



Genomic Resources for Orphaned Andean Crops – Amaranth and Quinoa

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Chenopodium quinoa

FAO/UN - 2013 the International Year of Quinoa



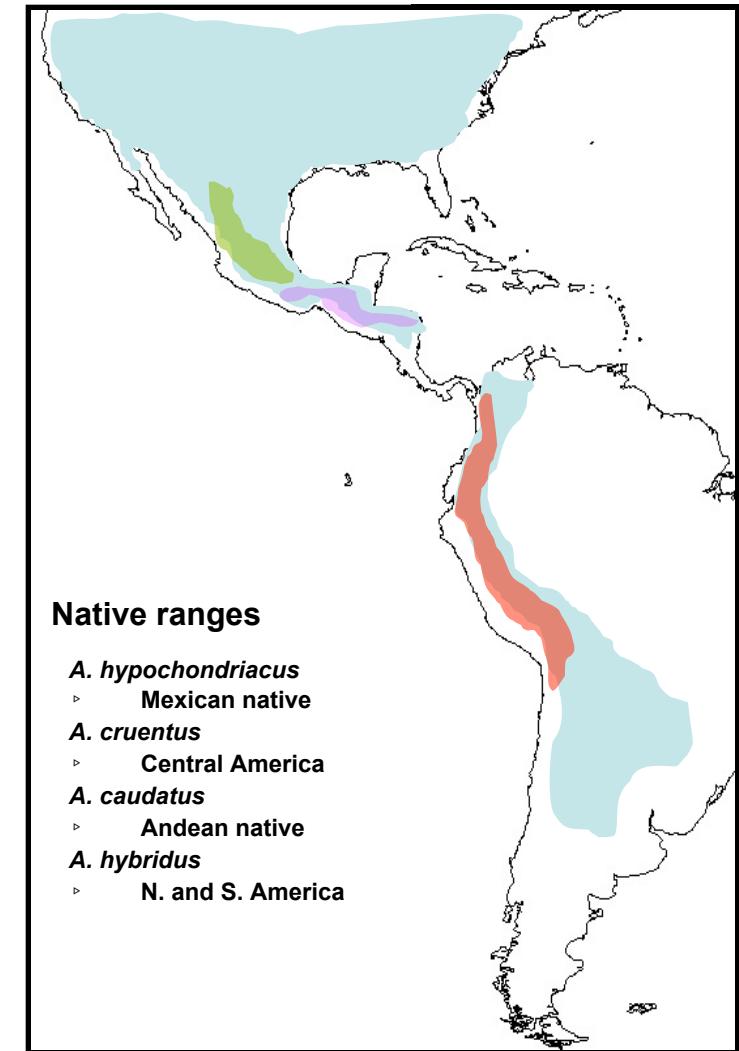
- ▷ The Altiplano: 255,000 km² at 3500-3850m (~12,500 ft)
- ▷ 100–200 mm (4-8 inches) rainfall/yr, frequent frosts (-3 to 38°C)
- ▷ Allotetraploid ($2n = 4x = 36$)
- ▷ Grain crop, excellent balance of carbohydrates, lipids, and protein
 - ▷ Ideal balance of essential amino acids
 - ▷ Protein content: 7.5 - 22.1%
 - ▷ Oil content: 4.5 - 8.75%
- ▷ Anti-nutritional triterpenoid saponins
- ▷ Export crop for expanding organic food market
- ▷ Bolivian government views increased quinoa productivity as critical for improving domestic food security



Amaranthus

Before the Spanish conquest of the ancient Americas, the grain amaranths were one of the basic foods of the New World. Records indicate that nearly 20,000 tons were sent from 17 provinces to Tenochtitlan (present-day Mexico City) as annual tribute to the Aztec emperor Montezuma (NCR 1984). This is equal to the tribute expected of beans and maize! Oldest archeological evidence 5000 BCE (Puebla, Mexico)

- ▷ Botanical Description: Broad-leaved, annuals - 0.4 to 3.0m in height
- ▷ Inflorescences: Dichasial cyme - variety of colors: red, purple, orange, gold, etc.
- ▷ Flowers: Unisexual, First flower on a cyme is staminate, 100+ pistillate flowers - Favors self-pollination over outcrossing (Sauer 1993)
- ▷ Fruit: Dehiscent Pyxides
- ▷ Seed: Variety of colors: pale vs. dark, 1 to 1.5mm diameter, seeds eaten raw or cooked as pseudocereal, leafy vegetables (15% protein)



Germplasm bank – USDA, Ames, IA

David Breener

Amaranthaceae- Genera (6); Accessions (3,297)
Chenopodiaceae – Genera (5); Accessions (751)

<http://www.ars-grin.gov>



Wheat – 69,266 Ac
Maize – 30,761 Ac



Arguably the genus contains the most damaging weedy species in the U.S.!

Summary of major *Amaranthus* weeds in the United States

Species	Common name	U.S. distribution ^a	Primary habitat ^b	Sex strategy	Nuclear DNA content ^c (pg)
<i>A. retroflexus</i>	redroot pigweed	throughout	agronomic	monoecious	1.1
<i>A. hybridus</i>	smooth pigweed	eastern half	agronomic	monoecious	1.0
<i>A. powellii</i>	Powell amaranth	northwest	agronomic	monoecious	1.0
<i>A. spinosus</i>	spiny amaranth	southeast	pastures	monoecious	1.0
<i>A. albus</i>	tumble pigweed	throughout	rangelands	monoecious	1.1
<i>A. blitoides</i>	prostrate pigweed	throughout	vegetable	monoecious	1.1
<i>A. tuberculatus</i> ^d	tall waterhemp	eastern half	agronomic	dioecious	1.3
<i>A. palmeri</i>	Palmer amaranth	southern	agronomic	dioecious	0.9

^aData from Horak et al. 1994.

^bAlthough a primary habitat is given, most of these species can be found in numerous habitats.

^cData from Jeschke et al. 2003 and Rayburn et al (In press).

^d*A. tuberculatus* and *A. rudis* are now classified as one species, *A. tuberculatus* (Pratt and Clark 2001; Mosyakin and Roberson 2004).



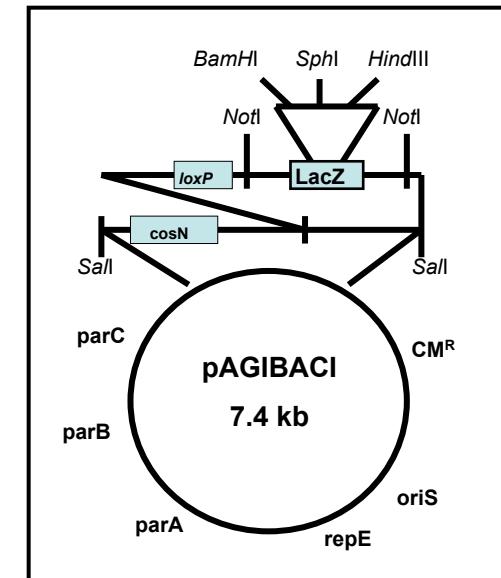
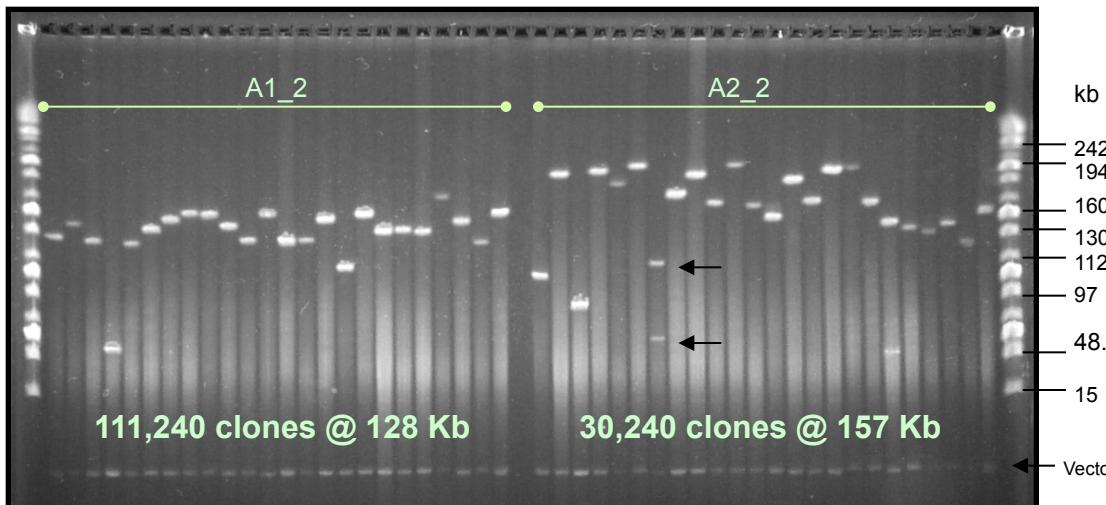
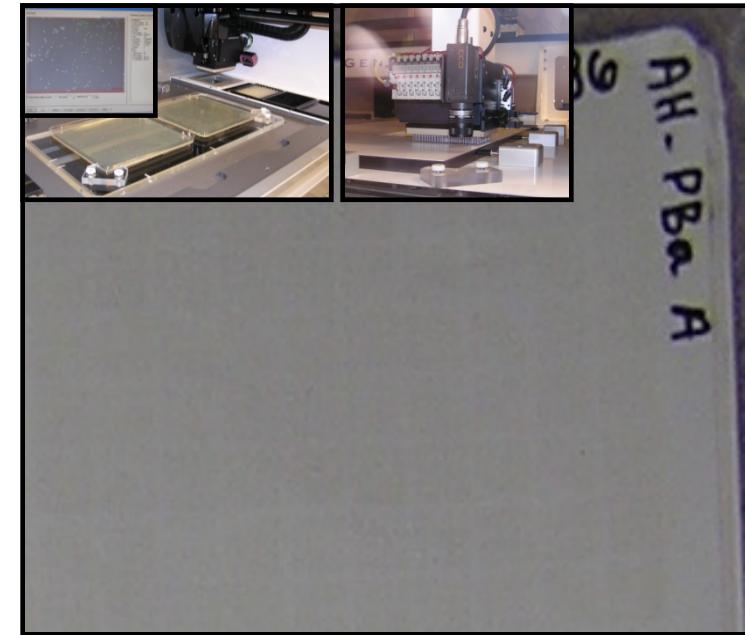
Round-up resistant Palmer amaranth (superweed) in a field of GMO Soybean

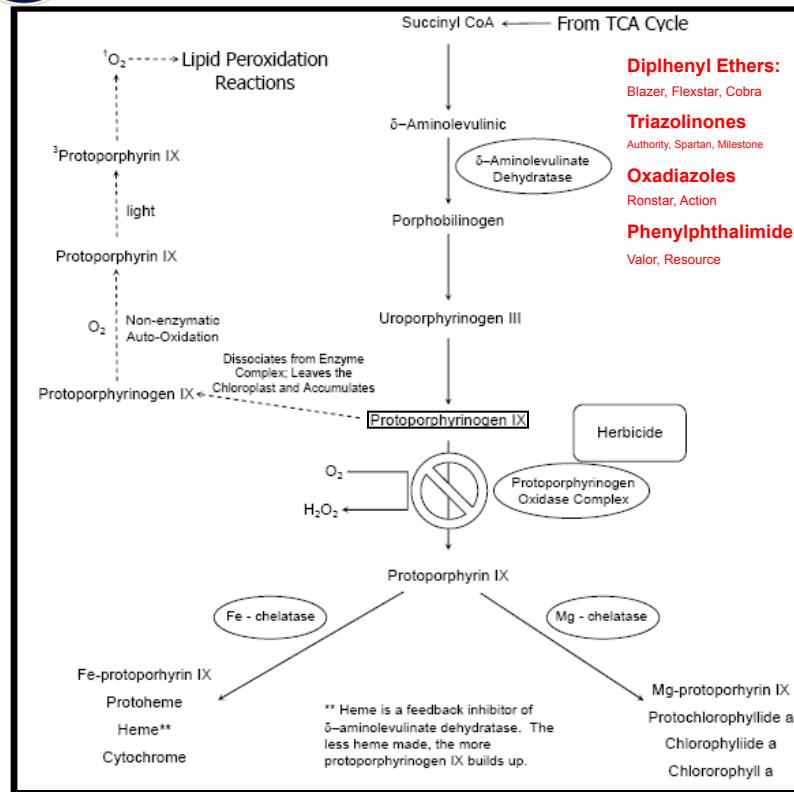
- ✓ Redroot pigweed and slender amaranth, are among the most widely distributed weeds in the world (Holm et al. 1997)
- ✓ Smooth pigweed and spiny amaranth are ranked among the 18 most serious weeds in the world (Holm et al. 1991)
- ✓ The pigweeds are notorious for their ability to develop resistance to herbicides. Resistance to triazine, acetolactate synthase, dinitroanilines (e.g. trifluralin), bipyridyliums (e.g., paraquat), and protoporphyrinogen oxidase-inhibitors (e.g., acifluorfen) have all been reported (Heap 2004)
- ✓ The pigweeds (and the grain amaranths) use the C4 photosynthetic pathway
- ✓ Pigweeds use two contrasting strategies of sexual reproduction

BAC Library Construction

Maughan et al. (2008) *Crop Sci.* 48:85-94

- ▶ Predicted average insert size of 139 kb/insert - should be near a 10X library.
- ▶ The entire library (AH_Pba) was double spotted on to two (2) filter membranes, where each contained 36,864 double spotted clones.





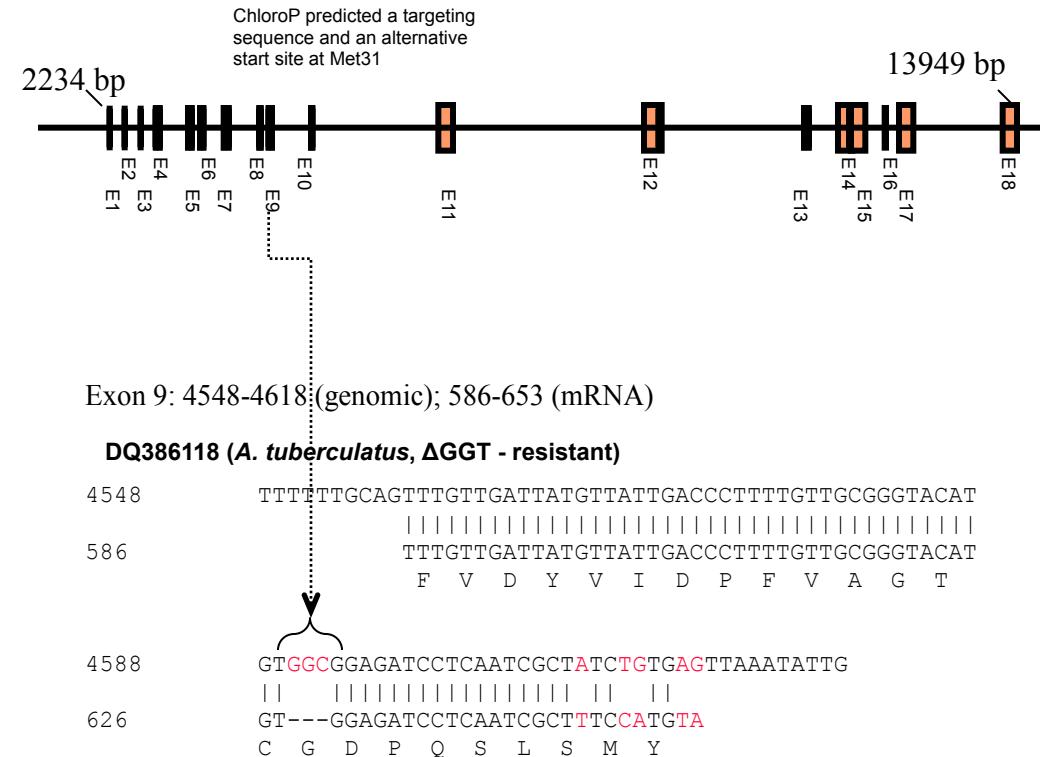
Protoporphyrinogen oxidase (PPO) is the last common enzyme in the tetrapyrrole biosynthetic pathway that produces heme.

