Sentences you will probably never read in a published paper:

"We were totally surprised it worked!"

"We just thought it'd be a neat thing to do."

"I'm only doing this to get tenure."

"Oops."

"Previous work by XXX et al. is actually pretty good!"

"To be honest, we came up with the hypothesis after doing the experiment."

"The results are just 'OK'."

"Future work will... ah, who are we kidding? We won't get more funding to do this." ALLER CONTRACT

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Genetic analysis of Fusarium head blight resistance in Tunisian-derived durum wheat populations

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# **Fusarium head blight**

- *O* Caused by *Fusarium graminearum*
- *Ø* Yield and quality reduction
- **Ø** Mycotoxin accumulation
- Ø Damage due to loss in North Dakota alone is in excess of billions of dollars





www.uky.edu/Ag/GrainCrops/Presentations/LEE\_Wheat\_5\_Diseases.ppt



# **Characteristics of FHB resistance in wheat**

- Sensitive to environmental conditions (i.e., difficult to measure accurately)
- Many genes with varied effect (4-20% of phenotypic variation)
- *Additive gene and some non-additive (dominance and epistatic) action*
- *It is a set of the se*



# **Characteristics of FHB resistance in wheat**

- Several QTL regions for Type II resistance have been identified (e.g., major QTL, *fhb1*, on 3BS) in hexaploid bread wheat sources (e.g., Sumai3, Wangshuibai)
- Transfer of resistance from identified sources into hexaploid wheat have been successful with release of varieties such as 'Alsen'
- Few QTL regions for Type II resistance have been identified (e.g., 3AS and 7AL) in durum or tetraploid wheat from *T. dicoccoides*
- Transfer of resistance from identified sources into durum wheat cultivars have met with limited success
- Number of introgression lines from wild tetraploid species have been developed with good identifiable resistance



- 1. Utilizing lines developed by the North Dakota durum wheat breeding program derived from Tunisian sources of FHB resistance
- 2. Lines are part of the breeding program (i.e., no need to incorporate them later)
- **3**. Fewer individuals need to be genotyped for prior selection on the trait by the breeding program
- 4. Allows finer mapping due to more opportunities for recombination than bi-parental populations



# **Data collection**

- *Ø* Both replicated greenhouse and field evaluations
- *Inoculation performed using a mixture of three isolates (R010, R1267, R1322)*
- Some measurements of FDK, and various DON
- **Ø** Genotyped using DArT, and SSR markers





The average of infection rate for durum cultivars and the Tunisian resistant sources D87450 was used as the susceptible and Sumai3 and ND2710 were used as the resistant controls The letters on each column indicates the Duncan grouping of means at the probability level of 0.05

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#### **Diversity analysis of Tunisian resistant and North Dakota durum cultivars**



Genetic distance (D=-LN J; J=Jaccard coefficient) dendrogram of Tunisian sources of FHB resistance and durum wheat cultivars

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# Association analysis of Tunisian derived populations



Frequencies of minor alleles in the populations is maximized at 0.2 due to the effect of selection (A). The estimates of  $r^2$  versus the genetic distances of the markers according to Tun34 × Lebsock genetic map (B). The LD decay is around 40 cM considering the critical value of 0.06.

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# **Association analysis of Tunisian derived populations**

