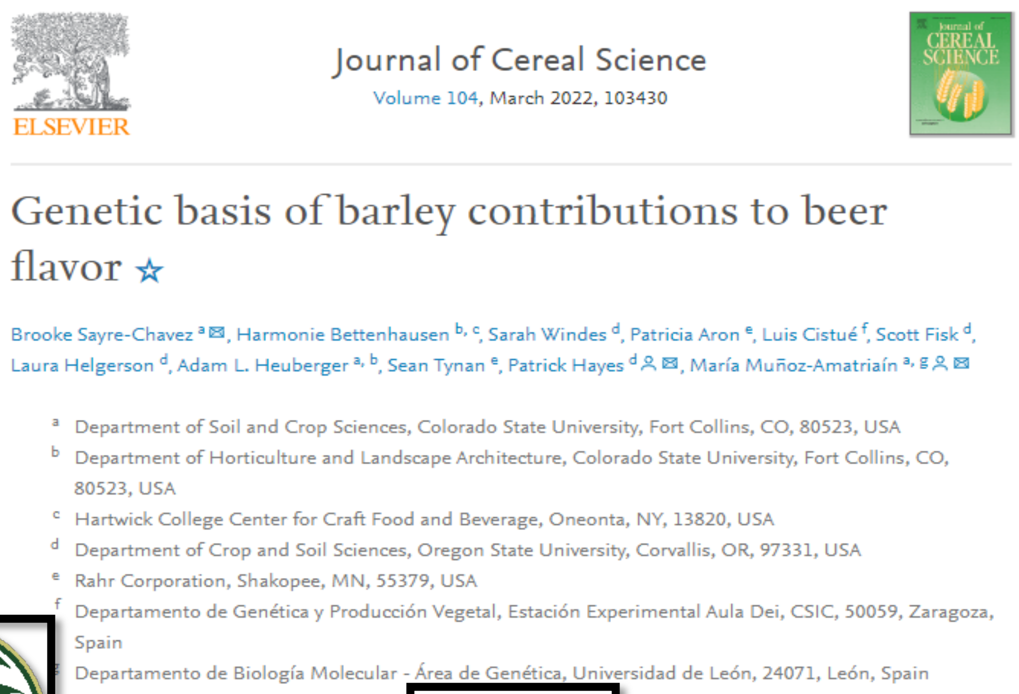
Genetic basis of barley contributions to beer flavor

Dr. Harmonie Bettenhausen
Director, Hartwick Center for Craft Food and Beverage

ASBC
THE SCIENCE OF BEER

BREWING SUMMIT 2022
Providence, Rhode Island | August 14–16



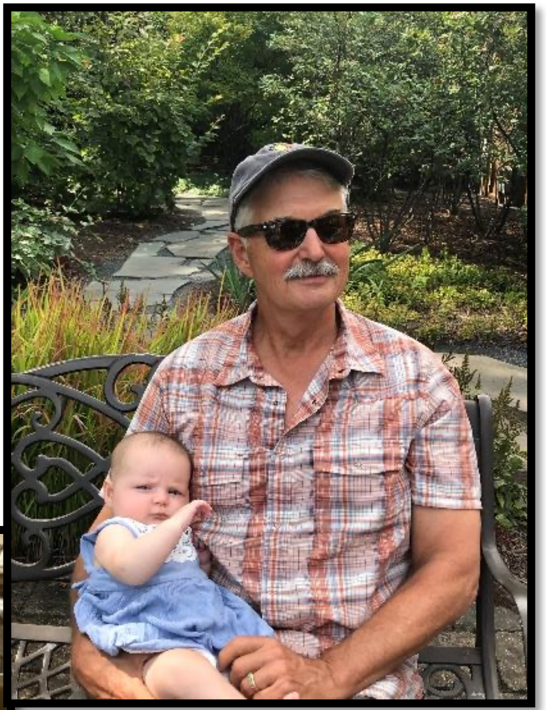
ELSEVIER

Journal of Cereal Science
Volume 104, March 2022, 103430

Genetic basis of barley contributions to beer flavor ☆

Brooke Sayre-Chavez [a] ✉, Harmonie Bettenhausen [b, c], Sarah Windes [d], Patricia Aron [e], Luis Cistué [f], Scott Fisk [d], Laura Helgerson [d], Adam L. Heuberger [a, b], Sean Tynan [e], Patrick Hayes [d] 👤 ✉, María Muñoz-Amatriaín [a, g] 👤 ✉

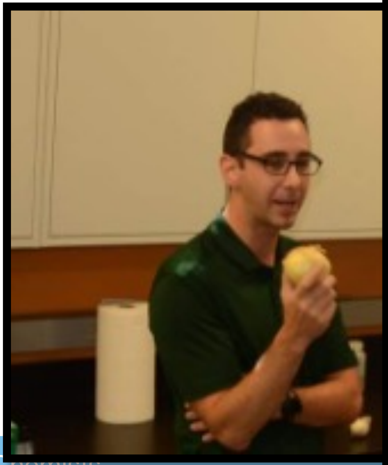
[a] Department of Soil and Crop Sciences, Colorado State University, Fort Collins, CO, 80523, USA
[b] Department of Horticulture and Landscape Architecture, Colorado State University, Fort Collins, CO, 80523, USA
[c] Hartwick College Center for Craft Food and Beverage, Oneonta, NY, 13820, USA
[d] Department of Crop and Soil Sciences, Oregon State University, Corvallis, OR, 97331, USA
[e] Rahr Corporation, Shakopee, MN, 55379, USA
[f] Departamento de Genética y Producción Vegetal, Estación Experimental Aula Dei, CSIC, 50059, Zaragoza, Spain
[g] Departamento de Biología Molecular - Área de Genética, Universidad de León, 24071, León, Spain

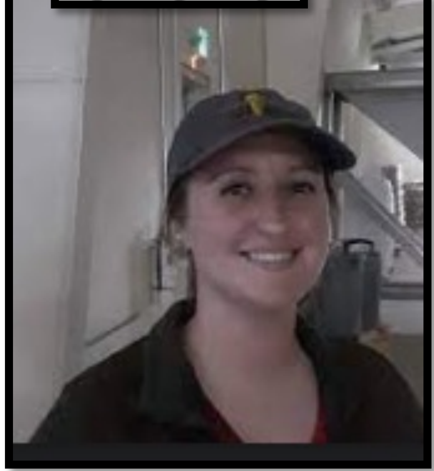


1847 RAHR MALTING CO.

EEAD

OSU





Behind the music: Pat Hayes Edition

Journal of the American Society of Brewing Chemists
The Science of Beer

ISSN: 0361-0470 (Print) 1943-7854 (Online) Journal homepage: https://www.tandfonline.com/loi/ujbc20

Effects of Barley (*Hordeum Vulgare* L.) Variety and Growing Environment on Beer Flavor

