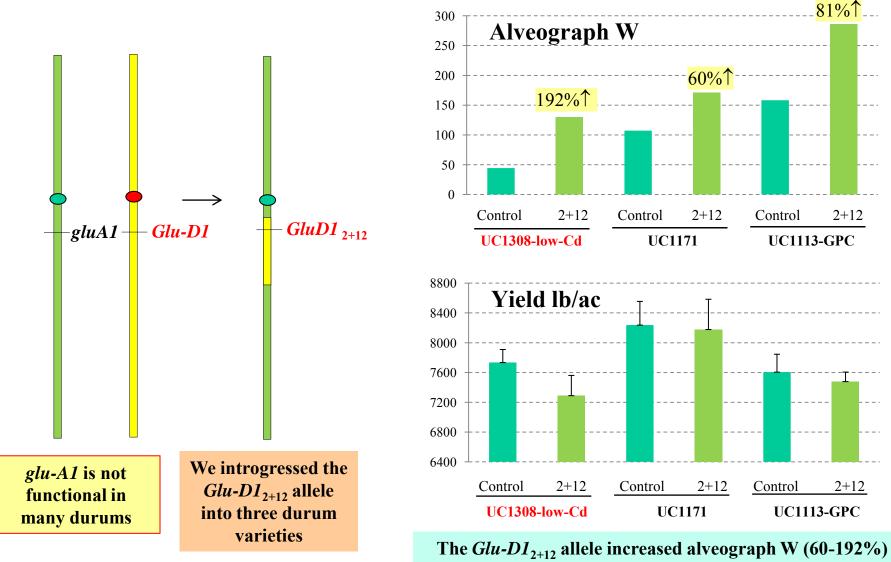


New CAP marker BF474090 completely linked and codominant! We can now see heterozygous *Cdu1* plants *Cdu1* is being introgressed in

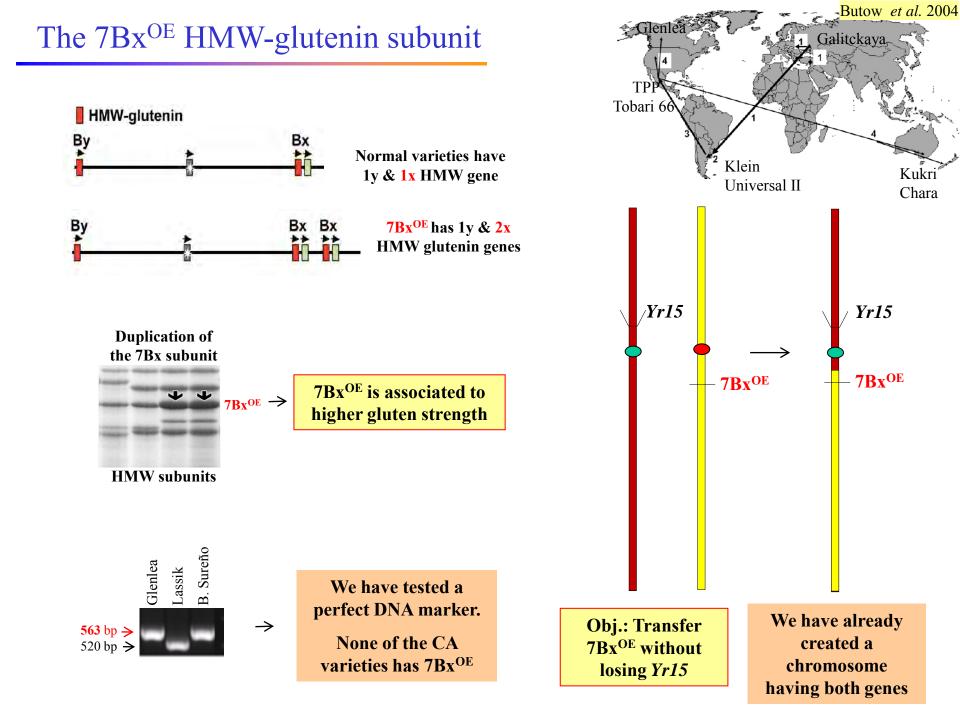
- * UC1113 (BC₄)
 -) * Kronos (BC₄)
- * Desert King (BC₁)

- * Tipai (BC₁)
- * D99-425_{APB} (BC₄) * D04AZ-335_{APB} (BC₄)

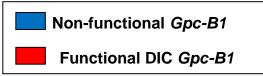
Replacement of pasta non-functional glu-A1 by Glu-D1

