CIOA 3 – Carrot Improvement for Organic Agriculture:
Leveraging On-Farm and Below Ground Networks

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Introduction/Overview

Organic growers need vegetable varieties that are adapted to organic growing conditions and have market qualities desired by organic consumers. In carrots, weed competition, nutrient acquisition, parasitic nematodes, and disease pressure are particularly critical challenges to fresh market carrot production. The CIOA 3 project seeks to deliver carrot cultivars with improved disease and parasitic nematode resistance, improved nutrient acquisition, seedling vigor and weed competitive traits, increased marketable yield, superior nutritional value, flavor and other culinary qualities, and storage quality for organic production. New cultivars developed to organic conditions will enhance organic vegetable production and organic farmer economic returns, thus facilitating expansion of organic agriculture in the USA. Publicly available breeding lines, including germplasm with nematode and Alternaria resistance, will support organic seed industry development of additional new cultivars. The development of improved carrot varieties with greater tolerance to biotic and abiotic stress will bring broad environmental benefits as well.

Main focus areas for CIOA 3 work:
1) Cultivar and breeding population development and release
2) Expand participatory variety trial and plant breeding network
3) Investigate links between microbiomes, nutrient uptake, pathogen resistance, and root nutritional quality and storability among carrot genotypes
inform growers about cultivar performance to maximize organic carrot production, markets, and organic seed usage;
4) Utilize molecular marketers to improve nematode resistance
5) Evaluate and improve carrot flavor, texture and color

This project builds upon accomplishments of the CIOA 1 & 2 projects funded by the USDA OREI

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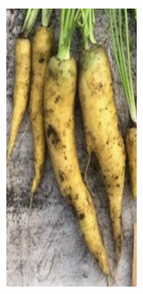
Cultivar Development

During this project, ***we are advancing all stages of the carrot breeding "pipeline" from initial screening of material to delivering finished cultivars.*** Several populations evaluated in CIOA 1 & 2 hold promising combinations of traits, including visual appeal, flavor, and agronomic potential, but they are still too phenotypically diverse for commercial release. These populations are being refined in CIOA2 with the goal of delivering elite materials for future cultivar development by the end of the project. Each year advanced lines and breeding populations are trialed at multiple University and collaborator research farms, seed companies, and organic farms across the nation.

Project Activities:

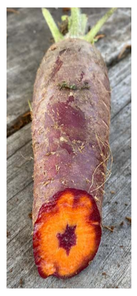
Cultivar releases – six open-pollinated and five hybrid varieties. Promotion through outreach, participatory trials and culinary evaluations.
Advance and release breeding populations – refine promising materials from CIOA 2 with the goal of publicly releasing 11 breeding populations/pools for participatory plant breeding and cultivar development.
Create new breeding populations – develop new breeding populations that combine flavor, novel visual appearance, disease, nematode and cavity spot resistance, seedling vigor and early canopy establishment, and high nutrient concentration. Breeding methods will utilize marker assisted selection, be shared with the participatory plant breeding network, and collaborative breeding efforts of project partners.
Germplasm screening and regeneration – The USDA collection includes over 700 accessions of geographically and genetically diverse cultivated carrot germplasm. Very little of this material has been evaluated under organic conditions or for traits prioritized for organic production. Project partners will screen and advance approximately 75 entries per year of this collection for use in cultivar and breeding population development.

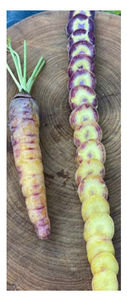

'Carnelian' released in CIOA 2


Y1246 released in CIOA 2


POr1129 to be released in CIOA 3


Novel Rainbow population




New novel populations

Participatory Trialing and Breeding Network

We are conducting on-farm trials with organic farmers and organic seed companies in five regions across the US to assess variety performance under diverse environments, solicit farmer input to inform breeding efforts, and train farmers in on-farm variety evaluation. These sites serve as a national testing network for evaluating cultivars that are ready for release and elite materials across highly diverse climates. Farmers and seed company representatives participate in evaluation of these trials to facilitate variety descriptions and recommendations for release and provide feedback on additional improvements for regi

We train farmers in carrot breeding methods by having farmers participate in root selection and on-farm plant breeding. The CIOA 3 team will train farmers in trialing methods and scale and on-farm appropriate plant breeding techniques for carrots. The goal is to empower farmers and small seed companies to steward and improve diverse carrot genetics and facilitate adoption and commercialization of new and improved carrot varieties.

Flavor

Assessment of carrot flavor is being integrated into all germplasm evaluations and breeding activities. Flavor is a priority trait necessary for the successful adoption of new cultivars with quality agronomic traits. Sensory analysis, including flavor, texture and culinary quality, is being conducted on advanced materials harvested from replicated research station trials in Wisconsin and Washington. Flavor analysis is conducted each year on all entries. A more comprehensive sensory analysis is conducted each year on a selection of entries from the research station trials in Wisconsin and Washington.

