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Exploring morphological diversity in carrot (Daucus carota l.) germplasm: A multivariate analysis approach

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ABSTRACT

This study looks at how quantitative parameter variation changes over two years in different carrot accessions and the genetic diversity that exists in a group of accessions that come from different parts of the world. An analysis of variance (ANOVA) was conducted to assess the significance of different factors, including the block effect, treatment effect, and check effect. The result indicated a significant mean sum of squares across various sources of variation. Both adjusted and unadjusted block and treatment effects were significant for all traits, while the check effect was significant for more. During both years, there was moderate to high variation in quantitative parameters for numerous quantitative traits among carrot accessions. Mature leaf length showed the highest variation, followed by mature leaf width, leaf area, root weight, leaf weight, and plant biomass. Principal component analysis revealed distinct patterns in the contributions of parameters during both years. Plotting carrot accessions on the scatter plot using the first three principal components also revealed an informative spread of accessions. A notable increase in variation during the second year was observed. Moreover, accessions from different continents displayed varying degrees of genetic diversity, with Asian accessions exhibiting the highest levels. These findings underscore the importance of global germplasm collection in breeding programs aimed at enhancing crop resilience and productivity. Leveraging this genetic diversity through advanced breeding strategies holds promise for developing cultivars capable of adapting to changing environmental conditions and meeting the demands of sustainable agriculture.

Contribution/Originality: The current study provides a holistic view of the genetic diversity profile of a diverse group of carrot accessions representing more than 20 countries. This profiling lays the groundwork for future crop improvement strategies aimed at increasing carrot quality and yield.

1. INTRODUCTION

Carrot (Daucus carota) is one of the most significant root herbal crops. It is a diploid species belonging to the Umbelliferae family, with nine relatively short, uniform-length chromosomes (2n = 18). The Umbelliferae family comprises about 250 genera and contains about 2,500 species, such as caraway, dill, chervil, cumin, fennel, coriander, parsley, parsnip, celery, and anise. In the Umbellifera family, carrot is the major genus; regarding the new assessment, it comprises a further 25 species (Que et al., 2019). Vegetables are the greatest source of essential micronutrients for the human body. Compared to other vegetables, carrot is a short-duration crop. It occupies the 3rd position among winter vegetables grown in Pakistan (Noor et al., 2020).

Carrot is a vital food in human nutrition because its bioactive ingredients may be useful to a larger number of users. Cultivation of carrots for medicinal purposes started about 2000–3000 years ago, and consumption dates to 600 A.D (Keser et al., 2020). It contains healthy antioxidants, which include carotenoids and hydrophilic compounds (Hager & Howard, 2006). Carrots also provide carbohydrates and minerals like Fe, Ca, Mg, and P (Sharma, Karki, Thakur, & Attri, 2012). Carrot is very sensitive to biotic and abiotic stress. Various abiotic and biotic features mark the quality of carrots throughout the entire production chain, from seed to feasting (Ahmed et al., 2005).

In Pakistan, carrot occupies a prime position among the winter vegetables. Carrot area and production are expanding; the economic study, however, showed that carrot production is a profitable business and provides the farmers with a return. In good years of production, carrot production yields higher returns (Majoka, Panghal, Duhan, Kumar, & Rani, 2019). Pakistan cultivates a vast array of vegetables. The main vegetable species contain potatoes, chilies, carrots, and tomatoes. Vegetables are an excellent source of vital micronutrients for the human body, as well as a great source of profits for farmers. Like other cereal crops, maize, wheat, and pulse vegetables bring higher returns but are difficult to grow (Adil, Chattha, Hassan, & Maqbool, 2007).

Vegetable production is a laborious activity that can also be useful to create employment in the rural economy. Thus, it is a complex activity that can aid the economy in numerous ways due to its maximum yield potential, higher return, great nutritional value, and highly labor-intensive features (Tahir & Altaf, 2013). Diversity analysis is an important process to clearly identify the genetic similarity of the available genetic resources. Current objectives in plant breeding might be attained through trait evaluation in genetic resources to better conserve and utilize genomic resources (Giraldo, López-Corrales, & Hormaza, 2010). The most significant method for the enhancement of various crop plants is diversity analysis. Local landraces are most significant for local agro-economic systems. Morphological traits are important parameters for the identification and selection of favorable genotypes, as plant breeders can use this information to develop breeding populations (Greene, Gritsenko, & Vandemark, 2004).

Major et al. (2022) suggest that by providing complete morphological and chemical features, we can suitably preserve and assess these landraces, in addition to commercial varieties. Identification of the morphological features, which comprise quantitative and qualitative features among carrot accessions, is very important. Generally, qualitative parameters are useful for varietal identification, while quantitative parameters are required for the development of new varieties (Luitel et al., 2018). Although there are some reports about the morphological traits of carrots, they are limited to a few germplasms (Chen, Ma, & Yang, 2020). In those cases, the study only focused on quantitative parameters. To fully understand the range of both qualitative and quantitative factors, it is necessary to do a detailed analysis of the morphological variability in the carrot core collection. This characterization of morphological parameters is considered an important step in the description and classification of germplasm (Tabor, Yesuf, Haile, Kebede, & Tilahun, 2016). Therefore, it is important to find essential morphological characters in compatibility with the environment; it allows breeders to compile favorable genes into commercial varieties (Tiruneh, Omonhinmin, Conrad, Feyissa, & Dagne, 2015). Characteristics such as yield and root quality are essential for crop development in carrots. The correlation between several factors, mainly traits for yield enhancement, is significant as it might offer a complete source for more development in the carrot (Arif, Amir, & Siddiqui MFKSU, 2020).

Therefore, we carried out the present investigation to characterize and assess the morphological variability of carrot accessions collected from different continents. Through this study, we successfully identified various morphological characters that aid in distinguishing between different carrot accessions.

2. MATERIALS AND METHODS

2.1. Experimental Site

The Plant Genetic Resource Institute (PGRI) and the National Agriculture Research Centre (NARC) in Islamabad provided the agro-climatic conditions for the current experiment. During 2016 and 2017, latitude was 33.6982 and longitude was 73.0393.

2.2. Seed Material

The accessions consisted of a set of thirty-three carrot accessions, representing collections of diverse origins, collected from the National Gene Bank of Pakistan (NGP), Bio-Resources Conservation Institute, and the National Agriculture Research Centre (NARC) in Islamabad. Table 1 provides the list of carrot accessions along with their origin and passport details.