Dustin Herb, Tanya Filichkin, Scott Fisk, Laura Helgerson, Patrick Hayes, Brigid Meints, Rebecca Jennings, Robert Monsour, Sean Tynan, Kristi Vinkemeier, Ignacio Romagosa, Matthew Moscou, Daniel Carey, Randy Thiel, Luis C

e-Xtra*

Malt Modification and Its Effects on the Contributions of Barley Genotype to Beer Flavor

Dustin Herb,[1] **Tanya Filichkin, Scott Fisk, Laura Helgerson, and Patrick Hayes,** *Crop & Soil Science Dept., Oregon State University, Corvallis, OR U.S.A.;* **Amanda Benson and Veronica Vega,** *Deschutes Brewery, Bend, OR U.S.A.;* **Daniel Carey and Randy Thiel,** *New Glarus Brewing Co., New Glarus, WI U.S.A.;* **Luis Cistue,** *Estación Experimental Aula Dei, CSIC, Zaragoza, Spain;* **Rebecca Jennings, Robert Monsour, Sean Tynan, and Kristi Vinkemeier,** *Rahr Malting Co., Shakopee, MN U.S.A.;* **Yueshu Li, Andrew Nguygen, and Aaron Onio,** *Canadian Malting Barley Technical Centre, Winnipeg, MB Canada;* **Brigid Meints,** *Dept. of Crop & Soil Sciences, Washington State University, Mt. Vernon, WA U.S.A.;* **Matthew Moscou,** *The Sainsbury Laboratory, Norwich Research Park, Norwich NR4 7UH U.K.;* **Ignacio**
Dundee

JOURNAL OF THE AMERICAN SOCIETY OF BREWING CHEMISTS
2020, VOL. 78, NO. 2, 136-152
https://doi.org/10.1080/03610470.2019.1706037

Taylor & Francis
Taylor & Francis Group

Open access

Variation in Sensory Attributes and Volatile Compounds in Beers Brewed from Genetically Distinct Malts: An Integrated Sensory and Non-Targeted Metabolomics Approach

Harmonie M. Bettenhausen[a], Amanda Benson[b], Scott Fisk[c], Dustin Herb[c], Javier Hernandez[c], Juyun Lim[d], Sue H. Queisser[d], Thomas H. Shellhammer[d], Veronica Vega[b], Linxing Yao[e], Adam L. Heuberger[a], and Patrick M. Hayes[c]

[a] Department of Horticulture and Landscape Architecture, Colorado State University, CO, U.S.A.; [b] Deschutes Brewery, Bend, OR, U.S.A.; [c] Department of Crop and Soil Science, Oregon State University, Corvallis, OR, U.S.A.; [d] Department of Food Science and Technology, Oregon State University, Corvallis, OR, U.S.A.; [e] Proteomics and Metabolomics Facility, Colorado State University, Fort Collins, CO, U.S.A.

JOURNAL OF THE AMERICAN SOCIETY OF BREWING CHEMISTS
2021, VOL. 79, NO. 3, 281-305
https://doi.org/10.1080/03610470.2020.1843964

Taylor & Francis
Taylor & Francis Group

Open access

Comprehensive Analysis of Different Contemporary Barley Genotypes Enhances and Expands the Scope of Barley Contributions to Beer Flavor

S. Windes[a], H. M. Bettenhausen[b], K. R. Van Simaeys[c], J. Clawson[c], S. Fisk[a], A. L. Heuberger[b], J. Lim[c], S. H. Queisser[c], T. H. Shellhammer[c], and P. M. Hayes[a]

JOURNAL OF THE AMERICAN SOCIETY OF BREWING CHEMISTS
2021, AHEAD-OF-PRINT, 1-14
https://doi.org/10.1080/03610470.2021.1952509

Taylor & Francis
Taylor & Francis Group

Open access

Continued Exploration of Barley Genotype Contribution to Base Malt and Beer Flavor Through the Evaluation of Lines Sharing Maris Otter® Parentage

Campbell P. Morrissy[a], Michael Féchir[b], Harmonie M. Bettenhausen[c], Karli R. Van Simaeys[b], Scott Fisk[a], Javier Hernandez[a], Kyle Mathias[d], Amanda Benson[d], Thomas H. Shellhammer[b], and Patrick M. Hayes[a]

Contents lists available at ScienceDirect

Journal of Cereal Science

journal homepage: www.elsevier.com/locate/jcs

ELSEVIER

Genetic basis of barley contributions to beer flavor☆

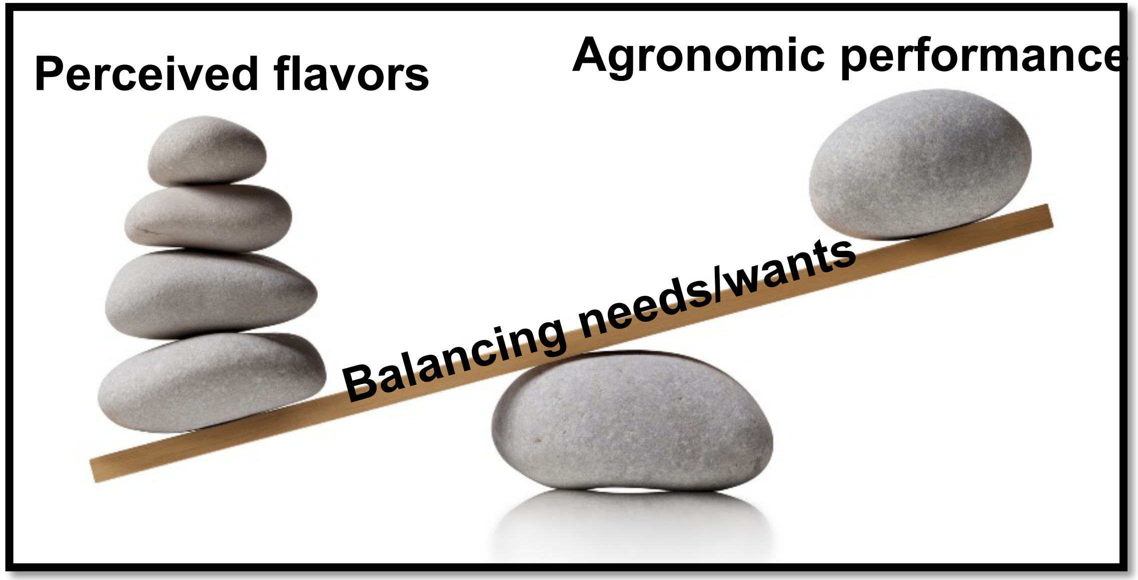
Brooke Sayre-Chavez[a], Harmonie Bettenhausen[b,c], Sarah Windes[d], Patricia Aron[e], Luis Cistué[f], Scott Fisk[d], Laura Helgerson[d], Adam L. Heuberger[a,b], Sean Tynan[e], Patrick Hayes[d,*], María Muñoz-Amatriaín[a,g,**]

What have we learned?

