Plant Breeding & Genetics Group

Shaun Townsend
Co-Director PBG

OSU
Oregon State
UNIVERSITY

Outline

- Introduction
- PBG Genetic Research
  - Program personnel
  - Research areas
- Questions

Introduction

- PBG is one part of a larger genetic research component at OSU
- Plant-based genetic research
  - Primarily in support of plant breeding efforts
- Initially formed by members of Crop & Soil Sciences and Horticulture

Hops

- Two programs:
  - Shaun Townsend, OSU, Aroma Hops
  - John Henning, USDA, Aroma and bittering hops

Hop Challenges

- Expensive production system
  - Infrastructure & labor
- Plants immature until third growing season
- Brewing chemistry extremely complex
- Dioecy

Genetic Approaches

- Traditional (statistical)
  - Heritability, co-inheritance, BLUP
- Induced mutations
- Molecular biology
  - Marker development, genetic diversity, gene discovery
  - Possibly gene editing and transformation

OSU Aroma Hops

- Task is to develop new aroma hop cultivars suitable for the craft beer industry and adapted to Oregon growing conditions.
- Traits include yield, maturity date, disease resistance, brewing profile

Traditional Approaches

- Understanding heritability of important traits
  - **B**est **L**inear **U**nbiased **P**redictor (BLUP)
  - Provides information to guide breeding strategy by partitioning observed or measured variation for a trait into genetic and non-genetic causes
  - Superior male genotypes identified

Traditional Approaches

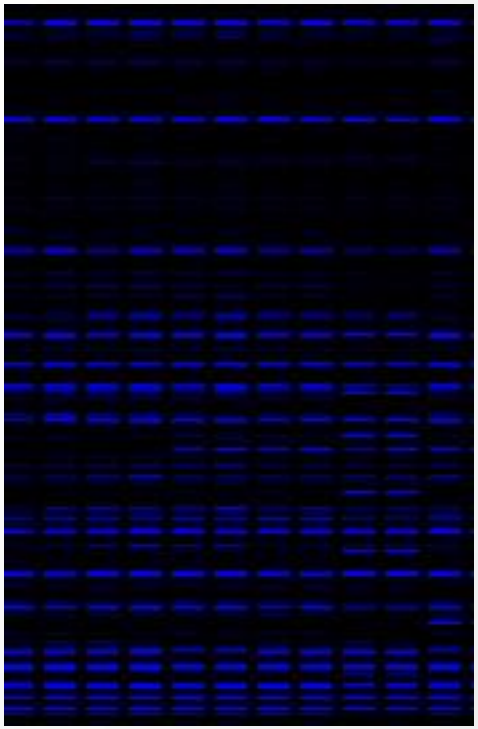
- Induced mutations
  - Subtle changes
  - Limited genetic change
  - Replacement hop cultivars

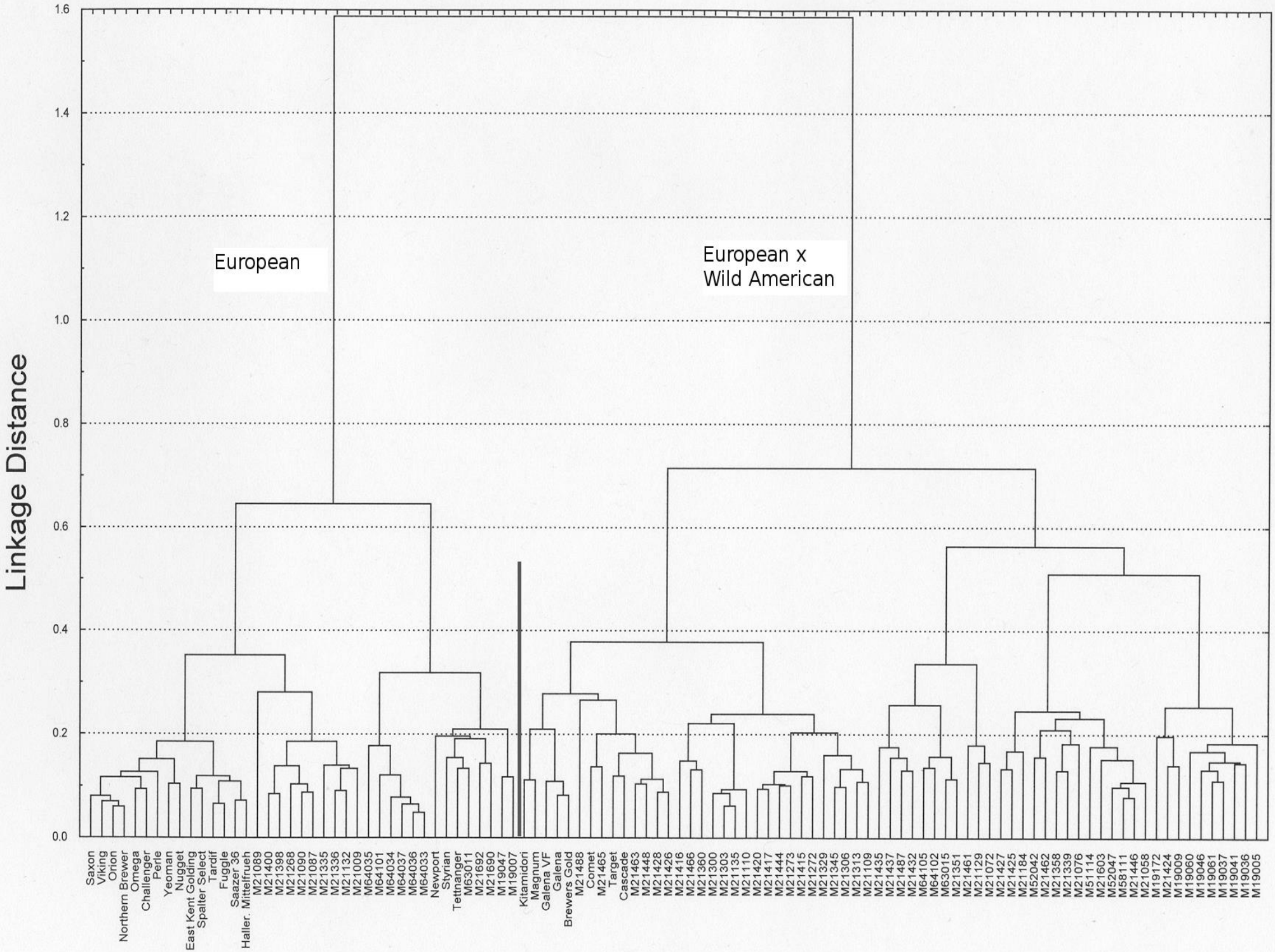
USDA Hops Program

- Led by John Henning
- Started in 1933
- Most public hop cultivars developed by this program

