

Breeding of drought tolerant canola: from lab to field

Guangyuan Lu

Oil Crops Research Institute , CAAS



中国农业科学院油料作物研究所

OIL CROPS RESEARCH INSTITUTE CHINESE ACADEMY OF AGRICULTURAL SCIENCES

诚信为本 创新惟先 本领过硬 业绩一流



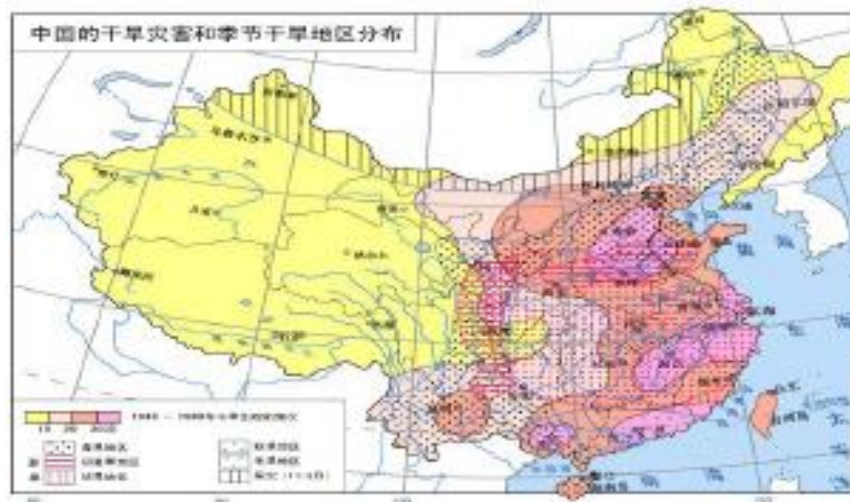
Rapeseed : The most important oil crops in China

- Major rapeseed growing area
- 4 million tons of edible oil



Drought cause severe yield lost

- Global warming, extreme climate
- Nation-wide reduction of yield per up to 15%
- Breeding of drought tolerant variety is an urgent task.



Severe drought in 2009 winter

- Area: Northern and central China
- Duration: 70—112 d
- Acreage: 0.9 m ha
- Severity: $DI=0.47$
- Impact: ~60% yield lost

Lingering drought threatens harvest across C China

By Guo Rui (China Daily)
Updated: 2011-05-05 07:19

[Comments\(0\)](#) [Print](#) [Mail](#) [Large](#) [Medium](#) [Small](#)



Huang Shenghua, a farmer in Xingguang village, Xiaochang county, in Central China's Hubei province, inspects a field of dying rapeseed plants on Tuesday. The province has endured a severe drought for the past five months. [Photos/Xinhua]

Question 1

Genetic variation for drought tolerance?

Question 2

Genes responsible for the tolerance?

Question 3

How to breed drought tolerant variety?

PART I

Discover of tolerant genotypes



Screening method

Method 1: 10% PEG6000

- 10% PEG6000 → Germination at RT → 5 d
Germination power, 7 d germination
percentage
- Indicator: relative germination rate