- ▶ **P. Tranel** (UI) provided primers for the amplification of a PPX probe from *A. hypochondriacus*.
 - ▶ PCR product was sequenced, labeled and probed against the BAC library.
 - ▶ Two positive hits were identified and then selected for primer walking.

Protoporphyrinogen oxidase

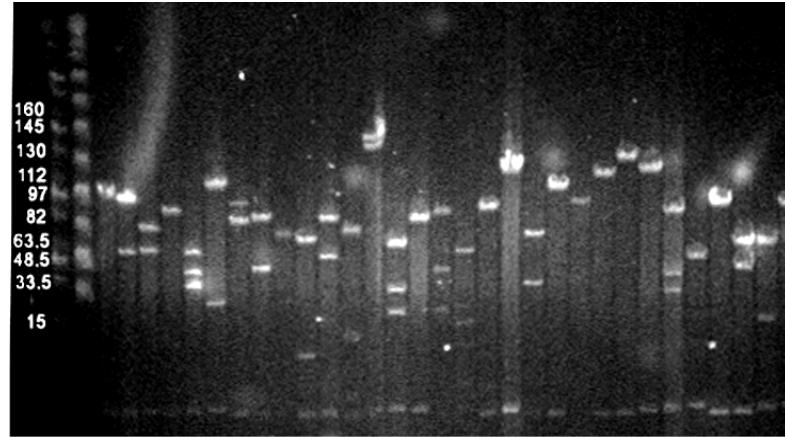
Patzoldt et al. (2006) A codon deletion confers resistance to herbicides inhibiting protoporphyrinogen oxidase. PNAS 103:12329-12334

Spidey Alignment: mRNA coverage: 100% - Overall percent identity: 97.0%
1608 bp of coding - 10108 bp of intron sequence (535 AA)



Bacterial Artificial Chromosome (BAC) Library

- ▷ Contains approximately 70,000 clones
- ▷ 10 X genome coverage
- ▷ Average fragment size in the first half of the library is 112 kb
- ▷ Average fragment size in the second half of the library is 130 kb
- ▷ In storage for public use at the Arizona Genomic Institute

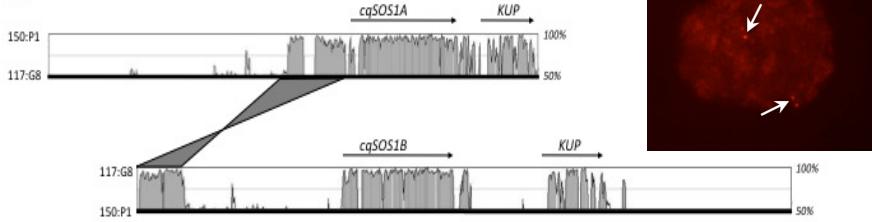


Stevens et al. (2006) *Theor. Appl. Genet.* 112:1593-1600

Characterization of salt overly sensitive (SOS1) gene homoeologs in quinoa

Maughan et al. (2009) *Genome* 52:647–657

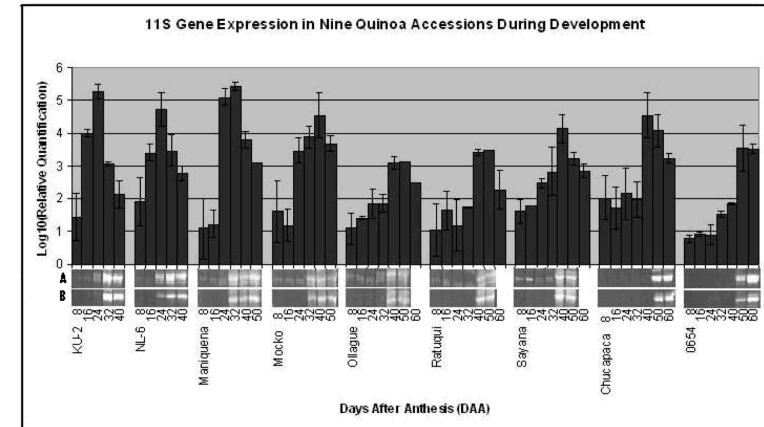
A.



	Number of Exons	Coding Length	Average Exon Length (Range)	Average Intron Length (Range)	Nucleotide Identity	Variable nucleotide sites	Insertion sites	Protein Identity	K _s (# of sites)	K _a (# of sites)	K _a /K _s ratio	Nucleotide diversity (Pi)*
SOS1A	23	3477	152	812 (78-2124)	96.9%	100	1 (9 bps)	96.5%	0.077 (62)	0.0145 (38)	0.186	0.0293
SOS1B	23	3486	(45-312)	842 (75-1998)								

Expression and evolutionary relationships of the *quinoa 11S* seed storage protein gene

Balzotti et al. (2008) *Int. J. Plant Sci.* 169:281-291



Glimpsing the Genome: BAC end sequencing & SSR development

Maughan et al. (2008) *Crop Sci.* 48:85-94

Four samples from each of the 96 384-well plates – bidirectionally sequenced.

Number of Samples*	768
Number of Successful Samples	748
Ave. No. of HQ Bases	747 bp
Total Length of Sequence	563 Kb
GC content:	35.12%
Retroelements:	37 (2.84%)
LINE (L1/CIN4)	5
LTR (Copia/Gypsy)	32
DNA transposons:	3 (0.07%)
Hobo, En-Spm, MuDR	
Simple sequence repeats: (TA) _n ; (TAA) _n ; (TAAA) _n ; (TAAAA) _n	66

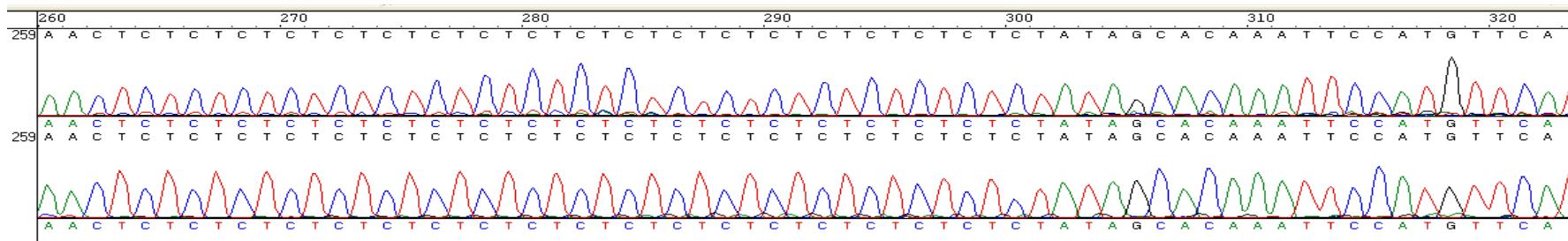
Transposable elements:

The fraction of predicted repeat sequences identified is low for a complex genome and suggests that *Amaranthus* may possess unique repeated sequences not detected by comparisons to *Arabidopsis* repeat database.

SSR development:

Enriched three libraries: AAT, AAC, AC

- ▷ 1457 clones sequenced (2% not readable)
 - ▷ 487 AAT library (45% unique)
 - ▷ 482 AAC library (27% unique)
 - ▷ 488 to the AC library (28% unique)
 - ▷ High number of AAT motifs observed in related species: *C. quinoa* and *B. vulgaris* (Mörchen et al. 1996, Mason et al. 2005, Jarvis 2006)



Microsatellites (SSRs) - Cost and limited availability

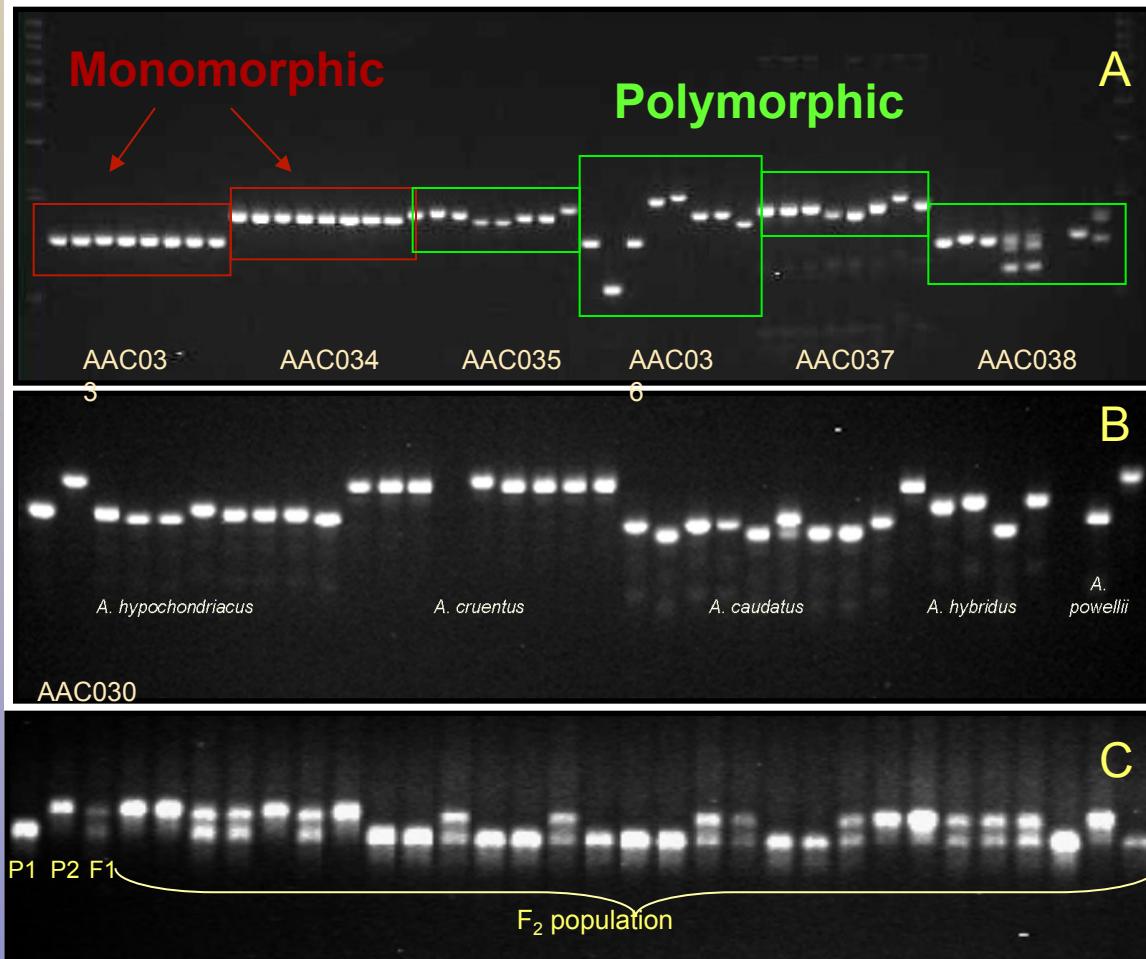
Mallory et al. (2008) *Crop Sci.* 48:1098-1106

Screened all SSRs 8 panel of grain amaranths (Panel A)

Ran polymorphic primers on full panel of 36 amaranth accessions (Panel B)

Quantified polymorphism using data from gels and checked for normal Mendelian segregations (Panel C)

Analyzed phenetic relationships among species using PAUP

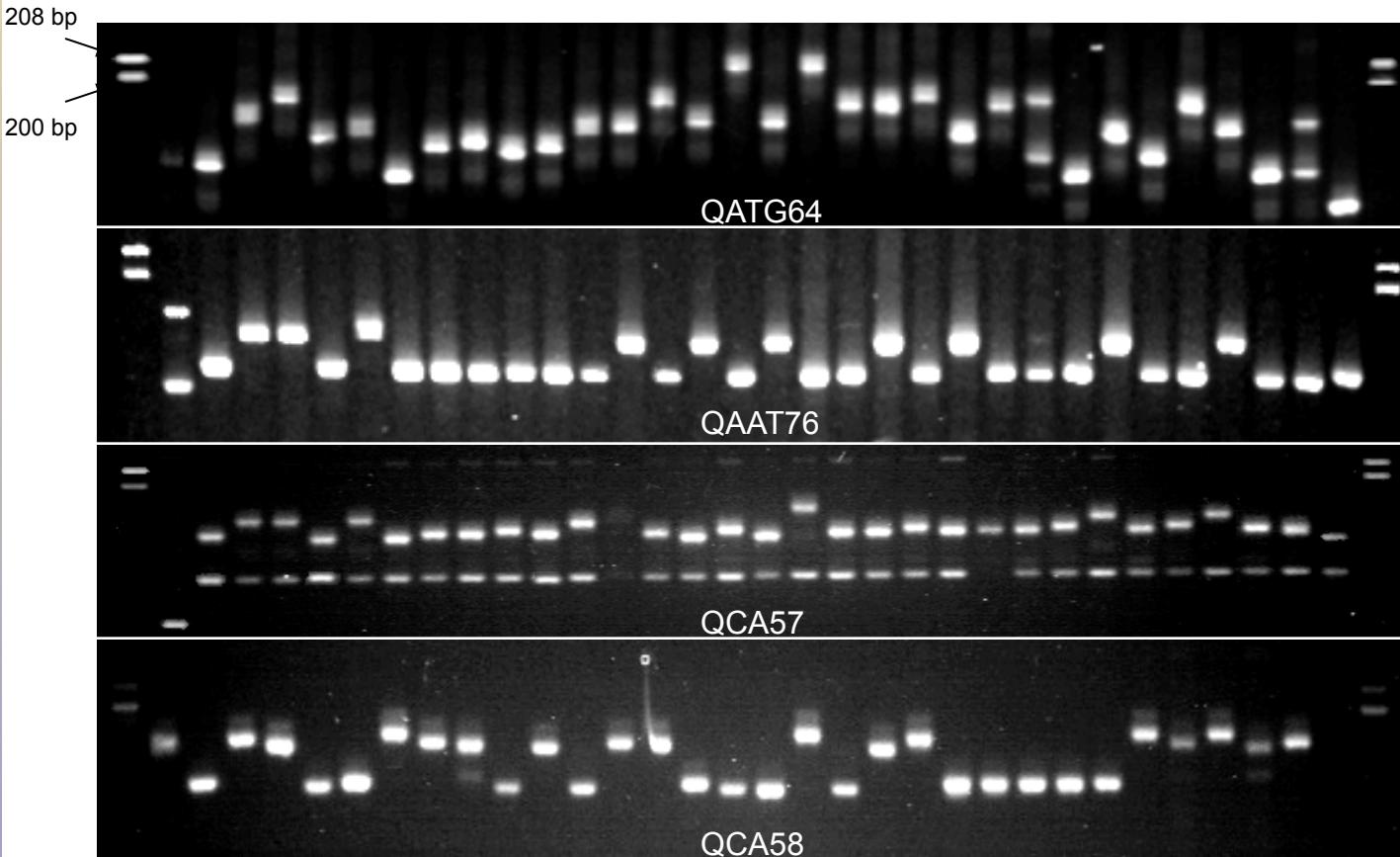


SSR development:
Library enrichment & sequencing (\$5000)
SSR dpt cost: \$0.35-0.50

