

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."



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

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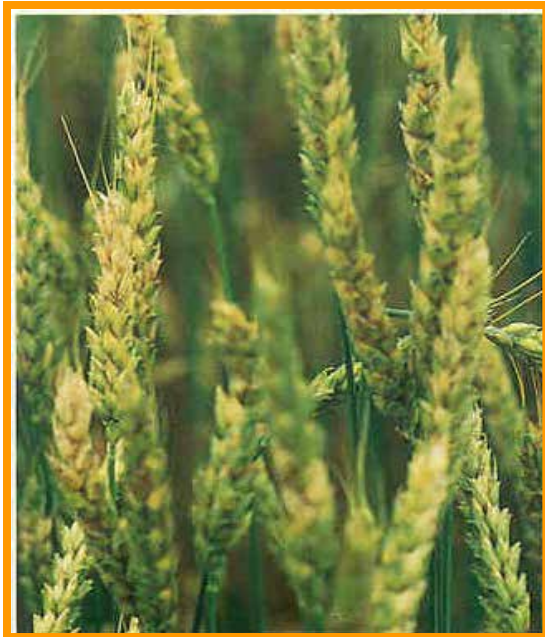
* Recently named University Distinguished Professor



WGC
NDSU

Fusarium head blight

- ∅ Caused by *Fusarium graminearum*
- ∅ Yield and quality reduction
- ∅ Mycotoxin accumulation
- ∅ 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
- ∅ Heritability for resistance ranges from 28% to 86%



Characteristics of FHB resistance in wheat

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

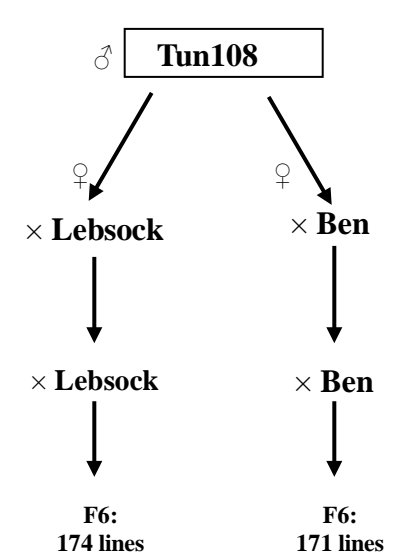
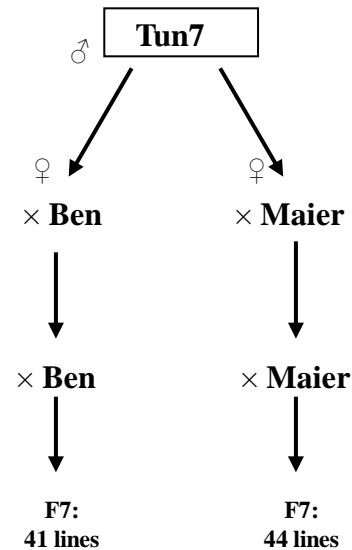
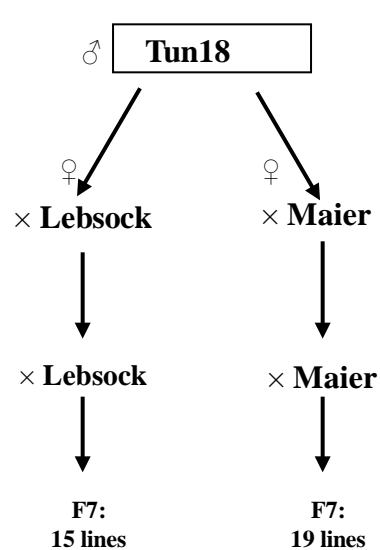
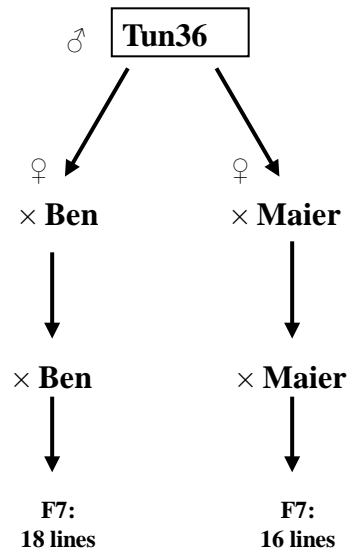
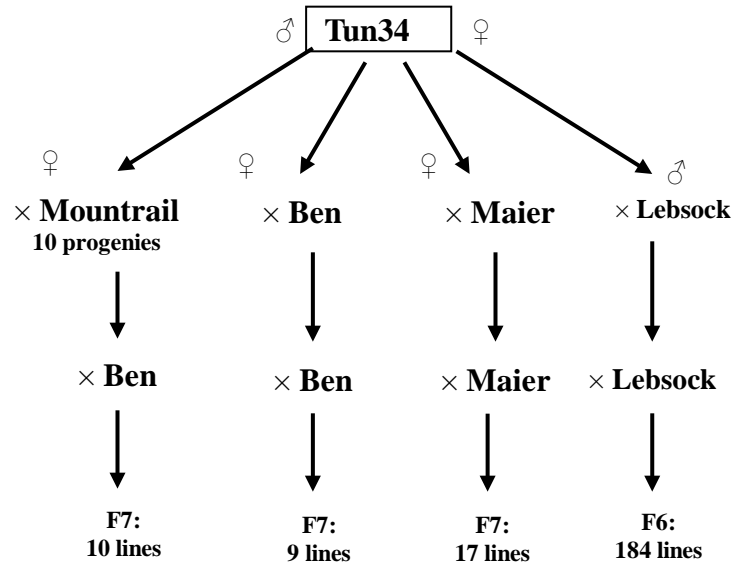


