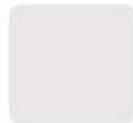
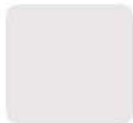
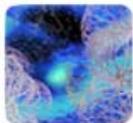




Transcriptome maps of *Populus tomentosa*: Characteristics and its applications

PhD. Li bo & Pro.Zhang Zhiyi

2008.10.29





Discrete traits and quantitative traits

Discrete trait: color,
gender, et al



Quantitative trait: size,
production, height, et al.
Most economic traits





Quantitative traits: big trouble



**Simpson, Tell me why
ones are small and
the others are so big?**

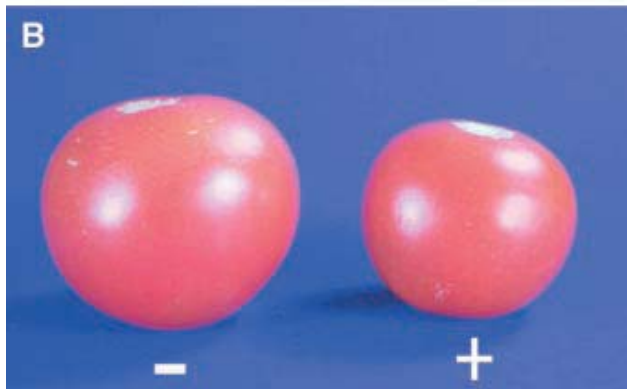
**Ho.....,you'd better
ask God!**

- Controlled by many genes
- Genes with minor effects
- Complicated to decipher





QTL mapping can answer ????



fw2.2: A Quantitative
Trait Locus Key to the
Evolution of Tomato
Fruit Size

Frary, et al. Science, 2000, 289
(7), P85-87



Development of Linkage Map in *Populus*

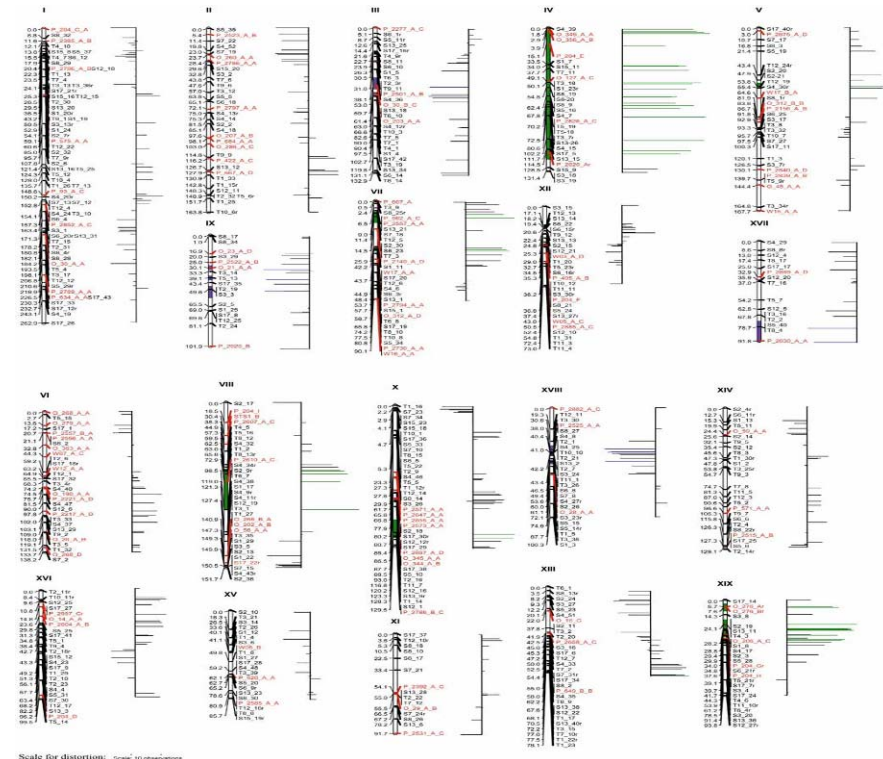
Summary:

>12 projects

>9 species or hybrids

most under pseudo testcross strategy

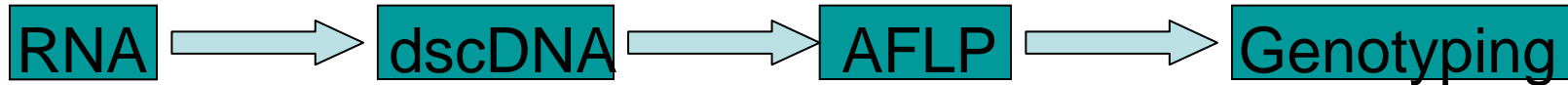
Most based on genomic DNA markers, RAPD, AFLP, SSR, ISSR et al.





Transcriptome map

Protocol:



Examples:

Potato (Brugmans,2002)

Arabidopsis (Brugmans,2002)

Cotton (Pan,2007)

Potato (Ritter,2008)

