



ERECTA, salicylic acid, abscisic acid and jasmonic acid modulate quantitative disease resistance of *Arabidopsis thaliana* to *Verticillium longisporum*

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Petr Karlovsky, Elke Diederichsen**



V. longisporum / Oilseed Rape (*Brassica napus* L.)



Susceptible



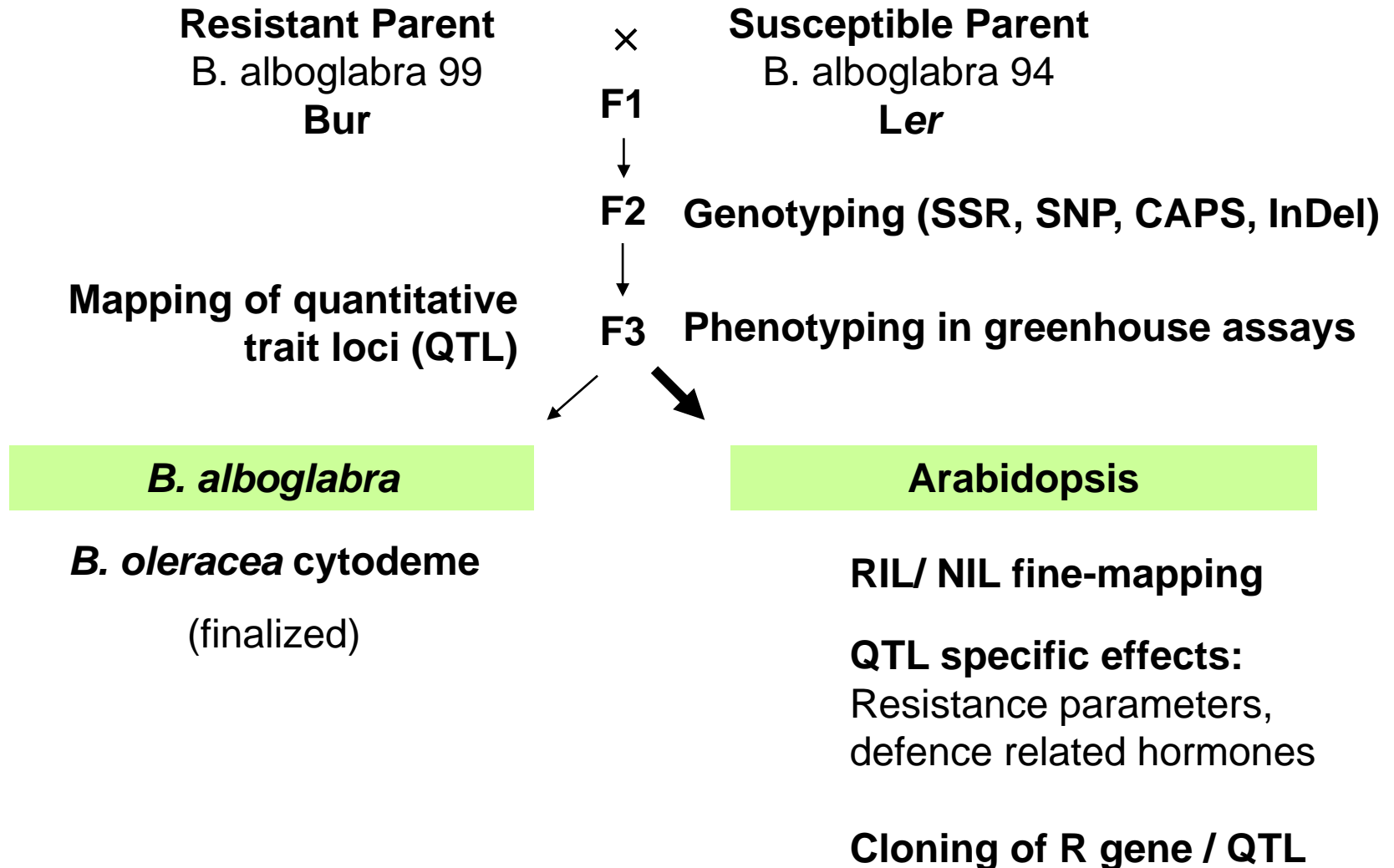
Resistant

- **Soil-borne, vascular pathogen**
- **Specialised on crucifers**
- **Colonises the whole shoot system at the onset of flowering**
- **Causes premature senescence, chlorosis, and reduced TKW, stunting in greenhouse**
- **Fungicides not efficient**

Project Goals

- ❖ Identify genetic factors controlling natural variation for resistance, relevance of resistance parameters (Brassica, Arabidopsis)
- QTL mapping of different resistance parameters and development, map based cloning of a major resistance QTL from Arabidopsis
- ❖ Physiological basis of resistance reaction for general understanding and to support candidate gene selection (Arabidopsis)
- Detect changes in hormonal status after infection depending on host genotype in Arabidopsis
- Study gene expression changes after infection depending on host genotype

QTL Mapping to dissect *Verticillium* resistance

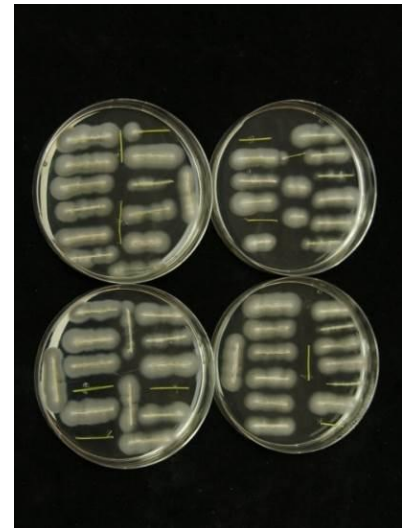


Determining resistance parameter

- **Greenhouse assay**
 - Root dip-inoculation
 - Mock vs. *V. longisporum* 43
 - % chlorotic leaves (Arabidopsis)

Final assessment **at maturity of 1st pod:**

- Shoot fresh weight
- Shoot length
- Systemic colonisation in stem segments from the shoot apex (malt agar plate assay and qPCR)
- Development (dpi, scoring of development)

