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United States Department of Agriculture
Project Initiation

Project Data for Accession Number 1030200

Project Title

Breeding for improved kernel quality characteristics in American hazelnut

Project Details

Sponsoring Institution National Institute of Food and Agriculture	**Project Status** ACTIVE
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Grant No. 2023-67013-39614	**Cumulative Award Amt.** $649,604.00
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Non Technical Summary

Hazelnuts represent a high value crop for growers and a crop with significant environmental benefits and present a unique opportunity for the Upper Midwest. American hazelnut, Corylus americana, is native to Eastern North America. It has strong tolerance to Eastern Filbert Blight, a devastating disease for European hazelnuts, C. avellana, the majority of US and global production. It is also cold hardy in the Upper Midwest. For these reasons it is both a well-adapted crop for our growers and a potential source of important traits for breeders of European hazelnut. The main drawback is its small nut size, meaning that it is primarily used for processing markets. To capture high value processing markets, cultivars with improved protein and oil composition and increased shelf life are needed. We propose to develop high throughput phenotyping methods and genomic prediction models for these traits. We will phenotype a population of American hazelnut and three interspecific crosses of American and European hazelnut for nutritional quality characteristics. We will then use hyperspectral NIR imaging to develop prediction models to reduce

the cost of acquiring this data in a breeding program. We will conduct genetic mapping for the nutritional quality traits using genome wide association analysis in the representative population of American hazelnut and F1 linkage mapping in the interspecific crosses. Finally, we will test genomic prediction models for kernel quality traits to develop tools for parental and progeny selection, which will greatly aid in selecting for these traits in a long lived perennial crop.

Animal Health Component

Animal Health Component: 0%

Research Effort Categories

Basic	0%
Applied	80%
Developmental	20%

Classification

Knowledge Area (KA)	Subject of Investigation (SOI)	Field of Science (FOS)	Percent
201	1210	1081	70%
202	1210	1081	30%

Knowledge Area

[201] Plant Genome, Genetics, and Genetic Mechanisms

[202] Plant Genetic Resources

Subject Of Investigation

[1210] Filbert

Field Of Science

[1081] Breeding

Keywords

genomic prediction, high throughput phenotyping, nutritional quality, american hazelnut

Goals / Objectives

Objective 1 Understand the phenotypic diversity of nutritional composition and kernel quality characteristics present in American hazelnut (C. americana) and interspecific crosses (C. americana xC. avellana).Objective 2 Develop high throughput phenotypic assays for assessing kernel nutritional quality traits, using results from Objective one to validate prediction models.Objective 3 Test genome wide association analysis and linkage mapping to better understand the genetic architecture of nutritional quality traits. Develop genomic prediction models for kernel and nutritional quality to be used in breeding and validate on experimental populations.

