GENETIC DIVERSITY, POPULATION STRUCTURE AND MARKER-TRAIT ASSOCIATION FOR ROOT TRAITS BY ASSOCIATION ANALYSIS IN CARROT (*Daucus carota* L.)

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CERTIFICATE

This is to certify that the thesis entitled "**GENETIC DIVERSITY, POPULATION STRUCTURE AND MARKER-TRAIT ASSOCIATION FOR ROOT TRAITS BY ASSOCIATION ANALYSIS IN CARROT (*Daucus carota* L.)"** submitted by **Ms. CHAITRA C. KULKARNI** bearing **ID. No. UHS15PGM583** for the degree of **MASTER OF SCIENCE (HORTICULTURE)** in **BIOTECHNOLOGY AND CROP IMPROVEMENT** to the University of Horticultural Sciences, Bagalkot is a record of research work carried out by him during the period of his study in this University, under my guidance and supervision, and the thesis has not previously formed the basis of the award of any degree, diploma, associateship, fellowship or other similar titles.

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"Every effort is motivated by an ambition and all ambitions have inspirations behind"

BAGALKOT

JUNE 2017 **(CHAITRA C. KULKARNI)**

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LIST OF ABBREVIATIONS

AFLP	: Amplified Fragment Length Polymorphism
AM	: Association Mapping
AMOVA	: Analysis of Molecular Variance
UPGMA	: Unweighted Pair Group Method with Airthmetic Mean
CV	: Coefficient of Variation
D	: Gene Diversity
F:	: *Fixation Index (inbreeding coefficient)*
Fst	: Subpopulation within the Total population (genetic divergence among subpopulations)
Fit	: Individual within the Total population (overall inbreeding coefficient)
Fis	: Individual within the Subpopulation
LD	: Linkage Disequilibrium
MAS	: Marker Assisted Selection
Ne	: Effective Population Size
QTL	: Quantitative Trait Loci
R^2	: squared correlation coefficient, measure of LD
SNP	: Single Nucleotide Polymorphism
SSR	: Simple Sequence Repeat
GLM	: Generalised Linear Model
Cm	: Centimeter
C.D.	: Critical difference
Df.	: Degrees of Freedom
G	: Grams
Ha	: Hectare
Kg	: Kilogram
Max	: Maximum
Min	: Minimum

M.S.S.	: Mean Sum of Squares
No	: Number
PCV	: Phenotypic coefficient of variation
PCA	: Prinicipal component analysis
SEm	: Standard error mean
%	: Percentage
Na	: Number of different alleles
Ne	: Number of effective alleles
I	: Shannon's Information Index
He	: Expected Heterozygosity
Ho	: Observed Heterozygosity
DM	: Days to Maturity
NP	: Number of Petioles
SL	: Shoulder Length
PH	: Plant Height
RWD	: Root Width
SWD	: Shoulder Width
VW	: Vegetative Weight
FPVW	: Five Plants Vegetative Weight
XW	: Xylem Width
PW	: Phloem width
HI	: Harvest Index
TSS	: Total Soluble Solids
RS	: Reducing sugar
Beta	: Beta carotenoid
RW	: Root Weight
FPRW	: Five Plants Roots Weight

1. INTRODUCTION

Carrot (*Daucus carota* L.), an ancient cool season root vegetable, is a member of the family Apiaceae (Peirce, 1987). It is a diploid species having chromosome number of 2n =18, with a relatively small genome of 480 Mb (Iorizzo *et al.*, 2016). It is considered to be native of Afghanistan (Banga, 1976). Roots are used for making soups, stews, curries, pies, pickles and for salad purposes. Carotenoid composition determines the white, yellow, orange or red root colour in the carrot (Nicolle *et al.*, 2004; Surles *et al.*, 2004).

It contains appreciable amount of carotene (10 mg/100 g), thiamin (0.04 mg/g) and riboflavin (0.05 mg/g) (Sharfuddin and Siddique, 1985) and it is an excellent source of iron, carbohydrate, vitamin-B, vitamin-C and sugar (Yawalker, 1985). In carrot roots, sucrose is most abundant with endogenous sugar contents, 10 times more than those of glucose and fructose. Carrot roots play an important role to protect the blindness in children by providing vitamin A. It increases the quantity of urine and helps in elimination of uric acid. It has a cooling effect and hence, is beneficial for people suffering from gall stones, constipation and heat troubles (Bose and Some, 1990).

Morphological characteristics lead to a division of the cultivated carrot (*Daucus carota* L.) into two botanical varieties: *Daucus carota* var. *atrorubens* and *Daucus carota var. sativus* (Small, 1978). Variety *atrorubens* refers to carrots originating from the East, exhibiting yellow or purple storage roots and poorly indented, grey-green, pubescent foliage. Variety *sativus* refers to carrots originating from the West and exhibiting orange, yellow or sometimes white roots, and highly indented, non-pubescent, yellow-green foliage (Small, 1978). Many intermediate variants exist between these two types. Despite this taxonomic differentiation between geographical groups, no population structure has been found in carrot germplasm by examining random molecular markers such as isozymes (St. Pierre and Bayer, 1991; St. Pierre *et al.*, 1990); random, amplified, polymorphic DNA (RAPD; Grzebelus *et al.*, 2002; Nakajima *et al.*, 1998, 1997); amplified-fragment length polymorphism (AFLP; Bradeen *et al.*, 2002; Nakajima *et al.*, 1998; Shim and Jørgensen 2000); and inter-simple sequence repeats (ISSR; Bradeen *et al.*, 2002). This lack of structure, despite the

morphological evidence, has been explained by the outcrossing mating system and frequent crossings within carrot germplasm (Bradeen *et al*., 2002).

The biennial nature of carrot makes it a challenging crop for improvement. But being a highly cross pollinated species, with seed producing nature and its broad genetic base make this crop a great interest to a breeder. Heterosis can be very well exploited in terms of hybrids or by developing synthetics by combining superior inbreds having higher general combining ability (GCA). Inbreeding depression is the main draw back in inbred development. Among the carrot root morphology, uniformity in root shape, size, external root color (uniform orange), core size (small), internal color (uniform orange xylem and phloem) are some of the most important characters (Peterson and Simon, 1986; Rubatzky *et al*., 1999). Since, the genetic control of these traits has not yet been reported and selection based on phenotype is the only way to identify the superior lines. But because of the environmental influence on phenotype, visual selection shows less effectiveness and more laborious.

Much of the carrot breeding work has been concentrated to temperate regions in carrot due to its suitability as a cool season vegetable. Few number of varieties suitable to tropical regions are also developed by private and public sectors in India, but due its vernalization requirement and a biennial nature makes the breeding effort more complex and laborious. Moreover, least efforts are made to explore the available germplasm lines suitable to tropical conditions although greater diversity is present within the germplasm pool with respect to adaptability, less vernalization requirement etc. Hence, it is most important to screen the large number of germplasm lines for the nutritional quality, root morphological traits, productivity traits for crop improvement of carrot.

Molecular markers play vital role in carrot breeding and various molecular markers *viz.*, AFLPs, RAPDs and SSRs have been used to assess genetic diversity in germplasm collections and biparental linkage mapping (Vos *et al*., 1995 and Welsh and McClelland, 1990). The detailed genome sequencing of carrot has been completed (Irizzo *et al.*, 2016), which paves the ways for carrot breeder to explore the genomic resources in molecular breeding, variety characterization, diversity studies, population structure determination, marker-trait association and marker assisted selection in carrot and other *Apiaceae* family members.

Characterization of carrot 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.

Microsatellite or simple sequence repeat (SSR) markers proved to be useful in the assessment of genetic diversity of populations occurring in natural habitats and large gene bank collections, as well as in revealing relationships between crop plants and their wild relatives (Varshney *et al*., 2010; Kalia *et al*., 2011). In *Daucus*, most molecular techniques used to date could not uncover clear population structure (Bradeen *et al*. 2002), although delimitation between cultivated carrots and wild populations using AFLP markers was achieved for a small number of accessions (Shim and Jørgensen 2000). Identification of SSR loci in carrot was initiated by Niemann (2001) for linkage mapping dispersal in wild carrot populations (Umehara *et al*., 2005; Rong *et al*., 2010). Recent results of Clotault *et al.* (2010) indicated that SSR markers were helpful in evaluation of genetic diversity in the cultivated carrot.

Carrot breeding success has relied upon the availability of diverse germplasm to improve numerous traits. Expansion of carrot germplasm collections and systematic evaluation of them will be vital to future breeding success. Local carrot landraces that have been grown in isolated areas may soon become extinct as they are replaced by commercial cultivars. A more concerted effort to collect, maintain and evaluate these potentially valuable materials should be initiated.

In order to understand the genetic variability, diversity and to search for the marker-traits associations in the carrot germplasm pool collected from all over India, the following objectives have been put forth.

1. Phenotyping of diverse carrot genotypes for qualitative and quantitative root traits
2. Genotyping of diverse carrot genotypes with various carrot specific molecular markers
3. Genetic Diversity, Population Structure assessment and Marker-Trait association for economic root traits by Association Analysis

2. REVIEW OF LITERATURE

The information on Asiatic carrots (tropical type) in India and probably abroad is very scanty because tropical types has not received ample attention for its genetic improvement. Therefore in the present study 96 germplasm lines representing both tropical and temperate types were evaluated in order to compare their genotypes for various horticulture traits, along with the molecular markers for understanding of these traits and incorporation these traits through breeding strategy.

Available literature pertaining to various aspects included in the present studies has been reviewed under the following sub-heads.

2.1 Genetic diversity for root morphological characters

2.1.1 Genetic variability

2.1.2 Correlation Coefficient

2.1.3 Gene action, Carrot breeding and heritability components

2.2 Genetic variation for nutritional quality traits

2.3 Genomics and molecular marker diversity in carrot

2.4 Population Structure

2.5 Marker-trait association for economic traits in carrot

2.1 Genetic diversity for root morphological characters

Species from family *Apiaceae* (also known as *Umbelliferae*) are generally herbaceous plants which are growing in temperate and tropical regions. The botanical family *Umbelliferae* consists of around 250 genera and about 2800 species (Rubatzky, *et al*., 1999).

Central Asia is considered as a centre of origin of cultivated carrot. Primitive purple and yellow carrots evolved to the modern edible carrot of Eastern and Western

types, which differ mainly in leaf morphology, root colour and shape of the root (Bradeen and Simon, 2007; Simon *et al*., 2008).

Rubatzky and Yamaguchi (1997) divided the cultivated carrots into two groups *viz.*, 1) Asian group which has unique traits such as yellow or purple root color, slightly soft texture, low sugar, bolt easily, pubescent leaves which gives a green gray appearance, and adapted to warm temperature (2) European group that has orange, yellow, red or white root in color, firm textured, sweet, less pubescent green leaves, slow bolting and acclimated to cool temperature.

Among the carrot root morphology, uniformity in root shape, size, external root color (uniform orange), core size (small), internal color (uniform orange xylem and phloem) are some of the most important characters (Peterson and Simon, 1986; Rubatzky *et al*., 1999).

Uniformity in root appearance is more important both for raw consumption as well as for processing of roots than just as a marketable yield. Genetic uniformity contributes substantially for the success of refined cultural practices such as seed coating, precision planting, irrigation and fertilization (Peterson and Simon 1986).

Carrot root shape is sometimes affected by environmental conditions. Root shape is one of the most important economic traits of carrot and the shape of the root is also influenced by soil conditions, genotype (Chaitra 2016). Hence considering these, IPGRI developed the carrot descriptors for the root shape (IPGRI, 1998).

European genotypes of carrot were characterized by Ramesh *et al.* (2011) based on principle component and regression analysis for root economic traits. Genotypes were characterized into four principle components explaining 83.86 % total variation. A first component accounted for about 39% of the total variation with the contribution of characters such as root diameter, root weight, marketable root yield, core diameter, flesh thickness, shoulder thickness, and days to marketable maturity. It was also concluded that based on the multiple linear regression model that, average root weight can be predicted on the basis of leaf length, shoulder thickness, crown diameter, marketable root yield per plot, forking and cracking percentage.

Western carrots appear as a more advanced group, better adapted for co mmercial production and processing. They usually develop roots of cylindrical or tapered cylindrical shape favored by the food processing industry, have less pubescent leaves and show little tendency for bolting (Rubatzky *et al*., 1999).Western carrots are sweeter, having on average 18 % higher sugar content than Eastern carrots (Baranski *et al.* 2012).

Eastern carrots commonly grown in Asia produce rather thicker, shorter roots or narrow conical roots with a tendency for branching in some varieties. They often have pubes-cent leaves and tend to flower early, and hence they exhibit more primitive traits (Rubatzky *et al*., 1999).

Eastern roots are poor in provitamin A carotenoids and have yellow (lutein), purple (anthocyanin) or red (lycopene) color. In contrast to Western carrots, they are riche in phenolic compounds, resulting in higher radical scavenging activity of the root extract that is particularly well exhibited in purple roots containing anthocyanins (Leja *et al*., 2013).

2.2.1 Genetic variability

There is a continuous variation in quantitative characters which are heritable and non-heritable. Heritable variation may be the consequence of genotype whereas non heritable variation is due to the environmental factors (Fisher, 1918).

Low coefficient of variation for root length and diameter, core size and root shape was reported in Chantenay variety of carrot (Anon., 1970).

Low genetic variability and heritability for root size and shape and high for root cortex/core ratio was reported by Mazurkiewicz (1973). Gill and Kataria (1974) reported that carrots of European origin had high caroteneand total soluble solids, apparently due to high dry matter and smaller cores than Asiatic types. Dowker and Jackson (1977) studied four cultivars each of Chantenay type and Autumn King and reported that there are low coefficients of variation for root size and shape in carrot.

Dowker *et al*. (1978) studied root yield and root length root diameter ratio (LI) in a series of carrot cultivars grown at different sites and densities over two years. It was observed that interaction for yield and LI ratio was largely linear.

Brar and Sukhija (1980) observed highest genetic variability for the root to leaf weight ratio amongst the seven traits studied in carrot and heritabiiity estimates were apparently high for most characters.

Twenty one genotypes of carrot were evaluated by Prasad and Prasad (1980) and observed that the yield of carrot roots would increase with an increase in the length and diameter of root.

Studies on root morphological diversity in Iranian yellow cultivars of carrot showed the wider range of variation for important root characters such as length of the root (5-50 cm), weight of root (83.6-610gms), root diameter (1.0 to 10 cm) and TSS (4.4-14.7%) (Kasiri *et al*., 2013).

Natarajan and Arumugam (1981) reported that 89 per cent of total variation in yield of carrot was contributed by leaf number, top length and root weight.

Ten varieties of foreign origin were evaluated by Riad *et al.* (1981) and found that variety Rouge Nantes (Red Nantes) had the highest plant weight and root weight whileDanvers-126 was the best variety for total soluble solids and dry matter and Chantenay for carotene.

Kletskova (1982) reported that Gold Pak, Nantes, Berliamer Red Giant, Special Long Chantenay gave high and stable yields. Selection Danvers and Chantenay 2461 had high carotene. Bassett *et al*. (1983) studied Orlando Gold, a new carrot hybrid with intensively orange and uniform roots tapering gradually towards tip and good top growth and recorded a carotene content of 12 mg/100 g in roots.

Fedorova and Mugniev (1985) screened varieties for chemical composition and storage quality. Amsterdam Zoete Bak (100%) was the best for marketable roots after Autumn Winter followed by Nantes (97.9%), Caromba Amsterdam Forcing (96.8%) and Chantenay Red Cored (93.3%). Soviet varieties Khibino Kaya, Lesina Kasaya, Goranda-1129 and Ganger maintained high carotene content during storage.

Singh *et al.* (1987) studied 40 genotypes of Asiatic and European carrots for different characters and observed highest genetic variability for leaf length and the lowest for total soluble solids. Highest heritability was observed for all the characters.

Agwah *et al*. (1990) reported that the heaviest roots at harvest were produced by Dako (64.1 and 50.0 g) and Chantenay Red Cored (60.2 and 53.2 g) in the first and second season, respectively. Chantenay Long (14.3 tonne/faddan) gave the highest root yield inthe first season than the Chantenay Red Cored (9.97 t/fadden). Chantenay Red Cord andRoma had the highest carotene contents in both seasons.

Twetia and Dudi (1999) studied 26 genotypes of carrot and found highest variability for root weight, root-shoot ratio and shoot weight.

2.2.2 Correlation Coefficient

The association of characters is an important tool in the hands of plant breeder for making improvement in the crops. Most of the traits of economic importance are complex involving several related traits, therefore, the knowledge of degree of phenotypic and genotypic correlation of the traits is important (Robinson *et al*.,1951).

Thompson (1969) reported that cylindricality of carrot roots was associated with high plant density whereas in older carrots it was associated with low plant density. Andryvshenko and Syrovatskaya (1971) found that dry matter sugar and carotene were positively correlated with root size.

Correlation between the time of emergence of individual carrot seedling and final root size was established by Dowkar *et al.* (1978). He made attempts to find ways of reducing the spread of time to emergence in order to increase the uniformity of root size.

Bhagchandani and Choudhary (1980) showed positive and highly significant phenotypic correlation between root weight, top length and top weight, root length and root diameter and core diameter in a 6-parents diallel set of crosses in carrot. According to them, genotypic correlations were higher than the phenotypic correlations.

Sukhija and Nandpuri (1986) observed significant positive correlations of root weight with root length, root diameter, fresh thickness and core diameter and found that root diameter had the positive direct effect on root weight followed by root length.

Sazonova (1986) found that yield was correlated with mean root weight (r=0.54) and number of plants forming roots (r=0.85). Higher root yields tended to be associated with larger shoots (Mole *et. al*.**,** 1987). Bujdoso and Hrakso (1988) observed a close correlation between root weight and shoulder diameter (r=0.73) and moderate correlation between root weight and root length.

Singh *et al*. (1989) observed that the correlations of root yield with leaf length, root weight and root length were positive. Total soluble solids showed negative association with leaf length, root length and root yield.

Timin (1989) reported that root colour intensity in carrot was found to be correlated with carotene content.

A positive correlation between root length and yield was observed in early and mid early varieties (Mugniev, 1991).

Pariari *et al*. (1992) observed high positive and significant correlation with root diameter and top weight and moderate positive and significant correlation with leaf number and root volume.

Kasiri *et al*. (2013) reported a positive correlation among the root weight, outer and inner core thickness, root length ratio and root diameter and there was a negative correlation between root weight and dry matter per cent also between outer core thickness and TSS content.

2.2.3 Gene action, Carrot breeding and heritability components

The main objectives of carrot breeding programs are the improvement of yield (root and seed), uniformity in visible characteristics such as colour; shape, smoothness, freedom from defects, resistance to common diseases, non-bolting (Peterson and Simon, 1986).

Significant heterosis is usually connected with a good combining ability of the lines. The production of inbred carrot lines by self-pollination is laborious and time-consuming and requires the kind of attention that is typical in biennial, open-pollinated vegetables, so knowledge of combining ability could be helpful in speeding up the selection of parents for hybrids (Peterson and Simon 1986, Rubatzky *et al*. 1999, Simon *et al*. 2008).

Suh *et al*. (1999) and Jagosz (2011) documented carrot hybrid breeding based on inbred lines, in which crossing resulted in a heterosis effect, mainly for root yield. On the other hand, heterosis is very rare in the case of root quality traits, which was confirmed by Gauciene *et al*. (1999), Chira *et al*. (2008) and Jagosz (2011).

Knowledge about inheritance and the genetic basis of quantitative traits in European carrot is still incomplete. Duan *et al*. (1996) tested seven carrot lines and 12 hybrids from a diallel cross and indicated a high positive general combining ability for yield.

Chandel *et al*. (1994) carried out wide-ranging genetic studies of five quality characters in carrot. They observed strong additive effects for ascorbic acid content and domination for dry matter and total sugar, while both additive and non-additive gene effects were significant for total soluble solids and β-carotene.

Chandel *et al*. (1994) also revealed epistatic gene effects for all of the examined traits. The heritability and gene numbers in the inheritance of carotenoids were widely studied by Santos and Simon (2006). By testing two carrot crosses, they observed broad-sense heritability ranging from 28-48% for carotenes and from 44-89% for lycopene and phytoene in one of the crosses.

