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AGRO-MORPHOLOGICAL ASSESSMENT AND SEED PROTEIN PROFILING IN CARROT (*DAUCUS CAROTA* L.) GERMPLASM

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Abstract

Carrot (*Daucus carota* L.) is an important vegetable, and one of the main sources of dietary pro-vitamin A carotenoids. Carrot accessions were investigated for genetic diversity using qualitative and quantitative morphological traits and biochemical analysis at National Agricultural Research Centre (NARC), Islamabad, Pakistan. A set of 33 carrot accessions were subjected to agro-morphological evaluation which revealed that said genotypes have great variation for yield contributing traits (root's weight, length and width) and various quality attributes i.e., root shape and color. Relationship among various traits based on correlation analysis showed some key facts with significant ($p \leq 0.05$ and $p \leq 0.01$) negative and positive correlation indicating the utility of the existing carrot germplasm. Cluster analysis divided and placed the accessions in five clusters, showing variations among accessions collected from geo-climatically diverse localities of Pakistan. Multivariate analysis enunciated a deep insight to understand variability pattern and relationship among carrot germplasm acquired from diverse ecologies of Pakistan. However, low variability with monomorphic banding was observed based on sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis. Distinct root trait needs to be identified and further evaluation should be carried out on diverse genetic base on the basis of agro-morphological traits. Carrot accession 20238 from Khanewal area was found to be the most distinct accession as reflected by multivariate analysis. The promising genotypes could be used for the development of high yielding genotypes in future breeding program.

Key words: Genetic diversity, Evaluation, Agronomic traits, Targeted selection, Carrot

Introduction

Carrot (*Daucus carota*), a member of family Umbelliferae, is one of the most important winter vegetables. The history about first evidence of carrot (used as a food crop) can be found in the Iranian plateau (present day Afghanistan, Iran and Pakistan) and the Persian Empire in the 10th century AD (Stolakzyk & Janik, 2011). Therefore, Pakistan might be centre of diversity for said vegetable. It can be consumed raw or cooked in curries, also a part of sweet meals and pickles. Carrot is known as poor man apple as it contains many nutrients and has high market value. It is rich source of vitamin-A whose precursor is carotene. The vitamins viz. thiamine, riboflavin and some content of sugar are also found in carrot. Carrot is cultivated in many countries as temperate crop, and 15 to 20°C is the favorable temperature for its better growth (Illyas *et al*., 2013).

Daucus carota subsp. *sativus,* the only cultivated carrot, is the subspecies out of total 12 subspecies. Fairly large morphological variations have been observed due to more frequent hybridization within the *D. carota*. Genetic diversity among wild and cultivated carrot can be assessed by isozymes and morphological analysis (Nakajima *et al*., 1998).

Genetically diverse germplasm and its delineation for desired traits is necessary for meaningful crop improvement (Huma *et al.,* 2020). The evaluation supported by biochemical markers at protein level as well as morphological characterization make it more reliable and acceptable (Akbar *et al.*, 2010). Genetic diversity assessment based on phenotypic characterization has been considered to be a practical way for germplasm evaluation and utilization (Aamir *et al*., 2016).

Past studies revealed that the SDS-PAGE electrophoresis banding of total seed protein have been used successfully to resolve evolutionary and taxonomic problems in few plant species (Zahoor *et al*., 2015). An effective breeding program in any crop mainly depends upon the availability of genetic diversity. Among the biochemical techniques, SDS-PAGE is most widely used due to its simplicity and validity in determining genetic structures of crop germplasm (Ghafoor *et al*., 2005). Protein analysis by SDS-PAGE has been extensively used for identification of seed protein profile of a number of plants as it is hardly affected by environment. It not only helps in characterization and identification of the variability in genotypes and wild species but also play important role in determination of phylogenetic relationships and rate of out-crossing (Zahoor *et al*., 2015).

In light of the above facts, the present study was planned with the objectives i.e., a) investigation of the genetic variability in cultivated *Daucus carota* for agro-morphological traits, total seed protein, b) correlation associations among different agro-morphological variables and c) the degree of dissimilarity or similarity among carrot accessions using Multivariate statistical approach.

Materials and Methods

The present research work was conducted during 2016-17 at Bio-resources Conservation Institute (BCI), National Agriculture Research Centre (NARC), Islamabad, Pakistan. Thirty-three carrot accessions were obtained from National Gene-bank of Pakistan (NGP), BCI, NARC, Islamabad, Pakistan. The list of accessions along with passport data is provided in Table 1. Sowing was done manually on September 25, 2016. Each accession was planted in two rows of 3 m length with row to row distance of 75 cm. The data were collected for different 18 each qualitative and quantitative characters (Table 2). The data for plant height and seed relating parameters were recorded at maturity during April-2017.

