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Categorization and Quantification of Hop Aroma for Targeted Aroma Breeding

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ABSTRACT

The changing climate has profound implications for economically important hop varieties, causing decreased yields and compromised substantial differentiation between the hop phenotypes. PCA, based on rat-ity. The current study addressed these concerns by conducting a coming across various categories, provided valuable insights into the diverse prehensive sensory evaluation of 153 hop genotypes using hop teas as primary aroma characteristics displayed by different hop genotypes. a representative medium. The objective was to investigate the potential connection between hop genotype primary aroma characteristics. A trained panel analyzed hop teas, and the resulting data were analyzed via principal component analysis (PCA) to uncover patterns and relation-

Keywords: climate change, hop aroma, hops, sensory, targeted aroma breeding

Introduction

Hops are essential for beer production, providing secondary plant compounds necessary for flavor and aroma. Climate change-related factors, such as increased temperatures, precipitation pattern changes, and extreme weather events, are affecting hop yields and quality, and chemical composition. This has significant implications for both the hop industry, the taste of beer, and fluctuations in the availability and cost of specific hop varieties. To address this issue, breeders must develop climate-adapted hop varieties capable of maintaining high quality despite changing weather conditions. A deeper understanding of hop aroma expression is critical for targeted breeding efforts. This article highlights the importance of studying hop aroma expression for breeding purposes and stresses the need to develop climate-adapted hop varieties to ensure the continued supply of high-quality hops in the future.

Breeding plants that can withstand the challenges of a changing climate is critical to ensuring food security and economic stability (6). This is particularly important for hop breeding, as hop plants are highly sensitive to temperature and precipitation changes (5). Small shifts in weather patterns can have significant impacts on hop yields and quality, making it essential to develop new hop varieties that are more resilient to environmental stresses such as drought, heat, and extreme weather events. Targeted breeding involves selecting plants with desirable traits such as disease resistance and drought and heat tolerance. By developing more resilient hop varieties, breeders can ensure the long-term viability of the hop industry and the quality of the beers made from them.

To ensure newly developed hop varieties are both climate resilient and desirable to brewers, plant breeders must target agronomic improvement in conjunction with aroma selection. Targeted aroma breeding is a strategy that utilizes both sensory evaluation and genetic analysis to create new plant varieties with desired aroma profiles. Genetic analysis utilizes genome-wide association study (GWAS) to identify genetic markers linked to the aroma trait of interest, which is then confirmed by sensory panels evaluating the aroma profiles of plant varieties (2). This method can produce plants with diverse aroma profiles, including those with high levels of specific flavor compounds or complex flavor profiles (1).

To further optimize the breeding process, genomic selection (GS) can be used on immature plants, as well as on male plants—where the trait is not visible—to specifically identify aroma traits and improve breeding efficiency (3). GS predicts the breeding value of plants using genomic data, allowing breeders to select plants with desirable traits before they reach maturity and without sensory evaluation. This approach can significantly reduce the time and cost involved in plant breeding (4).

By utilizing both GWAS and GS, breeders can predict aroma profiles of plants and accelerate the targeted aroma breeding process, increasing efficiency in creation of new plant varieties with desired aroma profiles.

Categorization and Quantification of Hop Aroma

Hop Tea Preparation

The first step in sensory analysis of hops was to select a diverse range of hop genotypes for evaluation. A total of 153 hop genotypes were chosen, including commercial varieties (i.e., Cascade, Centennial, Perle, Herkules, CTZ, etc.), wild types, and breeding lines sourced from various countries around the world. The hop genotypes were selected based on their known sensory

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characteristics, chemical composition, and commercial significance.

To gain a better understanding of the complex aroma and flavor characteristics of hops, it is crucial to extract hop compounds in a way that mimics its performance in beer. One common method for this purpose utilizes hop tea.

Hop tea was created by steeping hops in hot water, allowing the compounds to be released into the liquid and volatile headspace. For tea preparation, 200 mL of boiling water was added to 6 g of hop pellets or cones, which is equivalent to 7.5 lb/bbl. The mixture was then steeped for 24 h in a closed container to ensure maximum extraction of hop compounds (Fig. 1). The resulting liquid was decanted, and samples of the extract were distributed to panelists for sensory evaluation.