Traka-Mavrona (1996) obtained quite high heritability values (h^2 = 0.42-0.86) for skin quality, colour of shoulders, petiole attachment and root colour when observing some morphological traits of carrot.

The research provided by Michalik *et al*. (1988) showed genetic differences in dry matter, sugar content and nitrate accumulation between the tested carrot lines. The study suggests a very high role of additive gene effects and high heritability for these

compounds, especially nitrates, which are one of the undesirable compounds found in roots.

The relationship between major root carotenes, root colour and several other root morphological traits based on correlation and path analysis was revealed by Santos *et al.*, (2005), while working with two F2 carrot populations. Root weight had a positive significant correlation with leaf length, root length, top and middle root diameter. Path analysis of beta carotene synthesis in the B493 x QAL population suggested that selection for root carotenes had little effect on plant morphological traits.

Ahmed and Tanki (1992) reported high heritability estimate for shoot length and shoot weight followed by root weight, flesh thickness and root length while it was lowest for root yield. According to them, all the characters except root girth showed high genetic advance. Pike *et al*., (1991) evaluated Imperator 58 and found that roots of Texas Gold Spike were more uniformly orange in cross section and noted higher carotenoid contents.

Chandel *et al*. (1993) reported additive gene effects were prevalent for root yield and length, while the preponderance of both additive and non-additive genetic components of variance were detected in root and leaf weights, root top ratio and leaf length. They also observed over dominance for root girth.

Startseva (1990) reported that least environmental variation existed for leaf shape, leaf colour and days from planting to onset of maturity stage for which the heritabilityestimates were 0.97, 0.65 and 0.58, respectively.

Ragheb *et al.* (1989) found a wide range of phenotypic variation, with high valuesfor the genetic coefficient of variation, heritability and genetic advance for root freshweight, lop fresh weight, root/top ratio.

Peterson *et al*. (1988) observed Beta III as an improved source of carotene for providing vitamin A. It was also a promising population from which breeder could select inbred lines to exploit heterosis. Vyrodova *et al*.,(1988) observed that beta-carotene in carrot variety ranged from 3.6 to 9.8mg/100 g on fresh weight basis.

Timin (1987) found the coefficient of broad sense heritability to be 0.60-0.68 for root colour intensity and for root weight it was 0.15 to 0.36.

Singh *et al.* (1987) studied 40 genotypes of Asiatic and European carrots for different characters and observed highest genetic variability for leaf length and the lowest for total soluble solids. Highest heritability was observed for all the characters.

Brar and Sukhija (1981) evaluated 14 cultivars of carrots for seven characters, including yield components and observed that the genotypic and phenotypic coefficient of variability was lowest for the number of days to maturity and highest for leaf weight. Highest broad sense heritability and genetic advance were observed for leaf length, leaf weight and root weight.

Prasad and Prasad (1980) observed high estimates of heritability and genetic advance in 21 varieties of carrot for leaf number per plant, leaf length and width, weight of aerial parts and root length, diameter and fresh weight.

2.3 Genetic variation for nutritional quality traits

Carrot contains appreciable amount of carotene (10 mg/100 g), thiamin (0.04 mg/g) and riboflavin (0.05 mg/g) (Sharfuddin and Siddique, 1985) and it is an excellent source of iron, carbohydrate, vitamin-B, vitamin-C and sugar (Yawalker, 1985).

Vitamin A is essential for a variety of biological processes, many of which are related to growth cellular differentiation and interactions of cells with each other or with extracellular matrix. Its deficiency, even in its relatively early stage, results in impairments in linear growth, cartilage and bone development and epithelial cell differentiation and function (Roberts and Sporn, 1984; Deluca, 1991).

The most widespread and important carotene is β- carotene which is found abundantly in some plants. The essential role of β-carotene as a dietary source of vitamin A has been known for many years (Britton, 1995).

Among the provitamin A Carotenoids in food namely beta-carotene, alpha-carotene, gamma-carotene and beta-cryptoxanthin, beta-carotene is the one that is most efficiently converted to retinol (Olson *et al*., 2000).

Carotene of major importance is β-carotene which is present in almost all vegetables and fruits, and it is a very important precursor of vitamin A. During digestion carotenes in food are subjected to the action of different enzymes (esterases, lipases). Most of carotenoids are cleaved to retinoids (vitamin A) or to a lesser degree, absorbed intact (Simpson, 1983). Peto *et al* (1981) suggested that β-carotene might be the primary anticancer agent in fruits and vegetables.

Higher dietary intake of β-carotene has inverse relationship on various cancers, predominantly one of the aero digestive tract (Van Poppel, 1996). It has same influence on coronary heart disease (Gey, 1993).

Vitamin A is important factor in human growth and i mmunity. Daily needs for vitamin A of an adult person are around 600-700 μg, having in mind rate that for 1μg of vitamin A are needed 6 μg of β carotene or 12 μg of some other carotenoid. According to Heinonen (1990) carrot cultivars, in most of the cases, contains 1200 – 2300 μg/100 g of provitamin A in root fresh matter, providing enough pigment to satisfy daily needs.

Carotenoid pigments play an important role in human diet, as humans cannot synthesize carotenoids and depend on dietary sources for making their retenoids, such as retinal (the main visual pigment), retinol (vitamin A) and retenoic acid (a substance controlling morphogenesis) (Naik *et al*., 2005). β-carotene deficiency in human diet causes symptoms ranging from night-blindness to those of xerophthalmia and keratomalacia, leading to total blindness. They are also beneficial in reducing chronic conditions related to coronary heart diseases, certain cancers and macular degeneration (Mayne, 1996).

Carotene distribution inside the carrot root is not uniform. Carotene formation is much higher in older tissues in comparison with young one. That is why amount of carotene is decreasing longitudinally from the upper root part to the tip. Usually phloem root part has more carotene than xylem. During the maturation, carotene accumulation is raising inside root, improving it colour intensity (Gabelman, 1974).

There is a positive correlation between carotene content and colour. Carotene content increased with the age and size of the root (Fritz and Weichmann, 1979; Rosenfeld, 1998).

Santos and Simon (2002) studied the heritability and gene number in the production of provitamin a in carrots from the F_2 segregating population.

Environmental factors like normal water content in soil can reduce amount of carotene, while more fertilizer applications can increase synthesis of these pigments. For optimum increase of carotene amount it was reco mmended fertilization of 80 to 150 kg ha^{-1} of nitrogen. Optimum temperature for carotene synthesis is in the range of 15 ^{0}C up to 21 ^{0}C (Rubatzky *et al*., 1999).

It is believed that one medium sized carrot (60g) provides enough provitamin A carotene to fulfill adult vitamin A needs for one day (Simon, 1990). Recently twenty carotenoid biosynthetic structural genes have been cloned and sequence characterized in carrot (Just *et al.*, 2007) and hence provides a foundation for PCR based expression studies to characterize the varieties of carrot for carotenoids.

The modern cultivated carrot genus (*D. carota* spp. *sativus*) is genetically diverse and is further subdivided into two groups, namely, carotene (*D. carota* ssp. *sativus var. sativus*) and anthocyanin groups (*D. carota* ssp. *sativus* var. *atrorubens* Alef.) (Pistrick, K., 2001).

Majority of the carrot species belongs to carotene carrot cultivars which are the most important sources of carotenoids and provitamin A and have been cultivated as root crops since 1100 years, whereas anthocyanin group carrots have the history of 3000 years (Ka mmerer *et al*., 2003 and Iorizzo *et al.*, 2011).

Carrot is a significant source of vitamin A accounting for an estimated 30% of the dietary vitamin A in the diet (Simon, 1992). Carotenoids, including α and β carotene, are abundant in carrot and they account for both high provitamin A content and familiar orange color. Carrots contain approximately 150 ppm carotenoids. Darker orange carrot strains containing 300 ppm carotene and found to be suitable in temperature and highland tropical areas (Simon, 1990). Methods for selecting carotene content are well-established (Simon and Wolff, 1987). Visual selection is moderately successful for improving carrot carotene content up to 200 ppm but laboratory analysis is necessary for accurate selection at higher levels.

Transcriptional regulation is thought to be the major factor in carotenoid accumulation in the organs. Clotault *et al* (2008) studied the expression of eight genes encoding carotenoid biosynthesis enzymes during the development of various coloured carrots such as white, yellow, orange and red carrot roots. The genes chosen encode phytoene synthase (PSY1 and PSY2), phytoenedesaturase (PDS), z-carotene desaturase (ZDS1 and ZDS2), lycopene *e*-cyclase (LCYE), lycopene b-cyclase (LCYB1), and zeaxanthinepoxidase (ZEP). All eight genes were expressed in the white cultivar even though it lacks carotenoids.

By contrast, with fruit maturation, the expression of carotenogenic genes began during the early stages of development and then progressively increased for most of these genes during root development as the total carotenoid level increased in coloured carrots. The expression of genes encoding LCYE and ZDS was high in yellow and red cultivars, respectively, which could be associated with accumulation of lutein and lycopene, respectively. The accumulation of total carotenoids during development and the accumulation of major carotenoids in the red and yellow cultivars might partially be explained by the transcriptional level of genes directing the carotenoid biosynthesis pathway.

Malgorzata *et al*. (2006) explained about the presence of various carotenoids in different root colours of carrot such as orange carrots contain predominantly β-carotene (45-80%) followed by α-carotene that together constitute up to 95 % of total carotenoids. In yellow carrot, lutein and β-carotene are mainly found, but traces of α-carotene are also present. Significant amounts of lycopene are present only in red roots that contain also β-carotene while α-carotene is usually below the detection limit. Purple roots can possess a similar carotene composition as orange roots, but the presence of dark anthocyanins masks the orange colour.

The organoleptic quality directly depends upon the biochemical compounds like sugars, polyacetylenes and phenolics (Alasalvar, 2001 and Czepa and Hofmann, 2004) and High sensory quality and sweetness of carrot positively correlate with sugar content (Talcott *et al*., 2001).

Ahmed *et al*. (2011), studied the influence of location on nutritional and orgnaoleptic qualities of carrot such as reducing and total sugars, TSS, polyacetylenes,

phenols *etc.* most of these traits are highly influenced by environments. The same cultivar when grown in different districts in Pakistan showed significant variation in reducing sugars and non-reducing sugars concentration.

Carrot has medium to high energetic value (47 cal/100g) with balanced content of carbohydrates (8 – 9%). These carbohydrates are increasing the nutritional value of the carrot and they are giving sweet taste to this vegetable.

Carrot root and leaves have balanced amount of sugar compounds, their quantity can be partly influenced by environmental factors like plant density on the field and exposure to the light. Dry matter amount in the root can be under great influence of plant density, it decreases with higher density (Hole *et al.*, 1983).

Accumulation of glucose and fructose is dominant during the initial growing period of the root. That is connected with high activity of enzyme invertase in that growing stage. Amount of sucrose is increasing later, in mature plant. Capability of parenchyma tissue to stores sucrose enables drastically increase of it concentration inside carrot root (Rubatzky *et al.*, 1999).

Sugars distribution inside the root is not equal. Usually parenchyma of phloem has more carbohydrates per dry matter than root core part. Similar results are seen in sucrose distribution, less present in core part (Phan and Hsu, 1973).

Rosenfeld (1998) suggested that higher temperature influences on carrot root length and amount of glucose. Under higher temperature root length is shorter and amount of glucose is decreasing.

Fertilization as environmental factor, up to level of 140 kg/ha may influence on the increase of carbohydrates synthesis in certain varieties of carrot (Hochmuth *et al.*, 1999).

Sugar content ranges from 3 to 7% for carrots grown in organic soil (Sto mmel and Simon, 1989). Production of carrots on mineral soils can yield carrots with 7 to 16% sugar. Realized heritability for sugar content is 40 to 45%. In addition to the quantitative variation for total sugar content, a single gene controls sugar type (sucrose vs. reducing sugar) in carrots (Freeman and Simon, 1983).

Simon (1985) reported that a single gene *Rs* stands for reducing sugar seems to be controlling the type of sugar in the root. When dominant allele (*Rs*) is present, there will be accumulation of the reducing sugars *fructose* and *glucose*. When both the alleles *(rsrs*) are recessive sucrose concentration will be high.

2.4 Genomics and molecular marker diversity in carrot

To date, the origin(s) of carrot domestication has not been studied, and only a small number of studies have used molecular markers to examine carrot genetic diversity. Thus far, molecular data have not been able to uncover any clear population structure in carrot (Bradeen *et al.*, 2002).

A distinction was detected between cultivated and wild carrot accessions using amplified fragment length polymorphism (AFLP) markers (Shim and Jørgensen, 2000).

A wider characterization of cultivated carrot, using simple sequence repeat (SSR) markers and single nucleotide polymorphisms (SNPs) revealed a moderate separation between eastern and western cultivated carrots (Clotault *et al*., 2010 ; Baranski *et al*., 2012).

Carrot has not been studied extensively and molecular tools facilitating genome analysis and breeding of the crop remain underdeveloped. Only recently has a more systematic approach towards developing such tools been carried out, resulting in a set of simple sequence repeat (SSR) and single nucleotide polymorphism (SNP) markers identified through sequencing of carrot bacterial artificial chromosome (BAC) ends (Cavagnaro *et al*. 2009) and comparative analysis of three carrot transcriptomes (Iorizzo *et al*. 2011).

Iorizzo *et al*. (2013) provided clear evidence for diversification between wild and cultivated accessions, supporting previous reports based on amplified fragment length polymorphism markers (Shim and Jørgensen 2000; Bradeen *et al*. 2002).

Analysis of SSR markers by Baranski *et al*. (2012) showed evidence for the separation of the cultivated germplasm into two distinct groups, the Eastern (Asian) and Western (European and American) gene pools.

A recent study based on *D. carota* plants of different origin genotyped with more than 3,300 SNP markers suggested that Central Asia is the center of origin of cultivated carrot, and that orange-rooted carrots of the Western type were selected from the yellow domesticated carrots (Iorizzo *et al*., 2013).

Bradeen *et al*. (2002) reported that large amount of phenotypic and molecular diversity is available in carrot and this diversity has been important in improving nutritional value and consumer quality; disease and pest resistance; and yield characteristics important for growers.

Cavagnaro *et al.* (2011) evaluated 65 carrot cultivars including cultivated and wild species for 10 selected SSR markers to study the molecular diversity. For this germplasm they found 190 different alleles, with lengths ranging from 144 to 433 bp, were identified. All the loci examined were highly diverse. The average number of alleles per SSR was 19.1 with a range of 10-29, whereas the mean expected heterozygosity was 0.84, and ranged from 0.77 for GSSR9 to 0.91 for GSSR4. The most polymorphic loci were GSSR4 (NA = 29; He = 0.91) and GSSR6 (NA = 19; He =0.89) and the least polymorphic was gssr65 (NA =10; He = 0.79).

Baranski *et al.* (2012) evaluated carrot for genetic diversity in 88 accessions using 30 SSR markers. Based on the Bayesian approach, these accessions were clustered in two groups comprising of Asian and Western types and genetic diversity of Asian types was higher than the Western types. All thirty SSR markers were polymorphic with 227 alleles and an average of 7.6 per locus. Most of the alleles (66%) had frequencies below 0.1 and only 9% occurred with frequencies above 0.4. About half of the alleles (51%) were rare (freq. < 0.05) and were detected in all except one locus. In 12 loci, 19 unique alleles were identified (8.4% of all alleles). The observed heterozygosity (Ho=0.33) was, on average, much lower than the expected heterozygosity (He = 0.63). In this study SSR markers were selected from Cavagnaro *et al.* (2011), Rong *et al.* (2010) and Niemann (2001). PIC value was higher for the farmer author (0.67 ± 0.03 s.e.) followed by Rong *et al.*, 2010 (0.50 ± 0.06).

Bradeen *et al*. (2002) reported that large amount of phenotypic and molecular diversity is available in carrot and this diversity has been important in improving

nutritional value and consumer quality; disease and pest resistance; and yield characteristics important for growers.

Despite the taxonomic differentiation between geographical groups, no population structure has been found in carrot germplasm by examining random molecular markers such as isozymes (St. Pierre and Bayer, 1991; St. Pierre *et al*., 1990); random, amplified, polymorphic DNA (RAPD; Grzebelus *et al*., 2002; Nakajima *et al*., 1998, 1997); amplified-fragment length polymorphism (AFLP; Bradeen *et al*., 2002; Nakajima *et al*., 1998; Shim and Jørgensen 2000); and inter-simple sequence repeats (ISSR; Bradeen *et al*., 2002).

Bradeen *et al*. (1998) reported that Y2 locus controls carotene accumulation on the root xylem core. In F2 mapping using bulked segregant analysis 6 AFLP fragments were linked to Y2 by generating co-dominant PCR –based markers from dominant AFLP fragments using Y2 linked AFLP fragment as a module.

Just *et al*. (2009) reported a major QTL such as Y and Y2 loci on Linkage group 2 and 5 respectively for carrot colour which were linked to several carotenoid biosynthetic enzyme sequence tagged sites. The Y locus was closely linked to the STS marker for CHXE gene, the STS marker for NCED2, and more distantly linked to the STS marker for the PDS gene. In carrot, for accumulation of large amount of orange colour carotene pigments, these two loci must be in recessive state.

Clotault *et al*. (2012) showed that CRTISO gene has undergone through selection events in cultivated carrot but the polymorphism pattern was observed among partial CRTISO sequence (only 700–1,000 bp). The particular status of this gene and preliminary results suggest that CRTISO gene could be a good candidate for selection signature research. The analysis of the nucleotide polymorphism and the LD among the complete CRTISO sequence will enable to clarify the selection pattern, depending on the gene structure and in relation with colour types.

Carotenoid Isomerase (CRTISO) has emerged as a regulatory step in the carotenoid biosynthesis pathway and could be a good candidate to show how a metabolic pathway gene reflects a species genetic history (Soufflet-Freslon *et al.*, 2013).

Vivek and Simon, 1999, used a population of B9304 x YC7262 identified a locus of Y2 -Differential xylem/phloem carotene levels, Rs-Sugar type (reducing/non-reducing) in roots, P1 - Purple/yellow pigment accumulation in roots.

Transposable elements play an important role in shaping the plant phenotypes in carrot which conditions the type of sugar in storage taproot (Yau and simon 2003; Kurilich *et al*., 2005). Iorizzo *et al*., 2011 by *de novo* assembling of transcripts observed a range of functional TE transcripts suggesting the members of many TE families are potentially be active in carrot and MITEs and D cmaster related transposable elements are highly polymorphic in carrot and MITEs in the carrot genome are mainly associated non-coding regions of genes.

Polymorphism of simple sequence repeat (SSR) loci was assessed in a collection of 88 carrot (*Daucus carota* L. subsp. *sativus* Hoffm.) accessions. The collection comprised cultivars and landraces mainly from Asia, Europe, and North America. Plants were grown in the glasshouse and characterized for root color and shape. Thirty SSR loci were fully characterized using parameters derived from allele frequencies, *i.e.* the number of total, effective and rare alleles, the observed and expected heterozygosity and fixation index. Using a Bayesian approach, two clusters of 17 and 61 accessions were distinguished, which comprised of the Asian and Western type accessions, respectively. Genetic diversity of the Asian gene pool was higher than that of the Western gene pool.

Identification of SSR loci in carrot was initiated by Niemann (2001) for linkage mapping. The intervarietal variability and genetic distance of carrot germplasm both in cultivated and wild types was revealed by Clerc and Briard (2003) by using 70 AFLP and SSR markers. Based on this study, they concluded that the high level of variability was possible with the molecular markers in comparison with morphological characters and they are the best tools for variability studies but cannot replace the morphological characterization.

2.5 Population structure

Population structure is a constraint that can create false associations in association mapping studies. Population structure is formed by non-random mating within a species, which can cause changes in allele frequencies (Ersoz *et al.* 2007). The

non-random mating may be due to events such as genetic drift and domestication bottlenecks. This can inflate the presence of certain marker alleles resulting in over representation in a population, which in turn cause them to be falsely associated with a phenotype (Pritchard *et al*., 2000). There are different statistical approaches for controlling population structure in association mapping. In population based studies, two approaches are used: genomic control (GC) and structured association (SA) (Yu and Buckler, 2006). Genomic control calculates the non-independence of loci, which corrects for any population structure (Ersoz *et al*., 2007). The significance tests, or P-values, are then adjusted to account for the population structure. However, as Mackay and Powell (2007) noted, corrected P-values result in a loss of statistical power, especially when there are higher levels of population structure.

