



NEMATODES: FIRST STEPS TOWARD THE IDENTIFICATION OF MARKERS RELATED TO RK2 RESISTANCE GENE AGAINST *M. ARENARIA* AND *M. JAVANICA*.

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NEMATODES IN TOBACCO

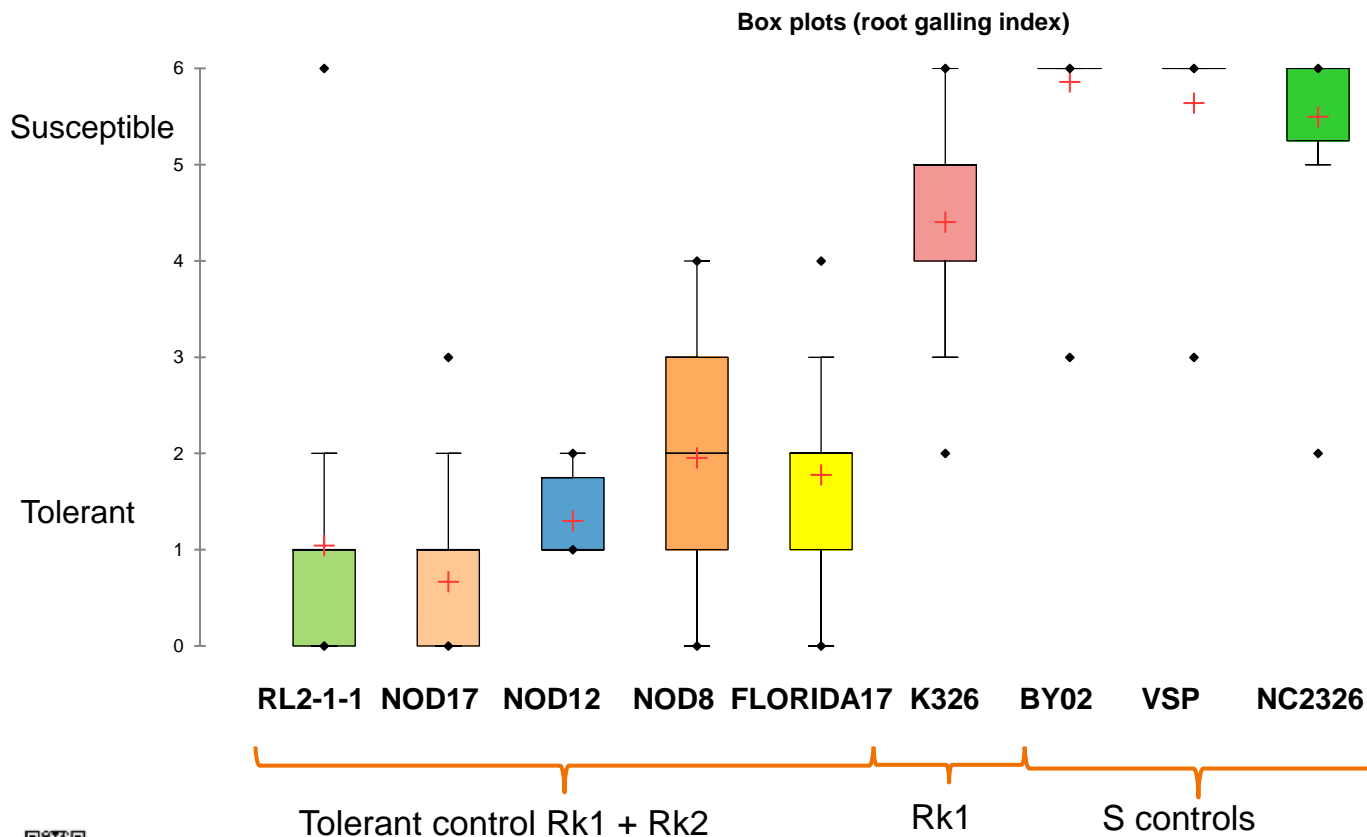
Nematodes are an important pathogen for many crops, including tobacco.

- Rk1 gene: resistance to *Meloidogyne incognita* races 1 and 3 and *Meloidogyne arenaria* race 1
 - This gene has an impact on giant cell formation and was mapped on chromosome 17
- A second locus, Rk2, identified in Zimbabwe induces partial resistant to *M. javanica*. Rk2 was incorporated in commercial varieties together with RK1 for increased efficiency, against *M. javanica*, and for *M. arenaria*.

