

BG-101

Selecting carrots with increased resistance to cavity spot

M. R. McDonald¹ K. Vander Kooi¹ and P. W. Simon²

¹ Department of Plant Agriculture, University of Guelph, Guelph ON, Canada N1G 2W1.

² USDA- ARS, University of Wisconsin, Madison, WI, USA

Cavity spot of carrot is caused by several species of *Pythium*, especially *P. violae* and *P. sulcatum*. The disease can be very difficult to control with fungicides. The most effective management method is the use of resistant cultivars. Carrot breeding lines were screened to identify differences in susceptibility to cavity spot in order to support the carrot breeding program of the USDA. Trials were conducted on high organic matter soil (organic matter ~ 72%, pH 5.7 – 6.0) in the Holland Marsh, Ontario, Canada (44°15` N, 79°35` W). Cavity spot occurs regularly at this site. A total of 56 breeding lines and several commercial cultivars were grown each year. Carrots were assessed for cavity spot incidence and severity in August (mid-season) and after harvest in October. Carrots were also assessed for forking, as a symptom of *Pythium* root dieback, in 2016, and for leaf blight severity and for bolting in 2015-2016. Cavity spot incidence ranged from a maximum of 30 -97% over the four years of the trial. Several lines with purple (anthocyanin) pigments and cv. Purple Haze had consistently low incidence of cavity spot. Cultivar Atomic Red was highly susceptible all years. Four orange breeding lines were identified that had consistently low cavity spot. These have been incorporated into the breeding program.

BG-102

The Search for Salinity Tolerance in Diverse Carrot Germplasm

Adam Bolton¹ and Phil Simon^{1,2}

¹ Department of Horticulture, Plant Breeding and Plant Genetics Program, University of Wisconsin, Madison; ² Vegetable Crops Research Unit, USDA, Agricultural Research Service, Department of Horticulture, University of Wisconsin Madison, WI

Carrot is a widely grown and economically important vegetable worldwide that provides a rich dietary source of vitamin A, but production is restricted by salt-affected soil in many parts of the world. There is evidence that carrots are especially salt sensitive relative to other crops, but little research has been devoted to evaluating genetic variation and breeding for salt tolerance in carrot. A promising source of genetic diversity for salt tolerance is the USDA Plant Introduction (PI) Collection, many of which grow natively in areas with relatively high levels of salt. In a preliminary study, we have evaluated 292 carrot accessions at seed germination and found evidence for a low incidence of salt tolerance. Salt tolerance will also be evaluated during plant growth and carrot populations, to map the inheritance and combine multiple genetic sources of resistance, will be developed.

BG-103

Characterization of a genomic region under selection in cultivated carrot (*Daucus carota* subsp. *sativus*) reveals a candidate domestication gene

Alicja Macko-Podgórn¹, Gabriela Machaj¹, Katarzyna Stelmach¹, Douglas Senalik², Ewa Grzebelus¹, Massimo Iorizzo³, Philipp W. Simon², Dariusz Grzebelus¹

¹*Institute of Plant Biology and Biotechnology, Faculty of Biotechnology and Horticulture, University of Agriculture in Krakow, Krakow, Poland*

²*Vegetable Crops Research Unit, United States Department of Agriculture-Agricultural Research Service, Department of Horticulture, University of Wisconsin–Madison, Madison, WI, USA*

³*Plants for Human Health Institute, Department of Horticultural Science, North Carolina State University, Kannapolis, NC, USA*

No systematic knowledge about the molecular mechanisms involved in the domestication syndrome in carrot are available, however, the ability to form a storage root is the essential transition from the wild *Daucus carota* to the cultivated carrot. We mapped a previously identified region under selection to the distal portion of the long arm of carrot chromosome 2, confirmed that it had been selected, as reflected in both the lower nucleotide diversity in the cultivated gene pool, as compared to the wild and high F_{ST} . We delimited the region to ca. 37 kb and identified a candidate domestication syndrome gene carrying three non-synonymous single nucleotide polymorphisms and one indel systematically differentiating the wild and the cultivated accessions. This gene, *DcAHLc1*, belongs to the AT-hook motif nuclear localized (AHL) family of plant regulatory genes which are involved in the regulation of organ development, including root tissue patterning. AHL genes work through direct interactions with other AHL family proteins and a range of other proteins that require intercellular protein movement. Based on QTL data, we speculate that *DcAHLc1* might be involved in the development of the carrot storage root, as the localization of the gene overlapped with one of the QTLs. We propose that the 'cultivated' variant of *DcAHLc1* has been selected from wild Central Asian carrot populations upon domestication. However, some primitive eastern landraces and the derived B7262 purple inbred line still carry the 'wild' variant, reflecting complexity of the genetic determination of the formation of carrot storage roots.

