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Detection of QTLs linked to leaf and smoke properties in *Nicotiana tabacum*





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From gene to smoke



Gene expression





Enzymatic reactions

Field practices



Curing

SMOKE Organoleptic quality Undesirable compounds (PAH, TSNA...)



Combustion

Physical properties tissu quality

Molecular markers for tobacco improvement

- Development of markers is recent in tobacco
 - \rightarrow Amphidiploid (2n=48) and large genome (4500 MB)
 - → Low levels of polymorphism
 - → Markers developed for simple traits (resistance to pathogens)
- Smoke is a complex product
 - → Lots of environmental factors from the field up to the smoke
 - → Tobacco quality results from complex traits => Quantitative Trait Loci

The identification of QTLs for these traits will help elucidate their genetic control.





Possibility to modify its expression ?



Characteristics of tobacco plants selected for mapping

	4K78	ITB32	P value
Leaf harvesting time	86.2	111.71	0.0000
Total weight	2.09	2.73	0.0014
Reducing sugars	13.97	19.32	0.0032
Total alkaloid	2.7	1.7	0.0038
Nitrogen	2.72	2.19	0.0078
Nicotine	2.46	1.38	0.0015
B[a]P in smoke	11.2	21.5	0.0008





VD x 72C18



Development of 114 F6 Recombinant inbred lines (RILs)

Obtention of polymorphic markers on ITB32 and 4K78

184 polymorphic markers

- 146 AFLP (Amplified Fragment Length Polymorphism)
- 13 ISSR (Inter Simple Sequence repeat)
- 20 SSAP (Sequence Specific Amplified Polymorphism)
- 3 SCAR (Sequence Characterized Amplified Region)





- Low polymorphism in agreement with previous studies
- Partial map
 - → 75 % of detected markers are mapped
 - → 47 % of mapped markers are on 2 linkage groups
 - → A resistance factor is present on these two linkage groups (PVY^N and black root rot resistances)
- Presence of high degree of polymorphism in interspecific regions already described in tobacco (introgression from *N. debneyi*)
- High distorsion segregation (46%) mainly on three linkage groups
 - → Structural reasons (interspecific introgression)?
 - → Type of cross involved ?

QTLs detection



Variation of chemical and physical traits

Characters	Mean	SD	Range	Transgressive RILs	Heritability
Leaf harvesting time	95.9	9	44	no	0.77
Weight after curing	2.1	0.35	2	yes	0.50
Suckers production	1	1.1	5	yes	0.69
Total alkaloids	2.23	0.47	2.13	yes	0.47
Anatabine	0.2	0.08	0.43	yes	0.72
Tar *	19.97	1.67	8.6	no	0.54
Chlorine *	0.65	0.1	0.56	no	0.07
Citric acid *	0.56	1.23	1.39	yes	0.21
Tobacco weight/cig	900	74.32	411	yes	-
B[a]P	15.66	3.62	18.29	no	-

* NIRS prediction

Correlation between characters



Phenotypic variance explained with Composite Interval Mapping detection



> 30 %				
from 20	up	to	30	%
from 15	up	to	20	%
from 10	up	to	15	%
< 10 %	•			

Character	Variance (%)	LOD
Suckers	33.0	3.6
Leaf harvesting time Stalk 1	30.0	3.2
Suckers	21.5	6.7
Anabasine	27.0	8.1
Proline	18.3	5.6
Polyphenols	16.9	5.7
Polyphenols	12.7	4.4
Anabasine	9.6	3.8

Examples of QTLs detected on linkage groups 1, 3, 4, 9 and 12



To conclude :

- First work on quantitative traits related to agronomical and industrial quality of the tobacco plant
 - → 75 QTLs detected explaining from 8 up to 41.5 % of trait variation
- Major QTLs detected
 - → Anatabine (27 %), anabasine (27,8 %) (TSNA precursors)
 - → Benzo[a]pyrene (19 %) (PAH)
 - → Suckers (33,3 %, 21,5 %, 8,2 %)
 - → Leave harvesting time (30 up to 44 %)
- QTLs detected have to be confirmed
 - → On several years
 - → On different geographical localizations

Outlooks

- Development of new markers to complete the genetic map
 - → Use of ESTs databank (ATC-ITB) to look for microsatellites markers and SNP (Single Nucleotide Polymorphism) markers
 - www.estobacco.info
- Development of a genetic map based on Burley type tobaccos
 - → detection of QTLs linked to Burley specific characters
 - → More characters studied (heavy metals, aromatic amines...)
- Development of a consensus linkage map based on the Flue-cured and on the Burley genetic maps thanks to common markers

Thanks

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