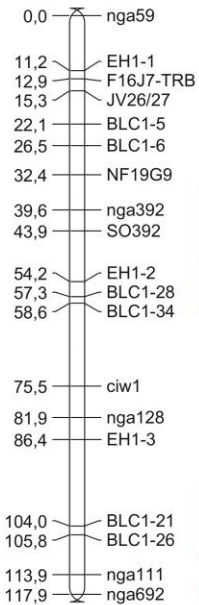


Status of Genetic Analysis in Brassica and Arabidopsis

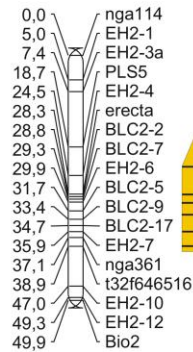
- Different resistance parameters studied: Resistance to systemic colonisation of shoot upon flowering, resistance to stunting, resistance to chlorosis
- Mapping in F2/F3 and BC in *B. oleracea alboglabra*: QTL that control colonisation resistance and stunting resistance colocalize on C1 and C3, differences in mode of inheritance indicate linkage, linkage to development QTL occurs for QTL on C3
- Inheritance in Arabidopsis more complex: QTL that control different resistance parameter can occur on different chromosomes, also linkage between QTL for different parameters (F2/F3 mapping, Häffner et al 2010 BMC PI Biol)

Fine-mapping in Bur x Ler RIL population

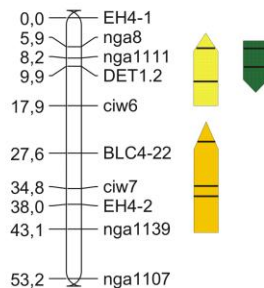
Chromosome 1



Chromosome 2



Chromosome 4



Confirmation of most QTL from F2/F3 mapping.







QTL for colonisation resistance reproducible in both RIL tests, stunting and chlorosis depending on season as in F2/F3

Explained trait variance per QTL:

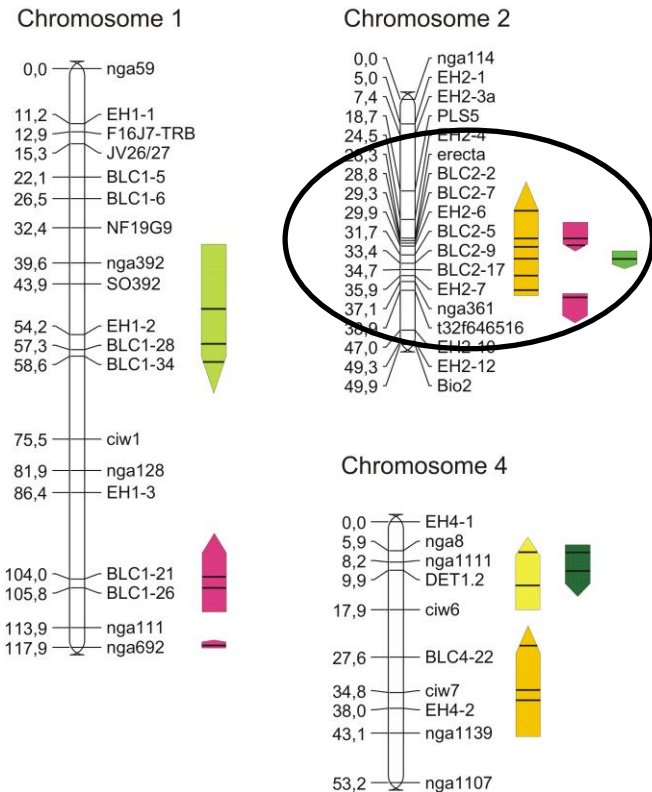
Colonisation resistance 15 – 19%

Stunting resistance 18 – 32 %

Resistance to chlorosis 13 – 27%

-  Trait: Systemic Colonisation, Parameter: % Colonised Shoot Segments, Experiment 1
-  Trait: Systemic Colonisation, Parameter: % Colonised Shoot Segments, Experiment 2
-  Trait: Stunting Resistance, Parameter: Performance Height, Experiment 1
-  Trait: Chlorosis, Parameter: Number of Yellow Leaves, Experiment 1
-  Trait: Chlorosis, Parameter: % Yellow Leaves, Experiment 1
-  Trait: Chlorosis, Parameter: Difference in yellow leaves, Experiment 1

„Candidate QTL“ on chromosome 2



Erecta-region:

-Contains QTL for different resistance traits

-Major QTL for colonisation resistance: *vec1*

-Is *Erecta* a *Verticillium* resistance gene?



Dissect region in near-isogenic lines, study more *erecta* mutants

- Trait: Systemic Colonisation, Parameter: % Colonised Shoot Segments, Experiment 1
- Trait: Systemic Colonisation, Parameter: % Colonised Shoot Segments, Experiment 2
- Trait: Stunting Resistance, Parameter: Performance Height, Experiment 1
- Trait: Chlorosis, Parameter: Number of Yellow Leaves, Experiment 1
- Trait: Chlorosis, Parameter: % Yellow Leaves, Experiment 1
- Trait: Chlorosis, Parameter: Difference in yellow leaves, Experiment 1

erecta mutants and corresponding wildtypes

La-0

Ler

Col-0

er-105

er-108

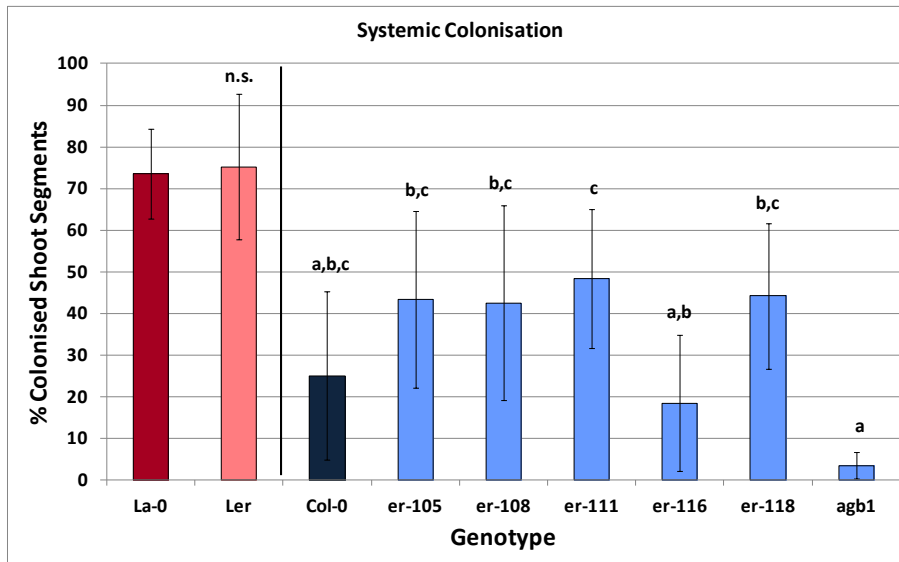
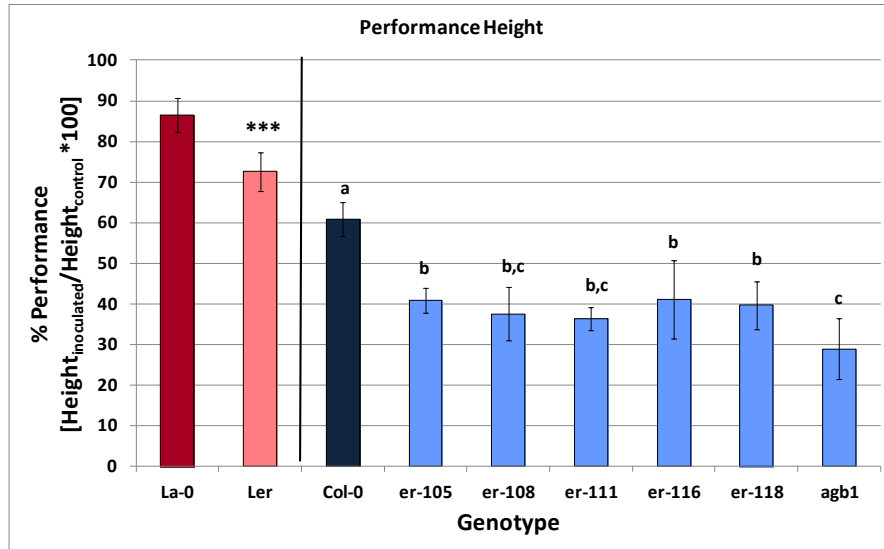
er-111