Utilization of hop tea as a method for sensory analysis offers several advantages. It provides a consistent and standardized approach for extracting hop compounds, ensuring that each sample has a similar level of extraction efficiency. This consistency enables better comparisons between different hop varieties, facilitating identification of aroma distinctions, than the more common method of hand rubbing, such as is used during annual hop selection.

Panel Training

The recent study focused on six aroma categories: fruity, citrusy, floral, herbal/spicy, resinous, and onion/garlic. These categories were chosen because they are commonly associated with hops and have the potential to differentiate between hop varieties. To standardize the sensory evaluations, the panelists underwent a training process using aroma references from DÖHLER GmbH that covered all six aroma categories. The panelists were provided with detailed descriptions of the characteristics of each category and asked to rate the intensity and quality of each aroma in multiple rounds. Any discrepancies in the ratings were resolved through repetition to ensure agreement among the panelists (described in the following section). This facilitated consistency in the evaluations of hop genotypes, which were standardized across all panelists.

Sensory Assessment

After the completion of training and standardization, sensory evaluation of hop genotypes commenced. The hop samples were prepared as hop tea using the previously described method. Each day, a set of 15 samples was randomly selected and assessed by the panelists. The panelists evaluated samples based on aroma categories employed during the training and standardization phase. Panelists indicated whether they detected the aroma for each category and rated the samples as either present (1) or absent (0).

To ensure quality assurance, 20 samples were repeated for each category. The repetition of these samples was employed to assess the suitability of the methodology for sensory evaluation of

hop aroma. The rating scheme was designed to ensure that panelists focused specifically on the aroma reference and only cast their votes for the respective category when they were completely certain. Once the sensory evaluation was completed, the mean value for each phenotype was calculated based on the panelists' ratings.

The density distribution of different categories showed significant variations and was not normally distributed, which was expected as distribution depends on the composition of the sample set. As anticipated, the onion/garlic category had a high density of low ratings due to the selection against this aroma expression in practice, but some phenotypes were still clearly assigned to this category. In contrast, some samples scored very high in the citrusy and fruity categories, while other categories did not have scores that were as high. According to the panelists, the citrusy, fruity, and onion/garlic categories were the easiest to evaluate. The density distribution of different aroma categories for all 153 genotypes is illustrated in Figure 2.

The density distribution of sensory evaluations across all categories were analyzed for the 153 tested hop genotypes. The sensory evaluation results were used to determine the frequency and distribution of ratings within each category. By examining the density distribution, we can gain insights into the overall patterns and variations in sensory perceptions for the tested hop ge-

otypes. These findings shed light on the sensory characteristics of the hop genotypes within specific categories. The normal distribution observed in citrusy and herbal/spicy aromas suggests a broader range of sensory perceptions, potentially providing more flexibility for breeders to select desired profiles within these categories. In contrast, the polarized distribution in the garlic aroma category highlights a distinctive and dichotomous sensory perception that breeders may need to consider when developing hop varieties with specific flavor profiles.

To investigate the sensory outcomes of each phenotype's differentiation, a principal component analysis (PCA) followed by t-SNE (partition around medoids) clustering was performed. PCA helps reduce the dimensions in multivariate data sets. The principal components PC1 and PC2 describe more than 66% of the variation in the data set (Fig. 3), with the categories citrusy, fruity,

and onion/garlic having the clearest influence on the differentiation, as indicated by the arrow lengths (loadings). The clustering reveals three clear demarcations for the six categories and eight clusters: a cluster for onion/garlic and resinous and a combined cluster for citrusy and fruity. The assignment of phenotypes to clear clusters within the floral and herbal/spicy categories is somewhat limited. However, the phenotypes in the floral and

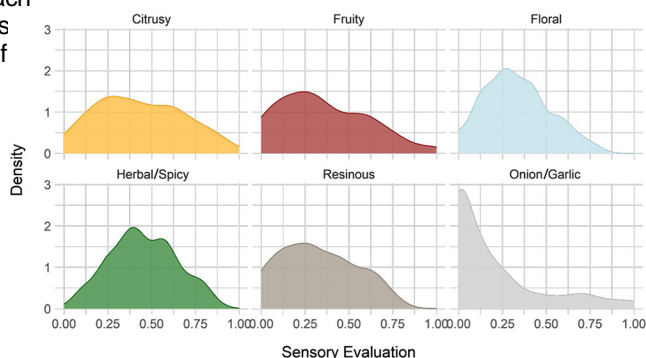


Figure 2. Density distribution of sensory evaluations of all categories of 153 tested hop genotypes.