More recently, structured association has been the method of choice to correct for population structure in most association studies. For structured association, random unlinked markers are used to calculate and assign individuals into population substructures (Pritchard, 2000). The program STRUCTURE (Pritchard, 2000) is often used to calculate population structure. This program uses a MCMC Bayesian algorithm to calculate the proportion of an individual's genome that originated from different inferred populations. The individuals are then clustered into different groups based on their genome characterization. STRUCTURE assumes that all individuals are unrelated and come from populations in Hardy-Weinberg equilibrium. STRUCTURE allows users to calculate the degree of population admixture of each individual. Principle component analysis (PCA) has also been used to calculate population structure (Price *et al*. 2006). This method can be much quicker than using STRUCTURE which has been suggested by Zhao *et al*. (2007) as effective.

There are two different types of models that apply structured association. The first is a GLM model which uses the subpopulations (Q) as covariates in a regression model, and then correlates the genotype with phenotype (Thornsberry *et al*. 2001). However, this model along with the GC model may not control false positives or have a low statistical power due to familial relatedness (Yu *et al*. 2005). The Q+K, or unified mixed model, still assigns subpopulations (Q) as covariates, but it also uses a kinship matrix (K) as a covariate in the regression (Yu *et al*. 2005). This method accounts for both population structure and familial relatedness. A number of studies have

demonstrated that the Q+K model can be more effective than just the Q model. For example in Arabidopsis, Zhao *et al*. (2007) found that when cumulative P-values were plotted for flowering time, the Q+K model corrected for more false associations than the Q model. As a result, the Q+K model is a popular choice in most GWAS.

2.6 Marker-trait association for economic traits in carrot

Association mapping (AM) is a method that can address the shortcomings of linkage mapping. This method allows the use of many diverse individuals, which increases the number of alleles examined and samples multiple historical recombination events. As a result, properly chosen AM panels have a greater frequency of alleles that encompass the genetic variation of the crop species. This can reduce the time, along with the costs, to identify markers linked to quantitative traits. Association mapping capitalize on the historical levels of recombination accumulated in natural populations, landraces, breeding material and varieties, which results in higher QTL resolution than linkage mapping. These advantages have made AM a valuable method in marker-trait associations.

There are two different types of association mapping reported in the literature: genome-wide association studies (GWAS) and candidate gene association mapping. The GWAS method scans the entire genome to determine if any association between markers and phenotypes exists. This method requires that there are enough markers to cover the genome based on the expected rate of linkage disequilibrium (LD) decay. The other method, candidate gene AM, requires prior knowledge of candidate genes that could be associated with a phenotype. This knowledge may have been gained through QTL linkage mapping, GWAS or from work in related species. Instead of a whole genome scan, only markers within those candidate genes are analyzed for associations.

As the whole genome of carrot is already been sequenced (Iorizzo *et al*., 2016), there is a great scope for utilization of this information in robust genomic platforms such as mapping, genomics assisted selection, expression profiling *etc* for carrot and other Apiaceae members to facilitate the identification of simple molecular markers and discovery of genes associated with traits of breeding interest.

Bradeen *et al*, (1998) identified Y2 locus which controls carotene accumulation on the root xylem core. In F2 mapping using bulked segregant analysis 6 AFLP fragments were linked to the Y2 by generating co-dominant PCR –based markers from dominant AFLP fragments using Y2 linked AFLP fragment as a module.

A major QTL such as Y and Y2 loci on Linkage group 2 and 5 respectively for carrot colour were linked to several carotenoid biosynthetic enzyme sequence tagged sites. The Y locus was closely linked to the STS marker for CHXE gene, the STS marker for NCED2, and more distantly linked to the STS marker for the PDS gene. In carrot, for accumulation of large amount of orange colour carotene pigments, these two loci must be in recessive state (Just *et al*., 2009).

Clotault *et al.* (2010) used Seven candidate genes involved in the carotenoid biosynthesis pathway which have been analysed from a sample of 48 individual plants, each one from a different cultivar of carrot (*Daucus carota L. ssp. sativus*). A high single nucleotide polymorphism (SNP) frequency of 1 SNP per 22 bp (mean psil = 0.020) was found on average within these genes. The analysis of genetic structure from carotenoid biosynthesis gene sequences and 17 putatively neutral microsatellites showed moderate genetic differentiation between cultivars originating from the West and the East (FST = 0.072) which was being consistent and not evident before by molecular markers. Carotenoid biosynthesis genes did not exhibit decay of LD (mean r^2 = 0.635) within the 700–1,000 bp analysed, even though a fast decay level of LD is expected in outcrossing species.

Carotenoid Isomerase (CRTISO) has emerged as a regulatory step in the carotenoid biosynthesis pathway and could be a good candidate to show how a metabolic pathway gene reflects a species genetic history (Soufflet-Freslon *et al.,* 2013).

Clotault *et al*. (2012) showed that CRTISO gene has undergone through selection events in cultivated carrot but the polymorphism pattern was observed among partial CRTISO sequence (only 700–1,000 pb). The particular status of this gene and preliminary results suggest that CRTISO gene could be a good candidate for selection signature research. The analysis of the nucleotide polymorphism and the LD among the complete CRTISO sequence will enable to clarify the selection pattern, depending on the gene structure and in relation with colour types.

Vanessa *et al*. (2013) conducted a study to know the nucleotide polymorphism and the linkage disequilibrium among the complete *CRTISO* sequence, and the deviation from neutral expectation were analysed by considering population subdivision revealed with 17 microsatellite markers. A sample of 39 accessions, which represented different geographical origins and root colours, was used. This species was divided into two genetic groups: one from Middle East and Asia (Eastern group), and another one mainly from Europe (Western group). The Western and Eastern genetic groups were suggested to be differentially affected by selection: a signature of balancing selection was detected within the first group whereas the second one showed no selection. A focus on orange-rooted carrots revealed that cultivars cultivated in Asia were mainly assigned to the Western group but showed *CRTISO* haplotypes common to Eastern carrots.

3. MATERIAL AND METHODS

Carrot being a highly cross pollinating nature has a greater diversity for various roots morphological and biochemical components. In India, due to a vast geographical diversity and varied agro climatic conditions both Asiatic and European types of carrots are grown suitable to temperate and tropical conditions. Farmers have also made their own selections from these collections and maintained them as landraces or local types. To know the genetic diversity both at the morphological and biochemical and at the molecular level and to identify the pattern of the population Structure among these germplasm lines, collected all over the country have been evaluated with various qualitative, quantitative traits and with the molecular markers consisting of genic and genomic SSRs, InDels (insertion-deletion markers) and SCARs. The details of the plant material, experimental details, methodology followed and various statistical analysis applied to fulfill the objectives of the present investigation is presented below.

3.1 Experimental Site

The field experiment was conducted at Udyanagiri Campus of University of Horticultural Sciences, Bagalkot, Karnataka, India during 2016 (Plate 1). Bagalkot is located in the northern region of Karnataka and positioned at 16°12′N, 75°45′E the average elevation in this area reaches approximately 610 m. The climate is warm and dry throughout the year and rainfall is scarce with an average annual rainfall of 318 mm and belongs to semi arid tropical region.

Lab experiments including biochemical estimation and molecular marker screening were carried at Plant Molecular Biology Lab in the Department of Biotechnogy and Crop Improvement with the future goal of exploring the available carrot germplasm lines for breeding and crop improvement in tropical region.

3.2 Plant Materials

Ninety six *Daucus carota* L. germplasm lines were used, including Asiatic and European cultivated accessions. This panel represents a large diversity present in carrot especially for the colour *viz.,* white, yellow, red, orange, Dark orange, purple and Black. The genotypes were collected from all over India, comprising of open-pollinated

Plate 1: The research plot at Udyanagiri Campus of University of Horticultural Sciences Bagalkot, Karnataka, India during 2016

Table 1: Details of 96 Carrot Genotypes and their description

Sl. No.	Name	UHSBC-Nomenclature	Collection site
1	VANNUR LOCAL 1	UHSBC-1	Local cultivar
2	VANNUR LOCAL 2	UHSBC-2	Local cultivar
3	VANNUR LOCAL 3	UHSBC -3	Local cultivar
4	CENTURY EARLY NANTES	UHSBC-7	Ooty collections
5	KANKANAKOPPA LOCAL-1	UHSBC-14	Local cultivar
6	KANKANAKOPPA LOCAL-2	UHSBC-15	Local cultivar
7	GHATAPRABHA LOCAL-1	UHSBC-16	Local cultivar
8	GHATAPRABHA LOCAL-2	UHSBC-17	Local cultivar
9	HANGARAKI LOCAL	UHSBC-18	Local cultivar
10	BLACK WONDER	UHSBC-19	Online Collection
11	BAGALKOT LOCAL	UHSBC-20	Local cultivar
12	MAHARASHTRA LOCAL	UHSBC-21	Local cultivar
13	JATT LOCAL	UHSBC-22	Local cultivar
14	VRCAR-1	UHSBC-23	IIVR Collection
15	VRCAR-1	UHSBC-23-1	IIVR Collection
16	VRCAR-2	UHSBC-24	IIVR Collection
17	VRCAR-5	UHSBC-25	IIVR Collection
18	VRCAR-7	UHSBC-26	IIVR Collection
19	VRCAR-8	UHSBC-27	IIVR Collection
20	VRCAR-9	UHSBC-28	IIVR Collection
21	VRCAR-11	UHSBC-29	IIVR Collection
22	VRCAR-13	UHSBC-30	IIVR Collection
23	VRCAR-17	UHSBC-31	IIVR Collection
24	VRCAR-20	UHSBC-32	IIVR Collection
25	VRCAR-20	UHSBC-32-2	IIVR Collection
26	VRCAR-22	UHSBC-33	IIVR Collection
27	VRCAR-25	UHSBC-34	IIVR Collection
28	VRCAR-25	UHSBC-34-1	IIVR Collection
29	VRCAR-25	UHSBC-34-2	IIVR Collection
30	VRCAR-26	UHSBC-35	IIVR Collection
31	VRCAR-29	UHSBC-36	IIVR Collection
32	VRCAR-32	UHSBC-37	IIVR Collection
33	VRCAR-35	UHSBC-38	IIVR Collection
34	VRCAR-40	UHSBC-39	IIVR Collection
35	VRCAR-42	UHSBC-40	IIVR Collection
36	VRCAR-45	UHSBC-41	IIVR Collection
37	VRCAR-45	UHSBC-41-1	IIVR Collection
38	VRCAR-54-1	UHSBC-42	IIVR Collection
39	VRCAR-59	UHSBC-43	IIVR Collection
40	VRCAR-59	UHSBC-43-1	IIVR Collection
41	VRCAR-62	UHSBC-44	IIVR Collection
42	VRCAR-63	UHSBC-45	IIVR Collection
43	VRCAR-66	UHSBC-46	IIVR Collection
44	VRCAR-68	UHSBC-47	IIVR Collection
45	VRCAR-70	UHSBC-48	IIVR Collection
46	VRCAR-74	UHSBC-49	IIVR Collection
47	VRCAR-77	UHSBC-50	IIVR Collection
48	VRCAR-80	UHSBC-51	IIVR Collection

Contd…

Sl.No	Name	UHSBC-Nomenclature	Collection Site
49	VRCAR-81	UHSBC-52	IIVR Collection
50	VRCAR-85	UHSBC-53	IIVR Collection
51	VRCAR-124	UHSBC-54	IIVR Collection
52	VRCAR-153	UHSBC-55	IIVR Collection
53	VRCAR-171	UHSBC-56	IIVR Collection
54	INDAM KURODA	UHSBC-58	Temperate
55	VAISHALI SEEDS (PUSA KESARI)	UHSBC-59	Released Variety (IARI, New Delhi)
56	PUSA PAYASA	UHSBC-63	Released Variety (IARI)
57	PUSA RUDHIRA	UHSBC-64	Released Variety (IARI)
58	PUSA MEGHALI	UHSBC-65	Released Variety (IARI)
59	PUSA ASITA	UHSBC-66	Released Variety (IARI)
60	PUSA VRISHTI	UHSBC-67	Released Variety (IARI)
61	AKSHAY-1	UHSBC-68	Bangalore Market
62	NEW KURUDA	UHSBC-69	Ooty collections
63	GADDANAKERI CROSS	UHSBC-71	Local cultivar
64	BELGUM ROOTS	UHSBC-73	Local cultivar
65	NAGANUR ROOTS	UHSBC-76	Local cultivar
66	SANGALI ROOTS	UHSBC-77	Local cultivar
67	BAGALKOT LOCAL-2 ROOTS	UHSBC-78	Local cultivar
68	GOLDEN ROD	UHSBC-79	Tamilanadu Collection-Temperate
69	ORANGE CARROT	UHSBC-85	Online collection
70	RUEBLI NANTAISE-2	UHSBC-89	Tamilanadu Collection-Temperate
71	FLAKKEER LANG	UHSBC-90	Online Collections
72	IMPERIAL HYBRID DARK RED	UHSBC-92	Online Collections
73	PRADHAM CARROT	UHSBC-93	Online Collections
74	SUPER KURUDA	UHSBC-94	Online Collections
75	BLACK CARROT	UHSBC-95	Collection from Farmer (Punjab Seeds)
76	DHENU SEEDS	UHSBC-96	Private Sector Seeds
77	KODAIKENAL NEW KURUDA	UHSBC-97	Kodaikenal
78	F1 ELI NANTES	UHSBC-98	Ooty Market (Private Sector Hybrid)
79	IMP KURUDA	UHSBC-99	Tamilanadu Collection-Temperate
80	HYB KURUDA	UHSBC-100	Ooty Market
81	SK KURUDA	UHSBC-101	Tamilanadu Collection-Temperate
82	OOTY KURUDA	UHSBC-102	Ooty Collections
83	NEW FIELD EARLY	UHSBC-103	Tamilanadu Collection-Temperate
84	EARLY NANTES	UHSBC-104	Tamilanadu Collection-Temperate
85	VIGRO KURUDA	UHSBC-105	Tamilanadu Collection-Temperate
86	UNIGEAN KURUDA	UHSBC-106	Tamilanadu Collection-Temperate
87	TOKITA EARLY NANTES IMPROVED	UHSBC-107	Tamilanadu Collection-Temperate
88	PAHAJA EARLY	UHSBC-108	Tamilanadu Collection-Temperate
89	SPLENDOUR BLACK SEEDS	UHSBC-110	Online Collection
90	SPLENDOUR KURUDA ORANGE SEEDS	UHSBC-111	Tamilanadu Collection-Temperate
91	SPLENDOUR DESI RED SEEDS	UHSBC-112	Tamilanadu Seeds
92	OCEAN SEEDS DARK RED	UHSBC-113	Tamilanadu Collection-Temperate
93	CARROT SUPER KURUDA	UHSBC-114	Tamilanadu Collection-Temperate
94	CARROT NANTES	UHSBC-115	Tamilanadu Collection-Temperate
95	MUKTESHWAR NORTH	UHSBC-116	North Indian –Temperate type
96	KULARKOPPA LOCAL	UHSBC-117	Local cultivar

Table 2: Augmented Block Design Field Experiment for morphological traits evaluation

S No	Check & Genotypes	BLOCK-I	BLOCK-II	BLOCK-III	BLOCK-IV	BLOCK-V	BLOCK-VI
1	Check-I	Ghataprabha Local	Pusa Vrishti	Ghataprabha Local	Vigro Kuruda	Ghataprabha local	Pusa Vrishti
2	Check-II	Pusa Vrishti	Vigro Kuruda	Vigro Kuruda	Ghataprabha Local	Vigro Kuruda	Ghataprabha Local
3	Check-III	Vigro Kuruda	Ghataprabha Local	Pusa Vrishti	Pusa Vrishti	Pusa Vrishti	Vigro Kuruda
4	Genotype 1	UHSBC1	UHSBC26	UHSBC41	UHSBC56	UHSBC77	UHSBC102
5	Genotype 2	UHSBC2	UHSBC27	UHSBC42	UHSBC58	UHSBC78	UHSBC103
6	Genotype 3	UHSBC3	UHSBC28	UHSBC43	UHSBC59	UHSBC79	UHSBC104
7	Genotype 4	UHSBC7	UHSBC29	UHSBC43-1	UHSBC63	UHSBC85	UHSBC105
8	Genotype 5	UHSBC14	UHSBC30	UHSBC44	UHSBC64	UHBC-89	UHSBC106
9	Genotype 6	UHSBC15	UHSBC31	UHSBC45	UHSBC65	UHSBC90	UHSBC107
10	Genotype 7	UHSBC16	UHSBC32	UHSBC46	UHSBC66	UHSBC92	UHSBC108
11	Genotype 8	UHSBC17	UHSBC32-2	UHSBC47	UHSBC67	UHSBC93	UHSBC110
12	Genotype 9	UHSBC18	UHSBC33	UHSBC48	UHSBC68	UHSBC94	UHSBC111
13	Genotype 10	UHSBC19	UHSBC34	UHSBC49	UHSBC69	UHSBC95	UHSBC112
14	Genotype 11	UHSBC20	UHSBC35	UHSBC50	UHSBC71	UHSBC96	UHSBC113
15	Genotype 12	UHSBC21	UHSBC36	UHSBC51	UHSBC73	UHSBC97	UHSBC114
16	Genotype 13	UHSBC22	UHSBC37	UHSBC52	UHSBC76	UHSBC98	UHSBC115
17	Genotype 14	UHSBC23	UHSBC38	UHSBC53	UHSBC 34-1	UHSBC99	UHSBC116
18	Genotype 15	UHSBC24	UHSBC39	UHSBC54	UHSBC 34-2	UHSBC100	UHSBC117
19	Genotype 16	UHSBC25	UHSBC40	UHSBC55	UHSBC 41-1	UHSBC101	UHSBC23-1

Table 3a: List of qualitative traits recorded in 96 genotypes in carrot

S. No	Characters	Details
1	Plant growth habit (score)	3-prostrate, 5-Semi-erect, 7-Erect
2	Root position in soil (score)	3-Shallow, 5-Medium, 7-Deep, 9- Very deep
3	Shoot Attachment (score)	1-Single, 2-Multiple
4	Leaf type (score)	1-Celery, 2-Normal, 3-Fern
5	Root branching (score)	1-Absent, 3-Sparsely, 5-Intermediate, 7-Dense
6	Root Hairiness (score)	1-Absent, 2-Very Low, 3-Low, 4-Moderate, 5-High, 6-Very high
7	Root cracking (score)	1-Absent, 2-P, 3-Low, Intermediate-4
8	Root tapering (score)	1-Blunt, 2-Pointed
9	Root texture (score)	1-Smooth, 2-Course, 3-Dimpled, 4-Ridged
10	Root shape (score)	1-Round, 2-obovate, 3-Obstrangular, 4-oblong, 5-tapering, 6-others
11	Root colour (score)	1-White, 2-Yellow, 3-Yellow orange, 4- Green Yellow, 5- Orange Yellow, 6-Orange/, 7-Dark Orange, 8-Light pink/Pink Yellow, 9-Pink/Purple Pink/Black Pink, 10-Dark Pink, 11-Red, 12-Purple, 13-Light purple, 14-Deep Purple, 15-Black Pink/Black/Black purple.
12	Shoulder colour (score)	1-Absent, 2-Green, 3-Orange, 4-Dark orange, 5-Pink, 6-Red/deep/dark pink, 7-Light purple/purple pink, 8-Black/ black pink/dark purple/dark pink/black green
13	Xylem colour (score)	1-White, 2-Yellow/Light Yellow/White Yellow, 3-Dark Yellow, 4-Green, 5-Yellow Green/Light Green/Green Yellow, 6-Light Orange/Yellow Orange, 7-Dark Orange, 8-Pink, 9-Red/dark red, 10-Purple, 11-Black/Dark Purple.
14	Phloem colour (score)	
15	Cambium colour (score)	

Table 3b: List of quantitative traits recorded in 96 genotypes in carrot

S. No	Characters	Details
1	Days to maturity	No of days to harvest from the date of sowing
2	No of petioles	Petioles Counted
3	Shoot length (cm)	Measuring scale
4	Plant height	Measuring scale
5	Root length (cm)	Measuring scale
6	Petiole length (cm)	Measuring scale
7	Root width (mm)	Digital Vernier Caliper-converted to cm
8	Shoulder width (mm)	Digital Vernier Caliper- converted to cm
9	Vegetative weight/plant (gms)	Weighing Balance
10	Five Plants Vegetative weight	Weighing Balance
11	Xylem width (cm)	Measuring scale
12	Phloem width (cm)	Measuring scale
13	Harvest index (%)	Economic yield/biological yield
14	Total Soluble Solids (^{0}Brix)	Digital Refractometer
15	Reducing Sugars (%)	Dinitro Salicylic Acid (DNS) method
16	Beta Carotene Content (µg/100 mg)	Acetone Extraction Method
17	Root yield (gms)/plant	Weighing Balance
18	Five plants root weight	Weighing Balance

cultivars, local varieties, modern hybrid cultivars, released varieties. These germplasm lines have been evaluated in the present study after two years of sib-pollination (to constitute the homogeneity in the respective genotype). These genotypes were as UHSBC (University of Horticultural Sciences Bagalkot Carrot) collections. The nomenclature and the numbers are given as per the collection data. The details of the genotypes used in the present study are presented in the Table 1.

