13th Annual Solanaceae Conference

SolGenomics: From Advances to Applications

CONFERENCE PROGRAM

September 12 — 16, 2016

Davis, California USA

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Roger Chetelat, UC Davis • Benny Julissa Ordonez Aquinno, UC Davis

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Session IV • HIGH-THROUGHPUT PHENOTYPING

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Session V • GENE-EDITING AND NEW BREEDING TECHNOLOGIES

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Session VI • EPIGENOMICS AND METHYLATION

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Session VII • GENOMICS-ASSISTED BREEDING

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Session IX • ABIOTIC STRESSES

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Session X • RESISTANCE, PATHOGENS, PESTS AND MICROBIOMES

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Session XI • TUBERS AND ROOT SYSTEMS

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Session XII • FLOWERS, SEEDS AND FRUIT

James Giovannoni, USDA/BTI/Cornell • Kimberly Rodriguez, New Mexico State Univ.

Session XIII • PLANT DEVELOPMENT AND REGULATION

Neelima Sinha, UC Davis • Timothy Batz, Calif. State Polytechnic Univ., Pomona

Session XIV • METABOLITES, FLAVOR AND QUALITY

Cathie Martin, John Innes Centre • Sassoum Lo, UC Riverside

9:00 am

ANALYZING AND EVALUATING THE USEFULNESS OF EXISTING MOLECULAR MARKERS FOR BREEDING OF ELITE TETRAPLOID POTATO

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In a world with increasing demands for high quality potatoes, both for direct consumption and industrial processing, breeding companies struggle to keep up. Potato breeding is largely carried out as traditional selective breeding with crossing of two tetraploid parental lines, followed by extensive phenotyping and selection of offspring, which can take 10-15 years. Thus, there is a significant interest for enhancing traditional breeding as has been done for many other crops. However, this is complicated by the extreme genetic diversity of potato, few meiotic cell divisions since introduction into Europe, and its autotetraploid nature. Nonetheless, breeding enhancement might be obtained through the use of molecular markers in marker assisted selection programs or through predictive algorithms to shorten the selection process. Molecular markers for both simple and complex traits in potatoes have been around for 20 years, but have only been applied in breeding practices to a small extent, and largely only to follow dominant resistance genes against late blight. The reason for this is that more complex molecular markers have often been derived from populations with little relevance for elite breeding programs and because marker linkages to traits are frequently lost.

With publicly available molecular markers as starting point, we have designed a pipeline for conducting analysis and evaluation of the usefulness of markers in an industrially relevant population of breeding clones and cultivars from the Danish breeding company LKF Vandel. Using the potato genome model (DM v4.03), CLC Genomics Workbench 8.0 and Illumina high throughput sequencing of multiple amplicons enabled us to map, select and, if necessary, redesign markers for genotyping a small population of 48 potato plants. Sequencing and analysis of haplotypes from each individual plant enabled both evaluation of the markers for different traits simultaneously and calculation of a reasonably predictive value for each trait.