er-118

er-116

agb1-1

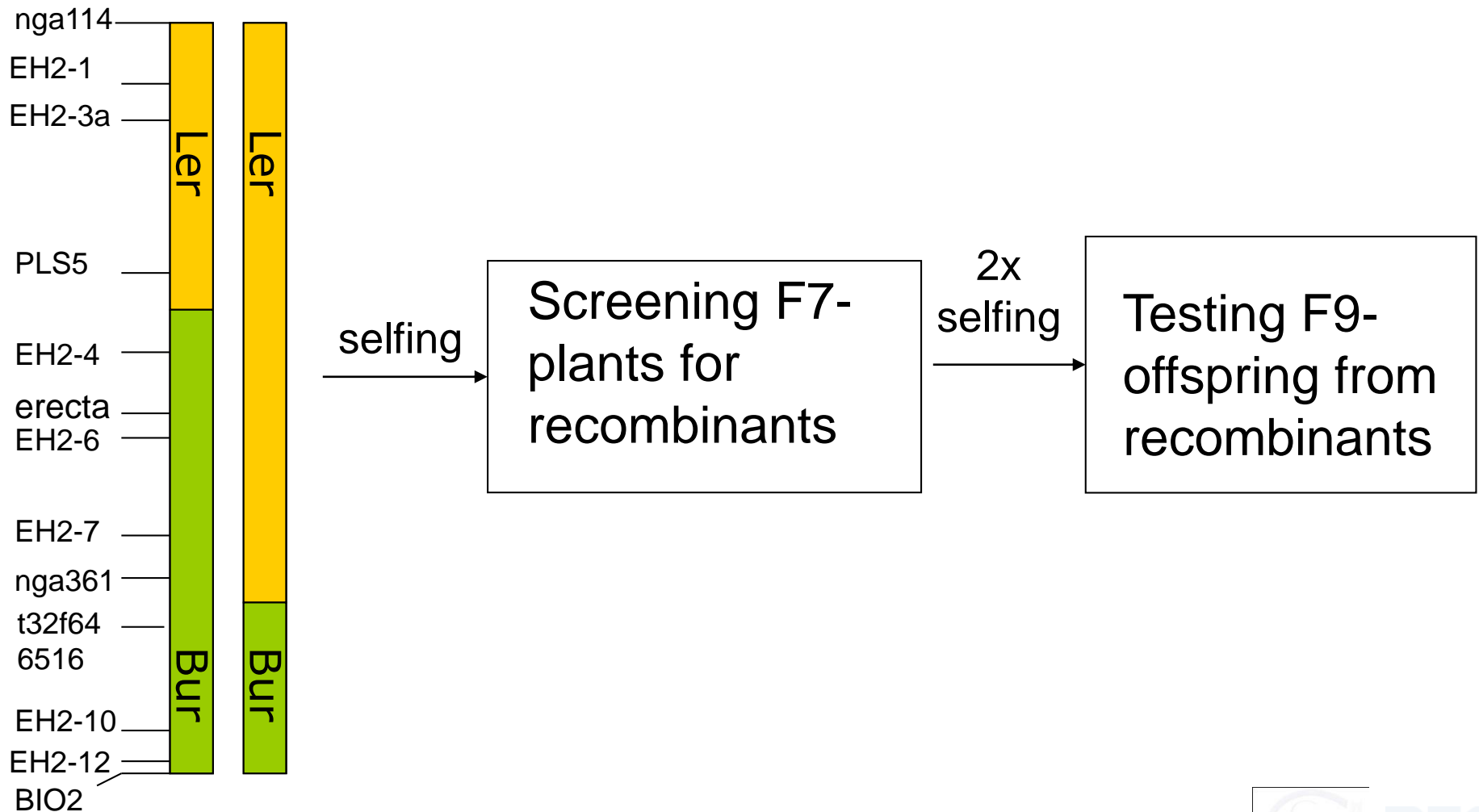
Verticillium reactions of erecta mutants



- Significant effect of *erecta* mutation on stunting
- Effect on colonisation only marginal
- Candidate for stunting resistance gene
- Confirmed in NIL mapping, close linkage to colonisation resistance locus *vec1*

Mapping in Near-Isogenic Lines (NILs)