MAF 0.14 0.11 0.11

0.12 0.14

Marker	Chro	om.	cM	Raw_p	pFDR	* 1	R <sup>2</sup>	N	1AF		
wPt-1876	1B	3	29	0.031	0.793	0.0	10	0	0.27		
wPt-9369	3A	1	45	0.002	0.174	0.0	20	C	0.08	A	ta
wPt-7992	3A	1	59	0.010	0.473	0.0	14	C	0.10		
wPt-6854	3A	1	44	0.010	0.473	0.0	13	C	0.10	ch	r
wPt-2305	5B	3	24	0.030	0.793	0.0	19	C	0.10	ro	
wPt-7663	6A	1	23	0.018	0.235	0.0	24	C	0.21	10	51
wPt-8554	6B	3	68	0.023	0.235	0.0	08	C	0.13	0	K
wPt-2162	6B	3	107	0.002	0.131	0.0	12	C	0.08		
wPt-9256	6B	3	115	0.016	0.235	0.0	03	C	0.09	RI	
wPt-4831	7A	1	122	0.032	0.793	0.0	16	C	0.13		
wPt-4025	7B	3	146	0.029	0.235	0.0	25	0	0.09		
wPt-8981	7B	3	149	0.014	0.561	0.0	28	0	0.24		
wPt-9665	7B	3	149	0.021	0.667	0.0	26	C	0.24		
wPt-4533	2A	1	18	0.033	0.235	0.0	02	C	0.06		
wPt-4021	2A	1	5	0.020	0.667	0.0	17	C	0.15		
wPt-4984	2A	1	21	0.039	0.812	0.0	27	0	0.26		
tPt-1041	2A	1	19	0.042	0.812	0.0	26	C	0.24		
wPt-7285	2A	1	5	0.049	0.812	0.0	14	C	0.13	T	16
tPt-6487	3B	3	22	0.048	0.812	0.0	11	C	0.20		_ `
wPt-6467	3B	3	0	0.002	0.188	0.0	04	0	0.32	th	e
wPt-4842	3B	3	22	0.014	0.235	0.0	09	C	0.20		
Marke	r	Ch	nrom.	cM	Rav	v_p	pFI	DR		<b>R</b> <sup>2</sup>	Γ
wPt-0054			5B	243	0.0	00	0.0	28	0.	061	
wPt-2885			5B	248	0.0	00	0.0	39	0.	050	
wPt-7400	)		5B	248	0.0	00	0.0	40	0.	045	
wPt-6910	)		5B	248	0.0	00	0.0	64	0.	058	
wPt-7279	)		5B	243	0.0	01	0.0	98	0.	055	
wPt-1121	6B	5	115	0.040	0.812	0.0	26		.15		
tPt-6107	6B	3	114	0.044	0.812	0.0	26	C	0.13		
tPt-9048	6B	3	151	0.044	0.812	0.0	20	C	0.23	Th	e
D4 9050					0.005	0.0		0	20		~
WP1-8059	6B	}	121	0.023	0.235	0.0	23	U U	1.20	ſ	

A total of 35 marker loci representing 10 chromosomes were associated to FHB Type II resistance based on the union output of the  $K_T$  and  $QK_T$  mixed model analysis of 537 markers in 340 RILs derived from 9 different crosses

#### The pFDR test just confirmed the association of the 5BL markers to FHB resistance

The QTL on 3AS, 3BS and 6BL seem promising or pFDR values close to significance







Frequency distribution of FHB severity among 169  $BC_1F_6$  wheat RILs of the Tun 34 × Lebsock cross measured in the two greenhouse seasons in 2006 and 2007

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#### **QTL analysis of Tunisian derived populations**

