BG-201

Paternity Analysis with Microsatellites in Carrot Polycross Breeding

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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

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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

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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

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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

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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

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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.

CP-201

Nitrogen fertilizer depth and timing effects on carrot and Powell amaranth

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Nitrogen (N) management in processing carrots grown on sandy soils in west Michigan typically involves 3 or 4 broadcast top dress applications of urea. These relatively frequent broadcast applications introduce N fertilizer to the system on the soil surface and create surges in N. Broadcast application also makes N vulnerable to loss through volatilization, leaching and uptake by weeds. Controlled release N fertilizer (CRN) including polymer coated urea (eg. ESN) may help to reduce the number of top dress applications and increase N efficiency. We hypothesized that deep placement of N would benefit carrots over Powell amaranth and that CRN would have a greater benefit for carrot than the urea system. We conducted a greenhouse study to test the effects of N fertilizer placement depth and CRN on both carrot and the problematic weed Powell amaranth. The study consisted of separate runs for carrot and Powell amaranth with three N fertilizer placement depths (surface, 7.62 or 15.42 cm) and two N delivery systems (urea at planting and simulated CRN). All treatments received the same N rate (40 lbs N/a equivalent); we simulated CRN by splitting the total N rate into four equal applications (at planting and every 10 days following). We found that carrot biomass was most responsive to N applications on the surface and that CRN simulation gave the greatest biomass. Contrary to our hypotheses, neither deep placement nor split applications reduced Powell amaranth biomass.

CP-202

Foliar-applied ethephon improves black carrot taproots as source of anthocyanin-based natural colorants

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The natural food colors market is a growing segment that accounts for more than 55% of the total food color market. Black carrots (*Daucus carota* ssp. *sativus* var. *atrorubens* Alef.) have strong bluish-purple color due to the anthocyanin pigments, which are used as natural food colorants due to their high pH, light, and heat stability. However, new production strategies of black carrots are required in order to increase pigment yield and, therefore, to improve profit margins.

Ethephon, an ethylene releasing compound, was used as elicitor of anthocyanin and phenolic compounds of 'Deep Purple F1' black carrots in field experiments conducted over three years in Denmark. Total monomeric anthocyanins and phenolic contents were measured spectrophotometrically, and anthocyanin composition was analysed by LC-MS/Q-TOF. The effect of ethephon was investigated on several quality parameters and on the expression of anthocyanin biosynthetic genes.

The results documented substantial increase in anthocyanin and phenolic contents upon foliar applications of ethephon, whereas root size remained unchanged. Five cyanidin-based anthocyanin forms were detected, with the acylated (more stable) anthocyanins being clearly predominant. New insight into the accumulation patterns of the different anthocyanins and phenolic compounds during root growth was provided. Moreover, a correlation between enhanced anthocyanin contents and decreased root dry matter and soluble sugar contents was found. In addition, the expression of the anthocyanin biosynthetic genes analyzed (*PAL1*, *PAL3*, *F3H1*, *DFR1*, *LDOX2*) increased upon ethephon-treatment. Collectively, our findings are of economic importance as they improve key parameters of black carrot for anthocyanin color production.

PM-201

The occurrence of psyllids on carrot and presence of *Candidatus* Liberibacter solanacearum in France

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The presence of psyllids has been known a long time ago in French carrot fields, but until recently, they don't make real damage in crops and are not well studied. It's not the same for Northern Europe, where *Trioza apicalis* is an important pest for carrot production and reduces both yield and quality of the carrots. Recently, the report of *Candidatu*s Liberibacter solanacearum (Loiseau et *al.*, 2014) and the presence of psyllid species in carrot fields are not well kwon, *Bactericera trigonica* (F. Villeneuve, unpublished results) in French carrot fields, leads us to make an inventory in order to know the species of psyllids present in French carrots fields. The transmission of proliferation symptoms to carrot by psyllids is not really new; Leclant et *al.* demonstrated the involvement of psyllids in the transmission of proliferation in carrot in 1974.
A survey was conducted during 2016 in carrot fields. Psyllid insects were collected in yellow traps and identified. The level of *B. trigonica* carrying the bacterium is also evaluated. Also, monitoring was set up from 2014 to 2016.
Different psyllid species are present and identified, the most frequent is *Bactericera trigonica*. This species seems to be present in carrot fields throughout the year. Detection tests to evaluate the presence of *Ca*. L. solanacearum in psyllid samples are in progress as of the end of 2016. Results of the survey carried out in carrot crops in France will be presented. The haplotype detected seems to be subservient to Apiaceae crops (carrot principally).
Acknowledgements:CaLiso project is conducted with a grant from the French Ministry of Agriculture (CasDAR project) and Marianne Loiseau, ANSES-Angers, is the project leader.

