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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

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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-malter. 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
Panelist	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 Panelist	0.005	0.504	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	0.55	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

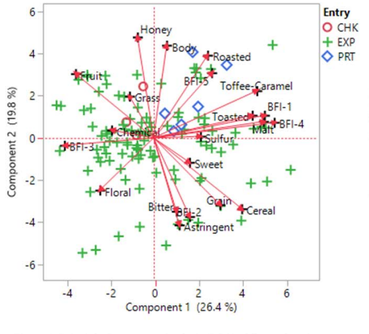

Figure 1. Principle Component Analysis (PCA) of flavor descriptors and barley flavor intensities (BFI) BLUPs against entries (EXP), parents (PRT), and checks (CHK).

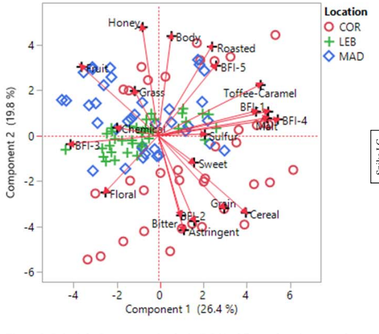

Figure 2. Principle Component Analysis (PCA) of flavor descriptors and barley flavor intensities (BFI) BLUPs against testing environment (COR=Corvallis, OR; LEB=Lebanon, OR; MAD=Madras, OR).

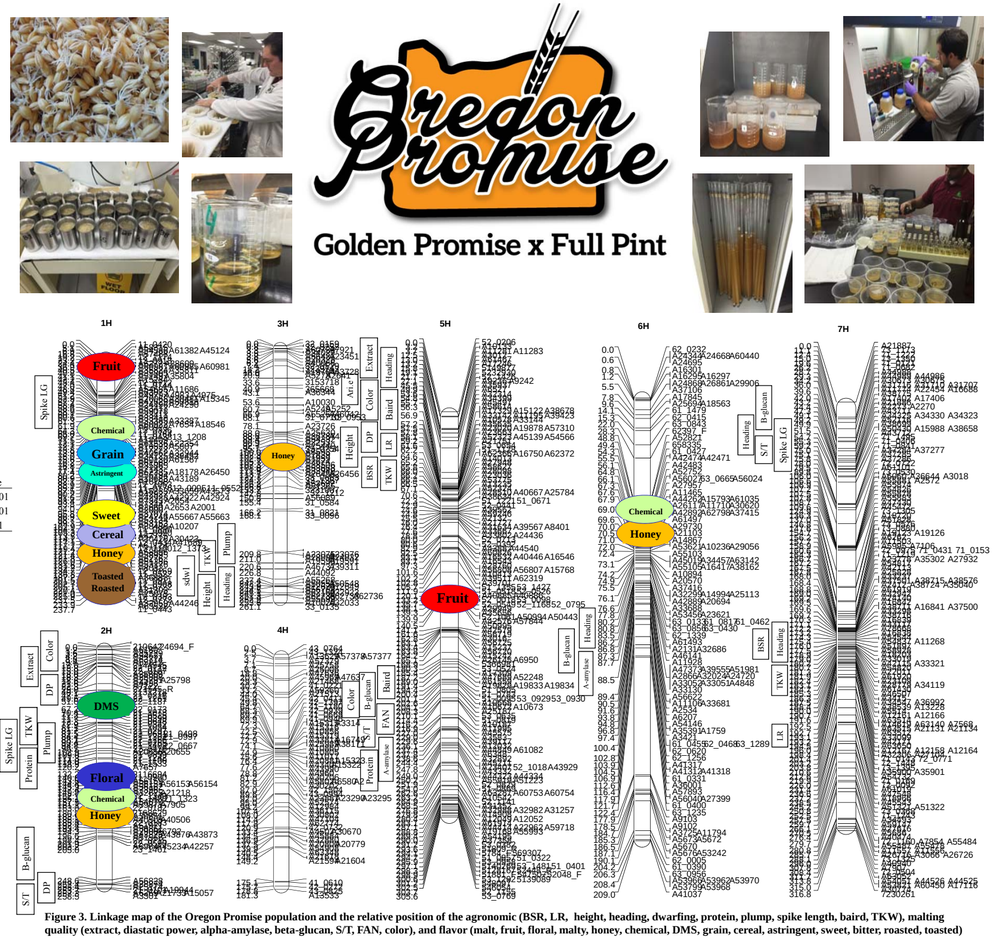

Figure 3. Linkage map of the Oregon Promise population and the relative position of the agronomic (BSR, LR, height, heading, dwarfing, protein, plump, spike length, baird, TKW), malting quality (extract, diastatic power, alpha-amylase, beta-glucan, S/T, FAN, color), and flavor (malt, fruit, floral, malty, 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.

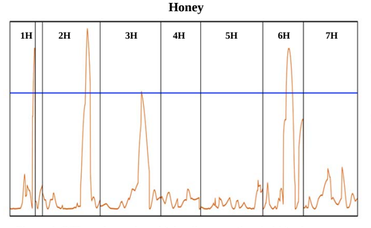

Figure 4. QTL peak graph and false discovery threshold for honey.

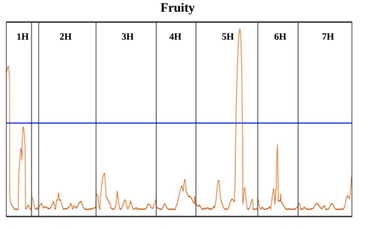

Figure 5. QTL peak graph and false discovery threshold for fruity.

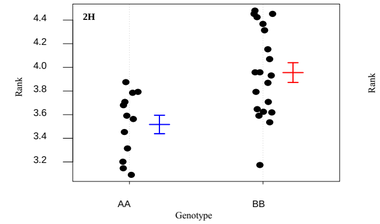

Figure 6. Allelic effect plot for significant markers controlling the honey QTL. Genotype AA is the paternal allele (Full Pint) and BB is the maternal allele (Golden Promise).

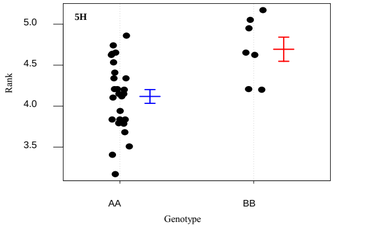

Figure 7. Allelic effect plot for significant markers controlling fruity QTL. Genotype AA is the paternal allele (Full Pint) and BB is the maternal allele (Golden Promise).

Acknowledgements

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