3.3 Experimental design

Seeds of the ninety six genotypes were planted and grown in Augmented Block Design (Table 2). The experimental site was divided in to six blocks and each block containing 19 sub plots. Three check varieties included in the design consisting of one tropical adapted released variety (Pusa Vrishti), one temperate adapted variety (Vigro Kuruda) and one local cultivar (Ghataprabha Local). These three check varieties were replicated and randomised in each the six blocks. The ninety six genotypes were distributed in all the six blocks such that each block consisted of 16 genotypes. Finally, each block carried total of 19 subplots carrying 3 check varieties and 16 genotypes to be tested. All the standard package of practices were followed to maintain healthy plots till the harvest of the roots.

3.4 Phenotyping for plant and root morphological characters

Phenotypic observations were recorded for total of six plants from each of the genotype and check varieties at the time of harvest. Total of 18 quantitative traits including three biochemical traits were recorded and the mean data of five plants were estimated and presented as per the SI units. A total of fifteen qualitative observations were also recorded based on the IPGRI descriptor (IPGRI, 1998). The list of observations recorded for the study is presented in Table 3a and 3b.

3.5 Biochemical estimation

The roots of all the genotypes after phenotypic evaluation for the morphological traits including qualitative and quantitative traits, were subjected to biochemical estimation such as total soluble solids (TSS), reducing sugars as it is an important component responsible for sweetness in carrot and also the biochemical estimation.

From all the ninety six genotypes, the roots were finely grinded after recording the observations for internal root characters such as xylem and phloem width and colour. The grinded samples were packed in an airtight tetra pack (Aluminium foil) covers and was stored in -20^0 C deep freezer till the estimations were carried out before using for biochemical estimation. From each of the genotypes, six biological replicates were made for all the biochemical components estimation and the average data was taken for the statistical analysis. For 3 check varieties, biochemical estimation was done for six replications from six blocks. From each replication, six biological replicates were made and the mean value for each replication was considered for the statistical analysis.

Total soluble solids: From the freshly ground samples juice was extracted and directly dropped on to a digital hand Refractometer and the values were recorded and expressed in ^{0}Brix. Similar procedure was followed for all the 96 genotypes as well as checks.

Estimation of reducing sugars: Reducing sugars were estimated by following the Dinitrosalicylic acid (DNS) method as it is a simple, sensitive and adoptable during handling of a large number of samples at a time. Reducing sugar was estimated by comparing the standard curve of glucose and expressed in percentage.

Estimation of ß – carotene : 100mg of carrot from each replication in each cultivar was homogenized with 5ml acetone in a pestle and mortar and centrifuged at 4000rpm and the supernatant was transferred to fresh tube and the volume was made up to 10ml with acetone. The solvent was i mmediately used for estimation of beta carotene along with the ß carotene standard as per the protocol of Harborne (1973).

Beta carotene was expressed in microgram/100 mg (µg/100 mg) after the estimation for each replication in each genotype and checks.

3.6 Molecular marker Profiling

3.6.1 DNA extraction

Young leaves and tissues from 2 months old seedlings were collected from all the 96 cultivars for the isolation of plant genomic DNA, using CTAB method of DNA extraction as per the procedure of Briard *et al*. (2000).

The detailed procedure is as follows

1. The young leaves and tissues were ground in to a fine powder in liquid nitrogen using a mortar and pestle and transferred up to 100 mg of the powder to a 2ml micro centrifuge tube and kept the sample on ice for i mmediate use or frozen at -20 ^{0}C until use.

2. To this 700 µl of 2% CTAB extraction buffer freshly prepared (60^0 C) was added and vortexed for 15s and incubated at 60 ° C for 30-45 min.

3. After cooling the tubes, equal volume (700 µl) of Phenol:Chloroform:Isoamyl Alcohol (25:24:1) was added and mixed by inverting for 8-10 times and centrifuged for 15 min at 14000 rpm at room temperature (27^0 C).

4. Supernatant was transferred to a fresh 1.5ml micro centrifuge tubes separately with proper labeling and added equal volume of approximately 500 µl of chilled isopropanol for precipitation of DNA, mix properly by inversion and incubate at -20^0 C for 30 min.

5. Following incubation, centrifuge for 15 min at 14000 rpm at 4^{0}C.

6. Carefully decant supernatant, wash pellet with 500 µl, 70% ethanol, incubate at room temperature for 15 min (longer is okay, even overnight), following incubation, centrifuge at 14000 rpm for 15 min.

7. Carefully remove all traces of ethanol and air dried the pellet.

8. After complete drying of pellet, dissolve it in 200 µl of Tris-EDTA (TE) and store at -20 ° C as a stock.

3.6.2 Quantification of DNA

The stock of DNA was tested for its quality and quantity in 0.8 % Agarose and nano-spectophotometer (Eppendorf India Ltd.).

3.6.3 DNA Quantification for Polymerase Chain Reaction

1. Based on the quantification (280nm/260nm) in nano-spectophotometer (Eppendorf India Ltd.), 50ng of working DNA was prepared for PCR for the 48 cultivars

2. For polymerase chain reaction (PCR), 108 microsatellite markers (listed in table 2 of Appendix) were diluted (10 Pico-moles) from the stock.

3. PCR was done for 10 µl reaction, the components used for PCR with the respective concentration and the protocol is presented in the Table 4a and 4b

Among the total of 80 carrot specific markers, 47 markers which were polymorphic in the carrot accessions were used for genotyping of carrot germplasm panel consisting of 96 genotypes. The detailed information of the list of molecular markers utilized in the present study is presented in the Table 5.

3.6.4 PCR Amplification, Electrophoresis and Marker scoring

A total of 80 published carrot specific molecular markers were selected for screening for polymorphism. These molecular markers included gene specific markers, genic and genomic microsatellite markers, indels, SCAR (Table 4). Among them, 47 markers showed polymorphism in the selected 96 carrot genotypes panel.

For PCR amplification, 2X master mix (Juniper life sciences) containing dNTPS, Taq polymerase enzyme, buffer and $Mg+^{2}$ was used. PCR protocol and programs were followed as per the published information and presented in Table 5.

PCR amplified products were size separated in 4.0% agarose gel electrophoresis for co-dominant markers, where as, for dominant markers, 2.0 % agaorse was used for gel electrophoresis. 5 markers were dominant types and remaining showed co-dominant banding pattern among the total 47 polymorphic markers. Dominant markers were scored as presence /absence (0/1 scores) polymorphism and the remaining 42 co-dominant markers were screened based on the allele sizing using the standard marker ladder of 100bp (Takara Life Sciences). However, dominant markers were dropped for further analysis due to incompatibility of software's for both the types of scores (presence/absence and allele sizing).

Table 4: List of polymorphic Microsatellite markers used in the present study for genotyping

List of Markers	Chromosome Position	Type of Marker	Optimal annealing temp. (ºC) ǂ	Expected amplicon size (bp)	References
BSSR128	-	Microsatellites	54	219	Baranski *et al*., 2012
BSSR76	-	Microsatellites	54		Cavagnaro *et al*. 2009
BSSR94	-	Microsatellites	54	181	Cavagnaro *et al*. 2009
DCM17	-	Microsatellites	55	191	Soufflet-Freslon *et al*., 2013
DCM2	9	Microsatellites	55	169	Soufflet-Freslon *et al*., 2013
ESSR114	-	Microsatellites	55	150	Cavagnaro *et al*. 2011
ESSR59	-	Microsatellites	58	283-294	Budahn *et al*., 2014
ESSR61	-	Microsatellites	57	195-204	Budahn *et al*., 2014
ESSR62	-	Microsatellites	57	236-245	Budahn *et al*., 2014
ESSR71	-	Microsatellites	56	168/171	Budahn *et al*., 2014
GSSR104	-	Microsatellites	54	330	Cavagnaro *et al*. 2011
GSSR111	-	Microsatellites	55	355	Niemann *et al*., 2001
GSSR122	-	Microsatellites	55	265-366	Cavagnaro *et al*. 2011
GSSR124	3	Microsatellites	55	349	Cavagnaro *et al*. 2011
GSSR138	-	Microsatellites	56	519	Niemann *et al*., 2001
GSSR14	-	Microsatellites	55	224	Niemann *et al*., 2001
GSSR152	-	Microsatellites	55	315	Cavagnaro *et al*., 2011
GSSR153	-	Microsatellites	56	255	Cavagnaro *et al*., 2011
GSSR154	2	Microsatellites	56	328	Cavagnaro *et al*., 2011
GSSR16	9	Microsatellites	57	212	Cavagnaro *et al*., 2011
GSSR17	3	Microsatellites	54	216	Cavagnaro *et al*., 2011
GSSR19	5	Microsatellites	54	231	Cavagnaro *et al*., 2011
GSSR39	4	Microsatellites	55	174	Cavagnaro *et al*., 2011
GSSR4	6	Microsatellites	55	314	Cavagnaro *et al*., 2011
GSSR44	-	Microsatellites	58	209	Cavagnaro *et al*., 2011
GSSR47	-	Microsatellites	55	183	Cavagnaro *et al*., 2011
GSSR54	-	Microsatellites	54	323	Cavagnaro *et al*., 2011
GSSR57	-	Microsatellites	54	329	Cavagnaro *et al*., 2011
GSSR6	4	Microsatellites	57	306	Cavagnaro *et al*., 2011
GSSR63	3	Microsatellites	54	336	Niemann *et al*., 2001
GSSR71	-	Microsatellites	55	290	Cavagnaro *et al*. 2011
GSSR85	7	Microsatellites	58	227	Cavagnaro *et al*. 2011
GSSR9	-	Microsatellites	54	316	Cavagnaro *et al*. 2011
GSSR93	-	Microsatellites	55	157	Cavagnaro *et al*. 2011
GSSR97	-	Microsatellites	58	245/236	Cavagnaro *et al*. 2011
GSSR98	8	Microsatellites	55	461	Cavagnaro *et al*. 2011
LCY (lycopene e-cyclase)	-	Gene Specific	57	2.5/3.0 Kb	Iorizzo *et al*., 2014
OPK9	-	RAPD converted SCAR	58	700/850	Ali *et al*., 2009
PSY (Phytoene Synthase)	-	Gene Specific	58	660/670	Simon *et al*., 2015
Q1/800	-	SCAR	59	759/544	Ali *et al*., 2009
Y2 (Carotenoid Y-Locus)	-	Gene Specific	50	172/310	Cavagnaro *et al* 2009
Y-Indel	5	Gene Specific	55	196-300	Iorizzo *et al*., 2016

Table 5a: PCR reactions for microsatellite and other molecular markers

Components	Concentration	PCR reaction (10 µl)
Primers (F+R)	10pM	0.5
PCR master mix (Takara)	2 X	5
Template	50ng/ µl	1
Deionizer water		3.5

Table 5b: PCR protocol followed for microsatellite primers for 96 carrot genotypes

Sl. No.	Steps	Microsatellite Primers		Cycles
		Temperature (^{0}C)	Time	
1	Initial denaturation	95	3 min	40 Cycles
2	Denaturation	94	20 sec	
3	Annealing	60	20 sec	
4	Primer extension	72	30 sec	
5	Store at	4	∞	

The morphological, biochemical observations recorded in the augmented design and the molecular markers data recorded for 42 markers across 96 genotypes were subjected to the following statistical analysis for the interpretation of the results.

3.7 Statistical Analyses

3.7.1 Analysis of variance (ANOVA) for Augmented Block design

Augmented Block Design introduced by Federer (1956) was used for field evaluation consisting of 96 genotypes and 3 check varieties distributed in six blocks. The phenotypic data consisting of 18 quantitative traits were subjected to Analysis of

variance (ANOVA), where the source of variation was divided in to Block (Ignoring Treatments), Genotypes + Checks (Eliminating Blocks), Checks, Checks +Gen vs. Gen, Error, Block (eliminating Check+ Genotypes), Entries (Ignoring Blocks), Genotypes, Checks vs. Genotypes. The analysis of variance (ANOVA) was carried out with the help of a software Windowstat (version 8.2).

3.7.2 Descriptive statistics (Mean, Range, SEm)

The mean data of all the 96 genotypes for 18 quantitative traits were subjected to descriptive statistic analysis such as mean, range, standard error using a statistical software SPSS (version 16.0).

3.7.3 Frequency distribution

Other 15 qualitative traits were subjected for frequency distribution based on the number of classes/categories in each of the traits using bar chart graph with the help of Microsoft Excel (Windows10.0).

3.7.4 Genetic variability and heritability parameters

Quantitative data for all the 18 traits were also subjected to genetic variability component analysis such as genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV) and to know the extent of heritability of these traits to next generation, the heritability components such as h^2 (heritability) in broad sense and the Genetic advance as percent mean (GAM) were estimated for each trait with the help of Windowstat (version 8.2) software and interpreted the results based on the following categorization as suggested by Sivasubramanian and Menon (1973).

For PCV and GCV the following classes were followed

0-10 % = Low

10-20 % = Moderate

20 % = High

Heritability (broad sense)

Heritability in broad sense was calculated as the ratio of genotypic variance to the phenotypic variance and expressed as percentage (Falconer, 1981).

$$\text{Heritability } (h^2) = \frac{\sigma_g^2}{\sigma_p^2} \times 100$$

The calculated heritability was classified into three groups as suggested by Johnson *et al* (1955):

0-30 % = Low

30-60 % = Moderate

60 % = High

Expected genetic advance (GA) Genetic advance was worked out by adopting the following formula given by Johnson *et al.* (1955):

$GA = k \times h^2 \times \sqrt{\sigma^2 p}$

Where,

h^2 = Heritability in broad sense

k = Selection differential, which is equal to 2.06 at 5% intensity of selection (Lush, 1949)

$\sqrt{\sigma^2 p}$ = Phenotypic standard deviation

Genetic advance as per cent of mean (GAM)

Genetic advance as per cent of mean for each character was worked out as suggested by Johnson *et al.* (1955).

$$GAM = \frac{GA}{X} \times 100$$

Where,

GA = Genetic advance

X = General mean

Genetic advance as per cent of mean was categorized as per the formula suggested by Johanson *et al*. (1955).

0-10 % = Low

10-20 % = Moderate

>20 % = High

3.7.5 Principal component analysis

It is a multivariate statistical technique to reduce the data with large number of correlated variables into a substantially smaller set of new variables, through linear combination of the variables that accounts for most of the variation present in the original variables. Principal components are generally estimated either from correlation matrix or variance-covariance matrix. When the variables are measured in different units, scale effects can influence the composition of derived components. In such situations, it becomes desirable to standardize the variables and the correlation matrix comes to the rescue. In the recent investigation, correlation matrix was used to extract the principal components. The same procedure was also followed in the present investigation to identify the principle components from among the 18 quantitative traits of carrot in 96 genotypes. The software package SPSS (Version 16.0) was used for the analysis in the present investigation.

For deciding number of principal components to be retained, Kaiser's (1958) suggestion of dropping those principal components of correlation matrix with Eigen roots less than one was followed in the study.

3.7.6 Correlation analysis

The correlation coefficients were worked out to determine the degree of association for a group of characters. The correlations were calculated for 18 quantitative traits including 3 biochemical and 15 morphological and yield related traits.

Phenotypic correlations were computed by using the formula given by Webber and Moorthy (1952).

$$r_p = \frac{\text{Cov } XY_p}{\sqrt{s_p^2 x \times s_p^2 y}}$$

Where,

r_p = Phenotypic correlation

Cov XY_p = Phenotypic covariance between the characters 'x' and 'y'

$s_p^2 x$ and $s_p^2 y$ = Phenotypic variance of the characters 'x' and 'y' respectively

Phenotypic correlation coefficients were compared against table value at (n-2) degrees of freedom at the probability levels of 0.05 and 0.01 to test their significance (Fisher and Yates, 1963).

3.7.7 Mahalanobis D^2 analysis for Phenotypic data

Mahalanobis (1936) D^2 analysis was used for assessing the genetic divergence among the 96 genotypes involving 18 quantitative characters using the software package Windowstat version 8.10. The generalized distance between any two populations is given by the formula:

Since, the formula for computation requires inversion of higher order determinant, transformation of the original correlated unstandardized characters (X's) to standardized uncorrelated variables (Y's) was done to simplify the computational procedure. The D^2 values were obtained as the sum of squares of the differences between pairs of corresponding uncorrelated (s) values of any two uncorrelated genotypes (Rao, 1960).

Cluster of D^2 values

All n (n-1)/2 D^2 values were clustered using ward minimum method described by Rao (1960).

Intra cluster distance-The intra cluster distances were calculated by the formula given by Singh and Choudhary (1977). The inter-cluster distances were calculated by the formula described by Singh and Choudhary (1977).

In brief, the steps involved for the estimation of D^2 values are as follows (Rao, 1952).

1. Pivotal condensation of error variance and co-variance matrix to obtain inverse matrix.
2. Transformation of original correlated data into un-correlated variables.
3. Calculation of mean values of the transformed characters.
4. **Calculation of D^2 values:** For each combination, deviation between the means was computed and the D^2 values were computed and arranged in the form of matrix.

Determination of group constellations: As such no standard rules are available for making the clusters because cluster is not a well defined term. The only criterion appears to be that any two genotypes belonging to the same cluster should at least, on an average, show a smaller D^2 value than those belonging to two different clusters. The D^2 values for all the combinations presented in the matrix form were arranged in increasing order of magnitude and clustering was done according to the method suggested by (Rao, 1952).

At first, the two most closely associated genotypes were chosen and then third genotype was located which had the smaller average D2 value as compared to the first two genotypes. Following this methodology, the subsequent genotypes were chosen which have smaller average D^2 value from the first three genotypes and change in D2value within a cluster due to inclusion of additional genotype was computed and so on. The new genotypes were added so long as the increase in average D2 value became abruptly high, then this genotype was not included in the former groups. The genotypes

of first cluster were omitted and rest were treated similarly for constructing new clusters.

Intra and inter cluster distance

The intra cluster D2 value was calculated as the sum of n (n-1)/2 D2 values among the genotypes within a cluster divided by n (n-1)/2. The single genotype always has zero intra cluster D2 value. For calculating the inter cluster D2 value, all possible D2 values between genotypes of two clusters were added and then divided by n1×n2, where n1 and n2 represented the number of genotypes in the first and second cluster, respectively. The intra and inter cluster distances were calculated by taking the square root of respective D^2 value between genotypes of a particular cluster and between genotypes belonging to two clusters, respectively.

Clusters mean value

The cluster mean of a particular character is the summation of mean values of the genotypes included in a cluster divided by number of genotypes in the same cluster. Cluster mean values for all the 18 traits were estimated to identify the superior clusters for the economic root traits of carrot.

3.7.8 Molecular marker analysis

3.7.8.1 Genetic parameters and allelic diversity estimation

The data set of 42 on 96 carrot accessions were used for statistical analysis using software package GENEALEX (version 6.503) for estimating basic statistics *viz.*, allelic richness as determined by the total number of the detected alleles, number of alleles per locus (Na), number of effective alleles (Ne), gene diversity as determinaed by observed hetreozygosity (Ho), expected heteriozygosity (He), occurrence of unique, rare, common alleles & Shannon's information measure (I) and the inbreeding coefficient as shown by Fixation index (F).

