BG-201

Paternity Analysis with Microsatellites in Carrot Polycross Breeding

Sabine K. Clausen and Søren K. Rasmussen
Department of Plant and Environmental Science, University of Copenhagen, Thorvaldsensvej 40, 1871 Frederiksberg C, Denmark

This project aims to develop new varieties of carrot with a high concentration of pigments (beta-carotene and anthocyanins). The improved plants could be used as raw materials in the production of natural food coloring. The carrots are developed through selection within existing purple and orange carrot varieties over several generations. Selected roots with a high content of pigment have been polycrossed by open pollination, and the progeny grown and compared in order to identify new cultivars with improved color concentration.

The polycross approach has been used to maximize the number of cross combinations that can be represented among the progeny. The polycross, however, lacks genetic control with complete loss of paternity information among the progeny. Simple sequence repeat (SSR) marker-based paternity analysis is proposed as an effective molecular tool for identifying paternity of progeny from a sixteen-parent polycross. Using previously described SSR markers, progeny from each polycross family is genotyped along with the parents. The objective of this study is to demonstrate that the paternity of polycross progeny can be determined by using polymorphic SSR markers. The ability to identify paternity of polycross progeny with microsatellite markers allow for a rapid assessment of diversity at the genome level and for a targeted selection of parental plants in carrot breeding programs.

BG-202

Solo-LTR of a *copia* retrotransposon is responsible for carotenoid accumulation in carrot roots

Alicja Macko-Podgórni, Justyna Bolek, Magdalena Klimek-Chodacka, Dariusz Grzebelus *Institute of Plant Biology and Biotechnology, University of Agriculture in Krakow, Al. 29 Listopada 54, 31-425 Kraków*

Recent identification and characteristics of a candidate gene (*Y*) controlling carotenoid accumulation in carrot roots revealed that a 212 nt insertion into exon 2 caused a frameshift mutation resulting in the yellow and orange root phenotypes. The insertion in the *Y* gene is a solo-LTR of a *copia* retrotransposon.

We utilized published carrot resequencing data to characterize the insertion in the *Y* gene and to reconstruct a full length *copia* LTR retrotransposon *DcC-Y* from *D. capillifolius*. The sequence of *DcC-Y* was used for blast search against 25 resequenced carrot genomes. A collection of *D. carota* accessions was PCR-screened for the presence of gag and RT domains, and LTRs of each of the retrotransposons. Retrotransposoncopy numbers were estimated via qPCR.

There was no full length copy of this retrotransposon in the DH1 genome, however, we reconstructed it from *D. capillifolius* contigs. We identified a related *copia* retrotransposon present as a single copy in the DH1 genome (named *DcC-DH*), showing 80.6% identity to *DcC-Y*. The gag and RT domains characteristic for *DcC-Y* were present mostly in wild carrots, while LTRs were identified in all cultivated carrots. In contrast, *DcC-DH* elements were predominantly present in cultivated carrots. The gag and RT domains of *DcC-Y* and *DcC-DH* were similarly distributed across the carrot diversity collection,

however, cultivated carrots carrying *DcC-Y* were identified. Both retrotransposons were present in low or moderate copy numbers, *DcC-Y* ranging from 4 to 68 copies while *DcC-DH* being less abundant and only in one plant reaching 15 copies**.**

BG-203

CarrotREP- Identifying phenotypes, markers, and genes in carrot germplasm to deliver improved carrots to growers and consumers

Philipp Simon, David Spooner
USDA-Agricultural Research Service and Department of Horticulture, University of Wisconsin, Madison, WI
Micaela Colley
Organic Seed Alliance, Port Townsend, WA
Julie Dawson, Laurie McKenzie
Department of Horticulture, University of Wisconsin, Madison, WI
Sherry Tanumihardjo
Department of Nutritional Sciences, University of Wisconsin, Madison, WI
Edgar Spalding
Department of Botany, University of Wisconsin, Madison, WI
Joe Nunez
University of California Cooperative Extension, Farm and Home, Bakersfield CA
Philip Roberts
Department of Nematology, University of California, Riverside, CA
Allen Van Deynze
Seed Biotechnology Center, University of California, Davis, CA
Daniel Sumner, William Matthews, Hyunok Lee
Agricultural and Resource Economics, University of California, Davis, CA
Massimo Iorizzo
Department of Horticultural Science, North Carolina state University, Kannapolis, NC
Lindsay du Toit
Washington State University, Mount Vernon, WA
Tim Waters
Area Extension, Commercial Vegetables, Washington State University, Pasco, WA
Jairo Diaz-Ramirez
Division of Agriculture and Natural Resources, University of California, Desert Research and Extension Center, Holtville, CA

A survey of U.S. carrot growers and seed industry stakeholders was conducted and a meeting was held in 2015 to identify key traits important for improved carrot quality and productivity anticipated to meet future market demands. This effort revealed that the carrot industry needs breeding stocks and genomic tools that can be used to develop carrots with improved field performance including disease and pest resistance, and abiotic stress tolerance; and improved flavor and nutritional quality to better meet consumer needs. Given this critical stakeholder input, the goals of the Carrot Research and Extension Project, or CarrotREP, are to: 1) phenotype diverse carrot germplasm and breeding stocks to discover and characterize previously uncharacterized variation for traits important for improving carrots for the US market; 2) develop an expanded carrot genomic and phenotypic database for breeders to catalogue genomic variation and track genes underlying important traits; 3) initiate the development of breeding pools from diverse germplasm and breeding stocks that include alleles for improved crop production and consumer quality traits, and test them on-farm with growers and for flavor and nutritional value for consumers; and 4) evaluate the market value and impact of carrot traits on grower and consumer decisions. A timeline of activities for this project has been developed.