2.3. Experimental Design

We sowed the crop in an augmented block design. There were seven blocks in the design, the distance between paths was 1.5 meters, and the non-experimental area was 2 meters. Every accession was grown in two rows of 3 m length with 8 cm plant spacing and a row-to-row distance of 75 cm, along with Check varieties Indian Desert Look and T.29. The checks were repeated before and after every 20 accessions.

2.4. Land Preparation

Since carrot seeds are small, we prepared a finely pulverized seedbed to speed up the germination process. The sowing was conducted in the last week of September, during 2016 and 2017. The maximum space between rows was 60 cm, and plant-to-plant was 3 to 4 cm. The first irrigation was done after five to six days of sowing and was continued when needed. Water was applied, and earthing was performed for the appropriate progress of roots and the elimination of weeds.

2.5. Harvesting and Data Recording

Once the roots at the upper end reached 2.5 to 3 cm in diameter, we harvested the carrots. The field was moderately irrigated regularly and slightly to avoid crust formation; ridges were not allowed to submerge in water. After the plants attained a size of approximately 5 to 7 cm, thinning was done so that the plants were 5 cm away from each other in rows. Thinning was carried out in two or three stages rather than all at once. The recommended chemical fertilizers—45 kg of P_2O_5, 62 kg of K_2O, and 52 kg of N—were applied earlier to seed sowing. After four to six weeks of sowing, left-over nitrogen was also used with successive irrigations. Hoeing was done in the initial phases of growth to keep the weeds under control a few days prior to harvesting. Since the tops wilted and began to decay first, we detached them all. Bioversity International (former International Plant Genetic Resource Institute) developed standard descriptors for wild and cultivated carrots, from which we selected 15 quantitative parameters. Five randomly selected plants provided the data Table 2 contains a trait list, trait descriptions, and measurement methods for various parameters. For most of the parameters, data were recorded when roots were at the edible stage, whereas the plant height data and traits concerning seed were noted at maturity in the month of April.

2.6. Data Analysis

To study the variability among different quantitative parameters, ANOVA (analysis of variance) and basic statistical analysis were conducted using R software (R Core Team, 2018). Additionally, we performed principal component analysis (PCA) using the computer program STATISTICA 13.0 (StatSoft, 2001).

Table 1. List of carrot (Daucus carota) accessions assayed in this study.

Accession	Origin	Accession	Origin	Accession	Origin
41300	India	41345	India	41390	Afghanistan
41301	Germany	41346	India	41391	Iran
41302	India	41347	India	41392	Iran
41303	US	41348	United State	41393	Denmark
41304	US	41349	United State	41394	Denmark
41305	US	41350	Turkey	41395	Denmark
41306	US	41351	Turkey	41396	Denmark
41307	US	41352	Belgium	41397	Japan
41308	US	41353	Belgium	41398	Mexico
41309	US	41354	Belgium	41399	Iran
41310	US	41355	Belgium	41400	Poland
41311	US	41356	Ethiopia	41401	Poland
41312	US	41357	Sweden	41402	Poland
41313	Iran	41358	Germany	41403	Poland
41314	New Zealand	41359	Russian Federation	41404	Poland
41315	New Zealand	41360	Tajikistan	41405	Poland
41316	South Africa	41361	Soviet Union	41406	Chile
41317	US	41362	Sweden	41407	Egypt
41318	Spain	41363	Sweden	41408	Egypt
41319	Spain	41364	India	41409	South Africa
41320	Netherland	41365	South Africa	41410	South Africa
41321	Netherland	41366	Denmark	41411	South Africa
41322	Japan	41367	India	41412	South Africa
41323	France	41368	Netherland	41413	New Zealand
41324	France	41369	Netherland	41414	Afghanistan
41325	France	41370	Netherland	41415	Afghanistan
41326	US	41371	Soviet Union	41416	Denmark
41327	US	41372	Sweden	41417	Denmark
41328	US	41373	Sweden	41418	Denmark
41329	France	41374	Poland	41419	Mexico
41330	France	41375	Poland	41420	Sweden
41331	France	41376	Poland	41421	Afghanistan
41332	France	41377	India	41422	Pakistan
41333	France	41378	India	41423	Pakistan
41334	France	41379	Pakistan	41424	India
41335	Japan	41380	Lebanon	41425	India
41336	Iran	41381	Afghanistan	41426	India
41337	Afghanistan	41382	Afghanistan	41427	India
41338	Afghanistan	41383	Afghanistan	41428	India
41339	India	41384	India	41429	India
41340	Pakistan	41385	Afghanistan	41430	Unknown
41341	India	41386	Afghanistan	41431	Unknown
41342	India	41387	Sweden	41432	Unknown
41343	India	41388	Sweden		
41344	India	41389	India		

Table 2. Descriptors used in evaluation of carrot accessions for quantitative traits.

S#	Traits	Scale	Description of the traits
1	Mature leaves per plant	(No)	The data for this parameter was recorded on the basis of a visualization of how many leaves are mature on a single plant. This data was recorded at 50% after germination.
2	Mature leaf length	(cm)	The leaf length data was collected by measuring the distance from the base to the tip of each individual leaf. Measurements were taken for three leaves from the main plant, and subsequently, an average was calculated.
3	Width of mature leaf	(cm)	The data for leaf width measured the widest part of the same leaf, which was selected to be used for leaf length.
4	Area of leaf	(cm^2)	The leaf area was recorded using the following formula: Leaf area = Length × Width × 0.75 (Elings, 2000)
5	Total umbels per plant	(No)	Data was recorded by counting the total umbels per plant in a single accession. Therefore, 5 plants were selected from each accession, and the total number of umbels on each plant was recorded.