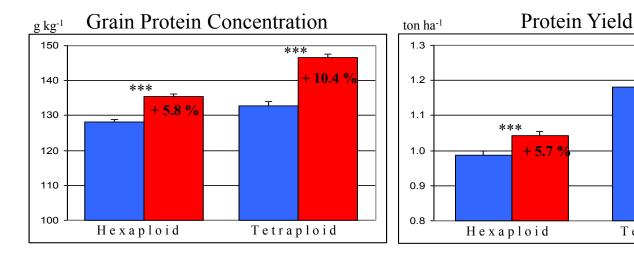


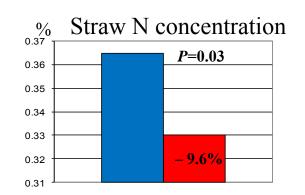
without significant penalty on yield (2010 results Imperial)



Deploy Gpc-B1 to increase grain protein







The *Gpc-B1* gene increases grain protein content by remobilizing more N from the leaves

- Field experiments
- 2 years

ns T

Tetraploid

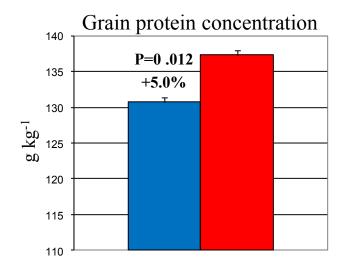
+ 5.1 %

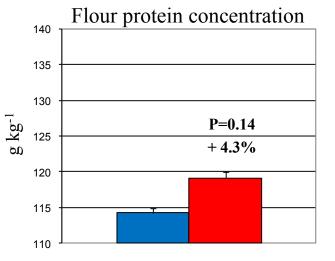
- 3 locations
- 5-10 replications
- 6 hexaploid lines
- 3 tetraploid lines

Crop Science 2010 Released varieties

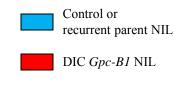
- Lassik
- Westmore
- Dessert King-High Protein

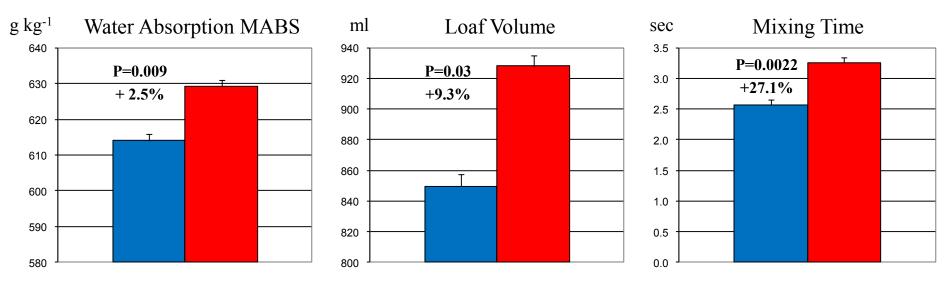
Effects of Gpc-B1 on bread-making quality