Molecular Approaches

- Marker development for **M**arker-**A**ssisted **S**election (MAS)
  - Disease resistance, plant sex
- Sequence the genome
  - Gene discovery
- Fix pedigree errors
- Assess genetic diversity




Linkage Distance
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
European
European x
Wild American
Saxon
Viking
Orion
Northern Brewer
Omega
Challenger
Perle
Yeoman
Nugget
East Kent Golding
Spalter Select
Tardif
Fuggle
Saazer 36
Haller. Mittelfrueh
M21089
M21400
M21398
M21268
M21090
M21087
M21335
M21336
M21132
M21009
M64035
M64101
M64034
M64037
M64036
M64033
Newport
Styrian
Tettnanger
M63011
M21692
M21690
M19047
M19007
Kitamidori
Magnum
Galena VF
Galena
Brewers Gold
M21488
Comet
M21465
Target
Cascade
M21463
M21448
M21428
M21426
M21416
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M63015
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M21462
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M21339
M21076
M51114
M21603
M52047
M58111
M21446
M21058
M19172
M21424
M19009
M19060
M19046
M19061
M19037
M19041
M19036
M19005

Barley

Hordeum vulgare

2n = 2x = 14

5.3 Gbp

~ 30,000 genes

Self-pollinated (hermaphroditic)

The OSU Barley Project

Crossing

Doubled haploids

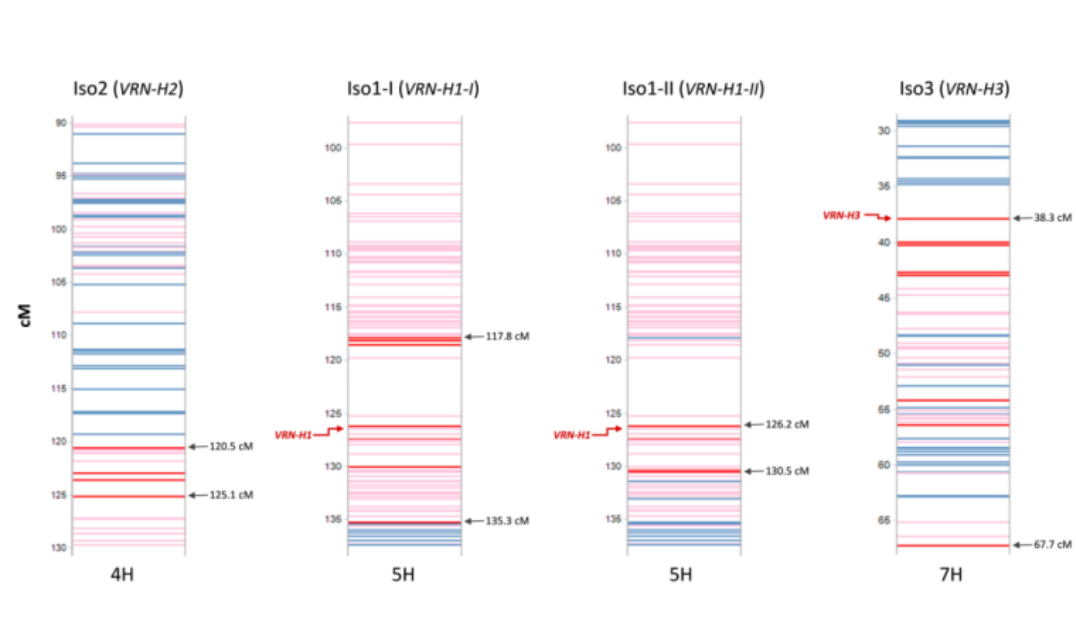
Genetics and Breeding

Publication, Variety/Germplasm release

The Relationships between Development and Low Temperature Tolerance in Barley Near Isogenic Lines Differing for Flowering Behavior. Cuesta-Marcos, A. et al. 2015. Plant and Cell Physiology Volume 56, Issue 12 Pp. 2312-2324.

Integrating genetics and breeding at a Land Grant University

Locus/alleles	Phenotype	Mechanism
Vrn1, Vrn2, Vrn3	Growth habit	Loss of function deletions
Ppd1, Ppd2	Flowering time	Loss of function deletions



Barley contributions to beer flavor

Deschutes + 6 and the Oregon Promise

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WORLD BREWING CONGRESS 2016

The Oregon Promise: A Tool for Understanding the Genetic Mechanisms Regulating the Expression of Flavor Traits in Barley (*Hordeum vulgare*) Important for Malting, Brewing, and Distilling

D. Herb[1], D. Carey[2], S. Fisk[1], P.M. Hayes[1], R. Jennings[3], C. Martens[4], B. Meints[5], R. Monsour[3], M. Moscou[6], I. Romagosa[7], W. Thomas[8], S. Tynan[3]

[1] Dept. Crop and Soil Science, Oregon State University, Corvallis, OR, USA; [2] New Glarus Brewing, New Glarus, WI, USA; [3] Rahr Malting Co. Shakopee, MN, USA; [4] USDA-ARS Cereal Crops Research Unit, Madison, WI, USA; [5] Dept. Crop and Soil Sciences, Washington State University, Mt. Vernon, WA, USA; [6] The Sainsbury Laboratory, John Innes Institute, Norwich, UK, [7] University of Lleida, Lleida, Spain; [8] James Hutton Institute, Invergowrie, Dundee, Scotland, UK

World Brewing Congress
August 13-17, 2016
Sheraton Downtown Denver
Denver, CO 80202, U.S.A.

Introduction

The Oregon Promise is a spring barley population derived from Golden Promise x Full Pint crosses. Golden Promise is an iconic variety for malting, brewing, and distilling developed in Scotland and Full Pint was developed by Oregon State University and is a contributor to the craft malt industry. The Oregon Promise will provide a valuable resource for extending current knowledge of malting and brewing genes to the frontiers of sensory assessment. The objectives of this study are to determine if there are flavor differences within modern barley varieties. If flavor differences are present: 1) describe the flavors, 2) map gene(s) controlling flavors, and 3) develop methods to select for flavors.