Table 1. Passport information of carrot (*Daucus carota*) germplasm.

S. No.	Accession No.	Location / District	S. No.	Accession No.	Location / District
1.	17378	Sahiwal	18.	20336	Gujranwala
2.	20063	Rawalpindi	19.	20354	Mianwali
3.	20098	Haripur	20.	20404	Multan
4.	20134	Attock	21.	20418	Vihari
5.	20146	Attock	22.	20450	Lodhran
6.	20159	Chakwal	23.	20466	Bahawalpur
7.	20170	Chakwal	24.	20471	Bahawalpur
8.	20192	Lahore	25.	20477	Mansehra
9.	20205	Sheikhupura	26.	27484	Sibi
10.	20218	Multan	27.	27486	Sanjawi
11.	20238	Khanewal	28.	27487	Mastung
12.	20244	Multan	29.	Local-1	Unknown
13.	20267	Bahawalpur	30.	Local -2	Unknown
14.	20280	Bahawalpur	31.	Local -3	Unknown
15.	20284	T.T.Singh	32.	Local -4	Unknown
16.	20289	Hafiz Abad	33.	T-29	Check variety
17.	20316	Mansehra			

DNA extraction: Ten seeds of each accession were crushed and grinded. Seed flour (10 mg) was taken in 1.5 ml Eppendorf tube. To extract protein from flour, 400 µL extraction buffer was added to the flour as an extraction liquid and mixed thoroughly. Sample tubes were centrifuged at 12000 rpm for 10 minutes, extracted proteins were recovered as clear supernatant and stored in a freezer (-20ºC). Seed protein was analyzed through slab type SDS-PAGE as per Laemmli (1970) using 12.25% polyacrylamide gel. Separation and staking gel were prepared and incorporated into apparatus. Electrophoresis was conducted at 150 V for approximately two hours until Bromophenol blue marker reached at the bottom of gel. After electrophoresis, the gel was stained in staining solution for one hour. After staining, the gel was de-stained overnight, and dried with cellophane sheets. Data were recorded for presence and absence of protein bands as '1' and '0', respectively.

Statistical analysis

The data recorded for quantifiable morphological traits was used for multivariate analysis. Distance matrix reflecting Euclidean dissimilarity coefficients was prepared that was further used to perform cluster analysis using numerical taxonomy-based software NTSYS-pc (Numerical Taxonomy System, version 2.0; Rohlf, 2005). Cluster analysis was performed to study the grouping pattern of various carrot genotypes. Descriptive statistics was employed to study the variance for different the quantitative characters in carrot accessions. The association among various traits was investigated using simple correlation coefficients (Aamir *et al*., 2016).

Results and Discussion

Agronomic traits evaluation is an important step in description and classification of crop germplasm as improvement depends on the magnitude of genetic variability. It also enables the researchers to plan and use the appropriate gene pools for specific attributes in crop improvement.

Variations among the accessions for various agro-morphological traits: The basic statistics for quantitative traits revealed moderate to high variation for various traits in carrot accessions (Table 3). Highest variation was recorded for root weight per plant, while substantial variation for mature leaf length (with/without petiole), mature leaf width, branch length and total umblets per plant. Considerable variability was also recorded for root length and root width (top and middle).

Carrot is normally grown for its edible root and significant variation was observed for said trait makes it prominent for its utilization in breeding. A varying degree of differences were depicted for yield contributing traits in carrot accessions. For seed length, seed width, primary leaf length and seed weight displayed low genetic variability in the germplasm assayed. The scope of selection based on the traits with low variation in the existing germplasm revealed narrow genetic base; hence large-scale testing of germplasm with diverse variability in these traits needs to established for sound breeding program. Yield factors are mostly influenced by genetic structure and environmental conditions like differences in soil moisture, light intensity, rainfall, temperature and day length (Manosa, 2011). Some characters are also specific to species i.e., specie dependent, as flower color and leaf characters are the features of taxonomic classification of species (Riaz *et al*., 2011). The morphological diversity among carrot genotypes shows that morphological differences are due to agronomic traits. Therefore, yield performance is related to vegetative characters (Fanlegue *et al*., 2017).

Table 2. Quantitative and qualitative parameters with their description.