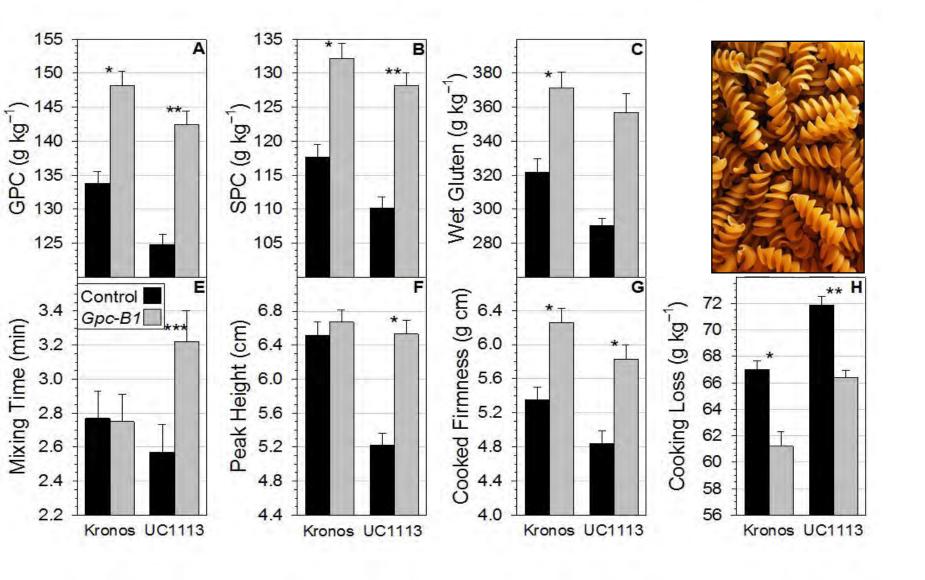




Effects of Gpc-B1 on pasta quality

Control or recurrent parent NIL

DIC Gpc-B1 NIL

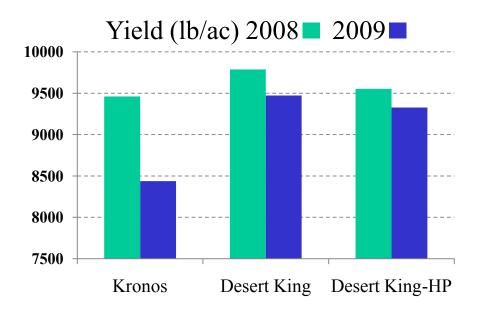


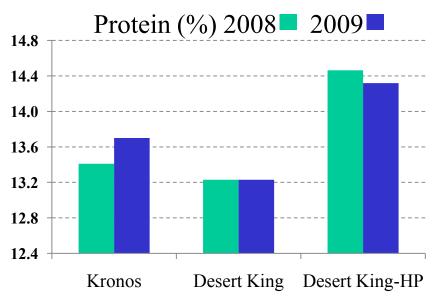
Durum variety 'Desert King High Protein'

Davis Elite trials	Yield lb/ac	Protein %
Kronos	8,950	13.6
Desert King	9,630	13.2
Desert King-HP	9,440	14.4



Desert-King-HP High protein >14% High-yield Foundation seed ready







Traditional breeding

Cross lines with complementary characteristics. Fix the variation by 6 generations of self-pollination. Select the best lines based on quality tests.

• Discovery of important genes

Cross lines with complementary characteristics. Find molecular markers close to the important quality traits. Use markers to accelerate the incorporation of these traits.

• Incorporation of new diversity from related species or mutants

Identify valuable genes.

Cross the wild germplasm with adapted varieties. Recover the adapted characteristics by backcrossing. Generate new variability by TILLING

Entries Common Wheat Collaborators 2010

							Yield 2010	
TEST #	Entry	Name	Туре	Parentage	Source	Sac	San Joaq.	Imperial
1	1651	WB SJ908-203	HWS	NOT RELEASED ELIMINATED	WB	5,610	6,320	6,190
2	1600	UCD 07013/30	HRS	UC1036 Yr5, Lr47, 2NS	UC	6,800	7,940	7,450
3	MIX-C	BL. GRANDE	HWS	EXPRESS//CLEO/2INIA66/4/PB775	RSI	6,500	7,750	6,870
4	1673	WWW CNBR9302	HRS		WWW	5,260	6,850	6,140
5	1660	RSI 05W90314	HRS		RSI	7,660	7,940	NT
6	1638	APB W11-6	HWS		APB	3,590	5,600	4,770
7	1599	UCD 07013/24	HRS	SUMMIT/3/HAHN/TURACO/2/TURACO	UC	7,260	7,930	6,860
8	MIX-A	BL.GRANDE	HWS	EXPRESS//CLEO/2INIA66/4/PB775	RSI	6,500	7,750	6,870
9	MIX-B	BL. GRANDE	HWS	EXPRESS//CLEO/2INIA66/4/PB775	RSI	6,500	7,750	6,870
10	1643	UCD 09014/16	HWS	UC896*4/ID0377S//KL*2/IDO377S/3/UC896 ₅₊₁₀	UC	5,440	7,180	5,970
11	1647	WB SJ908-186	HRS	NOT RELEASED ELIMINATED	WB	5,640	6,190	6,260
12	1650	WB SJ908-247	HRS	BC1 SOLANO YR15, YR17 WB-Rockland	WB	6,550	6,700	6,300
13	1616	UCD 0810/5	HRS	UC1357/KERN + <i>Yr17-Lr37</i>	UC	6,120	6,970	7,390
14	1642	UCD 09013/4	HRS	UC1357/EXPRESS + Yr17-Lr37	UC	6,130	6,730	6,270
15	1659	RSI 05W90192	HRS		RSI	6,640	7,480	6,390
16	1608	WWW CNBR9330	HRS	HRS MSFRS QUALITY POP	WWW	5,230	6,410	6,390