Materials & Methods

- *Germplasm:* 34 advanced lines selected based on desirable agronomic and malt quality traits from the Oregon Promise bi-parental population consisting of 200 doubled haploids.
- *Environments:* The advanced lines were grown in 2015 in two locations in Willamette Valley (Corvallis, OR & Lebanon, OR) and one in central Oregon (Madras, OR). Phenotype data recorded for disease resistance (barley stripe rust, leaf rust, scald), yield, lodging, brackling, plant height, dwarfing, flowering time, and ag score.
- *Micro-malting:* 250 g per entry including parents and check from each location were pale malted using a multi-sample micro-maltер. 75 g of each entry were used for quality analysis performed at Rahr Malting Co.
- *Nano-brewing:* 1 liter of each entry was brewed to a pilsner style using a step-mash protocol. Wort was filtered with Ahlstrom filters then dosed with iso-alpha-acid for balance. Samples were boiled to target °Plato (10°P). The wort was filtered again to remove denatured protein and trub before being pitched with flavor inert yeast to reduce confounding flavors. Samples fermented for 14 days at 12°C, then carbonated and bottled before being conditioned for 2 weeks.
- *Sensory & Analytics:* Blind tasting of a type II modified augmented design with 111 unreplicated entries and 59 replicated checks and controls (Golden Promise, Full Pint, Copeland, Rahr Pils, Miller High Life). 10 mL sample per entry collected for gas chromatography-mass spectrometry analysis
- *Genotyping:* The population was genotyped using the Eureka Genomics Next Generation Genotyping Barley SNP panel and genotyping-by-sequencing (GBS). Linkage maps were made in JoinMap 4.1 using manual curation and imputation. Quantitative trait loci (QTL) analysis was done using Windows QTL Cartographer 2.5.

Table 1. Analysis of variance (ANOVA) p-values, r-squared, and heritability estimates for barley flavor intensity* (BFI) flavors across the three testing environments (Corvallis, OR; Lebanon, OR; Madras, OR) in 2015.

Source	Corvallis					Lebanon					Madras				
	BFI (All)	Off-Flavors	Fruity	Malty	Taste	BFI (All)	Off-Flavors	Fruity	Malty	Taste	BFI (All)	Off-Flavors	Fruity	Malty	Taste
Entry	0.001	0.001	<0.001	<0.001	0.045	<0.001	0.187	0.055	<0.001	0.088	<0.001	0.344	<0.001	<0.001	<0.001
Parallel	0.134	0.094	<0.001	0.019	0.247	<0.001	<0.001	0.001	<0.001	0.02	<0.001	<0.001	<0.001	<0.001	<0.001
Entry x Parallel	0.005	0.564	0.271	0.025	0.297	0.075	0.006	0.831	<0.001	0.85	0.391	0.018	0.112	0.005	0.021
R^2	0.53	0.27	0.40	0.59	0.41	0.41	0.52	0.42	0.63	0.39	0.59	n/a	0.33	0.64	0.38
h^2	22.7	1.2	8.5	41.6	11.7	9.2	10.1	8.2	30.3	19.4	18.3	9.2	5.2	32.1	6.2

* BFI is an averaged estimate for groupings of similar flavors descriptors

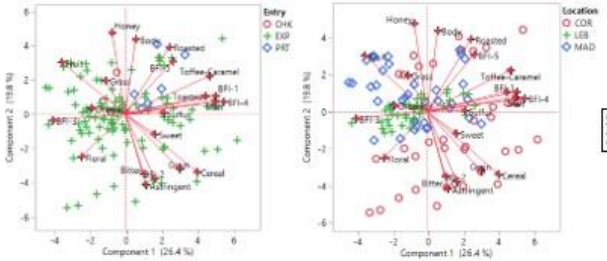


Figure 1. Principle Component Analysis (PCA) of flavor descriptors and barley flavor intensities (BFI) BLUPs against entries (EXP), parents (PAT), and checks (CHK).

Figure 2. Principle Component Analysis (PCA) of flavor descriptors and barley flavor intensities (BFI) BLUPs against testing environment (COR=Corvallis, OR; LEB=Lebanon, OR; MAD=Madras, OR).

Figure 3. Linkage map of the Oregon Promise population and the relative position of the agronomic (ESR, LR, height, heading, dwarfing, protein, plump, spike length, hairs, TKW), malting quality (extract, diastatic power, alpha-amylase, beta-glucan, S/T, FAN, color), and flavor (malt, fruit, floral, malty, honey, chemical, DMS, grain, cereal, astringent, sweet, bitter, roasted, toasted) QTLs across the 7 chromosome of barley.

Results

- Off-flavors were not significantly affected by genotype (Table 1).
- Significant genotype effect detected for malty flavor in all environments and fruity in Corvallis and Madras (Table 1).
- Taste BFI (sweet, bitter, body, astringency) had a significant genotype effect in Corvallis and Madras (Table 1).
- Significant GxE interaction detected in BFI (All), malty, and taste (Table 1).
- High h^2 estimates for malty (30-40%) in all environments and moderate h^2 estimates for BFI (All) (9-22%) in Corvallis and Madras and taste (6-19%) in Lebanon (Table 1).
- Differential flavor BLUPs between parental genotypes. Full Pint had the highest BLUPs in malty, toffee-caramel, toasted, and roasted flavors, while Golden Promise was highest in fruity, floral, grassy, and chemical flavors (Figure 1).
- Transgressive segregates for numerous flavors detected with the population (Figure 1).
- Significant environmental effect detected: Corvallis had the highest BLUPs in floral, bitter, astringent, grain, and cereal flavors, Lebanon was highest in chemical, grassy, and sulfur flavors, and Madras was highest in fruity, toffee-caramel, and roasted flavors (Figure 2).
- QTL detected for various flavors on chromosome 1H, 2H, 3H, 5H, and 6H (Figure 3).
- Flavor QTL on 1H, 5H, and 6H are not in association with malting, agronomic, or morphological QTL (Figure 3).
- Honey and fruity had the largest effect QTL. Detected three putative QTL for honey on chromosomes 1H, 2H, and 6H and two putative QTL for fruity on chromosomes 1H and 5H (Figures 3, 4, & 5).

