Project Title:
Validation of molecular markers associated with heat and water deficit adaptation in hop

Project Summary:

Selecting for tolerance to heat and water stress is a key objective in hop breeding, and wild hop adapted to variable climates may serve as an important source of genetic variation. In our recent analysis using environmental genome wide association study (eGWAS) we identified markers associated with environmental or climate variables in a collection of wild hop plants from 264 unique collection locations. The eGWAS can be a useful approach that predicts performance of germplasm based on the likely environmental variables encountered at the collection source. By applying this information to our breeding program, we were able to rank our germplasm by "most" and "least" adapted to heat and precipitation variables. Prior to applying these markers as a selection tool in breeding on a routine basis, we first need to validate that the method properly identifies genotypes that offer an advantage under heat and water deficit conditions. Thus, we propose exposing five of each of the "most" and "least" adapted genotypes to extreme temperatures in a controlled-environment growth chamber study, and under water deficit conditions in the seedling yard in Prosser, WA. The findings will provide insight into whether these markers may be effective for application in a genomic prediction model to improve heat and water deficit tolerance in hop.

Proposed Duration:
New proposal – 1 year

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Amount Requested:
$27,272

Other Prospective Funding Sources and Support:
NA

Send Funding To:
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24106 N Bunn Road, Prosser, WA 99350

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Statement of Problem:

Hop can be susceptible to abiotic stress, including water deficit and high temperatures (Eriksen et al., 2020) which have been shown to result in decreased yields (Gonzalez, 2025) (Nakawuka et al., 2017) (Gent et al., 2022) and quality issues (Mozny et al., 2023). Selection for tolerance to abiotic stressors in hop breeding will be important, especially as extreme heat events and interruptions to irrigation water availability in the Pacific Northwest growing region are expected to increase in frequency (Vano et al., 2010). Breeding for tolerance to these stressors first requires knowledge of the existing variation within hop germplasm, ideally across a large collection of parental material. Our previous work utilized an eGWAS approach to survey a collection of wild hop for beneficial alleles associated with variations in the environments (temperature and precipitation) from which they were collected. Before our program invests in selecting on these markers in a routine genomic selection scheme, the findings require validation under controlled-environment and real-world conditions to ensure the markers result in meaningful differences in plant performance under heat and water stress.

Justification and Importance of Proposed Research:

To make progress in breeding for improved tolerance to abiotic stress, breeders must first know which parents or germplasm pools offer advantageous alleles for these traits. So far, only a handful of female hop cultivars have been evaluated (Eriksen et al., 2020; Eriksen et al., 2021), which is helpful, but given the small sample size, the complexity of the traits, and the absence of knowledge of male genotypes, more research is needed to inform large-scale selection efforts for these traits. Studies like those conducted by Eriksen et al. (2020; 2021) or even field-based evaluations of different varieties to water deficit (i.e. Gent et al., 2022; Nakawuka et al., 2017) can be difficult, if not impossible to apply to large populations as would be required to identify beneficial parental material. One alternative approach is to utilize environmental genome-wide association study, or eGWAS on wild plants.

Plants, being sessile organisms, are required to adapt to their environment (and cannot escape), and over time, advantageous alleles increase in frequency as successful individuals reproduce more, and populations across geographical and climatic regions become increasingly distinct. eGWAS harnesses these differences by associating genetic marker data with average environmental variables (e.g. maximum temperature, precipitation, to name a few) at the collection site. We utilized this approach in our previous work with hop plants collected from 264 unique sites. We identified 46 markers across the hop genome associated with variation in environmental variables. Using this data, we determined which allele state at each marker offered an advantage, or was increased in frequency, in populations from hot and dry conditions. We then transferred this knowledge of marker location and advantageous alleles to our germplasm collection and ranked our germplasm by “most” and “least” adapted based on the proportion of sites that exhibited the beneficial alleles. Validation is required as a next step to leverage this largely theoretical approach into an applied breeding scheme. Furthermore, by utilizing the results from the eGWAS, we hope to narrow down our search to genotypes that are more likely to exhibit variation for abiotic stress tolerance rather than a relatively blind, large-scale, and time-consuming evaluation effort. If these markers we identified offer advantages under real-world settings, they could be readily implemented into a routine genomic prediction pipeline, allowing breeders to select for abiotic stress tolerance at the same time as other traits such as disease resistance or alpha acids content. Furthermore, existing cultivars for which sequence data exists could be evaluated for the proportion of advantageous alleles they carry. All data will be made public upon completion of the manuscript (previous work in progress) and from the proposed experiment.