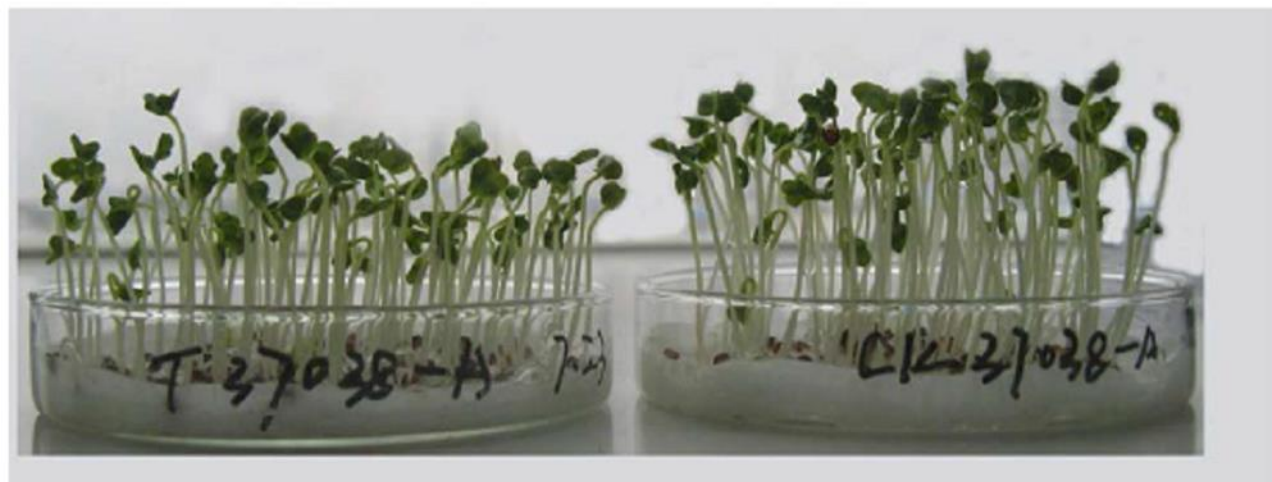
Susceptible



10% PEG-6000

CK

Tolerant



Method 2: Mimic raining

Direct seeding



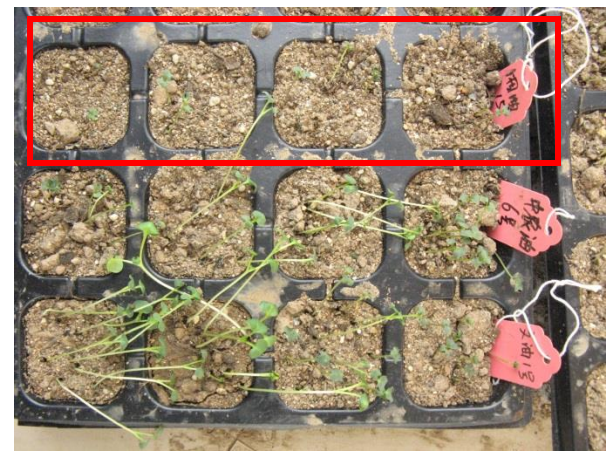
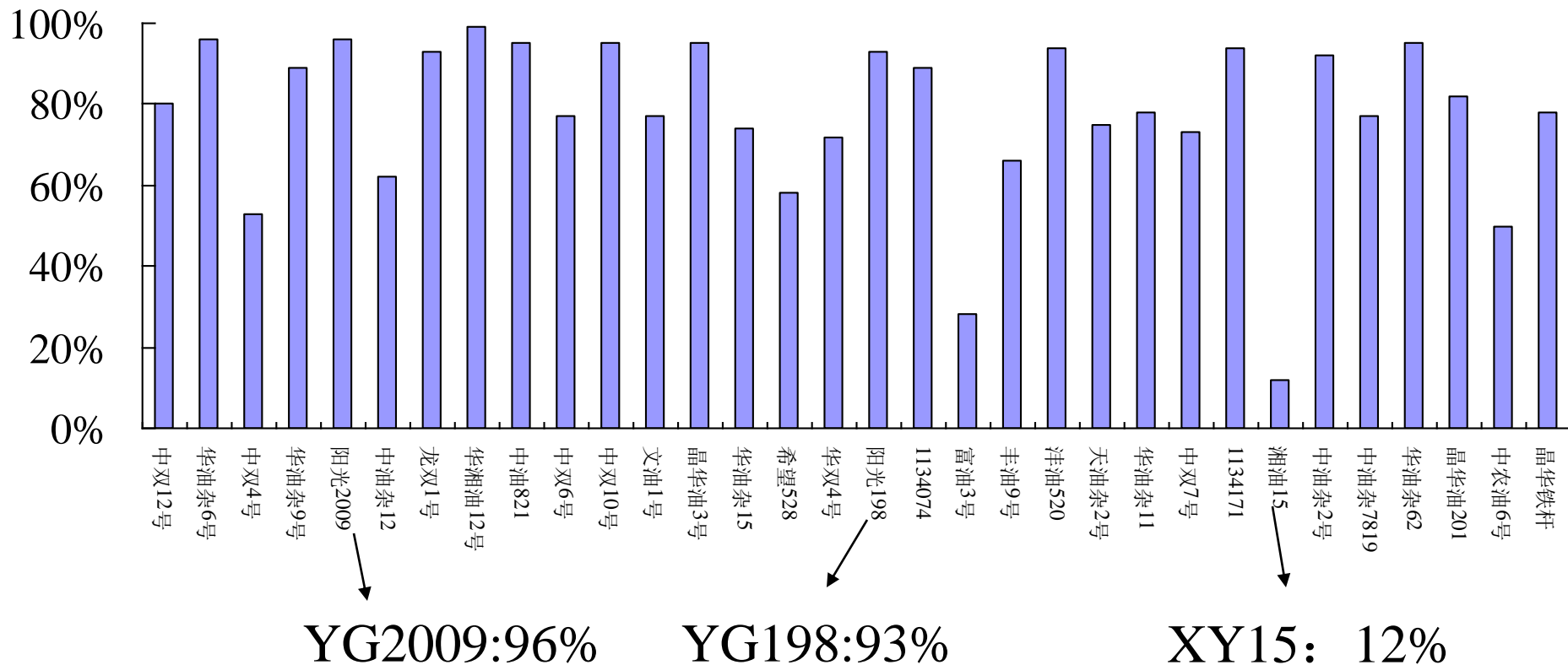
Mimic light rain (2mm precipitation)



Evaluation of germination



Germination rate



Method 3: Control watering in pot



Surviving rate in plastic box

Tolerant

Sensitive



Before drought treatment



After drought and re-watering

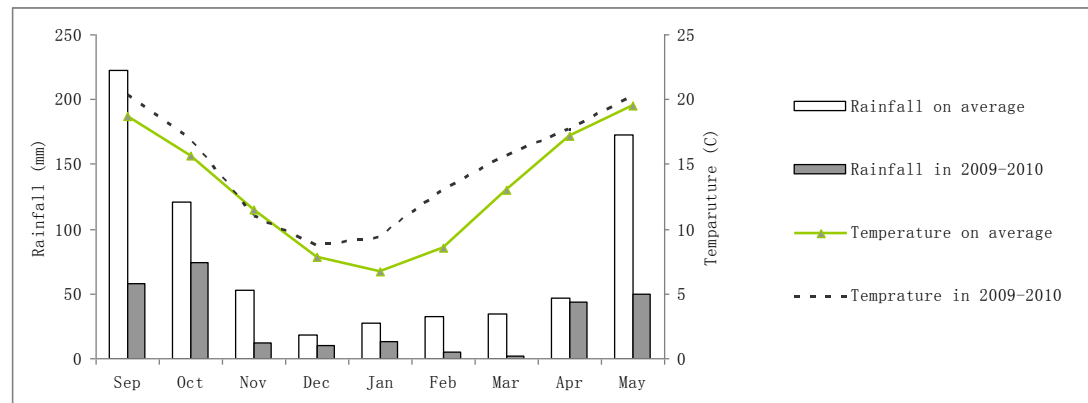
Method 4: Green house

- **Random blocks, stress at flowering stage**
- **Indicator: yield, roots, etc.**

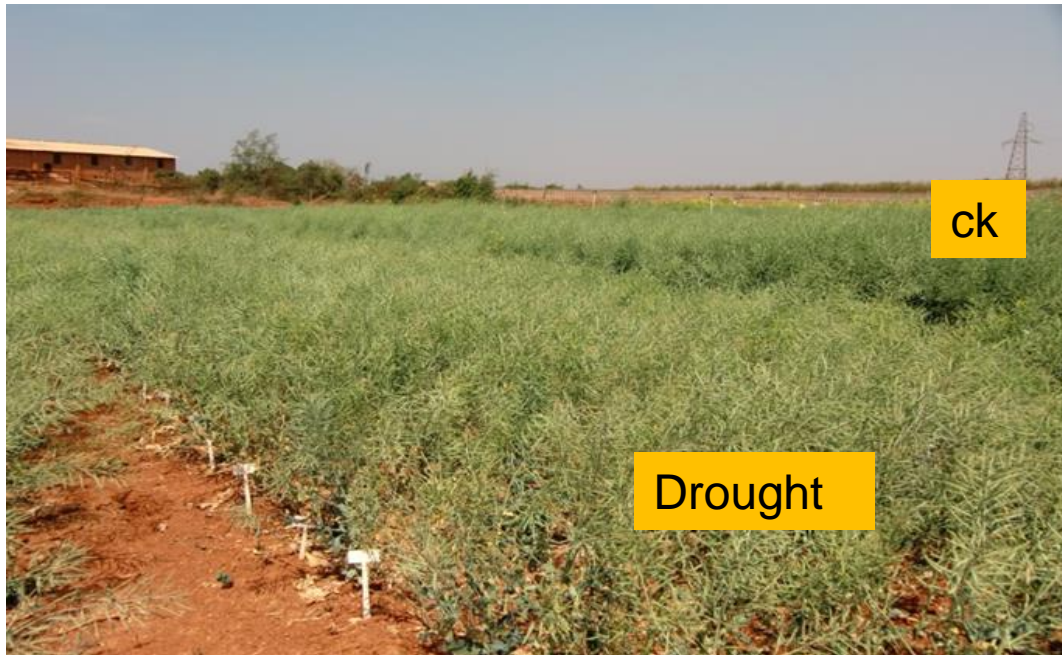


Method 5: Field test

- Genotype: 39 varieties
- Location: Luoping, Yunnan, China
- Design: 3 replicates, 15 m²