3.7.8.2 Analysis of Molecular Variance (AMOVA)

Analysis of molecular variance was estimated with the help of GENEALEX v. 6.503 where the two populations obtained from the population structure was considered

for analysis. For the 1st population 49 genotypes were there and in the second population, 47 genotypes were taken for AMOVA. The variation was partitioned into within individuals, within populations and between populations.

3.7.8.3 Molecular diversity and cluster analysis

To examine the genetic relationships among 96 genotypes of carrot based on 42 marker locus with 471 alleles, a dendrogram was constructed using UPGMA method of Cluster analysis and unrooted Neighbour-joining tree was constructed using software package DARwin 5.0 (Perrier and Jacquemoud-Collet 2006). The bootstrap value of 2000 was given while grouping the clusters.

3.7.8.4 Population structure

Analysis of the population structure and gene flow between carrot accessions was carried out using a model based clustering method (Bayesian approach) as implemented in the software program STRUCTURE v. 2.3.4 (Pritchard 2000). Co-dominant allelic data for 42 markers in the 96 carrot genotype panel was subjected to structure analysis. In this method, it is assumed that number of subpopulations exists in the sample analyzed. Each accession can have membership in different subgroups. The software was set to a length of burn-in period of 50,000 followed by 5000 Markov Chain Monte Carlo (MCMC) repetitions after burn-in. The optimum number of populations was determined by running admixture ancestry model with correlated frequencies starting from two populations. The number of subgroups (K) in the population was determined by running the progra mme at different K values with K varying from 1 to 10 with five independent runs for each K value. The number of true cluster of the population was identified based on the based on the delta K value, a procedure used by Evanno *et al.* (2005). The values for each Delta K were submitted to the STRUCTURE Harvester website which returned the delta K value (Earl *et al.*, 2012). The group that had the highest ad hoc statistic Delta K value was selected.

After identifying the delta K value and the number of populations obtained from among the genotypes, the genotypes were assigned to each population based on the Q value with >0.50. The genotypes consisting in each cluster were identified based on the

Q values of the population and the genotypes were catogorsied to two groups and their respective marker data of 42 markers were subjected to Analysis of moelcualr Variance (AMOVA) with 49 and 47 genotypes respectively in population I and Population II.

From the analysis of molecular variance (AMOVA), the total molecular variance was partitioned into variance among the populations and variance withing the populations between individuals and total variance using GENEALEX (version 6.503) software (Peakall and Smouse 2012).

3.7.9 Association of markers with economic traits of carrot roots

Association analysis was done by using phenotypic data of diverse carrot genotypes, genotypic data of 42 SSR marker data and population structure data (Q matrix) by using software TASSEL (Bradbury *et al.* 2007). The marker–trait association analysis was conducted using TASSEL 3.0 software following the general linear model (GLM) procedure.

The software program TASSEL (Bradbury *et al.* 2007) was used for the association analysis. To reduce false or spurious associations, population structure (Q) was calculated first. The generalized linear model (GLM) which takes the population structure (Q) into consideration was used for the analysis. A generalized linear model (GLM) was also used where only the Q was used as a covariate. The significant threshold value for the association was set at $P < 0.0001$. So the marker associated with the trait was considered highly significant when the marker p value was showing >0.0001.

4. EXPERIMENTAL RESULTS

Carrot is an important cool season vegetable and a rich source of provitamin A carotenoid. Numbers of varieties have been developed suitable to temperate climates. In India, greater variation is present both in Asiatic and European types. Many European varieties have been introduced in different parts of the country. Numbers of local varieties which are the selections by farmers adaptable to tropical and temperate climates are also available. Most of these Indian germplasm collections are least explored in breeding program as most of the breeding efforts are concentrated to development of temperate varieties. An investigation was carried out involving 96 carrot germplasm containing local varieties, landraces, released varieties, public and private sector varieties, IIVR germplasm collections. This germplasm panel was subjected to phenotypic evaluation for root morphological traits, productivity traits and important biochemical traits. The panel was screened for 42 DNA markers representing genic and genomic SSRs, SCARs and Indels. The genotypic data and phenotypic data of this carrot genotype panel was subjected for various statistical analysis to identify the variability and diversity among the phenotypes, their association pattern, principle components analysis, molecular genetic diversity, population structure and marker-trait association. The details of the results of the present investigation are presented in the following sub-headings.

4.1 Phenotyping for Root morphological and Biochemical traits

4.2 Molecular marker profiling for carrot population

4.3 Marker trait association

4.1 Phenotyping for Root morphological and Biochemical traits

All the 96 genotypes were subjected to phenotypic evaluation for important root morphological and biochemical traits. Total of 35 traits were evaluated for these 96 diverse carrot genotypes having different genetic background representing Indian carrot germplasm collections.

4.1.1 Frequency distribution for quantitative parameters

The qualitative parameters scored across the 96 genotypes with the help of IPGRI descriptor was utilized for frequency distribution analysis and presented in Figure1. The respective classes for each trait across the genotypes are given in X-Axis and the genotype frequencies are in Y-axis. The no of classes are depicted for the respective classes are depicted on respective bar charts. The details of the scores and classes for each parameter are mentioned in Table3a of Material and Methods.

For plant growth habit, there were mainly three classes, but more frequent in the present investigation was semi-erect type followed by erect. For shoot attachment, the single attachment was more common than the multiple types. Normal carrot type of leaf was more frequent in the present population; however, celery type and fern type of leaves were also present in the present study. Among the root colour parameters (external and internal), orange root colour was more common, although greater variation for colour was seen such as yellow, dark orange, pink, purple and black. For the internal root colour, xylem and cambium showed more of yellowish colour but the in phloem colour, the frequency of dark orange was more. Shoulder was mostly absent in many genotypes, however, green shoulder was common than other colours. Tapering root shape and coarse textured roots were more common in the population.

4.1.2 Analysis of variance (ANOVA)

A total of 96 genotypes were screened in the Augmented Block Design with 6 blocks and 3 checks. Each block containing 16 genotypes and 3 checks. The three checks were replicated and randomised in 6 blocks. The mean sum of squares with respect to all the 18 quantitative traits and its component characters are presented in the table 6a and 6b. Mean sum of squares for indicated significant differences among all checks for days to maturity, number of petioles, petiole length, vegetative weight, five plant vegetative weight, harvest index and root weight. Significant variation was also observed among the 96 genotypes for most of the traits except for biochemical traits indicating the existence of variation for all the morphological traits studied. Although biochemical traits did not show significance from ANOVA table, but the range of variation was very high and as they are important biochemical traits for crop

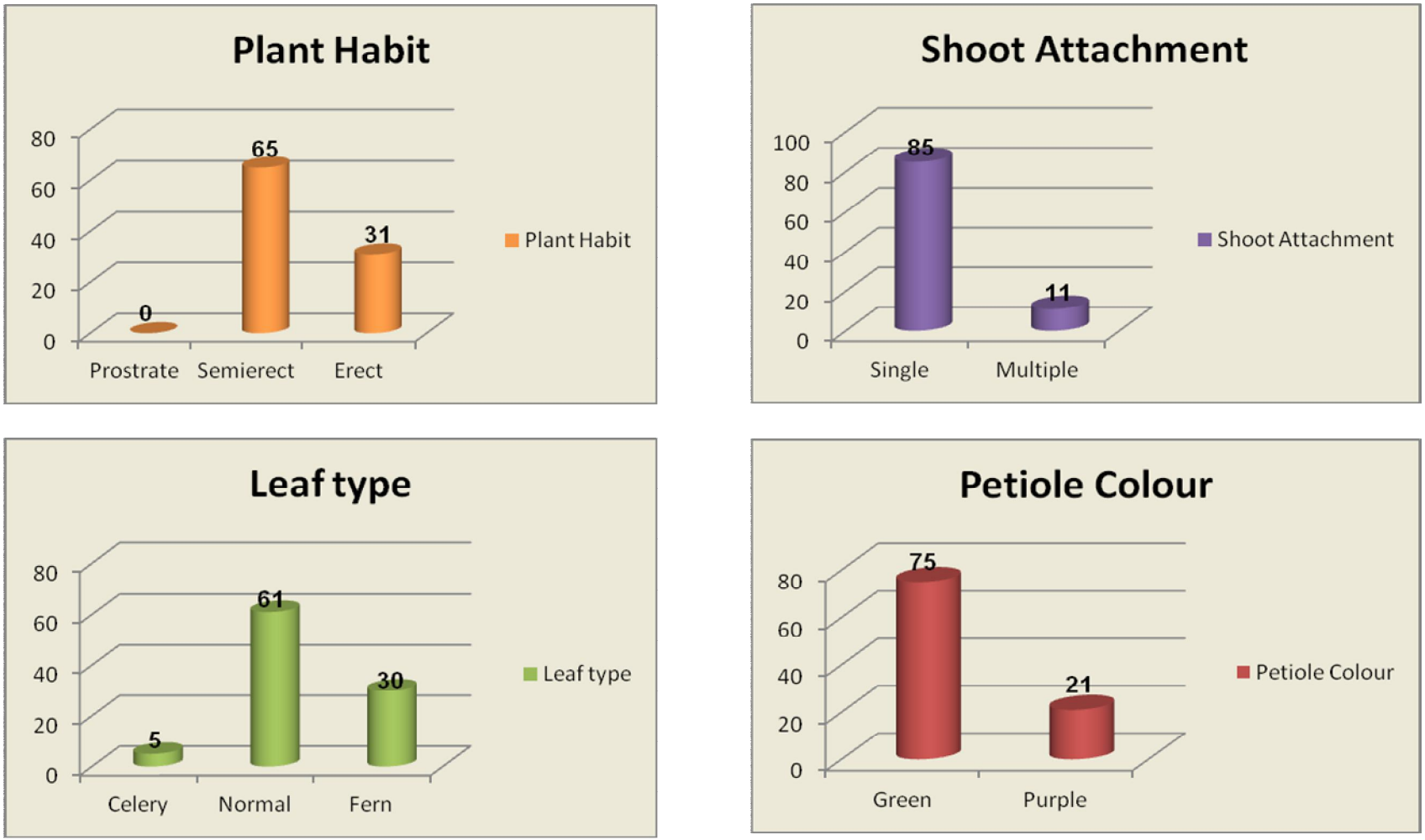
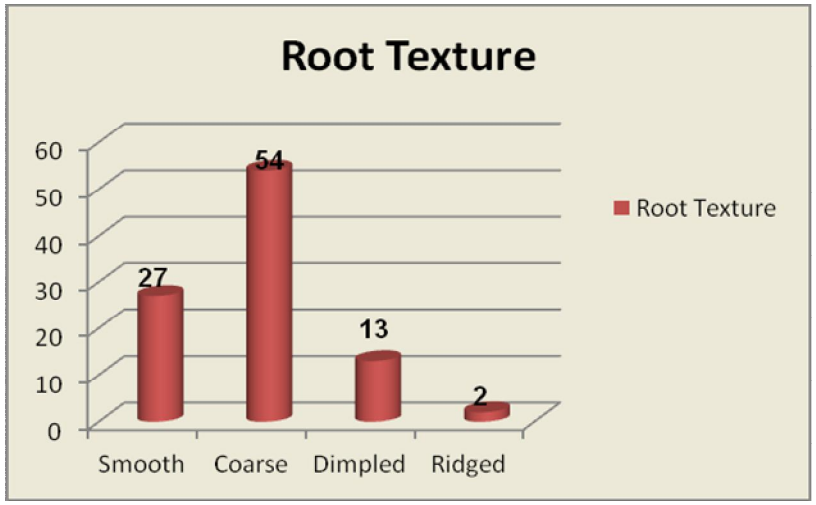
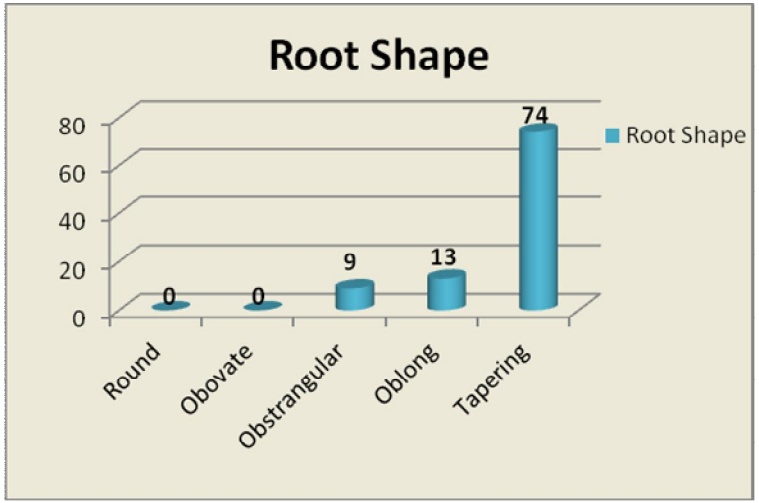
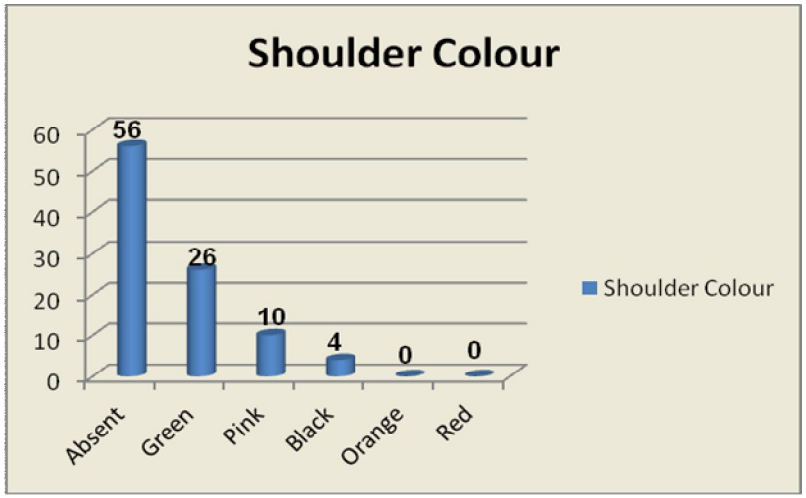


Fig. 1a: Frequency Distribution for qualitative characters recorded for 96 genotypes in carrot







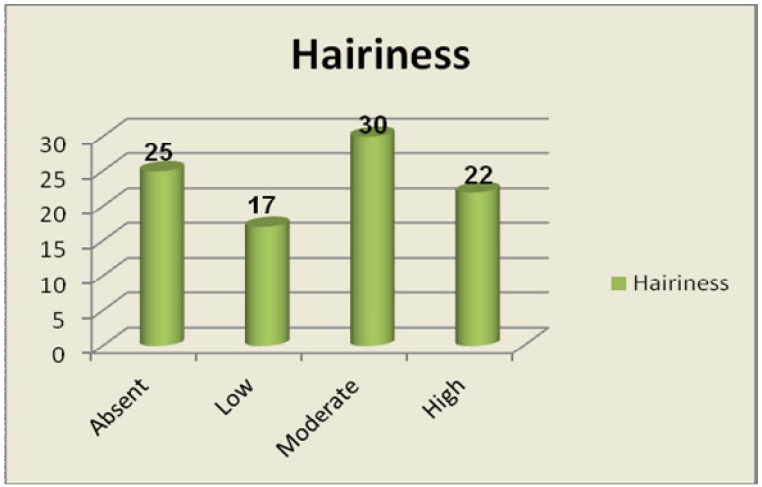
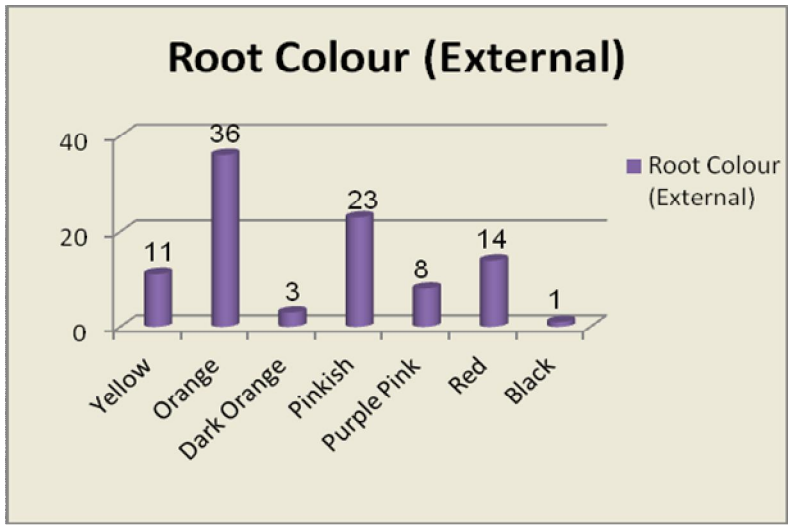
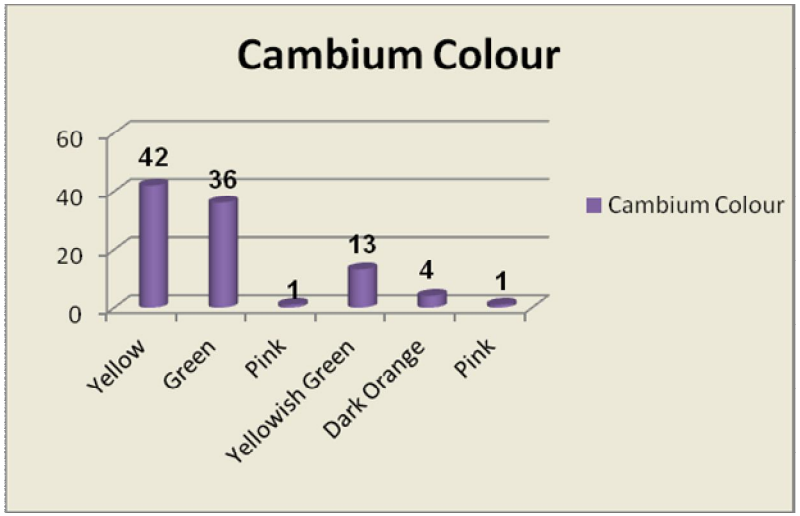
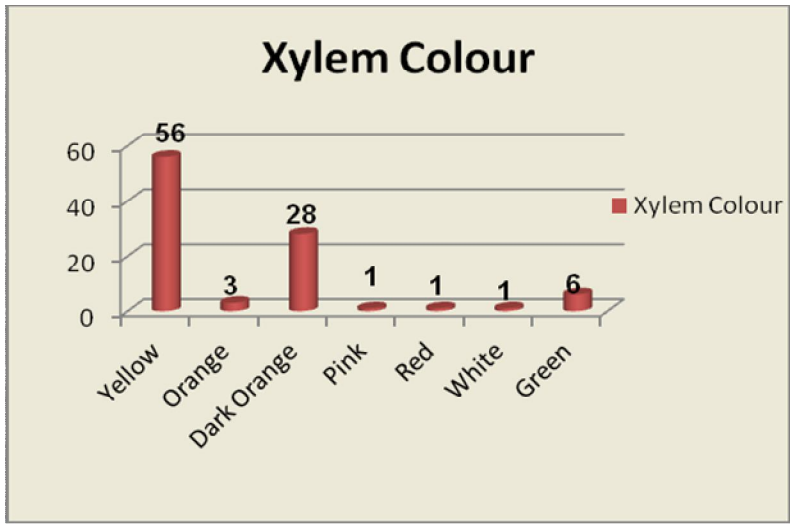


Fig. 1a: Frequency Distribution for qualitative characters recorded for 96 genotypes in carrot