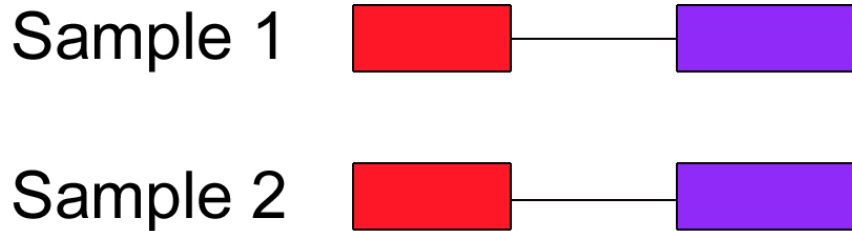
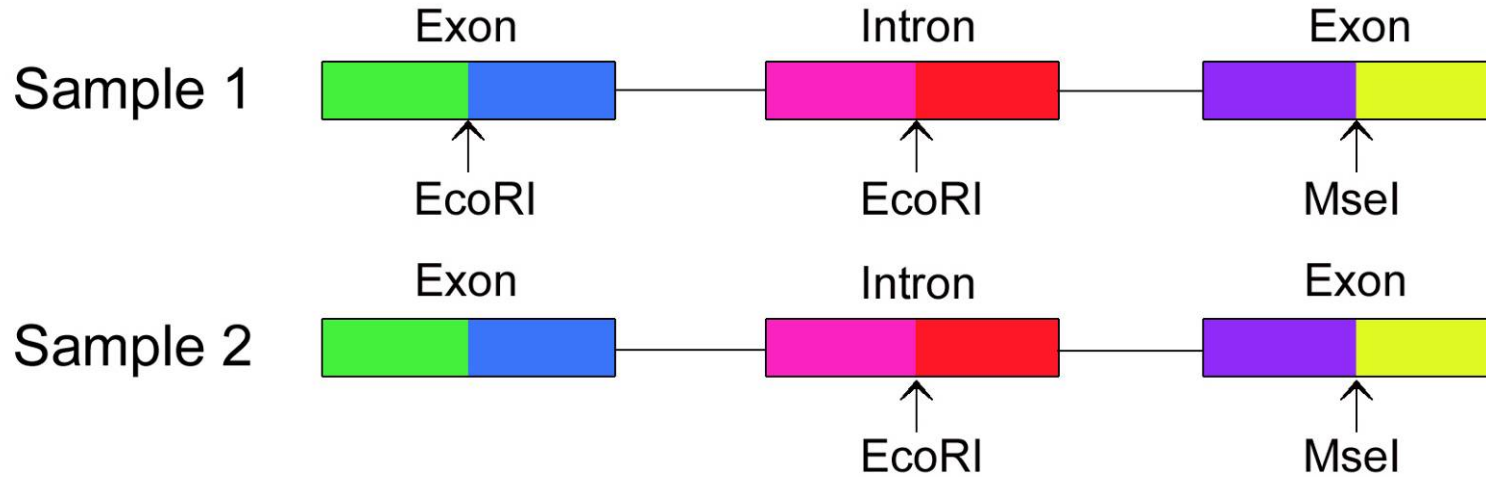


Differences between genetic map and transcriptome map

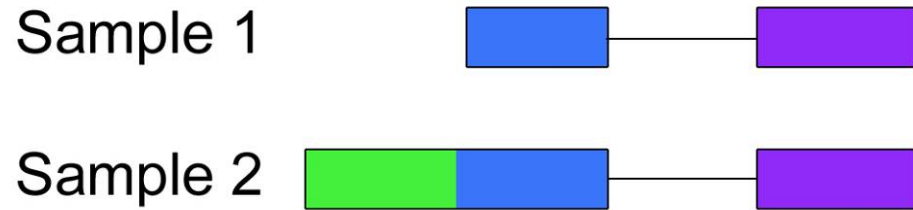
- Nature of the markers
- Reflection of specific tissue and development stage
- Novel markers: differential expression (rare)
cleavage site mutation