S. #	Qualitative traits	Description of the trait	S. #	Quantitative traits	Description of the trait
1.	Petiole shape	3. Slightly colored, 5. Intermediate, 7. Strongly color	19.	Length of basel primary leaflet (cm)	Recorded from the base of primary leaflet by Vernier caliper
2.	Petiole hairiness	3. Sparse, 5. Intermediate, 7. Dense	20.	Petiole thickness (mm)	The data was recorded with the help of Vernier caliper at the thickest point at the time of full development of the foliage.
3.	Leaf growth habit	1. Prostrate, 2. Erect, 3. Semi-erect	21.	Mature leaves per plant (No.)	The data of mature leaves of carrot were collected on the basis of visualization that how many leaves are mature on single plant. This data was recorded 50% after germination.
4.	Leaf color intensity	Explain from descriptor	22.	Mature leaf length (cm)	The distance from the base to the tip of the individual leaf will be measured. The data for leaf length will be measured on 3 leaves of the main plant and then average will be taken.
5.	Leaf dissection	3. Slightly dissected, 5. Intermediate, 7. Highly dissected	23.	Mature leaf width (cm)	Data for mature leaf width will be recorded by measuring the widest point of the same leaf which was selected to use for leaf length. The data will be recorded approximately 3 leaves and then calculate their average.
6.	Leaf color	Explain from descriptor	24.	Root length (cm)	This data was recorded by taking the length of root at three points at top, at shoulder and at bottom with the help of scale
7.	Root uniformity	1. Low, 2. Moderate, 3. High	25.	Root weight (g)	The data of root weight is taken by electronic balance in grams
8.	Root surface	1. Smooth, 2. Coarse, 3. Dimpled, 4. Ridged	26.	Root diameter at shoulder (cm)	The data was measure at 2-3 cm below the leaf attachment with the help of Vernier caliper
9.	Root branching	3. Sparse, 5. Intermediate, 7. Dense	27.	Inner core diameter at shoulder (mm)	The data was recorded at the widest point of the root with the help of vernier caliper
10.	Root shape	1. Round, 2. Obviate, 3. Obtriangular, 4. Oblong, 5. Tapering	28.	Total umbels per plant (No)	This was recorded by counting total umbel per plant in single accession. So, the data of total umbels were taken from 5 plants in one accession
11.	Outer core pigmentation /color	The data of pigmentation/color was recorded on visual basis at maximum diameter.	29.	Width of primary open umbel (cm)	This data was recorded by selecting the primary open umbel from the main stem and then take their width by scale
12.	Stem hairiness:	3. Sparse, 5. Intermediate, 7. Dense	30.	Leaves below the primary umbel (No)	Its mean how much leaves are below the umbel which are on the main stem. The data is recorded by selecting primary umbel and then count the leaves below the selected primary umbel
13.	Stem growth habit	Data on stem growth habit was recorded of five plants of selected accession by visualize.	31.	Seed width (mm)	The data for seed width was recorded by measuring the width of 3 seeds per umbel of selected plant at widest point by Vernier caliper and then calculate their average.
14.	Accession longevity**:**	3. Annual, 5. Biennial, 7. Both	32.	Seed length (mm)	Stem length was measured from the ground to the tip of the individual plant. The data for stem length was measured from the 5 plants of per accession in the field and then average will be taken.
15.	Flowering synchrony in plants	3. Low, 5. Intermediate, 7. High	33.	100-Seed weight (g)	It was determined by taking two umbels from single plant and collected their seed and count 100 seeds from single umbel and then calculate their average `` then weight the 100 seeds of single umbel.
16.	Flowering pattern with in plants	This depend on the flowering synchrony among plants if the flowering is low or intermediate then flowering pattern is indeterminate and if it is high then it became determinate. So, this data is taken on basis of flowering synchrony among plants	34.	Branches per plant (No)	This was recorded by counting the total number of branches on main shoot of the plant at the time of harvest.
17.	Umbel type	3. Simple, 5. Compound, 7. Both	35.	Mean branch length per plant (cm)	This data was recorded by selecting at least 3 branches on main stem and then calculate their average.
18.	Umbel shape	1. Convex, nest like umbel, 2. Flat-topped umbel with straight rays	36.	Mean stem length of leaves developed on stem (cm)	The data was recorded by measuring the length of leaves on main stem by scale. The data was taken at least three leaves from five plants per accession and then calculate the average of per plant.

Table 3. Basic statistic for different quantitative traits in carrot germplasm.