Pedigree based association analysis

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



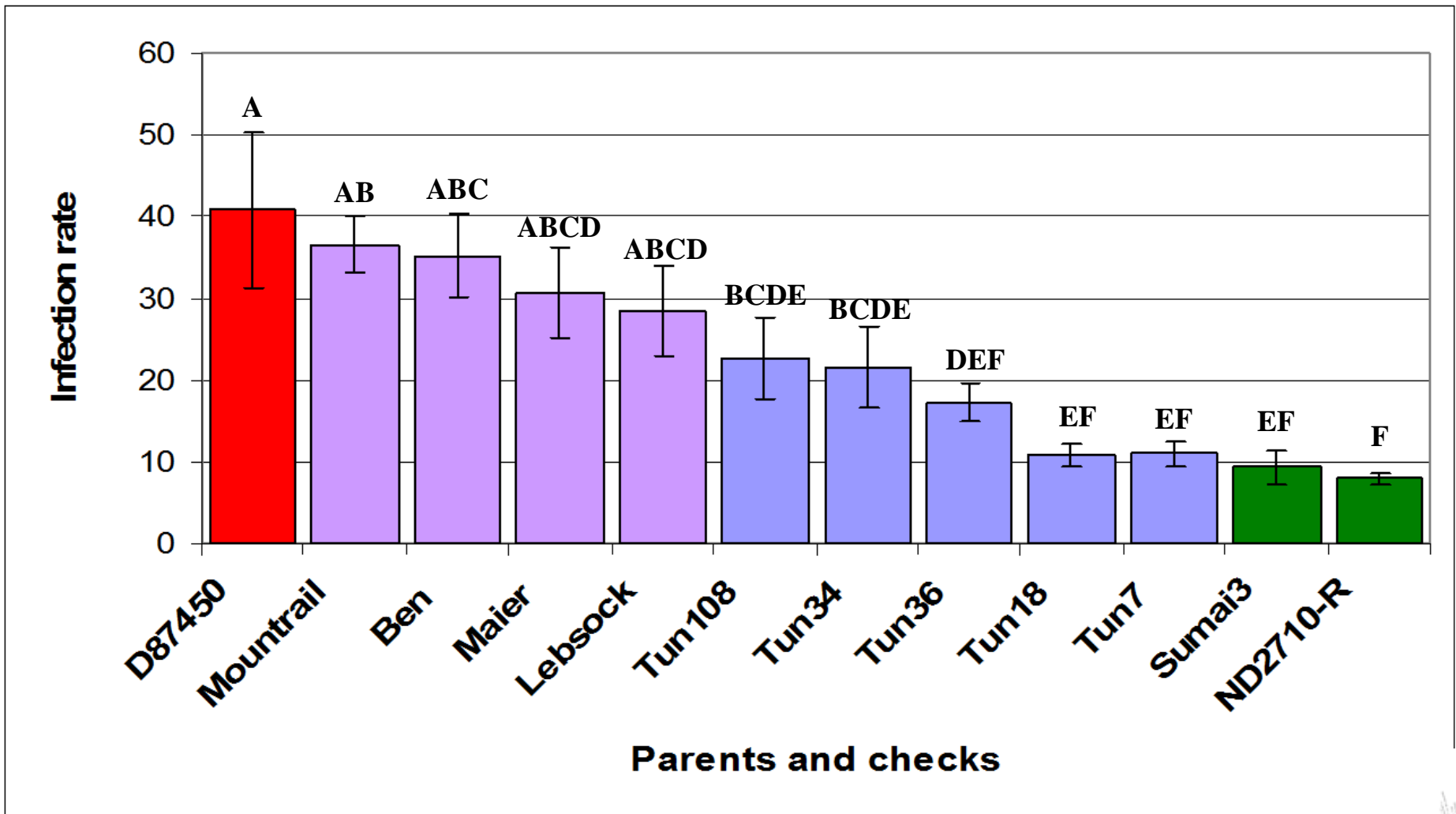
Pedigree based association analysis



Data collection

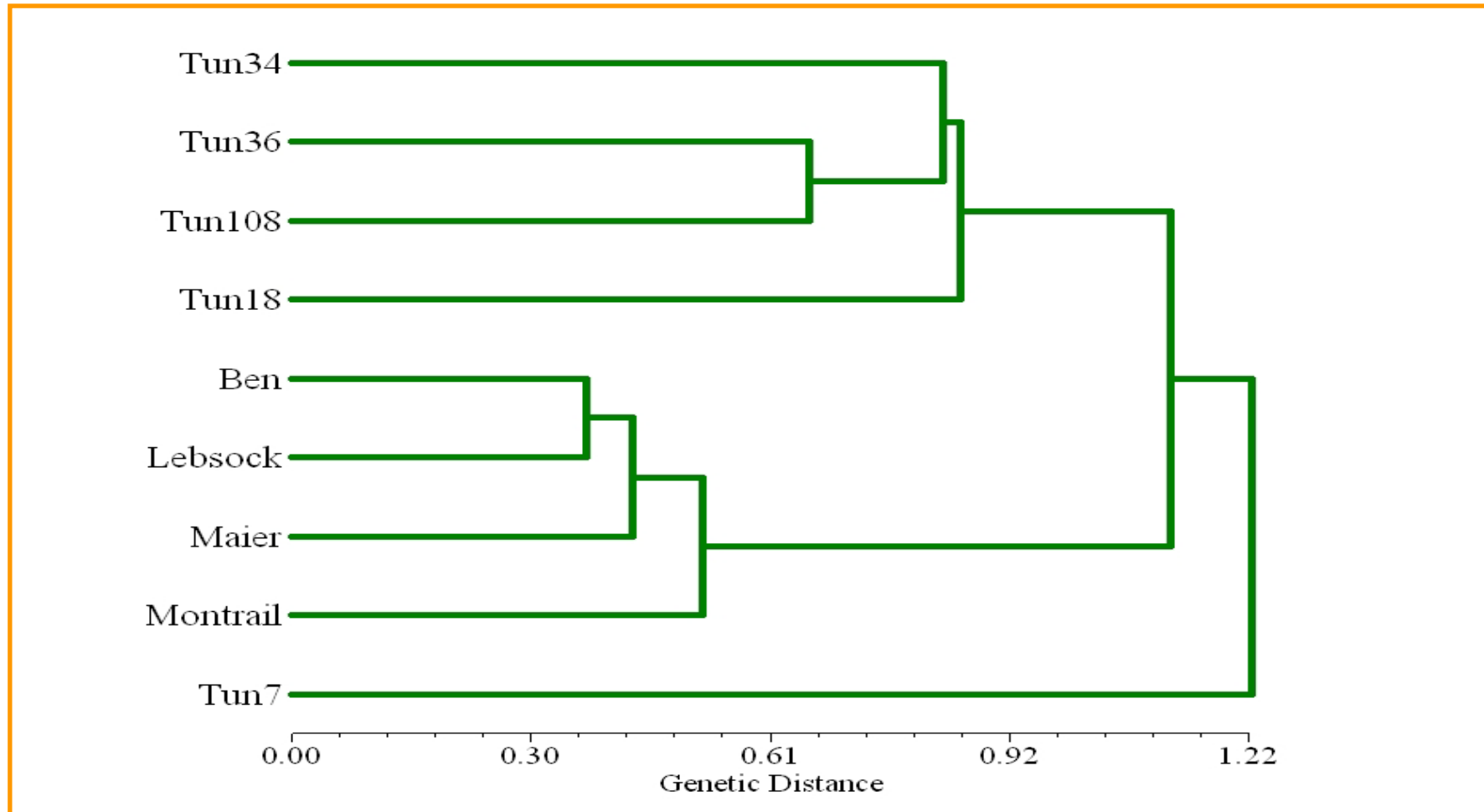
- ∅ Both replicated greenhouse and field evaluations
- ∅ Inoculation performed using a mixture of three isolates (R010, R1267, R1322)
- ∅ Some measurements of FDK, and various DON
- ∅ Genotyped using DArT, and SSR markers