	Grain amaranths*	<i>A. hypochondriacus</i>	<i>A. cruentus</i>	<i>A. caudatus</i>	<i>A. hybridus</i>
Number of Accessions	28	10	9	9	5
Polymorphic SSRs	179	129	123	136	160
Total Alleles	731	344	327	371	472
ONA Range	2 to 8	2 to 5	2 to 6	2 to 6	2 to 5
Average ONA	4	3	3	3	3
H-value range	0.14 to 0.83	0.18 to 0.74	0.12 to 0.78	0.10 to 0.77	0.18 to 0.80
SSRs H > 0.7	59	4	6	8	29
Average H	0.62	0.49	0.49	0.50	0.56

Development of SSR markers

32 genotypes – the CIP international nursery (A. Mujica)



Amplification of DNA from *C. berlandieri* and 31 quinoa accessions using primer-pairs QATG64, QAAT76, QCA57 and QCA58. Run on 3% Metaphor agarose for ~4 hours at 150V. Ladder bands in lanes 1 and 34 are 208 and 200bp. 'BaerII' is in lane 33 and *C. berlandieri* in lane 2

>400 SSR markers available

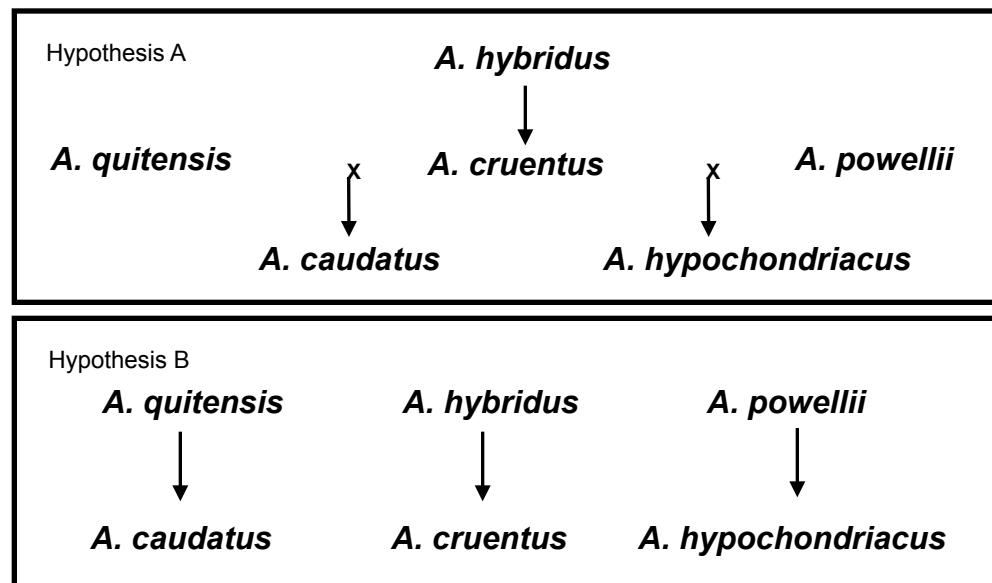
Mason et al. (2005) *Crop Sci.* 45:1618-1630
Jarvis et al. (2008) *J. Genetics* 87:39-51

- ▷ *C. berlandieri* Ames 6-5
- ▷ 0654
- ▷ KU-2
- ▷ Sayaña
- ▷ Real
- ▷ Ecu 420
- ▷ NL-6
- ▷ Jujuy
- ▷ Rataqui
- ▷ L-P
- ▷ Huariponcho
- ▷ Kamiri
- ▷ Kancolla
- ▷ Ingapirca
- ▷ Embrapa
- ▷ Baer
- ▷ G205-DK
- ▷ Ollague
- ▷ Surimi
- ▷ Chucapaca
- ▷ Mokco
- ▷ CICA 17
- ▷ CICA 127
- ▷ Maniqueña
- ▷ Illpa-INIA
- ▷ E-DK-4
- ▷ 3-21-79 BB
- ▷ 3-21-72 RM
- ▷ Nariño
- ▷ RU-2
- ▷ G-205-95
- ▷ Salcedo-INIA

Phylogenetics

Mallory et al. (2008) *Crop Sci.* 48:1098-1106

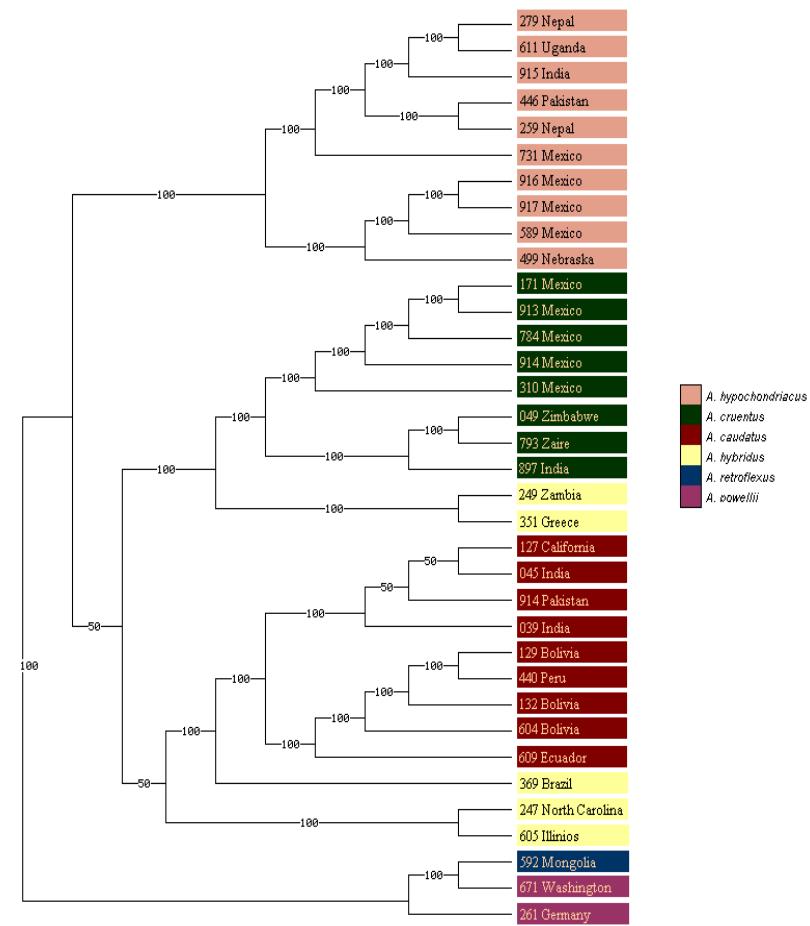
Two hypothesis proposed by Sauer (1950, 1967)



Other molecular and hybrid fertility studies

A. hybridus is closest putative progenitor
A. powelli is most divergent in the compl

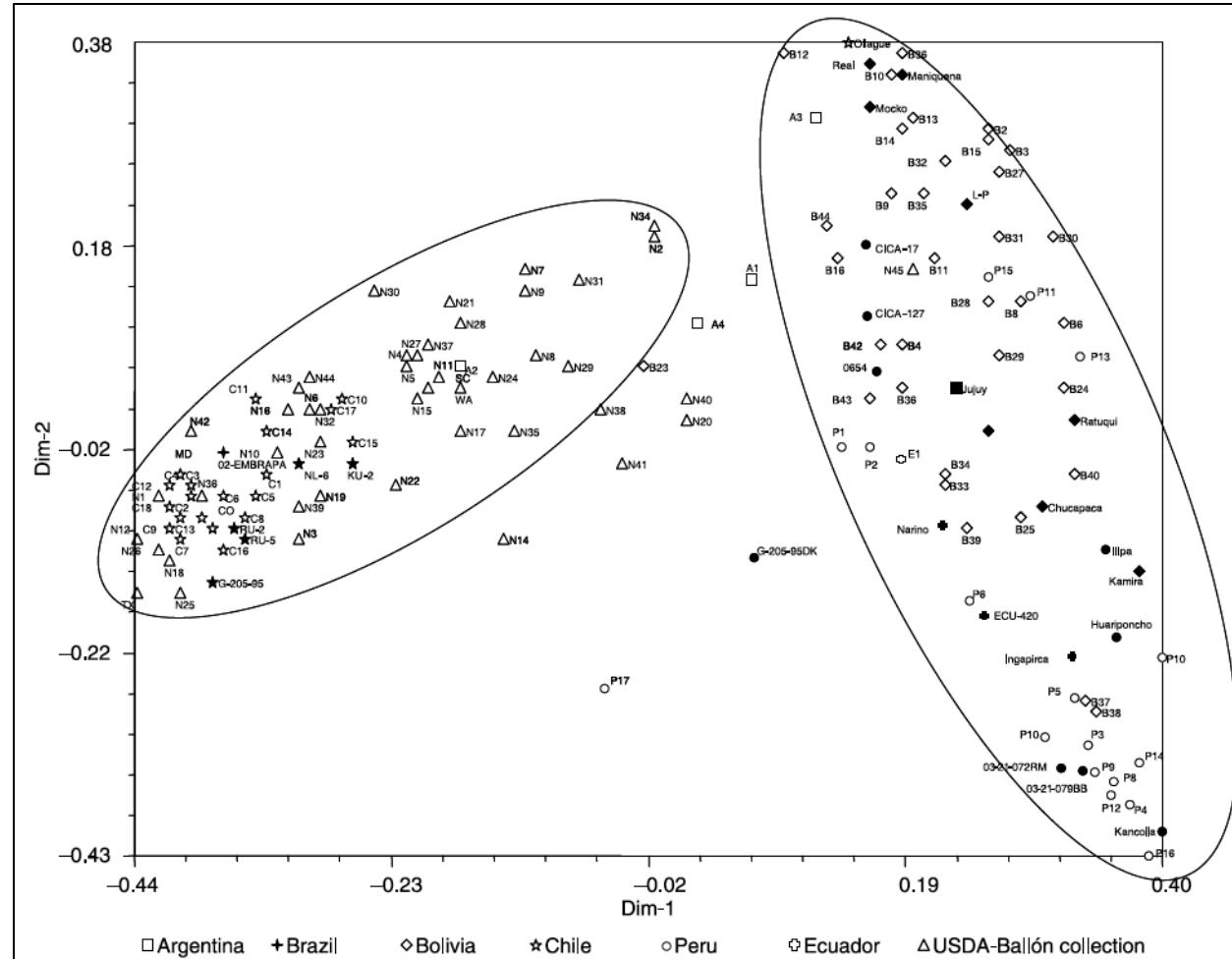
The dendrogram (NJ) shows monophyletic origin for all three grain species. The individuals in the tree are identified by the last three digits of the accession number, location, and are color-coded by species.



Actually appears that a new third hypothesis predicting independent domestication may explain the evolutionary origins of Amaranth!

Assessment of Available Quinoa Germplasm

- Used fluorescence-tagged SSRs and SNPs
- 152 accessions of *C. quinoa*
- 36 SSR loci comprising 432 alleles
 - ranging from 5 to 28 per locus
- 1st cluster consisted of accessions
 - Andean highlands of Peru, Bolivia, Ecuador, Argentina, and extreme northeastern Chile
- 2nd cluster contained accessions
 - Lowlands of Chile and those collected by Emigdio Ballón
- The CIP-FAO collection represents quinoa's genetic diversity within the clusters

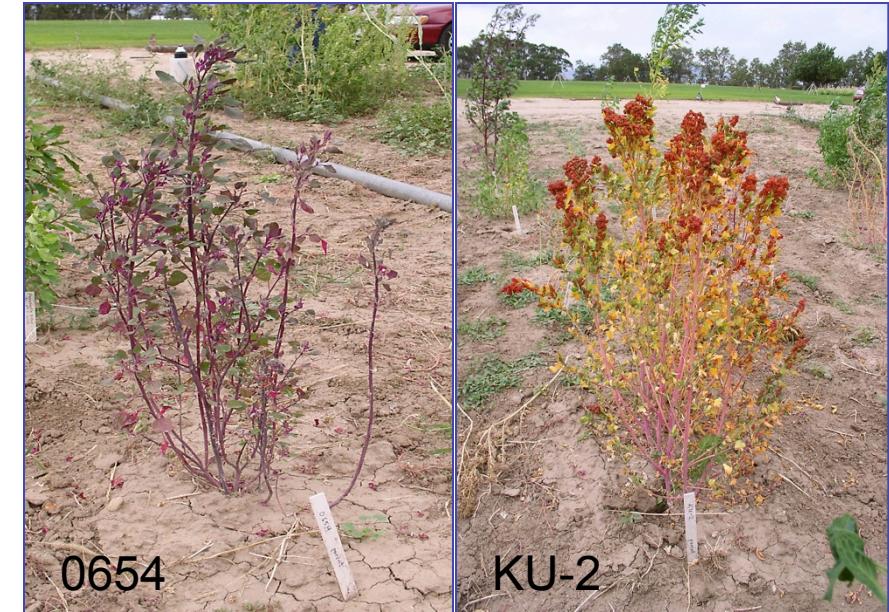


Fuentes et al. (2009) *Conserv. Genet.* 10:369-377

The two-dimensional scaling resulting from the PCA analysis of 150 *Chenopodium quinoa* accessions using genetic diversity data from 36 microsatellite markers. Circled areas represent the major highland and lowland clusters identified in the dendrogram. Accessions are coded for their country of origin as described in the legend. Blackened symbols indicate accessions from CIP-FAO and PROINPA collections.