Severe drought stress: only 30% rainfall



Impact on agronomic traits

Trait	CK	Drought	% reduction
Plant height (cm)	218.51	161.04**	26.3
Height of branch	134.76	91.02**	32.5
Primary branches	9.31	8.55	8.7
Main raceme length (cm)	43.64	33.51**	23.2
Pods/cm	1.78	1.63	8.4
Growth duration (d)	217.91	185.55 **	14.9
Pods/plant	431.64	308.41 *	28.5
Pods/silique	15.44	11.02 *	28.6
1000-seed weight (g)	3.99	2.56 **	35.8
Plot yield (kg)	4.66	1.47 **	68.5

Yield performance

	CK (kg/ha)	Drought (kg/ha)	DSI
Min	2833.5	44.9	0.35
Max	4633.5	2339.1	1.25
Mean	3475.3	743.2	1.00
C.V. (%)	11.8	76.4	19.6

$$DSI = \frac{(1 - Y_s/Y_i)}{(1 - \bar{Y}_s/\bar{Y}_i)}$$

where Y = mean of a characteristic; \bar{Y} = experimental mean;
 s = moisture stress and i = irrigated environment.

Identification of extreme tolerant genotypes by seed yield (kg/ha)

Genotype	CK	Drought	DSI	Type
09S301	3150.1	2281.8	0.35	Tolerant
09S204	4183.5	2339.1	0.56	Tolerant
09S103	3003.4	1558.5	0.61	Tolerant
09S202	4166.8	1779.6	0.72	Tolerant
09S208	3516.9	1463.5	0.74	Tolerant
09S305	3516.9	256.6	1.17	Sensitive
09S309	3433.5	220.0	1.19	Sensitive
09S111	3133.5	162.4	1.20	Sensitive
09S213	3875.2	181.5	1.21	Sensitive
09S101	2933.5	44.85	1.25	Sensitive

Impact on seed quality

Treatment	Oil content %	Glucosinolate umol/g	Protein %
Drought	37.71	26.39**	26.21**
CK	45.12**	21.95	23.32

Note: n=39

PART II

Genetic study



QTL mapping of drought-related traits

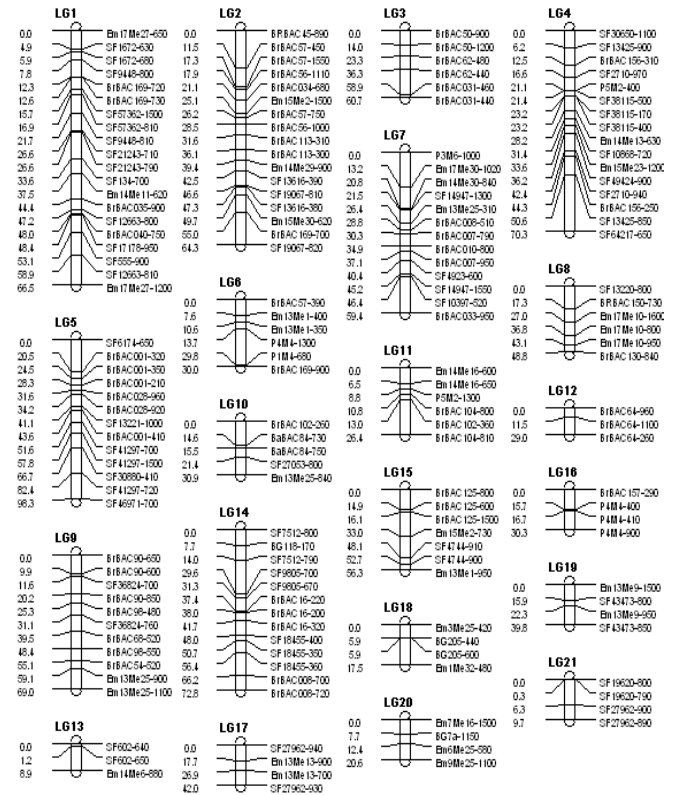
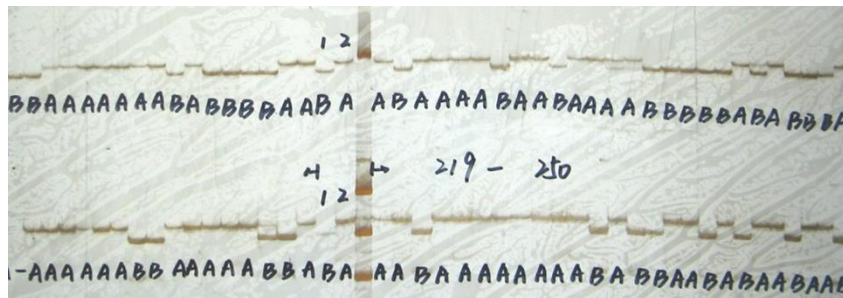
Trait	Environment	Parent	
		QY8	HY16
height	well-watered	60.4	20.0
	water-stressed	47.6	8.2
plant fresh weight	well-watered	137.7	122.1
	water-stressed	65.4	43.7
leaf wilting index	well-watered	0.99	0.98
	water-stressed	0.80	0.36