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 indicate the Duncan grouping of means at the probability level of 0.05.

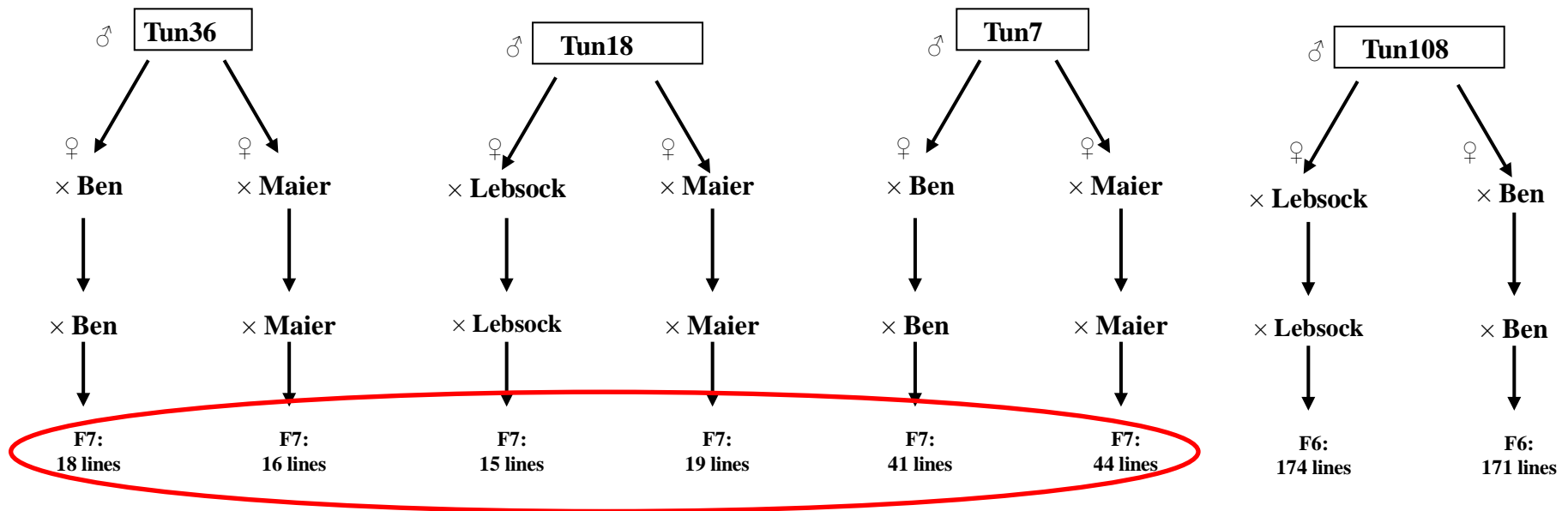
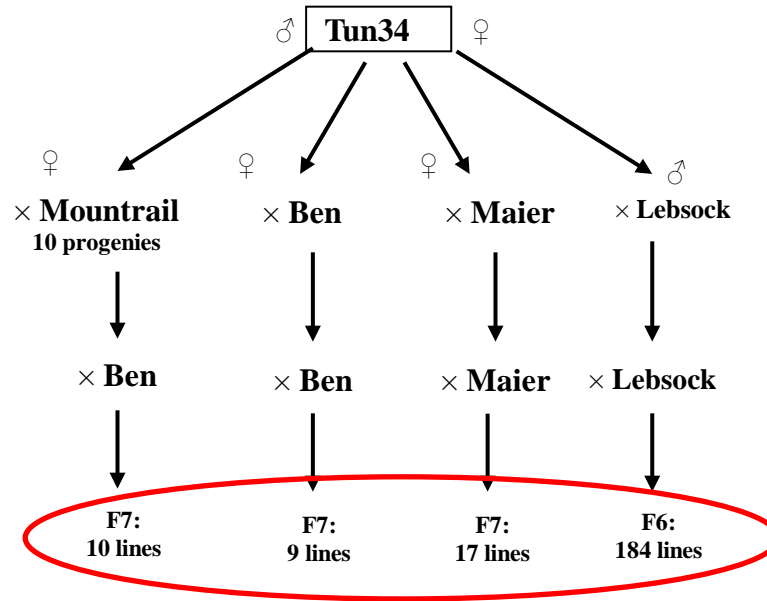
Diversity analysis of Tunisian resistant and North Dakota durum cultivars