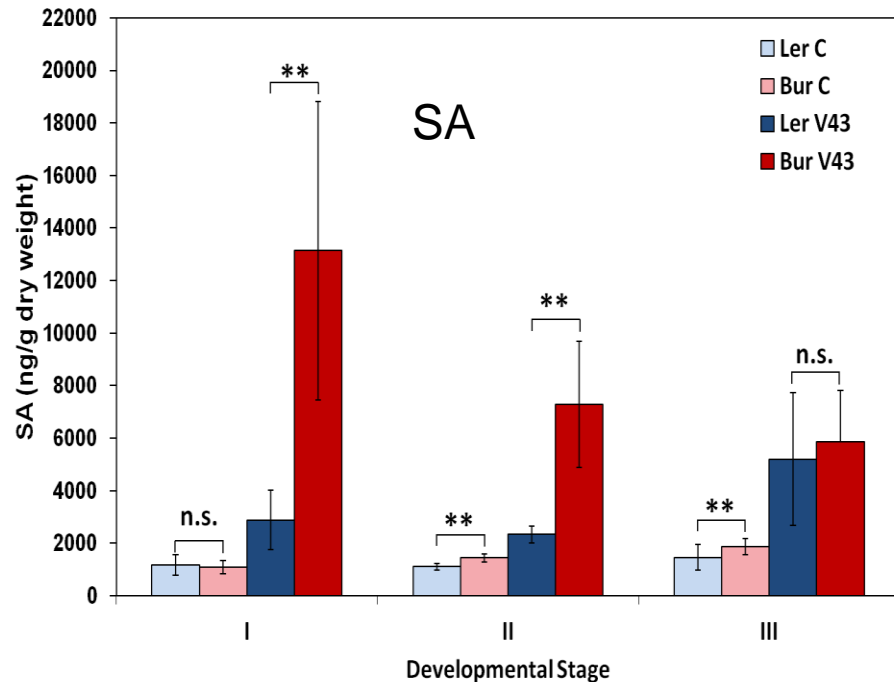
RI-Line 21 (F6): Heterozygous 3Mb-region on Chromosome 2



Selection of NIL5 and NIL9 to study defence

Marker	KB	5	103	236	256	340	338	171	125	57	178	202	237	6	41	157	151	118	216	47	225	122	9	54	69	98	283	284	335	85	230	240																									
EH2-4	10050	B	B	B	B	H	B	B	B	B	B	B	B	B	B	(B)	H	B	B	L	B	H		L	L	L	L	L	L	H	L	L	L																								
BLC2-1/ER	11212	(B)						(B)		(B)	(B)					(B)			(B)	(B)	(B)			(L)																																	
BLC2-2	11295	(B)						(B)		(B)	(B)					(B)				(B)	(B)			(L)																																	
BLC2-7	11400	B	B	B	B	H	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	L	L	L	L	L	L	H	L	L	L																									
EH2-6	11587	B	B	B	B	H	B	B	B	B	B	B	B	B	B	B	B	B	B	L	L	L		L	L	L	L	L	L	H	L	L	L																								
BLC2-18	11689																																																								
BLC2-19	11725	(B)	B	B	B	H	B	B	B	B	B	B	B	(B)	B	B	B	L	L	L	L	L		L	L	L	L	L	L	H	L	L	L																								
BLC2-20	11730																																																								
BLC2-22	11753	B	B	B	B	H		B		B	B	B	B	B	B	B		L	L					L	L	L	L																														
BLC2-24	11799																																																								
BLC2-25	11799	B	B	B	B	H	B	B	B	B	B	B	B	B	B	B	B	L	L	L	L	L	L	L	L	L	L	L	L	H	L	L	L																								
BLC2-27	11853	B	B	B	B	H	B	B	B	B	B	B	B	B	B	B	B	L	L	L	L	L	L	L	L	L	L	L	L	H	L	L	L																								
BLC2-28	11904																																																								
BLC2-29	11914																																																								
BLC2-31	11969	B	B	B	B	H	B	B	B	B	B	B	B	B	B	B	L	L	L	L	L	L	L	L	L	L	L	L	L	H	L	L	L																								
BLC2-35	12115	B	B	B	B	H	B	B	B	B	B	B	B	B	B	B	L	L	L	L	L	L	L	L	L	L	L	L	L	H	L	L	L																								
BLC2-36	12141																																																								
BLC2-5	12180	B	(B)	B	B			(B)	B	(B)	(B)	(B)	B		L	(L)		L	(L)			L		L	L	L	L	L	L	L	L	L	L																								
BLC2-9	12377	B	(B)	B	B	H	B	B	L	L	L	L	L		L	(L)	L	L	L	L	L	L	L	L	L	L	L	L	L	H	B	B	B																								
BLC2-11	12397	(B)						(L)	(L)	(L)	(L)	(L)	(L)		(L)	(L)			(L)					(L)																																	
BLC2-17	12594	(L)						(L)		(L)	(L)	(L)	(L)		(L)	(L)			(L)					(L)																																	
EH2-7	12651	B	L	L	L	L	H	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	B	B	B	H	H	B	B	B	B																								
nga361	13222	B	L	L	L	L	H	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	B	B	B	H	H	B	B	B	B																								
Test:																																																									
F9/1	(%	25	39					40		24	47									32	41	24		65	53	24																															
F9/2	Colon.)	27	33	28				22		24	35	30	31			28			40		56			64	60	17	30				62	31	40																								
F9/3		11	19					20		17	25	8	13			6			32		31			42																																	
V15/VI43		11																						79																																	
V15/Fe-09		29																																																							
<i>Erecta</i> WT																																																									

Role of salicylic acid (SA)



Ler (sus) and Bur (res) differences for SA content in stem depend on development:

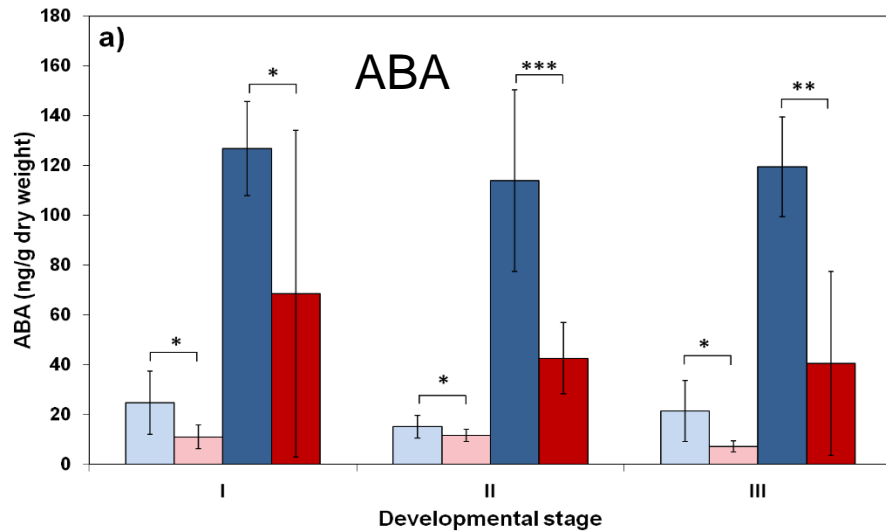
-At onset of flowering (stage I) strong SA induction by VI in Bur