AMERICAN
MALTING
BARLEY
ASSOCIATION

BREWING AND
MALTING BARLEY
Research Institute

Golden Promise
Klages
Maris Otter

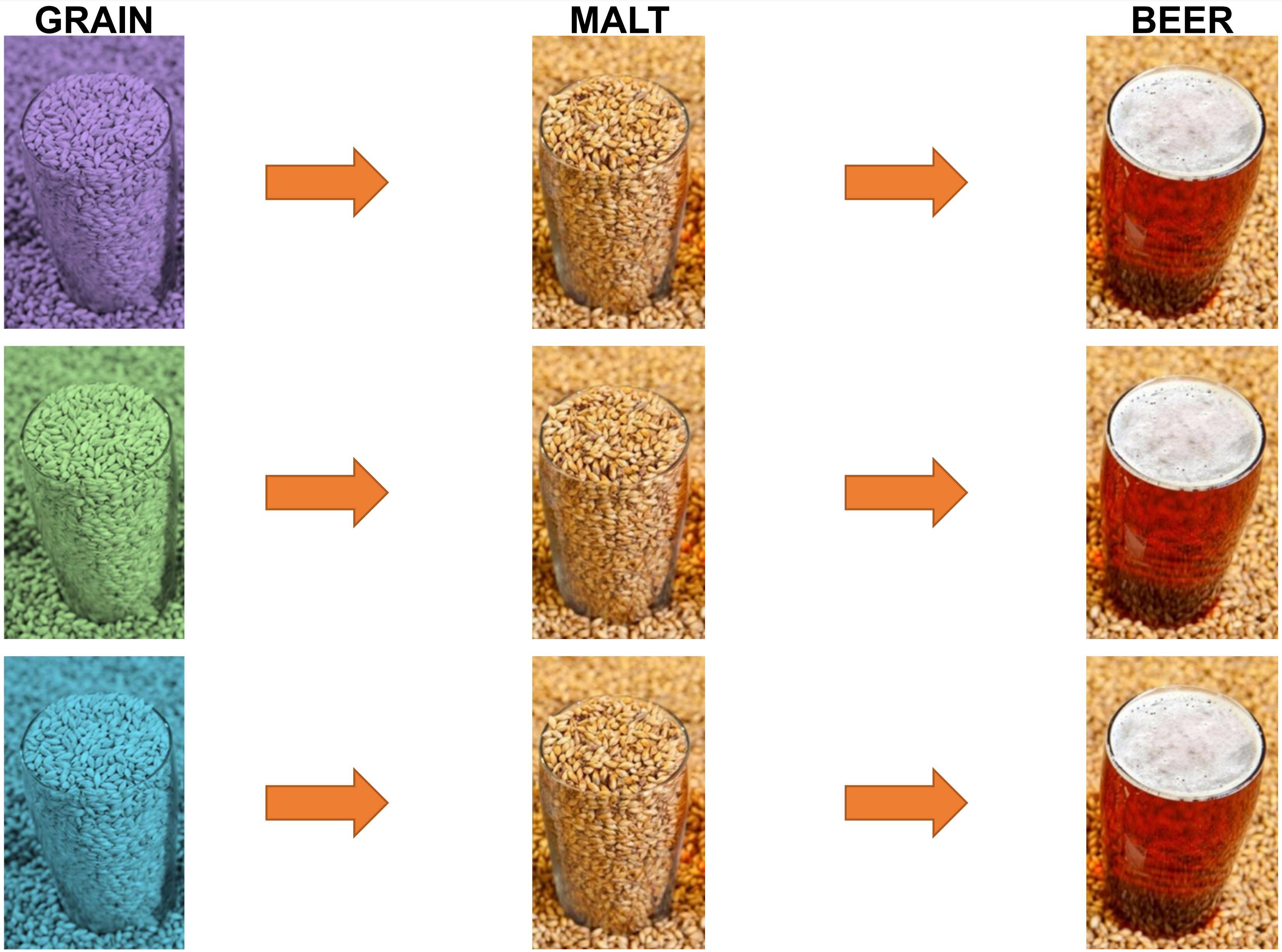


Limited work so far has found:

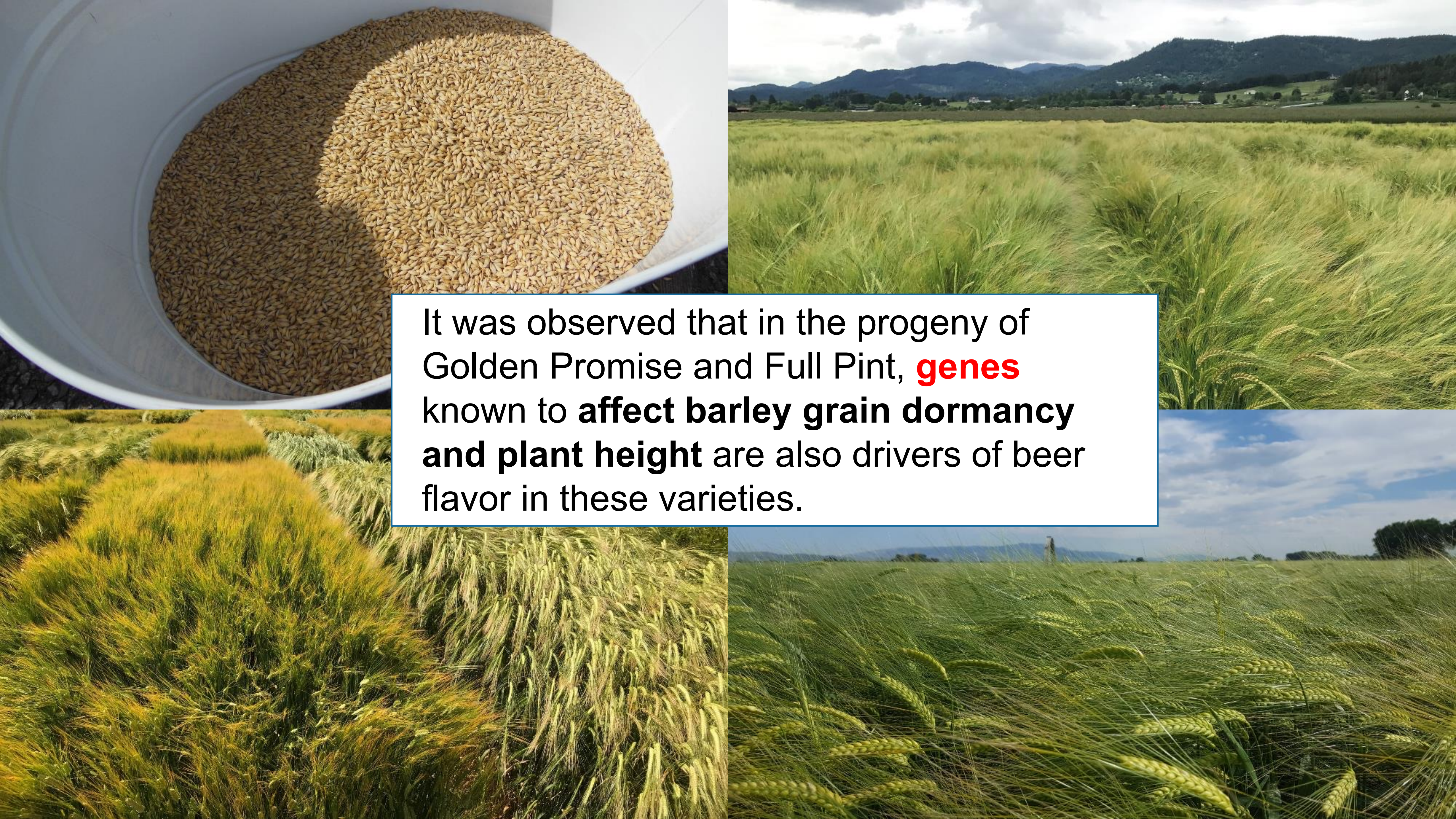
- Barley genotypes can differ in their contributions to beer flavor
- There is a genetic basis to these contributions
- Differences in sensory attributes and metabolite profiles of malt are affected by degree of modification of malt, but that is not the whole story.