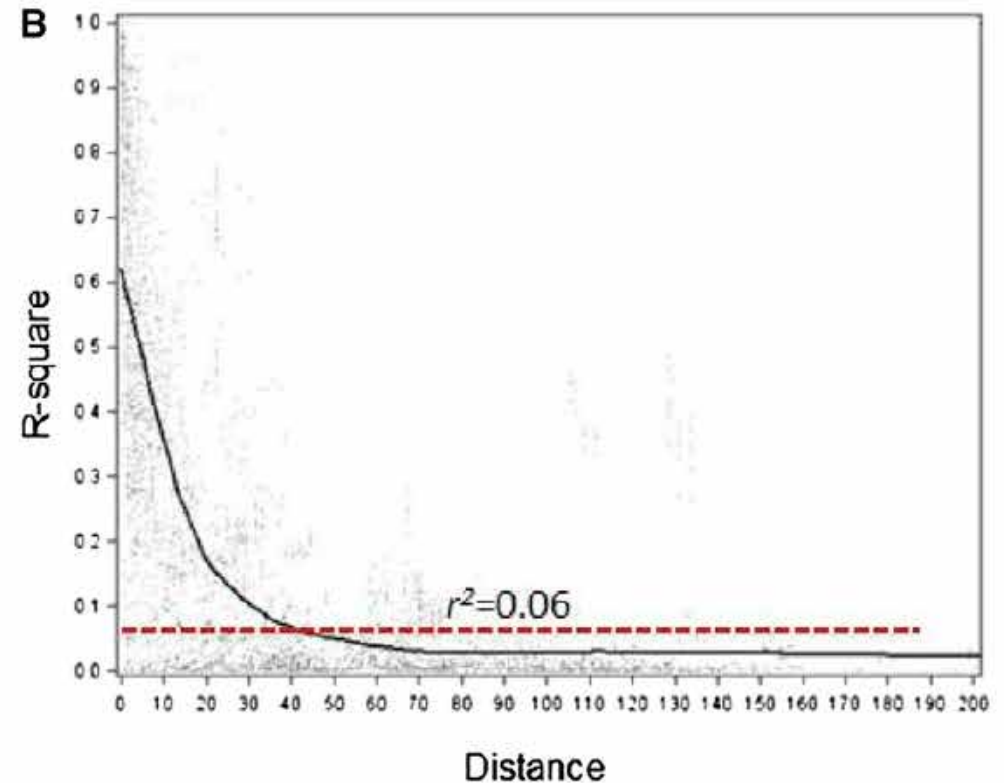
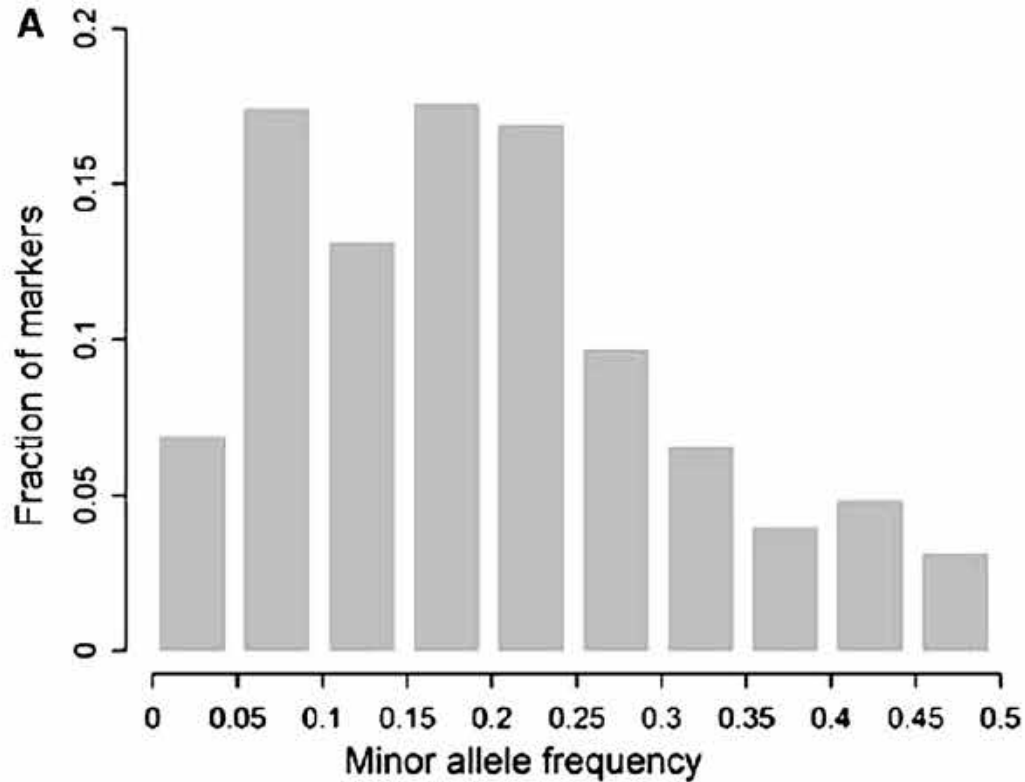
Genetic distance ($D = -\ln J$; $J = \text{Jaccard coefficient}$) dendrogram of Tunisian sources of FHB resistance and durum wheat cultivars



Pedigree based association analysis



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 \times Lebsock genetic map (B). The LD decay is around 40 cM considering the critical value of 0.06.



Association analysis of Tunisian derived populations

Marker	Chrom.	cM	Raw_p	pFDR	* R^2	MAF
wPt-1876	1B	29	0.031	0.793	0.010	0.27
wPt-9369	3A	45	0.002	0.174	0.020	0.08
wPt-7992	3A	59	0.010	0.473	0.014	0.10
wPt-6854	3A	44	0.010	0.473	0.013	0.10
wPt-2305	5B	24	0.030	0.793	0.019	0.10
wPt-7663	6A	23	0.018	0.235	0.024	0.21
wPt-8554	6B	68	0.023	0.235	0.008	0.13
wPt-2162	6B	107	0.002	0.131	0.012	0.08
wPt-9256	6B	115	0.016	0.235	0.003	0.09
wPt-4831	7A	122	0.032	0.793	0.016	0.13
wPt-4025	7B	146	0.029	0.235	0.025	0.09
wPt-8981	7B	149	0.014	0.561	0.028	0.24
wPt-9665	7B	149	0.021	0.667	0.026	0.24
wPt-4533	2A	18	0.033	0.235	0.002	0.06
wPt-4021	2A	5	0.020	0.667	0.017	0.15
wPt-4984	2A	21	0.039	0.812	0.027	0.26
tPt-1041	2A	19	0.042	0.812	0.026	0.24
wPt-7285	2A	5	0.049	0.812	0.014	0.13
tPt-6487	3B	22	0.048	0.812	0.011	0.20
wPt-6467	3B	0	0.002	0.188	0.004	0.32
wPt-4842	3B	22	0.014	0.235	0.009	0.20