-At onset of seed maturation (stage III) no significant difference between Bur and Ler after infection

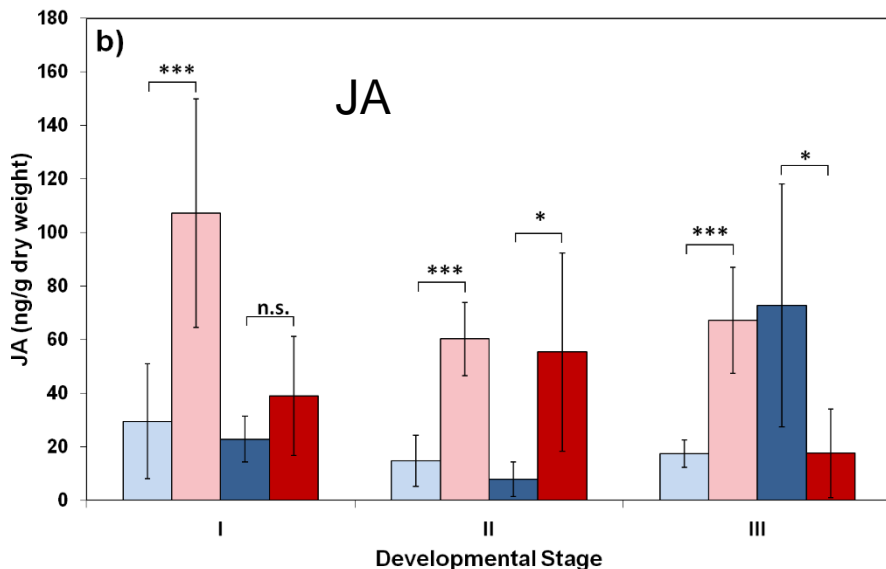
-**No difference in NILs and *erecta* mutants (not shown)**

Conclusion: SA not involved in resistance controlled by QTL in *Erecta* region.
Possible role in stunting: SA hyperinduction can induce stunting in light intensity dependent manner, Bur shows strong stunting after infection in winter

Roles of abscisic acid (ABA) and jasmonate (JA)



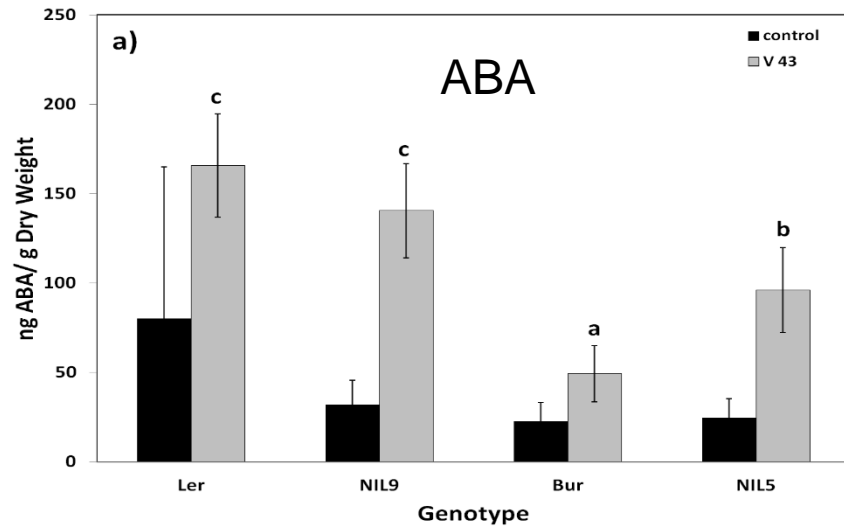
Ler (blue) shows much stronger induction of ABA after infection than Bur (red), no clear effect of development



JA content decreases in Bur and increases in Ler at onset of seed maturation after infection

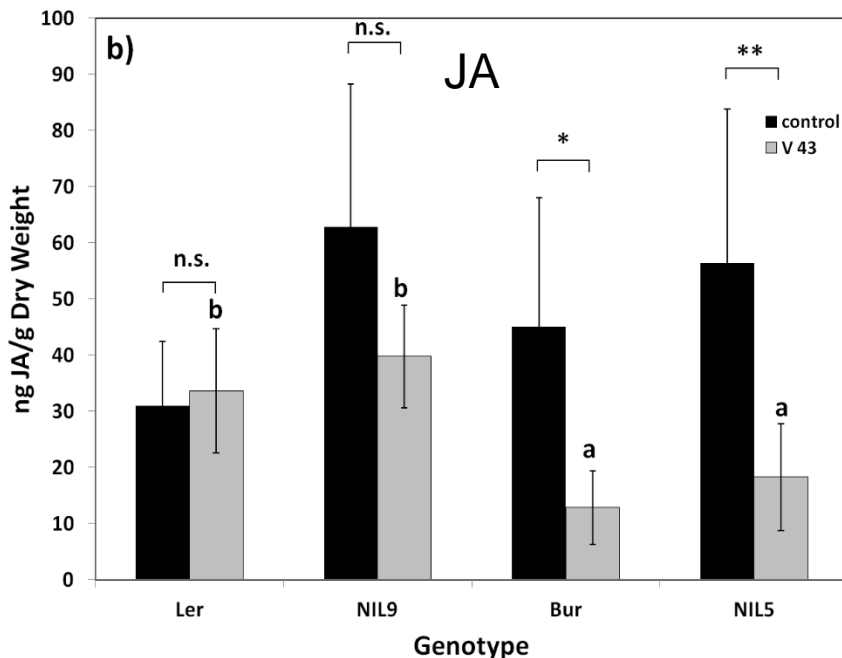
Bur has constitutively higher levels of JA than Ler

ABA and JA induction in NILs



NILs that differ in QTL region on chr. 2 show similar ABA and JA contents than the parent from which they received their alleles in the polymorphic region:

NIL9 (Ler alleles) has a similar ABA content as Ler after infection, while NIL5 (Bur alleles) shows a less strong increase



NIL5 shows strong JA decrease as Bur, while difference between control and inoculated variant was not significant for NIL9 and Ler

Both hormones seem to play a role for resistance controlled by QTL from polymorphic region

Summary

- Complex inheritance in Arabidopsis, multiple genes, linkage of colonisation resistance with stunting resistance, chlorosis and development
- New function for *erecta*: Stunting resistance
- QTLs on chromosome 2 depend more on ABA and JA signalling than SA signalling
- Häffner et al 2014 BMC Plant Biology
- Gene expression studies and cloning of a colonisation resistance locus from Arabidopsis is ongoing, resistance physiology supports candidate selection in polymorphic region

Announcement

11th Conference of the European Foundation for Plant Pathology

8-13 September 2014

Cracow, POLAND

Workshops on Brassica diseases:

Blackleg

Clubroot

<http://www.kongres-fitopatologiczny.pl/>

Acknowledgements

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Federal State of Berlin

Thank you for your attention!