S#	Traits	Scale	Description of the traits
6	Leaf weight	(g)	The leaf weight was measured by an electronic balance.
7	Petiole thickness	(mm)	The data was recorded with the help of a Vernier caliper at the thickest point at the time of the full development of foliage.
8	Root length	(cm)	The root length was measured by taking the length of roots at three points: at the top, at the shoulder, at the bottom, with the help of a scale.
9	Data of root weight	(g)	The root weight data was measured by an electronic balance.
10	Root diameter	(mm)	The root diameter data was measured from the center of the root with the help of a Vernier caliper
11	100-seed weight	(g)	It was determined by taking two umbels from a single plant, collecting their seeds, counting 100 seeds from the single umbel, calculating their average, and then weighting the 100 seeds from the single umbel.
12	Root diameter at shoulder	(mm)	The data was measured at 2-3 cm below the leaf attachment with the help of a Vernier caliper
13	Plant biomass	(g)	The data on plant biomass was determined by measuring the whole weight of the plant in grams with the help of an electronic balance.
14	Seed length	(mm)	The data for seed length was recorded by determining the length of 3 seeds per umbel of the chosen plant by Vernier caliper, and then the average was calculated.
15	Seed width	(mm)	Seed width data was noticed by measuring the width of three seeds per umbel of the plant at the broadest point with a Vernier caliper, and then the average was calculated.

Source: IPGRI (1998).

3. RESULTS AND DISCUSSION

Assessing agronomic traits is critical for understanding and categorizing crop varieties, because improvements depend on how much genetic variation exists. This evaluation helps researchers decide which gene pools to use to enhance specific characteristics in crop development.

3.1. Analysis of Variance (ANOVA)

The ANOVA displayed significant mean squares sums for various sources of variation in all studied traits. The effect due to checks was significant for most of the traits, while the block effect (unadjusted) and the treatment effects (adjusted as well as unadjusted) were significant for all the traits during consecutive years (Table 3). Numerous traits underwent analysis of variance, revealing a significant mean sum of squares among the traits, indicating a remarkable influence of the source of variation on the experimental results. Particularly, the block effect, both adjusted and unadjusted treatment effects, as well as the effect due to checks, were all found to be significant for the studied traits.

3.2. Variability among Quantitative Traits in Carrot Accessions

The accessions assayed during both years showed a high to moderate variance for numerous agronomic traits. In general, the vast variability observed for various traits revealed a broad genetic base of carrot germplasm investigated (Table 4). The significant amount of genetic diversity present among carrot germplasm can be exploited for further crop improvement in carrot.

3.2.1. Leaf Traits

During the second year, the mean values for various leaf traits were high as compared to the first year, indicating the impact of different environmental factors or developmental changes over time. The data on petiole thickness showed significant variation, ranging from 1.12 to 18.86 with a mean value of 5.0 mm in 2016 and from 1.39 to 20.2 mm with a mean value of 5.5 mm in 2017. The variation in petiole thickness may have been caused by genetic or environmental factors such as nutrition and water availability. In 2016, we observed a minimum number of mature leaves per plant in genotype 41397 (Japan), and a maximum number of mature leaves per plant in accession 41377 (India). In 2017, the genotype 41362 from Sweden exhibited a minimum number of four mature

leaves per plant. Accession 41377, originating from India, demonstrated the highest number of mature leaves per plant at 57. These results are in accordance with the findings of Koike, Smith, Cahn, and Pryor (2017).

Carrot accessions also showed a significant difference in leaf length and width. Genotype 413629 (Sweden) showed the lowest leaf length (5.5 and 6.76 cm) for two years, while genotype 41426 (India) displayed the highest leaf length (69 and 71.4 cm) in both years. Similarly, variation in leaf width ranged from 2.0 to 20 mm, with a mean value of 9.5 mm during 2016. However, leaf width varied from 3 to 22 mm, with a mean value of 10.3 mm in 2017. The difference in number of leaves, leaf length, and leaf width may occur due to variability in genetic profile or environmental factors. Parameters such as leaf area and leaf weight also play an important role in plant development. In the present study, the leaf area ranged from 12 to 94.9 cm^2 and from 22.35 to 97.14 cm^2 during both years. In both years, accession 41362 (Sweden) displayed a low leaf area, while genotype 41426 (India) demonstrated the highest leaf area. Similarly, we noted that accession 41396 had the highest leaf weight, while accession 41396 had the lowest; both accessions originated from Denmark. These results highlight the importance of variability in leaf characters among various carrot genotypes; highlighting different physiological variations. The findings underscore the significant variability in leaf characteristics among different carrot genotypes, reflecting diverse physiological modifications and possible consequences for yield and biomass accumulation.

3.2.2. Root Morphology and Yield Attributes

Root parameters are important factors for carrot productivity. Our study revealed considerable diversity in root length, ranging from 4 to 29.6 cm with a mean value of 13.6 cm in 2016, while during 2017 it varied from 4.8 to 30 cm with a mean value of 14.8 cm. During both years, we observed maximum root lengths in Accession 41321 (Netherlands) and minimum root lengths in Accession 41331 (France). These values correlate with the previous work (Majkowska-Gadomska & Wierzbicka, 2010). Root diameter, one more vital parameter that influences the yield and quality of carrots, varies from 5.5 to 45.2 mm with a mean value of 18.7 mm and from 6.3 to 41.99 mm with a mean value of 19.2 mm during both years. Over a two-year period, genotypes 41432 and 41384 recorded the highest root diameter, while accession 41406 and 41392 exhibited the lowest root diameters.

Furthermore, the diameter of the root at the shoulder ranged from 72.65 to 73.1 mm during both years. These results highlight the presence of genetic variation in root parameters among carrot genotypes, which plays a critical role in root yield and commercial value. Root weight, a direct method for evaluating root weight and productivity of yield, varies from 6.7 to 276.53 g and 7.7 to 295.5 g during both years. Accessions 41397 and 41424 displayed the lowest root weight, whereas accessions 41424 exhibited the highest root weight. These outcomes are in accordance with earlier studies by Kozik, Nowak, Nowakowska, and Dyki (2012) and Majkowska-Gadomska and Wierzbicka (2010).

3.2.3. Plant Biomass Traits

Plant biomass, a cumulative measure of vegetative growth, varied considerably among carrot genotypes, with genotype 41377 (India) showing the highest value and genotype 41397 (Japan) exhibiting the lowest across both years. These findings highlight different growth patterns and biomass allocation strategies among carrot accessions. This may influence overall yield and productivity. The number of umbels per plant, a key reproductive trait, ranged from 2 to 42 in 2016 and from 4 to 47 in 2017. The main umbels typically exhibit larger seeds with maximum germination and vigor, as noted by Merfield (2006). The mean number of umbels per plant was 22.2 in 2016 and 24.5 in 2017, with genotype-specific variation observed.