Cleavage site mutation within exons



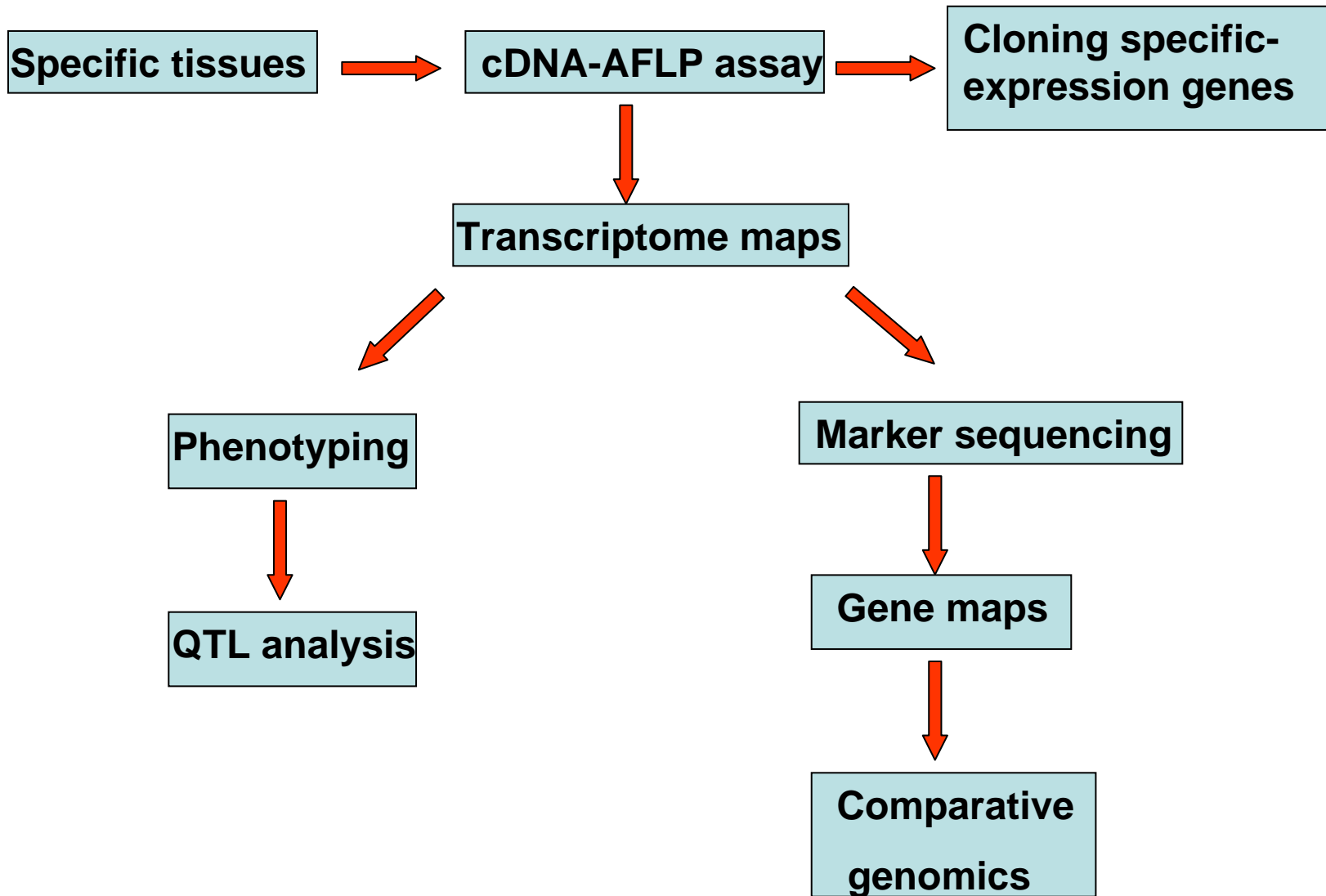
DNA level
No Polymorphism



RNA level
Polymorphism marker detectable



Work flow





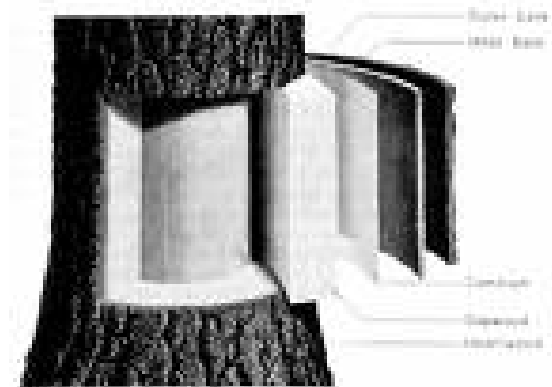
Mapping pedigree and materials

Mapping pedigree :

$(P.tomentosa \times P.bolleana) \times P.tomentosa$

7 years old, 132 genotypes

Materials : total RNA
from developing xylem





Marker systems

cDNA-AFLP analysis :

RNA → cDNA → dscDNA → AFLP → Genotyping

SSR analysis :

DNA → PCR → PAGE gel testing → Genotyping



Preparation (1): Primer selection

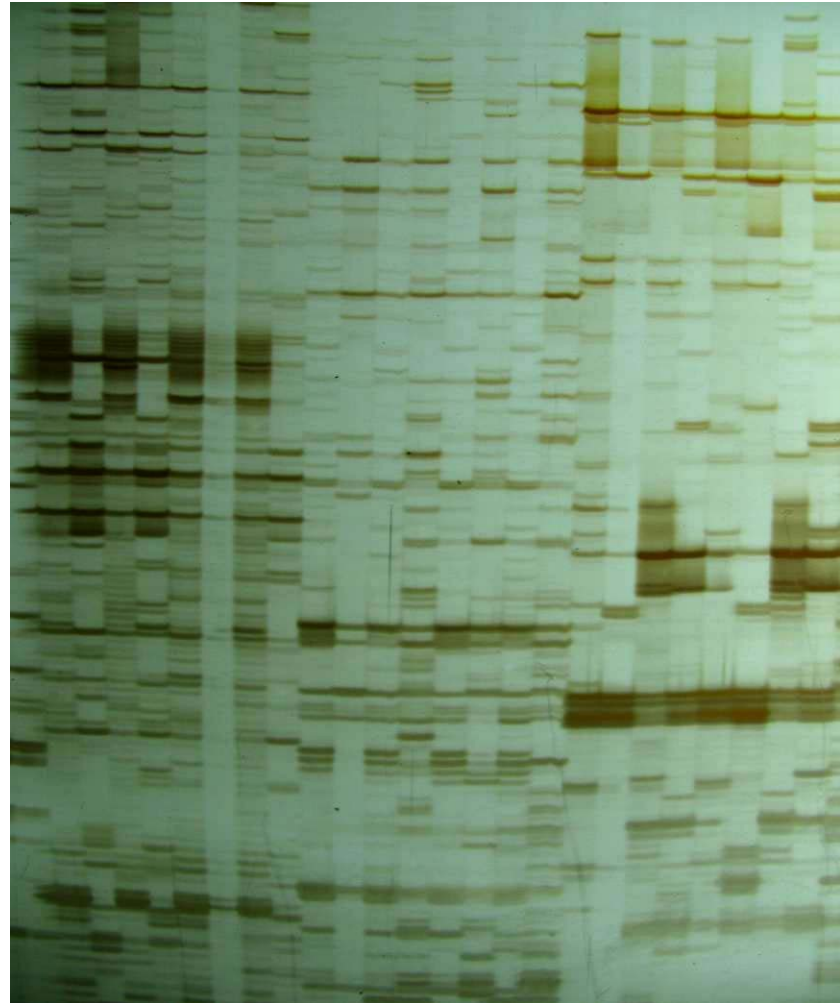
E02/M02 E02/M03 E03M02 E03M03

Pre-amplification:

E+0/M+0, E+0/M+1,
E+1/M+0, E+1/M+1,
NO obvious difference

Sel-amplification:

E+2/M+2, E+2/M+3, E+3/M+2,
E+3/M+3
E+2/M+3 or E+3/M+2
is ideal for mapping





Preparation (2): Polymorphism estimation

Polymorphism
Analysis with
21 random chosen
primers

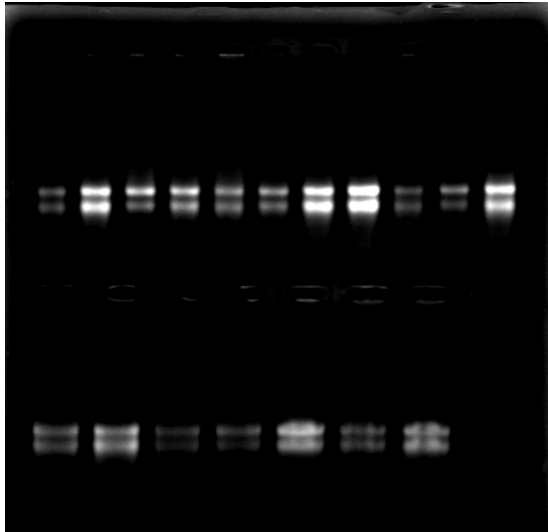
E11M33 E13M40 E15M56 E16M56



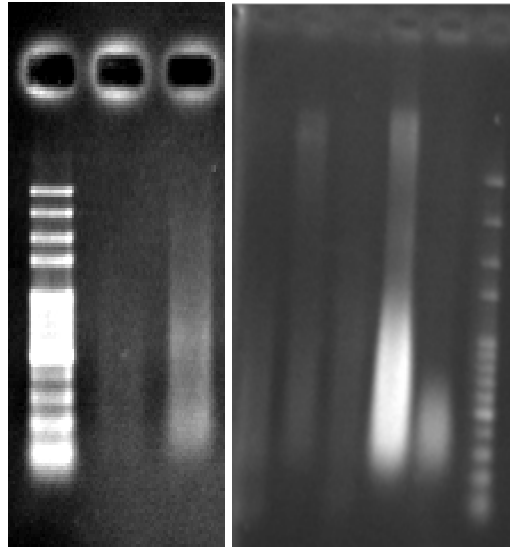


Polymorphism analysis of (*P. tomentosa* × *P. bolleana*) and *P. tomentosa*

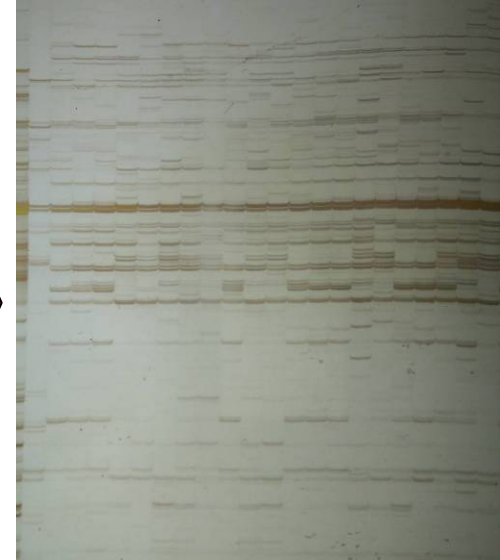
Primer combination	Total number of amplified band	Polymorphic marker of female parent	Polymorphism of female parent (%)	Polymorphic marker of male parent	Polymorphism of male parent (%)
E11M33	35	8	22.9	8	22.9
E13M40	27	7	25.9	10	37.0
E15M56	20	6	30.0	3	15.0
E16M56	23	6	26.1	10	43.5
E17M68	34	13	38.2	11	32.4
E19M88	25	6	24.0	10	40.0
E20M88	22	8	36.4	6	27.3
E31M11	40	6	15.0	14	35.0
E31M12	46	10	21.7	10	21.7
E40M14	26	7	26.9	9	34.6
E51M15	33	9	27.3	11	33.3
E51M16	34	8	23.5	8	23.5
E56M17	20	4	20.0	5	25.0
E56M18	14	3	21.4	3	21.4
E72M19	16	2	12.5	7	43.8
E21M88	22	2	9.1	3	13.6
E22M88	20	2	10.0	6	30.0
E23M40	17	4	23.5	4	23.5
E31M21	28	3	10.7	4	14.3
E31M22	20	2	10.0	3	15.0
E40M23	26	6	23.1	3	11.5
Total	548	122	22.3	148	27.0