Yet, questions remain!

Different genotypes



Differences in flavor?



It was observed that in the progeny of Golden Promise and Full Pint, **genes** known to **affect barley grain dormancy and plant height** are also drivers of beer flavor in these varieties.

Experimental design – but we'll come back to this

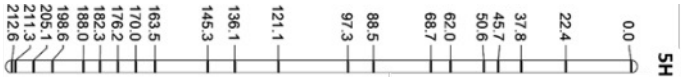
Golden Promise x Full Pint

Mapping population

Oregon Promise doubled haploid population
n = 228 DH lines

Genotyping

iSelect 50k SNP chip



Linkage map construction

Micro-malting

Rahr Malting Co.,
Shakopee, MN
n = 162 DH lines

Nano-brewing

Rahr Malting Co.,
Shakopee, MN
n = 162 DH lines

Malt quality analysis

@ USDA Cereal
Crop Research
Unit, Madison, WI
n = 150 DH lines

Beer sensory

Trained
sensory
panel
n = 162 DH
nano-brews

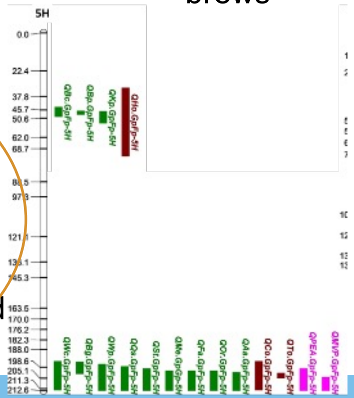
Color, sweet,
cereal, malty,
honey,
caramel/toffee,
grassy, floral, fruity,
toasted

Beer metabolomics

CSU ARC-BIO, Fort
Collins, CO
HS/SPME-GC-MS
n = 155 DH nano-brews

QTL analysis
Candidate genes

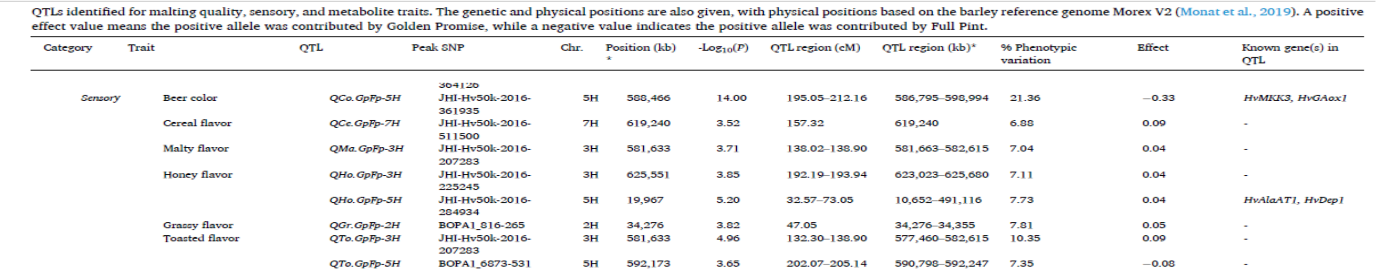
Mixed model method
implemented in R



QTL Regions explain

- Dormancy and dwarfing genes associated with flavor
- Degree of modification - a driver of flavor
- The hormone driver (of modification) in the background: **GA**

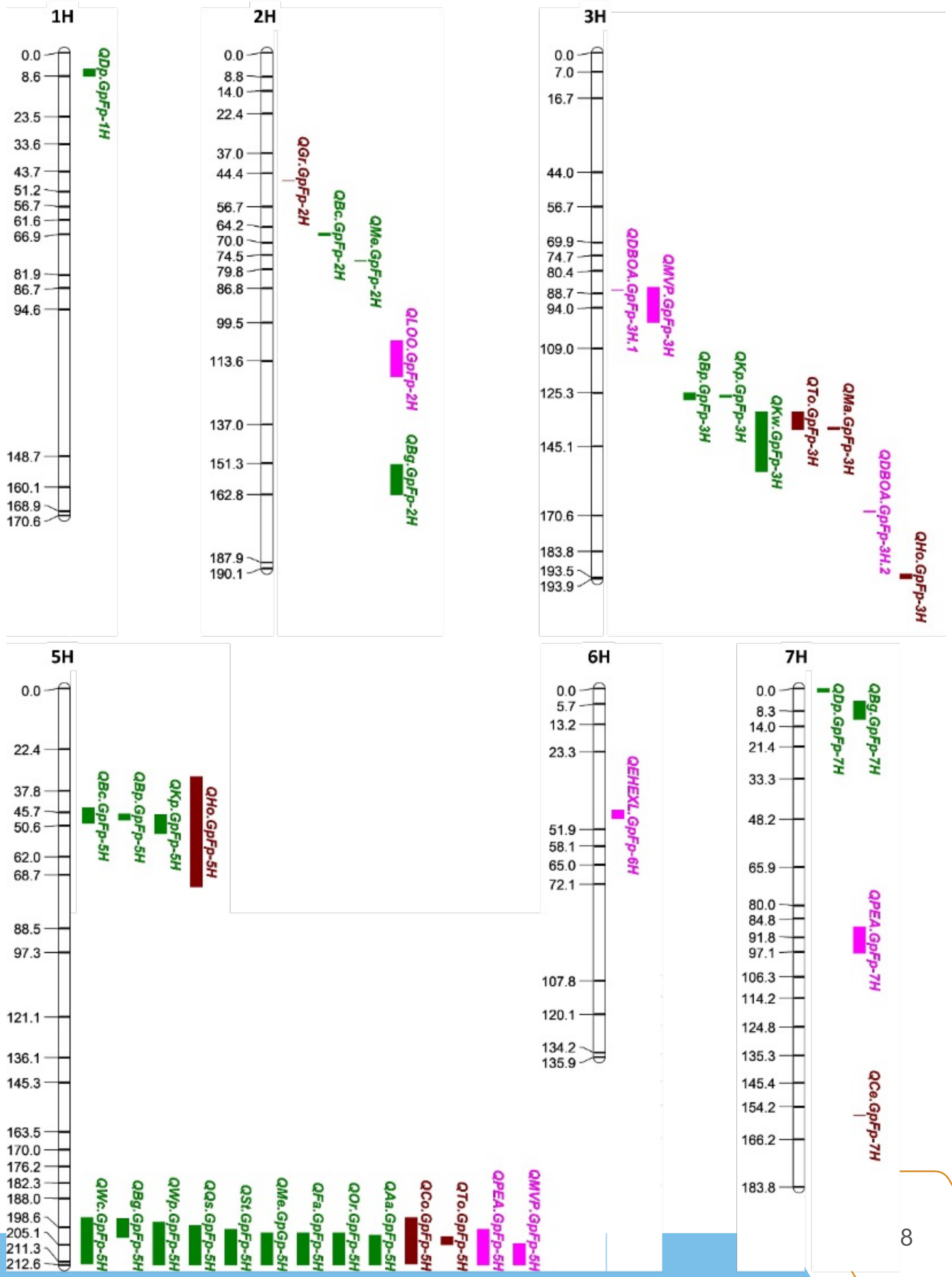
Caveats: micro-malts, range of modification, nano-beers



