Research Journal of Agricultural Sciences 2010, **1**(2): 80-84

Genetic Relationship among Carrot (*Daucus carota* L.) Breeding Lines Revealed by RAPD Markers and Agronomic traits

Asima Amin, Yogesh Vikal*, T. S. Dhillon and Kuldeep Singh*
*Department of Vegetable Crops, *School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana-141 004, Punjab, India*
e-mail: aasima_ameen@yahoo.co.in

ABSTRACT

Genetic variability is the pre-requisite for genetic improvement of a crop. Thirteen morphological markers, four biochemical markers and twenty RAPD primers were employed to estimate genetic diversity and to characterize 48 carrot genotypes possessing special attributes. The analysis based on morphological or field observations, biochemical constituents and RAPD primers revealed wide genetic diversity in the germplasm evaluated. The RAPD primers generated 254 bands of which all (100%) were polymorphic. The polymorphic information content (PIC) for the 20 primers ranged from 0.83 in Oligo-679 to 0.94 in OPS-13. The similarity coefficient analysis revealed five clusters. These clusters were further classified in sub- clusters. Cluster I has 4 sub-clusters, cluster II has 2, cluster III has 3 and cluster IV has 1 sub-cluster. The RAPD analysis proved helpful for estimating the magnitude of genetic diversity and for establishing genetic relatedness among germplasm.

Key words: Carrot, Molecular markers, RAPDs, Genetic diversity

Diverse germplasm are the most valuable basic materials for crop breeder to meet the current and future needs. Characterization of carrot (*Daucus carota* L.) varieties or genotypes using morphological markers requires collection of extensive field data. Using morphological markers, it is easier to characterize the germplasm at the species level, but identification of genotypes within a species based on morphological markers alone is relatively difficult. Among molecular markers, random amplified polymorphic DNA (RAPD) markers are cost effective and do not require prior information of the genome (William *et al*., 1990) Optimization of PCR conditions and scoring only reproducible bands improves efficiency of RAPDs analysis (Yonemoto *et al*., 2006).

In carrot, various molecular markers viz., AFLPs, RAPDs and SSRs have been used to assess genetic diversity in germplasm collections and for germplasm characterization (Vos *et al*., 1995 and Welsh and McClelland, 1990). RAPD has proven sensitive to experimental conditions for reliable reproducibility (Paul *et al*., 1997) RAPD has the advantage of being technically simple and rapidly facilitated and has been used for plant genetics and phylogenetic studies (Yamamoto *et al*., 1994 and Demeke *et al*., 1996). The present investigation was undertaken with the objectives of estimation of genetic diversity and characterization of important genetic stocks of carrot using morphological, biochemical and RAPD markers.

MATERIALS AND METHODS

Plant material:

The experimental material comprised 48 genotypes of carrot possessing important quality traits viz; high carotene content, high juice content, high dry matter content, high TSS content and the ones possessing abiotic stress tolerance. The genotypes viz; Hybrid-501, JKC, Early Nantes, Amity's carrot, KTCTH-7, KTCTH-8 and Nantes belong to temperate regions and rest of the genotypes belongs to the tropical regions. The horticultural traits evaluated included top height (cm), plant weight (g), root length (cm), root weight (g), root girth (cm), flesh thickness (cm), total yield (kg/plot), total soluble solids (TSS%) (using hand refractometer), dry matter content (%), β-carotene (mg/100g) (AOAC, 1970) and juice yield (ml/kg).