Genetic linkage map

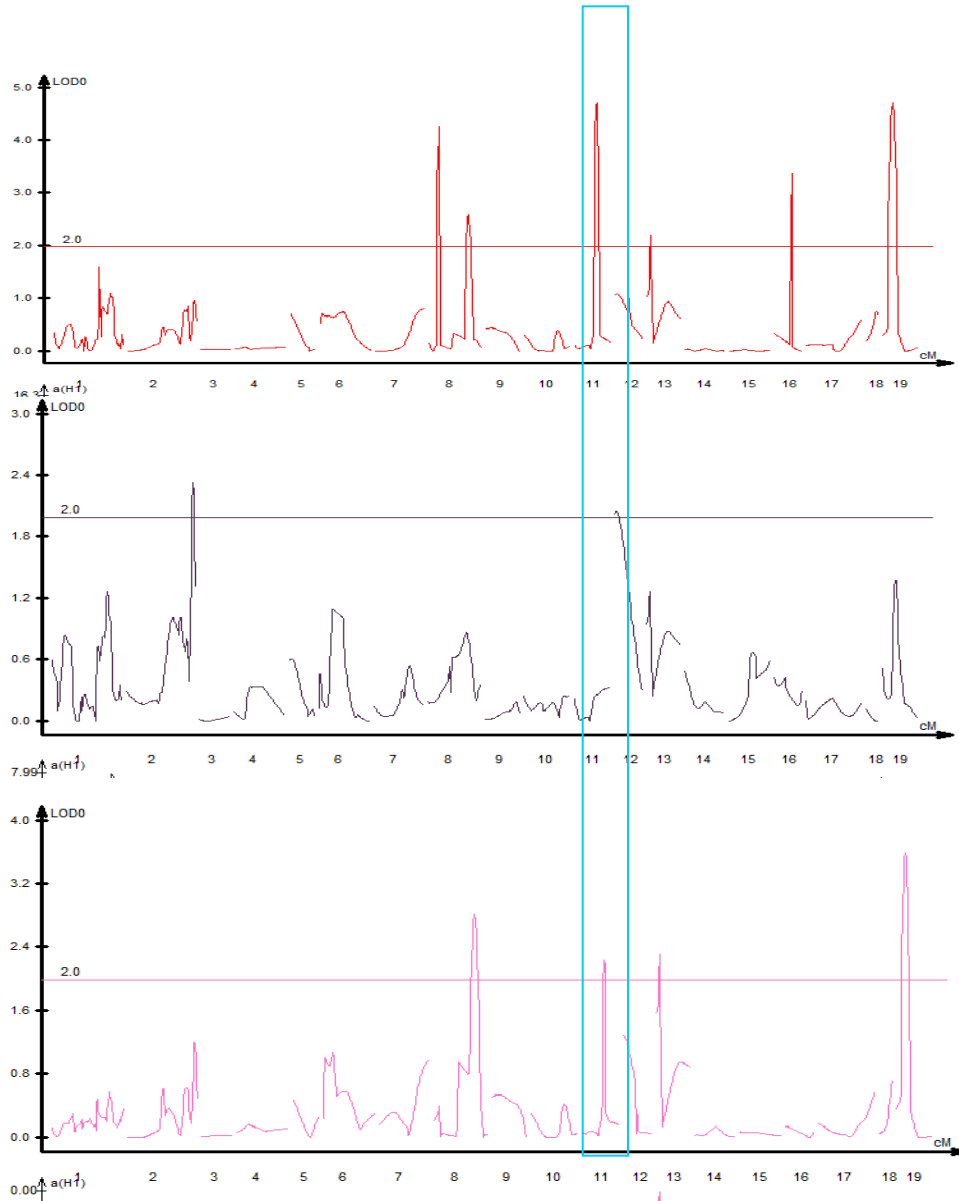
- 236 SSR, SRAP markers
- Linkage map

Total: 2010.49 cM

Average: 6.89 cM

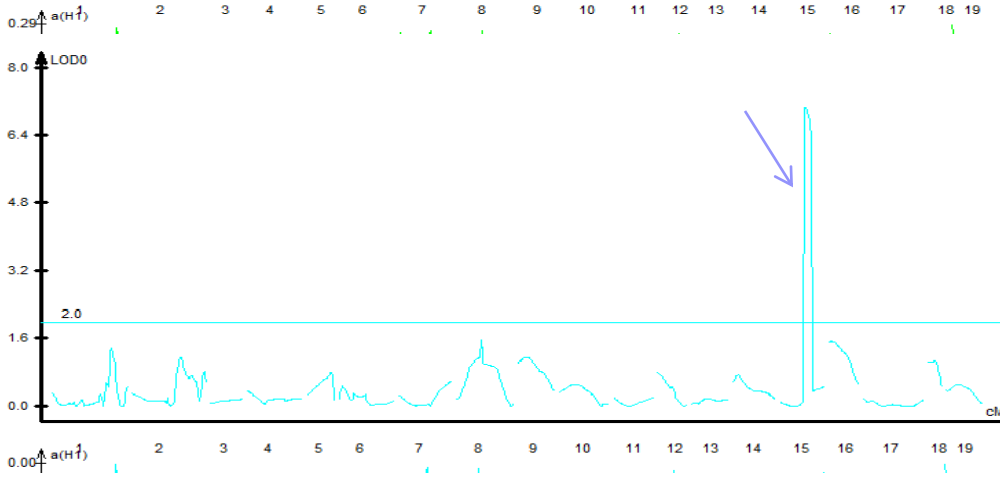
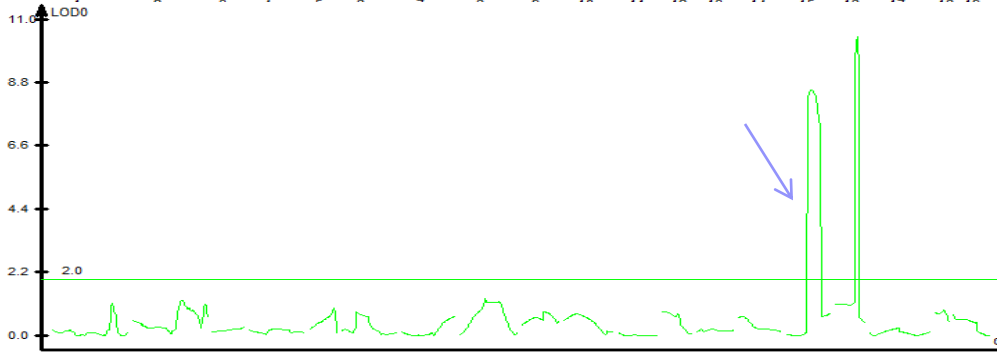
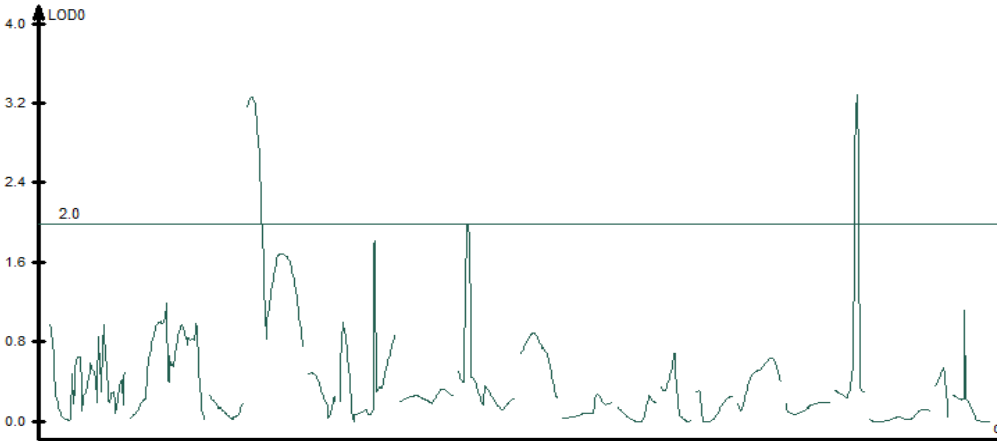