BG-104

New carrot germplasm from Spain and its use in breeding, genetic, and taxonomic studies

David M. Spooner¹, Philipp W. Simon¹, and Fernando Martínez-Flores²

¹*USDA-ARS, Vegetable Crops Research Unit, Department of Horticulture, University of Wisconsin, USA.*

²*Universidad de Alicante, P. O. Box 99, ES-03080 Alicante, Spain*

The Apiaceae (Umbelliferae) family contains 455 genera and over 3500 species, and is one of the largest families of seed plants. The genus *Daucus* contains carrot (*Daucus carota* L. subsp. *sativus* Hoffm.), the economically most important cultivated member of Apiaceae in terms of economic importance and nutrition. The latest genus-level treatment of *Daucus* by Sáenz Laín (1981) used morphological and anatomical data and recognized 20 species. Rubatzky et al. (1999) later estimated 25 species of *Daucus*, and Banasiak et al. (2016), using DNA sequences from nuclear ribosomal ITS and the three plastid markers redefined and expanded the genus *Daucus* to include representatives of nine other genera and now containing 53 species and winged fruits in addition to its traditionally recognized spiny fruits. The genus *Daucus* has a center of endemism in the Mediterranean Region, with several species occurring in North America, South America, and Australia. We have been expanding the available germplasm collections strategically, with Spain of critical importance because of its many species not available as germplasm. We conducted three expeditions to Spain in 2016, including the Balearic Islands (June 5-18, Aug 23-Sept 6, and Oct 7-10), designed to collect mature fruits of species differing in phenology. We collected over 120 accessions of *Daucus* (*Pseudorlaya pumila*, *D. (Pseudorlaya) miniscula*, *D. arcanus*, *D. aureus*, *D. carota* subsp. *carota*, *D. carota* subsp. *gummifer*, *D. carota* subsp. *maximus*, *D. crinitus*, *D. durieua*, and *D. setifolius*). These new germplasm resources, in concert with existing germplasm, are allowing much more comprehensive studies of the breeding and genetic value of these taxa, and taxonomic relationships, to be reviewed in this presentation.

BG-105

The influence of organic and conventional production environments on breeding for carrot top size

Charlene Grahn¹, Erin Silva², Philip Simon^{1, 3}

1. Graduate Research Assistant, Department of Horticulture, University of Wisconsin- Madison

2. Assistant Professor, Department of Plant Pathology, University of Wisconsin- Madison

3. Professor and Plant Breeder, USDA- Agricultural Research Service, Vegetable Crops Research Unit, University of Wisconsin- Madison

Carrot top morphology influences crop weed competitiveness and ease of mechanical harvesting; however, top morphology traits are seldom the focus of carrot breeding projects and research. In addition to genetic influences, carrot top size is influenced by environmental conditions and management practices. These GxExM interactions make carrot top size an ideal model trait for studying the impacts of selection under organic and conventional management. This study seeks to elucidate the heritability of carrot top size while answering the question: "Is selection for carrot top size in organic production systems effective in a conventional environment, or must selection be made in an organic environment if it is to be relevant to organic growers?" To accomplish this goal, four diverse F2 and F3 segregating carrot populations were evaluated in 2015 and 2016, respectively, in adjacent certified organic and conventionally managed fields at the University of Wisconsin- Madison West Madison Agricultural Research Station. Trait heritability and response to selection were compared between the two environments by selecting individual F2 plants within different top size categories in 2015 and evaluating the top size of their F3 progeny in 2016. F3 progeny were also evaluated in El Centro, CA in 2017 to determine the relevance of carrot top size evaluation in Wisconsin to California carrot growers. The results of this research will inform breeders about the heritability of carrot top size in organic and conventional environments and the need to manage breeding trials organically when developing new cultivars for organic growers.