3.2.4. Seed Parameters

The seed characteristics—length, width, and weight—play a crucial role in determining germination potential and ultimately affecting crop yield. In our study, significant variability was observed in seed length among carrot

accessions for both years. Seed length ranged from 0.18 to 5.88 mm in 2016 and from 0.38 to 5.16 mm in 2017, with mean values of 2.4 mm and 2.7 mm, respectively. Genotypes 41324 (France) and 41429 (India) exhibited maximum seed length, while genotypes 41371 (Soviet Union) and 41359 (Russian Federation) showed the lowest seed length across the consecutive years. These findings highlight the genetic diversity in seed morphology among carrot accessions, with potential implications for germination and seedling establishment. The observed mean value of seed length (2.4–2.7 mm) aligns with previous studies by Nikolay (2010) indicating consistent pattern across different carrot cultivars. However, the wide range of seed variability highlights the influence of environmental factors such as elevation, latitude, soil moisture, temperature on seed development and maturation (Kimura et al., 2020; Roy, Thapliyal, & Phartyal, 2004). Similarly, the seed width also showed a notable difference; accession 41300 showed the maximum seed width (0.06 to 3.41 mm) with a mean value of 0.6 mm to 0.7 mm over the period of two years. However, accessions 41308 and 41332 from the US exhibited the lowest seed width for consecutive years. These findings shed light on the diverse genetic backgrounds of carrot genotypes and demonstrate the capability of selective breeding to improve seed features for preferred agronomic traits.

The 1000 seed weight is a crucial parameter influencing seedling vigor and establishment. In our study, variation in 1000 seed weight ranged from 0.432 to 7.30 g in 2016 and from 0.589 to 7.3 g in 2017. Genotype 41324 (France) displayed the highest value (7.30 g) for seed weight with a mean value of 3.2 g, and for 2017, it ranged from 0.589 to 7.3 g with a mean value of 3.3 g. Genotype 41324 (France) showed the highest value (7.30 g), while genotype 41419 (Mexico) and 41418 (Denmark) showed the minimum value for both years. These findings indicate significant seed variability. The result does not conform to the findings of Panayotov, Kuneva, and Trayanov (2022) who explored variation related to 1000 seed weights that deviated from 1.74 to 1.91 g. Present investigations indicate that such variability in seed weight may be a result of environmental fluctuations due to genetics or during the entire reproductive and vegetative phases of growth.

Judging agronomic traits is an important part of understanding and grouping crop accessions. This is because crop improvement depends on the amount of genetic variation. It also helps the investigators design and use a suitable gene pool for specific features in crop enhancers. Both years observed moderate to high variation for numerous quantitative traits among carrot accessions. Mature leaf length showed the highest variation, followed by mature leaf width, leaf area, root weight, leaf weight, and plant biomass. Substantial variation in seed length, seed weight, yield, and root quality traits is commonly considered important for production and crop improvement in carrots (Teli et al., 2017). Both years' accessions exhibited varying performance, which could potentially be attributed to climatic fluctuations that occurred during both seasons. The differences in weather during cropping season affect crop growth (Nikolay, 2010).

The observational variation in quantitative parameters during the first year compared to the second year suggests a dynamic response within the studied population over time. Various factors, including environmental influences, genetic drift, and epigenetic modification, could be responsible for these phenomena. The significant increase in variation during the second year highlights the importance of long-term monitoring to capture the full spectrum of changes within a population. Furthermore, the collection of accessions from diverse geographical regions introduces a wide range of genetic backgrounds and environmental adaptations. In particular, Asian accessions exhibit the highest level of variation, indicating the rich genetic reservoir present within this region. This observation underscores the importance of conserving and utilizing genetic diversity from Asian germplasm in breeding programs aimed at enhancing crop resilience and productivity. The identification of high levels of variation among accessions underscores the potential for targeted breeding strategies to harness this diversity for crop improvement. By leveraging genomic tools and breeding techniques, researchers can exploit the unique attributes present in the diverse germplasm to develop cultivars with enhanced agronomic traits and adaptability to changing environmental condition.

Table 3. Analysis of variance (ANOVA) for qualitative traits of carrot accessions observed during 2016 and 2017.

ANOVA (Treatment adjusted)	Df	La	Lw	Mll	Mlp	Mlw	Nu	Pb	Pt	Rd	Rds	Rl	Rw	Sw	Tsw
2016															
Block (Ignoring treatments)	6	135833.61**	11028.01**	658.58**	55.27**	29.86**	56.02	39409.27**	8.4	139.39**	181.81	10.4	9189.78	0.25	14.02**
Treatment (Eliminating blocks)	131	24544.98*	4140.21**	148.21*	59.88**	9.82**	107.39	13206.9**	15.36	61.55*	141.42	19.2	4190.91	0.49	2.39
Treatment: Check	1	417877.6**	21307.92**	1184.7**	10.62	102.21**	924.62**	257974.64**	454.75**	0.04	2331.26**	0.14	112777.08**	14.44**	30.64**
Treatment: Test and test vs. check	130	21519.35*	4008.15**	140.24*	60.26**	9.11**	101.1	11324.07**	11.98	62.02*	124.58	19.3	3355.63	0.39	2.17
Residuals	8	6124.37	487.08	46.92	8.07	0.24	67.69	6136.51	16.69	14.9	58.63	13.9	3086.96	0.64	1.22
2017															
Block (Ignoring treatments)	6	168293.56**	11369.77**	769.25**	60.15	36.97**	53.81	42254.32**	12.55	87.1**	194.95	16.2	9653.94**	0.2	14.28**
Treatment (Eliminating blocks)	131	25036.42**	4588.97**	136.97*	61.17**	9.91**	104.25	12607.06**	20.42	55.06*	131.38	21.1	3311.54*	0.51	2.4
Treatment: Check	1	257877.86**	17985.47**	501.91**	9.49	77.54**	434.61*	78884.05**	586.83**	29.95	1738.9**	9.92	15096.66**	27.84**	29.01**
Treatment: Test and test vs. check	130	23245.34**	4485.92**	134.16*	61.56**	9.39**	101.71	12097.24**	16.07	55.26**	119.01	21.2	3220.88*	0.3	2.2
Residuals	8	4659.85	399.35	39.49	9.95	1.08	75.65	2128.9	23.65	11.4	39.32	13.1	929.7	0.85	1.64

Note: LA= Leaf area, LW= Leaf weight, MLL= Mature leaf length, MLP= Mature leaf per plant, MLW= Mature leaf weight, NU= Number of umbels per plant, PB= Plant biomass, PT= Petiole thickness, RD= Root diameter, RDS= Root diameter at shoulder, RL= Root length, RW= Root weight, SW= Seed width, TSW= Thousand seed weight,
(*): Significant at 5% level of probability,
(**): Significant 1% level of probability

Table 4. Variability in carrot accessions based on different morphological traits for the year 2016 and 2017.