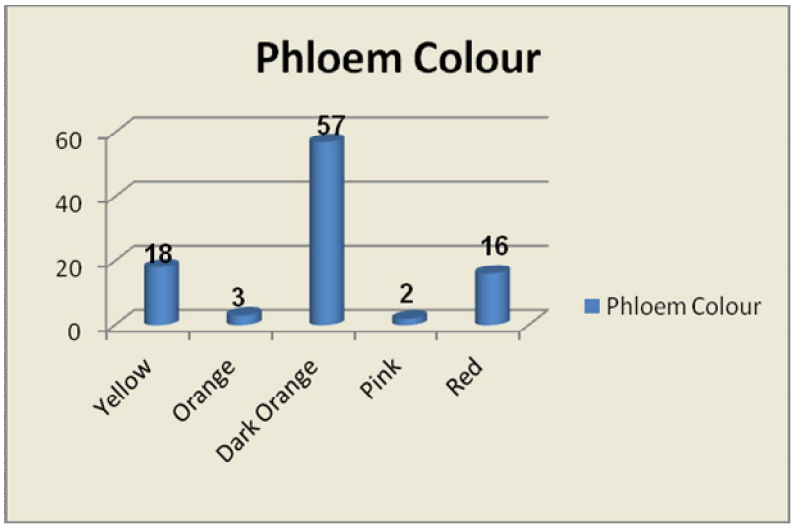
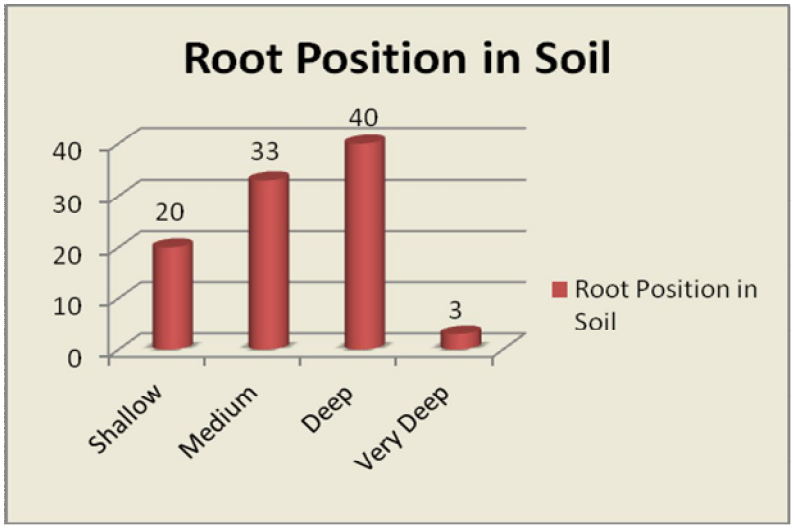
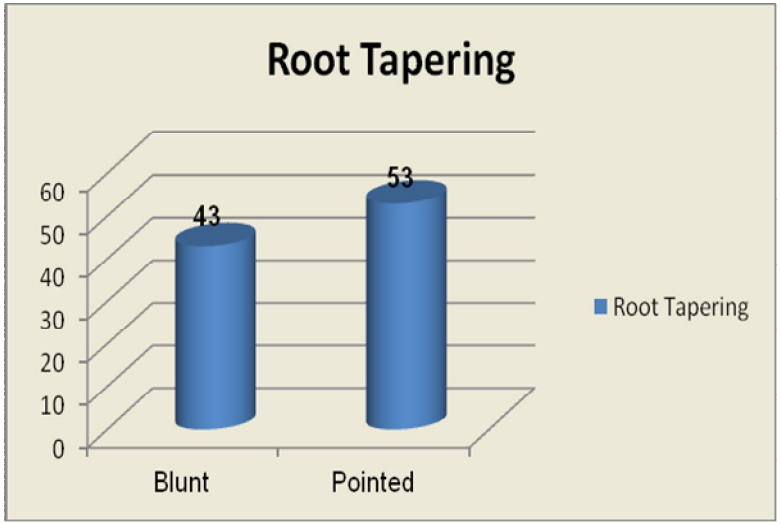


Fig. 1a: Frequency Distribution for qualitative characters recorded for 96 genotypes in carrot





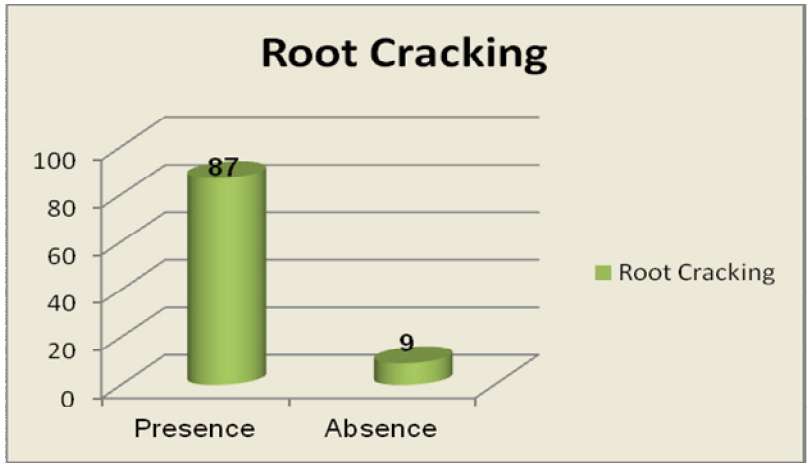


Fig. 1a: Frequency Distribution for qualitative characters recorded for 96 genotypes in carrot

Table 6a. Analysis of variance (ANOVA) for 18 quantitative traits (Plant and root morphological traits and biochemical traits) in carrot

Source of variations	df	Mean sum of squares								
		DM	NP	SL	PH	RL	PL	RWD	SWD	VW
Block (Ignoring Treatments)	5	213.29**	38.54***	0.06	242.16**	18.19	26.60***	6.13	80.36**	3838.60***
Genotypes + Checks (Eliminating Blocks)	98	124.16**	12.02***	0.05	89.58*	30.18**	28.90***	10.89**	39.33*	778.98***
Checks	2	74.67	2.04	0.03	422.02**	8.09	68.77***	29.61***	303.38***	537.59*
Checks +Gen vs. Gen.	96	125.19**	12.23***	0.05	82.66	30.64**	28.07***	10.50***	33.83*	784.01***
Error	10	20.8	0.66	0.02	31.92	7.41	2.43	1.92	11.79	104.34
Block (eliminating Check+ Genotypes)	5	69.43*	0.99	0.02	25.90	6.03	3.57	9.39*	13.98	26.90
Entries (Ignoring Blocks)	98	131.50**	13.94***	0.05	100.62*	30.80**	30.08***	10.72***	42.71*	973.46***
Checks	2	74.67	2.04	0.03	422.02**	8.09	68.77***	29.61***	303.38***	537.59*
Genotypes	95	110.95**	13.58***	0.05	89.24*	29.77*	29.28***	10.29**	37.58*	920.14***
Checks vs. Genotypes	1	2197.92***	72.18***	0	538.87**	173.44***	28.92**	14.29*	9.03	6910.21***
Error	10	20.8	0.66	0.02	31.92	7.41	2.43	1.92	11.79	104.34

Contd…

Table 6b. Analysis of variance (ANOVA) for 18 quantitative traits (Plant and root morphological traits and biochemical traits) in carrot

Source of variations	df	Mean sum of squares								
		FPVW	XW	PW	HI	TSS	RS	Beta	RW	FPRW
Block (Ignoring Treatments)	5	57665.43***	0.08	0.03***	21.99***	2.22	0.49	827.25	264.02*	1860.41
Genotypes + Checks (Eliminating Blocks)	98	18627.99**	0.04	0.01*	22.67***	1.31	0.74	189.75	253.25*	7014.33*
Checks	2	8470.06	0.23**	0.05**	0.01	0.23	1.17	79.43	995.05**	32236.22**
Checks +Gen vs. Gen.	96	18839.62**	0.04	0.01*	23.14***	1.34	0.73	192.04	237.80*	6488.88
Error	10	2702.31	0.03	0.00	0.00	0.82	0.49	173.59	71.43	2607.14
Block (eliminating Check+ Genotypes)	5	903.55	0.01	0.00	0.01	1.00	0.49	79.85	124.31	3828.12
Entries (Ignoring Blocks)	98	21524.01***	0.05	0.01**	23.79***	1.38	0.74	227.88	260.38*	6913.94*
Checks	2	8470.06	0.23**	0.05**	0.01	0.23	1.17	79.43	995.05**	32236.22**
Genotypes	95	20243.29***	0.04	0.01*	24.52***	1.41	0.73	230.83	245.16*	6453.08
Checks vs. Genotypes	1	169300.25***	0.00	0.15***	2.24***	1.06	0.79	244.47	237.08	50.75
Error	10	2702.31	0.03	0.00	0.00	0.82	0.49	173.59	71.43	2607.14

Smooth type | Coarse type | Ridged type | Dimpled type

Plate 2: Degree/type of root texture screened across 96 carrot genotypes

Plate 3. Genetic Variability for External Colour in carrot genotypes

Plate 4. Genetic Variability for External Colour in carrot genotypes

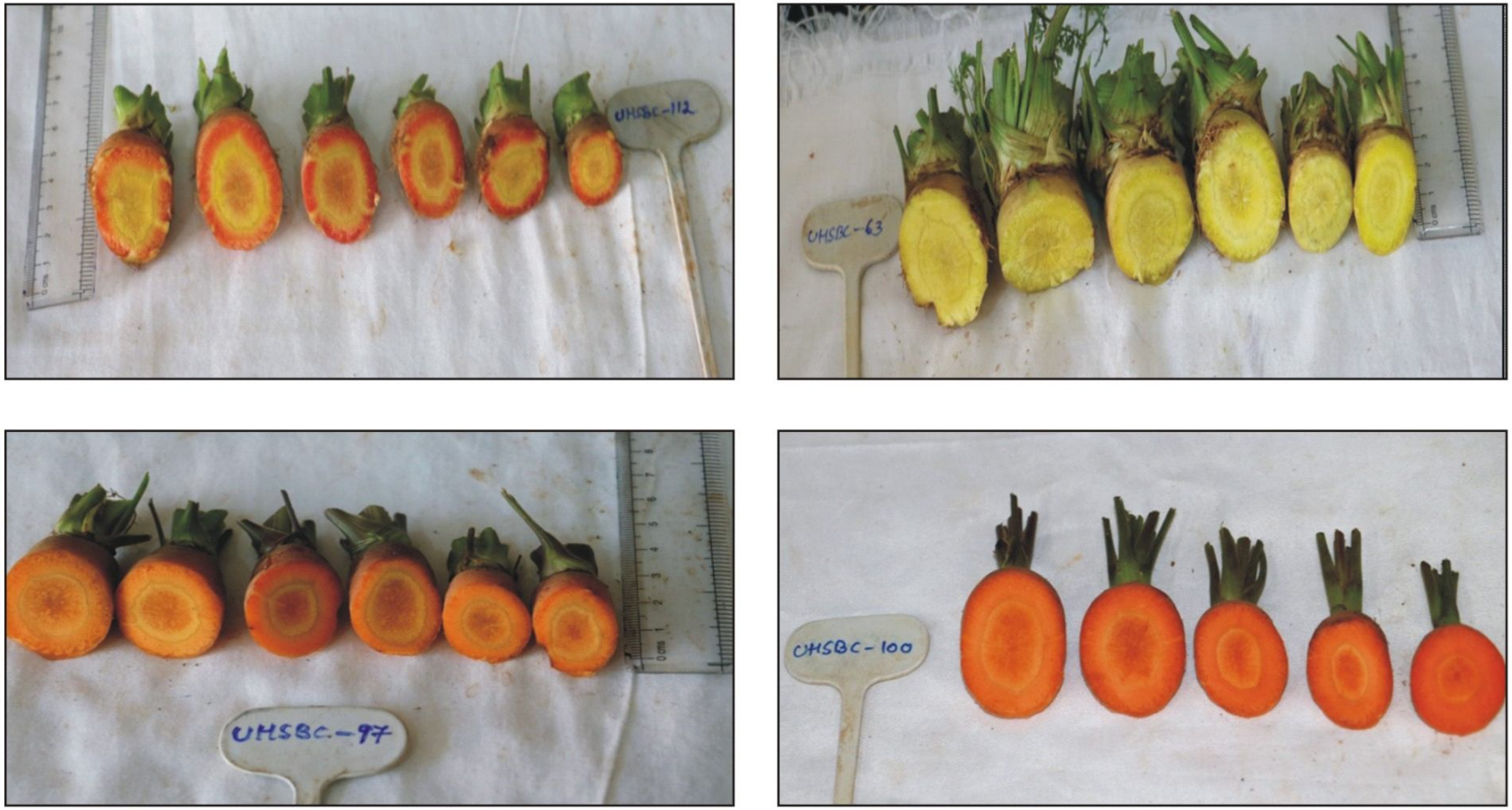


Plate 5. Genetic Variability for internal core colour (Xylem, phloem, cambium) in carrot Genotypes

Plate 6. Genetic Variability for External Colour, Size, Texture and Shape among Carrot Genotypes

improvement, hence to know the association between traits and between markers the data was further used for these traits.

4.1.3 Descriptive statistics for quantitative parameters

The mean, standard error of mean, range, genetic variability (GCV, PCV) and heritability components (h^2 and GAM) for 18 quantitative traits are presented in the table 7. The Individual genotypes mean for all the 96 genotypes are presented in Appendix-I.

The range of variation among the 96 carrot genotypes revealed high variation for the studied morphological traits indicating the existence of variation.

The mean number of days to maturity was 72.79 ranging from as early as 46 days to as late as 94 days. The genotype UHSBC-94 was the earliest genotype (46.0 days) followed by UHSBC-37 (47.0 days) and the genotype UHSBC-58 (94 days) was the genotype with highest number of day to maturity. Among the other plant morphological traits, number of petioles and the petiole length both showed wider range of variation of 6.60-28.80 and 6.50 cm-50.50 cm with the mean value of 10.84 to 13.70 cm respectively. The average plant height among the genotypes was 51.80 cm with the range from 30.50 to 72.80 cm.

The average vegetative weight (48.09 g) of the single plant among the genotype was higher than the average root weight (42.72 g). Same trend was also observed for five plants average vegetative weight (230.70g) and five plants root weight (205.20 g). The highest root weight/plant was recorded by the genotype UHSBC-71 with the root weight of 97.60 g, and the genotype UHSBC-85 showed the lowest weight of 16.0 grams. Mean root yield of five (single) plants was 205.2g ranging from 54.00 g to 470g/; root width was measured around a mean value of 18.28 ranging from 11.56 mm to 31.64 mm. Root length ranged from 10.38 cm to 59.34.

Exterior and interior colours are important characteristics receiving attention in all the carrot breeding projects. It is possible to achieve a deep uniform colour in both xylem and phloem and eliminate green colour. In this regard, the available germplasm

Plate 7. Genetic Variability for internal core colour among the carrot genotypes

Hybrid Vrishtirishti

Pusa Rudhira

Pusa Asita

VRCAR-42

Plate 8. Genetic Variability for leaf type (Fern, Celery, normal types) in carrot Genotypes

Celery type

Normal type

Fern type

Plate 9. Genetic Variability for leaf type (Fern, Celery, normal types) in carrot Genotypes

resources were evaluated for xylem and phloem colour along with the external root colours and presented as frequency distribution.

It is also important to achieve the higher phloem width than the cambium to get better root weight with the crispy textured carrot roots. As the width of the core/cambium increases, carrot root become tasteless as well as hardened texture in the root while cutting and eating.

Phloem width is an important trait contributing to economic part of the carrot root was ranging from 0.23 mm to 0.86 mm and xylem width ranging from 0.4 mm to 1.44 mm. The highest phloem width was recorded in the genotype UHSBC-18 (0.86 mm), where as the genotype UHSBC-100 showed the highest xylem width of 0.84 mm.

Many consumers purchase carrots for their vitamins, culinary quality and nutritive value rather than taste. In this regard, study conducted to evaluate β-carotene, TSS content and reducing sugars. The mean β-carotene content was 22.189 µg/100mg, ranging from 2.5 to 79.33 µg/100mg; and TSS ranged from 4.75 ◦Brix to 10.30^0 Brix. The results indicated that a wide range of genetic variability exists in almost all characters

4.1.4 Variability and heritability components estimates

The genetic variability and heritability parameters for the 18 quantitative traits are given in the Table 7 and Figure 2.0. It is clearly evident from the analysis that, considerable amount of variation was present among the genotypes for each character. Phenotypic coefficient of variation (PCV) was higher than the genotypic coefficient of variation (GCV) which indicates the role of environmental factors on the expression of various genotype traits studied in the present investigation.

Moderate to higher GCV and PCV was observed in most of the characters except TSS content and reducing sugars where GCV was lower although PCV was moderate indicating the role of environment/soil conditions/growth conditions on the expression of these traits. Yield parameters like single plant root weight and five plants root weight, vegetative weight, harvest index, showed higher GCV as well as PCV

Table 7: Mean, range, Genetic variability and heritability components for quantitative traits in carrot

Traits	Mean ± SEm	Min	Max	GCV	PCV	h^2 b.s	GAM (5%)
Days to Maturity	72.79 ± 1.08	46.00	94.00	12.23	13.74	0.79	22.43
No. of Petioles	10.84 ± 0.38	6.60	28.80	31.11	31.99	0.94	62.31
Shoulder Length (cm)	0.96 ± 0.02	0.10	1.57	17.78	23.44	0.58	27.77
Plant Height (cm)	51.80±0.96	30.50	72.80	13.71	17.52	0.61	22.1
Root Length(cm)	17.51 ± 0.56	10.38	59.34	25.33	29.72	0.73	44.47
Petiole Length(cm)	13.70 ± 0.55	6.50	50.50	35.46	37.24	0.91	69.53
Root width (mm)	18.28 ± 0.33	11.56	31.64	14.83	16.66	0.79	27.21
Shoulder Width (mm)	24.79 ± 0.63	11.69	40.43	19.22	23.69	0.66	32.11
Vegetative weight of single plant (g)	48.09 ± 3.10	6.00	173.60	55.71	59.62	0.87	107.22
Five plants vegetative weight (g)	230.70 ± 15.24	32.00	842.00	54.30	58.87	0.85	103.19
Xylem Width (mm)	0.76 ± 0.02	0.40	1.44	15.34	26.90	0.33	18.02
Phloem Width (mm)	0.45 ± 0.01	0.23	0.86	19.82	23.92	0.69	33.84
Harvest Index	0.50 ± 0.01	0.27	0.84	46.18	46.23	0.99	94.70
Total Soluble Solids (^{0}Brix)	7.77±0.13	4.75	10.30	9.21	14.86	0.38	11.75
Reducing Sugars (%)	4.79 ± 0.09	2.99	6.94	9.63	17.47	0.30	10.92
Beta Carotene (µg/gm)	22.19 ± 1.71	2.50	79.33	32.15	67.81	0.22	31.41
Root Weight of individual Plant (g)	42.72 ± 1.60	16.00	97.60	28.94	35.05	0.69	49.21
Five Plants Root Weight (g)	205.20 ± 8.43	54.00	470.00	28.82	38.43	0.56	44.72

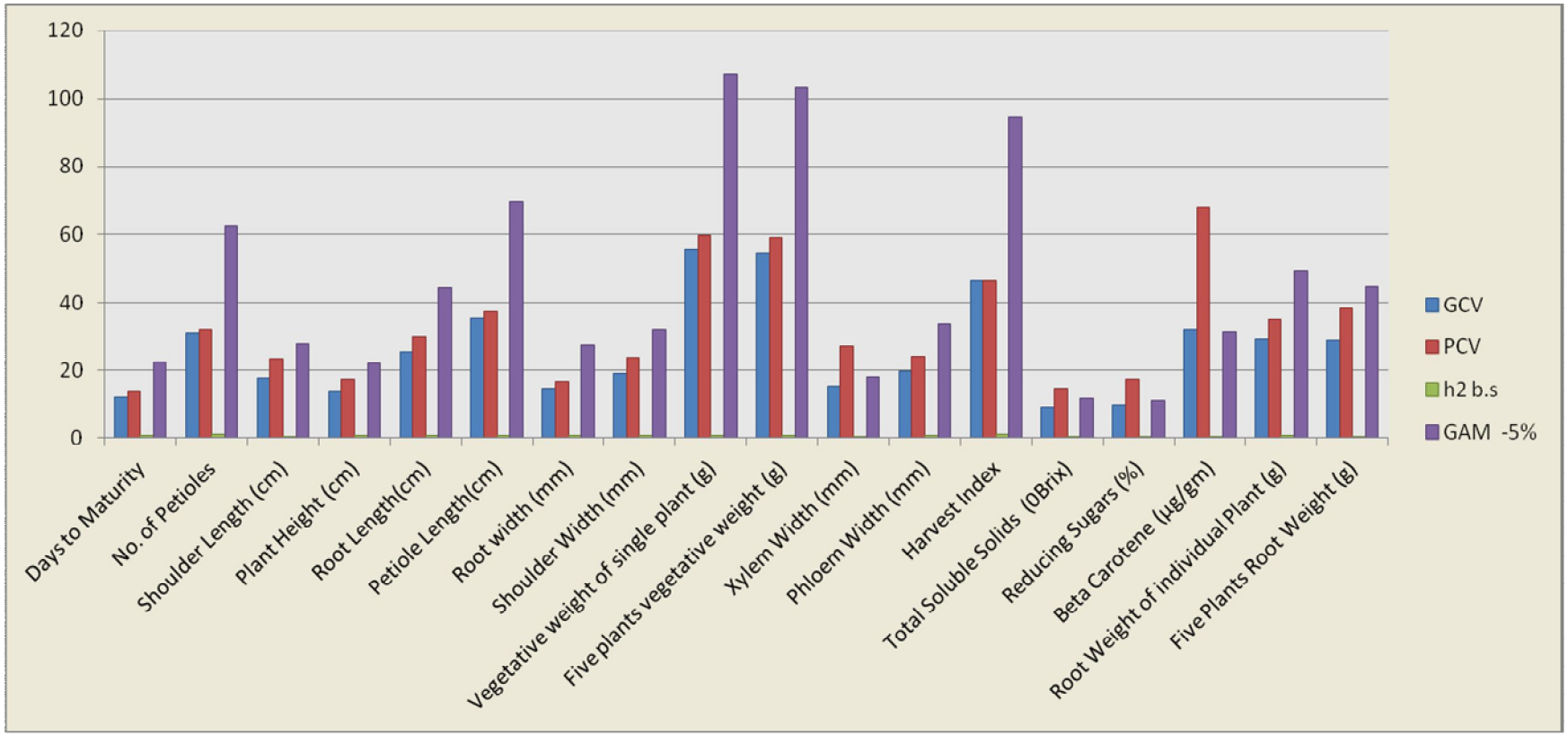


Fig. 2. Genetic variability (GCV, PCV) and heritability (h^2 and GAM) for quantitative traits in carrot genotypes

indicating the existence of grater variation among these characters in the genotype panel selected for the present investigation.