Fresh weight QTL



Treat.	QTLs	LG	% explained
CK	6	8	2.034
		8	4.687
		11	9.849
		13	11.607
		16	5.814
Drought	2	2	4.259
		11	8.781
Drought index	4	8	10.858
		11	11.201
		13	7.213
		19	5.072

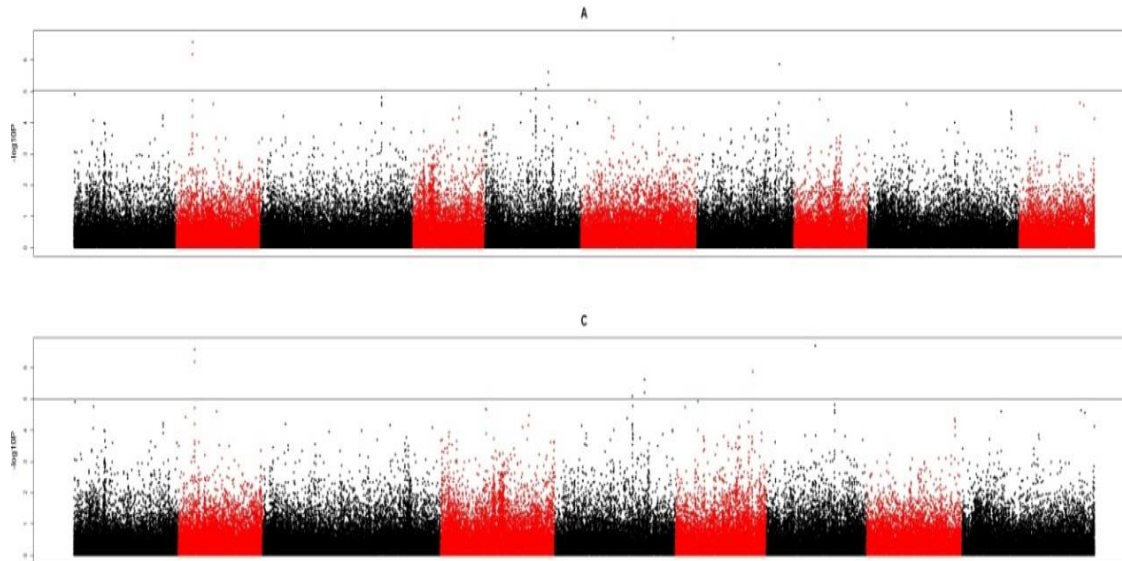
Wilting index



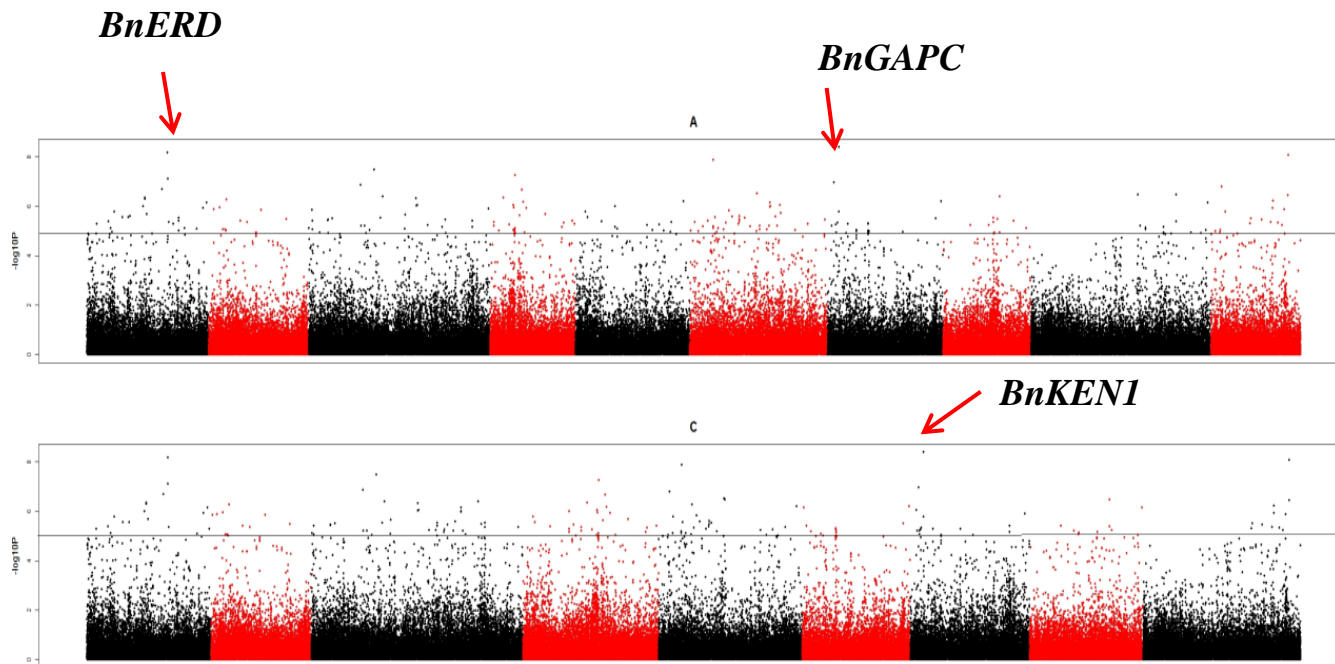
Treat.	QTL	LG	% explained
CK	3	4	13.51
		8	3.50
		16	11.187
Drought	2	15	5.226
		16	4.508
Drought index	1	15	11.182

GWAS for drought tolerance at seedling stage

- 101 lines from UK (Prof. I. Bancroft)
- ~140,000 SNPs derived from RNA-Seq data



Manhattan Plot for fresh weight



Manhattan plot for leaves wilting index

- In total, 186 significant SNPs ($P < 6.9 \times 10^{-6}$)
- 120 genes can be inferred, with annotation