Discussion

- The presence of transgressive segregates for traits with high h^2 indicate good selection potential for flavors in modern breeding programs.
- Some flavor traits may be mendelized, indicating simple(r) genetics structure (Figures 6 & 7).
- Insignificant QTL may be results of a complex trait or uninformative phenotype.
- Significant effects + highly heritable traits + significant QTL = micro-malting, nano-brewing, type II augmented design, and sensory are effective tools for determining barley flavor contributions to beer.
- *Next Step:* 1) Map the full Oregon Promise population including GC-MS analytic data, 2) association mapping of USDA barley world core collection, 3) selection of lines based off agronomic, malting quality, and flavor for large scale brewing/sensory validation, 4) characterize the environmental effects (i.e. soil type, rainfall, nutrients), and 5) flavorful barley variety development and release.

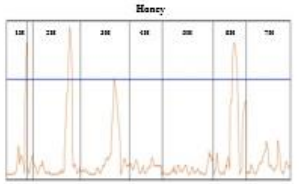


Figure 4. QTL peak graph and false discovery threshold for honey.

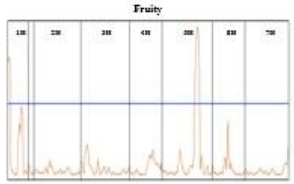


Figure 5. QTL peak graph and false discovery threshold for fruity.

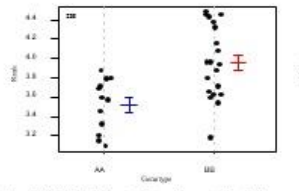


Figure 6. Allelic effect plot for significant markers controlling the honey QTL. Genotype AA is the paternal allele (Full Pint) and BB is the maternal allele (Golden Promise).

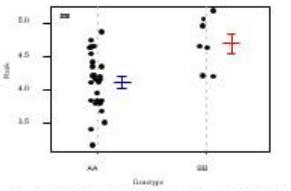


Figure 7. Allelic effect plot for significant markers controlling fruity QTL. Genotype AA is the paternal allele (Full Pint) and BB is the maternal allele (Golden Promise).

Acknowledgements

The Flavor 7-pack of breweries (Bells, Deschutes, Firestone Walker, New Glarus, Russian River, Sierra Nevada, and Summit), CISC-EEAD: Spanish Ministry of Science and Innovation (project AGL2015-69435-C3-2-R), IPSR: JSPS KAKENHI, grant numbers 24880025 and 16K18634 to HH, Brewers' Association, American Malting Barley Association, Rahr Malting Co., Mecca Grade Estate Malt

Hazelnut Program

- Led by Shawn Mehlenbacher
- Only hazelnut breeding program in the U.S.
- Hazelnut production is centered in Oregon

Hazelnut Breeding Objectives

A. Blanched kernel market (for chocolate, baked goods)
(93% of world crop is sold as kernels, 7% sold in-shell)

1. Bud mite resistance
2. Round nut shape
3. High percent kernel
4. Precocity
5. High yield
5. Easy pellicle removal
6. Few defects
7. Early maturity
8. Free-falling nuts

B. Resistance to eastern filbert blight (EFB)

1. Simply inherited resistance (‘Gasaway’ & >50 others)
2. Quantitative resistance (e.g. ‘Tonda di Giffoni’, ‘Sacajawea’)

Hazelnut Quantitative Traits

Trait	Heritability (%)
Good Kernels	42
Doubles	84
Moldy Kernels	61
Poorly Filled Nuts	25
Nut Length	68
Nut Shape Index	65
Nut Compression Index	88
Nut Weight	63
Percent Kernel	87
Fiber	56
Blanching	64
Relative Husk Length	91
Nuts per Cluster	67
Catkin Elongation Time	68
Nut Maturity	86

Most traits are highly heritable.

Mehlenbacher et al., 1993; Yao & Mehlenbacher, 2000

Eastern Filbert Blight

Fungus *Anisogramma anomala*, 2-year life cycle. Cankers girdle and kill branches.

We now have > 100 sources of resistance. We use single R-genes and quantitative resistance.

Sources of Very High EFB Resistance in *C. avellana* (greenhouse tests)

Accession	Origin	LG*	S-alleles
1. Gasaway	Unknown	6	3 26
1. Zimmerman	Barcelona x Gasaway	6	1 3
2. Ratoli	Spain	7	2 10
3. Georgian OSU 759.010	Rep. of Georgia	2	4 20
4. OSU 408.040	Univ. Minnesota	6	15 27
5. OSU 495.072	Southern Russia (VIR)	6	6 30
6. Culpla	Spain	6	9 10
7. Crvenje	Serbia	6	6 23
8. Uebov	Serbia	6	12 16
9. Moscow N02	Russia (Moscow)	?	6 20
10. Moscow N23	Russia (Moscow)	?	6 30
11. Moscow N26	Russia (Moscow)	?	1 29
12. Moscow N27	Russia (Moscow)	?	19 23
13. Moscow N37	Russia (Moscow)	?	1 6
14. Farris OSU 533.029	Lansing, Michigan	?	3 11
15. C. avellana COR 157	Finland	?	9 25
16. Amarillo Tardio	Chile (Chillan)	?	2 2

*Linkage Group assigned using microsatellite markers

Pacific Northwest Potato Breeding and Variety Development Program

Tri-State Potato Variety Development Team

USDA Agricultural Research Service
C. Brown, Prosser, WA
R. Novy, Aberdeen, ID
J. Whitworth, Aberdeen, ID
R. Navarre, Prosser, WA

Washington State University
M. Pavek, Pullman, WA
R. Knowles, Pullman, WA

University of Idaho
J. Stark, Aberdeen, ID
M. Thornton, Parma, ID
N. Olsen, Kimberly, ID
L. Ewing, Moscow, ID