9:15 am

GENOMICS-ASSISTED QTL MAPPING FOR AGRONOMICAL TRAITS IN PEPPER

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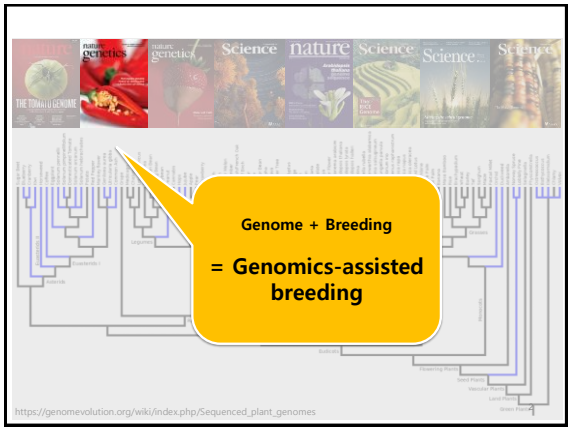
Pepper (*Capsicum annuum*) genome published in 2014 allows rapid identification of the genetic loci controlling agronomically important traits. To identify QTLs controlling pepper traits including plant height, fruit color, weight, and pungency, we constructed high-density maps by re-sequencing 120 recombinant inbred lines (RILs) derived from an intraspecific cross (*C. annuum* 'Perennial' × *C. annuum* 'Dempsey') and by genotyping-by-sequencing (GBS) of 96 RILs from an interspecific cross (*C. annuum* 'TF68' × *C. chinense* 'Habanero'). Single nucleotide polymorphisms (SNPs) were detected by aligning sequences obtained from resequencing and GBS to the *C. annuum* 'CM334' reference genome. To improve efficiency of genetic map construction, adjacent SNPs were combined to bins using sliding window approach. Genotypes of the bins were determined based on the ratio of SNPs with different genotypes. Also parental SNPs from GBS were imputated using a haplotype map derived from GBS of pepper germplasm. From resequencing of intraspecific population, 1.4 million SNPs were detected and a linkage map with a total map length of 1,372 cM was constructed using 2,578 SNP bins. A total of 17 horticultural traits including plant architecture, leaf, flower, and fruit were evaluated for three

GENOMICS-ASSISTED QTL MAPPING FOR AGRONOMICAL TRAITS IN PEPPER

Seoul National University
Horticultural Crops Breeding and Genetics Lab.
Koeun Han



Flower

Fruit

Quantitative Trait Locus

Plant architecture

Pungency

Plant materials

- 120 RILs from *C. annuum* 'Perennial' × *C. annuum* 'Dempsey'
- 85 RILs from *C. annuum* 'TF68' × *C. chinense* 'Habanero'

Cross	Casaicinoids contents (mg/gDW)	No. of lines (pungent lines)	Generation
C. annuum 'Perennial' × *C. annuum* 'Dempsey'	38.01 / 0 (placenta)	120 (56)	$F_7 \sim F_{11}$
C. annuum 'TF68' × *C. chinense* 'Habanero'	5.3 / 75.58 (placenta)	85 (85)	$F_8 \sim F_{10}$

<PD RIL>

<TH RIL>

Methods for phenotyping

- Plant growth - more than two environments

Population	Year	No. of RILs	Geological location	Cultivation
PD RIL	2011	170	Anseong	Ground
	2012a	162	Anseong	Ground
	2012b	162	Suwon	Pot
TH RIL	2013	102	Anseong	Ground
	2014	104	Anseong	Ground

- Morphological traits (PD RIL)
  - Plant architecture, flower, leaf and flower related traits
- Capsaicinoids contents (PD RIL and TH RIL)
  - Dissection of placenta from 5 fruits per each
  - Measurement of capsaicin and dihydrocapsaicin contents by HPLC

Methods for genotyping

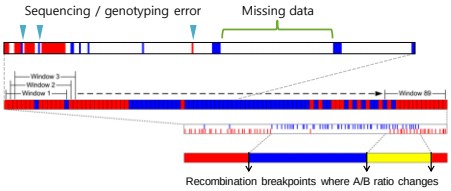
Sequencing
- Whole-genome re-sequencing of 'PD RIL' (Kim et al, 2014)
  - 18× for parents and 1× for RILs
- Genotyping-by-sequencing of 'TH RIL'
  - *PstI*/*MseI* double digestion with SBG v.2.0 kit (Keygene, Netherlands)

Genotyping
- SNP calling
  - *C. annuum* 'CM334' reference genome
  - BWA-mem, SAMtools, Picard, and GATK UnifiedGenotyper
  - SNPs with minimum QUAL 30 and depth 3

Bin map
- Sliding window approach
  - Imputation of missing data
  - Window length: physical length (PD RIL) or SNP number (TH RIL)

