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# Molecular and Transgenic Approaches to Improve Fiber Quality

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**Cotton Fiber Bioscience Research Unit  
USDA-ARS, New Orleans, LA**

**Sept. 19, 2013  
2013 Cotton Workshop**



# Mission of the CFB Unit

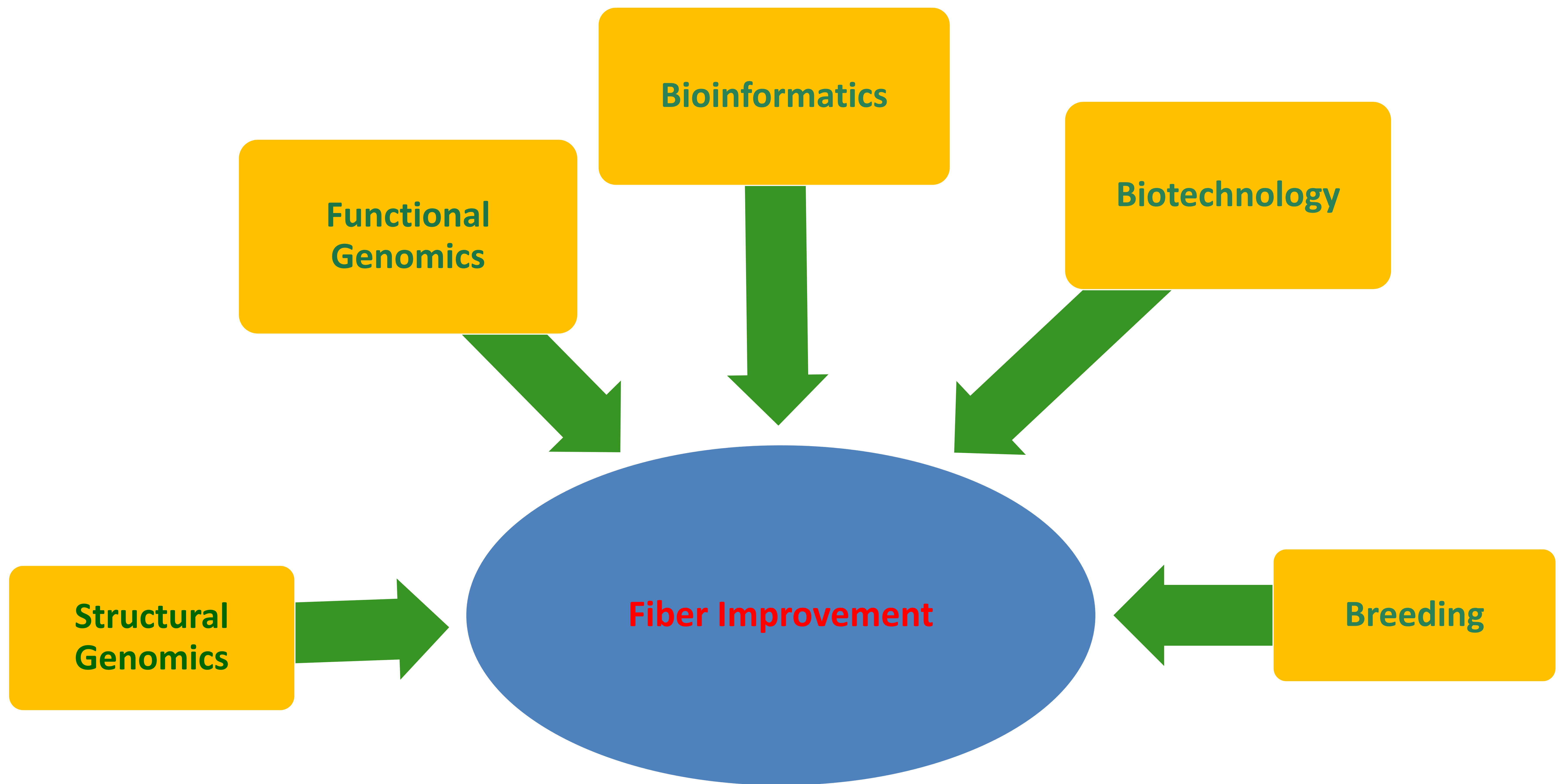
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- **Develop a broad base of knowledge on the fundamental cell biology and molecular genetics of the cotton fiber development.**
- **On the basis of these knowledge, develop and suggest approaches to improve fiber quality through biotechnology, breeding and production.**



# Overall Research Strategy

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# Genetic Mapping Fiber QTLs

➤ **Goal:** Identify molecular markers associated with fiber quality trait QTLs and use them in breeding.

➤ **Materials:**

1) MD90ne and MD52ne near-isogenic lines and populations.

2) RIL population derived from random-mating.



# Genetic Mapping Fiber Strength QTL in MD52ne

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- **MD52ne was developed by Dr. Bill Meredith at Stoneville, MS, and released in 2005.**
- **The fiber strength of MD52ne is about 10-30% higher than MD90ne, the NIL of MD52ne.**
- **An F2 population of 768 progeny between MD52ne and MD90ne was made and planted in 2012.**



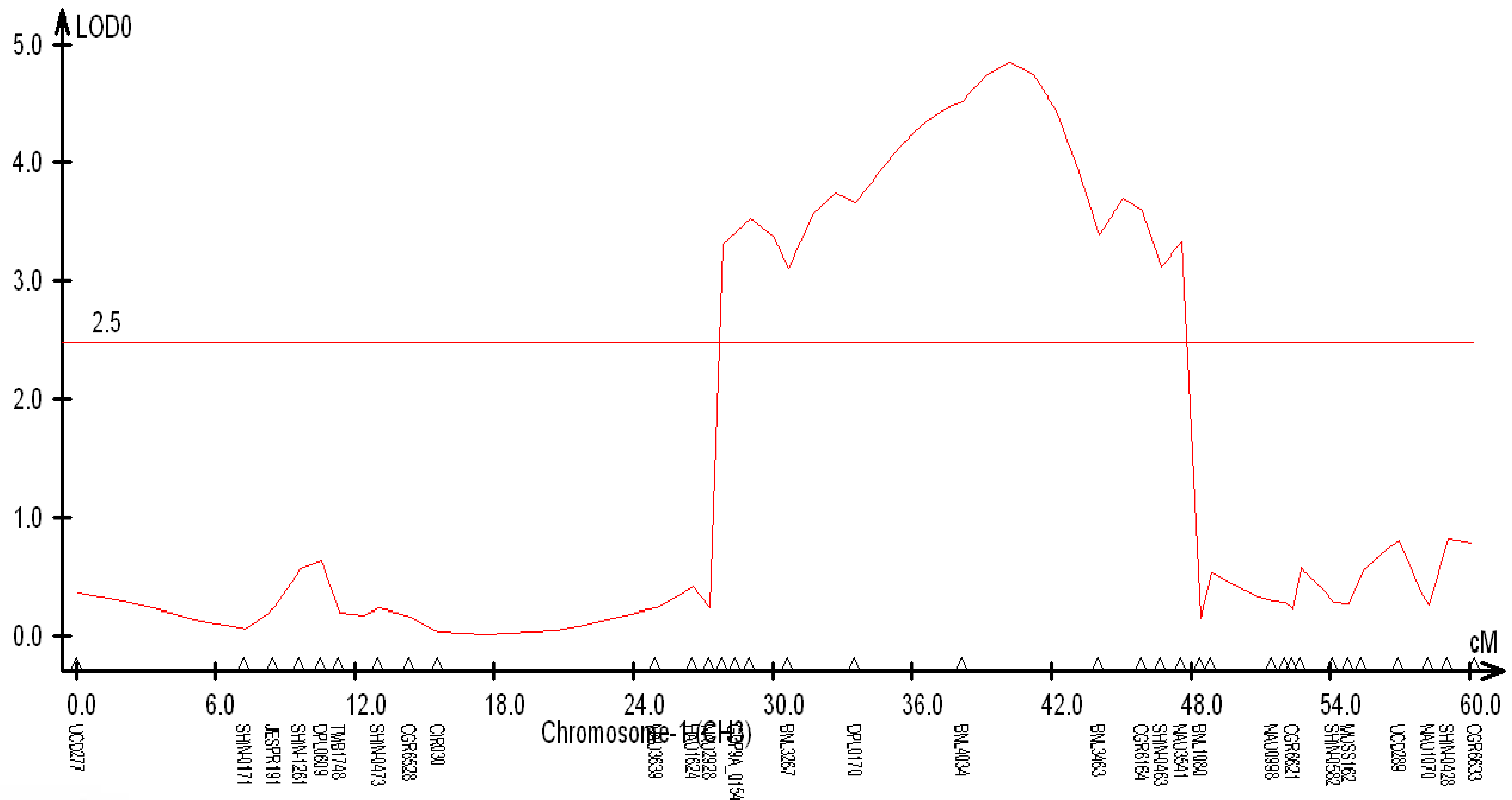
# Genetic Mapping Fiber Strength QTL in MD52ne

Trait	MD 52ne	MD 90ne	<i>p</i> value
FBS (g/tex)	39.95 ± 0.58	33.61 ± 0.51	8.22***
FE (%)	5.45 ± 0.06	5.96 ± 0.08	-5.19***
FM	0.89 ± 0.00	0.88 ± 0.00	1.72
MIC	4.94 ± 0.09	5.01 ± 0.20	0.77
UHML (in)	1.23 ± 0.01	1.17 ± 0.01	-4.53***
SFI	4.45 ± 0.19	6.34 ± 0.31	-5.17***

\*\*\*Significant at  $p = 0.001$



# Genetic Mapping Fiber Strength QTL in MD52ne



Fiber strength QTL on Chromosome 3



# Association Mapping Fiber QTLs Using Random-mated RILs

- Random mated population (RMUP-C5) involving six cycles of random mating beginning with a half diallel of 11 parents (COKER312, DP90, FM966, HS26, Ultima, M240, PSC355, SG747, Pyramid, ST474, ST825) developed by Dr. Jenkins and McCarty.
- 550 RILs ( $C_5S_6$ ) were planted in Starkville, MS during 2009-2012 to collect fiber quality and yield data.