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

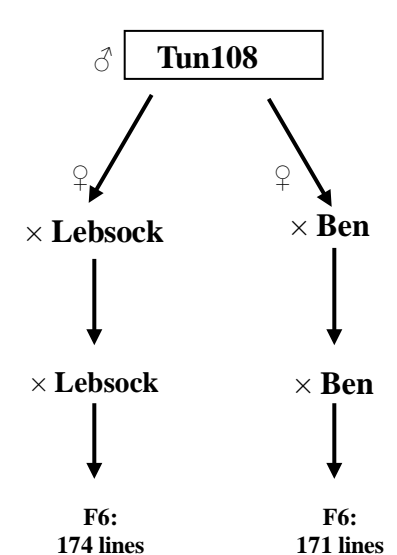
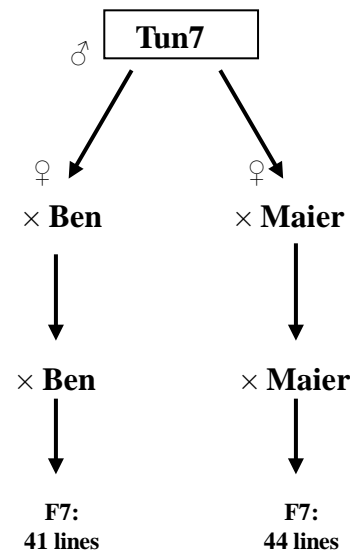
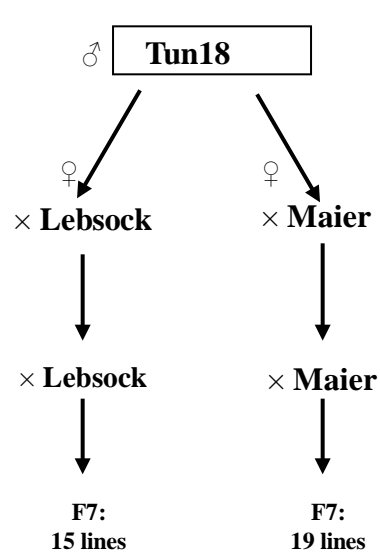
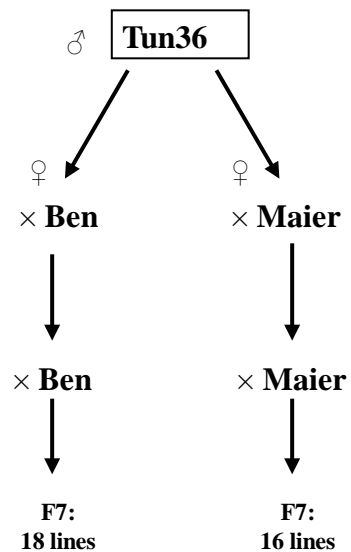
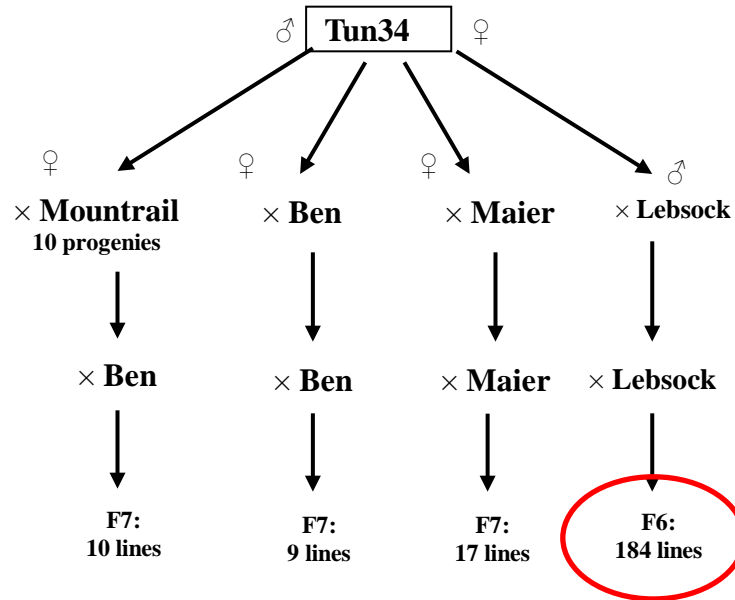
The pFDR test just confirmed the association of the 5BL markers to FHB resistance

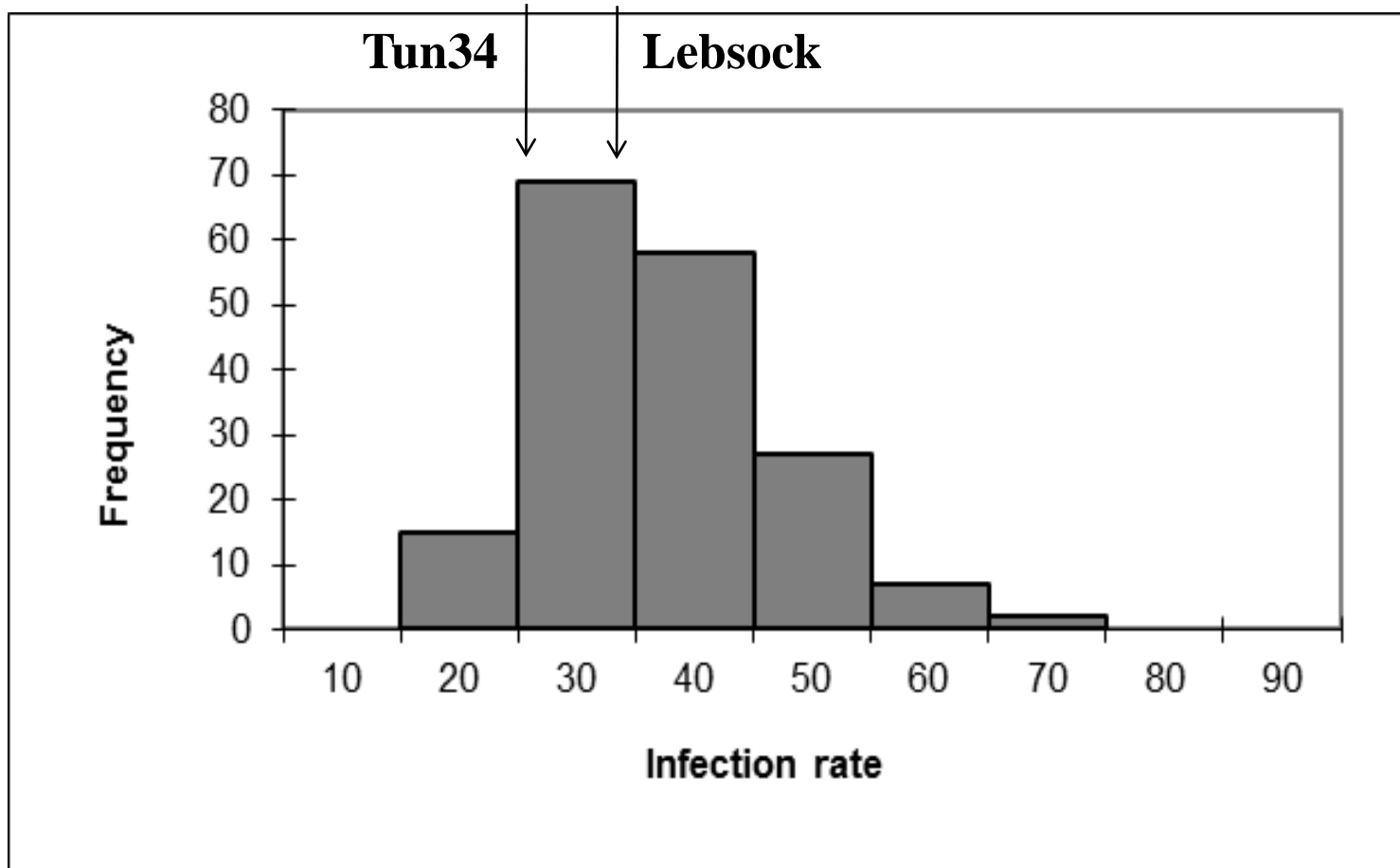
Marker	Chrom.	cM	Raw_p	pFDR	R^2	MAF
wPt-0054	5B	243	0.000	0.028	0.061	0.14
wPt-2885	5B	248	0.000	0.039	0.050	0.11
wPt-7400	5B	248	0.000	0.040	0.045	0.11
wPt-6910	5B	248	0.000	0.064	0.058	0.12
wPt-7279	5B	243	0.001	0.098	0.055	0.14
wPt-1121	6B	115	0.040	0.812	0.026	0.13
tPt-6107	6B	114	0.044	0.812	0.026	0.13
tPt-9048	6B	151	0.044	0.812	0.020	0.23
wPt-8059	6B	121	0.023	0.235	0.023	0.20
wPt-9241	6B	144	0.043	0.235	0.019	0.26