Figure 1. Hop tea preparation.

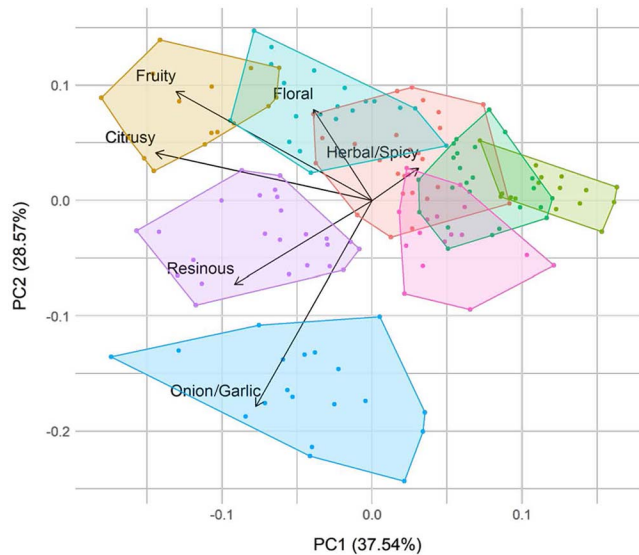


Figure 3. Principal component analysis (PCA) of sensory evaluations of each phenotype of 153 tested hop genotypes.

herbal/spicy categories can, to a limited extent, be assigned to clear clusters.

Discussion

As a preliminary or proof-of-concept study, this research highlights the importance of sensory evaluation in understanding and evaluating complex aromas of diverse hop genotypes. By conducting a comprehensive sensory analysis, breeders can utilize the insights gained to develop innovative hop varieties that align with market demands and consumer preferences. This study emphasizes the significance of sensory evaluation as a tool for introducing hop varieties that push the boundaries of traditional beer styles. Ultimately, the findings contribute valuable knowledge for the development of hop varieties with distinct aroma profiles that cater to evolving consumer preferences.

During sensory evaluation, distinct clusters emerged for certain aroma categories, such as onion/garlic and resinous, displaying clear separation and easy discernibility by the panelists. Similarly, citrusy and fruity aromas demonstrate a shared cluster, indicating some degree of similarity in their sensory profiles. The assignment of phenotypes to clear clusters becomes more challenging when considering the floral and herbal/spicy categories. The sensory characteristics within these categories exhibit greater variability and overlap, making it difficult to establish distinct clusters based solely on these attributes. Precisely categorizing samples into well-defined clusters within the floral and herbal/spicy categories posed difficulties for the panelists.

These observations suggest that the sensory evaluation of hop genotypes, particularly in relation to floral and herbal/spicy aromas, necessitates a more nuanced approach. Further research and analysis are required to better understand and differentiate the subtle differences within these categories. These insights highlight the complexity and intricacy involved in evaluating and char-

acterizing hop aromas, underscoring the need for continuous exploration and refinement in the field of sensory evaluation.

It is important to note that evaluating hop tea alone may not fully capture the entire aroma and flavor profile of hops in beer. Factors such as pH, alcohol content, yeast interactions during fermentation, and other beer-specific elements can impact the extraction of hop compounds. Thus, complementing hop tea evaluations with sensory assessments of beer is essential to obtain a comprehensive understanding of hop aromas.

Summary

The continued research on the sensory properties of hops serves as a foundation for advancing targeted aroma breeding techniques. By delving deeper into the complex world of hop aromas, researchers can uncover valuable insights that contribute to the development of new hop cultivars that align with ever-changing market demands and consumer preferences. This is crucial in an industry that is constantly seeking to push the boundaries of beer styles and flavors.

In addition, the integration of genomic selection techniques further optimizes the breeding process. By utilizing the aroma data generated from sensory evaluations, breeders can employ genomic selection in the early stages of plant development. This enables them to efficiently target specific aroma traits by predicting the breeding value of plants based on their genomic information.

The combined use of sensory evaluations, hop tea analyses, and genomic selection empowers breeders to accurately predict aroma profiles, streamline the targeted aroma breeding process, and create new hop varieties with desired aroma characteristics more efficiently. This integrated approach ensures that breeders stay at the forefront of innovation, enabling them to meet the evolving demands of the brewing industry and deliver exceptional flavors and aromas to beer enthusiasts around the world.

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