Traits	Year	Mean± SD	Variance	Range
Petiole thickness (mm)	2016	5.0 ± 2.8	8.1	1.11 (41366) - 18.85 (41300)
	2017	5.5 ± 3.4	11.6	1.39 (41396) - 20.1 (41367)
Mature leaf/Plant	2016	12.7 ± 7.9	62.2	3.5 (41397) – 56 (41377)
	2017	13.6 ± 8.0	63.8	4.33 (41362) – 57 (41377)
Mature leaf length (cm)	2016	28.3 ± 12.0	143.0	5.5 (41362) - 69.0 (41426)
	2017	29.7 ± 12.3	151.3	6.76 (41362) - 71.4 (41426)
Mature leaf width (cm)	2016	9.5 ± 3.2	10.1	2.0 (41396) - 20 (41428)
	2017	10.3 ± 3.2	10.4	3 (41396) - 22 (41428)
Leaf area (cm^2)	2016	219.6 ± 156.1	24381.4	12 (41362) - 94.9 (41426)
	2017	251.6 ± 168.9	28529.9	22.35 (41362) – 97.14(41426)
Root length (cm)	2016	13.6 ± 4.5	20.2	4 (41331) - 29.6 (41321)
	2017	14.8 ± 4.6	21.5	4.8 (41331) - 30.0 (41321)
Root diameter (mm)	2016	18.8 ± 8.0	64.6	5.6 (41406) - 45.3 (41432)
	2017	19.3 ± 7.5	56.5	6.4 (41392) - 41.98 (41384)
Root diameter at shoulder (mm)	2016	33.3 ± 11.0	120.7	9.59 (41312) - 72.66 (41377)
	2017	35.1 ± 11.0	120.2	11.85 (41310) - 73.26 (41377)
Root weight (g)	2016	90.8 ± 57.7	3329.7	6.80 (41397) - 277.42(41424)
	2017	97.4 ± 60.4	3643.3	7.8 (41397) - 296.6 (41424)
Leaf weight (g)	2016	52.2 ± 66.5	4421.9	1.84 (41396) - 492.7 (41377)
	2017	55.3 ± 70.2	4931.7	2.14 (41396) - 496.0 (41377)
Plant biomass (g)	2016	141.9 ± 111.3	12385.3	10.02 (41397) - 729.9 (41377)
	2017	151.8 ± 117.7	13864.0	11.02 (41397) - 734.71 (41377)
No. of umbels	2016	22.2 ± 8.9	79.7	2.66 (41431) – 42 (41340)
	2017	24.5 ± 9.2	84.8	4 (41431) - 47.5 (41340)
Seed length (mm)	2016	2.4 ± 1.0	1.1	0.18 (41371) - 5.88 (41324)
	2017	2.7 ± 1.1	1.1	0.38 (41359) - 5.16 (41429)
Seed width (mm)	2016	0.6 ± 0.5	0.2	0.06 (41308) - 3.22 (41300)
	2017	0.7 ± 0.5	0.2	0.16 (41332) - 3.42 (41300)
1000 seed weight (g)	2016	3.2 ± 1.6	2.7	0.432 (41419) - 7.30 (41324)
	2017	3.3±1.6	2.7	0.589(41418) – 7.2(41324)

3.3. Principal Component Analysis

By changing the number of relative variables to a smaller number of variables, the principal component analysis makes the complicated data into a simpler one (Wall, Rechtsteiner, & Rocha, 2003). Principal component analysis for 15 morpho-physiological traits in carrot accessions for the years 2016 and 2017 are shown in Table 5. Four components with eigen value > 1 were extracted. In 2016, PC 1 showed maximum eigen value of 6.26. The PC 1 also explained maximum variance 41.70% followed by PC 2 (11.23), PC 3 (8.78), and PC 4 (7.59) of total variance.

In 2017, PC 1 displayed the maximum Eigen value of 6.55. PC 1 accounted for 43.67% of the total variance, followed by PC 2 (11.12), PC 3 (8.51), and PC 4 (7.47%). The cumulative contribution was 76.21 % and 76.66% in 2016 and 2017, respectively.

Table 5. Principal components analysis of the 15 quantitative traits in carrot genotype assayed during 2016 and 2017 year.

Parameters	2016				2017			
	PC1	PC2	PC3	PC4	PC1	PC2	PC3	PC4
Eigenvalues	6.26	1.68	1.32	1.14	6.55	1.67	1.28	1.12
Cumulative eigen values	6.26	7.94	9.26	10.40	6.55	8.22	9.50	10.62
%total variance	41.70	11.23	8.78	7.59	43.67	11.12	8.51	7.47
Cumulative variance	41.70	52.93	61.72	69.30	43.67	54.80	63.30	70.77

3.4. Distribution Pattern of Various Quantitative Parameters on Scatter Plots

The principal component analysis (PCA) of data from two years in a row, 2016 and 2017, shows that the traits' effects on the overall variance in the dataset are very different from one another. Furthermore, by reducing the dimensionality of the data, PCA allows us to detect patterns and relationships among multiple variables. The scatter plot analysis of PC1 vs. PC2 during 2016 (Figure 1A) demonstrates a distinct separation of traits contributing to the variance, both negatively and positively. The parameters such as root length, root weight, root diameter at shoulder, and plant biomass exhibited a positive contribution, indicating a robust relationship and potential significance in determining overall plant performance. While traits like number of umbels, seed length, total seed weight, and seed width have a negative contribution, signifying a different set of relationships among these variables. This suggests a tradeoff between root characteristics and seed-related traits. These results align with the studies by Kurina, Kornyukhin, Solovyeva, and Artemyeva (2021).