Heritability and genetic advance are the components of heritability which gives the information about the extent of inheritance of value of the character to the next generation, which helps the breeder to rely on selection methods during breeding and handling of large segregating populations or germplasm lines. The extent of heritability >60.0% (>0.60) is considered to be high and in the present investigation, few traits especially the plant morphological traits (except xylem width and five plants root weight) showed very high heritability. Although for the biochemical components, higher heritability is expected, but in the present investigation, these traits showed moderate heritability as depicted by their moderate PCV but lower GCV indicating that, the expression of these traits are highly influenced by temperature, soil conditions as well as other environmental factors. Genetic advance as percent mean helps to compare the extent of heritability across the traits having different units, so that a breeder can go for selection of traits having higher GAM for effective selection in turn, overall crop improvement. Among the 18 traits studied, except beta carotene content, reducing sugars and the root width, almost all the traits showed higher GAM, where as in the farmer mentioned traits, the GAM was lower.

In general, the heritability and genetic variability components among the morphological and biochemical traits revealed significant variation within the germplasm panel of carrot selected for the study.

4.1.5 Correlation coefficient analysis

The results of Pearson's correlation coefficient across the 18 quantitative traits including 3 biochemical and 15 morphological and yield components is presented in table 8. Among the plant morphological traits, five plant vegetative weight; vegetative weight/plant had a strong positive correlation with number of petioles, petiole length and plant height and the shoulder width. With respect to the economic yield such as root weight/plant and root weight/five plants, they both were influenced by almost all the plant morphological characters as depicted by their strong correlation except with shoot length, days to maturity, harvest index and biochemical parameters. There was no

Table 8: Pearson's correlation coefficient analysis among root morphological and biochemical traits of carrot genotypes

Traits	DM	NP	SL	PH	RL	PL	RWD	SWD	VW	FPVW	XW	PW	HI	TSS	RS	β	RW	FPRW
DM	1.000																	
NP	-0.170*	1.000																
SL	0.018	0.055	1.000															
PH	-0.137	0.282**	0.058	1.000														
RL	-0.107	0.165*	0.182*	0.273**	1.000													
PL	-0.072	0.000	-0.039	0.527**	0.063	1.000												
RWD	0.221**	0.000	-0.091	0.193*	0.042	0.254**	1.000											
SWD	0.082	0.084	0.049	0.691**	0.069	0.507**	0.558**	1.000										
VW	-0.203*	0.726**	-0.009	0.699**	0.135	0.312**	0.174*	0.481**	1.000									
FPVW	-0.216	0.743**	0.061	0.670**	0.137	0.235**	0.135	0.464**	0.910**	1.000								
XW	0.019	0.124	0.106	0.692**	0.097	0.432**	0.433**	0.869**	0.440**	0.448**	1.000							
PW	0.153	-0.178	-0.115	0.056	-0.059	0.200*	0.440**	0.450**	-0.166*	-0.092	0.387**	1.000						
HI	-0.126	-0.056	0.015	-0.210	-0.038	-0.117	-0.109	-0.204*	-0.145	-0.057	-0.167*	0.039	1.000					
TSS	0.237**	-0.043	-0.053	0.290**	-0.020	0.209*	0.155	0.252**	0.030	0.104	0.260**	0.119	-0.114	1.000				
RS	0.089	-0.049	-0.048	0.059	0.030	-0.012	-0.043	0.040	-0.030	-0.057	0.005	0.124	-0.107	0.079	1.000			
β-carotenoid	0.073	-0.124	0.068	-0.114	0.096	0.115	0.110	-0.021	-0.187*	-0.174	0.040	0.088	0.004	0.141	-0.084	1.000		
RW	0.109	0.289**	0.096	0.637**	0.255**	0.448**	0.675**	0.784**	0.538**	0.547**	0.726**	0.342**	-0.136	0.297**	0.054	0.105	1.000	
FPRW	0.082	0.272**	0.080	0.577**	0.246**	0.396**	0.670**	0.779**	0.459**	0.552**	0.722**	0.430**	-0.089	0.328**	0.005	0.129	0.894**	1.000

*Significance at 5% probability, ** Significance at 1% probability

significant association among the biochemical traits indicating the independence of each of the character accumulation in the roots. Root width was highly influenced by shoulder width, xylem and phloem width as shown by their strong positive correlation. Interestingly, the TSS content was positively correlated with days to maturity indicating that, higher TSS will be accumulated at the later stages of root growth. Other than the negative significant correlation between number of petioles and days to maturity, there was no strong negative character association between the traits studied.

4.1.6 Principle component analysis by factor analysis

Utilization of genetic resources is the sustainable solution to basic crop constraints which have been suggested from time to time, but these genetic resources could not be exploited fully due to their inherent problems of large size and lack of sufficient evaluation and classification (Dahberg, 1995). Germplasm maintenance, evaluation and characterisation for economically important traits are prerequisite for genetic improvement program of any crop. Principal components analysis is one of the important approaches which would resolve several phenotypic measurements even if large collections into a fewer, more interpretable and more easily visualized dimensions by the method of data reduction from large number of variables in to smaller principle components. Hence, the PCA analysis was applied to partition the 18 quantitative variables into few important principle components in the present study.

Principal component analysis characterised all eighteen quantitative traits into six components (Table 9) which helps in identifying the most relevant characters and present them in more visualized dimensions through linear combination of variables that accounts for most of the variation present in original set of variables.

In the present investigation, principal components with Eigen values greater than unity (1.0) were selected for interpretation (Kaiser 1958 and Jeffers 1967). The six principal components which had Eigen values more than one were retained in the analysis because of the substantial amount of variation by them. The other factors corresponding to Eigen values < 1.0 were not considered (Fig. 3a). These factors are ignored due to Guttmen's lower bound principle, according to which an Eigen value less than unity ($\lambda < 1$) should be ignored (Kaiser, 1958). The first six principal components

Table 9: Principle component analysis (PCA) showing the principle components with the Eigen value and the Variance explained by extraction method for 18 quantitative traits

Total variance explained									
Component	**Initial eigen values**			**Extraction sums of squared loadings**			**Rotation sums of squared loadings**		
	Total	**% of Variance**	**Cumulative %**	**Total**	**% of Variance**	**Cumulative %**	**Total**	**% of Variance**	**Cumulative %**
1	5.891	32.729	32.729	5.891	32.729	32.729	3.712	20.62	20.62
2	3.193	17.738	50.467	3.193	17.738	50.467	3.162	17.569	38.189
3	1.38	7.669	58.135	1.38	7.669	58.135	3.028	16.824	55.013
4	1.235	6.861	64.996	1.235	6.861	64.996	1.403	7.796	62.809
5	1.171	6.504	71.501	1.171	6.504	71.501	1.391	7.727	70.536
6	1.119	6.218	77.718	1.119	6.218	77.718	1.293	7.182	77.718
7	0.853	4.738	82.456						
8	0.798	4.436	86.892						
9	0.604	3.358	90.25						
10	0.516	2.865	93.115						
11	0.482	2.68	95.795						
12	0.247	1.373	97.168						
13	0.154	0.854	98.022						
14	0.138	0.765	98.786						
15	0.098	0.545	99.331						
16	0.064	0.354	99.684						
17	0.037	0.206	99.891						
18	0.02	0.109	100						
Extraction Method: Principal Component Analysis									

Table 10: Rotated Component Matrix of PCA analysis showing individual traits contribution in each principle components

Traits	Component					
	1	2	3	4	5	6
VW	0.925		0.280			
FPVW	0.908	0.146	0.324			
NP	0.883		-0.184			
HI	-0.724	0.468	-0.269	0.122	0.164	0.262
RWD		0.843				0.192
PW	-0.252	0.766	0.148			-0.157
FPRW	0.372	0.730	0.434	0.127	0.176	0.122
RW	0.373	0.675	0.443	0.201	0.211	
PH	0.451		0.769	0.130		-0.170
PL		0.151	0.727			0.262
XW	0.219	0.470	0.702			
SWD	0.235	0.586	0.687			-0.152
SL			0.153	0.746	-0.121	-0.130
RL				0.737		0.183
DM		0.121	-0.128		0.764	
TSS			0.437		0.690	0.156
beta	-0.223			0.163	0.187	0.784
RS	-0.122			0.394	0.383	-0.558

Extraction Method: Principal Component Analysis.
Rotation Method: Varimax with Kaiser Normalization.
a. Rotation converged in 9 iterations.

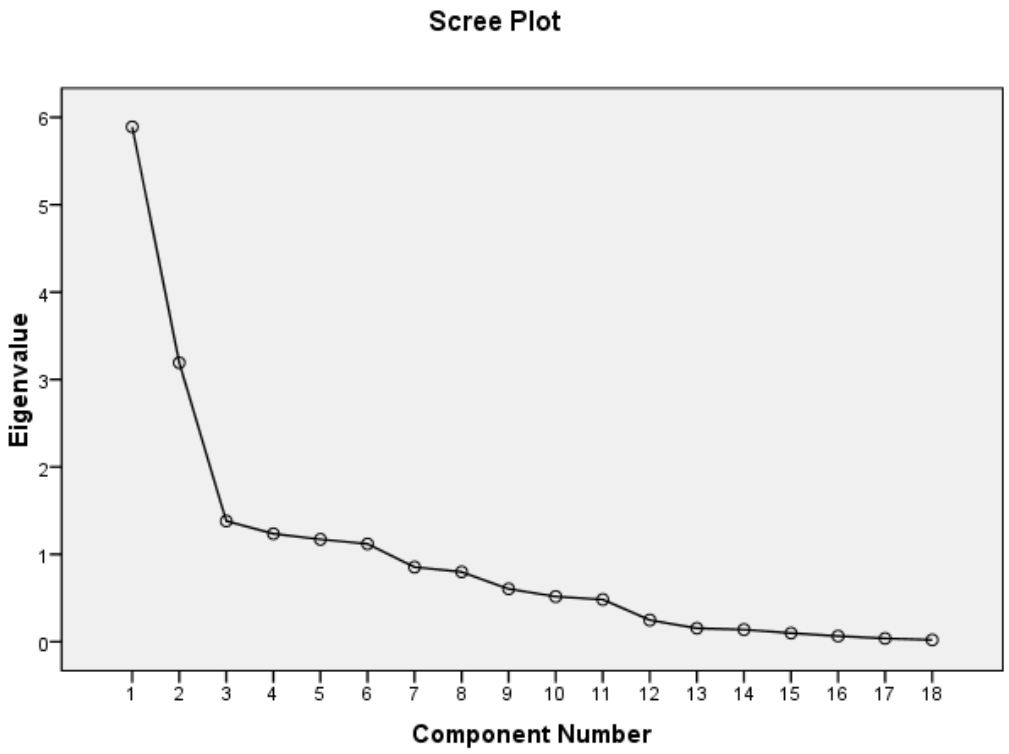


Fig 3a: Scree plot showing principle components in the Principle Component analysis (PCA) with Eigen value

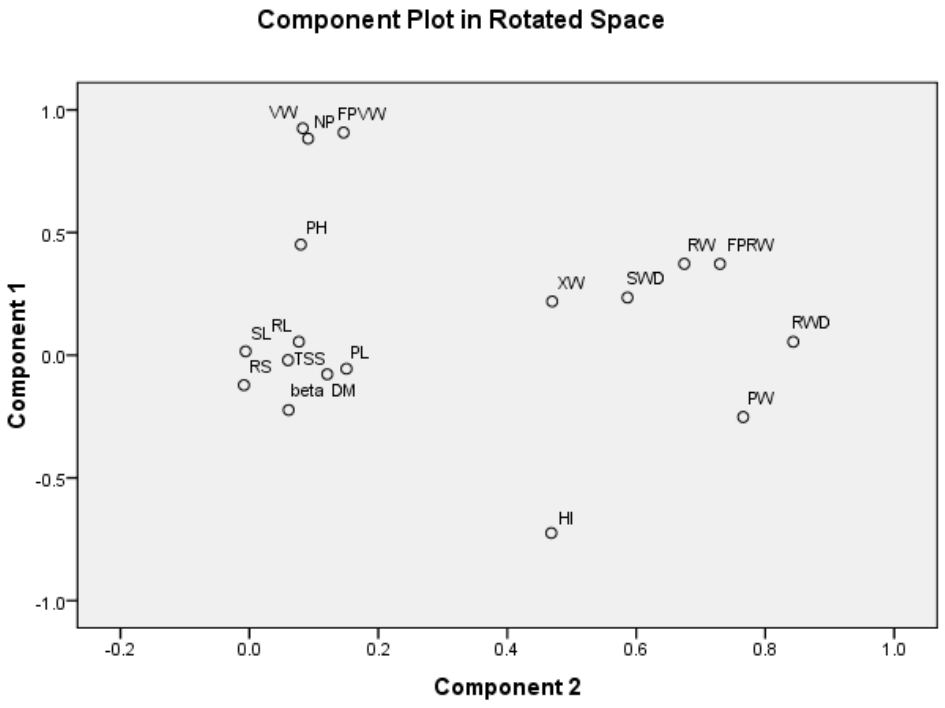


Fig 3b: Distribution of various quantitative traits among two principle components of the

had Eigen values greater than one and altogether explained 77.71% of total accumulated variability (Table 9).

The first principal component explained the maximum variance of 32.71% of the total variation. The remaining five principal components explained 17.73, 7.66, 6.86, 6.50, and 6.21 % of the total variance, respectively.

When the distribution pattern of the 18 traits among the first two principle components (higher Eigen Values >2.8) was studied (Fig 3b and Table 10), by keeping the PC1 as Y axis and PC2 as X-axis there was clear cut distribution of the traits studied. The first principal component extracted (PC1) had the combination of vegetative weight, five plants vegetative weight, number of petioles, root width, plant height and five plants root weight accounting 32.71 % of total variation. The second principal component extracted (PC2) explained harvest index, root weight, root width, phloem width accounting for 17.73 % of total variation. Among the remaining 4 PC, Third PC was a combination of plant height, petiole length, shoulder width, xylem width. The fourth PC extracted was a combination of shoot length, root length and reducing sugar. The fifth PC consists of days to maturity, TSS and beta carotenoid. In the sixth PC, combination of beta carotenoid and reducing sugar were present.

Based on the principle component analysis it was revealed that, plant and root morphological characters, especially, the yield components contribute maximum to the variance for the population and they can be accounted for selection criteria in breeding programs as they are also the major traits for overall productivity of the carrot.

4.1.7 Mahalanobis' D^2 statistics

To quantify genetic divergence between any two genotypes or group of genotypes, Mahalanobis' D^2 statistics (1936) as described by Rao (1952) was used and the genotypes were grouped into different clusters on the basis of ward's minimum variance method.

The mean sum of squares due to genotypes for all the traits studied were highly significant from the analysis of variance (Table 6a and 6b), thereby, revealing sufficient amount of genetic variation among the genotypes for all the eighteen characters studied.

Hence, these 18 traits were considered for D^2 statistics to know the extent of diversity among these carrot genotypes representing broad genetic background.

4.1.7.1 Contribution of different characters towards divergence

The diversity among 96 genotypes was measured by employing D^2 statistics. The table 11 shows the contribution of 18 quantitative characters towards genetic divergence. Out of these characters, contribution of five plants vegetative weight was maximum (63.2%), followed by five plants root weight (33.57%), whereas, the remaining characters like, beta carotenoid (1.05%), days to maturity (0.42%), harvest index% (0.31%) and few other traits contributed either very little or no contribution for divergence.

4.1.7.2 Cluster composition

All 96 carrot genotypes were grouped into clusters based on the relative magnitude of their D^2 values, in such a way that genotypes in each cluster had smaller D^2 value than between the clusters. Table 12 shows the distribution pattern of genotypes in different clusters. The genotypes were grouped into 12 different clusters. Cluster pattern revealed that cluster-I was the largest one with as high as 85 genotypes, and remaining 11 clusters formed solitary clusters. Most of these clusters which diverged from the cluster I were either IIVR collections or local varieties except for cluster 10 (Hybrid Kuruda-Public sector hybrid) and cluster 11(IARI, Released variety Pusa Payasa) indicating existence of higher diversity among the local cultivars and IIVR collections which are least explored in the breeding program for crop improvement.