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



Pedigree based association analysis

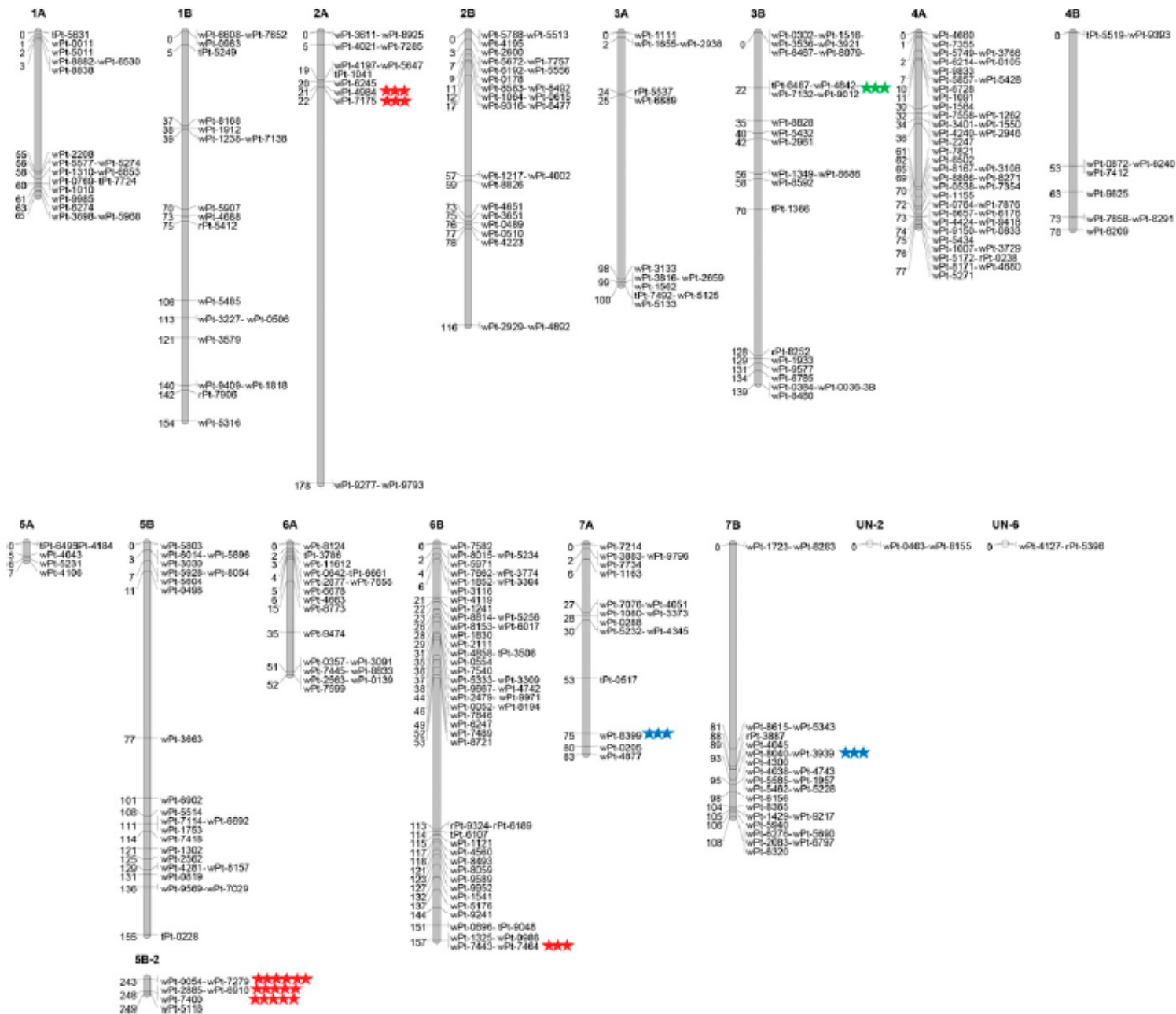




Frequency distribution of FHB severity among 169 BC₁F₆ wheat RILs of the Tun 34 × Lebsock cross measured in the two greenhouse seasons in 2006 and 2007



QTL analysis of Tunisian derived populations



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

Genomic regions associated with Fusarium head blight resistance in Tun34× Lebsock BC₁F₆ population

Group	Position (cM)	Locus	K*	P-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