Summary of GWAS

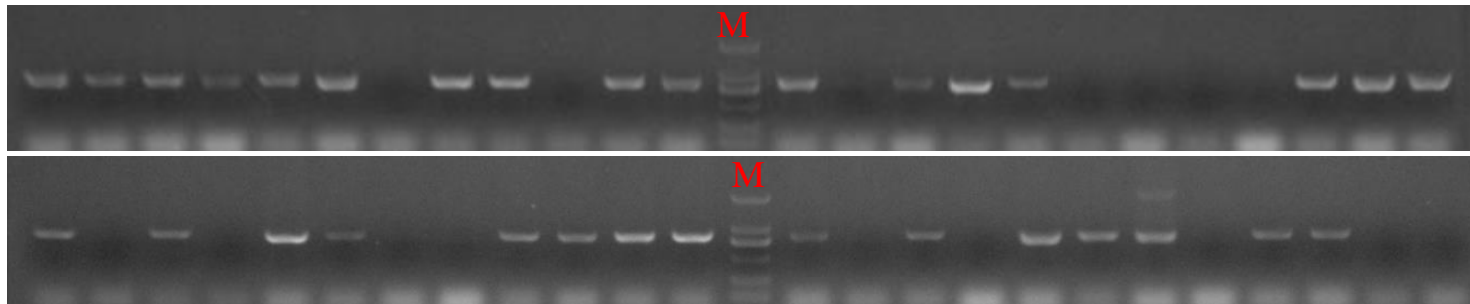
Trait	Gene model	SNP	-Log ₁₀ (P)	R ² (%)	Annotation
Fresh weight	AT5G16400.1	JCVI_2582:142	6.562	37.17%	electron transporter
	AT5G16390.1	JCVI_3013:946	6.167	34.54%	BCCP1
	AT3G14205.1	JCVI_5500:245	5.862	33.93%	phosphoinositide 4-phosphatas
	AT1G08930.1	JCVI_21135:1176	8.074	43.65%	ERD6
	AT2G44610.1	JCVI_17979:973	7.88	48.82%	RAB6
	AT1G58290.1	JCVI_1071:272	7.26	37.63%	HEMA1
	AT2G16950.1	JCVI_7977:682	7.13	35.82%	protein transporter
	AT2G30490.1	JCVI_19435:537	6.98	33.53%	ATC4H
Wilting index	AT3G48870.1	ES901006:388	6.79	42.22%	ATCLPC
	AT3G47000.1	JCVI_3396:688	6.68	40.66%	hydrolyzing O-glycosyl compounds
	AT2G24200.1	JCVI_16832:131	6.53	40.66%	aminopeptidase
	AT3G09160.1	JCVI_32526:230	6.48	35.29%	nucleic acid binding
	AT2G46020.1	JCVI_31893:781	6.47	34.13%	ATP-dependent helicase
	AT4G25650.2	EV208723:206	6.41	33.51%	oxidoreductase
	AT3G53710.1	JCVI_2368:42	6.36	32.72%	DNA binding
	AT3G07170.1	JCVI_23032:306	6.35	33.76%	transferring glycosyl groups
	AT4G25650.2	JCVI_9337:402	6.33	34.40%	oxidoreductase
	AT1G12430.1	JCVI_2368:78	6.3	32.82%	PAK
	AT5G15640.1	EV221762:391	6.28	39.33%	binding
	AT5G13300.1	JCVI_9220:758	6.28	36.90%	VAN3 (vascular network defective 3)
	AT1G79470.1	JCVI_12409:329	6.22	35.09%	IMP dehydrogenase
	AT4G38680.1	JCVI_9082:263	6.22	40.57%	GRP2
	AT3G04120.1	JCVI_21191:824	6.19	39.03%	GAPC (encodes cytosolic GADPH)
	AT1G01790.1	JCVI_18549:224	6.17	30.55%	KEA1 (k efflux antiporter 1)
	AT5G63780.1	JCVI_19909:96	6.17	39.72%	ubiquitin-protein ligase
	AT1G44180.1	EV093176:433	6.17	33.83%	aminoacylase/ metallopeptidase
	AT1G67090.1	JCVI_14139:758	6.16	41.20%	ribulose-bisphosphate carboxylase
	AT5G37370.1	EV127200:162	6.1	31.27%	response to salt stress
AT2G18300.2	JCVI_16411:908	6.07	39.59%	transcription factor	
AT3G07390.1	DY015167:493	6.06	31.40%	AIR12	
AT5G63810.1	JCVI_21686:110	6.01	38.35%	BGAL10	

➤ Development of functional markers

Primer design, PCR and re-sequencing of target gene fragments;

Detection of causative variation (InDel/SNP/CAPS)