Genomic DNA extraction and RAPD analysis:

The genomic DNA from fresh leaf tissues following CTAB method as described by Saghai-Maroof *et al*., (1984). Quantity and quality of DNA was checked by gel electrophoresis and spectrophotometer. Genomic DNA was amplified through PCR using RAPD primers in an Eppendorf Master Cycler. Initially 25 RAPD primers were used for amplification profile in 48 genotypes of carrot and 5 of the primers did not showed any amplification. The RAPD allele sizes were determined based on the position of bands relative to the ladder (Fermantas Gene Ruler 1 KB DNA ladder). Total no. of alleles was recorded for each of the 20 RAPD primers in all the 48 genotypes by assigning allele no. as 1,2,3,4 and so on. The amplified bands in the whole germplasm set were recorded in a binary matrix as 1 (band present) or 0 (band absent). The polymorphic information content (PIC) values for each of 20 primers were estimated using the formula:

$$PIC = 1 - \sum_{j=1}^{n} Pij^2$$

where Pij is the frequency of j^{th} allele in the i^{th} primer.

Cluster analysis:

The RAPD marker amplification profile of 48 genotypes was used to estimate genetic diversity or relatedness based on no. of shared amplified bands. The presence or absence of a particular amplification product was used as an index of genetic diversity or relatedness. The similarity matrix value based on Jaccard (Jaccard, 1908) coefficient of similarity was used to generate dendrogram. Clustering was done by UPGMA using SHAN module of NTSYSpc. Version 2.02e (Rohlf, 1998).

Table 1: Mean performance of carrot germplasm for various horticultural traits and biochemical constituents