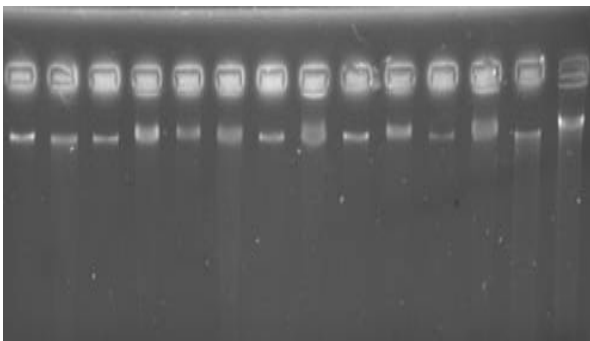
RNA isolation



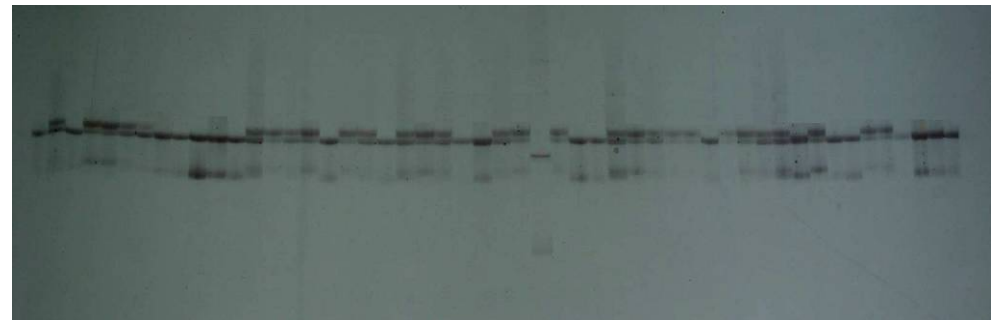
Ds cDNA syntheses



cDNA-AFLP analysis



DNA isolation



SSR analysis

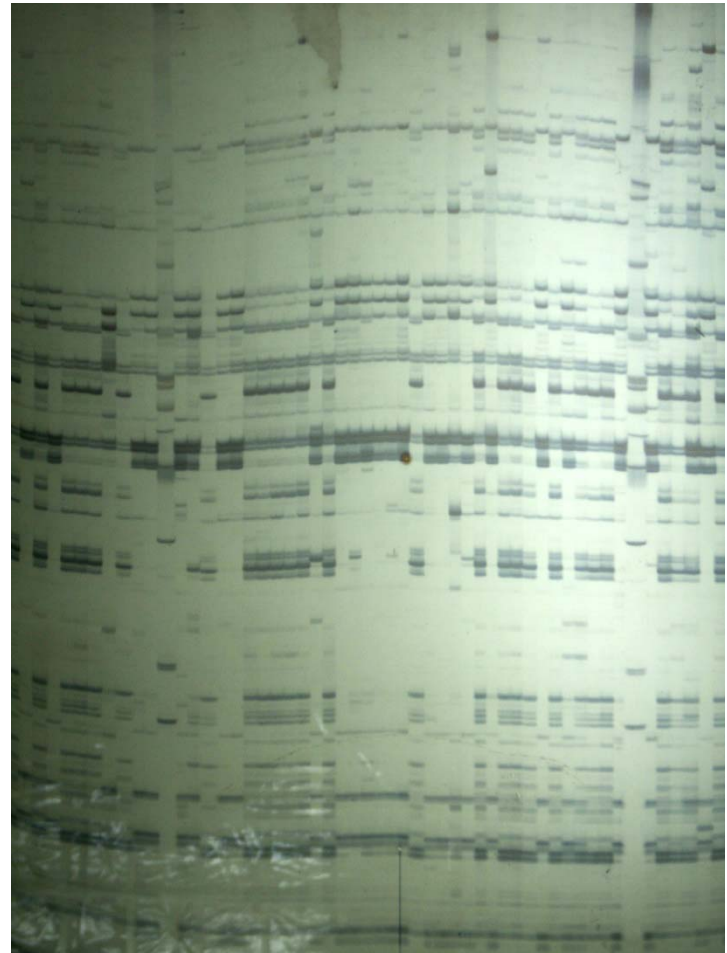


Transcriptome map construction

47 primer had been
selected from

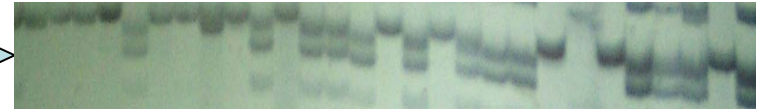
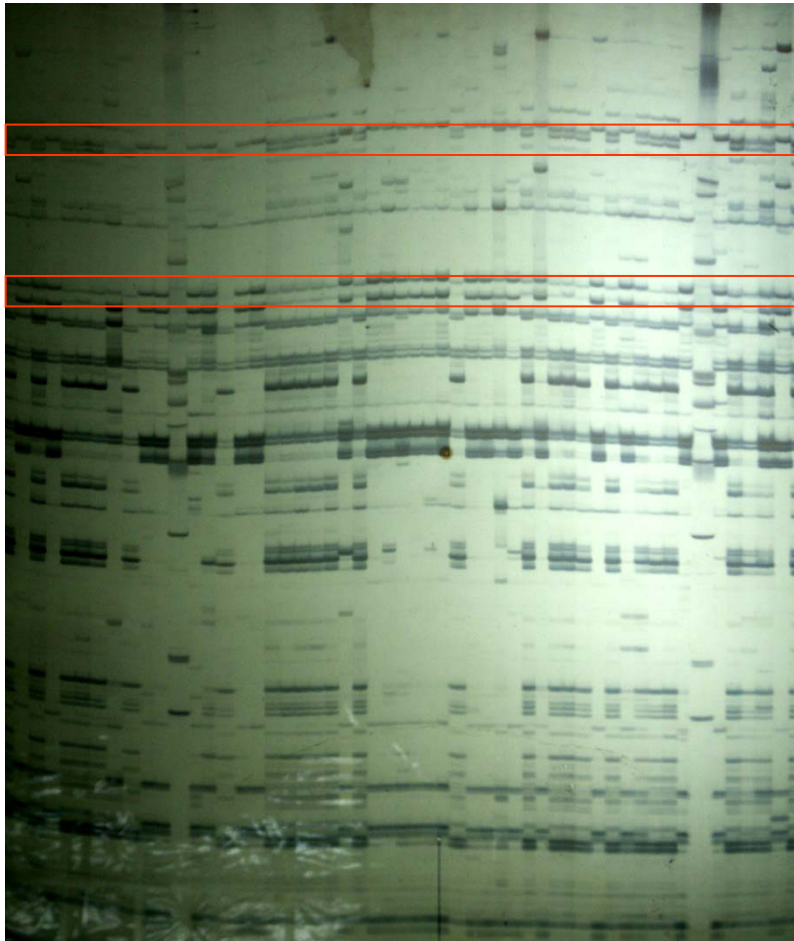
3 hundreds

And analyzed within BC1
population.