Entries Durum Wheat Collaborators 2010

			Yield				
TEST #	ENTRY	NAME	Sac	S. Joaq.	In	nperial	
1	MIX951 - C	KRONOS		5,430	7,290	8,350	
2	1585-Tipai	TIPAI		7,120	7,400	8,890	
3	1589	NORMANO ALLSTAR		2,890	NT	7,430	
4	1641	APB D2-97		6,340	8,390	8,830	
5	1435	WWW D8270		NT	7,030	7,970	
6	MIX 951 - B	KRONOS		5,430	7,290	8,350	
7	1628	UCD 08201/20		6,730	6,960	8,340	
8	MIX 951 - A	KRONOS		5,430	7,290	8,350	
9	1629	UCD 08201/21		7,170	8,200	<mark>8,970</mark>	
10	1644	UCD 09210/17		6,090	6,330	8,420	
11	1645	UCD 09213/30		8,100	8,560	8,310	
12	1583	SARAGOLLA ALLSTAR		5,830	NT	8,790	
13	1646	UCD 09220/135		6,500	8,170	8,740	
14	1640	APB D1-2		6,880	8,030	8,570	
15	1674	TANGO WWW		4,140	5,980	7,470	
16	1656	WB SJ807-006		5,540	7,430	NT	
17	1582	MAESTRALE ALLSTAR		6,180	NT	8,010	

Consistency common wheat collaborators 2010

		Protein					TEST WEIGHT					TKW					
ID Entry	Name	CWC ADM	Cereal Food	Bay State	Hor. Mil.	Conagra	CWC	ADM	Cereal Food	Bay State	Hor. Mil.	Conagra	CWC	ADM	Cereal Food	Hor. Mil.	Conagra
1 1651	WB SJ908-203	11.8	13.3	13.4	13.4	13.6	65.2		64.9	65.0	65.2	64.8	52.0		47.6	45.1	48.0
2 1600	UCD 07013/30	12.3 12.0	12.3	12.3	12.4	12.1	63.6	64.1	63.9	64.8	64.1	<mark>63.8</mark>	52.4	45.3	44.2	48.1	46.4
3 MIX-O	BLANCA GRANDE	11.9 12.3	11.9	12.0	12.0	12.1	64.4	64.8	64.5	65.0	65.1	64.7	49.7	46.1	42.8	43.3	45.2
4 1673	WWW CNBR9302	9.0 9.5	8.3	9.1	8.9	9.2	63.1	64.0	62.4	63.1	63.9	63.6	41.7	39.3	40.2	40.9	40.1
5 1660	RSI 05W90314	9.9 11.3	10.8	11.0	10.9	11.3	63.1	63.5	63.4	63.0	63.5	63.2	49.2	43.8	41.2	43.6	44.6
6 1638	APB W11-6	12.6 12.8	11.8	11.5	12.0	12.5	63.6	64.2	64.1	64.0	64.1	64.1	55.6	50.3	46.6	46.4	50.7
7 1599	UCD 07013/24	10.3 11.2	10.6	11.6	10.8	11.1	62.9	62.9	62.9	63.2	62.9	62.9	44.3	38.5	40.0	37.0	39.0
8 MIX-A	BLANCA GRANDE	11.8 12.5	11.9	12.4	12.0	12.3	64.5	64.9	64.6	64.9	64.8	64.7	49.6	46.4	42.4	44.4	45.4
9 MIX-E	BLANCA GRANDE	11.7 12.1	11.6	12.4	11.7	12.0	64.4	65.1	64.6	65.1	64.8	64.9	49.5	45.7	45.4	45.6	46.4
10 1643	UCD 09014/16	10.7 12.0	11.9	12.5	12.5	12.5	64.0	64.2	64.0	64.2	64.2	64.2	49.2	46.7	42.2	41.7	42.8
11 1647	WB SJ908-186	12.7	13.2	13.6	13.2	13.3	64.6		64.8	65.0	64.9	64.5	51.0		47.2	47.2	47.3
12 1650	WB SJ908-247	12.3 13.5	13.0	13.0	13.0	13.4	64.2	63.7	64.2	64.2	64.8	64.0	46.1	44.2	44.0	42.2	41.4
13 1616	UCD 0810/5	11.3	11.5	11.6	11.6	11.8	<u>63.5</u>		64.2	64.0	64.3	64.3	46.7		45.0	45.1	45.8
14 1642	UCD 09013/4	12.7 11.9	11.5	11.6	11.6	11.8	63.0	63.9	63.7	63.7	63.6	<u>63.5</u>	47.7	46.3	44.4	44.2	45.2
15 1659	RSI 05W90192	11.5 12.1	11.4	11.7	11.7	11.8	64.4	64.5	64.3	64.2	65.0	64.7	40.8	46.1	43.0	43.6	43.6
16 1608	WWW CNBR9330	9.1 9.0	8.4	10.7	9.0	9.0	63.4	64.1	64.2	64.4	63.9	63.8	42.4	41.5	39.6	40.1	39.8
		000/ 000/	0.50/	0.604	0.40/	0.40/	0.604	0.000	0.001	0.2.07	0.694	0004	5 20/	2 00/	2 00/	=00/	0.604
	AVERAGE	83% 92%	95%	86%	94%	94%	86%	82%	82%	83%	86%	<mark>89%</mark>	73%	79%	79%	79%	<mark>86%</mark>