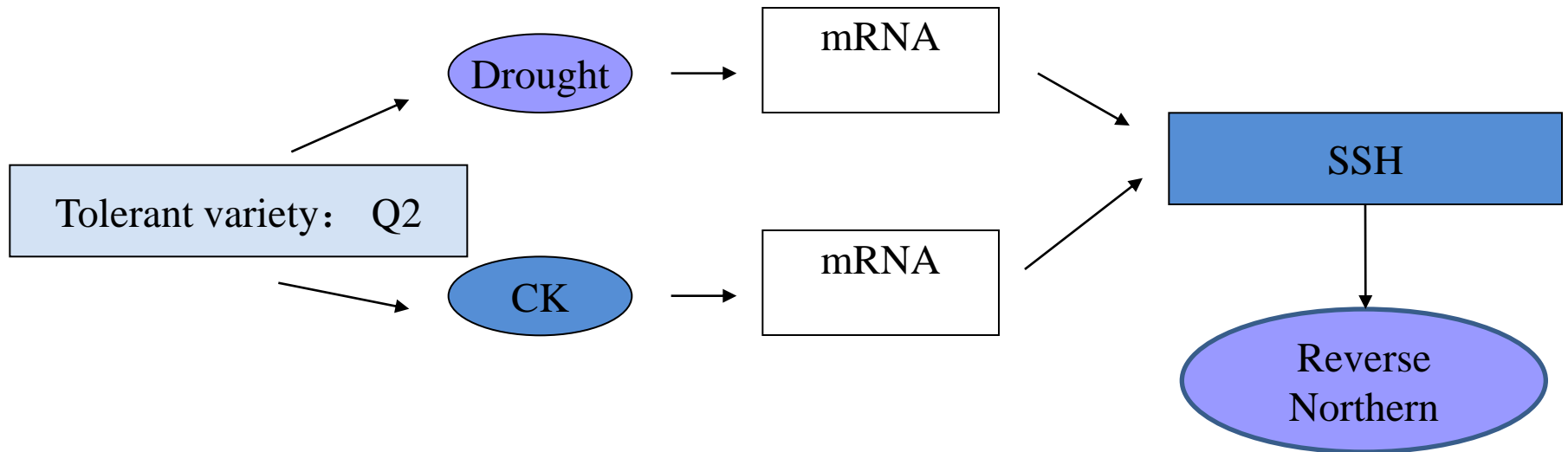
Conversion to new markers



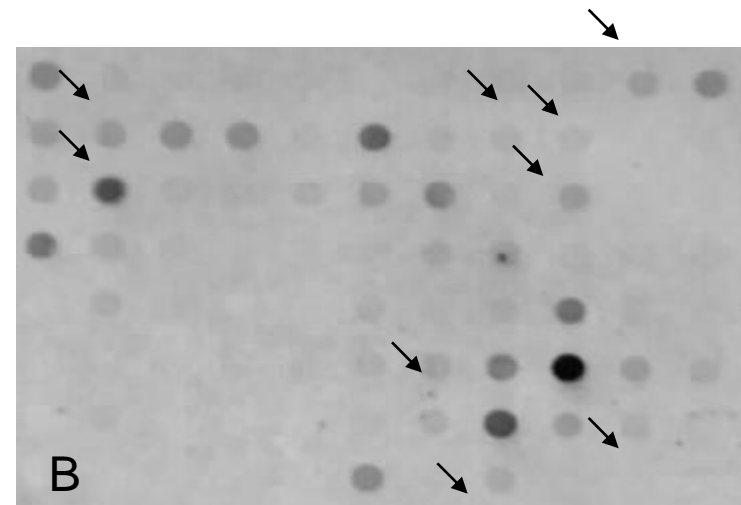
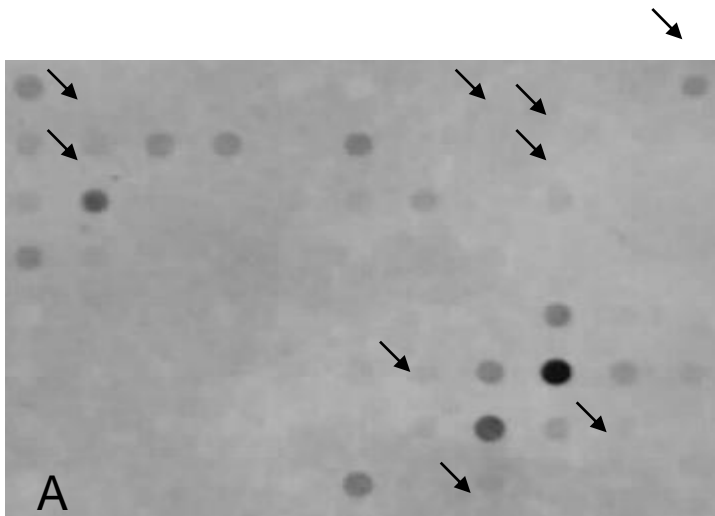
Functional genomics

Identification of differentially expressed genes using SSH

(Suppression Subtractive Hybridization)