Erecta a candidate gene?

Colonisation QTL

- ▶ reproducible in F2/F3 and RIL
- ▶ Ler allele confers susceptibility, Bur allele confers resistance

Stunting resistance QTL

- ▶ reproducible in both RIL tests
- ▶ Ler allele confers susceptibility, Bur allele confers resistance (RIL population)

- ▶ LRR-kinase involved in many physiological processes (morphogenesis, cell adhesion, resistance to *Plectosphaerella cucumerina* and *Ralstonia solanacearum*) (Torii et al. 1996, The Plant Cell 8, 735-746; van Zanten et al. 2009, Trends in Plant Science 14, 214-218)

erecta mutants and corresponding wildtypes

La-0

Ler

Col-0

er-105

er-108

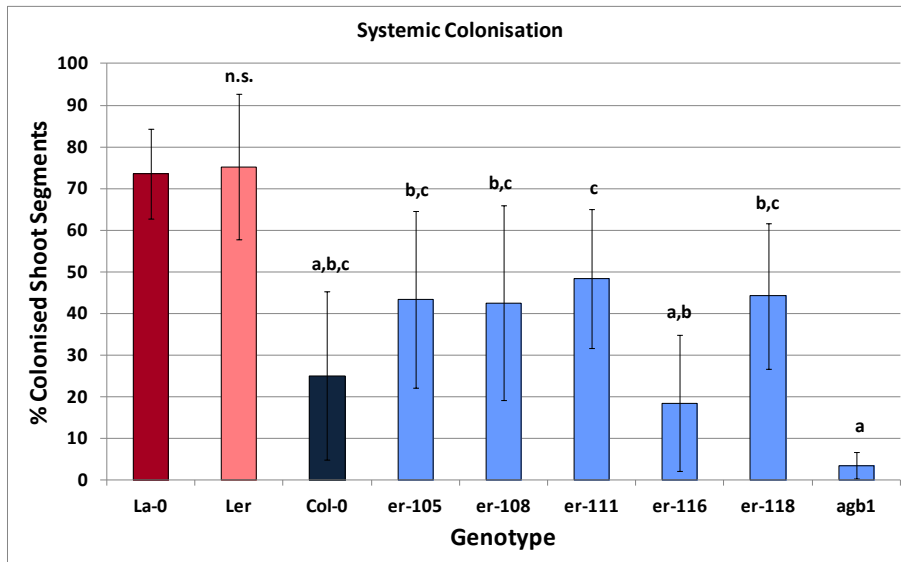
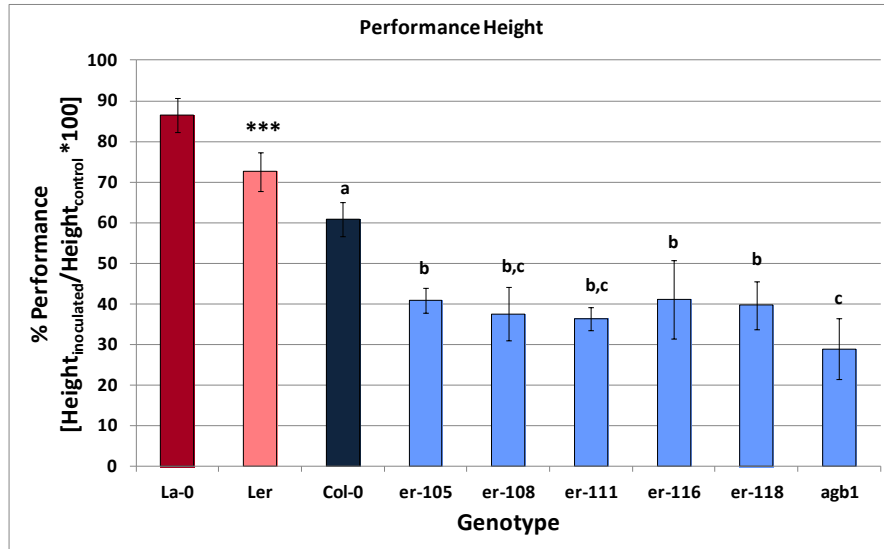
er-111