# Fiber Quality Results of RILs (means of 2009---2011 field results)

	ELO	MIC	UHM	UI	STR	SFC
RIL Mean	5.10 ± 0.15	4.73±0.13	1.06±0.02	83.01±0.53	30.01±0.75	7.80±0.32
RIL Min.	3.21	3.33	0.89	79.20	24.33	6.10
RIL Max.	7.53	6.25	1.27	85.79	39.06	10.30
Parents Mean	5.62±0.14	4.65±0.10	1.11±0.01	83.70±0.31	30.43±0.46	7.48±0.17
Parents Min.	4.10	4.03	1.02	82.74	28.13	6.96
Parents Max.	6.96	5.11	1.17	84.66	33.23	8.03



# Molecular Markers

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- **Analyzed about 16,000 SSR markers for polymorphism among parents.**
- **About 2200 markers were polymorphic.**
- **Selected 1582 SSR markers based on their genetic and physical positions and analyzed among 275 RILs.**
- **Conducted Association mapping using JMP Genomics 6.0, TASSEL and R package.**

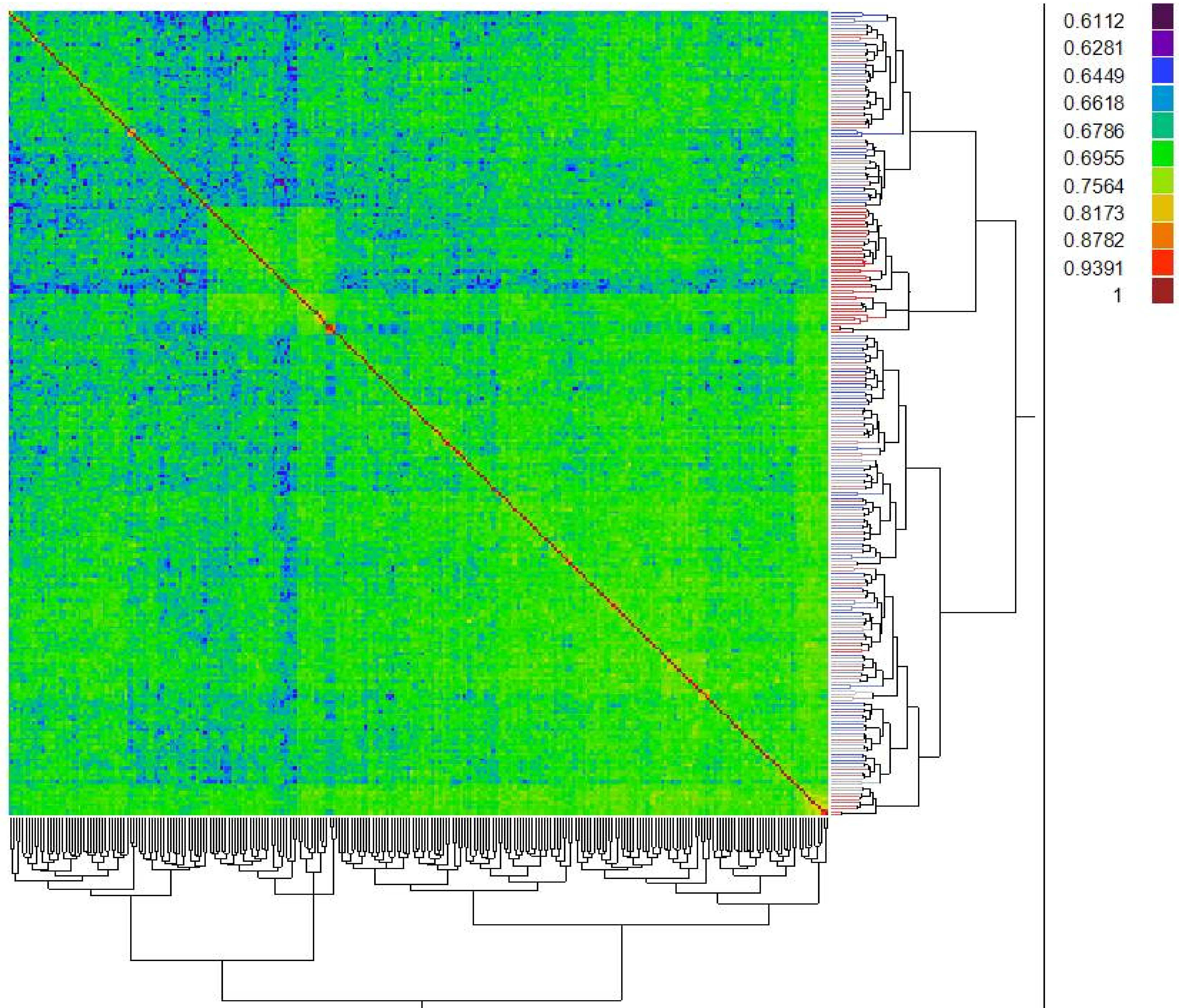


# SSR Markers Analyzed among 275 RILs

Chromosome	# Marker loci	Total cM covered by markers	Whole chromosome (cM)	% of the chromosome
Chr.01	59	125.34	155.10	81%
Chr.02	50	117.02	133.96	87%
Chr.03	80	132.90	170.81	78%
Chr.04	41	88.75	110.65	80%
Chr.05	126	122.27	148.31	82%
Chr.06	50	122.50	156.19	78%
Chr.07	67	136.58	168.73	81%
Chr.08	64	162.27	198.58	82%
Chr.09	78	118.86	146.06	81%
Chr.10	48	204.74	208.80	98%
Chr.11	75	190.40	228.17	83%
Chr.12	86	111.94	119.22	94%
Chr.13	61	112.78	134.35	84%
Chr.14	85	130.23	134.55	97%
Chr.15	96	127.76	230.77	55%
Chr.16	75	101.84	139.53	73%
Chr.17	44	122.07	142.87	85%
Chr.18	49	107.14	121.05	89%
Chr.19	117	161.00	201.23	80%
Chr.20	74	153.97	168.10	92%
Chr.21	72	142.06	173.91	82%
Chr.22	49	91.47	111.94	82%
Chr.23	67	113.42	170.92	66%
Chr.24	57	145.46	173.84	84%
Chr.25	50	121.76	154.44	79%
Chr.26	76	148.98	148.98	100%
unmapped	253			
Total <sup>§</sup>	2049	3413.51	4151.06	82%