The second plot was plotted between PC1 vs PC3 (Figure 1B). The parameters like leaf per plant, leaf weight, plant biomass, petiole thickness, number of umbels, and seed width show a positive contribution, while root diameter, seed length, and root length show a negative contribution, indicating an incomplete association between these variables. Similarly, the PC2 and PC3 (Figure 1C) indicate divergent links among traits such as seed width and number of umbels. However, the analysis of the 2017 data set revealed a notable shift in the dynamics. Especially the parameters like total seed weight, seed length, number of umbels, and leaf weight showed a positive influence in the first plot (Figure 2A), indicating a change in the significance of these variables compared to the previous year. Similarly, in the second plot (Figure 2B), parameters related to root features (root length, root weight, root diameter at shoulder, mature leaf weight) displayed a positive contribution, showing a potential emphasis on belowground morphology in driving the variability observed. The fact that root-related traits made some principal components better in both years supports the idea that belowground morphology is important for plants to get resources and stay healthy (De Deyn, Cornelissen, & Bardgett, 2008).

On the other hand, traits like plant biomass, leaf weight, and mature leaf per plant show negative variability, indicating a contrasting pattern compared to the prior year. The PC2 vs PC3 plot (Figure 2C) indicated a significant relationship with different traits, showing a diverse contribution to the observed variance. Overall, these findings highlight the dynamic nature of trait associations within plants and underscore the significance of considering temporal variability in trait contribution. The PCA results suggest potential genotype-environmental interactions driving phenotypic variation. The changes observed between the two-year data suggest possible environmental effects or genetic adaptations that allow additional investigation. Genome-environmental interaction plays an important role in shaping plant morphology and performance across heterogeneous landscapes (Franks, Hamann, & Weis, 2018). Additionally, knowledge about the associations among the traits and their implications for plant performance is important to provide valuable insight for breeding programs aimed at enhancing desired agronomic traits. According to principal component analysis, the positive contribution of various traits during both years suggests targets for selection in breeding programs focused on improving yield components and reproductive efficiency (Rebetzke et al., 2011). Moreover, the identification of negative traits highlights the need for breeding efforts that enhance resource allocation to maximize overall plant productivity under varying environmental conditions (Furbank & Tester, 2011).

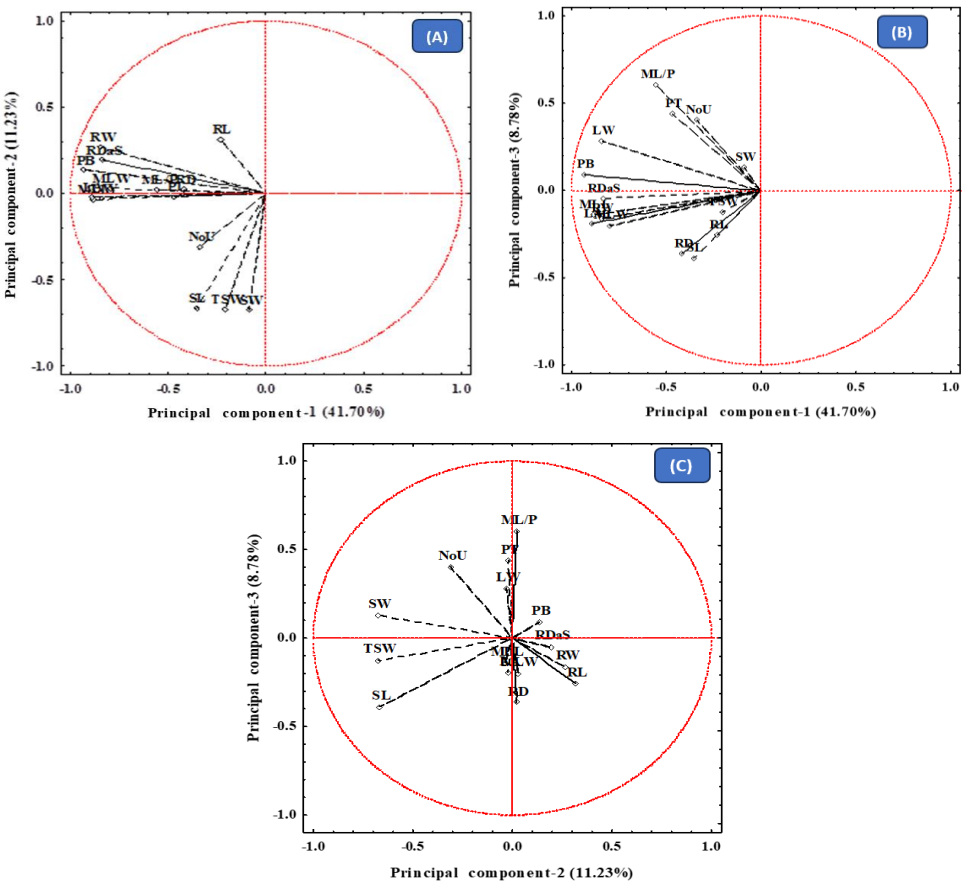


Figure 1. Distribution pattern of various quantitative parameters on scatter plot based on A) PC1-PC2, B) PC1-PC3 and C) PC2-PC3 during 2016.

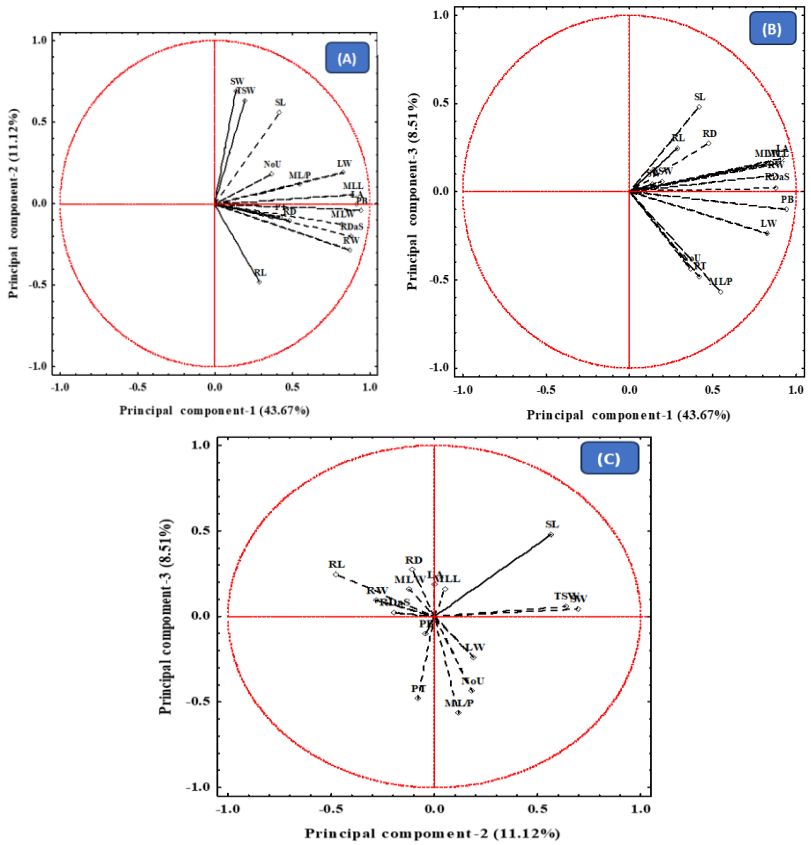


Figure 2. Distribution pattern of various quantitative parameters on scatter plot based on A) PC1-PC2, B) PC1-PC3 and C) PC2-PC3 during 2017.