BG-204

CIOA 2 - Carrot Improvement for Organic Agriculture with Added Grower and Consumer Value

Philipp Simon
USDA-Agricultural Research Service and Department of Horticulture, University of Wisconsin, Madison, WI
Micaela Colley, Laurie McKenzie, Jared Zystro, Cathleen McCluskey
Organic Seed Alliance, Port Townsend, WA
Lori Hoagland
Department of Horticulture & Landscape Architecture, Purdue University, West Lafayette, IN
Erin Silva
Department of Plant Pathology, University of Wisconsin, Madison, WI
Philip Roberts
Department of Nematology, University of California, Riverside, CA
Julie Dawson
Department of Horticulture, University of Wisconsin, Madison, WI
Lindsay du Toit
Washington State University, Mount Vernon, WA
Tim Waters
Area Extension, Commercial Vegetables, Washington State University, Pasco, WA
Joe Nunez
University of California Cooperative Extension, Farm and Home, Bakersfield CA

Carrot Improvement for Organic Agriculture 2 (CIOA 2) builds upon accomplishments of the CIOA 1 project funded by the USDA OREI. Plant breeding is long-term work and the proposed project will maximize impacts of prior research by delivering new, improved carrot cultivars and breeding lines to the organic seed trade; developing new breeding populations that combine critical traits identified during CIOA 1; expanding the screening of diverse carrot germplasm and field testing of finished cultivars and advanced materials in diverse organic environments; and advancing our understanding of positive genetic-soil microbial interactions, thereby expanding the potential to breed for nutrient use efficiency, disease resistance, and drought tolerance. The long-term goals are to: 1) deliver carrot cultivars with improved disease and 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; 2) determine how carrot genotypes interact with, or influence, the root microbiome to access key nutrients under limiting environments and limit heavy metal uptake; 3) inform growers about cultivar performance to maximize organic carrot production, markets, and organic seed usage; 4) inform consumers about the positive environmental impacts of organic production systems and about carrot nutritional quality, flavor and culinary attributes; and 5) train undergraduate, graduate, and post-doctorate students in critical organic agriculture issues. A timeline for project activities was developed.

BG-205

Unveiling Carrot Root Architecture Using 2D and 3D Image Analysis

Shelby Ellison
University of Wisconsin-Madison Department of Horticulture
Philipp Simon
University of Wisconsin-Madison Department of Horticulture, USDA Vegetable Crops Research Unit
Malcolm Bennett
The University of Nottingham Plant Sciences Division

Effective phenotyping in carrot is complicated by the fact that the agronomically valuable portion of the crop is underground. To better understand the genetics of carrot root architecture, novel approaches must be conceived and applied in the field of high resolution, high throughput phenotyping. One such approach is the use of microscale X-ray Computed Tomography (µCT). This technique allows for the non-destructive imaging of roots in soil conditions similar to those found in the field. Optimizing µCT for carrot will greatly increase the understanding of how storage root architecture develops across maturation. To better understand carrot root architecture we have used µCT to image four carrot cultivars, exhibiting extreme growth types, across five developmental time points. Further, we have utilized the 2D image analysis software, RootNav and SmartRoot, to measure carrot root architecture traits in an F_2 mapping population between wild and cultivated carrot. Phenotypic measurements will be used in conjunction with genotypic information to further understand the genetic basis of lateral root formation and root shape in carrot. Establishing a protocol for µCT and 2D image analysis in carrot, as model root crop, will facilitate the application of these technologies to other root and tuber crops such as sugar and table beet, potato, and cassava. Finally, increased knowledge pertaining to carrot root architecture can be used to design cultivars with better water and nutrient use efficiency.

BG-206

Utilization of Molecular Markers to Screen for Carotenoid Content within the USDA Carrot Germplasm Collection

Shelby Ellison
University of Wisconsin-Madison Department of Horticulture
Philipp Simon
University of Wisconsin-Madison Department of Horticulture, USDA Vegetable Crops Research Unit

Cultivated carrot (*Daucus carota* subsp. *sativus*) is an important vegetable crop that is popular around world. Carrots are well known for their nutritional value due to the large amount of provitamin A carotenoids (alpha- and beta-carotene) found in their storage roots. To date, there are a limited number of reliable, predictive DNA tests to screen for economically important traits in carrot. Recent research efforts have led to the identification of candidate genes for the *Y* and Y_2 traits in carrot, conditioning total carotenoid and beta-carotene accumulation, respectively. There are currently over 1,000 *D. carota* accessions at the North Central Regional Plant Introduction Station, including many that have yet to be characterized for root pigmentation. Utilizing recently discovered SNPs and InDels, several co-dominant markers for *Y* and Y_2 have been created to screen carrot Plant Introduction (PI) accessions for favorable combinations of alleles as well as to better characterize the collection. Furthermore, with markers for both *Y* and Y_2, it is now possible to select parents with the genetic potential to produce high-carotenoid accumulating progeny. Moreover, molecular markers will be tested across several cultivated carrot subpopulations showing high levels of population structure, including Eastern (Central Asia, East Asia, and the Middle East) and Western (South America, North America, and Europe) groups, to better understand domestication events and to test whether the newly developed markers have utility across different geographic populations.