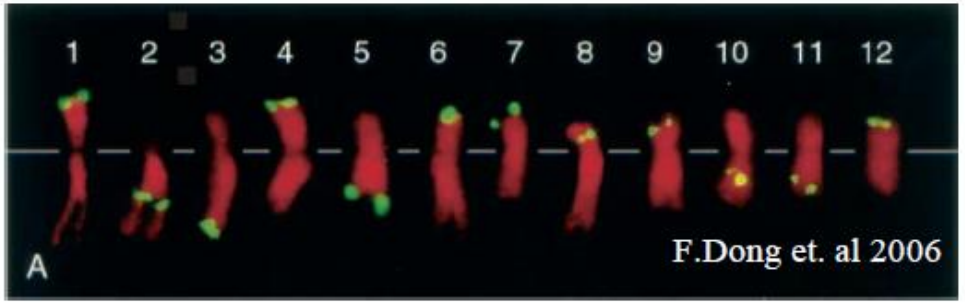
Oregon State University
V. Sathuvalli, Hermiston, OR
B. Charlton, Klamath Falls, OR
S. Yilma, Corvallis, OR
C. Shock, Ontario, OR

PVMI Potato Variety Management Institute
J. Debons, Bend, OR

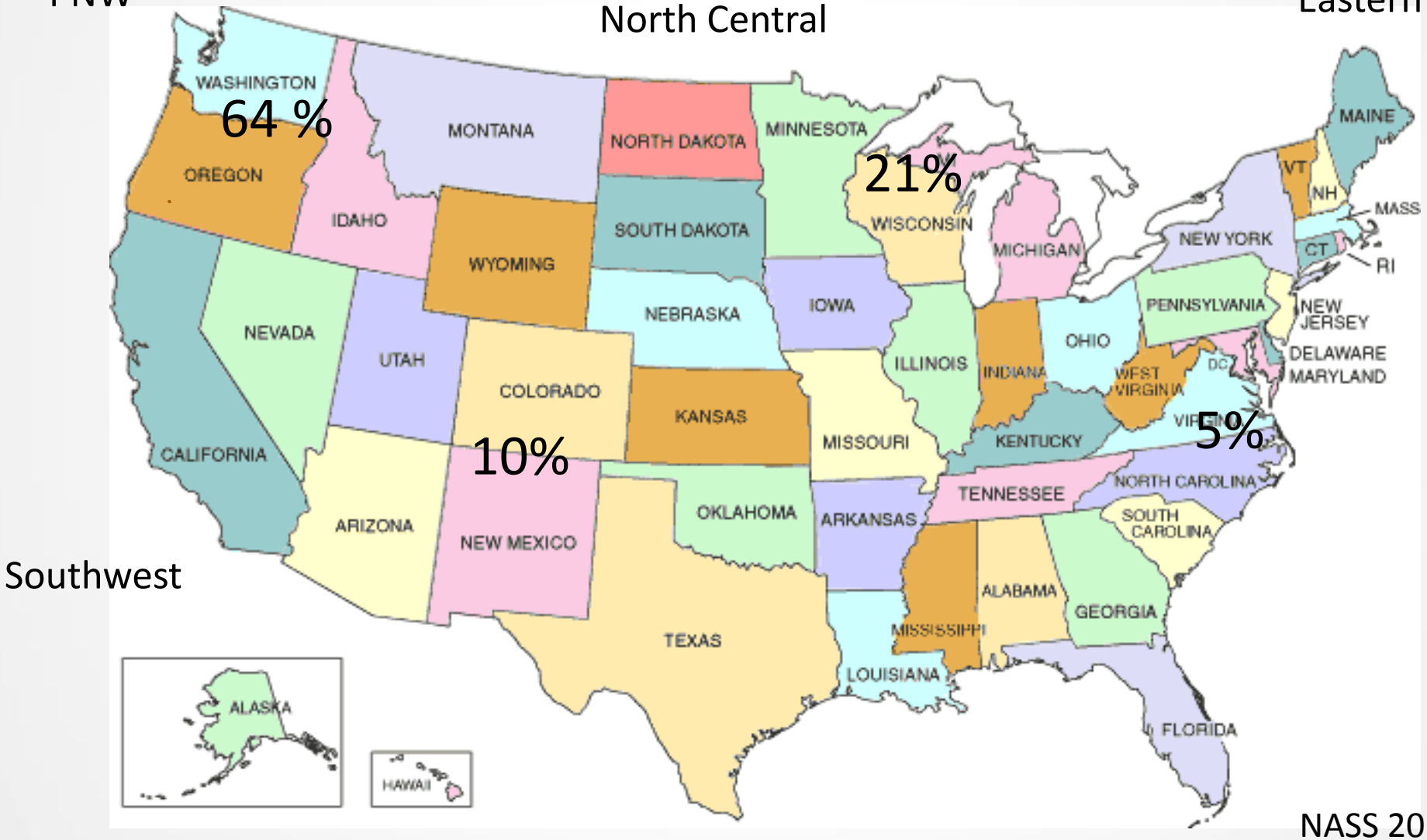
Jointly funded by USDA-NIFA & Potato Commissions of ID, OR & WA

Solanum sp.

- Range of ploidy: 2X, 3X, 4X and 5X
- Most cultivated potatoes are tetraploid (2n=4x=48)
- The basic chromosome number is 12
- Haploid genome size is ~900 mb



USA Potato Production 2014

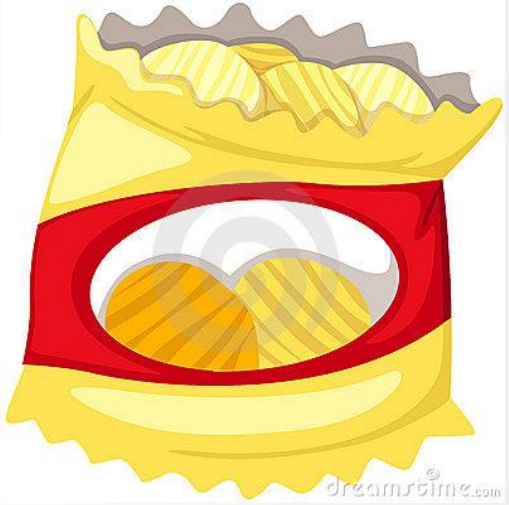


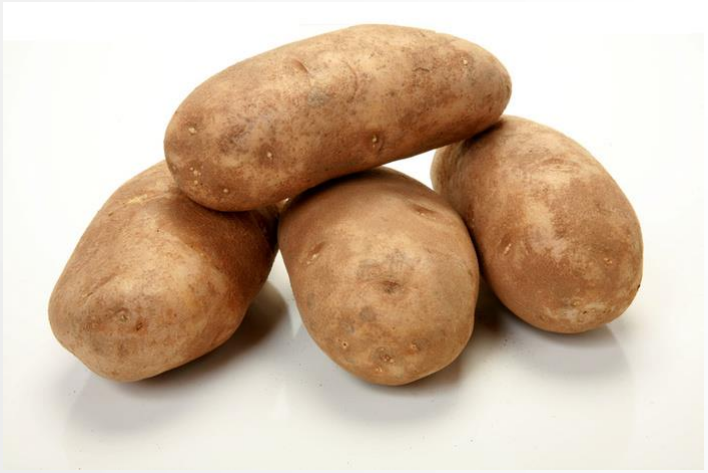
NASS 2014

Adapted from Knowles et al (2010)