Project Methods

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Project Initiation

Objective 1Physical phenotyping: Physical kernel traits will be measured following a protocol that has been developed in our lab. In-shell nuts are weighed then imaged on a grid to determine length along two dimensions. In-shell length in the third dimension is measured by digital bluetooth calipers. Following cracking, the mass and size of kernels are measured by the same procedure. Kernels are also visually inspected for insect damage or defects.Rapid chemometric phenotyping: We will determine the moisture content, total % protein, total % oil, and fatty acid profile of kernel samples. In-husk hazelnuts are dried to 6% moisture content post-harvest then de-husked and cracked to yield kernels. Moisture content of whole kernels is determined through oven drying. Kernels from each individual plant are pooled, flash frozen with liquid nitrogen, then ground and passed through a sieve to yield particles of uniform size. A portion of the ground kernel will be submitted to UW-Madison Grassland Ecology Lab for total nitrogen combustion analysis. A separate portion of ground kernel is subjected to room temperature extraction with hexane three times to defat the kernels. The extracted oil is dried and weighed to determine the total % oil of kernels. The oil extract is diluted in deuterated chloroform and analyzed by nuclear magnetic resonance (NMR) spectroscopy to determine the proportion of oleic, linoleic, linolenic, and saturated fatty acids in the sample.Additional chemometric phenotyping: For a subset of samples, GC analysis will be conducted and compared to results from NMR analysis to confirm that the methods are comparable; while NMR spectroscopy is faster and cheaper, it is unable to differentiate between saturated fatty acids of different chain length, unlike GC-MS. We propose that NMR analysis is sufficient for characterizing the breeding population.Polyphenol analysis: Spectrophotometric methods for determining the total phenol and proanthocyandin content of nuts will be used on a subset of samples representing genetic and phenotypic diversity.Oxidative stability: When stored under ambient conditions, hazelnuts develop oxidative rancidity which decreases their value. Selected hazelnut genotypes will be stored at accelerated aging conditions to induce lipid oxidation, consistent with our prior methods in almonds. Acidity and peroxide values will be assessed by titration of extracted oil.Objective 2We will develop a model using NIR spectroscopy to predict the moisture, total protein, total oil, and fatty acid contents of hazelnut samples. While analysis of in-shell hazelnuts or whole kernels would be ideal due to minimal sample preparation, we will also investigate use of halved kernel and ground kernel samples to ensure a robust model for predicting nutrient content is developed. While the chemometric phenotyping discussed earlier would enable analysis of hundreds of samples, an NIR procedure would dramatically accelerate nutrient analysis of hazelnut samples.A subset of hazelnut samples representing genotypic and phenotypic diversity will be selected. All NIR spectra will be collected on the hyperspectral flatbed scanner available at the Wisconsin Crop Innovation Center (WCIC) in Middleton, WI. In-shell hazelnuts will be arranged in a grid and scanned. These nuts will be re-scanned as whole kernels, halved kernels, and ground kernels to assess the role of sample preparation in influencing the accuracy of prediction models. The ground kernel material will be subjected to chemometric phenotyping described in Objective 1.A prediction model for each individual trait (moisture content, total protein, total oil, oleic acid, linoleic acid, saturated fatty acids) will be constructed using a subset of the collected chemometric and NIR data, and the remaining samples will be used to validate the model. Prediction models for compositional traits will be build using the 'pls' package in R. Dependent variables will be spectral data extracted from hyperspectral images and the independent variables are the trait measurements from objective 1 made on the same kernels.We expect that several of the nutritional quality traits of importance will have acceptable predictive accuracy for their use in high-throughput phenotyping in breeding. We will assess whether predictive models built on whole kernels are adequate or whether it is necessary to grind the samples. Once trait prediction models have been constructed we will use them for high-throughput screening of additional hazelnut samples. In the last year of the project, we will collect preliminary data on the variation due to genotype by environment interactions and intra-bush differences in nutritional quality and maturity. A full analysis of GxE will be conducted once replicated plantings of the same genotypes are mature in different locations in the upper midwest.Objective 3Two approaches to quantitative genetic analysis will be used in this stage of the project using high-throughput NIR-based phenotyping, which will enable the evaluation of larger sample sizes. In particular, we will leverage two populations of mature seedlings described above, which have previously been genotyped and validated as viable germplasm for conduction both QTL mapping, and genomic prediction.The first set of populations constitutes three F1 families, descended from full sib crosses between selections from the Oregon State breeding program and the University of Minnesota breeding program. These parents are phenotypically divergent for nut quality traits, and are thus ideal populations for carrying out linkage mapping analysis in the F1 generation. Being highly heterozygous,

recombination is evident in the F1 generation, and being phenotypically distinct, the F1 population is also segregating for a number of key traits. As such these families are well- suited to QTL analysis using linkage mapping and will help identify parental haplotypes that can be selected for using marker-assisted selection in future crosses.A second population composed of 632 wild C. americana selections represents a diversity panel of the phenotypic and genotypic diversity of C. americana in Wisconsin. This population is also mature, has been genotyped, and its utility in conducting genome- wide association analysis has been validated using morphological nut traits. This population is ideal from the perspective of improving pure C. americana, as well as identifying high- performing parents to use in interspecific crosses with C. avellana. We will use GWAS to identify QTL using mixed models which can control for population structure.Both of these populations will also be used to construct genomic prediction models using stage-wise analysis. While this form of analysis does not itself constitute a method for understanding the genetic architecture of the biosynthetic pathways underlying fatty acid synthesis directly, it has been shown to represent a highly effective strategy for making genetic gains within breeding programs. Particularly for highly quantitative traits with a complex, polygenic basis, genomic prediction offers a method for estimating additive genetic value (i.e., the breeding value of seedlings), using only molecular markers. The GBS data that has already been generated for these experimental populations will be sufficient to estimate the variance-covariance matrix for random additive genetic value using the realized relationship matrix. Accuracy of predicted breeding values can be measured in a number of ways, this study will utilize both a calculation of the prediction error variance (PEV), and a cross-validation approach.