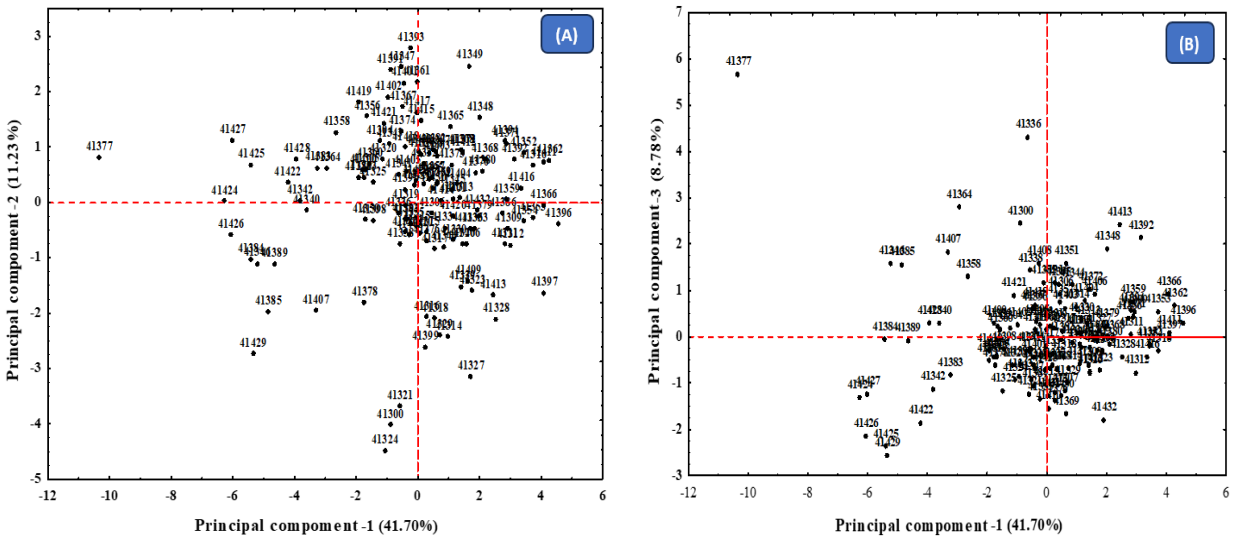
3.5. Distribution Pattern of Carrot Accessions on Scatter Plot

The scatter plot is often helpful for finding patterns of variation (Bro & Smilde, 2014). We plotted the first three PCs on the scatter plot to view the distribution of carrot accessions, revealing an informative spread of the accessions. In general, there was a clear distinction among the groups separated from each other based on plant biomass, seed weight, and seed length, number of umbels, root length, leaf weight, and root weight.

In 2016, we grouped the accessions with high mean values for those characters first, followed by those with medium and low mean values. However, accessions like 41377, 41427, 41425, 41424, 41426, 41385, 41429, 41397, 41327, 41349, and 41348 stood out as unique due to their noticeable divergence from the remaining accessions in PC1 and PC2 (Figure 3A). In the scatter plot of PC1 and PC3, specific accessions such as 41377, 41300, 41336, 41364, 41423, 41392, 41348, 41426, and 41342 exhibited divergence from the remaining accessions (Figure 3B). PC2 and PC3 made up the final scatter plot. The accessions 41377, 41336, 41300, 41324, 41321, 41429, 41426, 41425, 41393, 41348, 41392, and 41364 showed divergence. These accessions could be exploited for the improvement of carrots (Figure 3C).

However, in 2017, the distribution pattern and placement of accessions on the plot differed from those observed in 2016. In the first scatter plot between PC1 and PC2, twelve accessions—41385, 41300, 41377, 41328, 41397, 41366, 41352, 41394, 41349, 41356, 41427, and 41425—showed that they were different from the other accessions (Figure 4A). In the second plot, eleven accessions (41432, 41316, 41397, 41339, 41392, 41426, 41427, 4185, 41346, 41467, and 41377) were different from PC1 to PC3 (Figure 4B). While in the last plot, of PC2 and PC3, also eleven accessions (41425, 41361, 41356, 41367, 41377, 41336, 41300, 41385, 41393, 41309, and 41429) were showing divergence (Figure 4C). This observation aligns with previous research by indicating the genetic diversity and regional adaptations in the carrot populations (Iorizzo et al., 2016).

The observed divergence of carrot accessions across the first and second years, in particular, suggests magnificent changes in genetic grouping or environmental reactions within the population. Furthermore, the consistent divergence of accessions from the Asian continent, such as 41317, 41427, 41425, 41426, 41385, 41429, 41397, 41349, 41300, and 41336, across both years suggests a potential geographical influence on genetic differentiation.



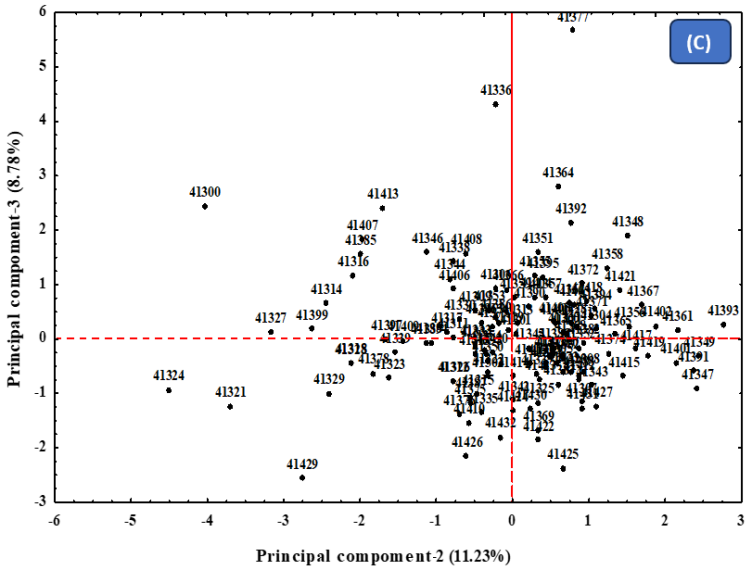


Figure 3. Distribution pattern of various carrot accessions on scatter plot based on A) PC1-PC2, B) PC1-PC3 and C) PC2-PC3 during 2016.