Variables	Mean + SE	Variance	Minimum	Maximum
Length of basal primary leaflet (cm)	13.840 ± 5.03	8.42	8.54	20.44
Petiole thickness (mm)	3.868 ± 0.101	0.34	2.92	5.00
Number of mature leaves per plant	9.726 ± 0.338	3.98	5.00	16.00
Mature leaf length without petiole (cm)	22.686 ± 1.748	90.60	0.00	34.54
Mature leaf length with petiole (cm)	48.354 ± 1.166	46.65	36.06	63.30
Mature leaf Width (cm)	27.919 ± 1.335	61.47	9.40	52.55
Number of branches per plant	6.303 ± 0.322	3.62	3.60	13.80
Mean branch length per plant(cm)	62.561 ± 1.456	74.03	50.60	83.13
Mean stem length of leaves developed on stem (cm)	20.342 ± 0.581	11.60	13.70	28.73
Root length (cm)	20.922 ± 0.611	13.09	12.20	26.66
Root Diameter (top) cm	5.219 ± 0.336	3.92	2.78	12.40
Root Diameter (middle) cm	3.949 ± 0.471	7.33	1.84	8.44
Root Diameter (bottom) cm	1.718 ± 0.170	0.95	1.16	5.26
Root weight per plant (g)	159.03 ± 11.187	4163.53	46.00	286.00
Outer core diameter at shoulder (mm)	5.046 ± 0.335	3.93	2.68	14.50
Outer core thickness at shoulder (mm)	14.560 ± 0.884	27.34	6.44	32.36
Inner core diameter at shoulder (mm)	20.017 ± 1.037	35.48	5.00	31.30
Total number of umbels per plant	15.829 ± 1.075	39.98	5.00	34.00
Width of primary open umbel (cm)	7.514 ± 0.303	3.10	5.00	12.80
No. of leaves below the primary umbel	6.382 ± 0.200	1.39	4.80	11.00
Seed length (mm)	6.382 ± 0.048	0.08	4.42	5.54
Seed width (mm)	1.827 ± 0.104	0.38	1.52	2.21
Total umblets per plant	49.300 ± 1.542	81.76	24.00	64.00
100-Seed weight (g)	0.476 ± 0.030	0.03	0.29	0.85

Frequency distribution of qualitative characters has been presented in Table 4. Accessions life cycle was biennial in all the accessions. Green color at shoulder was also not found in all accessions. The predominant leaf growth habit observed was erect whereas root skin was light to dark in color. Leaf color was green, flower pattern was intermediate. It was of interest to note that the observed root traits were much desired including absence of root splitting, and tapering root shape was also observed in almost all the accessions. Wide range of inner and outer core pigment comprising white, yellow, orange and red was recorded in the germplasm investigated revealing the diversity in pigmentation that need further detailed investigation on beta-carotenes and alpha-carotene types. Past findings revealed that carrot gets its characteristic, bright orange color from β-carotene, and lesser amounts of α-carotene, γ-carotene, lutein, and zeaxanthin are involved in its color depiction (Abdel *et al*., 2013).

Root uniformity was low to high, however, predominant was moderate. All the other characters not showed marked differences and revealed mixed frequency distribution of the accessions among these characters. Root attributes are important selection criteria for root crops like carrot being the major sole plant part used. The present germplasm also represented diversity in root shape, size, colour, and texture. The extent of genetic variation in germplasm relates to crop improvement, however, increased variability increases the chance of effective selection of desirable traits of germplasm (Meghashree *et al*., 2018).

Association among various traits in carrot: Correlation is considered as an important feature for breeding programs as it authenticates the probabilities for selection of desirable characters in a set of genotypes (Aamir *et al*., 2016). The relationship among various traits computed through simple correlation in carrot germplasm which revealed useful information on mutual trait association (Table 5). Various attributes depicted significant positive associations with each other for very useful yield related traits of roots that can impact targeted breeding and selection strategies positively. Root length was positively associated with length of basal primary leaflet, mature leaves per plant, mature leaf length with petiole and significant ($p \leq 0.01$) with mature leaf length without petiole. Similarly, another yield contributing trait i.e., root weight also displayed significant ($p \leq 0.01$) positive association among traits i.e., length of basal primary leaflet, petiole thickness, mature leaf length with petiole, mature leaf width, inner core diameter, and root length.

In case of other growth traits, the mature leaf has shown significant ($p \leq 0.05$) positive association with length of basal primary leaflet, and petiole thickness. Seed width also revealed significant ($p \leq 0.01$) positive association with total umbels per plant as well as leaf width, however, it was negatively correlated with total umblets per plant. Similarly, width of primary open umbel was significantly negatively associated with root weight showing the yield reducing impact of bolting and primary inflorescence development. Some characters also showed zero correlation by having no linear association with each other i.e., 100-seed weight with stem length and length of basal primary leaflet, root diameter (top) with mean branch length per plant, and root diameter (middle) with petiole thickness.

Table 4. Frequency Distribution of Qualitative plant traits in Carrot (*Daucus carota*).