*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

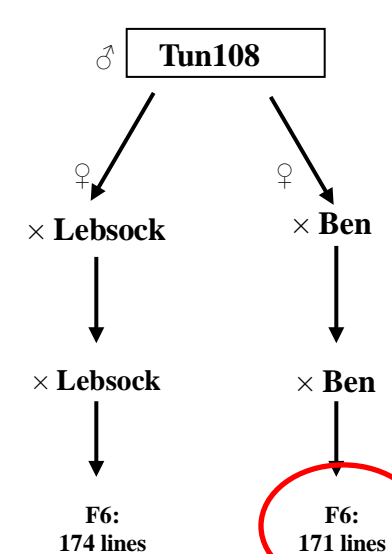
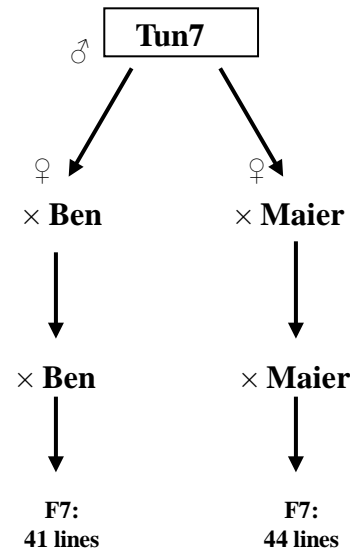
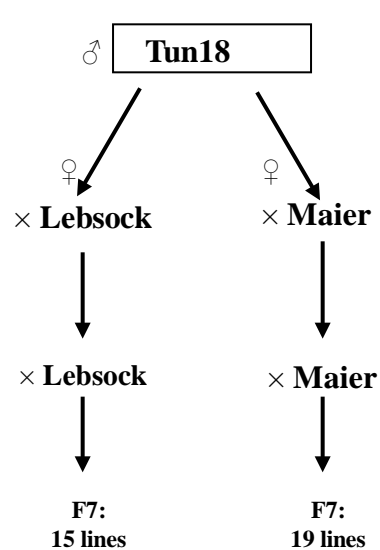
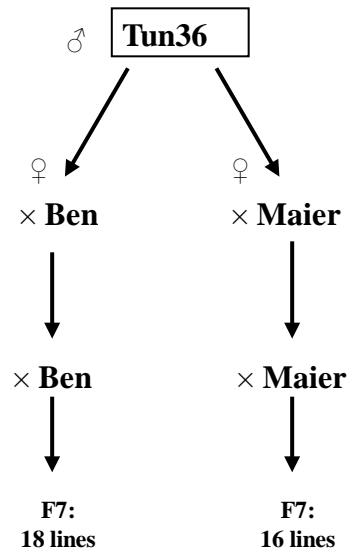
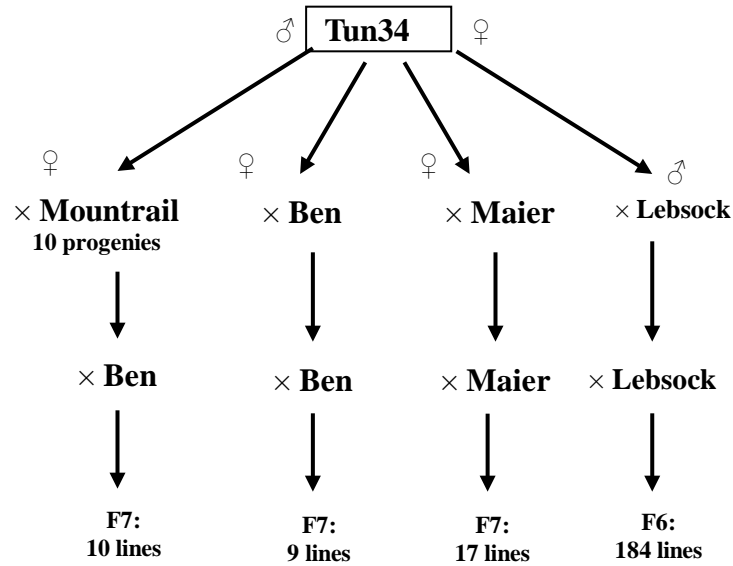


Summary of analysis for populations from Tunisian 34, 36, 18, and 7

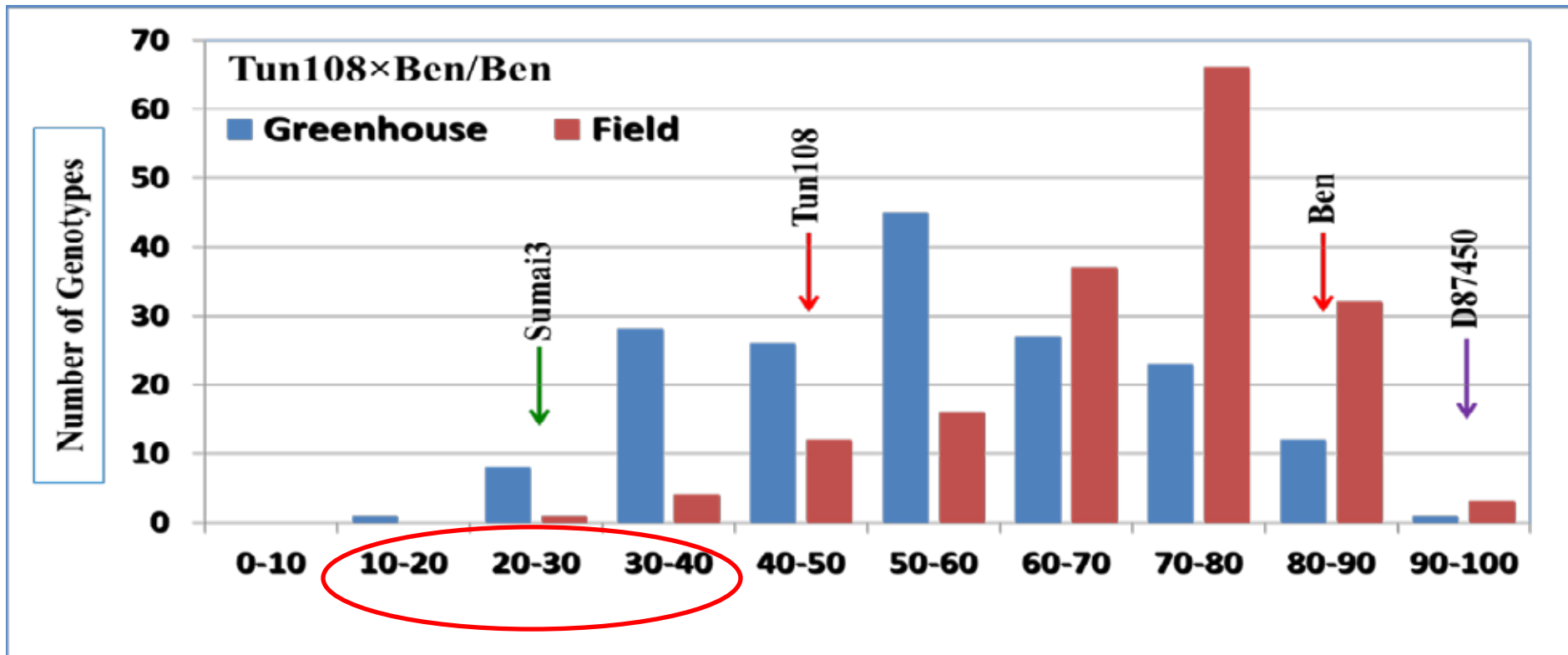
- Ø A significant QTL for FHB resistance on 5BL; *Qfhs.ndsu-5BL*, explaining up to 15% of the phenotypic variation was identified
- Ø Tunisian 34 derived material provided the most consistent result due to availability of large number of lines and ability to validate using several QTL mapping approaches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