S. No	Genotype	Top ht.	Plant wt.	Rt. length	Rt. wt	Rt. girth	Flesh thickness	Total yield	TSS	Dry matter	Carotene content	Juice
1	Amity's Carrot	54.8	333.67	21.64	139.50	3.15	2.03	3.49	7.13	7.33	6.00	479.17
2	CCA-05-01	69.1	225.17	21.06	123.64	2.83	1.83	6.18	7.38	7.55	4.63	466.67
3	CT-2	71.2	222.99	21.59	113.29	2.84	1.89	6.52	7.34	7.54	3.27	465.00
4	Early Nantes	58.9	377.50	20.16	148.00	2.98	1.82	3.89	7.15	7.24	5.30	415.83
5	HC-1	68.1	332.66	21.14	147.50	2.98	1.66	4.59	8.04	8.04	2.70	578.00
6	HC-100	61.8	185.33	22.88	110.83	2.58	1.52	4.26	8.68	8.50	3.23	575.00
7	HC-199-1	62.3	189.17	20.84	101.33	3.14	2.15	5.53	8.00	8.00	4.27	566.67
8	HCB-22-2	56.1	279.83	22.57	112.78	2.76	1.86	4.44	8.11	8.08	3.54	474.00
9	HCO-4-2	66.2	240.33	22.02	130.00	2.82	1.92	4.28	6.76	7.32	2.69	579.66
10	HCP-2	59.3	272.33	22.04	124.17	2.77	1.78	4.57	7.22	7.22	4.34	575.66
11	HCY-183-1	62.3	281.07	22.77	149.47	2.67	1.79	5.08	7.81	7.81	3.46	473.00
12	Hybrid-501	54.9	311.66	20.52	168.50	3.08	2.23	6.20	6.56	7.56	6.96	465.83
13	IPC-106	63.8	192.66	20.65	102.50	3.05	2.14	5.12	8.15	8.49	4.12	478.16
14	IPC-109	65.6	265.00	21.38	130.00	3.16	2.30	3.75	7.48	8.15	3.30	569.16
15	IPC-118	68.3	198.18	22.65	117.81	2.82	1.75	4.41	6.85	7.42	2.50	571.67
16	IPC-122	69.0	240.66	20.02	141.36	2.97	1.83	6.65	7.32	7.50	4.55	468.83
17	IPC-25	69.6	198.33	19.57	135.66	2.96	2.05	4.33	7.40	7.54	3.51	455.83
18	IPC-34	63.3	192.50	22.60	105.00	2.89	1.85	3.25	6.25	7.25	2.40	469.00
19	IPC-37	67.8	264.00	22.83	127.50	2.94	2.06	3.15	6.90	8.23	3.42	471.83
20	IPC-4	64.9	227.33	19.89	125.00	3.05	1.96	5.01	7.02	7.48	2.66	473.33
21	IPC-40	69.7	193.33	22.27	106.00	3.08	2.11	4.73	7.68	8.02	3.12	465.83
22	IPC-7	68.0	185.50	24.23	102.16	2.84	1.92	3.23	6.83	7.17	5.35	560.50
23	JKC	60.9	327.99	23.00	119.81	2.87	1.71	3.72	8.00	7.76	5.18	421.50
24	KTCTH-7	51.4	234.00	23.05	134.50	2.65	1.59	3.32	7.83	7.83	6.41	434.83
25	KTCTH-8	56.1	260.00	22.74	118.33	2.92	1.88	5.47	6.84	7.84	6.36	471.67
26	Nantes	50.4	323.33	20.81	132.50	2.82	1.96	5.21	7.79	7.79	5.44	484.17
27	PC-101	67.7	274.17	21.08	141.40	2.81	1.84	5.79	8.39	8.40	5.95	478.33
28	PC-15	62.1	390.00	22.39	162.50	3.15	2.15	4.34	8.01	8.15	2.97	479.00
29	PC-16	65.8	289.50	21.83	167.21	3.05	2.10	4.27	7.57	7.58	3.13	467.66
30	PC-34	70.7	241.18	22.09	120.00	2.87	1.87	6.41	6.74	7.24	4.72	460.00
31	PC-35-A	61.3	295.00	21.32	173.00	2.93	1.84	6.07	8.18	8.34	5.17	568.33
32	PC-41	70.3	335.00	21.40	154.83	2.91	1.81	5.69	7.98	7.94	2.68	526.17
33	PC-42	64.4	377.50	24.48	163.50	3.21	2.34	5.67	7.82	7.82	3.39	570.00
34	PC-43	68.1	304.83	20.12	159.50	2.74	1.89	6.33	7.87	8.16	2.66	478.33
35	PC-44	60.9	312.50	22.81	164.33.	2.63	1.87	6.17	7.42	7.55	3.33	478.33
36	PC-5	63.6	273.00	24.92	174.38	3.06	2.17	7.45	7.30	8.19	5.92	581.67
37	PC-50	64.0	380.12	24.03	182.00	2.94	1.88	7.56	7.71	7.66	6.56	579.17
38	PC-61	61.8	243.17	21.23	155.50	2.86	2.06	4.48	8.32	8.66	2.70	473.50
39	PC-76	65.7	226.67	21.93	110.59	3.04	2.09	4.66	6.43	7.27	2.70	463.33
40	PC-79	61.6	243.17	21.01	108.00	2.83	1.81	5.89	7.93	7.62	3.47	472.50
41	PC-81	60.6	198.28	21.65	112.33	2.96	1.96	7.35	8.23	8.34	4.11	505.00
42	PC-82	63.3	328.42	21.73	150.00	2.89	1.93	5.49	7.59	7.73	3.74	513.33
43	PC-83	55.5	227.33	22.69	110.00	2.97	1.99	5.03	8.78	8.78	3.54	573.33
44	PC-84	67.4	234.00	24.58	107.50	2.87	1.92	5.01	6.89	6.89	3.15	576.67
45	PC-87	68.7	185.83	21.13	105.50	2.73	1.71	4.25	6.89	7.33	4.50	481.83
46	PC-94	61.7	181.67	21.25	102.16	2.88	1.93	4.29	6.44	6.77	2.98	573.67
47	PC-96	61.9	225.17	23.57	115.66	2.92	2.00	5.83	7.19	7.20	2.81	517.00
48	PC-99	61.7	193.33	21.43	105.00	2.87	1.83	4.85	8.14	8.08	2.31	476.67
Range		50.48 - 71.21	181.67-390.00	19.57-24.92	101.33-182.00	2.58 - 3.21	1.52-2.34	3.15-7.92	6.25-8.78	6.77-8.78	2.31-6.96	455.83-581.67
CD at p=0.05		5.34	18.78	1.26	17.08	0.42	0.41	0.65	5.67	4.01	0.53	12.13