Moreover, other factors may exist and interact with Rk2. No marker has been proposed so far.



TOLERANT VARIETIES AVAILABLE FOR STUDY



Field trial evaluation
on % of root galled
under *M. arenaria*
infestation (France,
Charentes 2020/2021)



QTL APPROACH

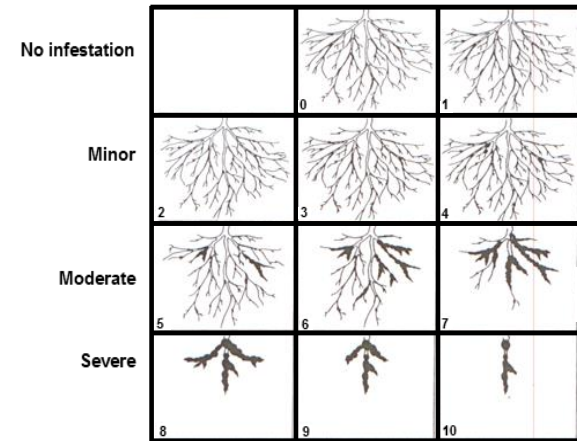
CROSS: NOD17 (Rk1/Rk2) x K326 (Rk1) to free the analysis from RK1 strong effect

POPULATIONS and PLACES:

- One pop with 110 F6 Recombinant Inbred Lines in Brazil (nursery) infested with *M. arenaria* and *M. javanica*, 2 years 2018 + 2019
- 2 pop F2 (170 + 288) in France, field trial with *M. arenaria*, years 2020 + 2021



France 2021



METHODOLOGY FOR SNPS ANALYSIS

1. Re-sequencing of parental varieties
2. Mapping of reads on K326 reference
3. SNPs detection with CLC Genomic Workbench
4. Polymorphic marker listing
5. Submission to LGC for SEQSNPS design
6. SEQSNPs analysis of segregating population (F2/F6)

NOVASEQ Analysis 26X (118 Gb) in 330 mi reads

75689 polymorphic SNPs between K326 and NOD17

Design of 2000 SNPs for SEQSNPs experiment spread on 24 chromosomes

1505 SNPs with normal distribution on F2 population

JOINMAP + MAPQTLs ANALYSIS



RESULTS

LINKAGE MAPPING WITH 170 F2 POP NOD17*K326 FIELD TRIAL 2020

Analysis with JoinMap

26 LG obtained with 1196 SNP markers

2 chromosomes on 2 LG (Nt14 and Nt17)

Chrom.	Markers	Length of Linkage Group	Chrom.	Markers	Length of Linkage Group
Nt01	44	102	Nt13	58	99
Nt02	50	99	Nt14	76/11	156/15
Nt03	42	119	Nt15	44	147
Nt04	51	175	Nt16	43	110
Nt05	49	115	Nt17	37/14	134/95
Nt06	85	139	Nt18	44	157
Nt07	43	106	Nt19	33	166
Nt08	61	138	Nt20	32	48
Nt09	55	143	Nt21	48	96
Nt10	46	127	Nt22	49	134
Nt11	49	131	Nt23	48	127
Nt12	58	147	Nt24	26	98



RESULTS ON F2 POPULATION

INTERVAL MAPPING ON ROOT GALLING INDEX (2020)