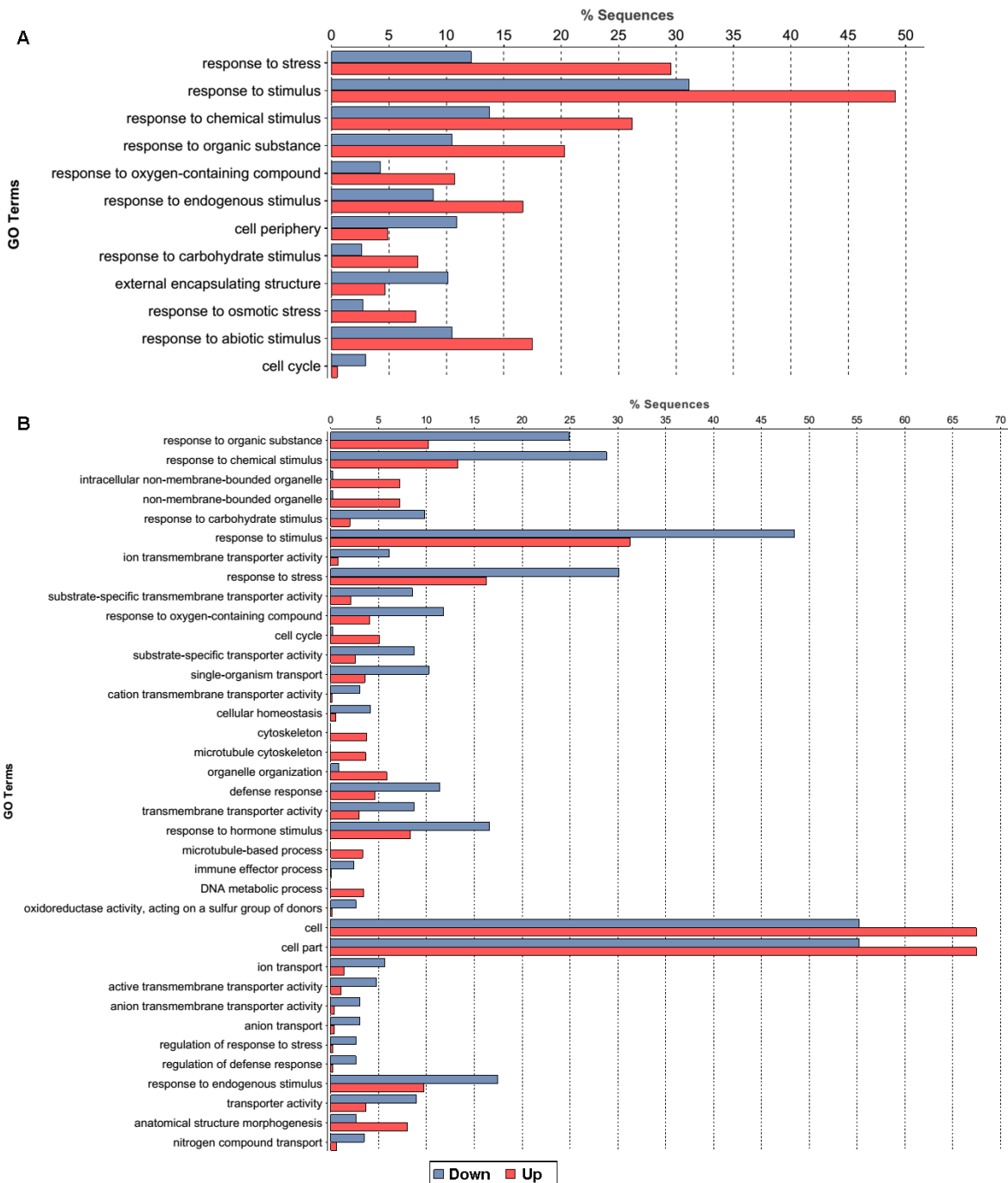
Reverse Northern blot



147 up-regulated genes

121 down-regulated genes

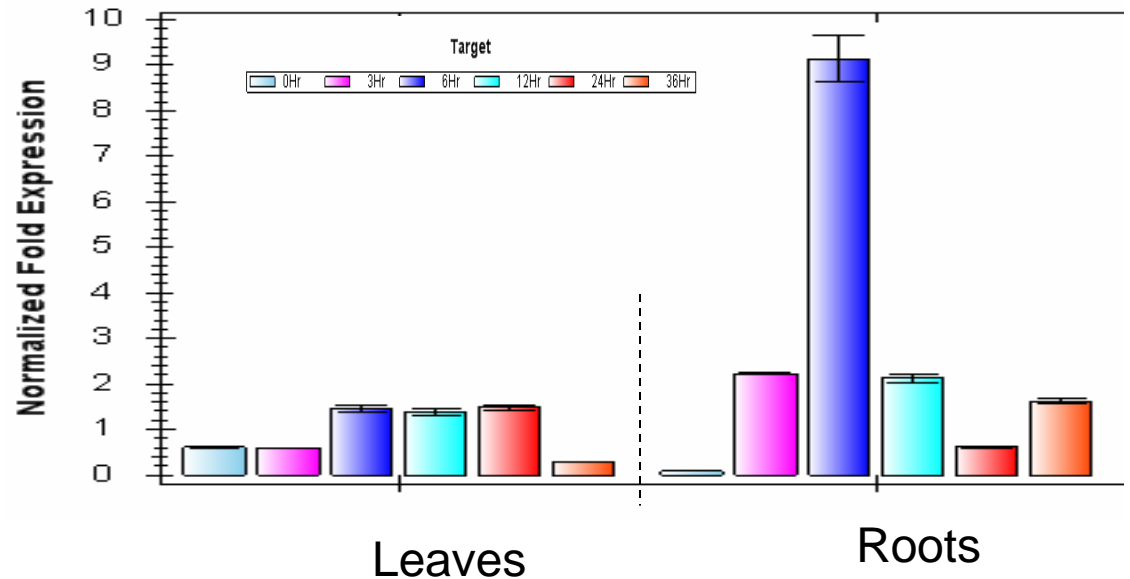
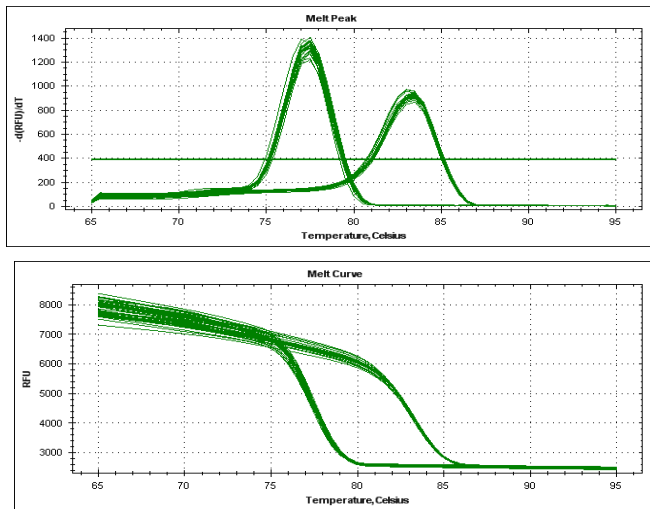
Differential GO-term Distribution



Candidate genes

NO.	Length (bp)	Putative Homologous genes	Identity
21	796	BR118232 root cDNA library KHRT Brassica rapa subsp. pekinensis	80/97%
23	722	BR042785 salt-treated Brassica rapa subsp. pekinensis	98/97%
28	530-760	h12_12na_2p.s 12na Brassica napus /BR121389 root cDNA library KHRT Brassica rapa /BR047648 etiolated mature leaf cDNA library	81-100%
29	466-590	BR104661 defected leaf cDNA library KHL D Brassica rapa /BR113232 ovule and silique cDNA library	92-99%

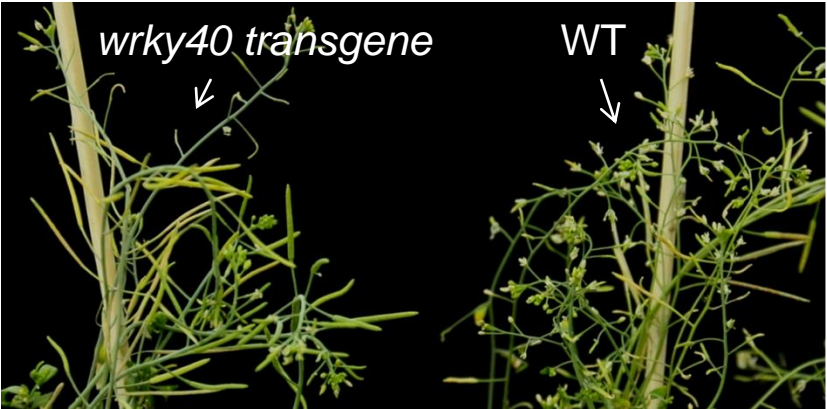
qRT-PCR of *WRKY40*



The expression of *WRKY40* was induced in roots under drought, but was not changed in leaves

Overexpression *WRKY40* in *Arabidopsis*

A vector construction



Over-expression of *WRKY40* in rapeseed

WRKY40

WT



WRKY40

WT



WRKY40



CK

300 mM
mannitol

WT





PART III

The Breeding

MAS breeding of drought tolerant variety

ZS6 × X22



F₁ ⊗



F₂

MAS Selection



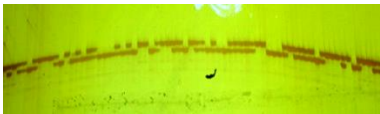
Evaluation of yield and drought tolerance



22038 (breeding line)



YG2009 (variety)



Performance of YG2009 in National Field Trial

- Yield: 2669.1 kg/ha , +5.17%
hybrid CK
- Oil yield: 1173.9 kg/ha , +8.73%
- Drought tolerant index: 0.62,
medium tolerance





YG2009 in Hunan Province (2015)



Acknowledgement