Table 2: Random Amplified Polymorphism DNA (RAPD) markers bands used in genetic diversity assessment

S.No	Primer designation	Primer sequence	Total number of bands amplified	Number of polymorphic bands	Polymorphic Information Content (PIC)
1	Oligo-625	5'-TTACCCACGC-3'	15	15	0.8906
2	Oligo-679	5'-AGTTCCAAGC-3'	11	11	0.8303
3	Oligo-688	5'-GAGGCTGGGC-3'	18	18	0.8525
4	Oligo-691	5'-TGAGTTGGGC-3'	10	10	0.8507
5	OPS-01	5'-CTACTGCGCT-3'	11	11	0.8287
6	OPS-03	5'-CAGAGGTCCC-3'	11	11	0.8421
7	OPS-04	5'-CACCCCCTTG-3'	11	11	0.8459
8	OPS-05	5'-TTTGGGGCCT-3'	10	10	0.8332
9	OPS-06	5'-GATACCTCGG-3'	10	10	0.8801
10	OPS-07	5'-TCCGATGCTG-3'	10	10	0.8548
11	OPS-11	5'-AGTCGGGTGG-3'	15	15	0.8916
12	OPS-12	5'-CTGGGTGAGT-3'	17	17	0.9205
13	OPS-13	5'-GTCGTTCCTG-3'	20	20	0.9371
14	OPS-14	5'-AAAGGGGTCC-3'	7	7	0.7805
15	OPS-15	5'-CAGTTCACGG-3'	13	13	0.8599
16	OPS-16	5'-AGGGGGTTCC-3'	19	19	0.9228
17	OPS-17	5'-TGGGGACCAC-3'	7	7	0.8378
18	OPS-18	5'-CTGGCGAACT-3'	10	10	0.7769
19	OPS-19	5'-GAGTCAGCAG-3'	14	14	0.8942
20	OPS-20	5'-TCTGGACGGA-3'	15	15	0.8998
Total			**254**	**254**	

RESULTS AND DISCUSSION

Evaluation of carrot germplasm for morphological and biochemical attributes:

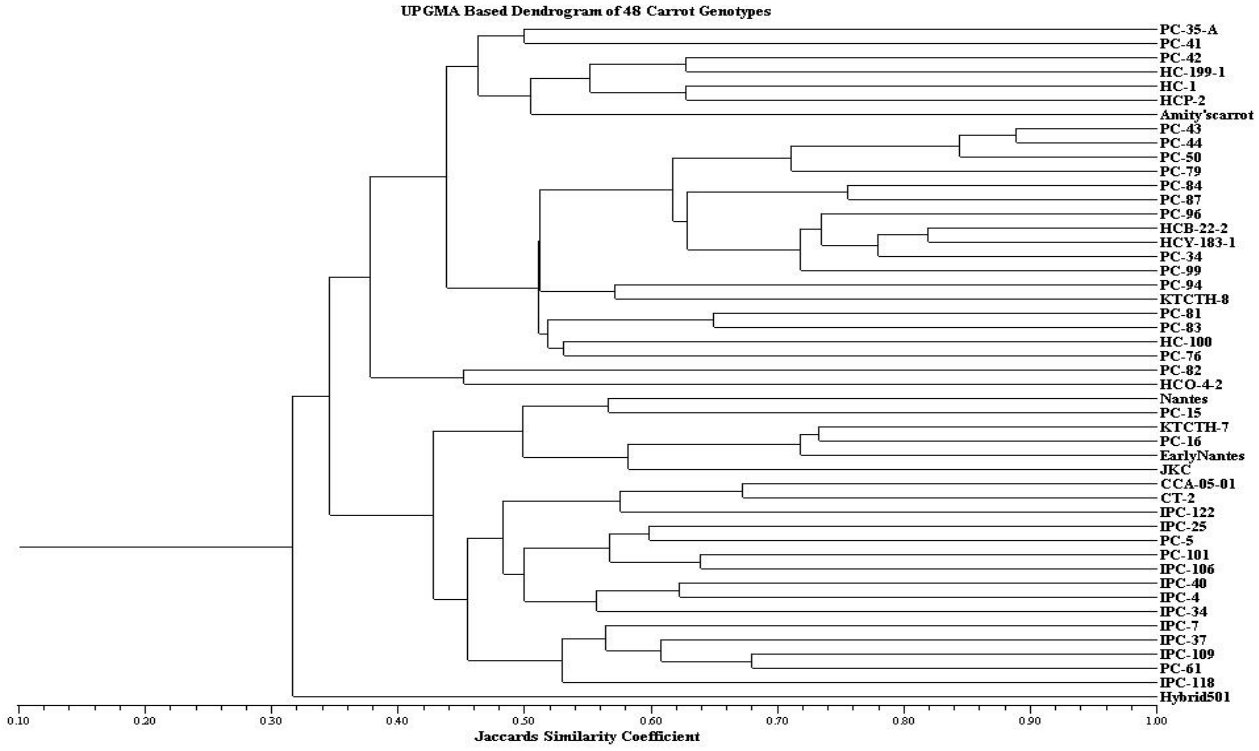
The analysis of variance for the characters evaluated revealed that the mean squares due to genotypes were highly significant for all the traits (data not shown). The mean values for various characters in 48 genotypes along with extent of diversity (range) and their respective LSD values are reported in Table 1. Minimum values for top height were recorded in Nantes (50.48 cm) genotype. Highest plant weight, root length, root weight, root girth, flesh thickness and total yield were recorded in PC-15 (390.00 g); PC-5 (24.92 cm); PC-50 (182.00 g); PC-42 (3.21 cm); PC-42 (2.34 cm) & PC-50 (7.56 kg/plot). Among quality attributes, the highest value for total soluble solids (TSS), dry matter content and juice yield were observed in PC-83 (8.78%); PC-83 (8.78%) and PC-5 (581.67 ml/kg) respectively. Beta carotene content varied from 2.03 in PC-61 to 6.96 mg/100g in Hybrid -501, a wide range in phenotypic means of both, morphological and biochemical characters was revealed. Earlier, Singh *et al.,* (2004) evaluated carrot germplasm for TSS and reported that TSS varied from 3.83% – 8.04%. Since, diversity between the parents is an important factor in determining extent of improvement. The present investigation revealed that there exists a tremendous scope for carrot genetic improvement through hybridization for most of the characters evaluated.

RAPD analysis:

Of the 25 primers screened, 5 primers amplified few bands and the bands were faint and not scorable unambiguously. On the basis of easily scorable

amplified bands, 20 primers were selected for final analysis. The primers used for genotyping the carrot germplasm along with their base sequence, total number of amplified bands and number of polymorphic bands generated by each of the primer is listed in table 2. The number of bands amplified was primer and genotype dependant and ranged from 7 in OPS-14 and OPS-17 to 20 in OPS-13. A total of 254 bands were amplified with 20 primers with an average of 12.7 bands per primer. Out of 254 bands amplified all were polymorphic (100%).

The PIC values for the 20 primers ranged from 0.83 in primer Oligo-679 to 0.94 in primer OPS-13 with an average of 0.88 for all 20 primers (Table 2). Primer OPS-19 amplified a total of 19 bands in 48 genotypes with PIC value of 0.92, whereas primer OPS-20 amplified only 15 bands with PIC value of 0.90. Thus, in the present set of genotypes, primer OPS-19 was more informative than primer OPS-20. The PIC values being high, thus the set of primers used was informative.