BG-106

Elicitation of anthocyanins in hairy root cultures of black carrots

Shih-Ti Chen¹, Gregorio Barba Espin¹, Trine Bundgaard Andersen¹, Bjarne Jørnsgård², Renate Müller¹, Henrik Lütken¹

¹University of Copenhagen, Faculty of Science Department of Plant and Environmental Sciences, Crop Sciences Section Højbakkegård Allé 9-13, DK-2630 Taastrup, Denmark

²Chr. Hansen Natural Colors A/S, Natural Colors Division
Agern Allé 24, DK-2970 Hørsholm, Denmark

Anthocyanins are water-soluble flavonoid pigments occurring in several tissues of higher plants. There is increasing demand for natural food colorants that can substitute synthetic colors due consumer concerns. Highly stable acylated anthocyanins may impart desirable color and stability for commercial food products. To date, over 600 different anthocyanins have been identified from plant sources comprising aglycones and various glycosylated and acylated compounds. Suitable sources of acylated anthocyanins are radishes, red cabbage, purple sweet potatoes and black carrots. Black carrots (*Daucus carota* ssp. *sativus* var. *atrorubens* Alef.) have particularly high anthocyanin levels in the taproot.

A promising approach to further increase the content of naturally present anthocyanins in black carrots is natural transformation by *Agrobacterium rhizogenes*. During *A. rhizogenes*-mediated transformation *root oncogenic loci (rol)*-genes are transferred from the T-DNA of the root-inducing plasmid. The stable introgression of the T-DNA causes hairy roots (HRs) to develop from the site of infection. HRs often produce increased levels of secondary metabolites compared to untransformed

roots and *in vitro* cell cultures. The first aim of this study is to obtain HR cultures for black carrots containing *rol*-genes as a platform for increased anthocyanin concentration per biomass.

Anthocyanins are regarded as secondary metabolites. The accumulation of secondary metabolites often requires elicitors, which usually act as signalling molecules of plant stress responses. The second aim is to use the HR cultures in elicitation experiments to further elevate the anthocyanin levels. Collectively, these approaches will potentially lead to future sustainable production of anthocyanins.

BG-107

Phenotypic, Genotypic, and Freedom to Operate Variation in US Carrot Cultivars

Claire Luby, Julie Dawson, Irwin Goldman
Department of Horticulture, University of Wisconsin-Madison

Increased use of intellectual property rights over plant germplasm has led to a complicated landscape for exchange among plant breeders. Our goal was to examine phenotypic and genotypic diversity present in commercially available carrot (*Daucus carota* L. var. *sativus*) germplasm in relation to the freedom to operate—the ability for plant breeders to access and use crop genetic diversity. A collection of 140 commercially available carrot cultivars were grown in replicated field trials in the Madison, WI area in 2013 and 2014. Phenotypic measurements were recorded for leaf and root characteristics. Illumina sequencing was used to conduct genotyping by sequencing analysis on all cultivars to understand the range of genetic diversity present. Additionally, the intellectual property rights associated with each cultivar was noted to determine the freedom to operate. We found that although one-third of the commercially available US carrot cultivars in our study are restricted through some form of intellectual property rights, the genetic and phenotypic variability of the protected cultivars does not represent a completely separate group from the available material. Phenotypic analyses including ANOVA and principal components analysis, suggest that many of the traits differed significantly based on market class, but not by whether the cultivar had freedom to operate. The principal components and *Fst* analyses on the genotyping by sequencing data revealed that carrot market classes (*Fst* = 0.065) and freedom to operate classes (*Fst* = 0.023) were not genetically distinct, and that principle components 1 and 2 account for only 10.1% of the total genotypic variation, implying that cultivated carrot germplasm in the US forms an unstructured population. Our findings suggest that the genetic diversity present in carrot cultivars that have freedom to operate is potentially large enough to support carrot breeding efforts in most market classes given present levels of intellectual property protection.