Genetic Linkage Map Construction: Population Selection

- ▶ Four phenotypically diverse potential mapping parents were screened AFLP markers to assess genetic diversity.
 - ▶ 597 polymorphic bands scored across the four potential parents.
 - ▶ 19 -52 bands detected/primer pair
 - ▶ ~6 polymorphic bands/pc
- ▶ Three diverse population derived from two way crosses of four parents were developed to produce a chain population.
 - ▶ KU-2 X 0654, 0654 X Chucapaca, Chucapaca X NL-6
- ▶ *KU-2 X 0654 was selected to be used to make the preliminary genetic map of quinoa using AFLPs*
 - ▶ All other populations are being selfed to form RIL populations

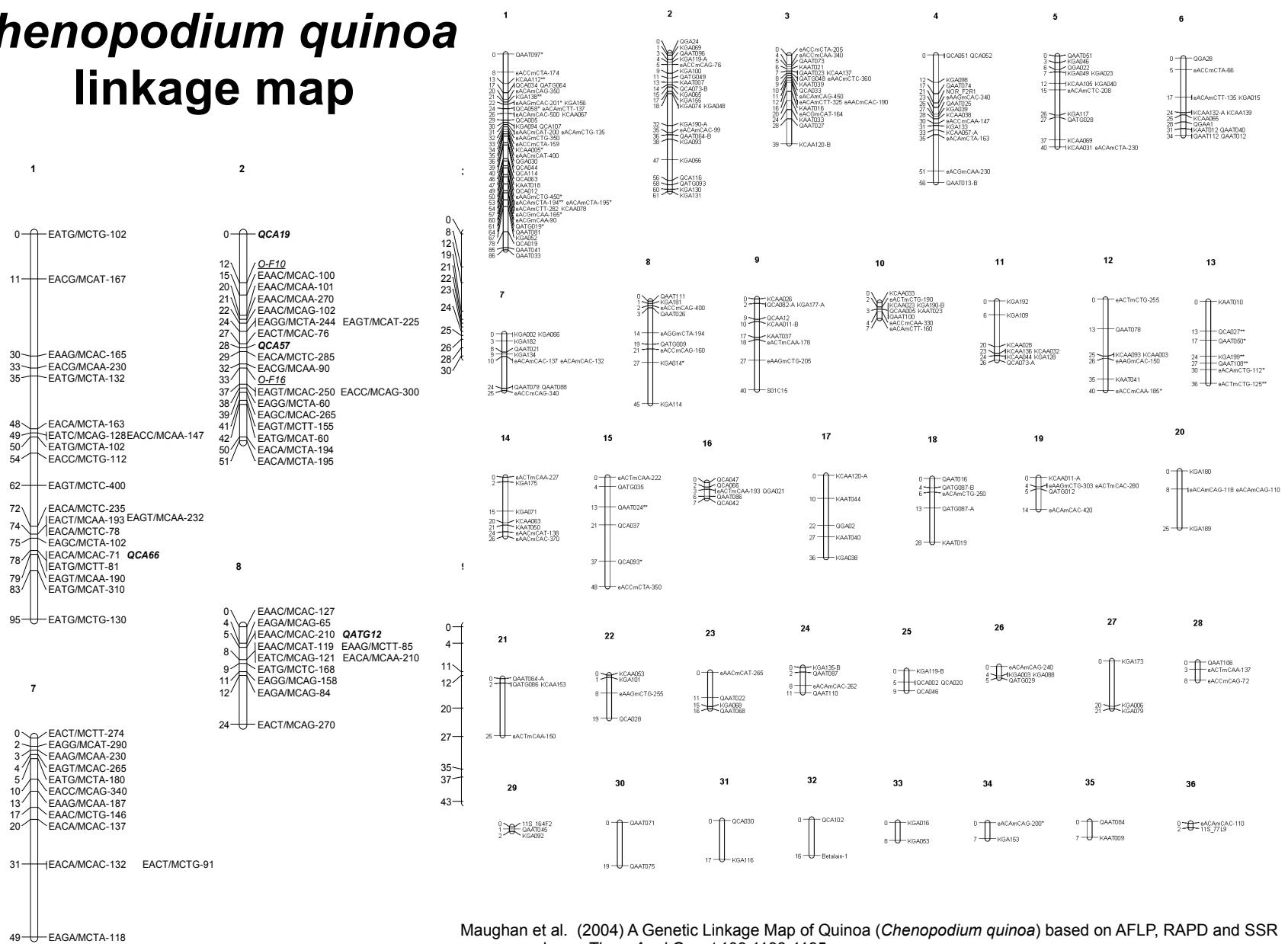


Plant characteristics of the genetic material utilized as potential mapping parents.

Similarity matrix of potential parents.

Genetic Material	Plant Color	Seed head type	Saponin (cc) ¹	Seed Size ²	Maturity ³	Country of Origin	0654	KU-2	Chucapaca	NL-6
0654	Red	Amaranthiform	10.0	0.31	150 days (Semi-late)	Peru (Altiplano)	-			
Ku-2	Green	Glomerulate	10.3	0.28	135 days (Semi-early)	Chile (Costal)	0.304	-		
Chucapaca	Red	Glomerulate	0.0	0.36	154 days (Semi-late)	Bolivia (Altiplano)	0.576	0.229	-	
NL-6	Purple	Glomerulate	10.5	0.22	130 days (Early)	Chile (Costal)	0.327	0.866	0.245	-

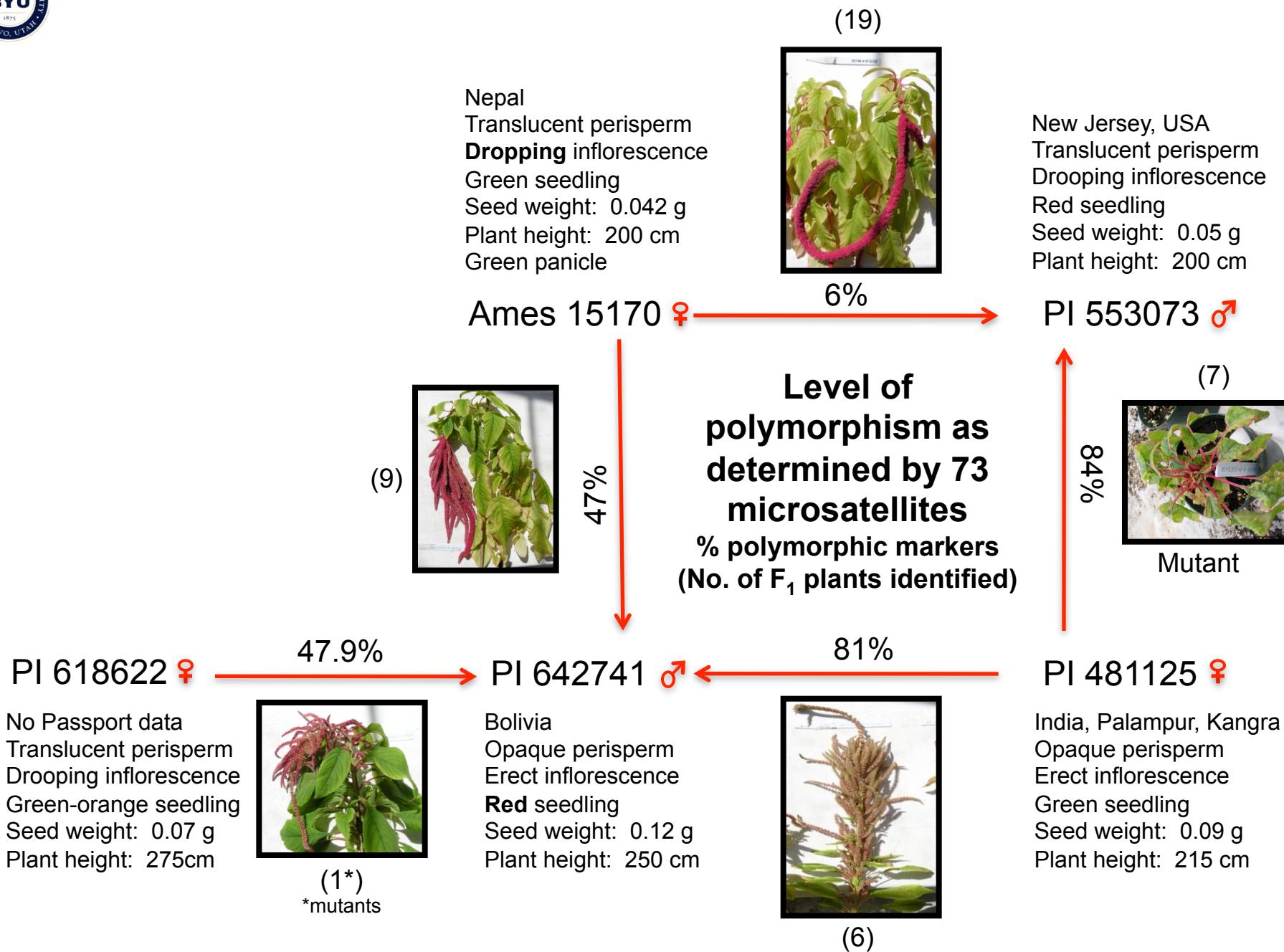
Chenopodium quinoa linkage map



Maughan et al. (2004) A Genetic Linkage Map of Quinoa (*Chenopodium quinoa*) based on AFLP, RAPD and SSR markers. *Theor Appl Genet* 109:1188–1195

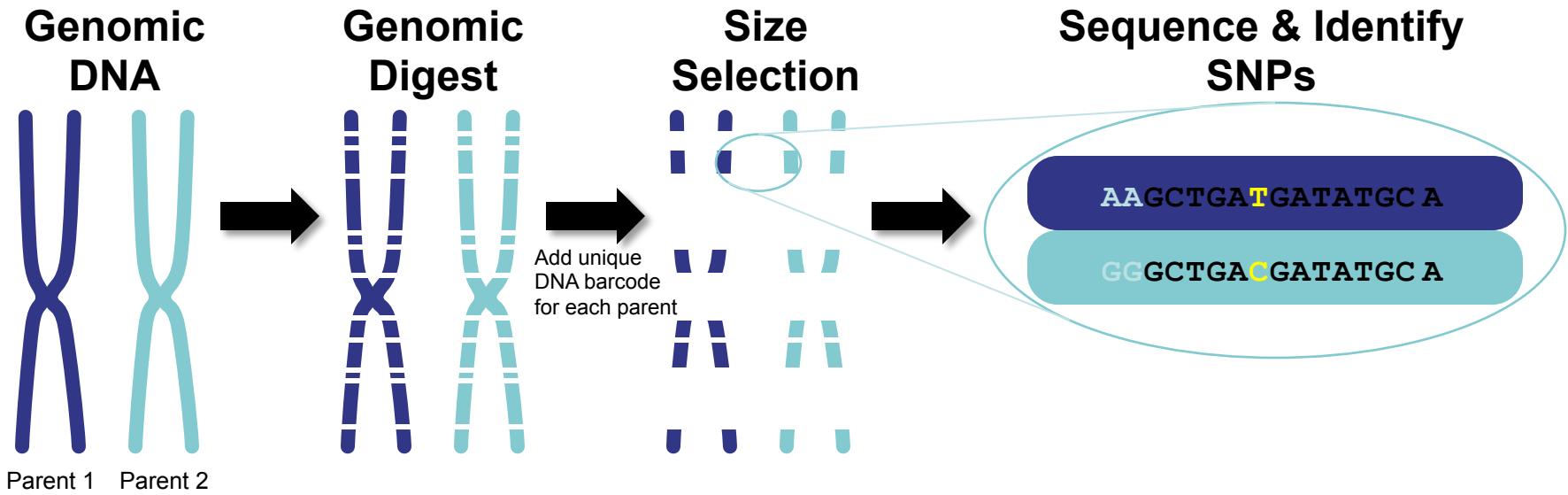
Jarvis et al. (2008) Simple Sequence Repeat Development, Polymorphism and Genetic Mapping in Quinoa (*Chenopodium quinoa* Willd.). *J. Genetics* 87:39-51





Genomic reduction & SNP discovery by sequencing

Maughan et al. (2009) *Plant Genome* 2: 260-270



Cronn R, Knaus B, Liston A, Maughan PJ, Parks M, Syring J, Udall J (2012) Targeted enrichment strategies for Next-Generation plant biology. *Am. J. Bot.* 99(2): 1–22

Well Categories

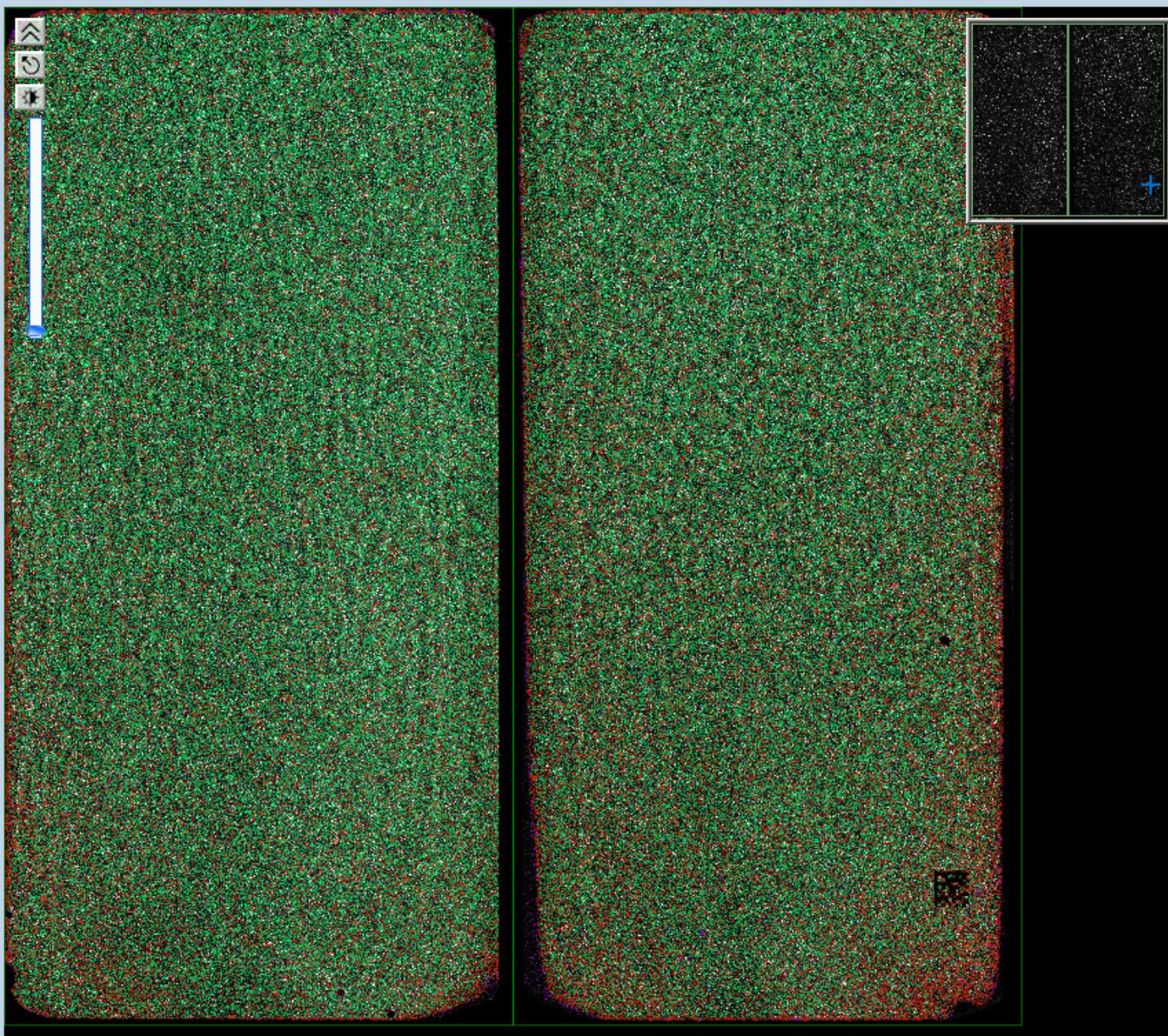
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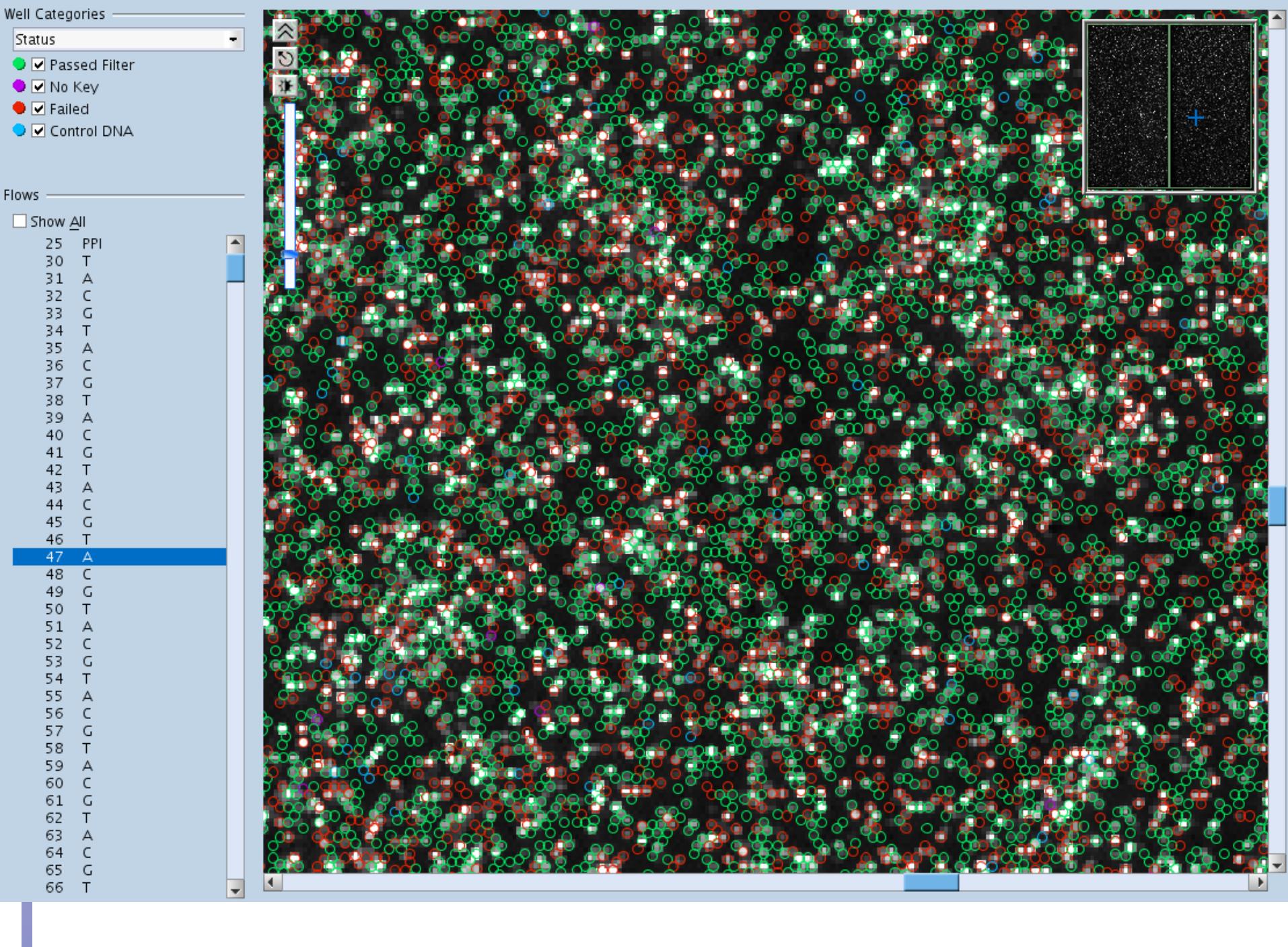
- Passed Filter
- No Key
- Failed
- Control DNA