Cluster analysis:

The genetic relationships among the genotypes are presented in the form of a dendrogram (Fig. 1). At 32% similarity level, the dendrogram revealed five clusters. The first cluster comprised of 26 accessions, second major cluster comprised of six accessions, third of ten accessions, fourth of five accessions and fifth cluster comprised of only one accession. The cluster five consists of only one genotype i.e. Hybrid-501 from temperate region and depicts diversity of 68%.

Cluster I having 26 accessions included germplasm from different regions viz. 17 from Punjab, 7 from HAU, Hisar, 1 from SKUAST Kashmir and 1 from IARI New Delhi. It is further sub-clustered into four groups, although all these exhibited an overall similarity of 42%.

Cluster II having 6 accessions included germplasm from different regions viz. New Delhi, Punjab and SKUAST with an overall similarity of 42%.

Cluster III having ten accessions viz. two from IIVR (Varanasi), seven from IARI (New Delhi), one from Punjab and exhibited an overall diversity of 50%.

Cluster IV having five accessions i.e. four from New Delhi and one from Punjab. They exhibited an overall diversity of 46%.

It was further revealed that the major group included the genotypes both from indigenous and the exotic sources. This indicated that the geographic distribution may not be the true index of genetic diversity in carrot. This could be attributed to the fact that so far genetic resources have been freely exchanged all over the world and were exploited for crop improvement programmes. Further, recent breeding trends towards a specific plant and root type seems to have contributed considerably to genetic uniformity among the modern cultivars.

LITERATURE CITED

A. O. A. C. 1970. Association of Analytical Chemists. Benjamin Franklin Station, Washington D.C.Ed.11.

Demeke, T., Lynch, D. R., Kawchuk, L. M., Kozub, G. C and Armstrong, J. D. 1996. Genetic diversity of potato determined by RAPD analysis. *Plant Cell Reproduction.* **15:** 662-667.

Jaccard, P. 1908. Nouvelles researches Sur la distribution florale. *Bull. Soc. Vaud Sci. Nat*., **44:** 233-270.

Paul, S., Wachira, F. N., Powell, W. and Waugh, R. 1997. Diversity and genetic differentiation among population revealed by RAPD markers. *Theoretical and Applied Genetics*. **94:** 255-263.

Rohlf, F. J. 1998. NTSYS-PC Numerical Taxonomy and Multivariate System, Version 2.0. Applied Biostatis Inc., New York.

Saghai-Maroof, M. A., Soliman, K. M., Jorgensen, A. R and Allard, R. W**.** 1984. Ribosomal DNA spacer length molymorphism in barley: inheritance, chromosomal location and population dynamics. *Proc. Nat. Acad. Sci., USA*. **81:** 8014-8018.

Singh, B., Pal, A. K., Sudhakar, P. and Rai, M. 2004. Genotypic variation for quantitative and qualitative traits in Asiatic carrot. *Indian Journal of Plant Genetic Resource*. **17(3):** 181-184.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., Vande Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M. and Zabeau, M. 1995. AFLP: a new technique for DNA fingerprinting. Nucleic *Acids Research*. **23:** 4407-4414.

Welsh, J. and McClelland, M. 1990. Fingerprinting genomes using PCR with arbitrary primers. *Nucleic Acids Research*. **18:** 7213-7218.

William, J. G. K., Kubelik, A. R., Livak, K. J., Rafalski, J. A. and Tingey, S. V. 1990. DNA Polymorphism amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research*. **18:** 6531-6535.

Yamamoto, T., Nishikawa, A. and Oeda, K. 1994. DNA polymorphism in Lactuca sativa L. amplified by arbitrary primed PCR. *Euphytica*. **78:** 143-148.

Yonemoto, Y., Chowdhury, A. K., Koto, H. and Macha, M. M. 2006. Cultivars identification and their genetic relationships in *Dimocarpus longan* subspecies based on RAPD markers. *Scientia Horticulturae*. **109:** 147-152.