QTL analysis
- Composite interval mapping
  - WinQTL Cartographer

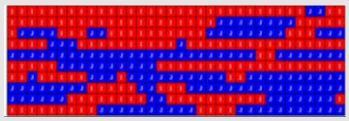
Imputation by sliding window approach



Bin map

Increased accuracy

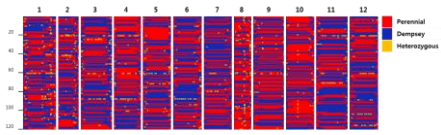
Useful for QTL analysis



(Huang et al, Genome Res, 2009)

Bin map of 'PD RIL'

- Whole-genome re-sequencing of 120 RILs

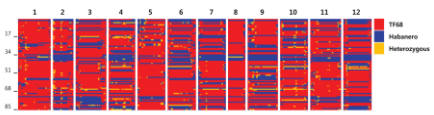


Chr.	Number of SNPs	Number of bins	Physical length of bin (Mb)		Genetic distance of bin (cM)	
			Mean	Total	Mean	Total
1	82,966	390	0.7	272.6	0.5	210.9
2	80,141	207	0.8	171.1	0.5	108
3	87,793	279	0.9	257.9	0.4	118
4	54,657	224	1.0	222.5	0.5	117.2
5	82,413	201	1.2	233.4	0.5	100.2
6	107,015	234	1.0	236.9	0.4	102.2
7	84,339	180	1.3	231.9	0.5	86.3
8	24,383	224	0.6	144.8	0.7	145.6
9	275,842	179	1.4	252.7	0.5	89.7
10	230,360	160	1.5	233.6	0.6	102.1
11	252,765	202	1.3	259.7	0.4	83.8
12	68,540	233	1.0	235.7	0.4	94.9
Total	**1,431,214**	**2,713**	1.0	2,752.8	**0.5**	**1,358.9**

(Han et al, DNA Res, 2016)

Bin map of 'TH RIL'

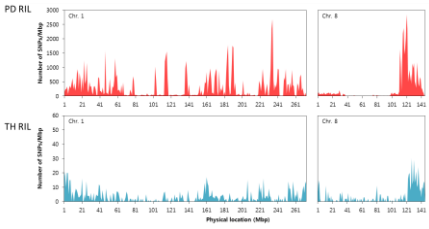
- Genotyping-by-sequencing of 85 RILs



Chr.	Number of SNPs	Number of bins	Physical length of bin (Mb)		Genetic distance of bin (cM)	
			Mean	Total	Mean	Total
1	920	118	2.3	272.7	1.3	155.2
2	804	93	1.8	171.1	1.2	112.1
3	1,060	127	2.0	257.9	0.9	111.7
4	657	102	2.2	222.6	0.8	81.7
5	672	105	2.2	233.5	0.7	78.2
6	794	101	2.3	236.9	0.9	89.9
7	679	78	3.0	231.9	1.0	80.5
8	541	35	4.1	145.1	1.1	39.6
9	614	88	2.9	252.8	1.0	88.5
10	589	83	2.8	233.6	1.3	105.4
11	613	88	3.0	259.7	1.0	87.3
12	644	71	3.3	235.7	1.4	97.2
Total	**8,587**	**1,089**	2.5	2,753.5	**1.0**	**1,127.3**

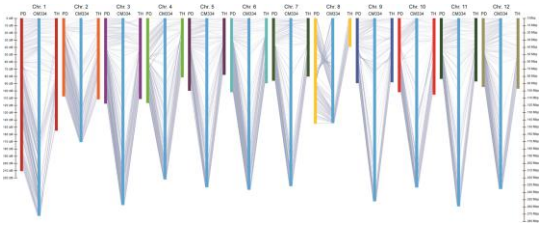
Bin map from WGS and GBS

	Population	
	PD RIL	TH RIL
Sequencing	**WGS**	**GBS**
Average number of SNPs per chromosome	119,268	716
Average distance between SNPs (bp)	2,713	328,662
Maximum distance between SNPs (bp)	1,590,868	10,432,679
Distance of LG constructed with SNP window (cM)	2,383	**1,127**
Distance of LG constructed with physical window (cM)	**1,359**	2,043