Flows

 Show All

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31	A
32	C
33	G
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35	A
36	C
37	G
38	T
39	A
40	C
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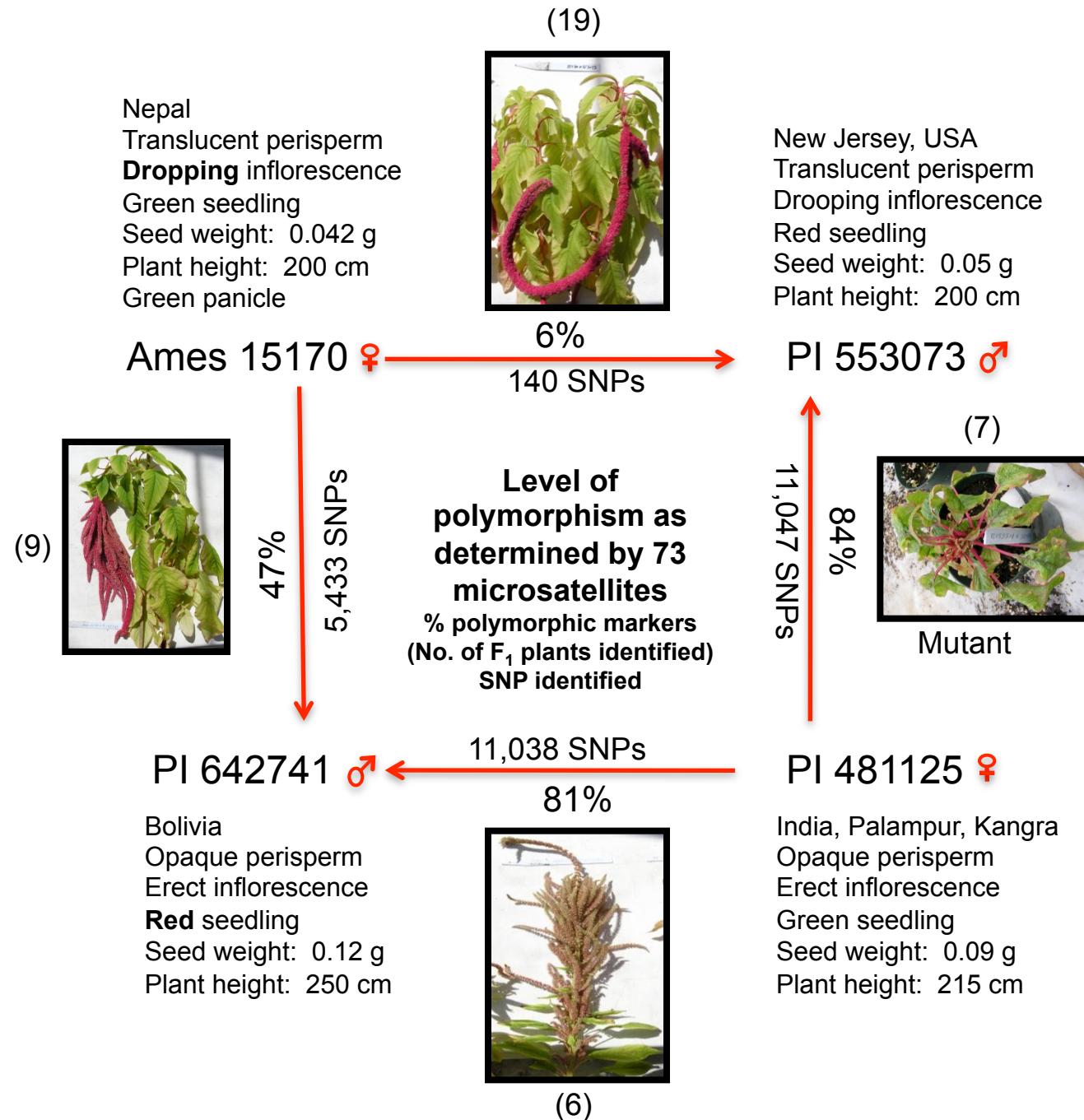
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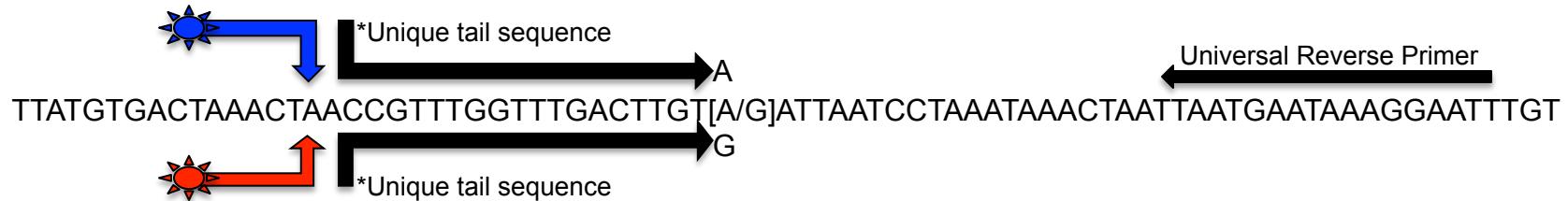
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-98101	SRR030257.2467	CAAGGCCGCCAACAAATGGTGGTGATAAGCGGGGGT							
+98102	SRR030257.7650	AAGGCCGCCAACAAATGGTGGTGATAAGCGGGGGTGG							
-98103	SRR030257.7695	AGGCCGCCAACAAATGGTGGTGATAAGCGGGGGTGGC							
-98104	SRR030257.2488	AGGCCGCCAACAAATGGTGGTGATAAGCGGGGGTGGC							
+98105	SRR030257.2806	AGGCCGCCAACAAATGGTGGTGATAAGCGGGGGGGG							
-98106	SRR030257.1881	GGCCGCCAACAAATGGTGGTGATAAGCGGGGGTGGCG							
+98107	SRR030257.1895	GGCCGCCAACAAATGGTGGTGATAAGCGGGGGTGGCG							
-98108	SRR030257.2251	GCCGCCAACAAATGGTGGTGATAAGCGGGGGTGGCGT							
+98109	SRR030257.3596	CGCCAACAAATGGTGGTGATAAGCGGGGGGGGG							
-98110	SRR030257.3066	GCCAACAATGGTGGTGATAAGCGGGGGTGGCGTGAT							
+98111	SRR030257.2170	CCAACAATGGTGGTGATAAGCGGGGGGGGG							
-98112	SRR030257.3282	CCAACAATGGTGGTGATAAGCGGGGGTGGCGTGATG							
-98113	SRR030257.4159	CCAACAATGGTGGTGATAAGCGGGGGTGGCGTGATG							
+98114	SRR030257.1502	CAACAATGGTGGTGATAAGCGGGGGTGGCGTGATGC							
-98115	SRR030257.2403	CAACAATGGTGGTGATAAGCGGGGGTGGCGTGATGC							
-98116	SRR030257.2498	CAACAATGGTGGTGATAAGCGGGGGTGGCGTGATGC							
+98117	SRR030257.2410	ACAATGGTGGTGATAAGCGGGGGTGG							
-98118	SRR030257.3463	ACAATGGTGGTGATAAGCGGGGGTGGCGTGATGCAT							
+98119	SRR030257.3446	CAATGGTGGTGATAAGCGGGGGGGGG							
-98120	SRR030257.1509	AATGGTGGTGATAAGCGGGGGTGGCGTGATGCATT							
+98121	SRR030257.2478	AATGGTGGTGATAAGCGGGGGTGGCGTGAT							
-98122	SRR030257.1708	ATGGTGGTGATAAGCGGGGGTGGCGTGATGCATTCC							
>	CONSENSUS -**-	AAAGGCGAGCACAAGGCCGCCAACAAATGGTGGTGATAAGCGGGGGTGGCGTGATGCATTCCGTCTCTTTCTGGTGGT							

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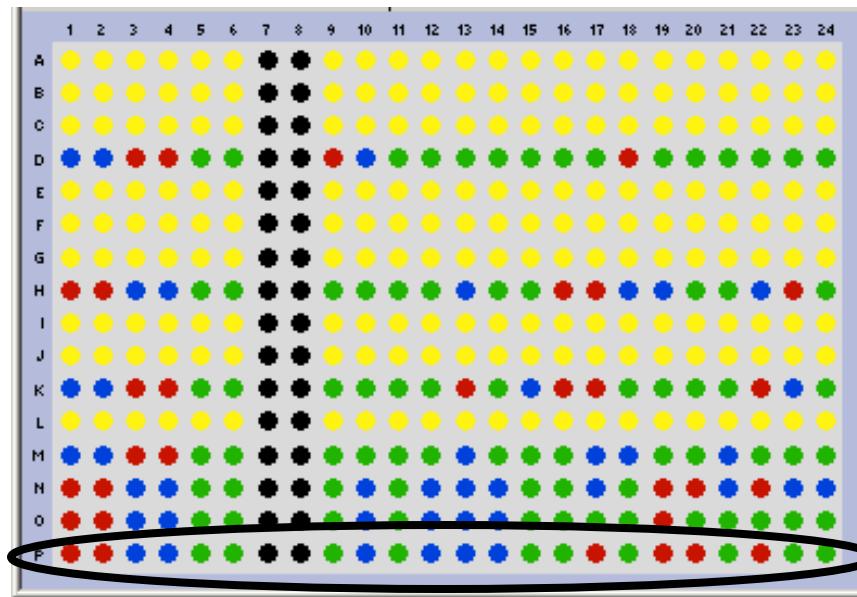


KASPar™ SNP Genotyping

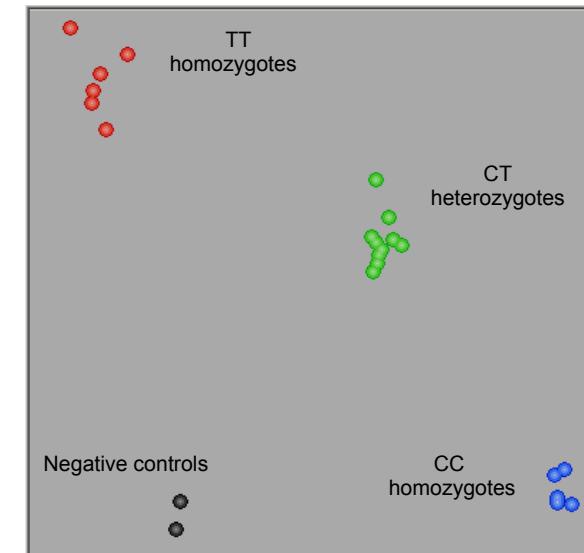
(Allele Specific PCR) 384-well, 4 uL volume (2uL DNA @ 25 ng/uL)



384-well plate analyzed by KlusterCaller (Kbiosciences):

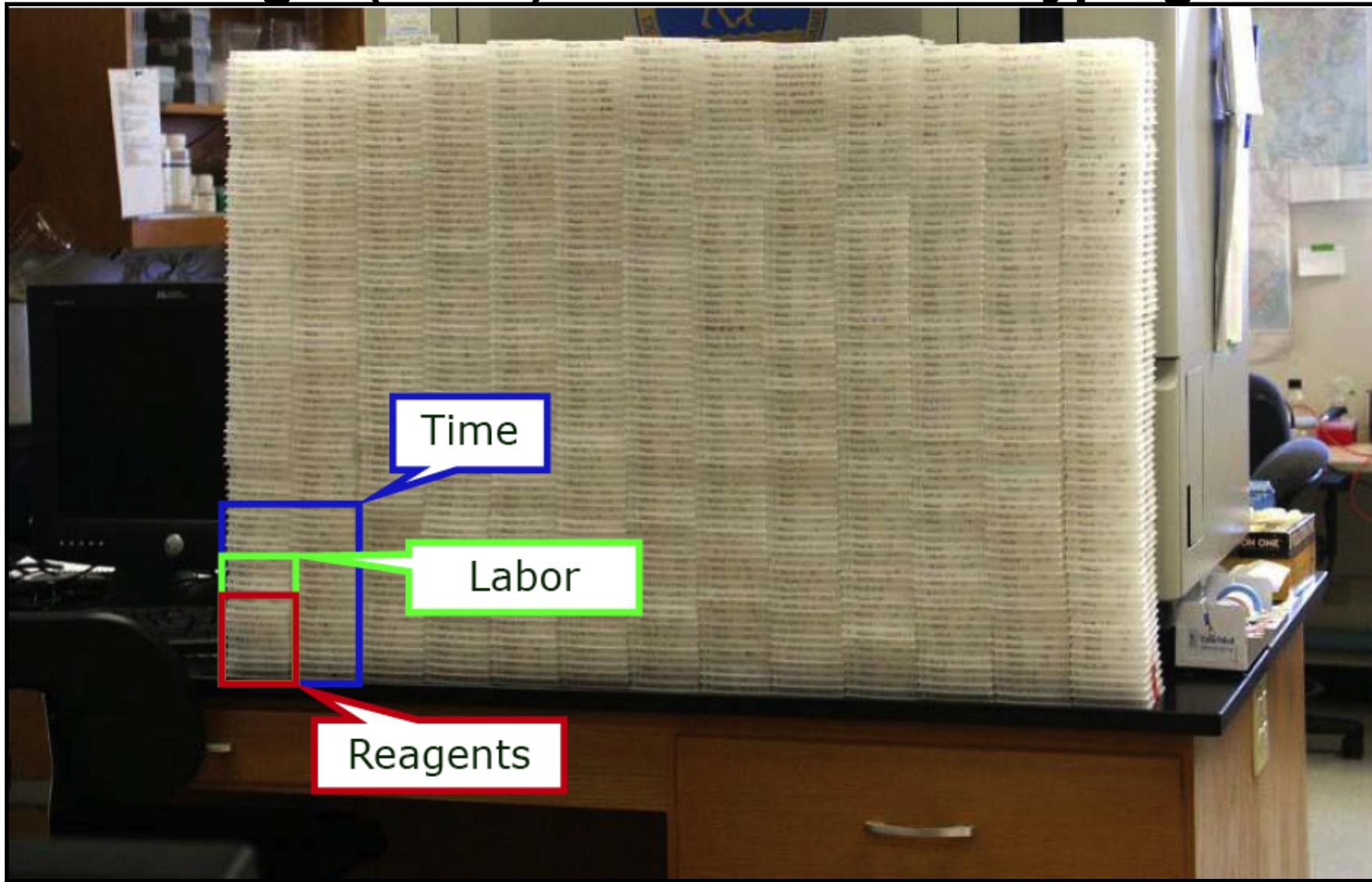


Row P: Primer- ID_AM27611 (C/T) SNP



No gel electrophoresis! Closed tube! Robot compatible!
Cost per datapoint: \$0.14 + initial primer cost of ~\$12
TaqMan: \$318/assay + taq polymerase costs