Parameters	Frequency (%)	Parameters	Frequency (%)
Petiole shape		Outer core pigment	
Round	37.85	White	42.94
Semi-round	53.11	Yellow	6.78
Flat	9.04	Orange	31.64
Petiole hairiness		Red	18.64
Absent	17.51	Inner core pigment	
Sparse	36.17	White	36.72
Intermediate	32.77	Yellow	55.93
Dense	13.56	Orange	2.82
Leaf growth habit		Red	4.52
Prostrate	1.69	Green color at shoulder	
Erect	93.79	Absent	177
Semi-erect	4.52	Present	0
Leaf dissection		Root uniformity	
Slightly dissected	16.95	Low	16.95
Intermediate	56.50	Moderate	68.93
Highly dissected	26.55	High	14.12
Leaf color		Root shape	
Absent	3.96	Obtriangular	2.82
Green	91.53	Oblong	2.82
Grey green	2.8	Tapering	94.35
Purple green	1.69	Convex	41.81
Leaf color intensity		Flat	58.19
Absent	15.82	Root shoulder shape	
Light	10.17	Flat	12.43
Intermediate	74.01	Flat to round	70.06
Stem growth		Round	17.51
Prostrate	21.47	Root splitting	
Erect	21.47	Absent	91.53
Semi erect	57.06	Low	3.95
Stem hairiness		Intermediate	4.52
Sparse	18.64	Flowering pattern	
Intermediate	31.07	Determinate	19.77
Dense	50.28	Indeterminate	80.23
Root surface		Flowering Synchrony	
Smooth	35.03	Low	18.64
Coarse	57.63	Intermediate	61.58
Dimpled	7.34	High	19.77
Root branching		Accession longevity	
Absent	44.06	Annual	0
Sparse	55.94	Biennial	100
Root skin color intensity		Umbel type	
Light	93.79	Simple	43.50
Dark	6.21	Compound	29.94
Flesh color intensity		Both	26.55
Pale/dull	13.56	Umbel shape	
Intermediate	68.49	Convex	41.81
Bright	16.95	Flat	58.19

Yield and root quality traits are very important factors that are mostly considered for crop improvement in carrot. Correlation among various parameters particularly for yield contributing traits is very important because this may provide the sound basis for further improvement in the carrot. Current study also provided the deep insight on the trait association that would surely be very helpful. Crop improvement is relatively proportional to magnitude of genetic variation in germplasm. Greater the variability greater are the chances of effective selection for desirable traits. Genotypic and phenotypic coefficients of variation are imperial in detecting the extent of variation in germplasm. Heritability is a portion of phenotypic correlation which is transmitted from ancestors. Heritability is foremost important in judging the variation for specific character whether it is due to environment or genotype (Teli *et al*., 2017).

Table 5. Trait association in carrot germplasm based on simple correlation coefficients.

Trait	LBPL	PT	ML/P	MLLP	MLL	MLW	B/P	MBL/P	MSL	RL	RD(T)	RD(M)	RD(B)	RW	OCDS	OCTS	ICDS	U/P	PUW	LBPU	SL	SW	TU/P
PT	0.65**																						
ML/P	0.15	0.11																					
MLLP	0.25	0.27	0.32																				
MLL	0.62**	0.46*	0.07	0.01																			
MLW	0.74**	0.47**	0.06	-0.14	0.58**																		
B/P	-0.20	-0.29	0.11	0.12	-0.12	-0.49**																	
MBL/P	-0.14	-0.08	-0.24	-0.04	0.04	-0.03	0.15																
MSL	0.12	0.07	0.17	0.10	-0.01	-0.02	-0.16	-0.34															
RL	0.42*	0.28	0.36*	0.54**	0.36*	0.04	0.28	-0.05	0.05														
RD(T)	0.03	0.01	0.33	0.15	-0.08	0.01	0.41*	0.00	-0.12	0.24													
RD(M)	0.03	0.00	0.06	0.06	0.03	0.03	0.43*	0.25	-0.19	0.15	0.72**												
RD(B)	-0.03	0.11	0.25	0.09	-0.10	-0.02	0.21	0.09	-0.01	0.16	0.69**	0.64**											
RW	0.66**	0.53**	0.31	0.28	0.56**	0.54**	-0.01	0.15	-0.11	0.58**	0.34	0.23	0.26										
OCDS	0.30	0.31	0.57**	0.08	0.36*	0.23	-0.07	0.02	-0.03	0.15	0.15	0.09	0.09	0.47**									
OCTS	-0.03	-0.04	0.24	0.18	-0.03	-0.29	0.60**	-0.06	-0.15	0.40*	0.03	0.00	0.09	0.12	-0.01								
ICDS	0.47**	0.29	0.36*	0.43*	0.30	0.47**	-0.10	0.25	0.00	0.35*	0.29	0.19	0.22	0.71**	0.49**	0.03							
U/P	0.19	-0.03	0.14	0.09	0.03	0.32	0.08	-0.20	-0.33	-0.12	0.23	0.07	-0.04	0.05	0.08	0.09	0.27						
PUW	0.10	-0.07	-0.06	-0.29	-0.05	0.13	-0.17	-0.09	-0.13	-0.33	-0.25	-0.22	-0.17	-0.37*	0.10	-0.08	-0.08	0.14					
LBPU	-0.04	-0.01	0.09	0.08	-0.08	-0.05	0.17	0.24	0.02	0.06	0.12	-0.06	0.12	0.08	0.09	0.04	0.20	0.15	-0.06				
SL	-0.03	0.17	0.26	0.18	-0.02	-0.01	0.04	-0.16	0.21	0.28	0.18	0.01	0.25	0.06	-0.10	0.21	0.06	0.07	-0.24	0.29			
SW	0.18	0.05	0.08	-0.43*	0.23	0.54**	-0.11	0.00	-0.05	-0.13	0.05	0.01	-0.07	0.16	0.02	-0.05	0.21	0.54**	0.05	0.11	0.06		
TU/P	-0.17	-0.01	-0.03	0.13	-0.12	-0.30	-0.02	0.07	-0.13	-0.05	-0.13	-0.22	-0.18	-0.12	0.23	-0.01	-0.02	-0.17	0.08	0.18	-0.15	-0.51**	
HSW	0.00	0.18	0.02	0.01	0.21	0.04	0.04	0.13	-0.23	0.10	0.18	0.11	0.09	0.39*	0.44*	0.27	0.21	-0.01	-0.25	0.06	0.00	-0.04	0.13