1A	1B	2A	2B	3A	38	4A	4B
0 PF-5831 1 PF-0011 2 PF-8882-wP1-6630 wPt-8030	0 wPL-6808-wPt- wPL-0083 sPt-5249	7652 0 wPI-3611-wPI-8929 6 wPI-4021-wP6-7280 19 wPI-4197-wPI-5647 20 wPI-6245	0 wPt-5788-wPt-55 3 wPt-2600 7 wPt-5672-wPt-77 9 wPt-6182-wPt-55 9 wPt-0178 11 wPt-8583-wPt-84	13 0 wPt-1111 2 wPt-1655-wPt-2930 565	8 0 wPt-0302-wPt-1516- wPt-3536-wPt-3521 wPt-6487-wPt-8070-	0 wP+4680 1 wP+7355 2 wP+5740-wP+3788 2 wP+6214-wP+0105 9 wP+6833 10 wP+5857-wP+5428 10 wP+578	0
55 (wPt.2208	37 38 39 39 wPt-1912 39 wPt-1238-wPt-	21 22 7138	12 WPI-1064 WPI-0 17 WPI-6316 WPI-6	115 26 CA-8869	22 wPt-7132-wPt-9012 35 wPt-8828 40 wPt-5432 42 wPt-2961	11 wP-1681 30 wP-1684 31 wP-7558 wP-1262 34 wP-7558 wP-1262 34 wP-7558 wP-1262 34 wP-240 wP-2946 36 wP-2247 61 wP-7521	
56 wPt.5577-wPt.5274 58 wPt.010-wPt.6853 60 wPt.0769-8747724 61 wPt.9845 63 wPt.0274 69 wPt.0598,wPt.5988	70		57 wPt-1217-wPt-40 50 wPt-8828 73 wPt-4851 75 wPt-3651	102	56	62 wPi-8167 wPi-3108 65 wPi-8167 wPi-3108 69 wPi-8868 wPi-8271 70 wPi-0538 wPi-7354 wPi-1155 72 wPi-0764 wPi-7370 71 wPi-0627 wPi-0176	53 Pt-0672-wPt-6240 wPt-7412 63 wPt-0625 79 pt-76258, wPt-9201
	75 - Pt-8412		78 77 78 wPt-0510 78 wPt-4223	98 wPt-3133	0	74 wP-4424 wP-4418 75 wP-6450 wPt-0833 vP-5434 vP-1607-wPt-3729 vP1-5172-rPt-0238 77 wPt-5171-wPt-4680 vP1-5271	78 P1-8209
	108 wPI-5485 113 wPI-3227- wPI- 121 wPI-3579	C506	116 wPt-2929-wPt-48	100 WPt-1592 100 WPt-5125 wPt-5133			
	140 - wPt-9409-wPt- 142 - Pt-7906	1818			128 (P1-4252) 129 (P1-4253) 131 (P1-4233) 131 (P1-4253) 134 (P1-6264) 139 (P1-6460)	ł	
	154 wPI-5316						
		178 H wP1-9277-wP1-979	1				
5A 56	в	6A	6B	7A	7B UN-	2 UN-6	
0 1691-849397-4184 0 5 wPL4043 3 6 wPL40231 3 7 wPD4105 -	wPt-5803 wPt-8014-wPt-5896 wPt-3030 wPt-5928-wPt-8054	0 wPt-8124 2 P1-3786 3 wPt-11612 wPt-0642-4P1-8661	wPt-7582 wPt-8015-wPt-5234 wPt-6971 wPt-7682-wPt-3774	0 wPt-7214 0 2 wPt-3803 wPt-9796 wPt-7734 wPt-1163	wPt-1723-wPt-8283 0	*wPt-0463-wPt-8155 0 *** wP	%-4127-rPt-5396
ú/	wPt-5504 wPt-0498	4 wPt-2077-wPt-7655 5 wPt-0678 6 wPt-4063 2	wPt-1852-wPt-3304 wPt-3116 wPt-4119	27 /wPt-7076-wPt-4051			
		35 wPt-9474 2	WP1-8814- wP1-5256 wP1-8153- wP1-8017 wP1-1830	28 wPt-1080-wPt-3373 wPt-0288 30 wPt-5232-wPt-4345			
		51 wPt-0357-wPt-5091 33	wPt-4858-tPt-3506 wPt-0554 wPt-7540				
		52 wPt-7509 3	7 wPt-5333- wPt-3300 wPt-9667- wPt-4742 wPt-2479- wPt-8971 wPt-0052- wPt-8194	53 691-0517			
77	wPt-3863	400	9 wP1-7846 9 wP1-6247 2 wP1-7480 9 wP1-7480	75 wPt-8399 total 81	wPt-8615-wPt-5343		
			, and a second	80 wPt-0205 81 83 wPt-4877 93	wPi-8040.wPt-3939 ****		
101	wPt-8902			95 10-	wPI-5685-wPI-1057 wPI-5482-wPI-5228 wPI-6156 wPI-6305		
111	wPt-7114-wPt-8892 wPt-1753 wPt-7418	11	3 rPt-9324-rPt-6189 4 tPt-6107 5 wPt-1121	10/ 10/ 10/	5 8 wPs-1429-wPt-8217 6 wPt-5940 wPt-526-wPt-5880 8 wPt-2083-wPt-6797		
1229	wPt-1302 wPt-2562 wPt-4281-wPt-8157 wPt-0819	11	7 wPt-4560 8 wPt-8493 1 wPt-8059 3 wPt-9589		wPt-6320		
136	- wPt-9569-wPt-7029	12 13 13	7 wPI-9952 2 wPI-1541 7 wPI-5176 4 wPI-9241				
155	1P1-0228	15	1 wPt-0696- IPI-9048 wPt-1325- wPt-0986 wPt-7443- wPt-7464	**			
5B	-2						
243 248 249	wPt-2885-wPt-8910 wPt-2885-wPt-8910 wPt-7400 wPt-5118						



### QTL (composite interval mapping) analysis of Tun34 x Lebsock/Lebsock population

Group	Position (cM)	Locus	K*	<i>P</i> -value
5BL	243-247	wPt-0054	17.115	10-6
5BL	243-247	wPt-7279	15.188	10-6
5BL	243-247	wPt-2885	14.543	10-5
5BL	243-247	wPt-6910	13.205	10-5
5BL	243-247	wPt-7400	12.652	10-3
2A	20-22	wPt-7175	8.037	10-3
2A	20-22	wPt-4984	6.823	10-2
6B	156	wPt-7443	6.93	10-2
7A	75	wPt-8399	7.287	10-2
7B	93	wPt-8040	7.463	10-2
7B	93	wPt-3939	6.745	10-2
7B	93	wPt-4300	7.584	10-2

# Genomic regions associated with Fusarium head blight resistance in Tun34× Lebsock $BC_1F_6$ population

\*Kruskal–Wallis test statistic (df=1)

CIM revealed a significant QTL (LOD=6.1) on chromosome 5B accounting for 18.1% of genetic variation for FHB