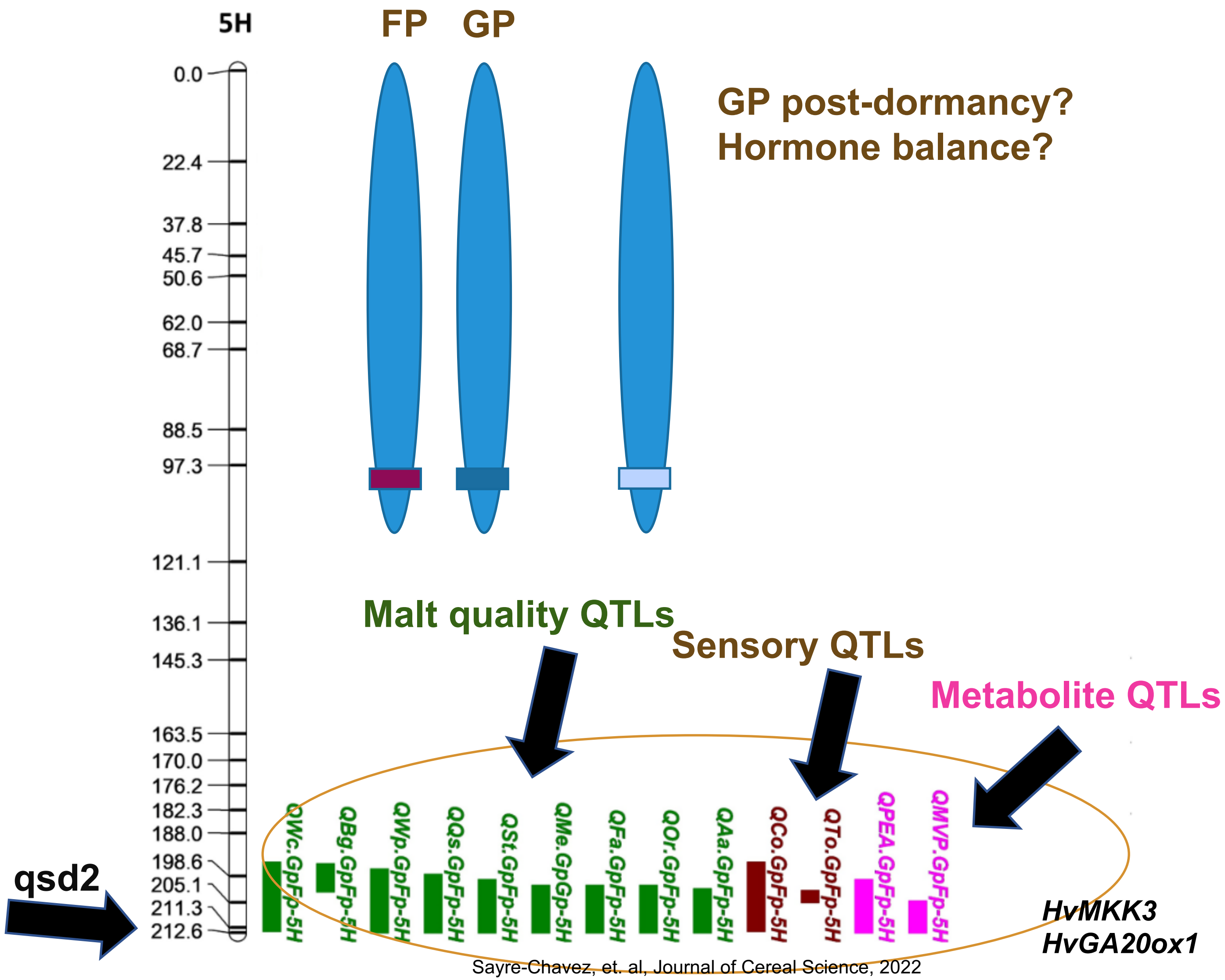
QTLs identified for malting quality, sensory, and metabolite traits. The genetic and physical positions are also given, with physical positions based on the barley reference genome Morex V2 (Monat et al., 2019). A positive effect value means the positive allele was contributed by Golden Promise, while a negative value indicates the positive allele was contributed by Full Pint.

Category	Trait	QTL	Peak SNP	Chr.	Position (kb) *	$-Log_{10}(P)$	QTL region (cM)	QTL region (kb)*	% Phenotypic variation	Effect	Known gene(s) in QTL
			364126								
Sensory	Beer color	*QCo.GpFp-5H*	JHI-Hv50k-2016-361935	5H	588,466	14.00	195.05–212.16	586,795–598,994	21.36	−0.33	*HvMKK3, HvGAox1*
	Cereal flavor	*QCe.GpFp-7H*	JHI-Hv50k-2016-511500	7H	619,240	3.52	157.32	619,240	6.88	0.09	-
	Malty flavor	*QMa.GpFp-3H*	JHI-Hv50k-2016-207283	3H	581,633	3.71	138.02–138.90	581,663–582,615	7.04	0.04	-
	Honey flavor	*QHo.GpFp-3H*	JHI-Hv50k-2016-225245	3H	625,551	3.85	192.19–193.94	623,023–625,680	7.11	0.04	-
		QHo.GpFp-5H	JHI-Hv50k-2016-284934	5H	19,967	5.20	32.57–73.05	10,652–491,116	7.73	0.04	*HvAlaAT1, HvDep1*
	Grassy flavor	*QGr.GpFp-2H*	BOPA1_816-265	2H	34,276	3.82	47.05	34,276–34,355	7.81	0.05	-
	Toasted flavor	*QTo.GpFp-3H*	JHI-Hv50k-2016-207283	3H	581,633	4.96	132.30–138.90	577,460–582,615	10.35	0.09	-
		QTo.GpFp-5H	BOPA1_6873-531	5H	592,173	3.65	202.07–205.14	590,798–592,247	7.35	−0.08	-