LBPL: Length of basal primary leaflet (cm), PT: Petiole thickness (mm), ML/P: Number of mature leaves per plant, MLLP: Mature leaf length without petiole (cm), MLL: Mature leaf length with petiole (cm), MLW: Mature leaf Width (cm), B/P: Number of branches per plant, MBL/P: Mean branch length per plant(cm), MSL: Mean stem length of leaves developed on stem (cm), RL: Root length (cm), RD(T): Root Diameter (top) cm, RD(M): Root Diameter (middle) cm, RD(B): Root Diameter (bottom) cm, RW: Root weight per plant (g), OCDS: Outer core diameter at shoulder (mm), OCTS: Outer core thickness at shoulder (mm), ICDS: Inner core diameter at shoulder (mm), U/P: Total number of umbels per plant, PUW: Width of primary open umbel (cm), LBPU: No. of leaves below the primary umbel, SL: Seed length (mm), SW: Seed width (mm), TU/P: Total umblets per plant, HSW: 100-Seed weight (g).

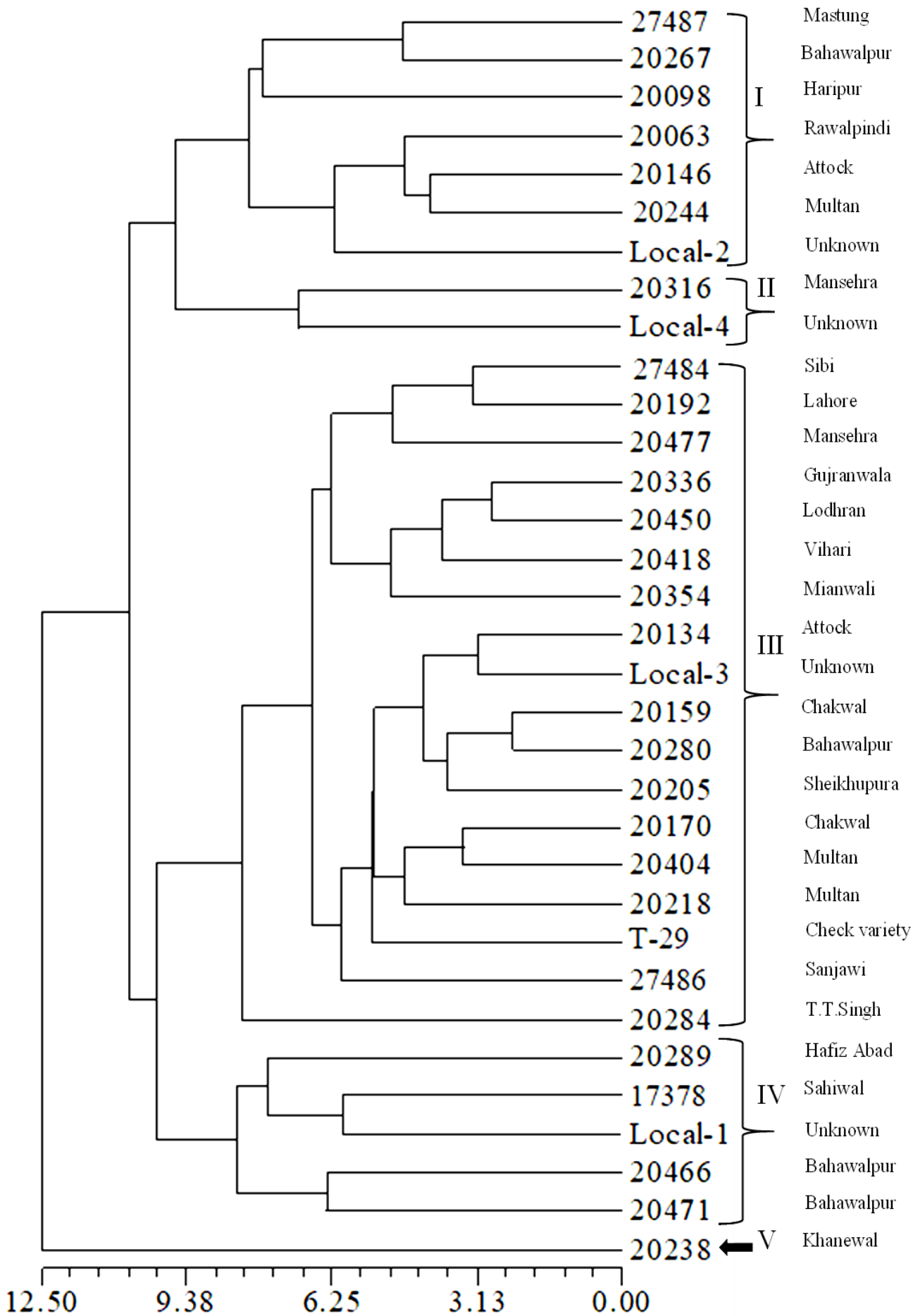

Fig. 1. Association among carrot germplasm using cluster analysis.

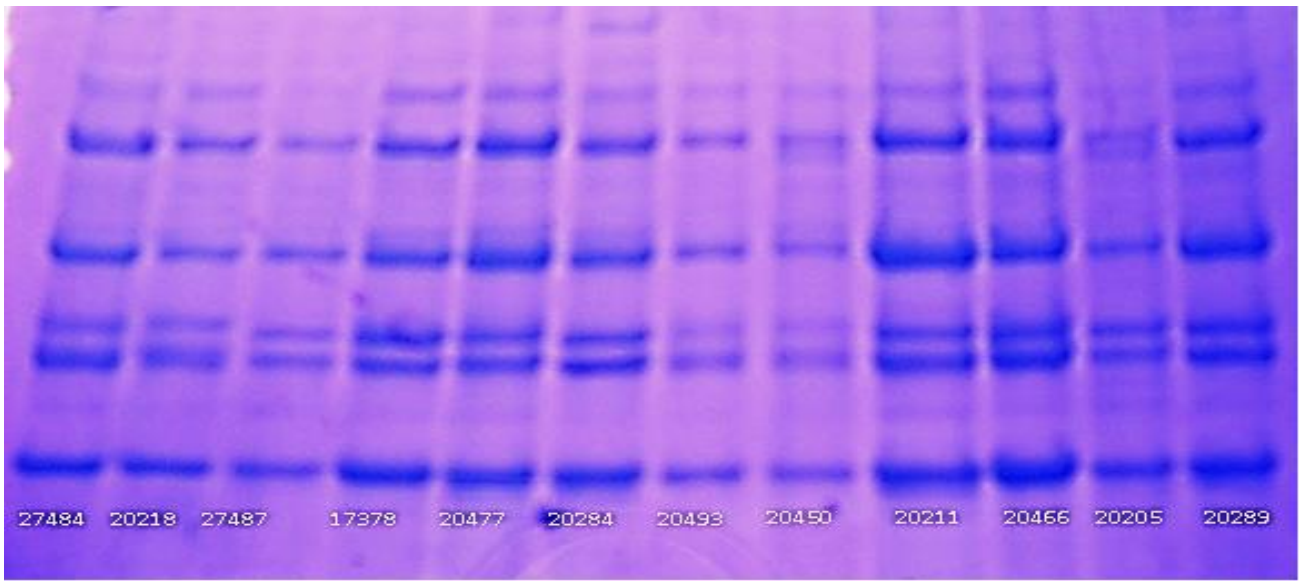

Fig. 2. Banding pattern of carrot germplasm revealed through SDS-PAGE Gel Electrophoresis.

Genetic relationship among carrot accessions: Cluster analysis divided all the carrot accessions into five major groups (Fig. 1). The number of accessions assigned to each cluster were i.e., cluster-I (7 accessions), cluster-II (2 acc.), cluster-III (18 acc.), cluster-IV (5 acc.), and cluster-V (1 acc.). The large number of 18 accessions were grouped in cluster-III while cluster-V comprised only one genotype. It was observed that the assignment of accessions into clusters was based on mutual similarities and not by their association with origin of collection. Thus it was not possible to ascertain the geographic origin of the four accessions of the unknown origin. The most closeness in the dendrogram was observed between accession 20159 and 20280 which were collected from Chakwal and Bahawalpur Districts, Pakistan respectively; though province is same but districts are quite apart from each other.