# The RIL Population Was Thoroughly Random-mated

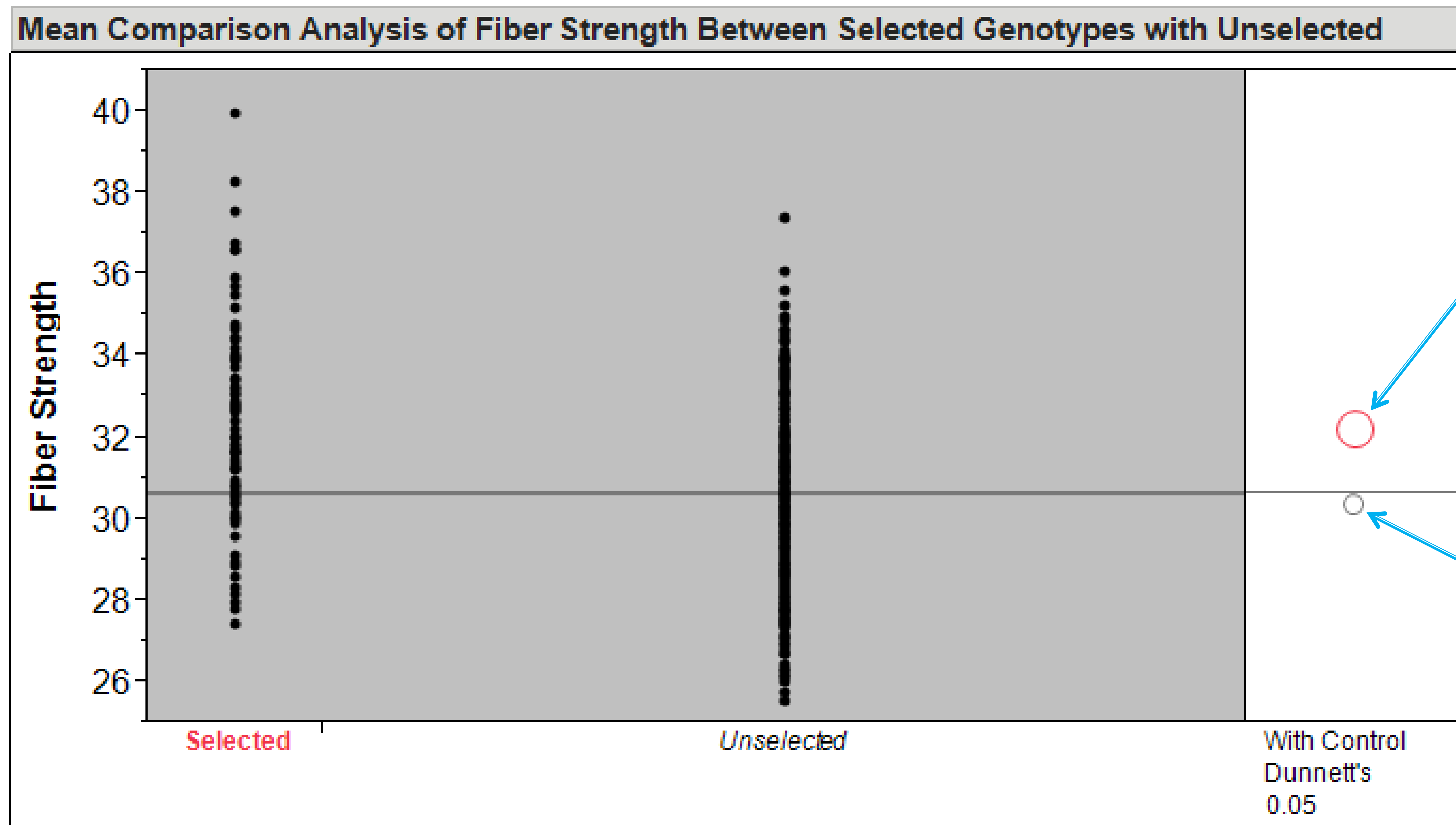


# Fiber Quantitative Trait Loci Identified

Trait	No. QTLs	No. Chromosomes
Elongation	13	11
MIC	11	6
SFC	8	7
Strength	12	12
UHM	11	9
Uniformity	8	5



# Example: Fiber Strength QTL

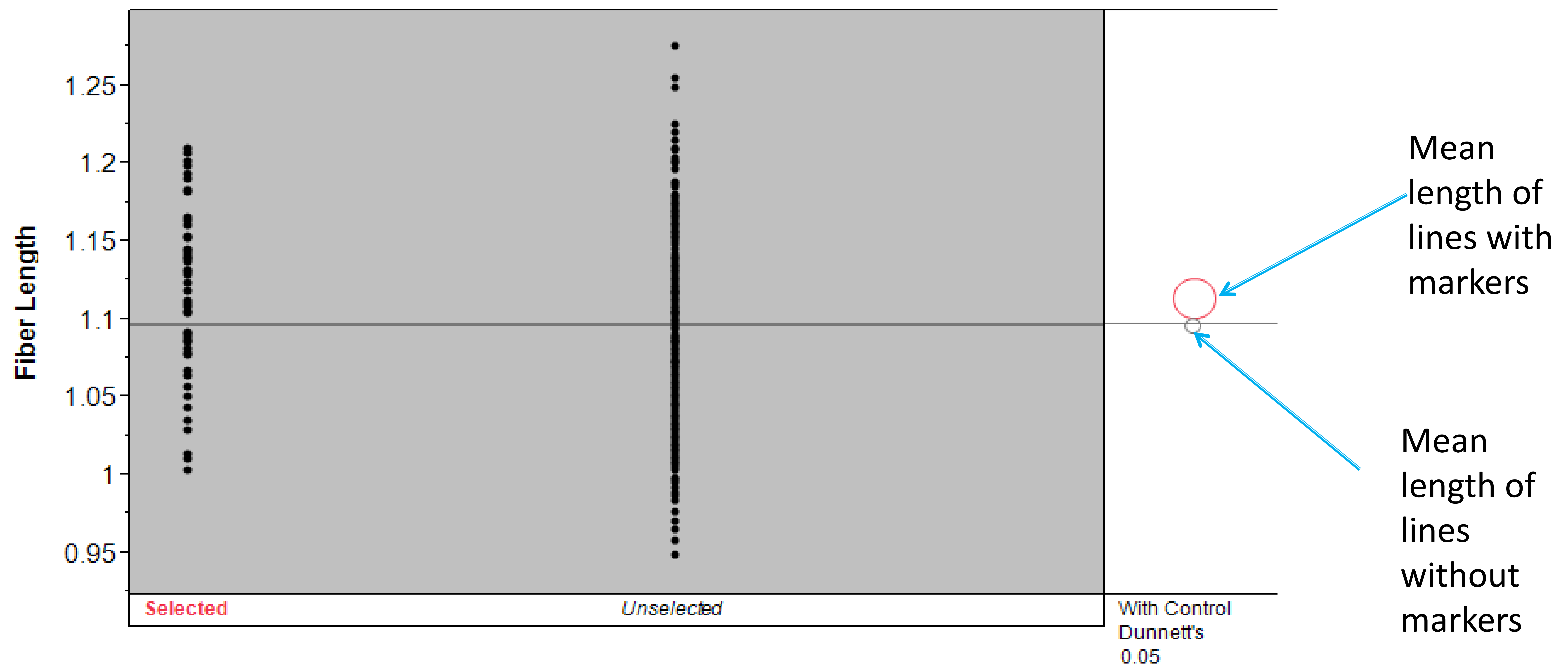


Mean strength of lines with markers

Mean strength of lines without markers



# Example: Fiber Length QTL



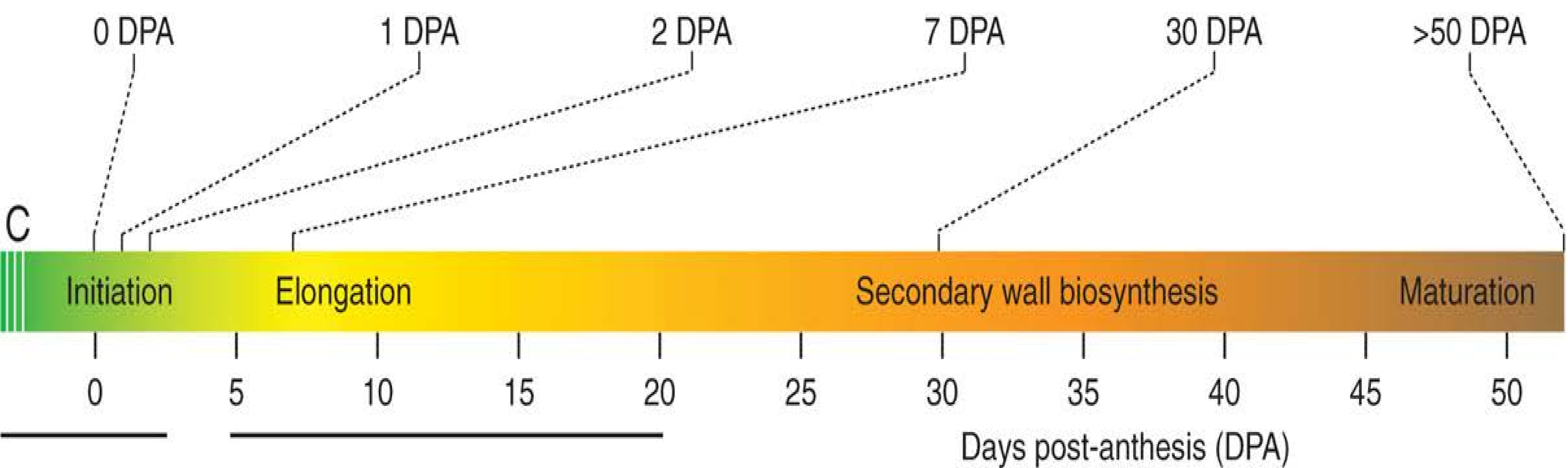
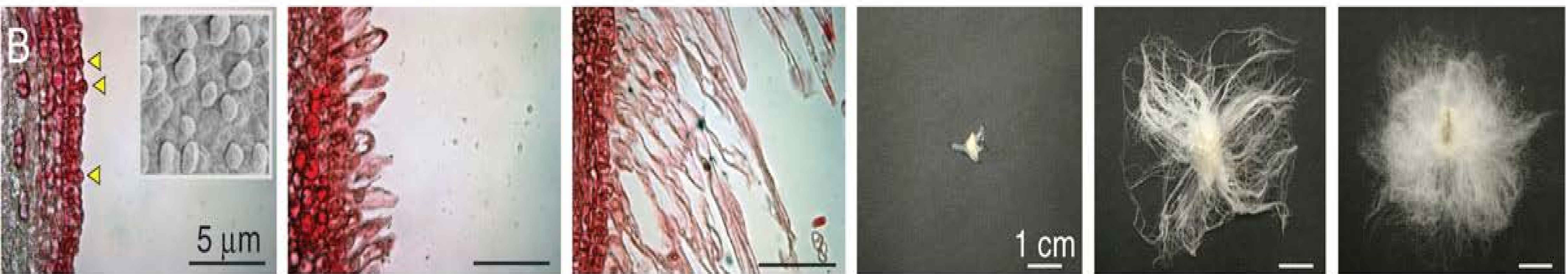
# Future Work