Objectives:

Determine whether hop genotypes with a high proportion of adaptive alleles to high temperatures and low precipitation, as identified in our previous work, offer an advantage in performance compared with individuals with a low proportion of adaptive alleles:

1) under high temperature conditions using a controlled environment growth chamber study; and
2) under water deficit conditions in a field setting.

Methods:

Objective 1:

1) Select five genotypes with a high proportion of adaptive alleles (Figure 1; 0.6 or greater), and five with a low proportion (0.4 or less) from our germplasm collection. Dig rhizomes, propagate via softwood cuttings, up-pot into 11.4 cm pots (15 per tray). Approximately fifteen plants of each genotype will be propagated for each treatment, with overage to allow for selection for uniformity once established.
2) Apply heat treatments using two Percival PGC-15 chambers with extended temperature capabilities. Previous work has determined, across all cultivars tested in their study, that photosynthesis begins to show signs of decline in hop at and above 41 deg C with optimal performance between 21 – 39 deg C (Eriksen et al., 2020), as such, the four treatments will include 28, 35, 42, and 49 deg C for 16 hr days with night-time temperatures decreasing to 12, 15, 21, and 26 deg C across all treatments for seven days. The first two treatments (max temperature 83 and 95 F) correspond to average daily temperatures in June and July in the Yakima Valley. The third treatment (max temperature 108 F) corresponds to common extreme heat events that occur in July and August. The fourth treatment (max temperature 120 F) expose plants to record breaking temperatures encountered by hops in this region. Plants of each genotype will be randomized within 10 flats per chamber, and sub-

irrigated in trays to maintain consistent water and nutrient availability throughout the treatments. Practice runs and a series of optimization attempts will be required to ensure growth chamber functionality, and stress response at the listed temperatures and durations, to help avoid the need for extensive contingency plans.

3) 3-4 leaves per plant will be measured using a Li-600 (LI-COR, Lincoln, NE) stomatal conductance and chlorophyll florescence meter designed for high throughput screening experiments in the context of plant breeding after 3 days of heat treatment, or Li-6800, depending on availability. Before and after treatments, the number of nodes and living leaves will be counted and potted plants will be imaged using the HopBox (Altendorf et al., 2023) imaging platform, and greenness values will be extracted from the images. Finally, aboveground biomass will be collected from each plant from each treatment. The experiment will be conducted in two growth chambers simultaneously, one treatment temperature per growth chamber (referred to as trial). The trials will be run twice (two reps per temperature treatment).
4) Due to the nature of the design (10 reps and 10 genotypes per trial) a Latin Square design will be employed within each growth chamber. This classical approach allows modelling of two directional environmental gradients (row and column). Genotypic differences within each treatment can be modelled simply by including row and column as random effects and the genotype as the fixed effect. Differences among temperature treatments are modelled through the incorporation of “trial.” Here, trial and trial by temperature interaction will be included as random effects along with row and column nested within trial. Estimated marginal means will then be calculated for each response variable in terms of genotypic performance within and among temperatures.

Objective 2:

1) Select five genotypes with a high proportion of adaptive alleles (Figure 1; 0.6 or greater), and five with a low proportion (0.4 or less) from our germplasm collection. Dig rhizomes, propagate via softwood cuttings, up-pot into 11.4 cm pots (15 per tray). Approximately fifteen plants of each genotype will be propagated for each replication, with overage to allow for selection for uniformity once established. Depending on snowpack and predicted irrigation shortages on the Roza Irrigation, the experiment may require water transport to the field site to establish the plants during a shut down. Our team is equipped to provide this on an as-needed basis, assuming staffing is not affected for reasons beyond our control.
2) Seedlings will be transplanted into the seedling yard (8’ height, 10’ spacing) at the Irrigated Agriculture Research and Extension Center in Prosser, WA using a randomized complete block design with 4 blocks and 6 plants per genotype per block. The seedling block was chosen due to ease of maintenance and because yield is not a trait of interest at this stage and would be more appropriate in follow-up study on fewer individuals. Maintain with standard 0.53 gph 24” Netafim drip tube for 1.5 months, until the plants are established and climbing, or until

approximately half-way up the string. Implement water deficit by replacing drip tube with solid tube for half of each block. Each block and treatment will be monitored for soil moisture using Zentra loggers.