Consistency common wheat collaborators 2010

			Farino; absorp			Farinograph peak							Loaf volume					
TES T#Entry	Name	CWC	ADM	Cereal Food	Bay State	Hor. Mil. C	Conagra	CWC	ADM	Cereal Food	Bay State	Hor. Mil. C	Conagra	CWC	ADM	Cereal Food	Hor. Mil. C	Conagra
1 1651	WB SJ908-203	68.0		68.9	66.1	65.8	67.4	6.5		8	9.8	6.75	8.0	875		3044	860	2350
2 1600	UCD 07013/30	63.0	64.4	60.7	61.9	61.4	59.3	6.0	7.0	5	8.5	5.25	4.5	920	2800	2985	858	<u>2500</u>
3 MIX-C		67.4	68.4	68.4	66.0	64.8	66.9	5.5	7.5	6	5.8	5.75	6.0	920	2750	3074	865	2525
4 1673	WWW CNBR9302	68.0	67.0	65.4	62.3	64.0	65.1	2.0	3.5	3	6	4.75	3.0	675	2500	2514	590	2175
5 1660	RSI 05W90314	64.2	66.8	63.8	62.3	62.9	62.7	3.5	5.5	5	6	4.50	4.0	775	2850	3015	820	2600
6 1638	APB W11-6	67.0	67.2	63.0	64.4	64.1	65.7	6.0	6.5	5	6.5	5.50	6.0	880	2800	2897	875	2575
7 1599	UCD 07013/24	65.2	65.4	62.1	62.7	63.6	<u>63.9</u>	3.5	5.0	5	5.3	4.00	4.5	800	2650	2897	805	2375
8 MIX-A	BLANCA GRANDE	67.6	70.2	68.2	66.3	65.5	67.6	5.5	12.5	5	5.2	5.50	6.0	920	2850	3103	848	2475
9 MIX-B	BLANCA GRANDE	67.8	70.0	65.0	65.6	65.7	67.6	5.0	12.0	5	4.5	4.50	5.5	920	2800	2926	913	2350
10 1643	UCD 09014/16	69.2	70.7	68.2	67.1	67.6	67.5	5.0	5.5	5	4.5	4.50	5.5	760	2550	2838	838	<u>2350</u>
11 1647	WB SJ908-186	70.4		68.5	68.2	68.1	67.4	8.8		8	9.5	7.50	8.5	900		3074	887.5	2375
12 1650	WB SJ908-247	69.8	72.4	68.9	66.0	67.0	68.4	4.8	8.0	6	4.8	6.50	5.5	890	2925	2838	863	2450
13 1616	UCD 0810/5	64.8		62.2	62.2	62.0	62.4	4.8		5	5	5.50	5.0	885		2985	863	2450
14 1642	UCD 09013/4	71.0	71.8	70.2	67.8	71.0	<u>68.8</u>	6.0	4.5	5	3.7	4.25	4.5	900	2600	2853	780	2425
15 1659	RSI 05W90192	64.0	65.8	62.4	62.4	63.2	62.4	5.0	5.0	5	4.5	4.50	4.5	850	2900	3044	825	2650
16 1608	WWW CNBR9330	69.0	72.0	68.5	65.6	66.3	68.6	2.5	2.5	4	2.2	2.50	2.0	625		2691	500	2200
	AVERAGE	92%	89%	89%	90%	88%	<mark>89%</mark>	74%	53%	75%	70%	73%	<mark>83%</mark>	70%	67%	72%	73%	66%