Sayre-Chavez, et. al, Journal of Cereal Science, 2022



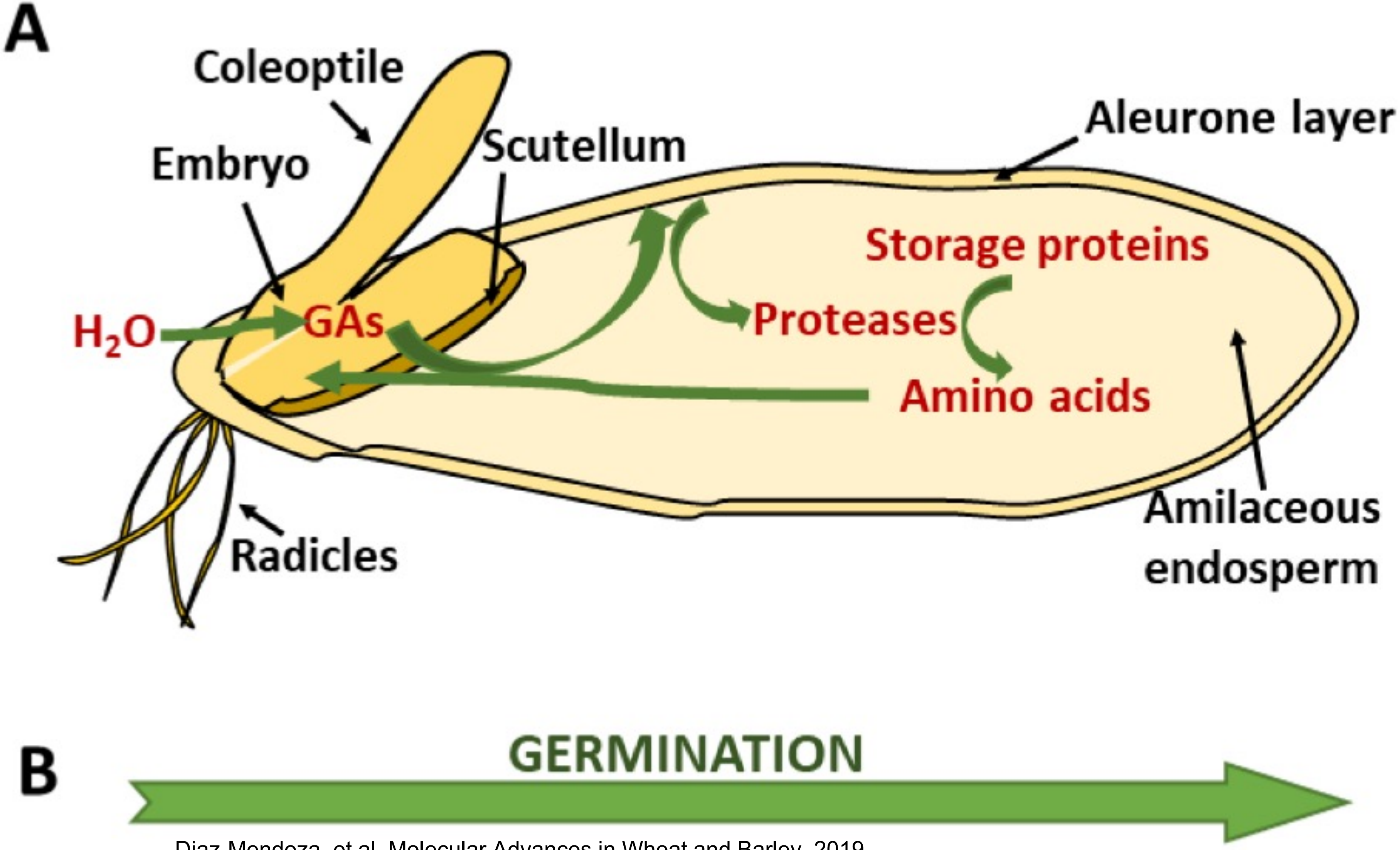
Is dormancy “f(l)avorable”?



Sayre-Chavez, et. al, Journal of Cereal Science, 2022

- Dormancy is usually binary (one dormant allele, one non-dormant allele)
- Full Pint– non-dormant allele/Golden Promise– dormant allele
- Golden Promise – is there lingering effect?
- Is there a “cost” in terms of modification (or lack thereof)?