PNW Potato Industry

- **Processing Industry**
- Fresh Market – Table stock Russets
- Chipping
- Specialty – Reds, yellows, etc.
- Dehy Industry – Potato starch, flour, etc.





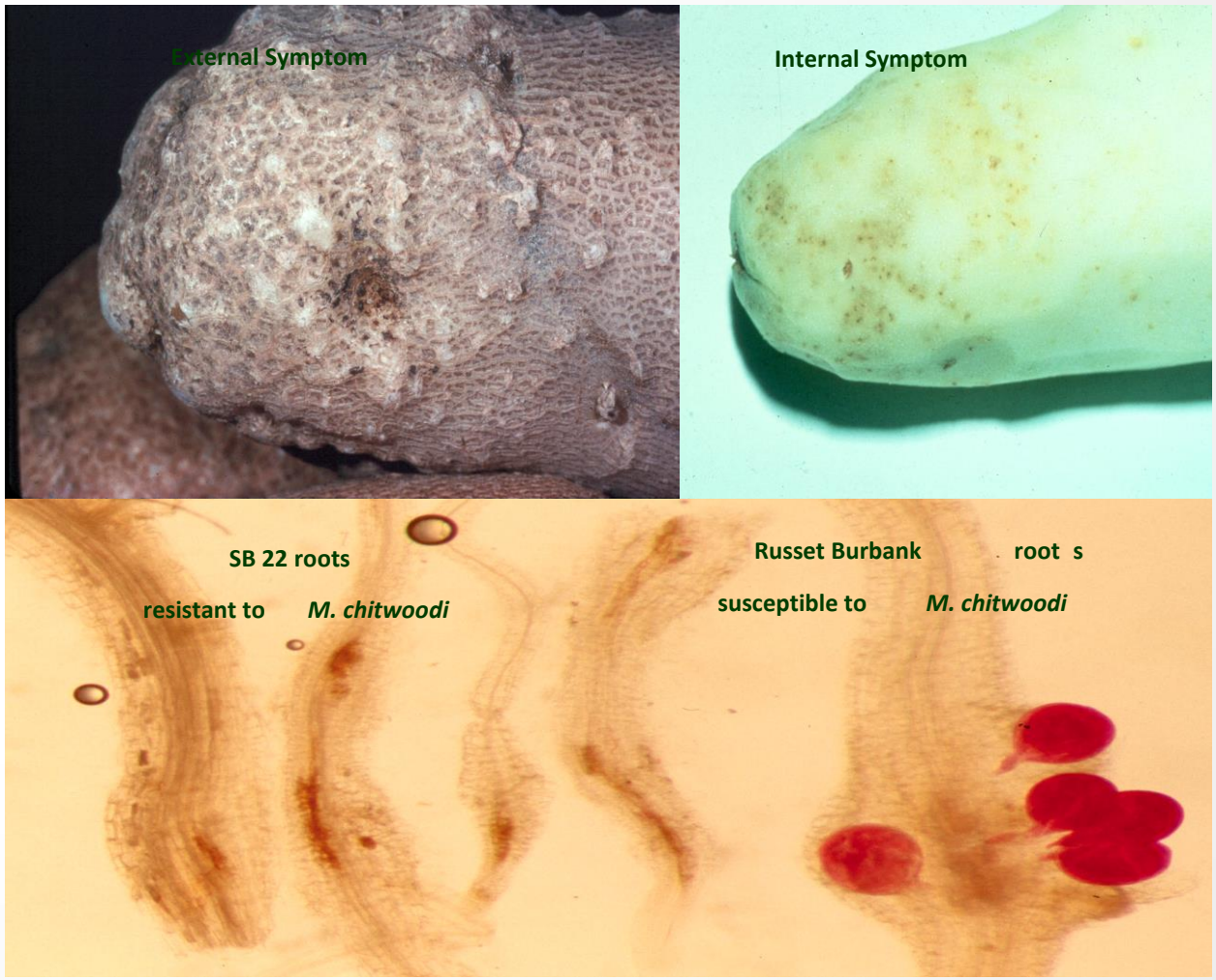
Breeding Objectives

- Develop new russet potatoes
  - Dual purpose russet varieties (ID)
  - Individual market oriented russet varieties (OR)
- Breeding for resistance to major pests and diseases
  - PVY, Verticillium wilt, Zebra Chip, TRV, PMTV, CRKN, Scab etc.
- Breeding of specialty potatoes
  - Reds/yellows/purples
  - High anthocyanins, minerals, carotenoids, Nutrients, Flavor
- Breeding for cold sweetening resistance and high nutrient efficiency
  - Low acrylamide, low N input

Overall Goal:
Release & commercialize new potato varieties that will directly benefit all segments of the PNW potato industry

Columbia Root Knot Nematode

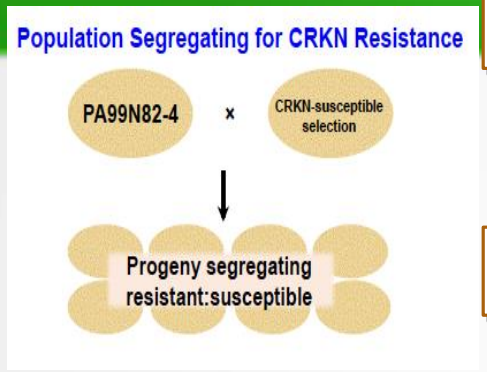
- Serious pathogen - cause severe disease on potato
- A gene, $R_{Mc1(blb)}$, controlling resistance derived from *Solanum bulbocastanum* has been identified and used in breeding resistant potato lines.