3) Soil moisture will be continuously monitored. This data along with climatic variables from the WSU Ag Weather Net station are Roza will be fed into a hop specific model provided by the WSU Irrigation Scheduler. This model predicts the severity of drought stress and the likely impact on cone yield. The optimum treatment will be held at high soil moisture. The stress treatment will hold the moisture level between the "first stress" and "dead" water contents. These two benchmarks are automatically calculated by the Irrigation Scheduler model. Once soil moisture drops between "first stress" and "dead" water contents in the deficit plots, 3-4 leaves per plant will be measured using a Li-600 (LI-COR, Lincoln, NE) stomatal conductance and chlorophyll florescence meter designed for high throughput screening experiments in the context of plant.
4) This analysis will determine the longitudinal impact of water deficit over time. A mixed model will include genotype, time, and genotype x time interaction as fixed effects. Block and block by genotype interaction will be included as random effects. Estimated marginal trends will be explored to determine whether there are linear or quadratic trends in the data. If quadratic trends are detected, segmented regression will be employed to determine at what point in time gas exchange ceases (stomatal closure and plant senescence). In either case (linear or quadratic), model coefficients will be used along with their standard errors to determine differences among genotypes and drought treatments.

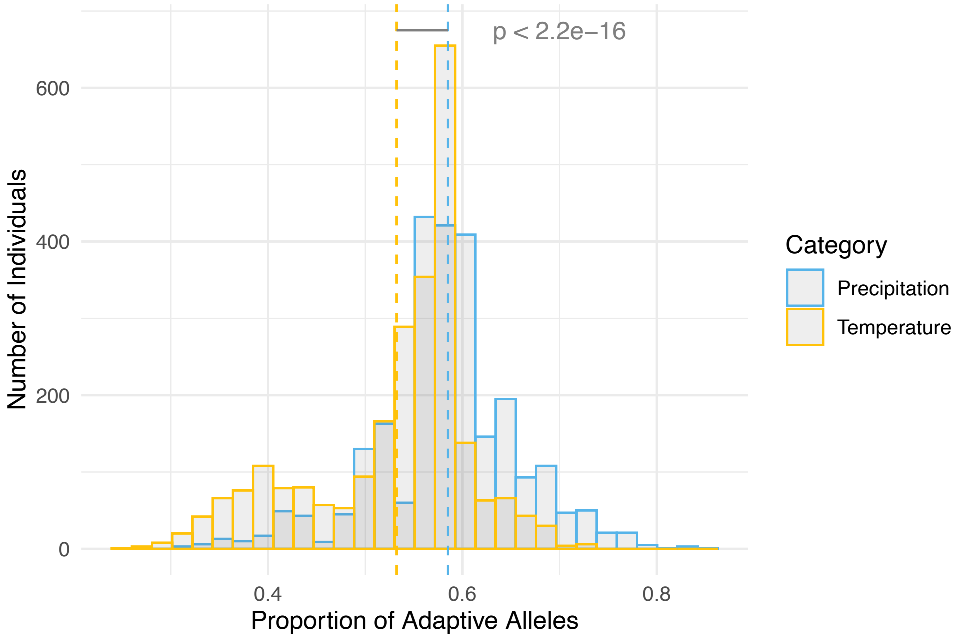

Figure 1. Distribution of hop germplasm in our collection and their proportion of adaptive or advantageous alleles for precipitation (blue) and temperature variables (yellow) (Altendorf & Havill, et al., in preparation).

Outcomes:
Objective 1: Dataset outlining performance of ten hop genotypes under temperature stress, knowledge of whether genotypes with high proportions of adaptive alleles as identified in our previous work perform better (i.e. higher greenness values, superior values for stomatal conductance, chlorophyll florescence) under heat stress.

