

An Affordable & Convenient Diagnostic Marker to Identify Male and Female Hop Plants - *a major boost for hop breeding efficiency using PACE genotyping*

Discover how researchers from the National Clonal Germplasm Repository, OR and Forage Seed and Cereal Research Unit, WA of the USDA-ARS, in collaboration with the University of Minnesota and Hudson Alpha Institute for Biotechnology, have developed an accurate, low-cost, and highly scalable diagnostic marker, poised to significantly enhance efficiency in hop breeding.

Hops: A Multi-Purpose Crop for Brewing and Beyond

Hops (*Humulus lupulus* L.) are a crucial ingredient in beer production, belonging to the Cannabaceae family. They contribute aroma, bitterness, and preservatives to the final beer product. The US, primarily the Pacific Northwest (Washington, Idaho, and Oregon), is the largest producer of hops globally (45% in 2022), with three of the five subspecies native to North America.

Hops plants are perennial, wind-pollinated, possess a diploid genome, and are dioecious – producing separate male and female plants. In commercial hop production, cones from female plants are exclusively utilised, while male plants contribute to generating novel variations in breeding programs through crossing. Currently, hop lacks a rapid and accurate diagnostic marker to determine a plant's sex, hampering breeding programs by relying on time-consuming visual observations alone.



Hop seedlings. Image courtesy of Kayla Altendorf



Female hop plants. Image courtesy of Ninh Khuu

The absence of a diagnostic marker can extend breeding programs by 1-2 years to determine the sex of new seedlings. Understanding seedling sex earlier in the breeding cycle would facilitate more efficient breeding strategies and selections.

Previous research on sex-linked markers has been limited to specific populations or breeding programs, with hindered progress due to restricted transferability and low scalability. Recent advances and reduced costs in long-read genome sequencing and assembly now make assembling XY pairs in complex species with large genomes, such as hops, feasible expanding the possibilities for genomic study.

The study

A diverse collection of 765 male and female hop genotypes and 20,861 biallelic SNPs was assembled for GBS and GWAS studies. Association mapping revealed a highly significant SNP marker on the sex chromosome predicting sex within the study population with 96% accuracy. The SNP is in a gene predicted to encode the basic helix-loop-helix transcription factor protein; a family of proteins previously implicated in male sterility in a variety of plant species.

The team developed a PACE genotyping assay for the diagnostic SNP, testing three quick extraction methodologies for a rapid, high-throughput genotyping workflow. The marker was validated in a separate population of 94 individual male and female seedlings from 15 independent crosses in the USDA-ARS hop breeding program, achieving 96% accuracy.



Sampling hop seedlings for screening. Image courtesy of Kayla Altendorf

"The marker was applied in the USDA-ARS hop breeding program in Prosser, WA in 2023 and enabled 25% increase in the ratio of female: male seedlings evaluated in the same field space as the previous year, and resulted in a 97.4% female yard."

Reference: Clare, Shaun J., Ryan M. King, Anna L. Tawril, Joshua S. Havill, Gary J. Muehlbauer, Sarah B. Carey, Alex Harkess, Nahla Bassil, and Kayla R. Altendorf. "An affordable and convenient diagnostic marker to identify male and female hop plants." *G3: Genes, Genomes, Genetics* (2023): jkad216.



Hop yard growing nearly all female first-year seedlings. Image courtesy of Ninh Khuu

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The methodology is widely accessible to breeders, combining rapid, in-house hot-shot DNA extraction and a marker assay utilising low-cost reagents, alongside equipment and instruments commonly found in molecular laboratories. It will allow hop breeding programs to boost efficiency (saving time, labour, money, and space) for earlier cultivar releases. It will also enhance genetic gains to counter challenges such as climate change, diseases, and addressing changing consumer trends, by liberating resources to other aspects of hop breeding programs.

This new marker is diagnostic, accurate, affordable, and highly scalable, with the potential to dramatically improve efficiency in hop breeding. It is also a compelling illustration of the benefit of Marker-Assisted-Selection (MAS) techniques for hop breeding.