BG-108

Genetic Analysis of Carrot Root Shape and Top Size Measured by Image Analysis

Sarah Turner¹, Nathan Miller², Edgar Spalding², Phil Simon^{1, 3}

¹ *Department of Horticulture, Plant Breeding and Plant Genetics Program, University of Wisconsin, Madison*; ² *Department of Botany, University of Wisconsin, Madison*; ³ *Vegetable Crops Research Unit, USDA, Agricultural Research Service, Department of Horticulture, University of Wisconsin Madison, WI*,

Many quantitative phenotypes of interest to carrot breeders remain difficult and time intensive to measure. These include storage root shape, which has been used to categorize orange carrot cultivars since the 1600s, and top size, which influences weed competitive ability and management costs. While genetic variation for carrot root shape and top size has been widely observed, models for the genetic control of these traits have not been reported due to limitations in phenotyping methods. To address this bottleneck, an image-based phenotyping platform was created to perform morphometric analysis

on harvested carrots. Image-extracted traits included digital root and shoot biomass, leaf number, petiole length, and root shape. Digital estimates were five times more efficient to collect and were well correlated with hand measurements. Additionally, image-extracted data is being used in subsequent genetic studies, which include diallel analysis and quantitative trait loci (QTL) mapping. Continued advances are being pursued through the development of field-based imaging systems. These improvements will allow the incorporation of non-genetic variables, such as developmental variation and planting density, which complicate genetic analyses.

BG-109

Marker Assisted Recurrent selection (MARS) for development of nutritionally rich, nematode resistant Carrot (*Daucus carota* L.) suitable to tropical region

Sarvamangala S. Cholin* Chaitra A. Poleshi, Manikanta D. S and Jagadeesha R. C.
University of Horticultural Sciences, Bagalkot-587103, Karnataka, India

Carrot (*Daucus carota* L.), a highly out crossing vegetable belonging Apiaceae family is known for its principle nutrient component carotenoids and also has incredible antioxidant and detoxifying properties. Despite, its importance as nutritionally rich vegetable, least efforts are made for the development of tropical varieties due to its biennial nature and high vernalization requirement. Nematode is another major disease which spoils the quality and productivity of roots; hence, resistance to nematodes is another major objective in development of varieties to warmer climates. Recent availability of whole genome sequence of carrot and gene specific markers for economic traits such as carotenoids, sugar type, nematode resistance, and other stress responsive genes will help a molecular breeder in precise selection of superior plants. MARS is one of the best approaches in carrot for rapid development of superior varieties by enriching the population with desirable alleles at multiple loci within few cycles of selection than by traditional breeding alone. A simple PCR can detect the plants carrying desirable alleles at different loci in early seedling stage in a large segregating population to ease in selection and crossing. Already available gene specific and tightly linked markers will be explored for development of varieties rich in sugar (*Invertase Isozyme II*), carotenoids (*Y locus*), nematode resistance (*Mi* and *Mj*), vernalization (*VRN*) and better stress tolerance (*AOX*). Two to three cycles' recurrent selection will be done for the allelic enrichment in the population. The new variety would help a farmer of the tropical region to produce nutritionally rich roots as well as to maintain the seeds for the next generations.

BG-110

Utilizing 2D Imaging to Map Root System Architecture QTL in Carrot (*Daucus carota* L.)

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

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 to scan roots and use 2D image analysis software to extract traits of interest. We have used the programs RootNav and SmartRoot to measure carrot root architecture traits in an F₂ mapping population between wild and cultivated carrot. Phenotypic measurements have been used in conjunction with genotypic information to better understand the genetic basis of lateral root formation and root shape in carrot. We have identified several QTL controlling the number of lateral roots and carrot root shape. Estimates of heritability and further QTL mapping are being conducted in F₃ families. Establishing a protocol for 2D image analysis in carrot, as a 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 to better meet farmer and consumer needs.

BG-111

Sources of Genetic Variation for Early Plant Growth in Diverse Carrot Germplasm

Adam Bolton¹ and Phil Simon^{1,2}

¹ Department of Horticulture, Plant Breeding and Plant Genetics Program, University of Wisconsin, Madison; ²Vegetable Crops Research Unit, USDA, Agricultural Research Service, Department of Horticulture, University of Wisconsin Madison, WI