Gibberellic Acid – the backseat driver



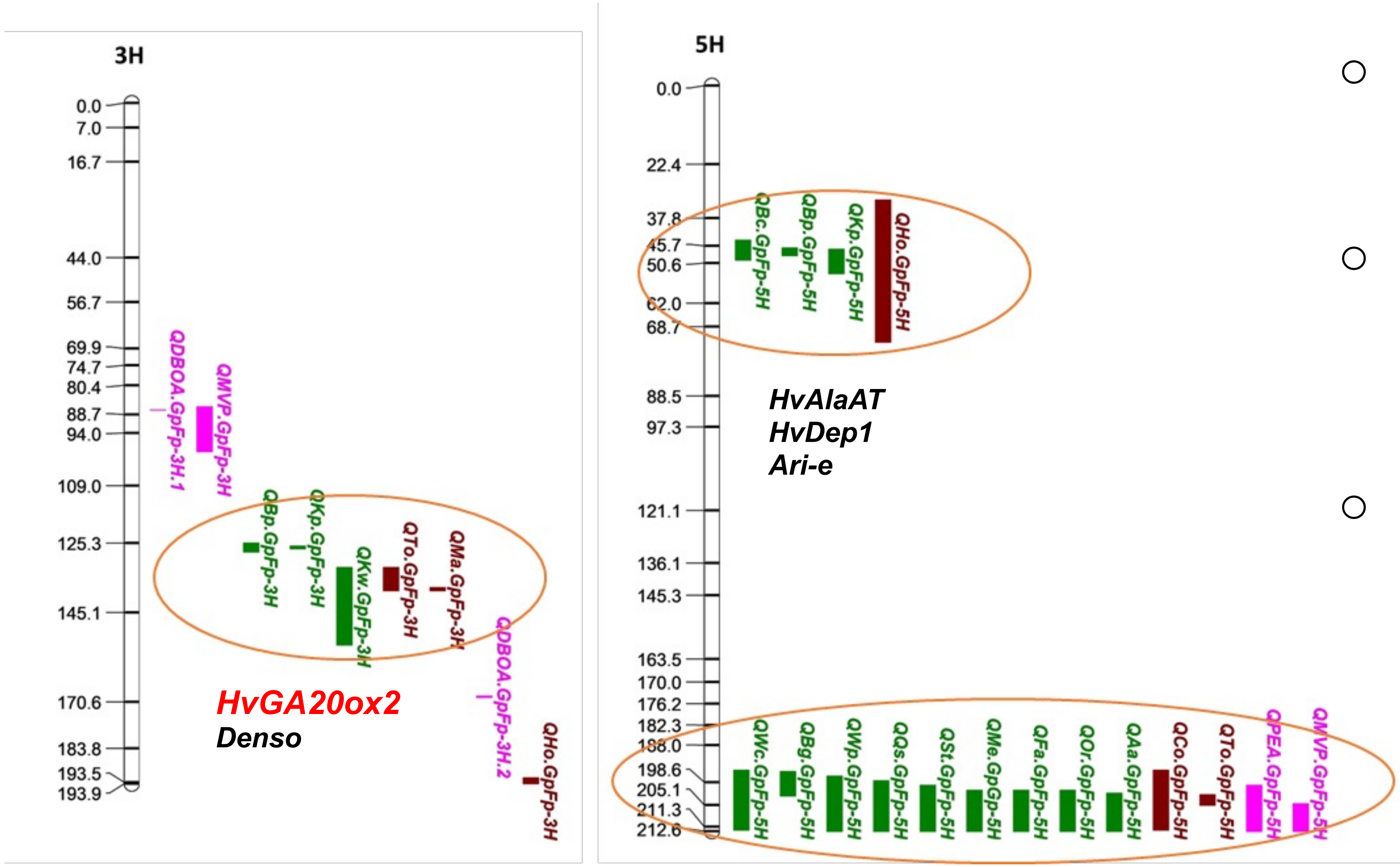
Diaz-Mendoza, et.al, Molecular Advances in Wheat and Barley, 2019

Gibberellins coordinate the **release from dormancy** so the seed can germinate

“It is tempting to speculate that the candidate gene(s) for SD2 are involved in dormancy, degree of dormancy, and PHS”

How are GA being affected by this and how is this affecting mod, flavor?

Dwarfing may have a big impact



Sayre-Chavez, et. al, Journal of Cereal Science, 2022

- Binary – one allele standard height, one is dwarf height
- Ari-e (5H) – GP dwarf allele/FP standard height allele (OP has FP alleles at both sites)
- Denso (3H) GP standard height allele/FP dwarf height allele (GP has favorable alleles for flavor, but higher protein)

Experimental design – we're back!

Oregon Promise

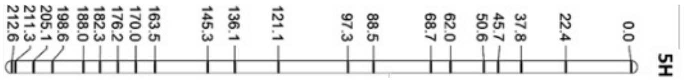
Golden Promise x Full Pint

Mapping population

Oregon Promise doubled haploid population
n = 228 DH lines

Genotyping

iSelect 50k SNP chip



Linkage map construction

Micro-malting

Rahr Malting Co.,
Shakopee, MN
n = 162 DH lines

Nano-brewing

Rahr Malting Co.,
Shakopee, MN
n = 162 DH lines

Malt quality analysis

@ USDA Cereal Crop Research Unit,
Madison, WI
n = 150 DH lines

Beer sensory

Trained sensory panel
n = 162 DH nano-brews

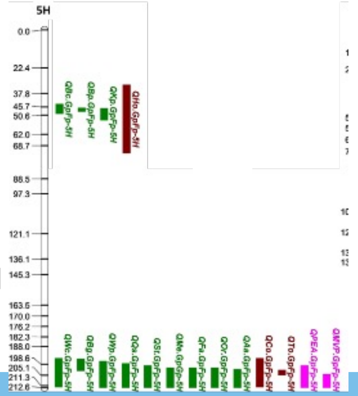
Color, sweet, cereal, malty, honey, caramel/toffee, grassy, floral, fruity, toasted

Beer metabolomics

CSU ARC-BIO, Fort Collins, CO
HS/SPME-GC-MS
n = 155 DH nano-brews

QTL analysis
Candidate genes

Mixed model method implemented in R



Malting Quality and Sensory Results

Category	Trait	Golden Promise	Full Pint	DH population	
				Mean	SE
Malt quality	Kernel weight (mg)	44.00	49.70	42.58	0.33
	Kernel plumpness (%)	41.00	100.00	91.77	0.62
	Barley color (Agtron)	96.00	43.00	46.53	0.56
	Malt extract (%)	77.80	78.10	77.51	0.13
	Wort color	2.00	2.60	2.13	0.03
	Barley protein (%)	11.90	13.90	12.78	0.09
	Wort protein (%)	3.64	5.07	4.51	0.06
	S/T (%)	32.20	36.40	36.78	0.45
	DP (°ASBC)	98.00	204.00	137.02	2.08
	AA (20°DU)	52.10	122.40	77.09	1.77
	BG (ppm)	677.00	421.00	361.25	13.15
	FAN (ppm)	172.00	245.00	179.29	3.58
	Quality score	29.00	42.00	38.84	0.94
	Overall rank	199.00	53.00	75.95	3.70
Sensory	Beer color (-4 – +4 scale)	-1.13*	-0.50*	-0.59	0.06
	Cereal flavor (-4 – +4 scale)	0.88*	0.75*	0.49	0.03
	Malty flavor (-4 – +4 scale)	0.38*	-0.13*	0.21	0.02
	Honey flavor (-4 – +4 scale)	0.38*	0.57*	0.41	0.02
	Grassy flavor (-4 – +4 scale)	0.75*	0.25*	0.80	0.03
	Toasted flavor (-4 – +4 scale)	-0.25*	0.00*	-0.04	0.02
Metabolites	2-methoxy-4-vinylphenol (a.u.)	3,745,686.15*	4,826,833.96*	3,513,127.46	62,018.71
	Acetic acid, 2-phenylethyl ester (a.u.)	279,516,231.50*	643,562,567.60*	790,741,728.85	19,694,988.91
	Linalool (a.u.)	6,103,479.69*	5,183,004.04*	4,152,971.83	94,007.80
	Ethyl hexonate-like (a.u.)	76,978.45*	21,639.55*	35,891.96	2,214.40
	Oxalic acid dibutyl ester (a.u.)	23,780,778.51*	21,752,774.06*	23,260,970.58	448,241.33