er-118

er-116

agb1-1

Verticillium reactions of erecta mutants



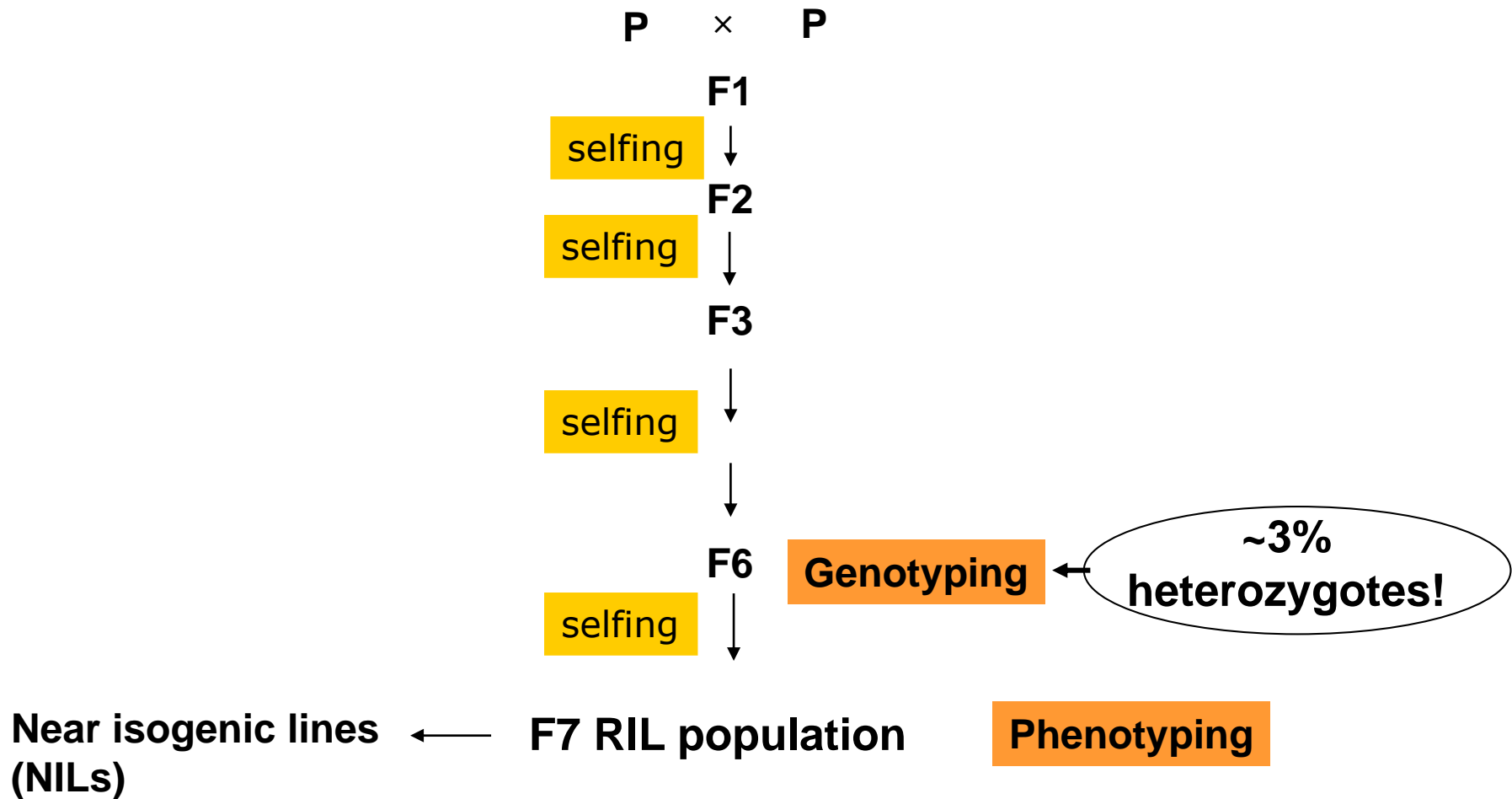
- Significant effect of *erecta* gene on stunting
- Effect on colonisation only marginal
- Candidate for stunting resistance gene
- Close linkage to colonisation resistance locus *vec1*

Screening results

Quantitative variation in *Brassica* and *Arabidopsis*
 Most contrasting genotypes selected for study

	<i>Brassica alboglabra</i> 99	<i>Brassica alboglabra</i> 94	<i>B. rapa</i> 121	<i>B. rapa</i> 111	<i>Arabidopsis</i> Bur	<i>Arabidopsis</i> Ler
Stunting	none	severe	little	severe	Depending on season	Depending on season
Colonisation	none	high	none	high	none	high
Flowering	slow	fast	slow	fast	slow	fast

Development of recombinant inbred lines (RILs)



Developmental implications

- Major disease symptoms indicate accelerated senescence
- Systemic colonisation often occurs at a certain development stage, ie the onset of flowering or maturity (Zhou et al. 2006 and Duncker et al. 2008 for oilseed rape, Häffner et al. 2010 for Arabidopsis)
- In greenhouse tests modified host development can be observed (mock vs. inoculated)
- Slow developing genotypes seem to be more resistant
- Is resistance controlled by genes controlling also development?

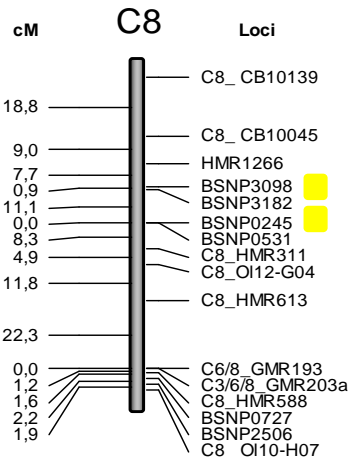
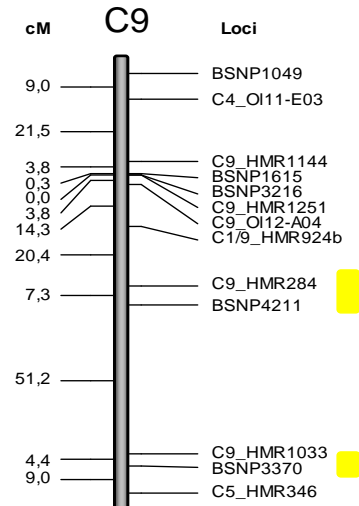
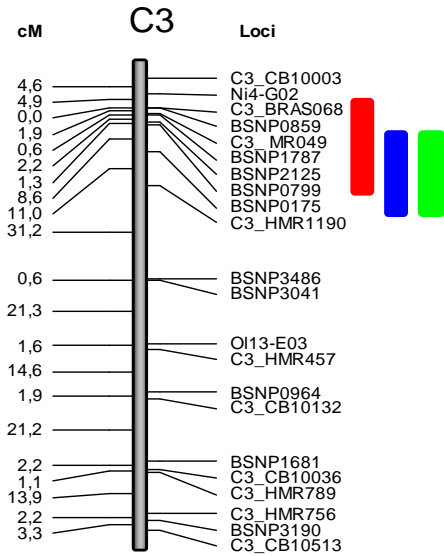
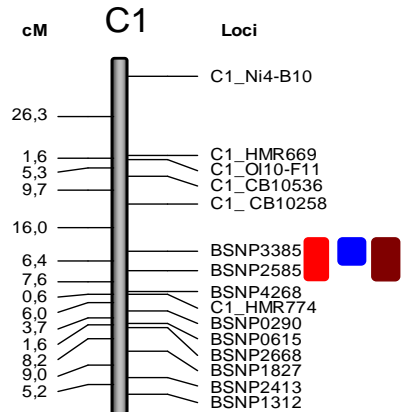
⇒ Parallel recording of development in bioassays, long day regime to induce flowering (suppl. Light in greenhouse) and stage-specific final assessment at the onset of pod maturity

Mapping results – *Brassica* (cont.)