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- **Genotyping the RILs with more DNA markers.**
- **Collecting more fiber and yield data.**
- **Develop a sub-set of highly informative markers and enabling technology (such as putting all good markers in a chip) that breeders can easily use in their breeding programs.**







(Lee et al, 2007)

# Identification of Genes Affecting Fiber Development

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- **Goal:** Identification of genes affecting fiber development.
- **Materials:** fiber mutants
- **Methods:** combination of microarray, RNA-Seq, and genetic mapping
- **Results:** important genes that can be used to manipulate fiber development via transgenic approaches.





**Lintless (Li1 & Li2)**



**Fiberless (N1 & n2)**

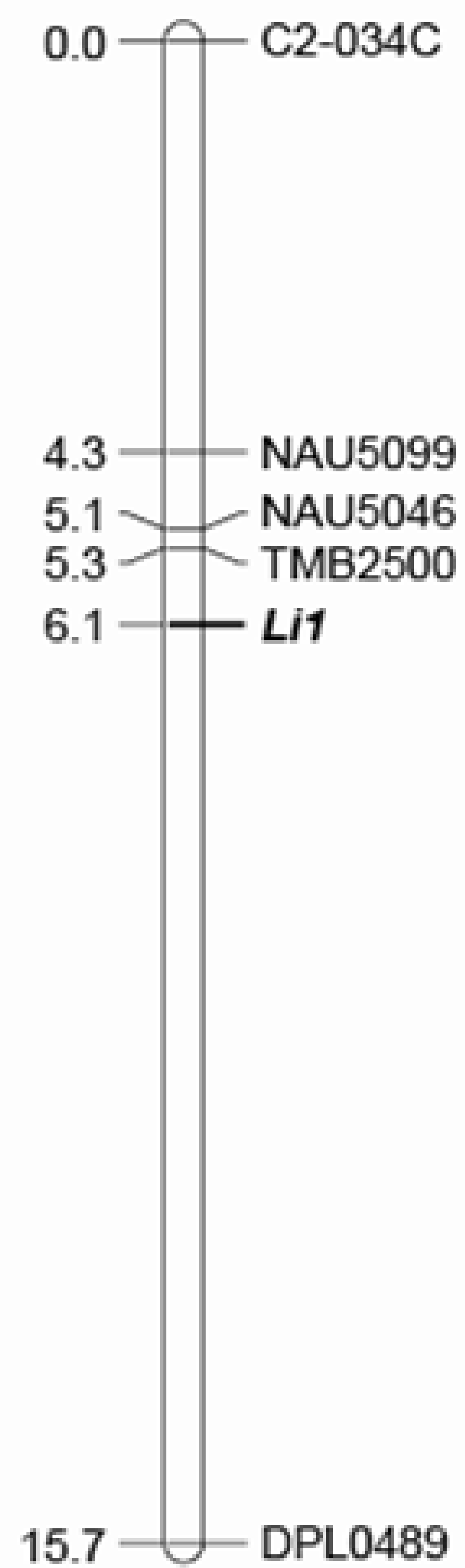


**Normal Cotton**

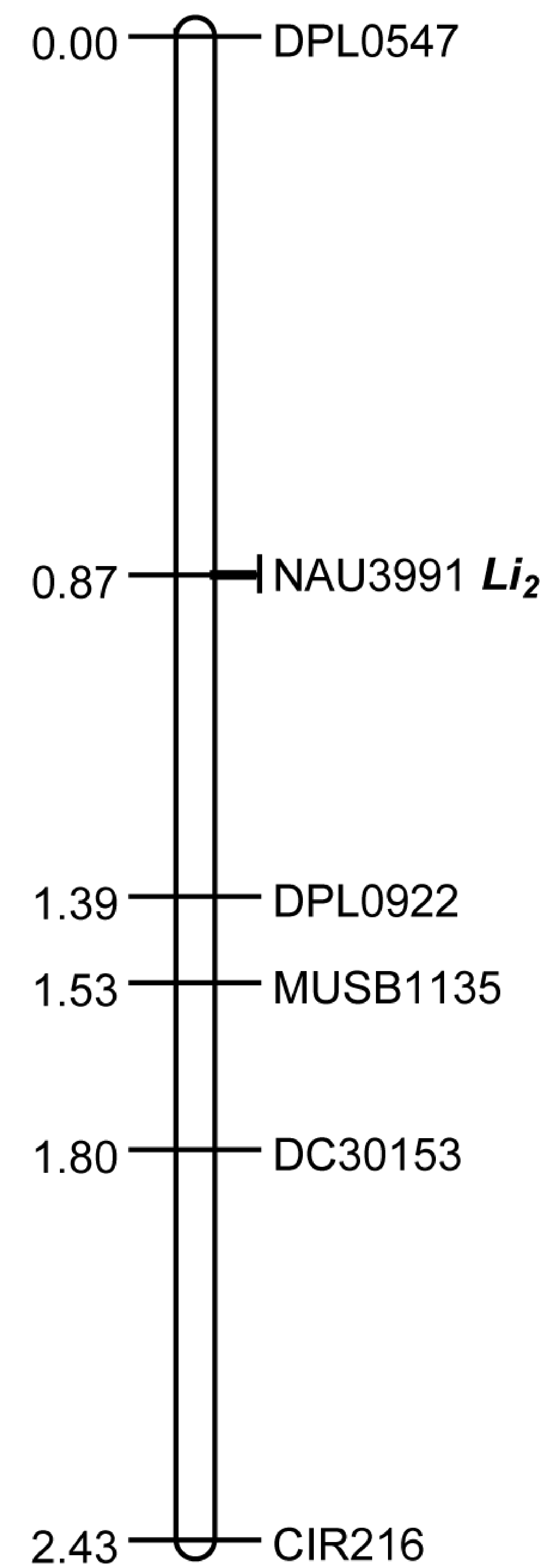
**(Courtesy of Dr. Turley)**

# Mapping Genes Affecting Fiber Length

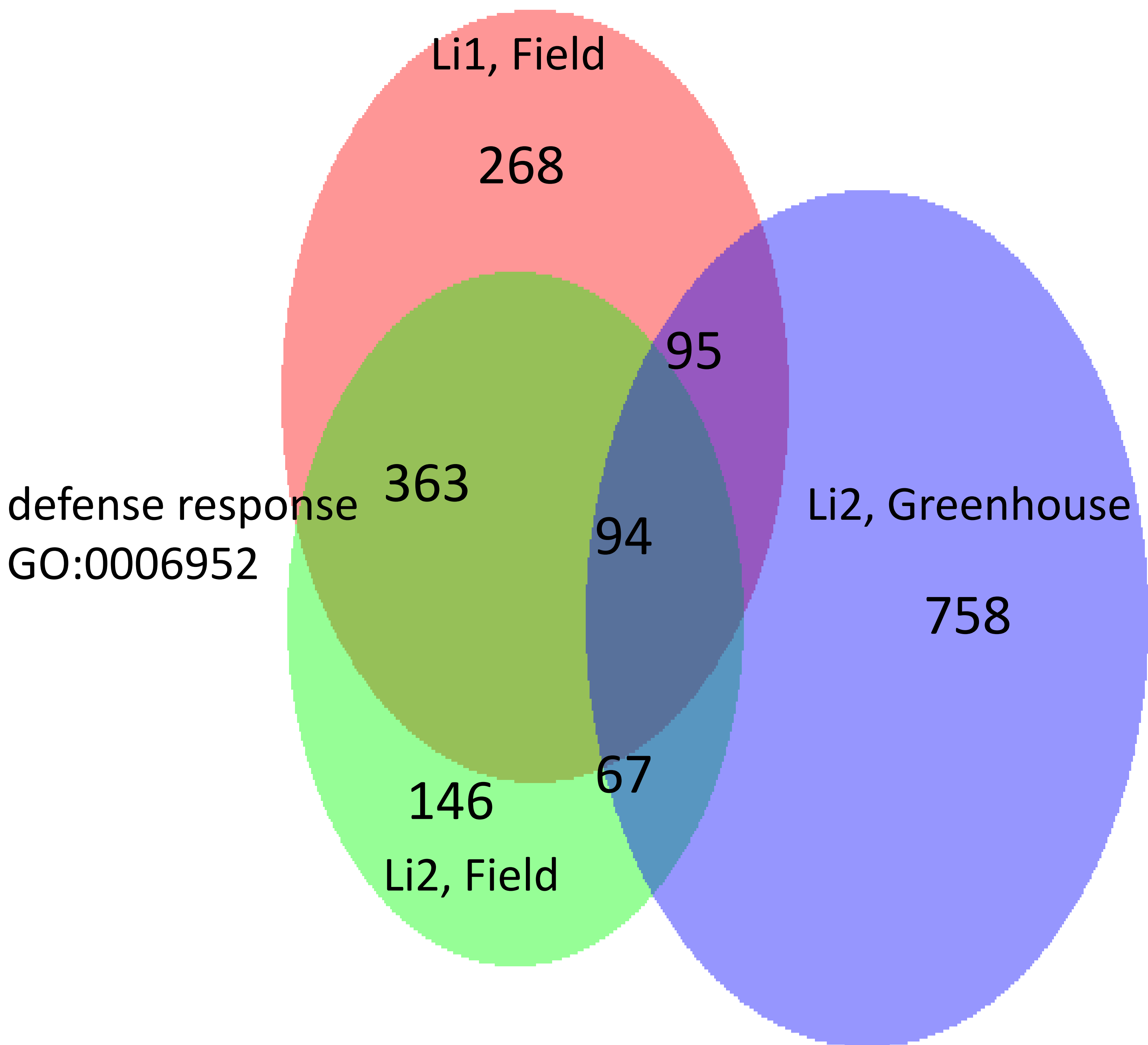
$Li_1$  Region  
Chr22



$Li_2$  Region  
Chr18



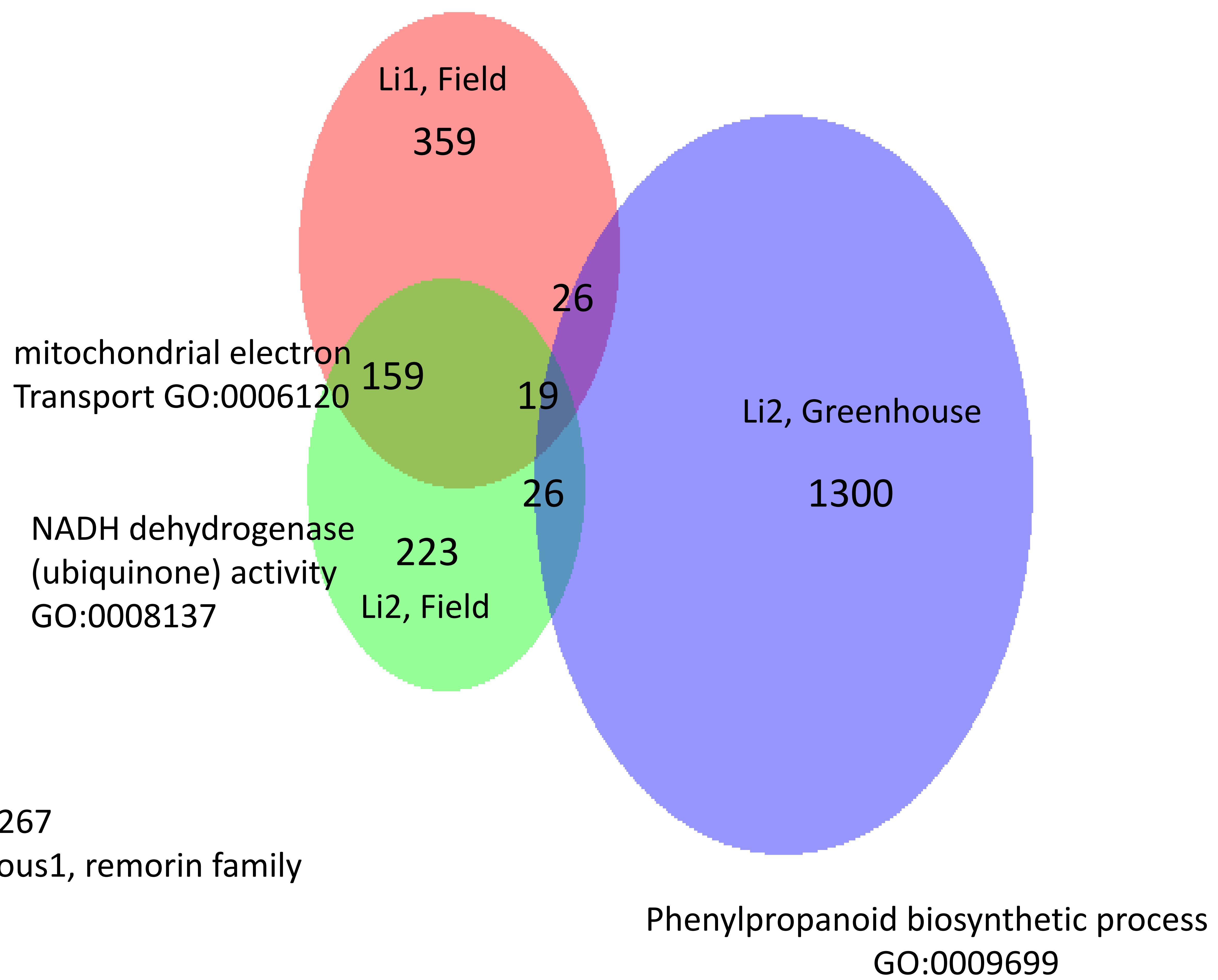
## Down-regulated



defense response  
GO:0006952

Cell-cell signal GO:0007267  
eg, glabrous1, remorin family

## Up-regulated



mitochondrial electron  