Carrot seed germination is often uneven for a given seed lot, sometimes occurring over a course of several days or more. This leads to variation in seedling emergence, early plant growth, and stand establishment, and may even account for variation in storage root size at harvest. Many non-genetic variables can account for variation in carrot seed germination and stand establishment including variation in seed maturity and cleaning, dormancy, disease attack, planting depth, soil conditions, weed competition, and microclimate, to name a few. Genetic variation has also been implicated in accounting for phenotypic variation in these early phases of plant growth, but is not well-documented. To begin to assess the genetic basis of this complex of traits, 736 carrot Plant Introductions (PIs) from the germplasm collection in Ames, Iowa, were evaluated for variation in seedling emergence in Hancock, WI. Emergence was preliminarily screened in the field and 20 PIs with uniform emergence of small plants (cotyledons only), and 20 PIs with uniform emergence but larger plants (2-3 true leaves) were evaluated in the lab. Seeds from both groups were germinated, transplanted to the greenhouse when radicles emerged, and growth measured for 56 days after transplanting. PIs with smaller seedlings in the field germinated slower in the lab, and the early plant growth rate of slower-germinating seed lots was slower for young plants in the greenhouse. Early plant growth in progeny will be evaluated and breeding pools will be developed.

BG-112

Deploying *Meloidogyne incognita* Nematode Resistance in Carrot

Phil Simon¹, Joe Nunez², William Matthews³, Phil Roberts³

¹USDA-ARS, Vegetable Crops Unit, University of Wisconsin-Madison Department of Horticulture;

²University of California Cooperative Extension, Farm and Home, Bakersfield CA; ³University of California-Riverside Department of Nematology

Root knot nematodes (*Meloidogyne* spp.) are a major pest attacking carrots (*Daucus carota*) worldwide. Root knot nematodes cause galling and forking of the carrot root, rendering an infected carrot unfit for market. The current management practices of applying broad spectrum soil fumigants works well, but is coming under continuously restricted regulations, is costly for growers, and is considered damaging to the environment. Genetic resistance to nematodes would be an ideal solution to eliminate or strongly reduce the use of broad spectrum soil fumigants in carrot production. Resistance to *M. javanica* has already been discovered and mapped to the *Mj-1* locus on chromosome 8 in a 'Brasilia' cultivar. Beyond *M. javanica* resistance, genetic resistance to *M. incognita* was discovered in three diverse sources of resistance, from Syria, Europe and South America, and resistance genes mapped. A consensus genetic map of the three populations revealed five non-overlapping QTLs for *M. incognita* resistance. Resistance was high in the original genetic backgrounds in which it was discovered and in mapping populations, but introgression of resistance genes into carrots with horticultural quality suitable for US commercial production has not yet been reported. In recent field trials new inbreds with *M. incognita* resistance genes from multiple genetic sources were

observed to have been a high level of resistance combined with long, smooth root shape and excellent horticultural quality. Steps to deploy resistance will be discussed.

BG-113

Marker development for genes of the carrot terpene biosynthesis

Thomas Nothnagel, Holger Budahn, Detlef Ulrich, Jens Keilwagen, Thomas Berner, Heike Lehnert and Frank Dunemann

Julius Kuehn-Institute, D-06484 Quedlinburg, Germany

Terpenes are an important group of compounds in carrot influencing significantly taste and aroma. Additionally, terpenoids play a considerable physiological role as phytohormones and photosynthesis pigments as well as for communication and defense of the plants. So it has been shown that terpenoids help to attract pollinators or predators of herbivores. Terpenoid secondary metabolites are abundant in many essential oils, resins and floral scents among them some with pharmaceutical relevance.

Aim of our research is the development of functional molecular markers for some important genes of the terpene metabolism of carrot on the basis of associations between candidate genes and the content of individual volatile organic compounds (VOCs). The availability of the assembled whole carrot genome sequence (Iorizzo et al., 2016) and some other plant genomes provided the possibility for a genome wide inventory of the carrot terpene synthase (TPS) gene family. Homology-based gene prediction (Keilwagen et al., 2016) utilizing RNAseq data (Iorizzo et al., 2016) resulted in 65 putative functional TPS candidate genes belonging to the known TPS subfamilies of angiosperms. In parallel the volatile profile was analyzed for individual carrot genotypes using a semi-quantitative headspace SPME gas chromatography. For the association of SNPs of selected TPS candidate genes to the VOCs in carrot leaves and roots we pursued two approaches i) a QTL based mapping approach by using a bi-parental F₂ mapping population and ii) a GBS based genome-wide association study (GWAS) by using a diversity set of 96 carrot genotypes. Preliminary results of our research will be discussed.