Solanum bulbocastanum Dunal

- Wild, diploid potato
- Source of late blight resistance genes
- Source of tuber resistance to Columbia Root Knot Nematode (CRKN)
- Accession SB22 (PI 275187)

Identification of Molecular Markers

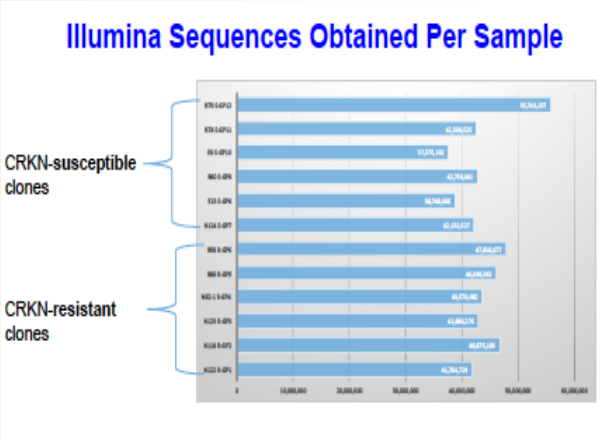


Resistant

Susceptible

Illumina Hi-seq 2000

SB22 genome



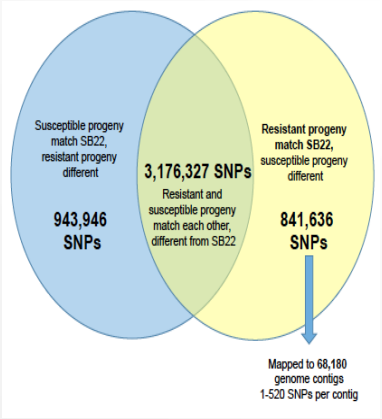
Genome alignments

Samtools

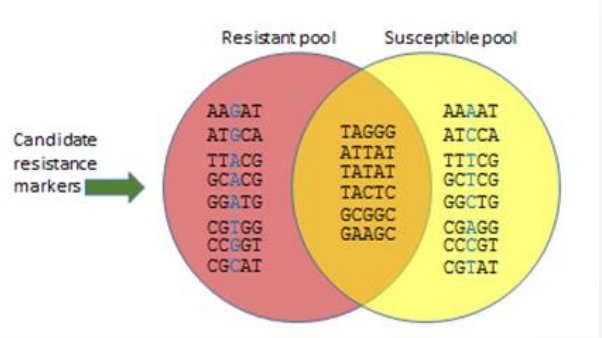
SNP calling

Resistant

Susceptible



68,180 contigs



Genetically Engineered Trees

Steve Strauss
Distinguished Professor
Oregon State University
Steve.Strauss@OregonState.Edu

OSU
Oregon State
UNIVERSITY

Focus in Strauss lab

- Genetic engineering approaches to tree breeding, with a focus on poplar (cottonwood) and eucalypts
  - Emphasis on containment for social and regulatory acceptance given wild relatives, long distance gene flow capability
- Genomic analysis of role of structural polymorphisms in poplar heterosis
- GWAS analysis of genes that control variation in capability for genetic engineering (major new, $4 million NSF project)

Study organisms: Poplar plantations

Rapid cycling eucalypts recently proven in Strauss laboratory

Plant Biotechnology Journal
Open Access

Plant Biotechnology Journal

Plant Biotechnology Journal (2016) **14**, pp. 808–819 doi: 10.1111/pbi.12431

FT overexpression induces precocious flowering and normal reproductive development in *Eucalyptus*

Amy L. Klocko[1], Cathleen Ma[1], Sarah Robertson[1], Elahe Esfandiari[1], Ove Nilsson[2] and Steven H. Strauss[1,*]

[1]*Department Forest Ecosystems & Society, Oregon State University, Corvallis, OR, USA*
[2]*Department of Forest Genetics and Plant Physiology, Umeå Plant Science Centre, Swedish University of Agricultural Sciences, Umeå, Sweden*

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accepted 10 June 2015.
**Correspondence* (Tel (541) 760 7357; fax (541) 737 1393; email steve.strauss@oregonstate.edu)

Keywords: Eucalypts, breeding, transgenic, forest biotechnology, *Flowering Locus T*, genetic engineering.

Summary

Eucalyptus trees are among the most important species for industrial forestry worldwide. However, as with most forest trees, flowering does not begin for one to several years after planting which can limit the rate of conventional and molecular breeding. To speed flowering, we transformed a *Eucalyptus grandis* × *urophylla* hybrid (SP7) with a variety of constructs that enable overexpression of *FLOWERING LOCUS T* (*FT*). We found that *FT* expression led to very early flowering, with events showing floral buds within 1–5 months of transplanting to the glasshouse. The most rapid flowering was observed when the cauliflower mosaic virus 35S promoter was used to drive the *Arabidopsis thaliana FT* gene (*AtFT*). Early flowering was also observed with *AtFT* overexpression from a 409S ubiquitin promoter and under heat induction conditions with *Populus trichocarpa FT1* (*PtFT1*) under control of a heat-shock promoter. Early flowering trees grew robustly, but exhibited a highly branched phenotype compared to the strong apical dominance of nonflowering transgenic and control trees. *AtFT*-induced flowers were morphologically normal and produced viable pollen grains and viable self- and cross-pollinated seeds. Many self-seedlings inherited *AtFT* and flowered early. *FT* overexpression-induced flowering in *Eucalyptus* may be a valuable means for accelerating breeding and genetic studies as the transgene can be easily segregated away in progeny, restoring normal growth and form.

Field trials: Coleopteran resistant Bt-cottonwoods in eastern Oregon field trial

RNA interference for sterility (suppression of endogenous flowering genes)

Policy analysis relevant to GE crops and trees – many lab contributions

Traces of the emerald ash borer on the trunk of a dead ash tree in Michigan, USA. This non-native invasive insect from Asia threatens to kill most North American ash trees.