Transport GO:0006120

NADH dehydrogenase  
(ubiquinone) activity  
GO:0008137

Phenylpropanoid biosynthetic process  
GO:0009699

# Genes Affecting Fiber Length

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- Of the 113 probes that are common between the 3 data sets, 25 show variation between field and greenhouse, meaning they may be environmentally regulated and not strictly elongation related.
- This leaves 88 total probes.
- Among them are (examples):
- Ghi.3235.1 GhUGT73C14 (UDP-glycosyltransferase)-Glycosylates ABA
- GhiAffx.33535.1 Gh actin depolymerizing factor 5 (ADF5)-established role in polymerizing/depolymerizing Actin
- Ghi.10822.1 Gh xyloglucan endotransglucosylase/hydrolase (XTH2) - restructures primary cell wall. There are many XTH's, thus elucidating which is important in cotton is relevant.



# Genes Affecting Fiber Length

- We identified a UDP-glycosyltransferase (UGT73C14) that was highly expressed in *Li2* mutant.
- Expression of UGT73C14 in *E.coli* showed enzymatic activity toward ABA and *trans*-zeatin.
- Transforming the UGT73C14 into Arabidopsis resulted in higher ABA glycosylation.
- This UGT may affect cotton fiber length development via regulation of ABA homeostasis