Objective 2: Dataset outlining field performance of ten hop genotypes under water deficit stress, knowledge of whether genotypes with high proportions of adaptive alleles as identified in our previous work perform better (i.e. superior values for stomatal conductance, chlorophyl fluorescence) water stress. This experiment also provides an opportunity to validate the WSU crop model that can be used by hop growers.

If not already publicly available, genotypes used in this study can be made available via breeding material transfer agreement and eventual germplasm release if the results warrant it.

Extension and Outreach Activities:
Results will be communicated through a peer-reviewed publication, and bi-annual presentations at Hop Research Council Meetings.

Time Frame for Objectives: Rhizomes will be dug in December of 2025 and propagation will begin in January of 2026. Growth chamber trial runs will commence in March 2026 with the experiment taking place in April-June 2026. Irrigation deficit experiment will begin in May 2026 with transplanting, and treatments will be applied in July 2026. Data will be collected in late-July or early August. Data will be analyzed and published in fall 2026 and into winter 2027.

Project Budget and Other Funding Sources and Support:

Expenditure:	**Description:**	**Amount:**
Salaries	To dig rhizomes, propagate and maintain plants, assist with data collection, monitor and maintain experiment, maintain and install sensor equipment, clean up, plant removal, field maintenance. 20 hrs per week, for 8 months at $24/hr.	$15,360
Employee Benefits	45% of salary	$6,912
Other	Pots, flats, fertilizer, string, clips, potting soil, bi-weekly beneficial insects to maintain plants in greenhouses and	$5,000

	growth chambers, irrigation tubing and solid tubing, miscellaneous fittings.	
	Total:	$27,272

Travel to conferences will be covered by in-kind project funds. Land rent, greenhouse use, Li-COR equipment use, sensors, vehicle use, growth chamber equipment and installation, facilities use, project supervision, all provided in-kind.

Literature Review:

Extreme heat events and interruptions to irrigation water availability due to reduced or early run-off of snowpack are expected to increase in frequency in the Pacific Northwest where most U.S. hop production takes place (Salathé et al., 2010) (Vano et al., 2010) (USDA-NASS, 2024). The year 2015 was the worst drought year on record for the Roza Irrigation District, which services a large portion of the Yakima Valley hop growing region (WSDA, 2017). This year, 2025, is slated to be worse yet for the Roza supply, especially for users with junior rights. 2025 is also the third consecutive drought year in a row, with users receiving only a portion of their allotment, as well as week-long or more shut offs to save water for later in the season. Water deficit has been repeatedly found to be associated with reductions in yield in hop, whether it takes place early or late in the growing season, but results are somewhat dependent on variety (Gonzalez et al., 2025; Nakawuka et al., 2017; Gent et al., 2022). Modeling work from Germany, Czech Republic, and Slovenia demonstrated a predicted decline in hop yield and alpha content with increased temperatures (Mozny et al., 2023). Elevated temperatures, water-stress, and the combination of the two stressors resulted in substantial changes to gene expression in hop, and limitations to carbon assimilation and to photosynthesis when evaluated under controlled conditions, especially above 41 deg C (Eriksen et al., 2021).

Breeding for increased tolerance to abiotic stress such as heat and water stress is one important way to mitigate the impacts of these factors on hop production. However, there is very limited information in the hop literature about where to begin for parental selection to meet these objectives. A few studies have evaluated small numbers of genotypes (females) in controlled environment studies (Eriksen et al., 2020; Eriksen et al., 2021; Gent et al., 2020; Nakwuka et al., 2017), but the information is inadequate for initiating large scale breeding efforts. A common way to identify promising parental material for breeding is to conduct large scale phenotyping projects to survey hundreds or thousands of genotypes for promising traits. Due to the complex nature of abiotic stress tolerance traits and the difficulty in phenotyping large experiments, many researchers have opted to utilize eGWAS as a more efficient method to narrow down the search by identifying germplasm that is more likely to carry advantageous alleles for abiotic stress traits (Ferrero-Serrano and Assmann, 2019). eGWAS has been used in cranberry (Neyhart et al., 2022) and rice (Wang et al. 2023), among other species. eGWAS studies are generally used to generate hypotheses (Lasky et al., 2023) and as is the case in the hop study, where results require validation and further study before implementing the markers into a breeding program. This research seeks to determine the reliability of the markers in an applied setting.

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