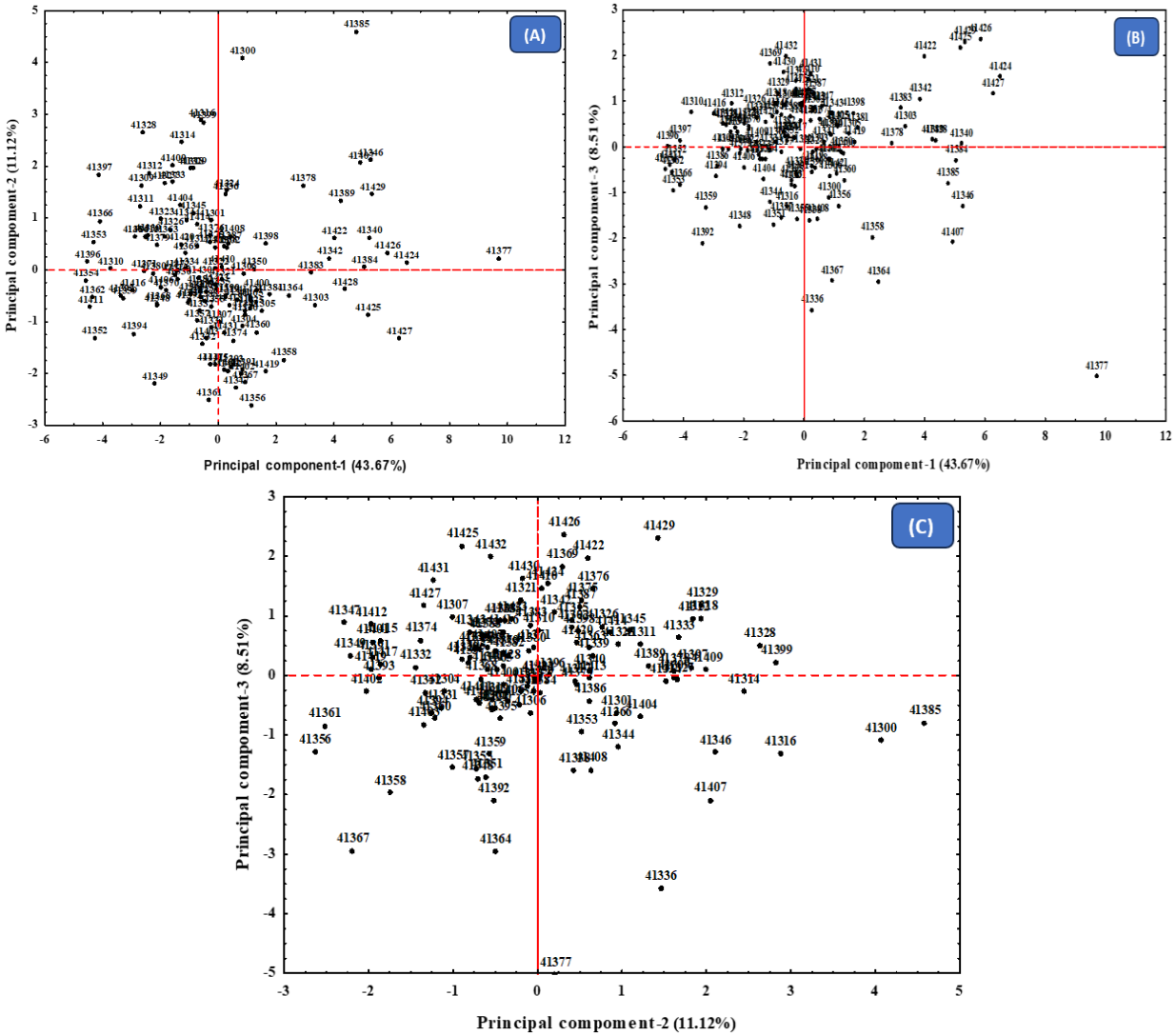


Figure 4. Distribution pattern of various carrot accessions on scatter plot based on A) PC1-PC2, B) PC1-PC3 and C) PC2-PC3 during 2017.

4. CONCLUSION

The significant mean square observed for all the traits across different sources of variation highlights the multifaceted nature of plant responses to experimental conditions. By acknowledging and accounting for these sources of variation, researchers can enhance the reliability and interpretability of their findings, thereby advancing our understanding of plant physiology and improving agriculture practices.

Variability in quantitative parameters in accessions highlights the vigorous nature of quantitative parameter variation in the population over time. The observed increase in variation during the second year emphasizes the need for longitudinal studies to comprehensively assess genetic dynamics and environmental influence. Furthermore, the diverse origins of the collected accessions, with Asian accessions exhibiting the highest variation, underscore the significance of the global germplasm collection for breeding programs. The genetic diversity present in these collections offers promising possibilities for crop improvement and sustainable agriculture.

PCA results offer valuable insight into the complex interplay between plant traits and environmental factors, with implications for ecological research, agricultural management, and breeding programs. By correlating these findings with existing literature, we can advance our understanding of plant biology and inform strategies for sustainable crop production and ecosystem management.

The scatter plot distribution of carrot accessions reveals a dynamic pattern of genetic divergence and geographic differentiation over time. These findings underscore the importance of considering temporal and spatial factors in genetic studies of crop populations. Future research could further investigate the underlying mechanisms driving these patterns and explore their implications for crop breeding and conservation efforts.

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Authors' Contributions: Conducted experimental work and drafted the manuscript, U.A.; monitored/supervised the experimental work, reviewed the manuscript and provided technical input, S.A.J. Both authors have read and agreed to the published version of the manuscript.

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