**TM-1**

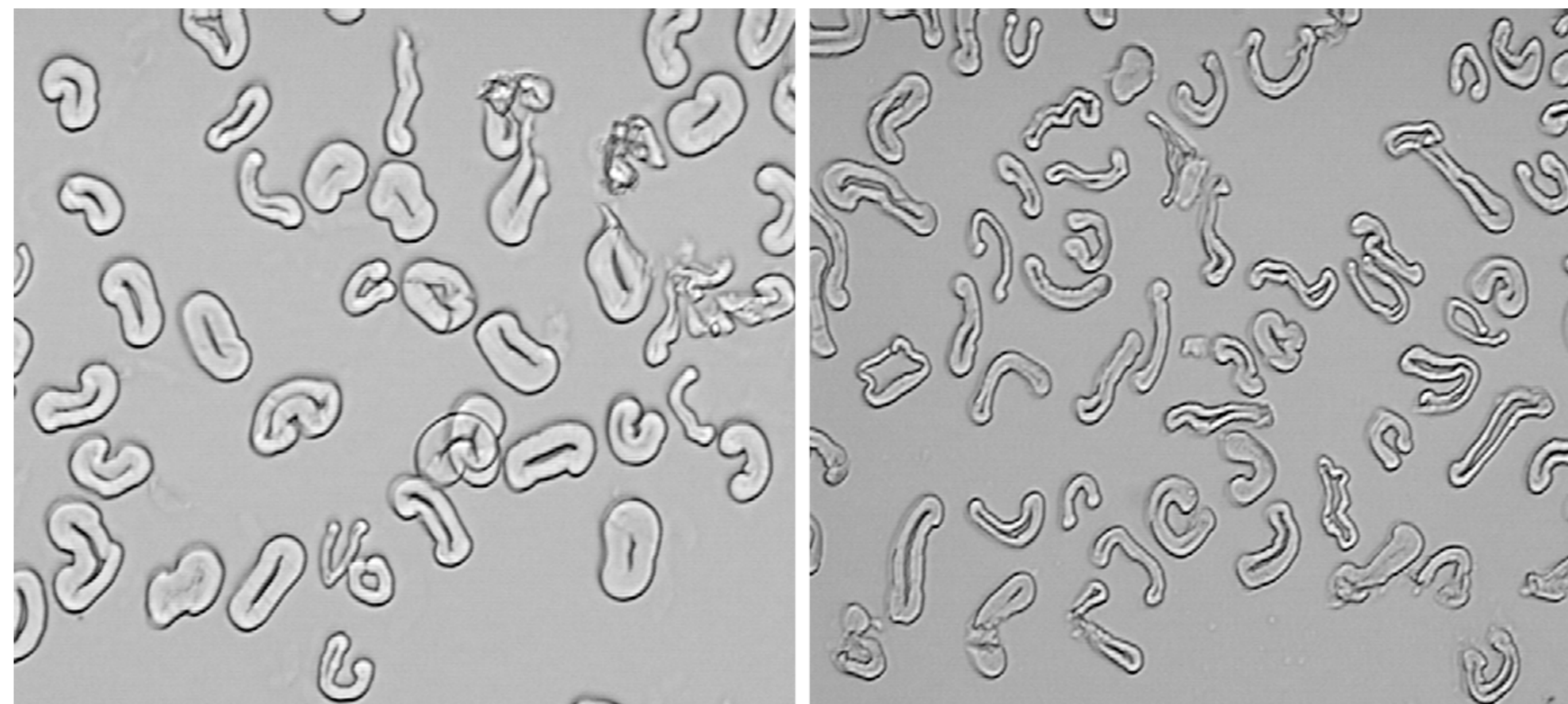
***im***





# Genes Affecting Fiber Maturity

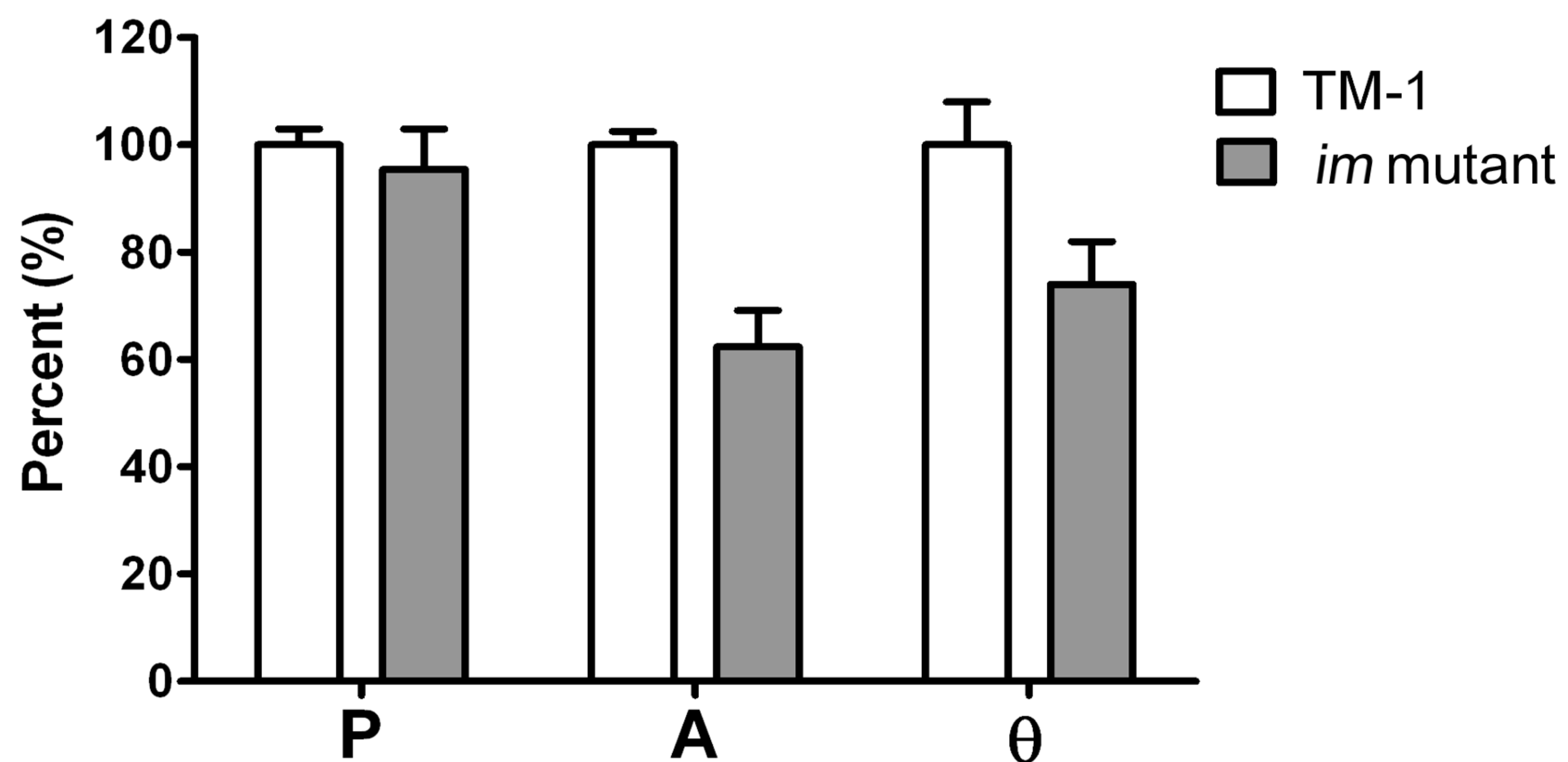
**a**



TM-1

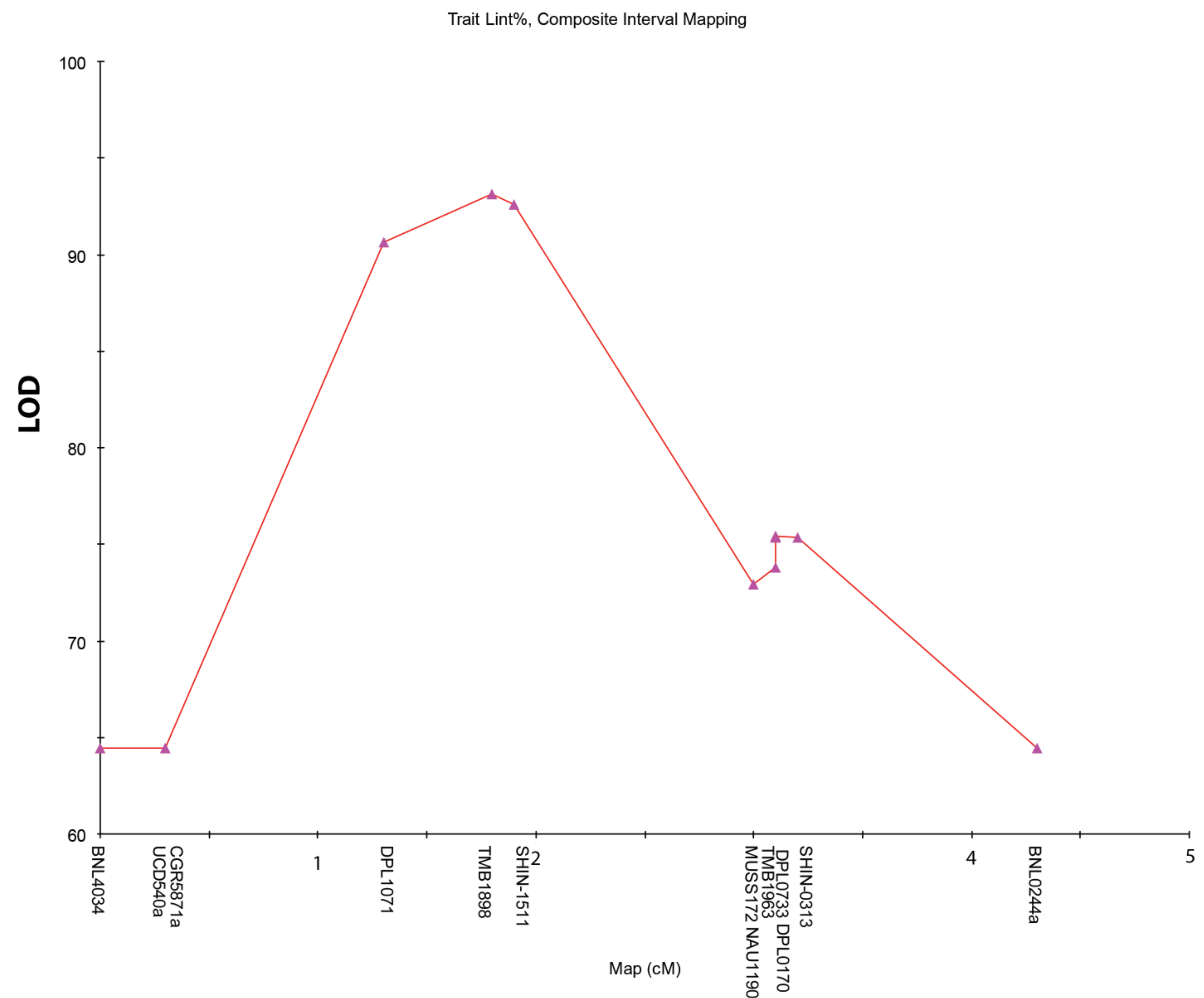
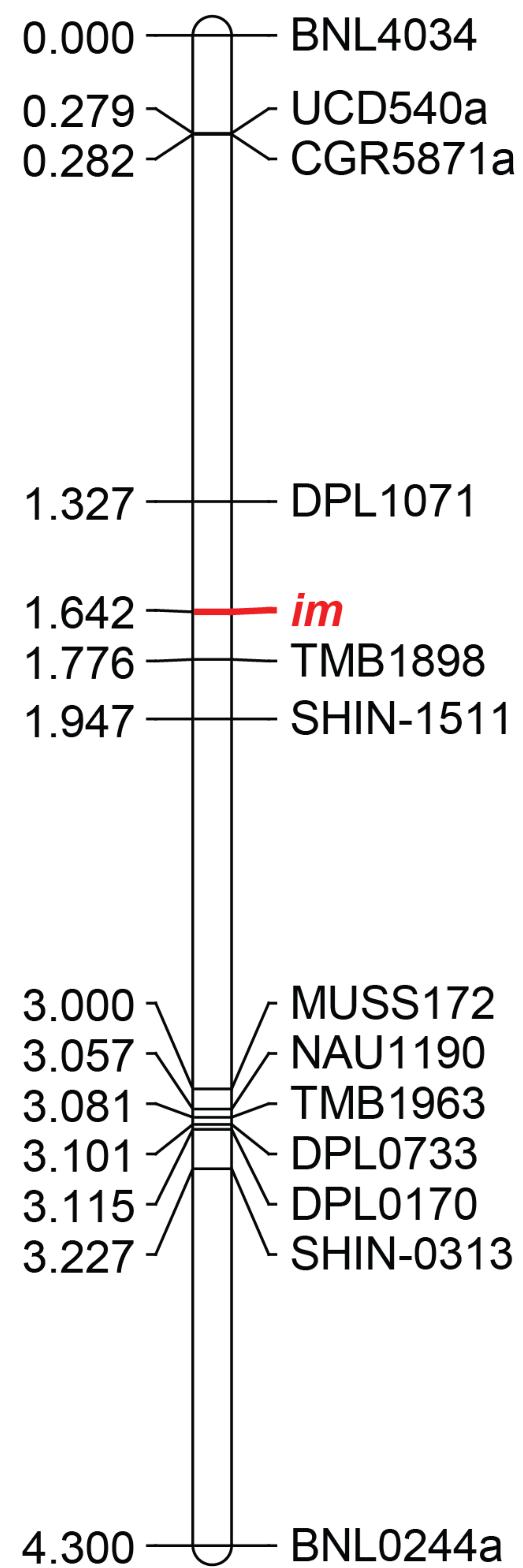
*im* mutant