Group	Position	Locus	LOD	mu_A	mu_H	mu_B	Variance	% Expl.	Additive	Dominance
Nt12	20,068	Nt12_6482810	3.00	2.55032	3.65313	3.75753	2.94338	7.8	-0.603603	0.499203
Nt12	24,532	Nt12_7913729	4.17	2.38095	3.75309	3.70213	2.85174	10.7	-0.660588	0.711546
Nt12	33,926	Nt12_10247746	3.73	2.43902	3.60714	3.88889	2.88575	9.6	-0.724932	0.443186
Nt12	34,222	Nt12_9709049	3.68	2.43902	3.62353	3.86364	2.88960	9.5	-0.712306	0.472199
Nt12	34,517	Nt12_9686107	3.33	2.47500	3.59302	3.86364	2.91713	8.6	-0.694318	0.423705
Nt12	35,111	Nt12_9781735	3.64	2.43902	3.63954	3.83720	2.89284	9.4	-0.699093	0.501432
Nt12	36,462	Nt12_9421936	3.41	2.47499	3.58010	3.91563	2.91136	8.8	-0.720321	0.384787
Nt12	37,814	Nt12_9324129	3.87	2.43902	3.57647	3.95455	2.87506	10.0	-0.757761	0.379686
Nt12	42,743	Nt12_15324411	3.82	2.33333	3.61538	3.83721	2.87882	9.8	-0.751938	0.530113
Nt14	3,644	Nt14_10679597	3.92	2.75556	3.25641	4.25532	2.87129	10.1	-0.749882	-0.249027
Nt14	7,371	Nt14_11823614	4.07	2.95553	3.14287	4.41460	2.85971	10.4	-0.729538	-0.542192
Nt14	12,54	Nt14_13397746	3.12	2.93766	3.22106	4.30777	2.93420	8.1	-0.685050	-0.401660
Nt07	81,973	Nt07_100310758	2.89	2.53656	3.72414	3.57143	2.95211	7.5	-0.517436	0.670151
Nt07	82,868	Nt07_99614230	3.37	2.45000	3.72093	3.63636	2.91402	8.7	-0.593182	0.677748
Nt07	83,581	Nt07_99822090	3.34	2.42102	3.69246	3.66027	2.91687	8.6	-0.619622	0.651811
Nt07	84,658	Nt07_99918482	3.36	2.45000	3.69318	3.69048	2.91525	8.7	-0.620238	0.622944

3 QTLs detected by IM and MQM on Nt12, Nt14 and Nt07



RESULTS ON F6 POPULATION

BRAZIL FIELD TRIAL 2018 AND 2019

Analysis with JoinMap :24 LG obtained with 1498 SNP markers

Analysis with MapQTL6 : MQM (with notations 2018)

Group	Position	Locus	LOD	mu_A	mu_H	mu_B	Variance	% Expl.	Additive
Nt12	22.568	Nt12_10633068	5.74	6.09478	6.92605	7.75732	2.14476	21.9	-0.831271
Nt12	21.601	Nt12_10802056	5.53	6.13075	6.95358	7.77642	2.16443	21.2	-0.822832
Nt12	22.360	Nt12_10868604	5.40	6.14870	6.95124	7.75378	2.17670	20.7	-0.802541
Nt12	20.907	Nt12_11138394	5.31	6.15807	6.96447	7.77086	2.18442	20.4	-0.806399
Nt12	24.380	Nt12_10050018	5.24	6.10381	6.91299	7.72216	2.19104	20.2	-0.809175
Nt14	11.719	Nt14_13173118	3.45	6.42683	7.01600	7.60517	1.87647	10.9	-0.589173
Nt14	10.480		3.40	6.42935	7.03266	7.63597	1.88026	10.8	-0.603310
Nt14	10.677	Nt14_13166149	3.33	6.44088	7.03300	7.62511	1.88574	10.6	-0.592113
Nt14	8.480	Nt14_12364492	3.27	6.43213	7.01129	7.59046	1.89119	10.4	-0.579163
Nt14	8.162		3.25	6.42156	7.00642	7.59128	1.89247	10.4	-0.584859
Nt14	7.162		3.05	6.41294	6.98870	7.56445	1.90924	9.7	-0.575752

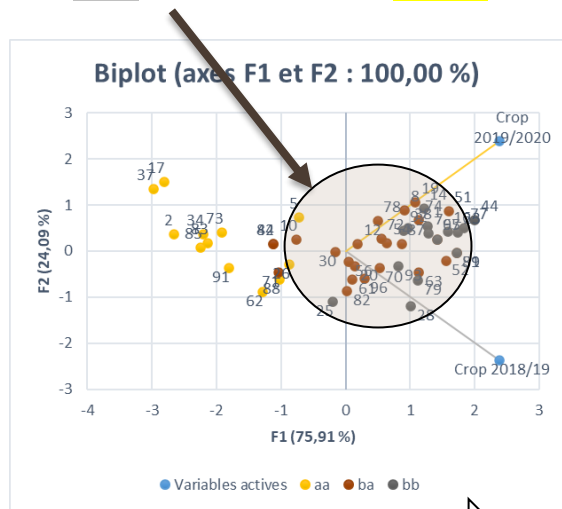
2018: 2 QTLs detected by IM and MQM on Nt12 and Nt14. No QTL on Nt07.