Selection for carrot flavor will be exercised in promising breeding lines. Organoleptic evaluation of the trial entries is being performed on all selected roots, scoring them on a 1-5 scale for sweetness (from not sweet to very sweet), harshness (mild to harsh or turpentiney) and texture (dry or tough to juicy). Selected carrots are being used for subsequent seed production, aiming to select a set of the 12 most promising lines. Between 900 and 1,000 breeding populations are being evaluated for flavor in 2018. Project collaborators receive training to perform flavor analysis and secondary flavor evaluation is being conducted for the selected lines in both Wisconsin and Washington.

A mixed model analysis of variance will be used to analyze flavor data. With taster and year considered random effects, and variety, location, and treatment considered fixed. Using the least squared means generated for the variety:location:treatment interaction through the mixed model ANOVA a principal component analysis will be performed to visualize the relationship among varieties using the entire quality profile. These evaluations will be used to produce a consensus map based on their comments and preference ratings to rate flavor of advanced carrot breeding populations in CIOA 3.

Root Microbiome Research

Plants are literally covered, inside and out, with a diverse assortment of microorganisms that are now commonly referred to as a plant's microbiome, or 2nd genome. Some of these microbes are pathogens that can negatively influence plant and human health, and reduce the quality and storage potential of produce. In contrast, other microbes can benefit plants by helping them acquire resources, withstand biotic and abiotic stress, and improve the nutritional quality and safety of produce. Consequently, understanding how to manage plant microbiomes has become an important area of research. Soils are generally the most important factor shaping the composition and activity of plant microbiomes so taking steps to promote soil health is critical. However, crop genotype has also been demonstrated to play a smaller, yet significant role in structuring plant microbiomes indicating that it may be possible to leverage the benefits of healthy soils by selecting for beneficial plant-soil-microbial relationships in breeding programs (Hoagland, 2015). For example, we have demonstrated that carrot genotypes differ in their potential to alter the composition and activity of soil and plant-associated microbes that make nutrients available for plant uptake, and help carrots withstand assault by pathogenic fungi and nematodes (Martin, 2016; Abdelrazek et al., 2020a; Abdelrazek et al., 2020b; Meija, 2020; Triviño, 2021;). We have also determined that carrot genotypes vary in their potential to accumulate toxic heavy metals such as cadmium (Cd) (Santos, 2019), and that the composition of arbuscular mycorrhizal fungal communities can influence Cd uptake (Komanapalli, 2021), indicating that these could be related. During CIOA3 we will follow up on these studies to determine whether these genetic differences hold up under diverse field environments. We will also determine how differences in the potential for carrot genotypes to recruit and host endophytic microbes can influence the nutritional quality and storability of carrot taproots. **This will test whether root exudates and rhizosphere communities are associated with carrot pigmentation, sugar accumulation, and/or nematode resistance as well as determining whether root-exudate associated rhizosphere microbiome recruitment influences carrot quality traits such as storability, nutritional quality, and flavor.**

Molecular Markers for Nematode Resistance

The molecular marker work in CIOA 1 and 2 resulted in the public release of five nematode resistant carrot populations and identified genes that confer nematode resistance.
CIOA 3 will build upon this success by utilizing these molecular markers to confirm the identity of resistance genes segregating breeding populations, derived from the populations developed in CIOA 1 and 2, and utilize those markers to select parental roots with resistance alleles from multiple genes to assure strong resistance during the breeding process. New and advanced populations with nematode resistant parentage will be evaluated and selected each year.

To accomplish our research aims, we are evaluating a diverse set of carrot genotypes in greenhouse trials and using organic substrates labeled with 15N to track mineralization and uptake of N, ICP-MS to quantify heavy metal uptake, and 16S/ITS amplicon sequencing to quantify root microbiomes. We are also working with engineers to identify spectral images that can detect heavy metal contamination in carrot foliage.

Outcomes and Impacts from CIOA 1 & 2

- More than 45 breeding populations advanced with combinations of priority traits
- New source of norther root-knot nematode resistance discovered in a purple CIOA breeding line from Syria
- Public release of three new open-pollinated varieties – two red and one yellow
- Public release of six nematode resistant breeding lines – five orange and one purple-orange
- Established extensive trialing and breeding network spanning 11 states, 4 commercial seed companies, 6 universities, and over a dozen farms
- Identified molecular nematode resistance - major *M.* hapla resistance gene and resistance traits *M. hapla, M. incognita, and M. javanica*
- Over a dozen and a half research updates, articles, webinars and project events published on the eOrganic CIOA website ( )
- 14 manuscripts published, including six graduate theses and one undergraduate thesis
- Numerous public field days and outreach events in WA, CA, IN, WI, and ME