**b**



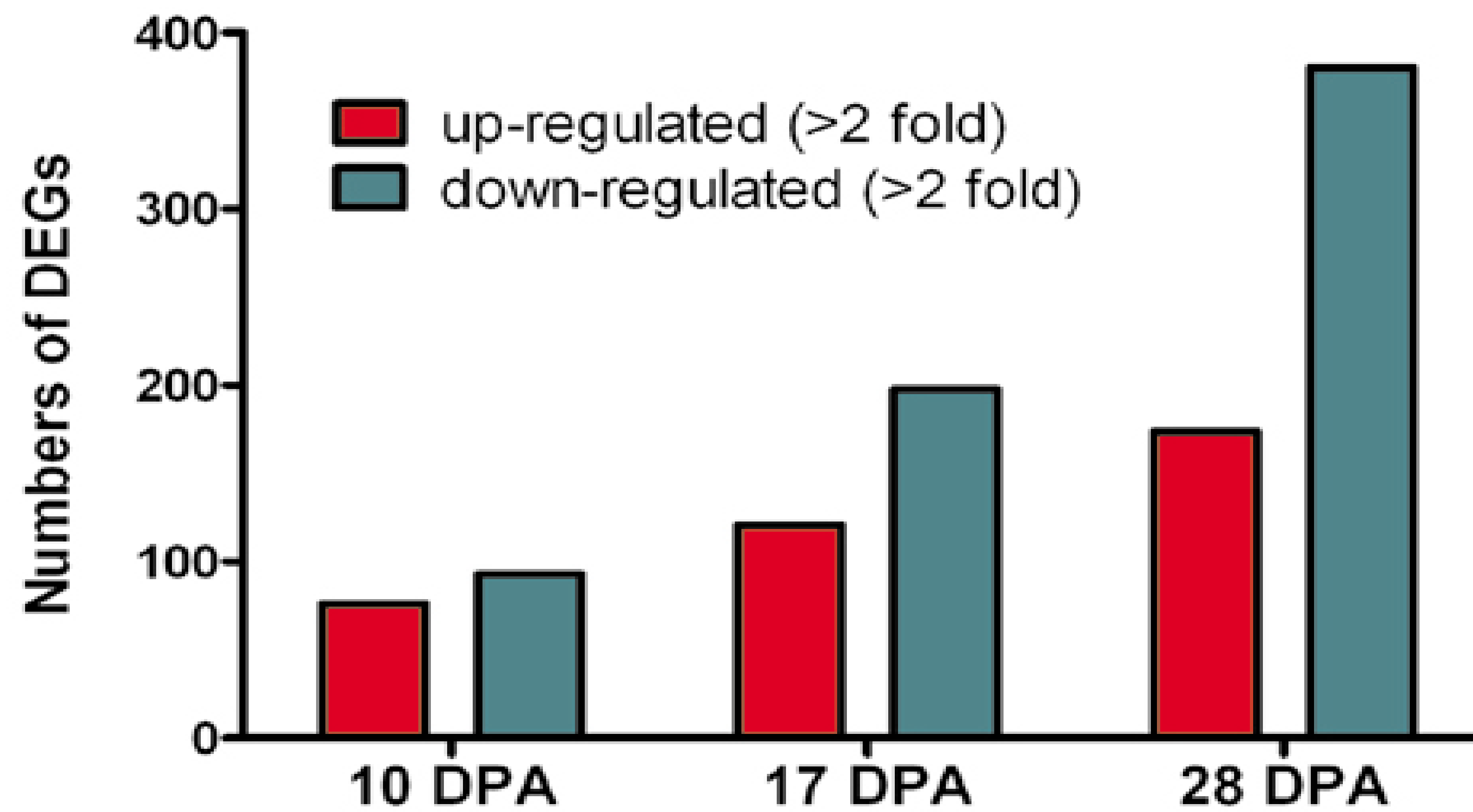
# Genes Affecting Fiber Maturity

## Chr.03

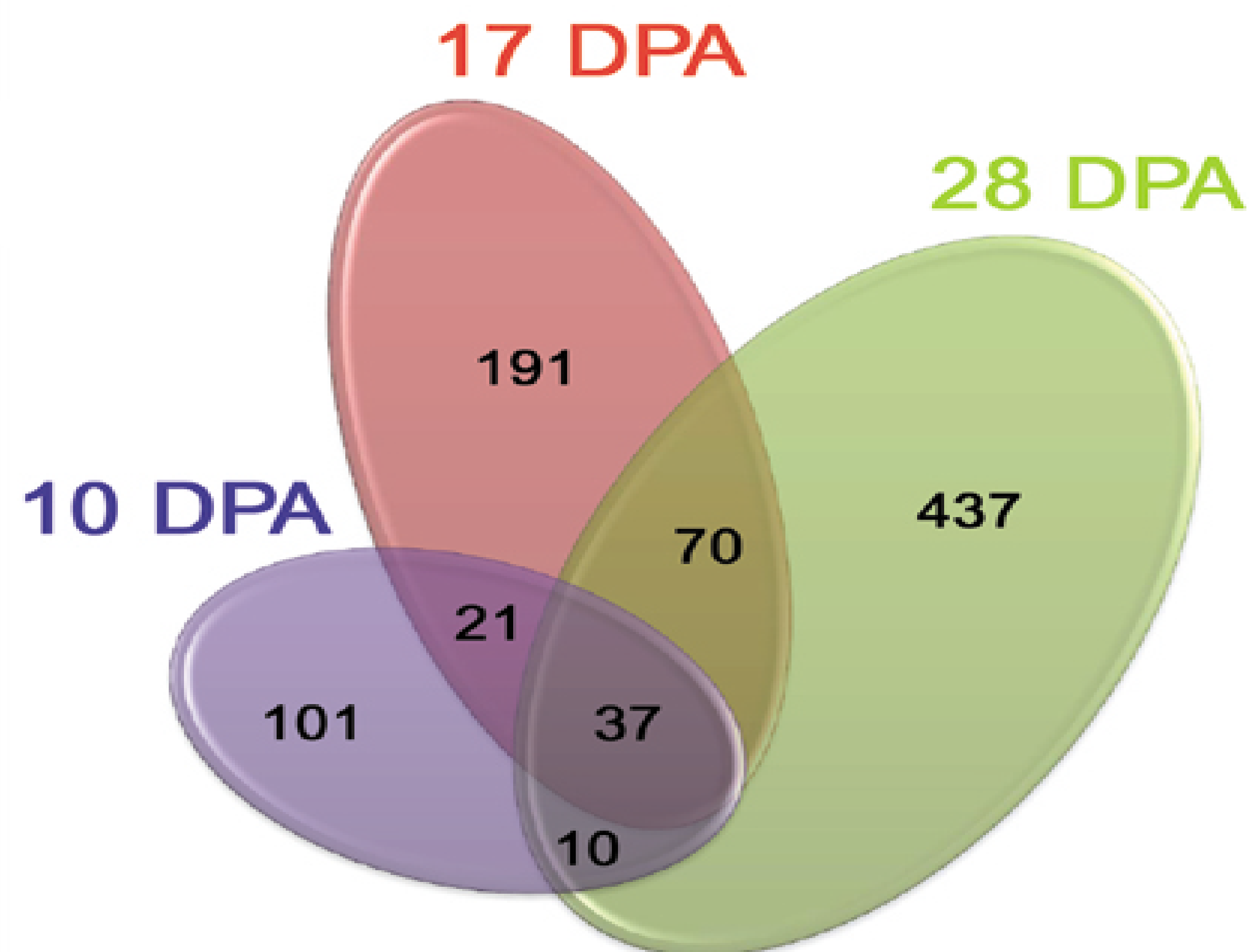


# Fiber Cell Wall Development is Associated With Sensitivity to Stress

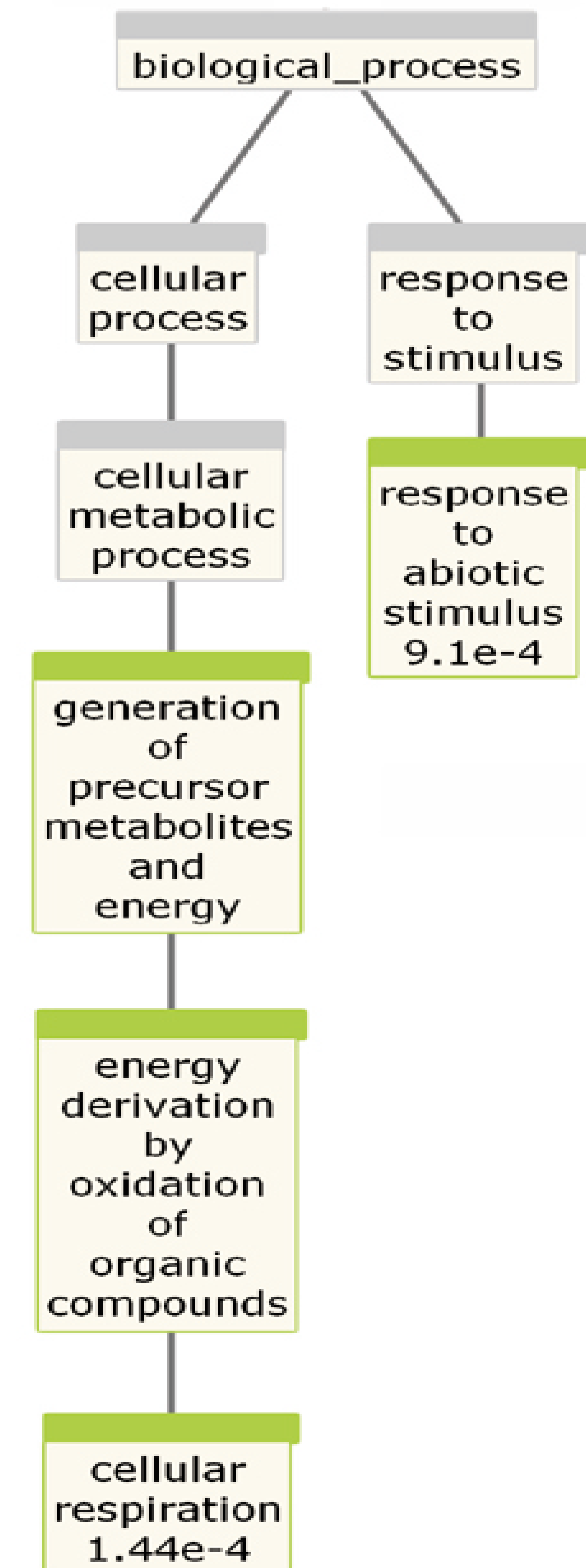
