Cultivated carrot germplasm resources attempt to evaluation of genetic diversity using molecular tools

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Introduction

The aim of the project is to evaluate the genetic variation of cultivated carrots of different origin by using a variety of molecular techniques. The initial stage of the analysis was carried out by searching for DNA polymorphisms using molecular markers: gene-specific, microsatellites and transposon insertions.

Material and Methods

One hundred accessions of cultivated carrot were grown in pots in a greenhouse and fully characterized according to IPGRI descriptors. The collected and freeze-dried leaves from individual plants were used to DNA extraction according to Rogers and Bendich (1988) method.

For the preliminary analysis we chose 42 accessions and performed PCR reactions by using pairs of primers anchored in the coding regions of genes. These genes encode enzymes that route the biosynthesis of various carotenoids as well as flavonoids (CHS) and phenylopropanoids (PAL). The same accessions were analyzed by using microsatellite markers and iPCR markers derived from the flanking sequences of trasposon insertions.

Products were separated in 1% agarose gels and visualized by ethidium bromide or in polyacrylamide gels and visualized by silver staining.

Polymorphic variants were numerically encoded and analysed with respect to the population diversity structure of the investigated accessions, sepaertely for each type of markers and jointly, using Structure v.2.2 and GenAlEx v.6.0.

Gene	Primer	Detected polymorphism
Geranyl geranylpyrophosphate synthase	GGPS1	-
Geranyl geranylpyrophosphate synthase	GGPS2	-
β-Ring carotene hydroxylase	CHXB1-3`	-
β-Ring carotene hydroxylase	CHXB1-5`	+
Carotenoid isomerase	CRTISO3`	-
Carotenoid isomerase	CRTISOm	-
Carotenoid isomerase	CRTISOi1	+
Phenyloalanine ammonialyase	PAL	-
Phenyloalanine ammonialyase	PALex2	-
Phytoene synthase	PSY1	-
Phytoene synthase	PSY2	+
?-ring carotene hydroxylase	CHXE5`	-
?-ring carotene hydroxylase	*CHXE3`*	+
Lycopene ?-cyclase	LCYE5`	-
Lycopene ?-cyclase	LCYE3`	+
Lycopene ?-cyclase(exon)	LCYBex	-
Phytoene desaturase	PDS5`	-
Phytoene desaturase	PDS3`	-
IPP isomerase	*IPI3`*	+
Violaxanthin deepoxidase	VDE	+
Chalcone synthase	CHS2	+

Transposon markers	Detected polymorphism	Microsatellite markers	Detected polymorphism
KL 1	+	DCM 5	-
KL 2	+	DCM 9	+
KL 3	+	DCM 10	-
KL 4	+	*DCM 12*	+
KL 5	+	DCM 23	-
KL 6	+	DCM 24	+
KL 7	+	DCM 37	-
KL 8	-		
KL 9	+		
KL 10	+		
KL 11	+		

Results

Transposon insertion based markers allowed discrimination of 3 groups (K=3, see diagram) with the highest Log Likelihood, while gene-specific and microsatellite markers revealed no apparent structure of genetic diversity. The grouping was confirmed by AMOVA, both for transposon based polymorphism and for the whole set of markers, variability among populations explained 28% and 14%, respectively. In the group 1, 18 accessions of diverse origin were included, group 2 contained 12 accessions, mainly originating from Central Asia, and group 3 comprised 8 accessions and included accessions of Japanese origin and colored F1 cultivars (see Figure showing PCA results).