On the other hand, accession 20238 was collected from District Khanewal, also remained distinct from rest of the germplasm. The cluster-III was the largest group of carrot accessions and contained genotypes from diverse ecologies of Pakistan belonging to provinces of Balochistan, Punjab and Khyber Pakhtunkhwa, along with T-29 (as check variety) was also found in the cluster-III. However, this cluster was further divided into two sub-clusters; sub-cluster III-A contained seven genotypes (27484,20192, 20477, 20336, 20450, 20148 and 20354) representing collection origin from three provinces. Sub-cluster III B contained ten genotypes (20134, Local-3, 20159, 20280, 20205, 20170, 20404, 20218, T-29, 27486). The Accession 20284 from T.T. Singh remained distinct from the whole cluster III hence remained separate from rest of the cluster. The accessions within the same cluster IV i.e., accessions 20466 and 20471, representing collection from District Bahawalpur, may share a high proportion of similarity for agronomic characteristics revealing common ancestry (Buckseth & Singh, 2016). However, such close relationship in clusters also indicated common lineage that may lead to narrow genetic basis when included in breeding programs (Aamir *et al.*, 2016). This fact is also observed by the presence of largest number of accessions in cluster III having the similar background as check variety T-29 being widely grown in Pakistan. Whereas, the material collected from Bahawalpur appeared in three separate clusters showing greater divergence. Genetic advancement, variability and heritability are effective indicators of genotype selection among highly variable germplasm on phenotypic basis. Heritability indicates the influence of environmental factors on genotype expression (Meghashree *et al*., 2018).

Though carrot germplasm under investigation comprising 33 accessions, however, the list of descriptors for which it was assayed is quite long enlisting more than 35 traits. The cumulative picture of the diversity profile, thus, makes it more reliable as it is not merely based on few field parameters. Multivariate analysis envisages a deep insight to understand variability pattern and relationship among germplasm acquired from diverse ecologies of Pakistan. It is important to note that three carrot genotypes collected from Balochistan were not grouped together and remained dispersed in different clusters. Similarly, four carrot genotypes with unknown collection origin were also appeared distinct and thereby appeared into four different clusters. It emphasis the need that the genotypes representing diverse base should be incorporated into carrot breeding on priority basis. The germplasm investigated is collected from Pakistan and diversity revealed is also due the fact that Afghanistan is the centre of diversity for wild carrot (Stolarczyk & Janick, 2011). Another past study also documented that Asian carrot gene pool has higher genetic diversity (Baranski *et al*., 2012). However, many wild relative populations of carrot exist in our country depicting that Pakistan is also the centre of diversity of carrot.

Seed protein profiling: The formation of banding pattern of seed protein is managed by genes and gene complexes, as protein is primary product of structural gene. Changes involved in coding base sequences, may lead to changes in primary structure of protein. Even the addition/deletion of a single amino acid could result in marked effects on

migration of protein under an applied electric field during electrophoresis (Buckseth & Singh, 2016). Total seed protein profile based on SDS-PAGE analysis of carrot germplasm generated 12 fragments (Fig. 2). The seed protein banding patterns exhibited low variability amongst genotypes assayed. Bands were variable in their intensities which denote the accumulation of protein peptides at those sites with particular molecular weight. The low variation for seed protein in genotypes might be due to the purity or homogeneity of genotype for these traits (Odeigah *et al*., 1999). These findings confirmed the narrow genetic base of accessions for total seed protein. Also, there is no documented information on carrot seed protein profile, therefore, it is not possible to draw final conclusion. However, further screening of large and diverse collection of carrot germplasm is suggested that may ascertain the seed protein variability in cultivated and wild carrot types.

Conclusion

The present study provides the cumulative picture of the diversity profile of carrot germplasm based on field as well as SDS-PAGE analysis. Substantial variation was revealed by the carrot germplasm for yield contributing traits (root's weight, length and width) and various quality attributes i.e., root shape and color based on agro-morphological evaluation. However, seed protein profile revealed low variability. Multivariate analysis and similarity coefficients provided a good insight into the relationship among various accessions and association among traits, respectively. However, agro-morphological traits were not found related to the geographic origin; which also limits to determine association based on origin of collection. This might be the first study that reports the diversity profile of carrot in Pakistan. Such information is quite valuable for identifying some particular accessions or traits for prioritizing those in future breeding programs.

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