Sayre-Chavez, et. al, Journal of Cereal Science, 2022

Malt quality

- ○ Under-modified
- ○ QTLs for 14 out of 15 malt quality traits

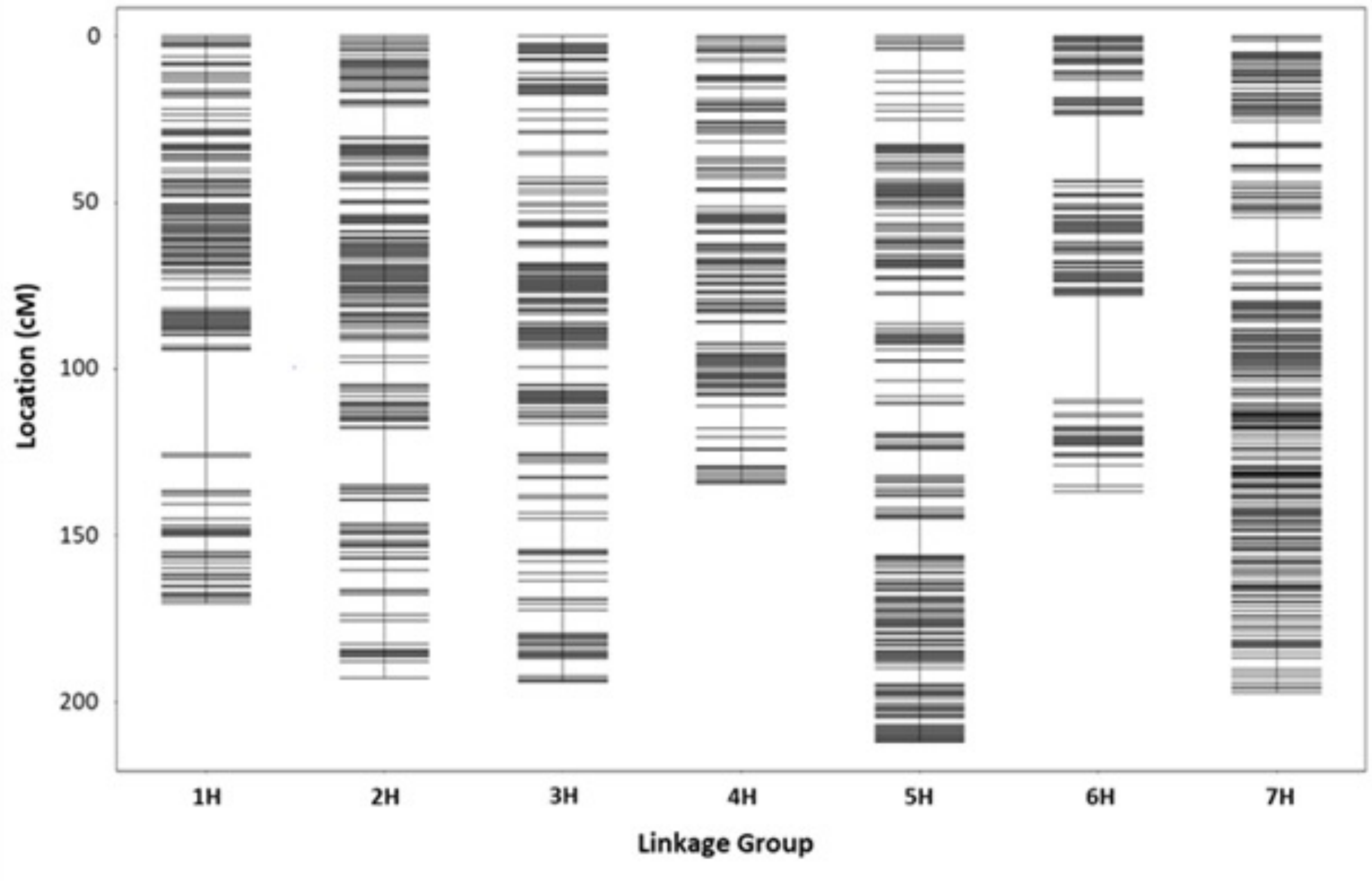
Sensory

- ○ QTLs for 6 out of 10 sensory attributes

Metabolites

- ○ 144 compounds annotated as metabolites
- ○ QTLs detected for **5** metabolites:
  - 2-methoxy-4-vinylphenol (MVP)
  - Acetic acid, 2-phenylethyl ester (PEA)
  - Linalool (LOO)
  - Ethyl hexonate-like (EHEXL)
  - Oxalic acid dibutyl ester (DBOA)

Single Nucleotide Polymorphism Party



12,453 SNPs (sequence variations) mapped across 7 chromosomes

Chr	1H	2H	3H	4H	5H	6H	7H	Total
Markers	1,512	2,284	1,487	1,310	2,145	1,492	2,223	12,453
Bins	143	174	151	128	194	102	181	1,073
cM	170.63	190.12	193.94	134.77	212.60	135.92	183.79	1,221.76

Sayre-Chavez, et. al, Journal of Cereal Science, 2022

Take Homes

Oregon Promise (selection from the mapping population) has "favorable" alleles and top rated for lager flavor by consumer sensory panel.

Potential for **clear signals** for the plant breeder - Rheostats driving more subtle quantitative variation where otherwise allelic variation = extremes (dormancy vs. pre-harvest sprouting).

Clear signals to maltsters and brewer - Mileage in manipulating modification in a spectrum of barley varieties.

Power of QTL mapping – coincidence validates correlation.
It is worth getting beyond the confines of Golden Promise and Full Pint…but $$$

The sensory and metabolite data sets are anchored in the malting quality data set

Unmalted barleys do not display notable flavor or aroma differences: it is the malting process that leads to these differences. Therefore, an analysis of the contributions of barley genotype to beer flavor is inextricably confounded by the style of malt, and how each genotype responds to the malting protocol used to make the malts.

Conclusions

Re-thinking "many barleys are called, but few are chosen"-PH

- Farmers love productive varieties
  - Storage a constant issue
  - How profitable is it?
  - Craft-malt and local supply chain: small %, but possible outlet for interesting, locally adapted varieties

Need a **high throughput pipeline** for assessment of barley contributions to beer flavor

- Automated micro-malting and metabolomics
- Markers based on
  - Hot steep sensory?
  - Nano-brew sensory?

Conclusions

Barley does contribute to beer flavor, via malt – how does climate change impact this?

If modification drives flavor and climate change forces maltsters to accept higher grain protein malt (among other things) → new or changing flavors?

Thank you!

Colorado State University:
Brooke Sayre-Chavez, without whom this work would not exist!
María Muñoz-Amatriaín
Adam Heuberger
Corey D. Broeckling
Amy Sheflin
Linxing Yao

Oregon State University
Patrick Hayes
Scott Fisk
Sarah Windes
Laura Helgerson
Tanya Filichkin
Javier Hernandez
Campbell Patrick Morrissy, Duke of pFriem

Estación Experimental Aula Dei
Luis Cistué

Rahr Malting Co.
Sean Tynan
Patricia Aron (Kalsec)

Funding:
Oregon State University Agricultural Research Foundation Barley Progress Fund

American Malting Barley Association


Q
&
A