2019: only Nt12 detected.



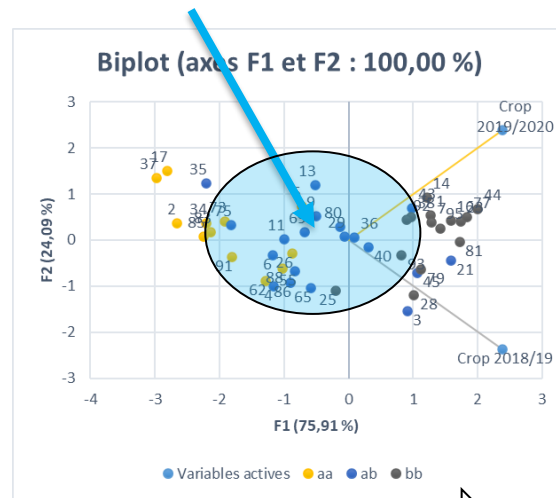
IMPACT OF THE PRESENCE OF SNPS ON NT12 AND NT14 AT CANDIDATE QTL ON F6 RILS

b/a = type K326 for Nt12 AND type NOD17 for Nt14



Tolerant Susceptible
 NOD17 type on Nt14: RILs group close to S

a/b = type NOD17 for Nt12 AND type K326 pour Nt14



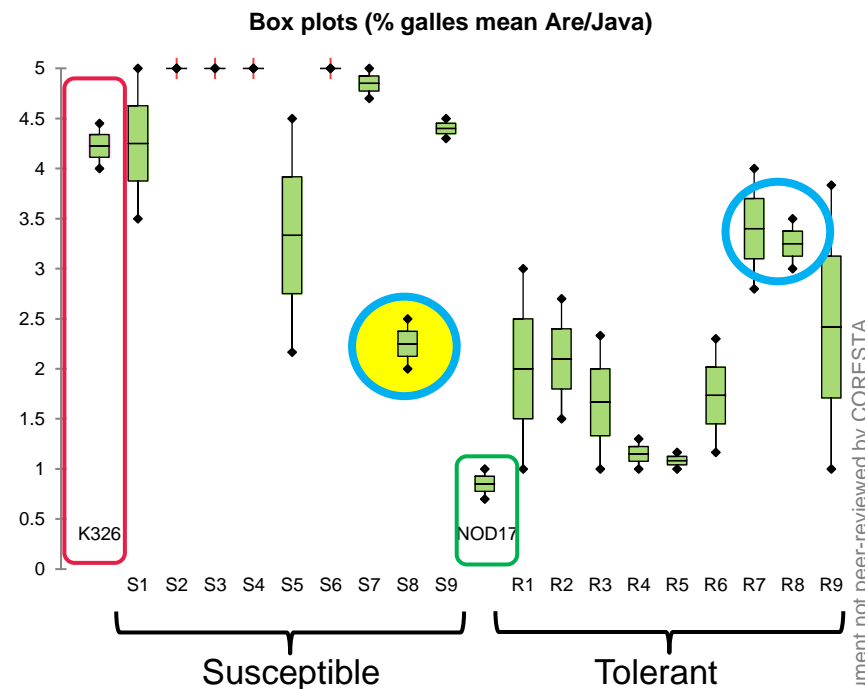
Tolerant Susceptible
 NOD17 type on Nt12: RILs group move to T