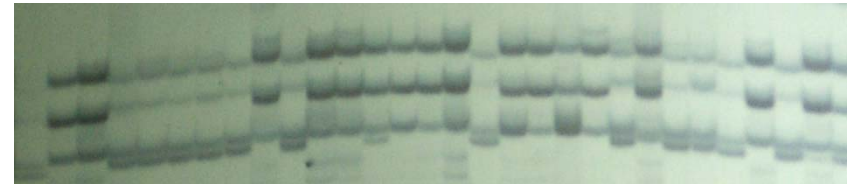




Display of marker polymorphism



1:1 segregation markers



Quantitative expression markers

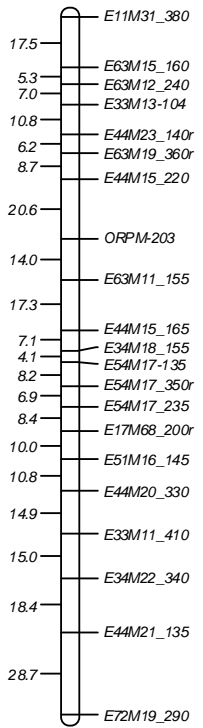
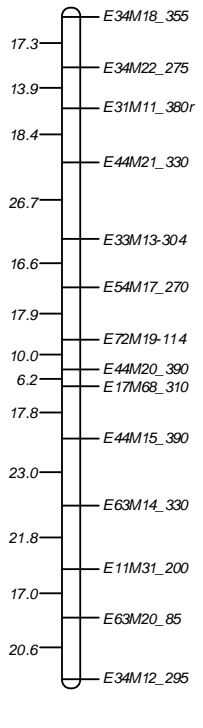
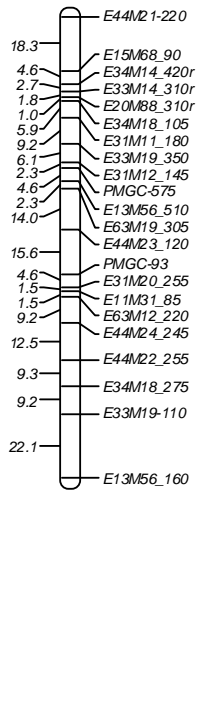
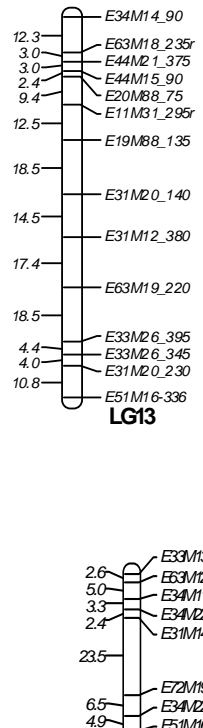
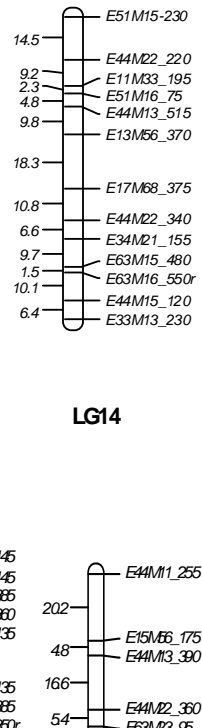
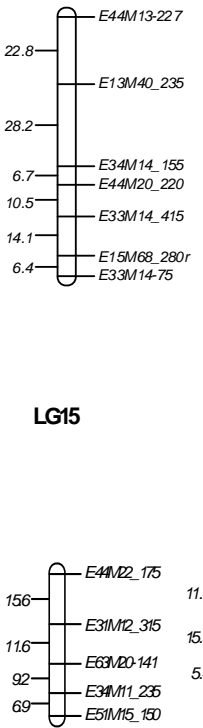
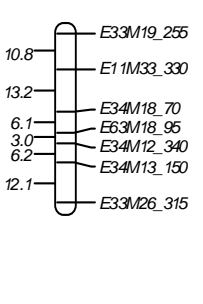
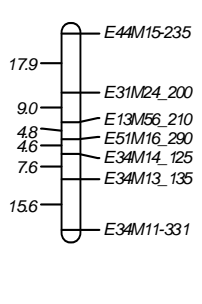
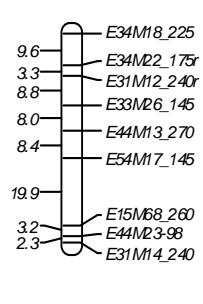
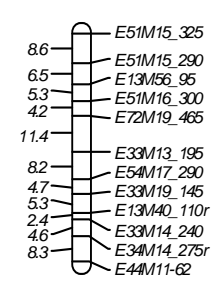
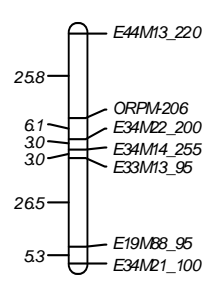
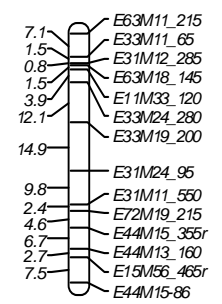
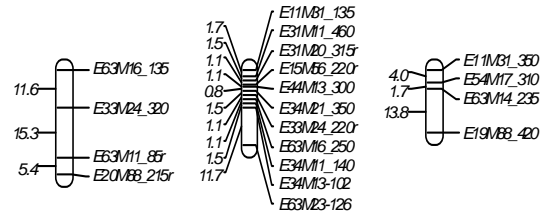
Polymorphism analysis of cDNA-AFLP markers

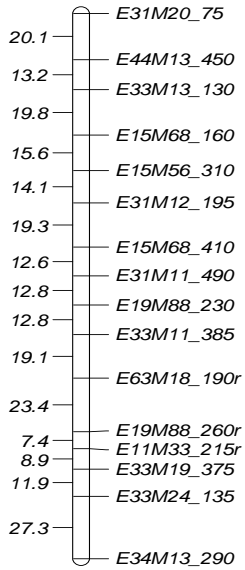
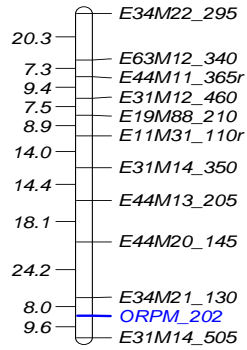
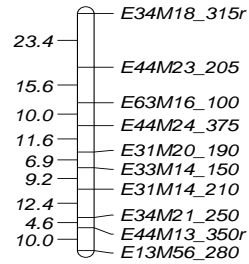
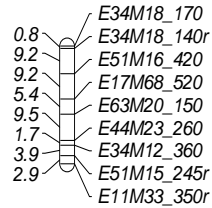
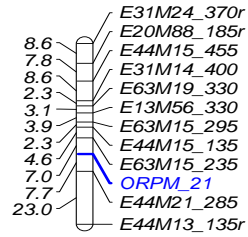
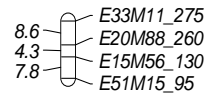
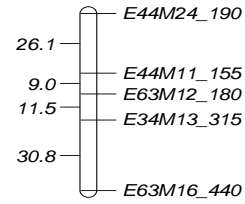
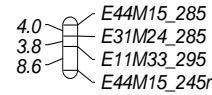
Primer combination	Total amplified bands	Polymorphic markers			Polymorphism level (%)	Testcross markers	
		Total	P1	P2		1:1	Distorted
E11M31	39	8	2	6	20.5	7	1
M33	33	6	3	3	18.2	6	0
E13M40	37	4	1	3	10.8	4	0
M56	38	7	2	5	18.4	7	0
E15M56	42	7	4	3	16.7	5	2
M68	40	6	3	3	15.0	6	0
E17M68	39	5	1	4	12.8	5	0
E19M88	38	6	3	3	15.8	5	1
E20M88	33	7	4	3	21.2	7	0
E31M11	32	7	2	5	21.9	6	1
E31M12	51	8	3	5	15.7	8	0
M14	39	7	5	2	17.9	7	0
M20	30	6	2	4	20.0	5	1
M24	28	4	2	2	14.3	4	0
E33M11	29	4	2	2	13.8	4	0
M13	27	6	1	5	22.2	4	2
M14	29	8	3	5	27.6	8	0
M19	32	7	1	6	21.9	6	1
M24	36	5	2	3	13.9	4	1
M26	36	4	0	4	11.1	4	0
E34M11	37	6	1	5	16.2	5	1
M12	31	5	2	3	16.1	3	2
M13	30	9	6	3	30.0	8	1
M14	25	10	2	8	40.0	9	1
M18	26	9	3	6	34.6	9	0
M21	25	7	3	4	28.0	7	0