**A**



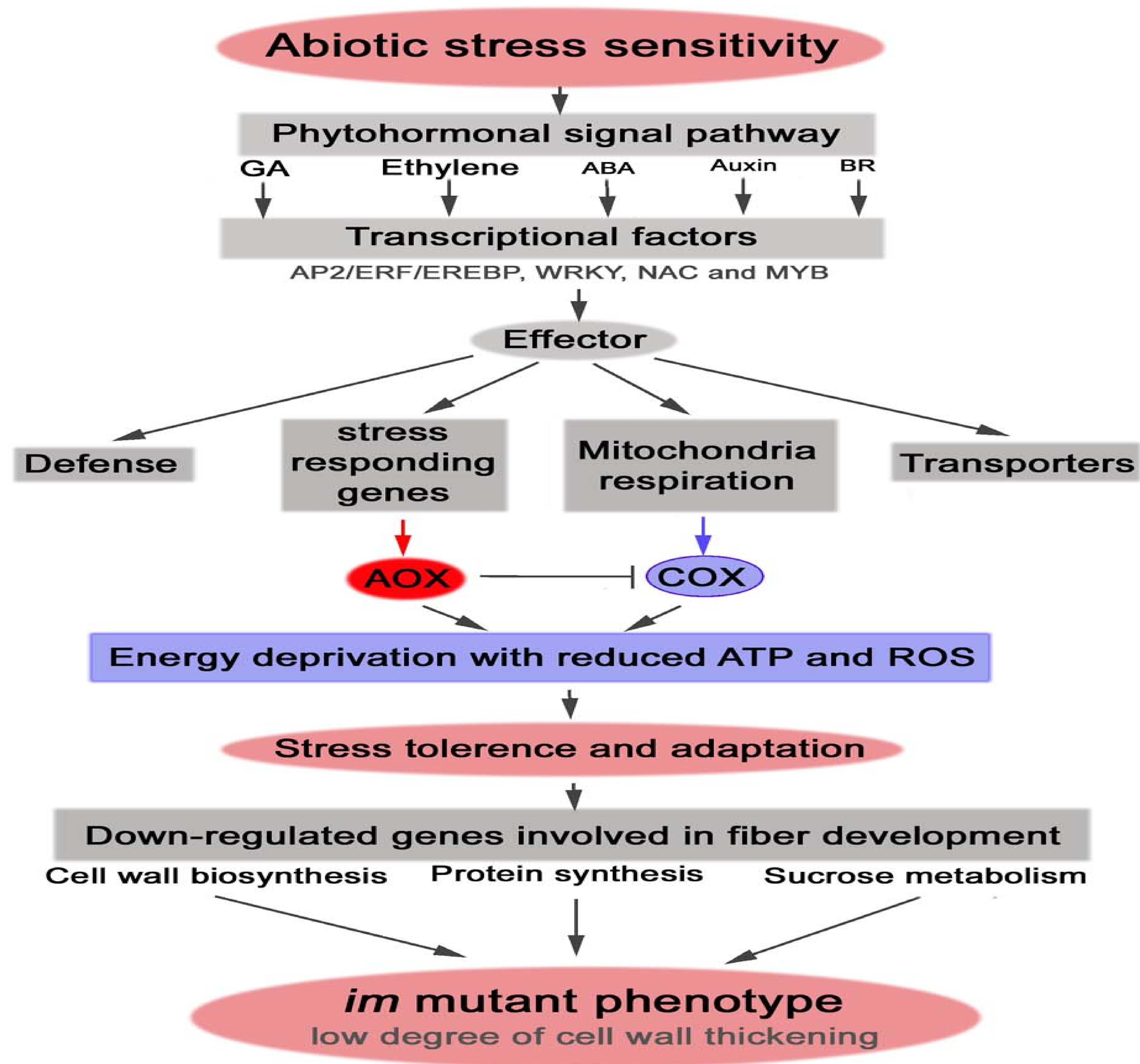
**B**



**C**



# Fiber Cell Wall Development is Associated With Sensitivity to Stress



# Acknowledgement

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**Mr. Chris Delhom**