QTLs on Nt12 seems more important for tolerance phenotype than Nt14



ANALYSIS OF EXTREME F6 RILS WITH ONE MARKER AT NT12 QTL AND ONE MARKER AT NT14 QTL

Name	Brazil field trial		France Greenhouse testing		NT12	NT14
	Crop 2018/19	Crop 2019/2020	% galls M.Arenaria	% galls M.Javanica		
K326	7,8	7,5	4,45	4,00	b	b
F6 ULT "S1"	8,3	7,8	3,50	5,00	b	b
F6 ULT "S2"	8,0	8,0	5,00	5,00	b	b
F6 ULT "S3"	8,3	7,3	5,00	5,00	b	b
F6 ULT "S4"	8,8	9,0	5,00	5,00	b	b
F6 ULT "S5"	8,5	8,8	2,17	4,50	b	b
F6 ULT "S6"	8,0	7,3	5,00	5,00	b	b
F6 ULT "S7"	8,3	7,8	5,00	4,70	b	b
F6 ULT "S8"	8,0	6,5	2,50	2,00	h	h
F6 ULT "S9"	9,0	7,3	4,50	4,30	b	a
NOD17	3,5	1,3	0,70	1,00	a	a
F6 ULT "R1"	4,3	1,5	3,00	1,00	a	a
F6 ULT "R2"	2,0	2,8	1,50	2,70	a	a
F6 ULT "R3"	2,0	2,3	2,33	1,00	a	a
F6 ULT "R4"	4,3	1,8	1,00	1,30	a	a
F6 ULT "R5"	2,5	2,0	1,17	1,00	a	a
F6 ULT "R6"	2,8	3,0	1,17	2,30	a	a
F6 ULT "R7"	4,5	2,5	2,80	4,00	a	b
F6 ULT "R8"	3,0	3,3	3,50	3,00	a	b
F6 ULT "R9"	4,3	2,5	3,83	1,00	a	a



Influence of combination of markers on Nt12 and Nt14



STUDY OF A NEW F2 POPULATION: FLORIDA 17 X K326 (2021 FIELD TRIAL IN FRANCE)

Analysis with JoinMap of 288 F2 individuals

24 LG obtained with 1865 SNP markers

Reference genome used : Zouine 2021

Analysis with MapQTL6

MQM analysis with root galling index Field trial, *M. arenaria* 2021

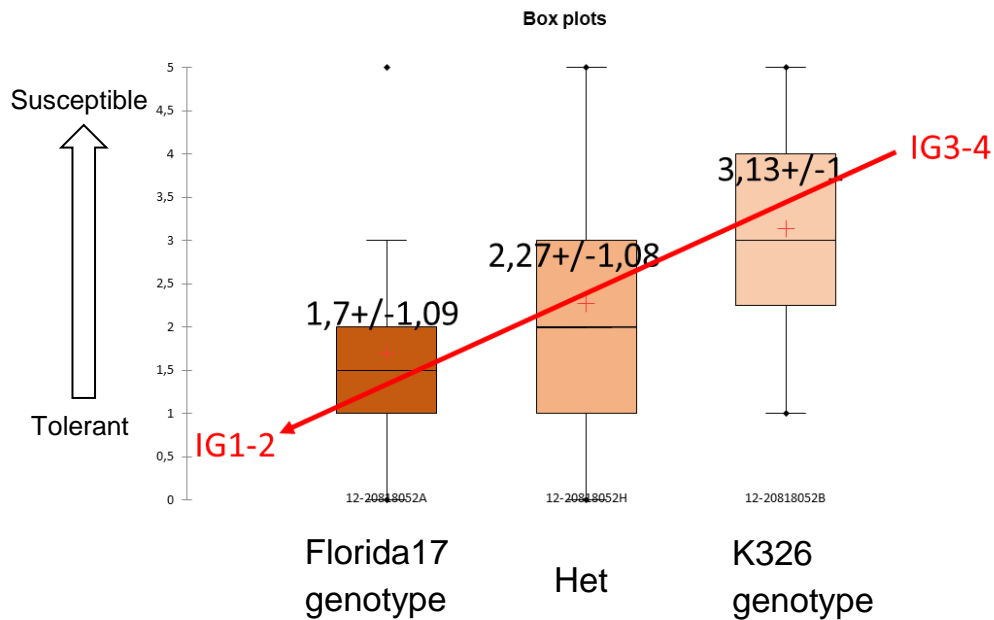
Chrom	Markers	Lenght of Linkage Group	Chrom	Markers	Lenght of Linkage Group
Nt01	103	188	Nt13	75	151
Nt02	75	111	Nt14	88	156
Nt03	95	187	Nt15	83	169
Nt04	72	168	Nt16	59	125
Nt05	77	150	Nt17	72	151
Nt06	90	188	Nt18	77	151
Nt07	70	149	Nt19	61	183
Nt08	80	162	Nt20	87	140
Nt09	84	139	Nt21	62	97
Nt10	74	130	Nt22	78	210
Nt11	61	125	Nt23	71	144
Nt12	82	176	Nt24	89	156