Fluidigm (96.96) Nanoscale Genotyping



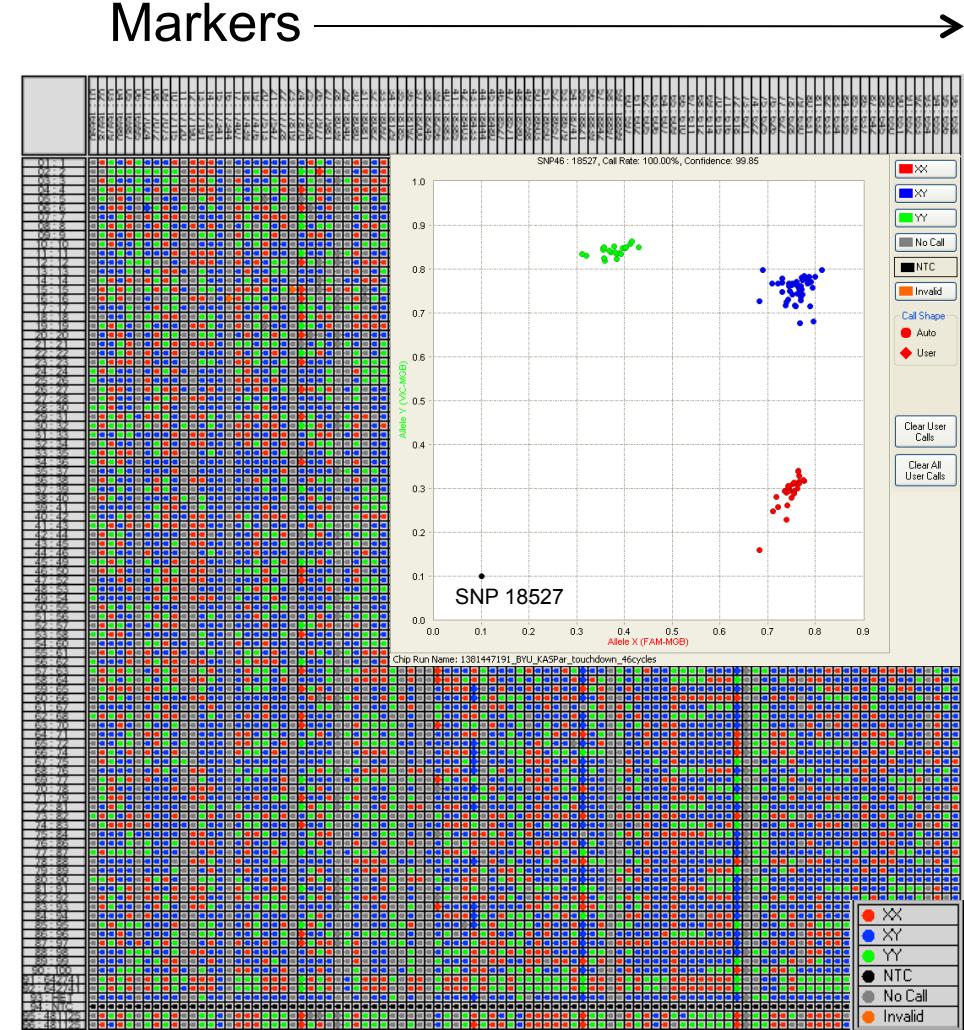
Fluidigm (96.96) Nanoscale Genotyping

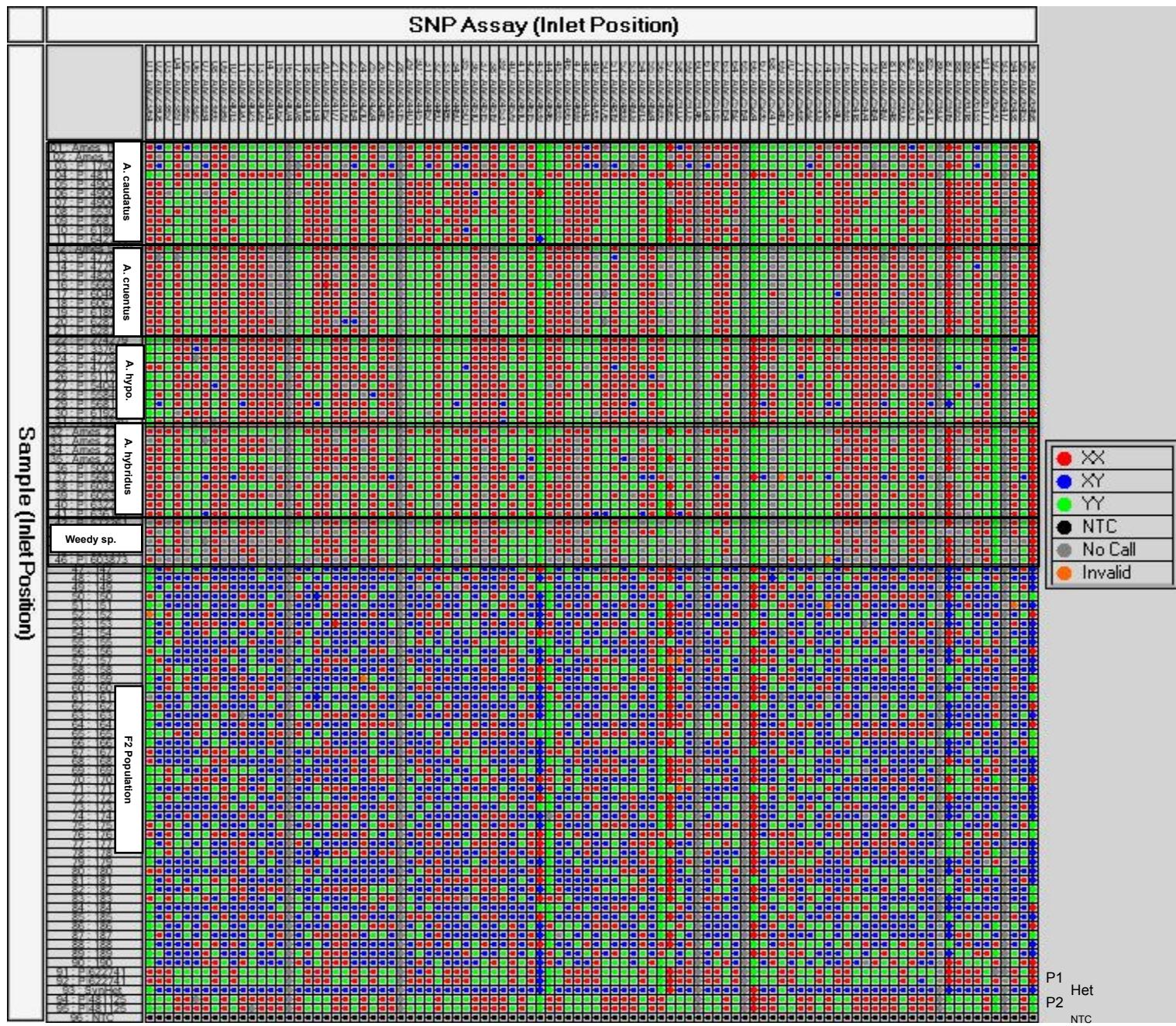


9,216 Simultaneous PCR Reactions

Reagent cost per datapoint: \$0.001
Chip + reagent datapoint: \$0.05
(Still an initial 15\$ per primer cost)

Samples ↓





P1 Het

P2 NTC

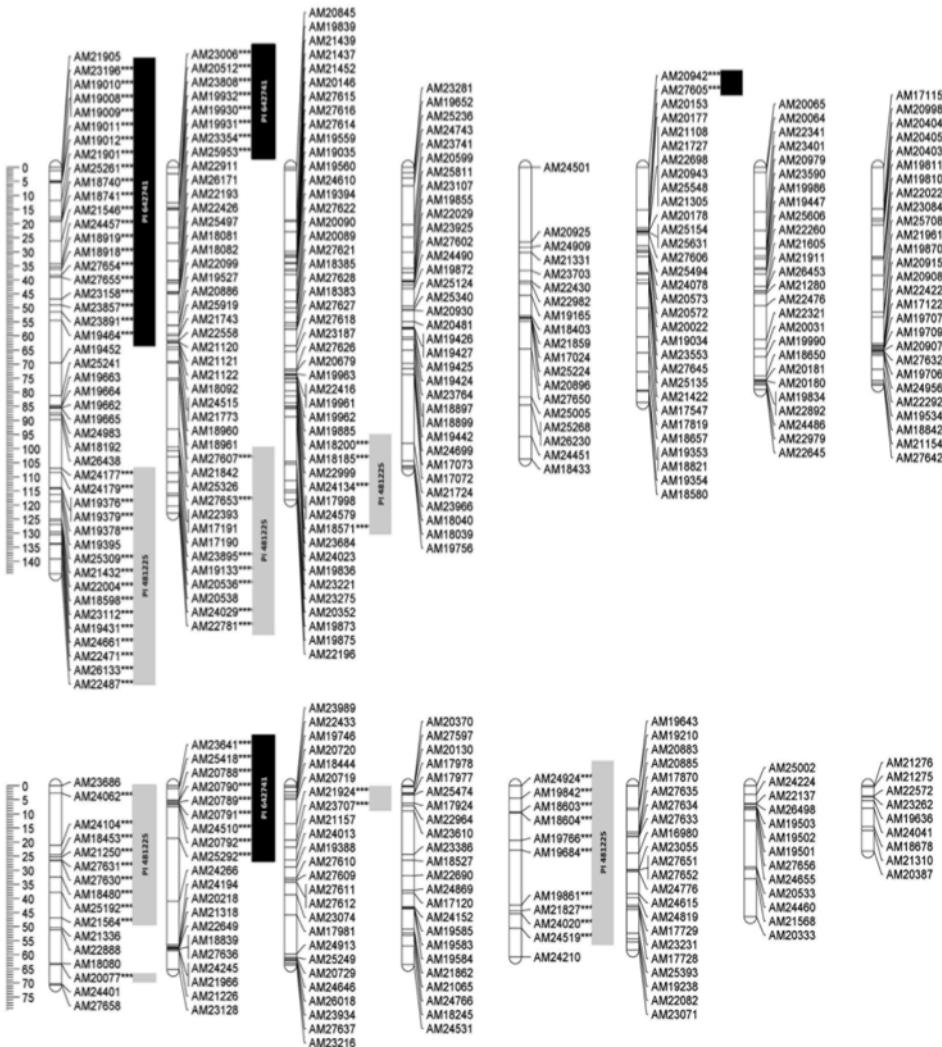
NTC

SNP Map and Diversity Results



PI481125 X PI642741

- ✓ Extracted DNA from 134 F₂ Individuals and 46 diversity individuals.
- ✓ 480 putative SNPs screened, 419 (87%) were successfully converted and genotyped using the Fluidigm platform.
- ✓ 1,072 dtps were run in duplicate => 2% mismatches.
- ✓ Linkage mapped spanned 1317cM across 16 linkage groups (2n=32).



A 16 group linkage map constructed from an interspecific *A. hypochondriacus* X *A. caudatus* F₂ population (2n=32). Distances are shown centiMorgans (cM). SNP loci showing segregation distortion ($P<0.001$) to PI 642741 or PI 481125 are identified with blackened or shaded boxes, respectively.

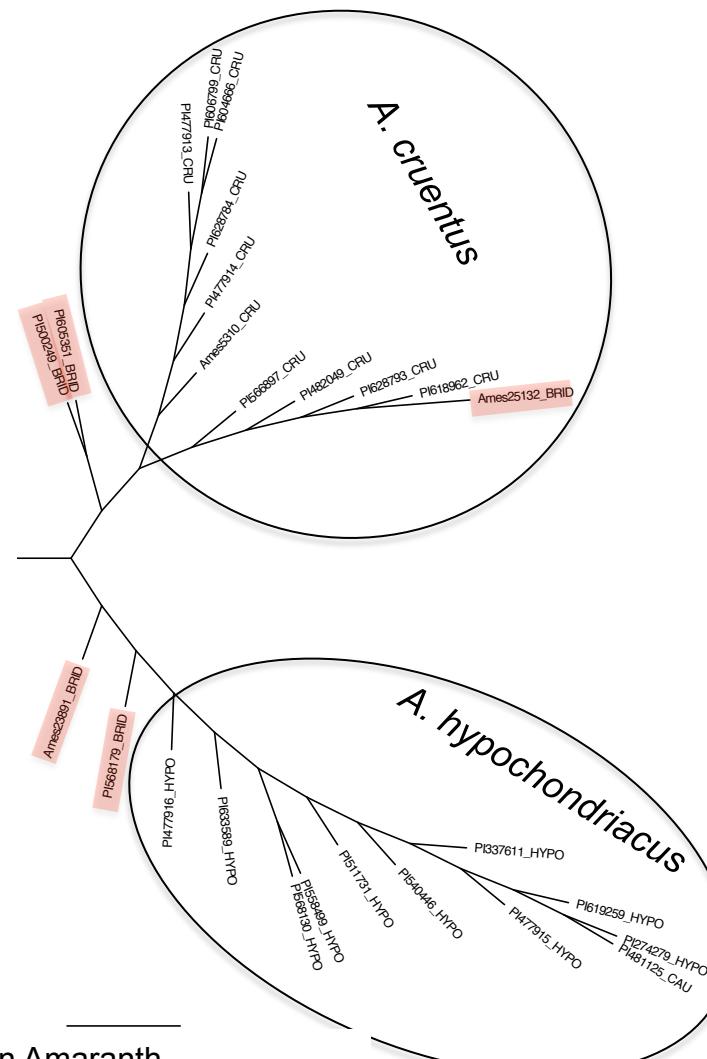
UPGMA Dendrogram and PCO analysis

A. hybridus

Sample Size	10
Total SNP Screened	480
Total SNP Pass QC*	414
Polymorphic SNP	252 (61%)
Highly Polymorphic	96
H Range	0.10 - 0.5
Average H	0.20



Assessment of Genetic Diversity in Peruvian Amaranth (*Amaranthus caudatus* and *A. hybridus*) Germplasm using Single Nucleotide Polymorphism Markers