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PM-202

Assessing new chemical control options for carrot weevil in the Holland Marsh, Canada

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The carrot weevil (CW), *Listronotus oregonensis* (LeConte), is a primary insect pest of carrot production in Ontario. Yield loss due to CW larval feeding can exceed 40% but is highly variable. In Canada, the CW has historically been univoltine and IPM recommendations consist of insecticide applications at the 2nd and 3rd true leaf stage, based on action thresholds. Imidan 70 WP (phosmet) was the only registered product for CW control in Canada until recently and is still the primary product used despite concerns of resistance. This study examined the efficacy of seed treatment and foliar applications to mitigate CW damage in the Holland Marsh, a region of intense carrot production in Ontario, Canada. Phosmet failed to reduce CW damage compared to untreated plots, while two insecticides (novaluron as a foliar, cypermethrin as a foliar and seed treatment) showed some efficacy in 2016. Another trial examined the impact of an insecticide synergist, piperonyl butoxide, on the efficacy of phosmet and cypermethrin, another prevalent insecticide in Canadian carrot production. Phosmet alone slightly reduced CW damage, though both insecticides performed significantly better with the addition of piperonyl butoxide. Overall, CW mitigation appears to be challenging with the insecticides available currently. However, future research using novaluron, cypermethrin, and piperonyl butoxide appears promising. In all trials presented here, increases in CW damage was found late in the growing season. This likely indicates that some second generation CW activity is occurring.

PM-203

Carrot cyst nematode survey in the Holland Marsh, Ontario, Canada 2016

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Carrots are grown in all regions of Canada, and the province of Ontario is the largest producer. *Heterodera carotae* Jones, is commonly known as the carrot cyst nematode. It is found in several countries in Europe, and Michigan, USA. It is considered an exotic plant pest to Canada and can cause serious damage to carrot crops. Carrots (*Daucus carota* L.) and wild carrots are the lone hosts. In recent years, carrots in commercial fields were found to have patches with poor growth, stunting, smaller and forked carrots with a proliferation of secondary roots, and cysts associated with the damaged roots. Thirty carrot fields in Holland Marsh region of Ontario, Canada were sampled in November 2016 following carrot harvest. Field size varied from 5 - 25 acres. Soil samples from the top 20 cm of soil were sampled in an X pattern in each field. Samples were analyzed for the presence of carrot cyst nematode. Veriform nematodes were extracted using a Baermann funnel and the Fenwick method for cysts. Second stage juveniles, males, and cysts were recovered. The species was confirmed as *H. carotae* using morphological and molecular methods. Carrot cyst nematodes were found in 90% of the samples and were widespread throughout the sampled area. Population densities of the carrot cyst nematode ranged from 0 to 16,100 juveniles/kg of soil. This confirms the presence of carrot cyst nematode in the Holland Marsh. Further studies are needed to assess the impact of this pest to carrot producers.

PM-204

Assessing new chemical control options for carrot weevil

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The carrot weevil (CW), *Listronotus oregonensis* (LeConte), is a primary insect pest of carrot production in Ontario, Canada. Yield loss due to the feeding of CW larvae feeding can exceed 40% but is highly variable. In Canada, the CW has historically been univoltine and IPM recommendations consist of insecticide applications at the 2nd and 3rd true leaf stage, based on action thresholds. Imidan 70 WP (phosmet) was the only registered product for CW control in Canada until recently and is still the primary product used, despite concerns of resistance. This study examined the efficacy of seed treatment and foliar applications to mitigate CW damage in the Holland Marsh, a region of intense carrot production in Ontario, Canada. Phosmet did not reduce CW damage compared to untreated plots. Two insecticides showed some efficacy in 2016: novaluron as a foliar application and

cyantraniliprole as a foliar and seed treatment. Another trial examined the impact of an insecticide synergist, piperonyl butoxide, on the efficacy of phosmet and cypermethrin, another prevalent insecticide in Canadian carrot production. Phosmet alone slightly reduced CW damage, though both insecticides performed significantly better with the addition of piperonyl butoxide. Overall, CW mitigation is challenging with the insecticides available currently. However, insecticides novaluron, cyantraniliprole and cypermethrin with piperonyl butoxide appear promising as replacements for phosmet. There were increases in CW damage, late in the growing season, in all of these trials. This suggests that some second generation CW activity is occurring.