Nr	Group	Position	Locus	LOD	# Iter.	mu_A	mu_H	mu_B	Variance	% Expl.	Additive	Dominance	Cofactor
2712	12	139.139	12_20818052	9.35	3	1.65789	2.29264	2.96122	1.20808	15.8	0.651667	-0.0169101	
2715	12	141.118	12_5940346	9.22	3	1.65404	2.30225	2.96491	1.21147	15.6	0.655436	-0.00723155	
2713	12	139.658	12_20693812	8.98	2	1.67568	2.27119	2.96610	1.21701	15.2	0.645213	-0.0497022	
2707	12	136.735	12_22366516	8.23	2	1.68000	2.29565	2.90164	1.23378	14.0	0.610820	0.00483250	
2709	12	136.914	12_22447014	8.02	2	1.68919	2.28448	2.90164	1.23854	13.7	0.606225	-0.0109315	
2708	12	136.914	12_6288616	8.02	2	1.68919	2.28448	2.90164	1.23854	13.7	0.606225	-0.0109315	
2727	12	151.366	12_8247813	7.42	3	1.67967	2.33726	2.81982	1.25134	12.8	0.570075	0.0875167	
2721	12	146.306	12_18909046	7.33	3	1.62687	2.36613	2.80397	1.25208	12.7	0.588549	0.150713	
2739	12	162.639	12_7639268	6.89	2	1.66197	2.35000	2.78333	1.26442	11.9	0.560681	0.127347	

Only one QTL on Nt12 detected.

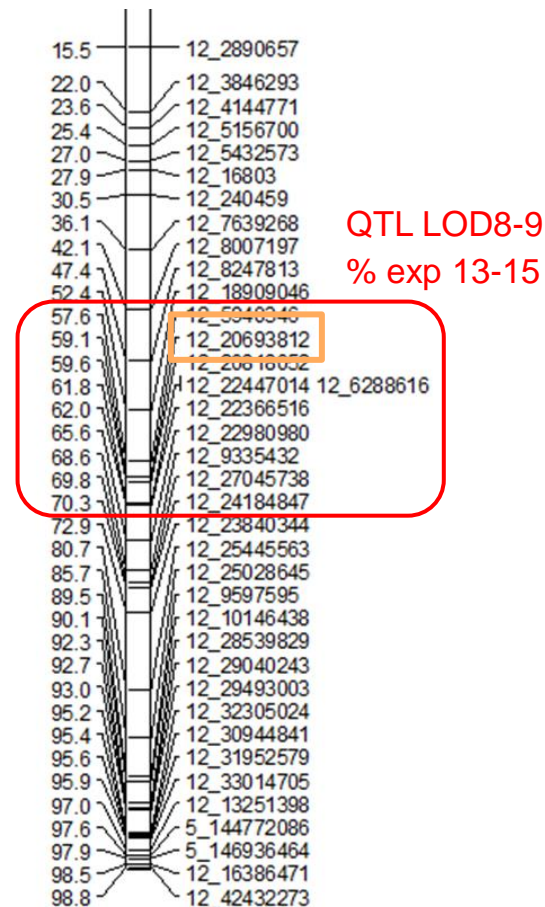


IMPACT OF ONE SNP GENOTYPE AT NT12 QTL



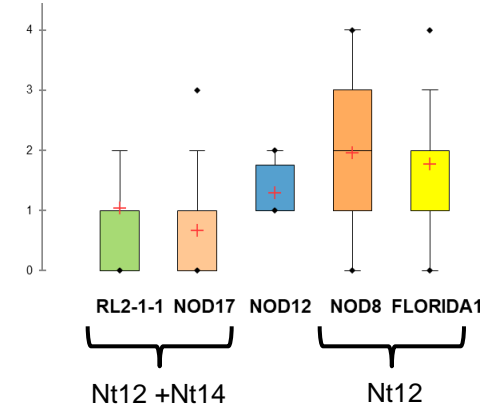
Box plot of best candidate SNP on CH12

Significant impact of marker on Nt12 on tolerance.



TO CONCLUDE

- The best candidate for Rk2 tolerance is located on Nt12.
- Rk2 seems to be under the influence of additional QTLs (at least on Nt14), not always detected according to the variety used and trial conditions (data investigation on RL211 and NOD8 not shown).
- Some candidate markers on Nt12 and Nt14 can be used to increase the chance to obtain the best plants for nematodes tolerance.
- Coming soon :
 - Analysis of F6 population with the same markers than F2 to compare F2 and F6
 - Confirmation of QTLs on F2 with 288 additional individuals (SeqSNP running, field trial 2021)





Thank you.



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