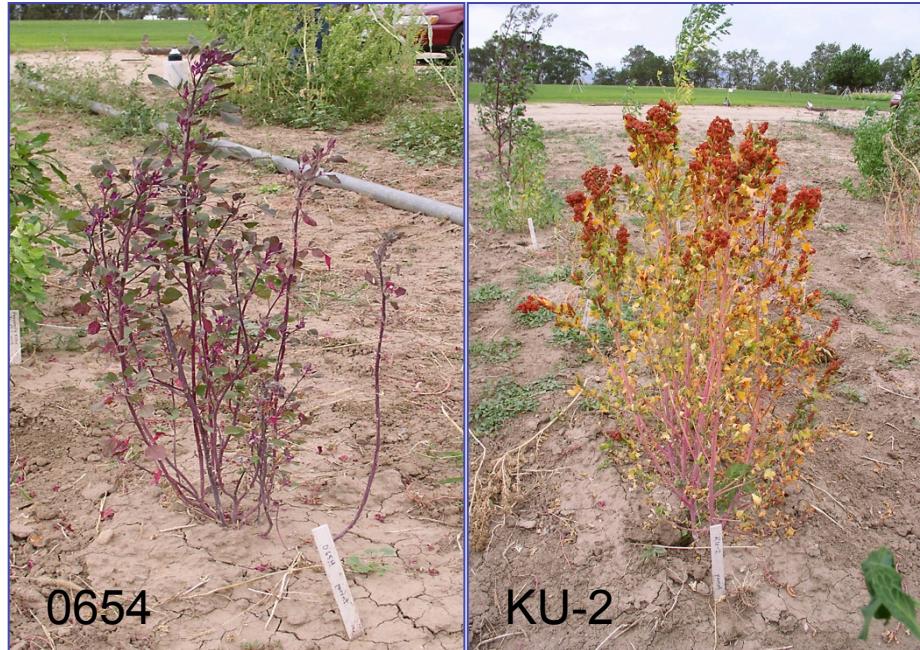


<i>A. caudatus</i>	Sample Size	10
Total SNP Screened	480	
Total SNP Pass QC*	414	
Polymorphic SNP	136 (28%)	
Highly Polymorphic	54	
H Range	0.10 - 0.5	
Average H	0.09	
<i>A. hypochondriacus</i>	Sample Size	11
Total SNP Screened	480	
Total SNP Pass QC*	414	
Polymorphic SNP	186 (39%)	
Highly Polymorphic	76	
H Range	0.09 - 0.5	
Average H	0.13	
<i>A. caudatus</i>	Sample Size	10
Total SNP Screened	480	
Total SNP Pass QC*	414	
Polymorphic SNP	35 (7%)	
Highly Polymorphic	10	
H Range	0.10 - 0.5	
Average H	0.02	

Across all species, 296 markers were highly polymorphic (average $H = 0.37$)

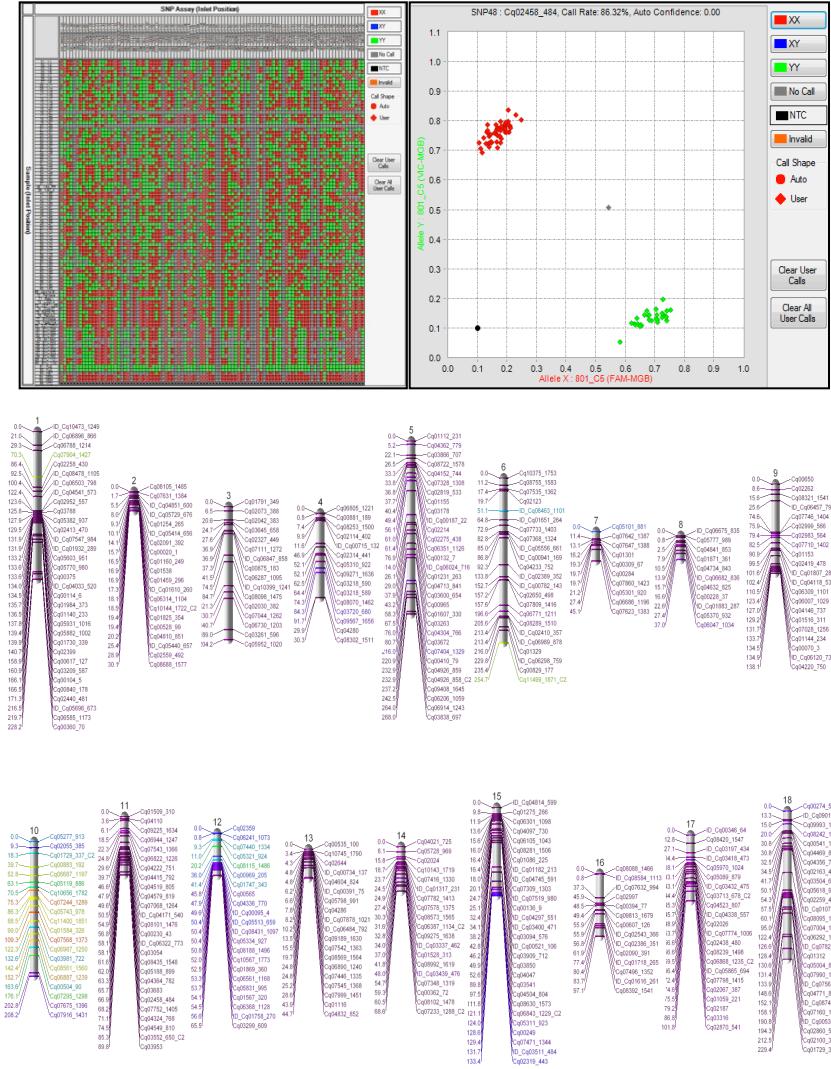
Quinoa Genetic Linkage Map Construction

- KU-2 X 0654 was selected to be used to make the preliminary genetic map of quinoa using AFLPs - other populations are being selfed to form RIL populations



Plant characteristics of the genetic material utilized as potential mapping parents. Similarity matrix of potential parents.

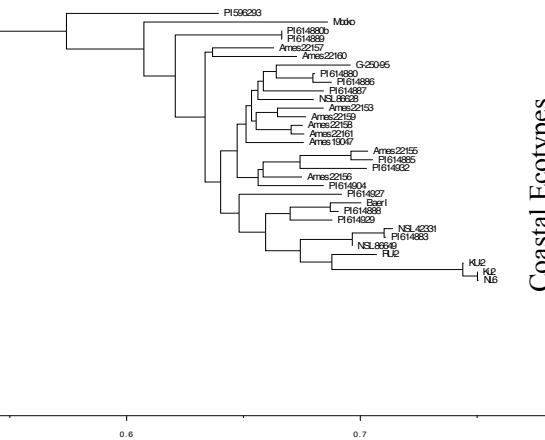
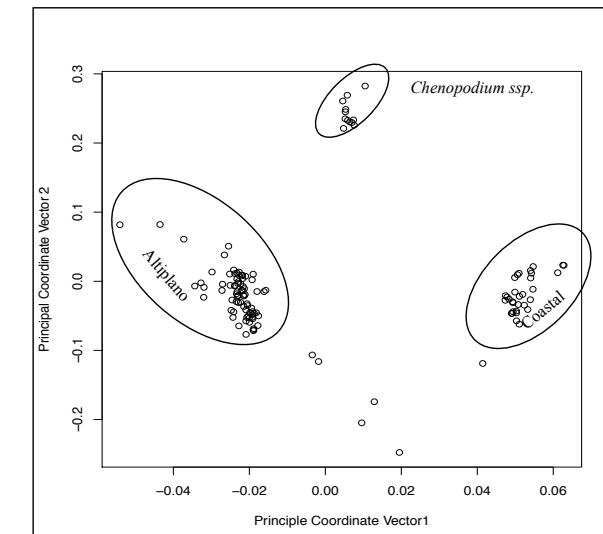
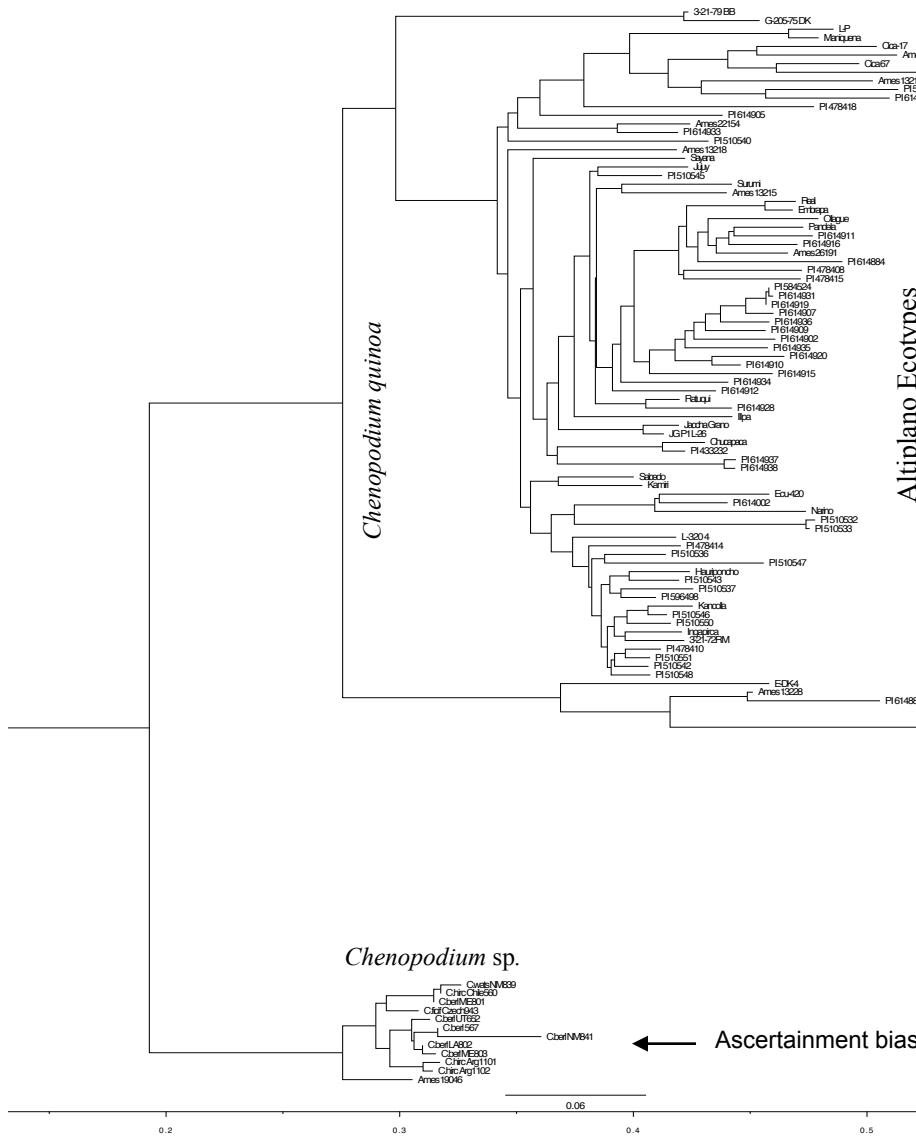
Genetic Material	Plant Color	Seed head type	Saponin (cc)	Seed Size	Maturity	Origin
0654	Red	Amaranthiform	10.0	0.31	150 days (Semi-late)	Peru (Altiplano)
Ku-2	Green	Glomerulate	10.3	0.28	135 days (Semi-early)	Chile (Costal)



An 18 group linkage map constructed by combining Ku-2 X 0654 RIL population. Segregation distortion is shown using the p-value from testing segregation distortion using a purple to red scale (yellow/orange/red colored markers are distorted).

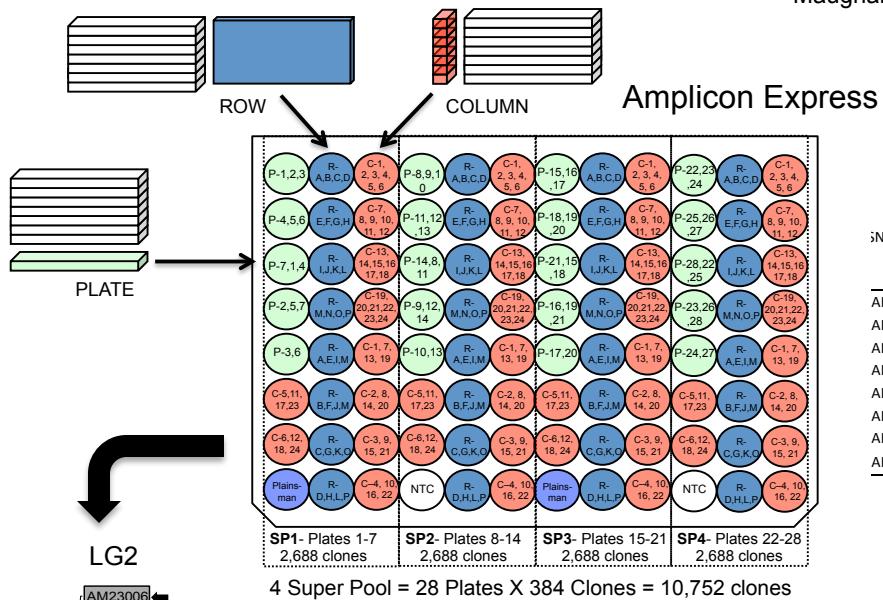


Genetic Diversity

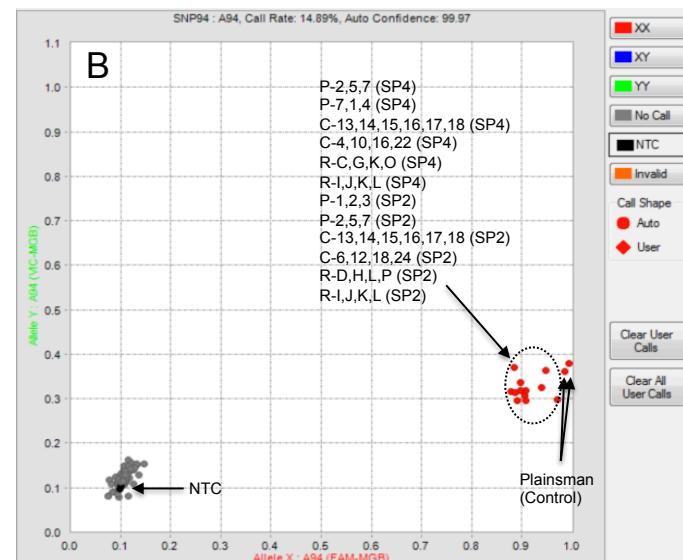
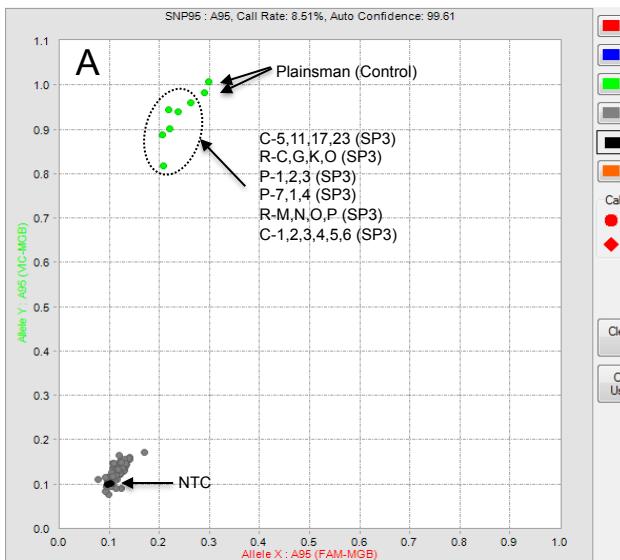