Primer combination	Total amplified bands	Polymorphic markers			Polymorphism level (%)	Testcross markers	
		Total	P1	P2		1:1	Distorted
E44M11	40	9	5	4	22.5	8	1
M13	41	12	4	8	29.3	11	1
M15	39	15	6	9	38.5	14	1
M20	37	4	1	3	10.8	4	0
M21	35	7	2	5	20.0	7	0
M22	31	5	0	5	16.1	5	0
M23	38	5	2	3	13.2	5	0
M24	38	4	3	1	10.5	4	0
E51M15	26	7	3	4	26.9	7	0
M16	26	9	3	6	34.6	9	0
E54M17	38	6	0	6	15.8	6	0
E63M11	38	4	1	3	10.5	4	0
M12	38	5	2	3	13.2	5	0
M14	38	6	3	3	15.8	4	2
M15	36	5	2	3	13.9	5	0
M16	30	6	3	3	20.0	4	2
M18	25	4	1	3	16.0	3	1
M19	24	5	2	3	20.8	5	0
M20	27	6	3	3	22.2	5	1
M23	27	3	1	2	11.1	3	0
E72M19	26	5	0	5	19.2	4	1
Total	1564	308	112	196	-----	282	26
Average	33.3	6.6	4.2	2.4	19.7	6	0.08

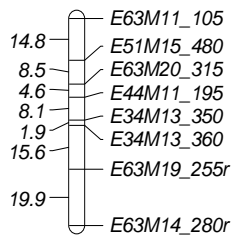
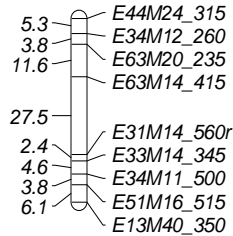
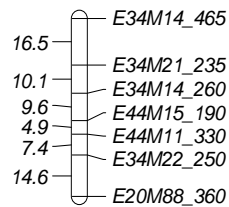
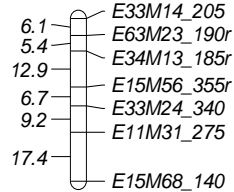
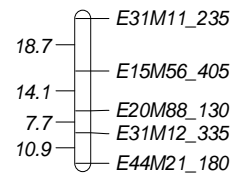
LG1**LG2****LG3****LG4****LG5****LG6**

Transcriptome map of *P. Tomentosa*

**LG7****LG8****LG9****LG10****LG11****LG15****LG16****LG17****LG18**

LG1**LG2****LG3****LG11****LG4****LG12****LG5****LG13**

Transcriptome
map of
P.tomentosa × *P.bolleana*

LG6**LG7****LG8****LG9****LG10**

Characteristics of transcriptome map of *P. tomentosa*

Linkage map	No. of markers	>20cM gaps	Min. distance	Max. distance	Total length	Average distance
LG1	21	2	4.1	28.4	239.9	11.4
LG2	13	4	6.2	26.7	209.9	16.1
LG3	22	1	1.0	22.1	158.3	7.2
LG4	14	0	2.4	18.5	130.7	9.3
LG5	13	0	1.5	18.3	104.0	8.0
LG6	7	2	6.4	28.2	88.7	12.7
LG7	14	0	0.8	14.9	75.5	5.4
LG8	7	2	3.0	26.5	69.7	10.0
LG9	12	0	2.4	11.4	69.5	5.8
LG10	9	0	2.3	19.9	63.5	7.1
LG11	7	0	4.6	17.9	59.5	8.5
LG12	7	0	3.0	13.2	51.4	7.3
LG13	9	1	2.4	23.5	50.6	5.6
LG14	5	1	4.8	20.2	47.0	9.4
LG15	5	0	6.9	15.6	43.3	8.7
LG16	4	0	5.4	15.3	32.3	8.1
LG17	11	0	0.8	11.7	23.1	2.1
LG18	4	0	4.0	13.8	19.5	4.9
Total	184	13	62.0	346.4	1536.4	147.6
Average	10.2	0.72	3.4	19.2	85.4	8.35

Characteristics of transcriptome map of (*P. tomentosa* × *P. bolleana*) × *P. tomentosa*

Linkage map	No. of markers	>20cM gaps	Min. distance	Max. distance	Total length	Average distance
LG1	16	3	7.4	27.3	238.3	14.9
LG2	12	2	7.3	24.2	141.8	11.8
LG3	10	1	4.6	23.4	103.7	10.4
LG4	12	1	2.3	23.0	79.0	6.6
LG5	5	2	9.0	30.8	77.4	15.5
LG6	8	0	1.9	19.9	73.4	9.2
LG7	9	1	2.4	27.5	65.1	7.2
LG8	7	0	4.9	16.5	63.1	9.0
LG9	7	0	5.4	17.4	57.7	8.2
LG10	5	0	7.7	118.7	51.4	10.3
LG11	9	0	0.8	9.5	42.6	4.7
LG12	4	0	4.3	8.6	20.7	5.2
LG13	4	0	3.8	8.6	16.4	4.1
Total	108	10	61.8	232.4	1030.6	117.1
Average	8.3	0.8	4.8	17.9	79.3	9.5



Characterization of transcriptome map

Genome estimation of *P. tomentosa* × *P. bolleana* and *P. tomentosa*

	<i>P. tomentosa</i>	<i>P. tomentosa</i> × <i>P. bolleana</i>
Total length of LGs (cM)	1536	1030
Genome length (Ge)	2186	1534
LOD _≥ 6.0		
N	184	108
X	28.7	30.8
K	442	232
Coverage (%)	72.3	67.1

Ge=N (N-1) X/K (Hulbert et al. 1988)



Marker distribution analysis across LGs

Linkage Group	length of LG	Length of Expected LG	Number of observed marker	Number of expected marker	Poission Value
LG1	239.9	263.9	21	25.8	0.201
LG2	227.2	262.2	14	25.6	0.009**
LG3	158.3	173.5	22	16.9	0.091
LG4	130.7	150.9	14	14.8	0.486
LG5	104.0	121.4	13	11.9	0.308
LG6	88.7	118.3	7	11.6	0.108
LG7	75.5	87.1	14	8.5	0.028*
LG8	69.7	92.9	7	9.1	0.312
LG9	69.5	82.1	12	8.0	0.064
LG10	63.5	79.3	9	7.8	0.259
LG11	59.5	79.3	7	7.8	0.481
LG12	51.4	68.6	7	6.7	0.356
LG13	50.6	63.2	9	6.2	0.099
LG14	47.0	70.6	5	6.9	0.465
LG15	43.3	64.9	5	6.3	0.558
LG16	32.3	53.9	4	6.2	0.259
LG17	23.1	27.7	11	2.7	0.0001***
LG18	19.5	32.5	4	3.2	0.219

* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$



Marker distribution analysis across LGs

Linkage Group	length of LG	Length of Expected LG	Number of observed marker	Number of expected marker	Poission Value
LG1	238.3	268.1	16	22.8	0.088
LG2	141.8	165.4	12	14.1	0.349
LG3	103.7	124.5	10	10.6	0.508
LG4	79.0	92.2	12	7.8	0.055
LG5	77.4	108.4	5	9.2	0.104
LG6	73.4	91.8	8	7.8	0.380
LG7	65.1	79.5	9	6.8	0.150
LG8	63.1	81.8	7	7.0	0.599
LG9	57.7	74.1	7	6.3	0.299
LG10	51.4	72.0	5	6.1	0.430
LG11	42.6	57.0	9	4.8	0.028*
LG12	20.7	31.1	4	2.6	0.123
LG13	16.4	24.6	4	2.1	0.062

* $P \leq 0.05$



Heterozygosity estimation within transcribed regions

Primer Combination	Total number of Amplified loci	Total number of Polymorphic loci	Polymorphism (%)	Heterozygosity level		Heterozygosity level	
				<i>P. tomentosa</i> <i>P. balsamifera</i>		<i>P. tomentosa</i>	
				Loci	Heterozygosity	Loci	Heterozygosity
E ₁₁₁ M ₁₁₁	39	8	20.5	2	0.051	6	0.154
	33	6	18.2	3	0.091	3	0.091
E ₁₁₀ M ₁₁₀	37	4	10.8	1	0.027	3	0.081
	38	7	18.4	2	0.052	5	0.132
E ₁₀₁ M ₁₀₁	42	7	16.7	4	0.095	3	0.071
	40	6	15.0	3	0.075	3	0.075
E ₁₀₀ M ₁₀₀	39	5	12.8	1	0.026	4	0.108
E ₀₁₁ M ₀₁₁	38	6	15.8	3	0.079	3	0.079
E ₀₁₀ M ₀₁₀	33	7	21.2	4	0.121	3	0.091
E ₀₀₁ M ₀₀₁	32	7	21.9	2	0.063	5	0.156
	51	8	15.7	3	0.039	5	0.098
	39	7	17.9	5	0.128	2	0.051
	30	6	20.0	2	0.067	4	0.133
	28	4	14.3	2	0.071	2	0.071
E ₀₀₀ M ₀₀₀	29	4	13.8	2	0.069	2	0.069
	27	7	22.2	1	0.037	6	0.222
	29	7	27.6	3	0.103	4	0.138
	32	7	21.9	1	0.031	6	0.188
	36	5	13.9	2	0.056	3	0.083
E ₁₁₁ M ₁₁₁	36	4	11.1	0	0.000	4	0.111
	37	6	16.2	1	0.027	5	0.135
	31	5	16.1	2	0.065	3	0.097
	30	9	30.0	6	0.200	3	0.300
	25	10	40.0	2	0.080	8	0.320
E ₁₁₀ M ₁₁₀	26	9	34.6	3	0.115	6	0.231
	25	7	28.0	3	0.120	4	0.160
	22	8	36.4	2	0.091	6	0.273
	40	9	22.5	5	0.125	4	0.300
	41	12	29.3	4	0.098	8	0.195
E ₁₀₁ M ₁₀₁	39	15	38.5	6	0.154	9	0.230
	37	4	10.8	1	0.027	3	0.081
	35	7	20.0	2	0.057	5	0.143
	31	5	16.1	0	0.000	5	0.161
	38	5	13.2	2	0.053	3	0.079
E ₁₀₀ M ₁₀₀	38	4	10.5	3	0.079	1	0.026
	26	7	26.9	3	0.115	4	0.154
E ₀₁₁ M ₀₁₁	26	9	34.6	3	0.115	6	0.231
	38	6	15.8	0	0.000	6	0.158

	M _{GC}	37	4	10.8	1	0.027	3	0.081
	M _{GG}	35	7	20.0	2	0.057	5	0.143
	M _{GT}	31	5	16.1	0	0.000	5	0.161
	M _{TA}	38	5	13.2	2	0.053	3	0.079
	M _{TC}	38	4	10.5	3	0.079	1	0.026
E _{CCA}	M _{CA}	26	7	26.9	3	0.115	4	0.154
	M _{CC}	26	9	34.6	3	0.115	6	0.231
E _{CCT}	M _{CG}	38	6	15.8	0	0.000	6	0.158
E _{GAA}	M _{AA}	38	4	10.5	1	0.026	3	0.079
	M _{AC}	38	5	13.2	2	0.053	3	0.079
	M _{AT}	38	6	15.8	3	0.079	3	0.079
	M _{CA}	36	5	13.9	2	0.056	3	0.083
	M _{CC}	30	6	20.0	3	0.100	3	0.100
	M _{CT}	25	4	16.0	1	0.040	3	0.120
	M _{GA}	24	5	20.8	2	0.083	3	0.125
	M _{GC}	27	6	22.2	3	0.111	3	0.111
	M _{TA}	27	3	11.1	1	0.037	2	0.074
E _{GGC}	M _{GA}	26	5	19.2	0	0.000	5	0.192
	Total	1564	307	-----	112	0.072	195	0.125
	Average	33.28	6.53	19.6	2.4	-----	4.1	-----

Comparison between whole genome and transcribed regions

	Whole genome		Transcribed region	
Average number of detectable bands	65.73		33.28	
Average number of polymorphic bands	17.87		6.53	
PCs with max detectable bands	E _{AAT} /M _{ATC} (110)		E _{AAA} /M _{AC} (51)	
PCs with min detectable bands	E _{AAG} /M _{AAC} (50) E _{AAG} /M _{TCT} (50)		E _{AAT} /M _{GT} (22)	
PCs with max polymorphic bands	E _{AAT} /M _{TCT} (26) E _{ATC} /M _{ATT} (26)		E _{ATC} /M _{CA} (15)	
PCs with min polymorphic bands	E _{AAG} /M _{TCT} (8)		E _{GAA} /M _{TA} (3)	
	<i>P. tomentosa</i> × <i>P. bolleana</i>	<i>P. tomentosa</i>	<i>P. tomentosa</i> × <i>P. bolleana</i>	<i>P. tomentosa</i>
Average Number of loci	5.57	12.30	2.40	4.10
Average heterozygosity(%)	8.47	18.71	7.16	12.47



Other work

- Transcriptome map from root-born seedlings

QTL analysis



sequencing the markers







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Thanks very
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