Comparison Durum Davis vs. Imperial

	D Imp	D Imp	D Imp	D Imp	D Imp	D Imp	D Imp	D Imp	D Imp
TES T# ENTRY NAME	PROT %	SEM. EXT.	ALVEO- GRAPH W	WET GLUT	DRY GLUT	COLOR "b" VALUE	COLOR SCORE	COOK LOSS %	FIRM (gcm)
1 MIX951 - C KRONOS	11.7 13.6	60.3 60.1	186 111	28.2 36.5	10.9 12.6	24.3 25.3	8.0 9.5	8.0 7.1	7.0 8.1
2 1585-Tipai TIPAI	9.9 13.1	65.9 63.9	144 90	24.4 26.8	9.4 10.1	26.0 28.0	8.5 10.0	7.7 7.4	6.8 8.3
3 1589 NORMANO	allstar 10.4 13.9	64.0 63.6	171 135	25.1 33.5	9.7 11.6	25.5 26.2	7.5 9.0	8.7 7.6	7.0 8.3
4 1641 APB D2-97	10.6 13.5	62.9 62.3	223 166	25.8 32.7	9.7 11.3	27.1 27.3	8.5 9.0	8.3 7.3	7.2 8.5
5 1435 WWW D8270) 12.4 13.6	62.6 60.4	247 113	32.5 34.7	11.3 12.9	25.7 26.2	7.5 8.5	7.0 7.5	7.6 8.1
8 1628 UCD 08201/2	0 10.1 13.9	63.8 63.4	183 173	23.5 35.0	8.3 12.1	25.9 26.9	9.0 10.0	8.7 7.3	6.7 9.1
10 1629 UCD 08201/2	1 11.0 14.5	65.6 62.0	182 58	26.6 36.0	9.6 12.3	24.8 26.6	8.0 8.5	8.2 7.9	6.4 8.2
11 1644 UCD 09210/1	7 11.0 13.6	63.2 64.2	158 <mark>96</mark>	27.2 35.6	9.6 11.7	25.3 26.5	9.0 9.5	8.2 8.2	7.4 8.0
12 1645 UCD 09213/3		60.4 61.6	101 104	19.0 34.4	7.0 11.9	26.0 27.3	10.0 9.0	8.2 6.8	5.8 8.5
14 1583 SARAGOLLA ALLSTAR	A 9.9 12.8	63.8 65.4	179 95	27.9 25.9	9.8 9.6	24.4 25.0	8.0 8.5	8.7 7.9	6.4 7.9
15 1646 UCD 09220/1	35 10.4 14.6	65.2 63.7	135 81	28.0 40.4	10.1 14.2	25.7 28.4	8.5 10.0	8.6 6.5	6.4 9.2
17 1640 APB D1-2	11.2 13.8	63.2 65.9	224 213	28.1 37.3	10.2 13.1	26.7 28.7	9.0 10.0	7.6 7.2	6.8 8.2
18 1674 TANGO WW		62.1 62.2	107 39	31.7 32.4	11.0 11.8	24.9 25.7	7.5 9.0	7.1 7.8	7.5 8.3
20 1582MAESTRALI20 1582ALLSTAR	E 9.6 15.1	64.7 60.5	108 98	22.7 43.4	8.2 15.0	22.1 25.5	7.5 8.0	8.1 8.3	6.2 8.3
AVERAGE	10.5 13.7	63.4 62.8	167.5 112.2	26.5 34.6	9.6 12.2	25.3 26.7	8.3 9.2	8.1 7.5	6.8 8.4
Correlation Davis vs. Impe	erial <u>24%</u>	38%	59%	-10%	-3%	71%	53%	-11%	-29%

Good correlation: semolina extraction, Alveograph W, and Color **Poor correlation**: protein content, wet and dry gluten, firmness and cooking loss. It might be better to run the durum collaborators directly in Imperial

EFFECT ON BREAD QUALITY

Reconstitution experiments: Substitution with highamylose wheat flour (HAF= **37.5% amylose SGP mutant**)

Dough properties:

- decreased peak and final viscosities
- doughs were weaker and less elastic
- doughs absorbed more water than those of the normal wheat flour.

After baking:

- Substitution of 50% flour with HAF increased
 - RS from 1% to 3% (db).
 - dietary fiber from 13% to 17% (db).
- RS increased gradually during storage for 1-5 d.
- loaf volumes of up to 30% HAF were not significantly different from control
- substitution with 50% HAF decreased loaf volume.
- During storage, the firmness of breadcrumb with 10% HAF substitutions was higher than control.

Conclusion: Substitution of up to 50% normal wheat flour with HAF (~31% amylose) results in bread with acceptable quality and significantly high amount of RS.