Simultaneous multi-clone identification: BAC 7-Plate Super Pool

Maughan et al. (2012) *J Biomed Biotechnol* doi:10.1155/2012/405940



AM23006
AM20512
AM23808
AM19932
AM19930
AM19931
AM23354
AM25953
AM22191
AM26171
AM22426
AM25497
AM18081
AM18082
AM22099
AM19527
AM20886
AM25919
AM21743
AM22568
AM21120
AM21121
AM21122
AM18992
AM24515
AM21773
AM18960
AM18961
AM27607
AM21842
AM25326
AM27653
AM22393
AM17191
AM17190
AM23895
AM19133
AM20536
AM20538
AM24029
AM22781



Sequence and verify

BAC Assembly Statistics

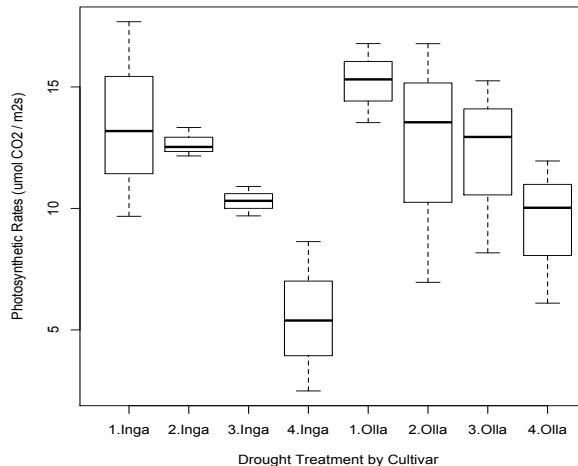
SNP Target Name	Positive BAC Clone Address	BAC Assembly Statistics					
		No. Reads	No. Bases	Contigs (>500 bp)	N50 Contig Size (bp)	Max Contig (bp)	Number of Bases
AM18081	1604	28417	11346335	14	52842	58444	187937
AM20886	1241	58578	21913991	15	25282	57023	181556
AM21120	3L14	12523	4717010	8	124794	124794	235849
AM21773	6M9	38937	14568922	15	23276	54030	125585
AM22193	13G11	13529	4999229	15	30565	40572	206077
AM23006	15O11	9887	3722293	9	30149	66008	184367
AM23895	9N2	26614	9798881	4	48808	56894	181431
AM25953	3L19	30301	11263931	7	147194	147194	164652
Average:		10.9	60364	75620	183432		

RNAseq and EST libraries - Greenhouse Set Up

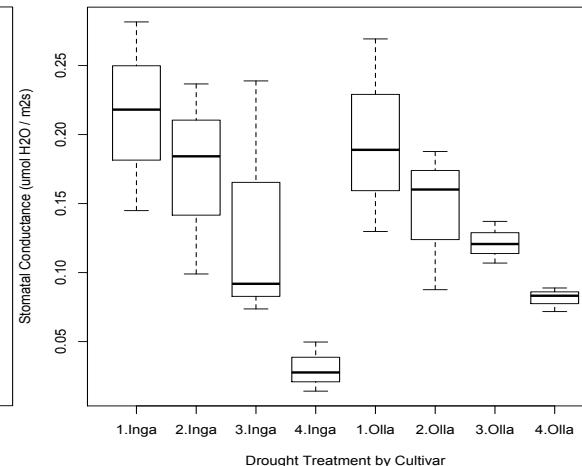
- ▷ Using a random block design I planted four pots of each cultivar: DS- Ingapirca (Ecuador) and DT- Ollague (Bolivia)
- ▷ Each of the four pots represented a estimated drought treatment: 100% Field Capacity (FC), 50% FC, 30% FC and 10% FC
- ▷ Each set-up was replicated three times
- ▷ Different drought treatments applied at day 23



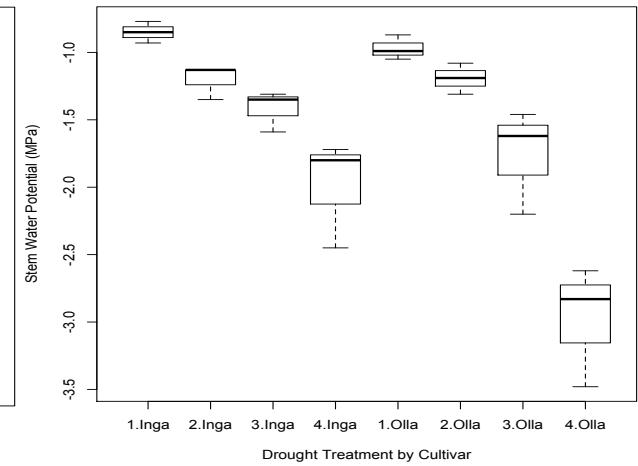
Photosynthetic rate



Stomatal conductance



Stem water potential



Labeled cDNA Libraries Sequenced

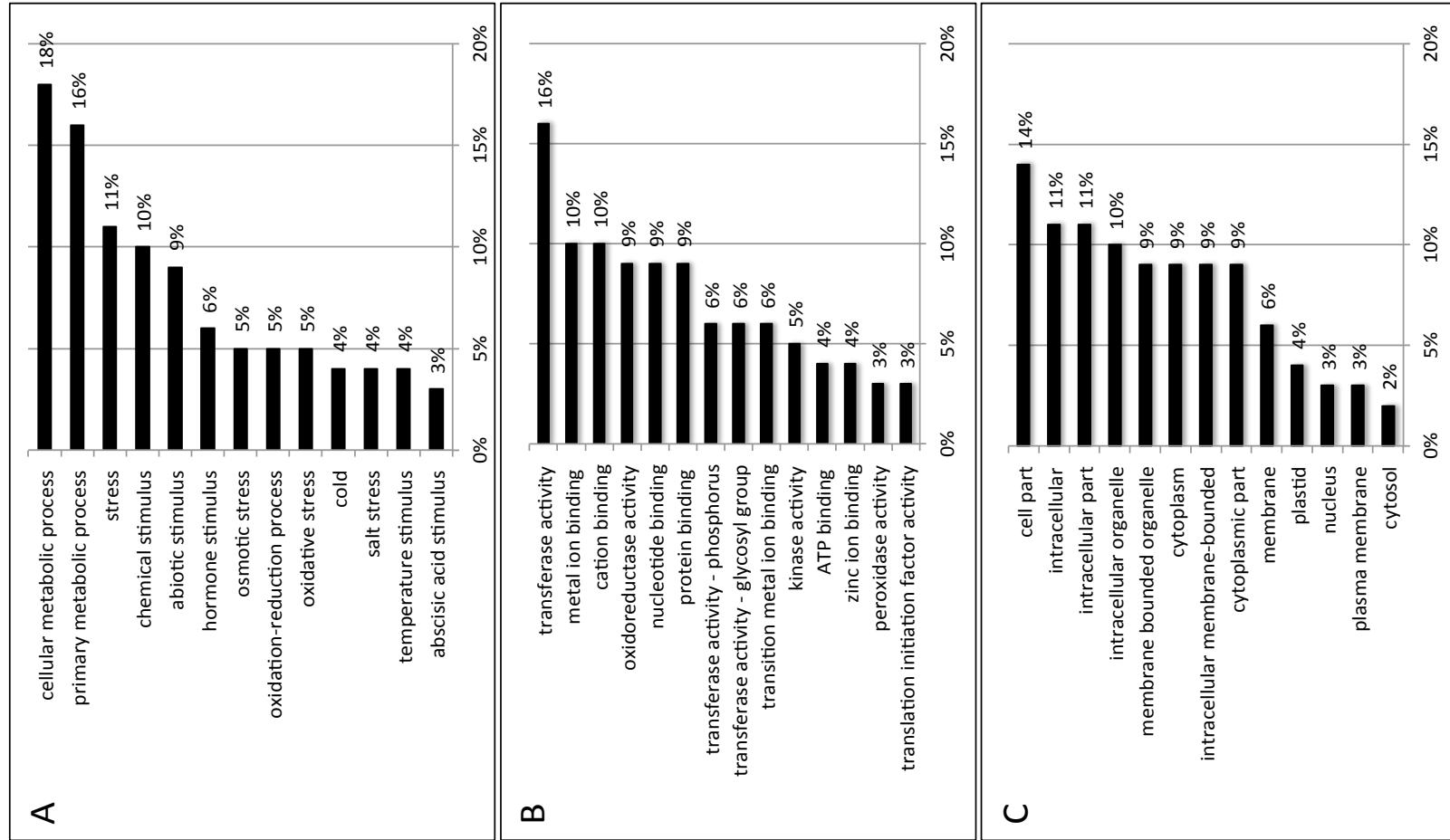
Replicate	Replicate 1								Replicate 2								Replicate 3								
	Ingapirca				Ollague				Ingapirca				Ollague				Ingapirca				Ollague				
Variety	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	
	5	5	5	5	5	5	5	5	5	3	5	3	5	3	3	3	5	3	3	3	3	3	3	3	
Treatment	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
	2	1	2	2	1	2	1	2	1	1	1	0	1	0	1	0	1	0	0	1	0	0	1	0	
Sequencing Lane	1	9	3	4	7	0	8	2	6	0	3	8	4	4	2	3	5	6	5	1	7	1	9	2	
	1	9	3	4	7	0	8	2	6	0	3	8	4	4	2	3	5	6	5	1	7	1	9	2	
cDNA construction sample number																									

	Reads	Total bp
Lane 3	191,304,208	
Lane 5	194,006,856	
Total	385,311,064	19.27 Gb
After trimming	373,835,465	

The combined assembly produced **20,337** unique consensus sequences (contigs), with contig read length ranging from 201 to 18,777 bp with an average length of 525 bp.
 (Publically available from GenBank SRA #SRR799899 and SRR799901).

462 genes were differentially expressed based on treatment

Raney et al. 2013 (M.S. Thesis, BYU HBLL)



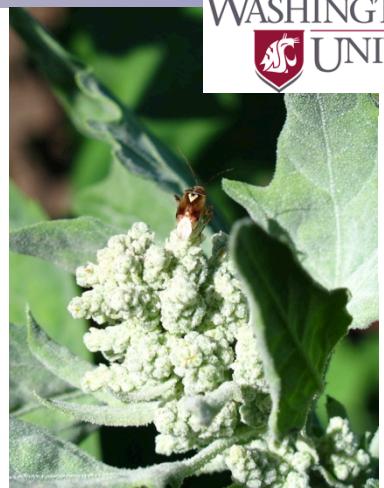
A: Biological process; B: Molecular function; C: Cellular component

27 Genes were differentially expressed between the varieties (example genes)

Gene	Product	Function
Comp42593_c0_seq1	Naringenin,2-oxoglutarate 3-dioxygenase	Catalyze intermediates used to synthesis flavonoids
Comp1839_c0_seq1	Serine threonine-protein kinase rbk-1-like protein	SnRK2.6/OST1 (<i>Arabidopsis thaliana</i>)- main effector in the hydric stress response (drought-tolerance mechanism)
Comp56807_c0_seq1	Chaperone-1-like protein	Response to high light intensity and heat
Comp42870_c0_seq1	Heat-shock protein	Associated with photosynthetic acclimation under drought stress
Comp1469_c0_seq1	Pathogenesis related gene protein	Increase of these protein types have been associated to various abiotic stress (drought included)



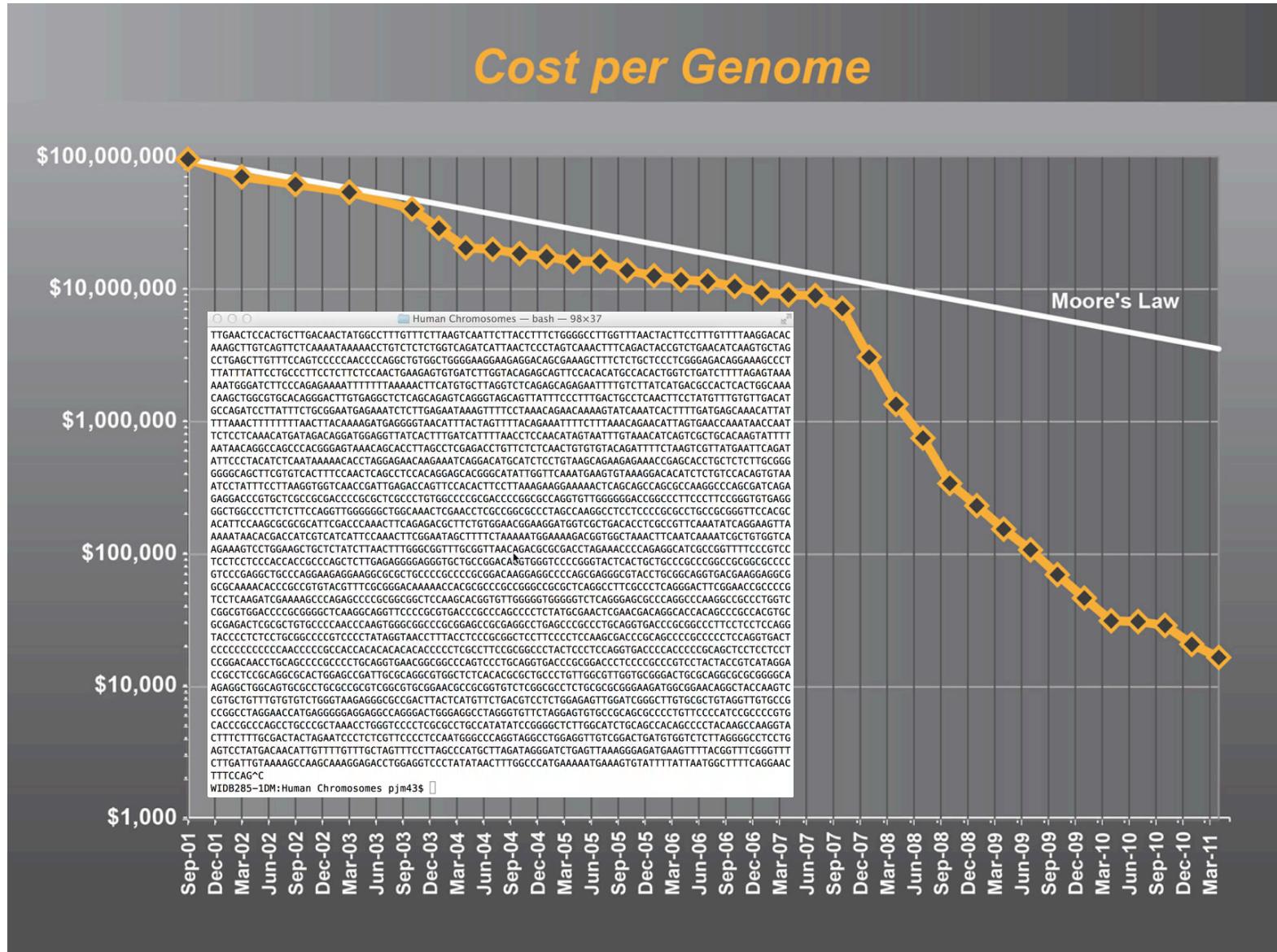
2013 – First year of field trials (WSU – Kevin Murphy); Three RIL populations







The Future (~18 months) – Complete Draft Genome Sequence (at least amaranth)



Come visit Utah!





Brigham Young University

Craig Coleman, Dan Fairbanks

Rick Jellen

Mike Stevens

Josh Udall

Melanie Mallory

Derrick Renyolds, David Elzinga

Rozura Vivas-Hall, Nate Barney

Kyle Nielson, Tina Lam

Scott Smith, Zac Danielson

Kyle Nielson, Alicia Barreda
(ETC.)



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