BIOTECHNOLOGY

Genetically engineered trees: Paralysis from good intentions

Forest crises demand regulation and certification reform

By **Steven H. Strauss[1]*, Adam Costanza[2], Armand Séguin[3]**

Intensive genetic modification is a long-standing practice in agriculture, and, for some species, in woody plant horticulture and forestry (*1*). Current regulatory systems for genetically engineered

recently initiated an update of the Coordinated Framework for the Regulation of Biotechnology (*2*), now is an opportune time to consider foundational changes.

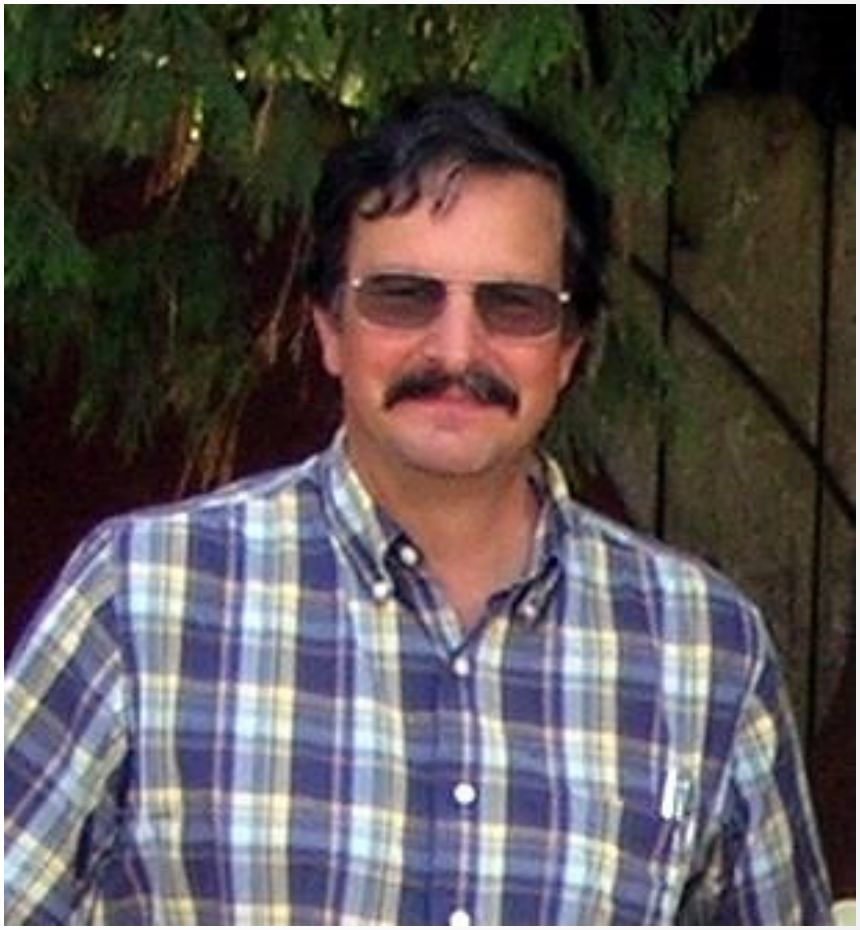
Difficulties of conventional tree breeding make genetic engineering (GE) methods relatively more advantageous for forest trees than for annual crops (*3*). Obstacles

Although only a few forest tree species might be subject to GE in the foreseeable future, regulatory and market obstacles prevent most of these from even being subjects of translational laboratory research. There is also little commercial activity: Only two types of pest-resistant poplars are authorized for commercial use in small areas in China and two types of eucalypts, one approved in Brazil and another under lengthy review in the USA (*5*).

METHOD-FOCUSED AND MISGUIDED. Many high-level science reports state that the GE method is no more risky than conventional breeding, but regulations around the world essentially presume that GE is hazardous and requires strict containment

Vegetable Breeding & Genetics

- Various species
  - Snap beans
  - Snap peas
  - Broccoli
  - Tomatos
  - Cucurbits
- Traditional and organic production



Disease Resistance in Bean

- Genetic resistance in beans to *Fusarium* root rot
- Screened 148 bean varieties in Oregon
- Associated morphological traits to resistance
- Used **S**ingle **N**ucleotide **P**olymorphism (SNP) to identify markers for MAS
- Created a linkage map

Indigo Rose Tomato

- Introgressed chromosomal segments from a wild relative into tomato
- High levels of healthful flavanoids

Ornamental Breeding & Genetics

- Various landscape ornamentals
  - Maples
  - Cape hyacinth
  - Sweetbox
  - Flowering currant
  - Many others

Plant Sterility

- Genetic work to support plant breeding effort
  - Ploidy manipulation to induce sterility (ie. triploids) in nonnative species
  - Mutagenesis via chemical and physical means
  - Traditional genetic research (ex. heritability)

Genetic Work

- Interspecific hybridization in Lilac
- Heritability of floral traits in *Hibiscus syriacus*
- Cytogenetics of various woody shrubs

Winter Wheat Breeding Program

- Soft white winter wheat
  - Cakes, cookies, pancakes
- Hard white winter wheat
  - Noodles, bread
- Hard red winter wheat
  - Bread, rolls, cereal

Bob Zemetra

Program Goal

- Increase profitability of growing wheat for Oregon producers
- How:
  - Boost production - yield
  - Decrease costs - disease resistance
  - Boost demand - high quality

Disease Resistance

- In some cases, genetic resistance is the only option
  - Barley Yellow Dwarf (BYDV)
  - Wheat Mosaic Virus (sbWMV)
- Viruses have a great impact on yield and quality

Barley Yellow Dwarf Virus

- 30-40% yield loss
- Resistance gene bdv2 from Oklahoma germplasm
- Moving gene into Oregon germplasm

Wheat Mosaic Virus

- Soil-borne
- Only control is genetic resistance
- sbwm1 gene from midwest and New York


Disease can also reduce quality
- Fusarium head blight
Infected seed
Non-infected seed

Fusarium head blight

- Fungal disease that infects the head and seed
- Disease reduces yield and seed quality
- Pathogen produces a toxin making the seed useless for animal and human consumption
- Source of resistance gene *Fhb1* and QTLs
– Michigan and New York germplasm
- Breeding program transferring *Fhb1* and QTL for FHB resistance into OSU germplasm

Other Programs

- Jennifer Kling - Quantitative genetics
- Kelly Vining - Mint breeding & genomics
- Laurent Deluc - Grape genomics
- Chad Finn - USDA, Berry breeding & genetics