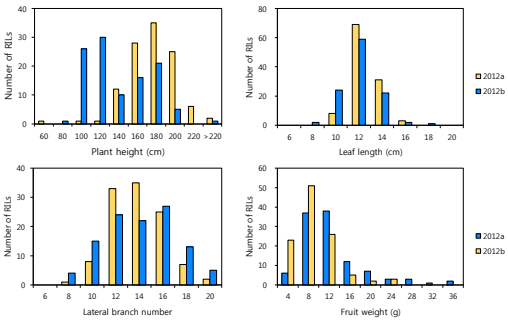


Comparison of bin maps

- Genetic location in bin map and physical location in 'CM334' reference genome
  - Collinearity of bins except centromeric regions and chromosome 8

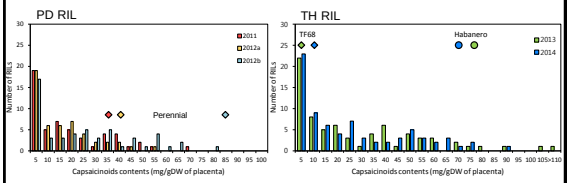


Variation of quantitative traits in 'PD RIL'



Capsaicinoids contents of RILs

- Transgressive segregation except 2012b of 'PD RIL'
- Positive skew
  - Epistasis between QTLs



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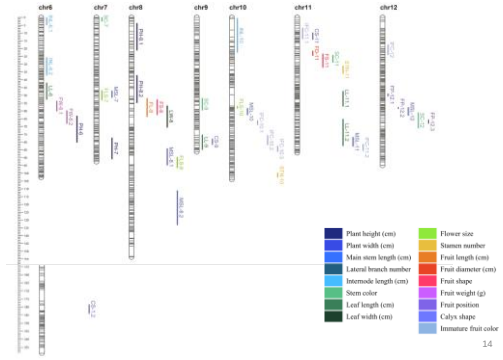
Major QTLs for morphological traits



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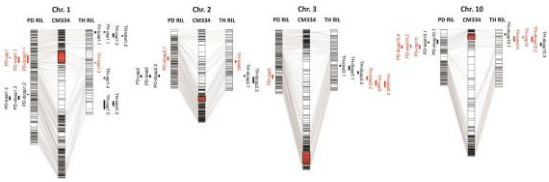
Major QTLs detected from both RILs

- Comparison of physical location of QTLs
- 4 QTLs on chromosome 1, 2, 3, and 10 were detected commonly



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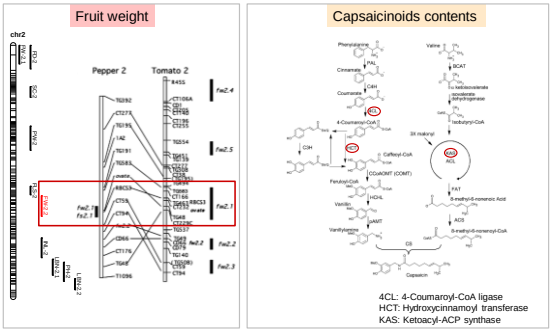
Commonly detected QTLs



(Zygier et al, TAG, 2005) (Lee et al, Plant Breeding, 2016; Ben-Chaim et al, TAG, 2006) 16

Summary

- High-density bin maps were constructed by WGS and GBS of RILs
- To use sliding window approach, window length determined by physical length and number of consecutive SNPs is appropriate for WGS and GBS data, respectively
- A total of 86 significant QTLs controlling 17 morphological traits and 4 major QTLs controlling capsaicinoids contents were detected
- Construction of bin map using low-coverage sequence is a powerful tool for QTL mapping

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