QTL	Chr.	Position (cM)	LOD	% Expl.	Means of F3-families with alleles from		
					94	Heterozygous	99
AUDPC_1	C1	59	3,88	26%	149,6 ^a	146,4 ^a	127,5 ^b
AUDPC_2	C3	15,6	4,97	36%	172,4 ^a	160,8 ^a	134,2 ^b
FW_1	C1	59	3,99	26%	2,6 ^a	3,4 ^b	3,7 ^b
SL_1	C3	24,1	3,71	28%	18,4 ^a	23,6 ^b	25,8 ^b
Col_1	C1	59	5,62	24%	18,1 ^a	4,9 ^b	2,5 ^b
Col_2	C3	24,1	5,23	37%	28,3 ^a	11,7 ^b	7,7 ^b

Dominant alleles in yellow

Mapping in *B. albobglabra* F2/F3 (99 res x 94 sus)



QTL in F2/F3

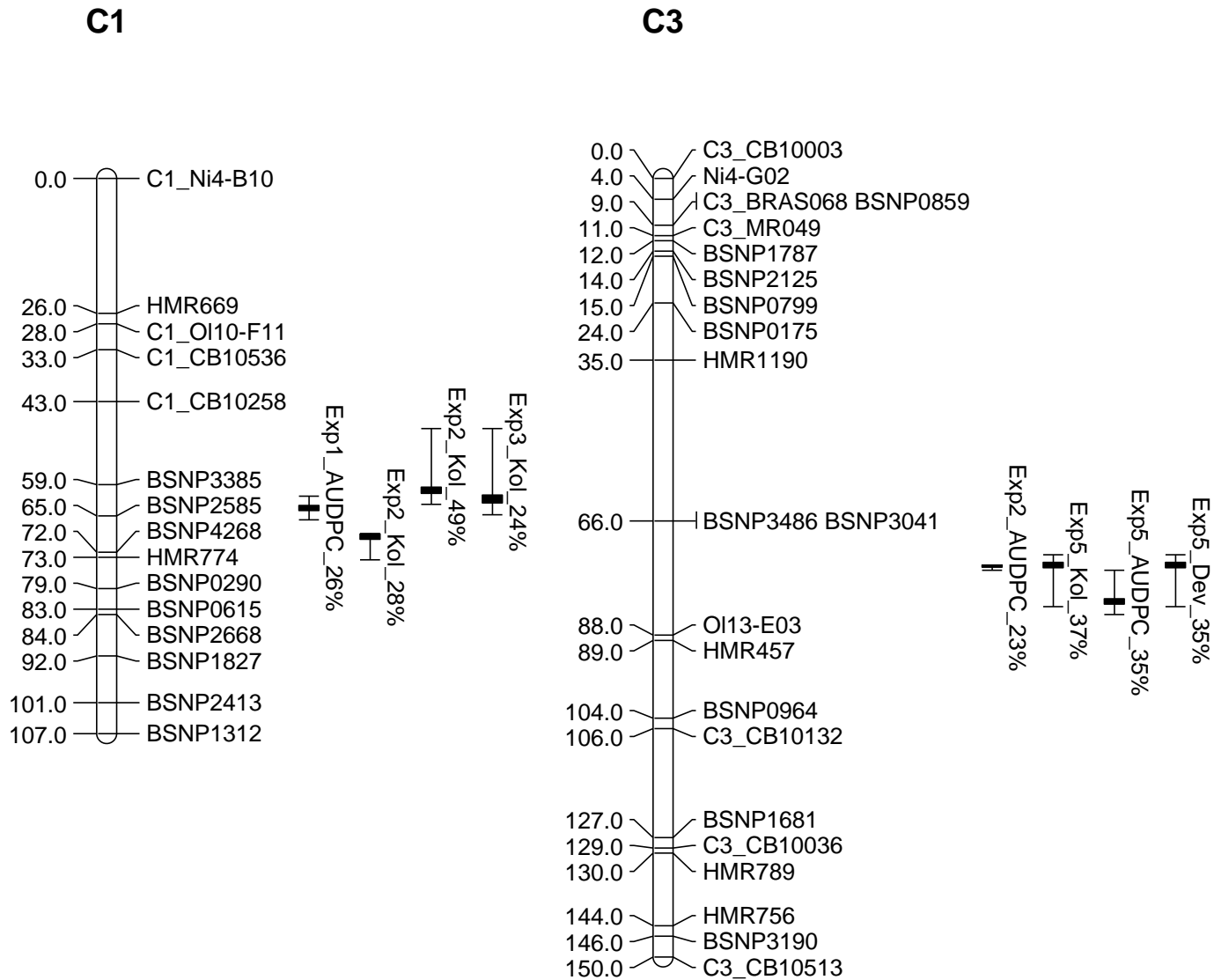
-  AUDPC_inoc.
-  Fresh weight_inoc.
-  Colonisation_inoc.
-  Stem length_inoc.
-  AUDevPC

**Genetic map of 135 SSR
or SNP markers**

10 linkage groups

Total map size: 965,24 cM

Resistance QTL in *B. alboglabra* F2/F3 (99 res x 94 sus)



Determining resistance parameter

- **Greenhouse assay**

- Root dip-inoculation
- Mock vs. *V. longisporum* 43
- Scoring of symptoms (*Brassica*)
- Area under disease progress curve AUDPC (*Brassica*)
- % chlorotic leaves (*Arabidopsis*)



Final assessment at maturity of 1st pod:

- Shoot fresh weight
- Shoot length
- Systemic colonisation in stem segments from the shoot apex (malt agar plate assay and qPCR)
- Development (dpi, scoring of development)



Mapping results – *Brassica*

QTL	Chr.	Position (cM)	LOD	% Expl.	Means of F3-families with alleles from		
					94	Heterozygous	99
Exp1_AUDPC	C1	63,8	3,9	26%	149,6 ^a	146,4 ^a	127,5 ^b
Exp2_AUDPC	C3	81,5	7,8	35%	172,4 ^a	160,8 ^a	134,2 ^b
Exp5_Dev	C3	74,5	5,4	35%	70,1 ^a	79,1 ^b	82,4 ^b
Dev_2, 3, 4	C9	13; 34; 75	3 major QTL on C9, „fast“ + recessive alleles from 94				
Exp2_Kol	C1	60,2	11,0	49%	123 ^a	0 ^b	0 ^b
Exp2_Kol	C1	68,5	7,3	28%	0 ^a	55 ^b	101 ^c
Exp5_Kol	C3	74,5	5,2	35%	28,3 ^a	11,7 ^b	7,7 ^b

Dominant alleles in bold, increase of resistance in yellow

Summary mapping results - *Brassica*

- Colocalisation of QTL with significant effect on resistance as determined as
 - disease scores (AUDPC)
 - systemic shoot colonisation
 - stunting: stem length (C3) or fresh weight (C1)
- Different inheritance indicates differences in physiology or close linkage of loci that control stunting and colonisation independently
- Correlation between AUDPC and colonisation ($r = 0,69^{**}$)
- A QTL for development on C3 colocalizes with resistance, other QTL for development show no interdependence to resistance (on C8 and C9)
- Confirmation of major QTL in BC population

Mapping in Arabidopsis (Bur x Ler) RIL population