Pedigree based association analysis



FHB severity means in Tun108 x Ben/Ben population



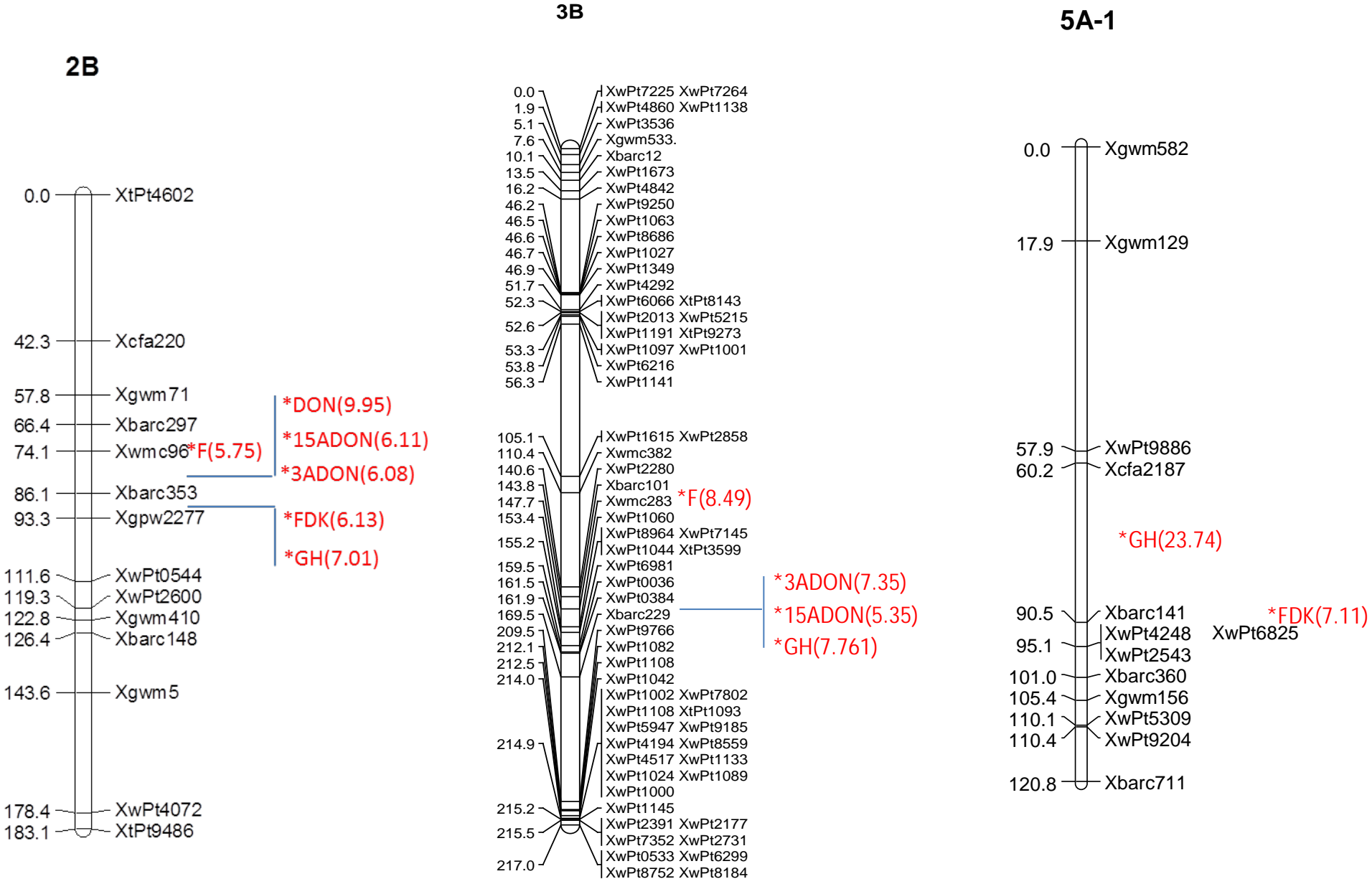
Average of values measured in two replicated greenhouse and two replicated field experiments

QTL analysis of Tun108 x Ben/Ben population

Phenotype	Environment	Location	Marker interval	positions	LOD	Additive effect	%R 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	5A-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
3ADON	Field 2011	2B	Xgwm71-Xbarc297	68.41	2.2079	0.0695	6.08
3ADON	Field 2011	3B	Xwpt0384-Xbarc229	161.91	3.1286	0.077	7.35
3ADON	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



QTL analysis of Tun108 x Ben derived durum population

- Ø Broad sense heritability for FHB infection rate was calculated to be around $40.4\% \pm 0.09$
- Ø Transgressive segregants (~5% in the field and ~25% in the greenhouse) for increased resistance to FHB relative to tolerant parent were observed
- Ø 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
- Ø Two significant QTL for multiple FHB resistance related traits were identified on chromosomes 2B and 3B in the Ben population



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 M₃ lines with ≤10% severity
- ∅ Presence of *fhb1* in Tunisian derived lines is being further investigated







QTL (composite interval mapping) analysis of Tunisian derived populations