# Summary of analysis for populations from Tunisian 34, 36, 18, and 7

- A significant QTL for FHB resistance on 5BL; *Qfhs.ndsu-5BL*, explaining up to 15% of the phenotypic variation was identified
- Tunisian 34 derived material provided the most consistent result due to availability of large number of lines and ability to validate using several QTL mapping approaches
- Surprisingly a region on 3BS in proximity of *fhb1* was also involved in FHB resistance
- Number of FHB resistant QTL are present in durum cultivars indicating a possible "suppressor of resistance" gene(s) or silencing mechanism
- *Inkage disequilibrium blocks extended up to 40 cM*





### FHB severity means in Tun108 x Ben/Ben population



Average of values measured in two replicated greenhouse and two replicated field experiments

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### QTL analysis of Tun108 x Ben/Ben population

Phenotype	Environment	Location	Marker interval	positions	LOD	Additive effect	% <b>ℝ</b> ²
Severity	GH 2010	2B	Xwmc96-Xbarc353	74.11	2.8928	0.692	6.12
	GH 2010	3B	Xwpt0384-Xbarc229	167.91	4.4399	0.8003	10.81
	GH2010	<b>5</b> A-1	Xbarc2187-Xbarc141	86.21	6.8814	1.1718	23.74
	GH 2011	5B-2	Xwpt5928-Xwpt5604	127.91	2	-0.497	5.05
	Ave across GH	2B	Xwmc96-Xbarc353	76.11	2.5227	0.5552	7.01
	Ave across GH 🔍	3B	Xwpt0384-Xbarc229	163.91	3.2172	0.5389	7.76
	Ave across GH	5A-1	Xbarc2187-Xbarc141	88.21	5.5385	0.9963	19.11
Incidence	Field 2011	2B	Xgwm71-Xbarc297	61.81	2.1271	0.6187	5.75
	Field 2011	3B	Xwpt0384-Xbarc229	142.61	3.5179	0.7141	8.49
	Field 2011	7B	Xgpw1054-Xwpt0884	47.91	4.3424	0.7856	9.68
	Field 2011	7B	Xwpt7975-Xwpt5846	3.91	2.1542	-0.4957	4.81
	Ave across filed	1B	Xwpt1818-Xwpt5061	156.81	3.0598	-0.746	16.32
	Ave across filed	7B	Xwpt7975-Xwpt5846	3.91	4.1823	-0.4348	9.46
DON	Field 2011	1A	Xwpt7784-Xwpt6853	146.81	2.0626	-4.5926	4.82
DON	Field 2011	2B	Xbarc297-Xwmc96	70.41	2.8868	7.7066	9.95
<b>3ADON</b>	Field 2011	2B	Xgwm71-Xbarc297	68.41	2.2079	0.0695	6.08
<b>3ADON</b>	Field 2011	3B	Xwpt0384-Xbarc229	161.91	3.1286	0.077	7.35
<b>3ADON</b>	Field 2011	6A-1	Xwpu0139-Xwpt2014	20.71	7.1348	0.2745	48.62
15ADON	Field 2011	2B	Xgwm71-Xbarc297	70.41	2.1548	0.1393	6.11
15ADON	Field 2011	3B	Xwpt0384-Xbarc229	161.91	2.3373	0.1285	5.36
FDK	Field 2011	1B	Xgwm264-Xwpt3451	81.31	5.1241	7.8064	11.7
FDK	Field 2011	2B	Xwmc96-Xbarc353	78.11	2.2732	6.4989	6.13
FDK	Field 2011	5A-1	Xbarc141-Xwpt4248	101.01	3.3051	-6.6074	7.11
FDK	Field 2011	5B-2	Xwpt6902-Xwpt5514	22.01	2.084	6.5267	7.82

#### QTL analysis of Tun108 x Ben/Ben population <sup>3B</sup> 5A-1

2B



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### QTL analysis of Tun108 x Ben derived durum population

- Broad sense heritability for FHB infection rate was calculated to be around 40.4%±0.09
- Transgressive segregants (~5% in the field and ~25% in the greenhouse) for increased resistance to FHB relative to tolerant parent were observed
- A significant QTL for FHB resistance on 5AL; *Qfhs.ndsu-5AL*, explaining up to 24% of the phenotypic or over 59% of genotypic variation for this trait was identified
- Two significant QTL for multiple FHB resistance related traits were identified on chromosomes 2B and 3B in the Ben population

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# **Future research directions**

- Additional backcrosses to further introgress QTL regions on group 5 chromosomes into advanced breeding material has been initiated
- Radiation induced deletions for overlapping segments of chromosome 2A have been generated to delete possible "suppressor of resistance" gene(s)
- Ten advanced durum cultivars (ave. severity >80%) were treated with 5-methyl azacytidine (prevents cytosine methylation) resulting in ~200  $M_3$  lines with  $\leq 10\%$  severity
- *Presence of fhb1* in Tunisian derived lines is being further investigated







### QTL (composite interval mapping) analysis of Tunisian derived populations