4. 1.7.3 Intra and inter cluster average D^2 values

The intra and inter cluster D^2 values among 96 genotypes are given in table 13. The results showed that inter cluster distances are more than intra cluster distances which indicates the presence of narrow genetic variation within a cluster, but very high divergence between the clusters as indicated by higher values of inter cluster D^2 values. The highest intra cluster D^2 value was observed for cluster number I as there were 85 different genotypes and remaining all other showed 0.00 intra cluster distance as they were solitary clusters consisting of single genotype. Diversity among the inter-clusters

Table 11: Mahalanobis D^2 analysis showing percent contribution of root morphological characters to diversity among 96 carrot genotypes

S No	Source	Times ranked 1st	Contribution %
1	Days to Maturity	19	0.42%
2	No. of Petioles		0.0 %
3	Shoulder Length (cm)		0.0 %
4	Plant Height (cm)	3	0.07%
5	Root Length(cm)	4	0.09%
6	Petiole Length(cm)		0.0 %
7	Root width (mm)		0.0. %
8	Shoulder Width (mm)		0.0 %
9	Vegetative weight of single plant (g)	54	1.18%
10	Five plants vegetative weight (g)	2882	63.2%
11	Xylem Width (mm)		0.0 %
12	Phloem Width (mm)		0.0 %
13	Harvest Index	14	0.31%
14	Total Soluble Solids (^{0}Brix)		0.0 %
15	Reducing Sugars (%)		0.0 %
16	Beta Carotene (µg/gm)	48	1.05%
17	Root Weight of individual Plant (g)	5	0.11%
18	Five Plants Root Weight (g)	1531	33.57%

Table 12: Cluster Composition showing the genotypes grouped into each cluster in Mahalanobis D^2 analysis using 18 quantitative traits of carrot

Clusters	No of Genotypes	Genotype Composition
Cluster 1	85	UHSBC-1, UHSBC-2, UHSBC-3, UHSBC-7, UHSBC-14, UHSBC-15, UHSBC-16, UHSBC-17, UHSBC-19, UHSBC-20, UHSBC-21, UHSBC-22, UHSBC-23, UHSBC-25, UHSBC-27, UHSBC-28, UHSBC-29, UHSBC-30, UHSBC-31, UHSBC-32, UHSBC-32_2, UHSBC-33, UHSBC-34, UHSBC-36, UHSBC-37, UHSBC-38, UHSBC-39, UHSBC-40, UHSBC-42, UHSBC-43, UHSBC-43_1, UHSBC-44, UHSBC-45, UHSBC-46, UHSBC-48, UHSBC-49, UHSBC-50, UHSBC-51, UHSBC-52, UHSBC-53, UHSBC- 34_1, UHSBC- 34_2, UHSBC- 41_1, UHSBC-77, UHSBC-56, UHSBC-58, UHSBC-59, UHSBC-64, UHSBC-65, UHSBC-67, UHSBC-68, UHSBC-69, UHSBC-71, UHSBC-78, UHSBC-79, UHSBC-85, UHBC-89, UHSBC-90, UHSBC-93, UHSBC-94, UHSBC-95, UHSBC-96, UHSBC-97, UHSBC-98, UHSBC-99, UHSBC-101, UHSBC-102, UHSBC-106, UHSBC-107, UHSBC-108, UHSBC-110, UHSBC-111, UHSBC-112, UHSBC-113, UHSBC-114, UHSBC-115, UHSBC-54, UHSBC-66, UHSBC-92, UHSBC-103, UHSBC-104, UHSBC-105, UHSBC-116, UHSBC-117, UHSBC-23_1
Cluster 2	1	UHSBC-18-Local Cultivar (Hangaraki Local)
Cluster 3	1	UHSBC-47-IIVR Collection
Cluster 4	1	UHSBC-73-Local cultivar (Belgaum)
Cluster 5	1	UHSBC-41-IIVR collection
Cluster 6	1	UHSBC-24-IIVR collection
Cluster 7	1	UHSBC-76-Local cultivar (Naganur)
Cluster 8	1	UHSBC-35-IIVR collection
Cluster 9	1	UHSBC-55-IIVR collection
Cluster 10	1	UHSBC-100 (Hyb Kuruda)
Cluster 11	1	UHSBC-63-Released varieties (Pusa Payasa)
Cluster 12	1	UHSBC- 26- IIVR collection

Table 13: Mahalanobis D^2 Analysis showing inter-intra cluster distances among the twelve clusters analyzed for 96 carrot genotypes

Clusters	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Cluster 6	Cluster 7	Cluster 8	Cluster 9	Cluster 10	Cluster 11	Cluster 12
Cluster 1	36227.53											
Cluster 2	91355.59	0.00										
Cluster 3	84352.95	29624.58	0.00									
Cluster 4	83582.09	4376.17	12787.12	0.00								
Cluster 5	79252.07	14530.97	3256.20	3477.88	0.00							
Cluster 6	112536.50	3331.50	36948.89	7504.90	19227.41	0.00						
Cluster 7	88565.80	5249.33	49384.45	13470.05	27838.94	5732.92	0.00					
Cluster 8	86820.52	51478.09	6048.01	28273.58	12491.81	58952.35	69638.55	0.00				
Cluster 9	163796.60	32538.49	22068.85	20269.64	18499.61	27221.05	53153.13	36317.88	0.00			
Cluster 10	87776.03	138674.80	210813.30	158188.90	178121.70	154027.10	102748.50	221859.00	283256.30	0.00		
Cluster 11	339242.70	93410.63	134827.70	99028.78	115300.60	71427.90	109410.00	166173.10	52730.13	411913.10	0.00	
Cluster 12	456310.80	209853.00	165322.80	184929.50	174429.50	191564.40	258766.80	180942.70	86098.05	671114.10	77268.62	0.00

showed a very wide range of D^2 value ranging from 671114.10 to 456310.80. The inter cluster distance was found to be highest between cluster X and XII (671114.10), followed by cluster I and XII (456310.80), cluster X and XI (411913.10). Whereas, the lower inter cluster distance was observed between cluster III and V, followed by cluster VI and II (3331.50). The higher inter cluster distance indicated the presence of more diversity among the genotypes included among these clusters.

4.1.7.4 Mean values of different clusters for 18 characters

The cluster means for all the 18 characters are presented in Table14. The data revealed considerable differences among all the clusters for most of the characters studied. It was evident that carrot root length was highest in cluster II (23.83 cm) and lowest in cluster XII (12.52 cm). Cluster I recorded the highest root diameter (32.41 mm) while cluster III recorded the lowest (15.43 mm). Root weight was minimum for cluster VIII (32.03 g) and it was maximum for cluster XII (57.83 g). Cluster X revealed the highest mean value for TSS (9.29^0Brix), whereas cluster III had the lowest mean value (5.74^0Brix). For harvest index, cluster I (0.98) had the highest mean value while cluster XII had the lowest mean value (0.24).

Cluster III showed the maximum mean value (54.283) for phloem colour and cluster VII showed the lowest mean value (47.822). For xylem colour, the highest mean value was possessed by cluster III (12.600) and the lowest value was possessed by cluster VII (9.289). Root colour was the highest in cluster VII (86.44) and the lowest in cluster IV (82.303). Cluster VII recorded the highest mean value of days to maturity (114.667) and the lowest in cluster IV (107.212). Five plants root yield was recorded maximum for cluster V (3.327) and the minimum for cluster I (2.237).

4.2 Molecular marker profiling for carrot populations

4.2.1 Genetic diversity parameters

All the 47 molecular markers including five dominant markers involving 471 alleles were analyzed for genetic diversity parameters among the 96 genotypes such as polymorphic information content (PIC), number of alleles for each locus (Na), number of effective alleles (Ne), Shanon's information Index (I), observed heterozygosity (Ho),

Table 14: Cluster means of 12 clusters for 18 quantitative traits analyzed for 96 genotypes of carrot

Traits / Clusters	DM	NP	SL	PH	RL	PL	RWD	SWD	VW	FPVW	XW	PW	HI	TSS	RS	Beta	RW	FPRW
Cluster I	73.04	10.22	0.95	50.71	17.40	13.19	17.97	24.02	42.58	199.12	0.73	0.45	0.95	7.65	4.85	21.49	40.08	185.89
Cluster II	48.83	10.92	1.07	75.55	23.83	20.18	19.30	38.59	78.72	397.72	1.40	0.85	0.46	6.74	4.45	4.14	69.10	358.22
Cluster III	62.50	21.19	1.03	50.63	17.46	9.01	15.43	22.41	91.34	451.06	0.72	0.35	0.33	5.74	2.70	16.53	38.90	203.56
Cluster IV	75.50	11.66	1.19	67.47	16.33	19.02	21.26	36.34	83.80	419.72	1.13	0.44	0.42	8.45	6.36	5.86	60.63	304.89
Cluster V	73.50	13.59	1.15	56.63	18.06	11.95	17.42	27.65	86.14	433.06	0.91	0.25	0.39	9.19	3.90	21.86	49.30	253.56
Cluster VI	77.83	13.92	0.75	58.25	20.61	11.80	32.41	32.20	82.72	423.72	0.90	0.47	0.47	8.04	3.44	12.19	74.30	388.22
Cluster VII	75.50	8.86	0.87	68.67	19.35	17.58	21.84	42.95	71.40	353.72	1.21	0.71	0.53	9.20	5.16	27.53	78.63	394.89
Cluster VIII	73.50	14.46	0.99	53.02	13.84	12.97	15.59	22.37	92.54	448.72	0.87	0.20	0.26	7.80	3.11	79.97	32.03	161.56
Cluster IX	88.50	20.79	1.07	59.23	16.80	12.11	11.65	23.88	93.34	559.06	0.89	0.39	0.36	8.44	3.65	16.36	58.90	299.56
Cluster X	82.17	7.06	1.05	52.30	21.08	51.50	24.65	29.87	9.67	46.39	0.83	0.71	0.91	9.29	4.58	79.53	77.03	383.56
Cluster XI	63.50	21.06	1.03	61.57	22.13	11.98	26.52	34.76	132.20	661.72	0.98	0.62	0.43	8.60	4.34	22.69	97.43	494.89
Cluster XII	58.50	28.66	1.03	58.62	12.52	16.43	21.52	27.16	174.94	838.72	0.61	0.57	0.24	6.45	5.39	13.14	57.83	289.56

expected heterozygosity (He) and a fixation index (F) using the software package GENALEX 6.503 version.

A total of 42 microsatellite loci were used to genotype 96 individuals of carrot (Table15) The genetic profiles detected 471 alleles from these 42 loci, which ranged between 2 (Y2, Y-indel, PSY and BSSR 128) to 24 (ESSR9) per locus. The PIC value of the markers used ranged from 0.0782 to 0.927. The average number of effective alleles (Ne) in the total population was 4.37 with the higher effective alleles in Population-1 (4.98) than population-2 (3.75) as presented in table 15. In the marker wise analysis, the maximum number of effective alleles were recorded by ESSR 59 (14.57) where the no of alleles were as high as 28. The minimum effective alleles was shown by a marker Y_2 (1.17) with only 2 alleles.

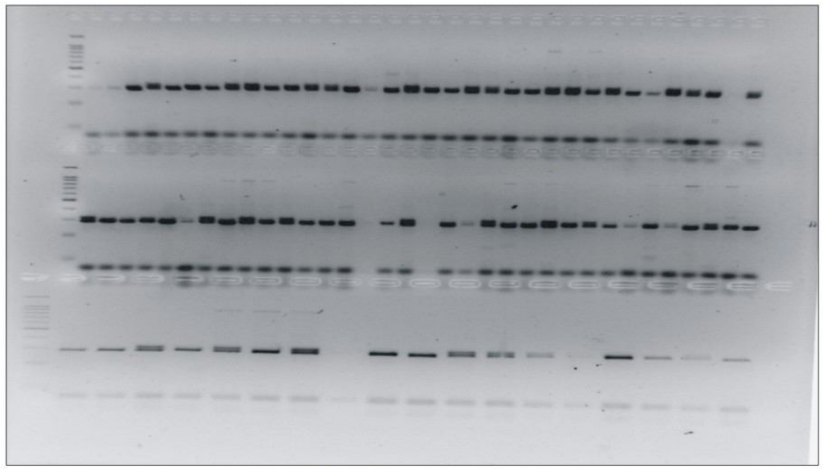
Shanon's information index (I) ranged from 0.18 to 2.98, with an average of 1.75. Among the populations, I was more for population-1 (1.63) than population-(1.34). The PIC (polymorphic information content) values of all the polymorphic markers across the 96 carrot genotypes varied from 0.54 to 1.00 at an average of 0.94 indicating the greater extent of polymorphism among the 96 genotypes with these set of molecular markers used in the present study which is also supported by highly out crossing nature of carrot.

Heterozygosity is an important measurement depicts the gene diversity. Among the two populations, the observed heterozygosity was comparatively higher in population-2 (0.14) than population-1 (0.13). In general, the observed heterozygosity was much lesser when compared with the expected heterozygosity in the population for almost all the loci indicating the deficiency of heterozygote's at these loci. Although carrot is a highly cross pollinated crop, in the present investigation, very high allelic diversity was observed with as high as 28 alleles per locus with the average no of alleles 4.37, but the reduced observed heterozygosity at these loci in the 96 germplasm panel of carrot indicates that, most of these loci have been fixed to homozygosity as depicted by higher fixation index for the respective loci, although greater allelic diversity is observed in most of these molecular markers. This indicates that, the genotypes utilized in the present investigation are mostly homozygous and could be utilized as parents in the breeding program. Since most of these genotypes have been evaluated after two

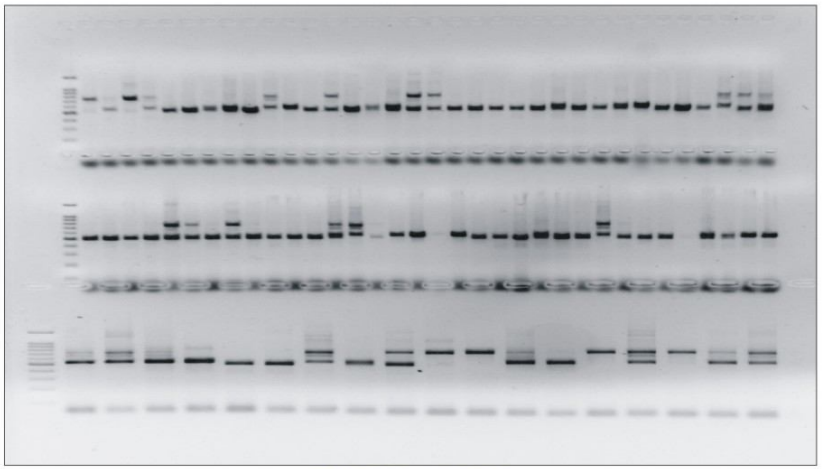
Table 15: Details of genetic parameters of molecular markers used for characterizing 96 genotypes of carrot

S No	Locus	Na	Ne	I	Ho	He	F	PIC
1	BSSR128	2.00	1.96	0.68	0.75	0.49	0.54	0.370
2	BSSR76	23.00	6.29	2.34	0.17	0.84	0.80	0.799
3	BSSR94	12.00	7.55	2.21	0.00	0.87	1.00	0.854
4	DCM17	4.00	2.95	1.13	0.06	0.66	0.92	0.595
5	DCM2	4.00	2.95	1.13	0.06	0.66	0.92	0.595
6	ESSR114	4.00	3.22	1.24	0.00	0.69	1.00	0.629
7	ESSR59	28.00	14.57	2.98	0.02	0.93	0.98	0.927
8	ESSR61	24.00	12.01	2.83	0.12	0.92	0.87	0.911
9	ESSR62	12.00	7.77	2.21	0.00	0.87	1.00	0.858
10	ESSR71	11.00	5.25	1.84	0.15	0.81	0.82	0.782
11	GSSR104	12.00	4.77	1.88	0.42	0.79	0.47	0.768
12	GSSR111	6.00	4.05	1.46	0.16	0.75	0.79	0.718
13	GSSR122	14.00	4.99	2.03	0.00	0.80	1.00	0.783
14	GSSR124	13.00	7.45	2.22	0.01	0.87	0.99	0.852
15	GSSR138	11.00	4.68	1.79	0.08	0.79	0.90	0.757
16	GSSR14	8.00	2.74	1.27	0.19	0.64	0.70	0.576
17	GSSR152	14.00	5.94	2.12	0.00	0.83	1.00	0.813
18	GSSR153	5.00	4.16	1.51	0.16	0.76	0.79	0.722
19	GSSR154	22.00	10.37	2.63	0.05	0.90	0.94	0.896
20	GSSR16	9.00	5.42	1.84	0.16	0.82	0.81	0.790
21	GSSR17	11.00	5.11	1.88	0.07	0.80	0.92	0.783
22	GSSR19	25.00	12.01	2.77	0.33	0.92	0.64	0.911
23	GSSR39	8.00	3.56	1.54	0.24	0.72	0.67	0.679
24	GSSR4	4.00	2.66	1.13	0.17	0.62	0.73	0.562
25	GSSR44	8.00	2.82	1.40	0.16	0.65	0.76	0.615
26	GSSR47	8.00	4.51	1.72	0.00	0.77	1.00	0.747
27	GSSR54	19.00	11.58	2.67	0.04	0.91	0.95	0.907
28	GSSR57	17.00	6.30	2.14	0.23	0.84	0.73	0.823
29	GSSR6	12.00	5.68	2.04	0.14	0.82	0.83	0.804
30	GSSR63	10.00	4.43	1.78	0.10	0.77	0.87	0.751
31	GSSR71	10.00	4.15	1.63	0.44	0.76	0.41	0.720
32	GSSR85	10.00	4.00	1.63	0.07	0.75	0.91	0.711
33	GSSR9	8.00	3.21	1.37	0.22	0.69	0.69	0.637
34	GSSR93	12.00	6.42	2.10	0.13	0.84	0.85	0.827
35	GSSR97	24.00	8.87	2.59	0.31	0.89	0.65	0.878
36	GSSR98	8.00	3.85	1.67	0.10	0.74	0.86	0.712
37	LCY	9.00	2.30	1.27	0.26	0.56	0.54	0.538
38	OPK9	21.00	12.64	2.74	0.00	0.92	1.00	0.915
39	PSY	2.00	1.86	0.66	0.00	0.46	1.00	0.356
40	Q800	3.00	2.11	0.87	0.12	0.53	0.78	0.449
41	Y2	2.00	1.17	0.27	0.00	0.14	1.00	0.133
42	Y-Indel	2.00	1.09	0.18	0.06	0.08	0.22	0.0782

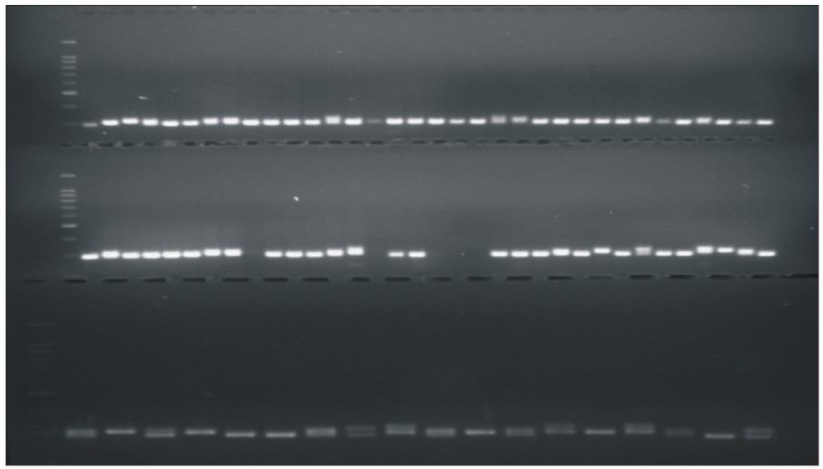
Na-Number of alleles, Ne-No of effective alleles, Ho-Observed heterozygosity, He-Expected heterozygosity, uHe-unbiased expected heterozygosity, I-Shanon's Information index, F-Fixation index.



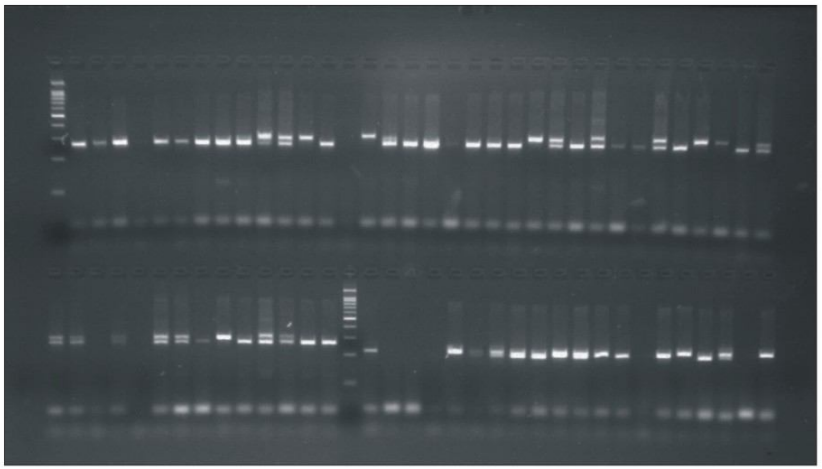
ESSR-61Microsatellite



Q1/800-SCAR



ESSR-6-Microsatellite



GSSR-9-Microsatellite

Plate 10. Genotypic profiling of Microsatellite and SCAR markers across 96 carrot genotypes

years of sib pollination, hence, the homozygosity is achieved in most of these loci. The superior genotypes of this germplasm panel are suitable for hybridization, as it takes longer time to develop inbreds in carrot due to its highly cross pollinating nature.

4.2.2 Genetic diversity by cluster analysis

To study the molecular diversity, the genotypic data of 96 carrot genotypes across 42 marker loci consisting of 471 alleles were subjected to cluster analysis with UPGMA neighbour joining tree using a software package DARwin 6.0. Population of 96 genotypes were grouped in to 3 main clusters but only one genotype was separated as 3rd cluster (UHSBC-69) which belongs to Ooty collection, New Kuruda. The divergence of this single genotype as solitary cluster need to be further tested with new set of molecular markers.

Further, in the first cluster, there were two sub cluster (sub cluster-I and sub cluster-II) with 26 and 22 genotypes respectively as shown in the table and in the second cluster there were again 2 sub clusters (I, II) with 30 and 17 genotypes respectively. The phylogenetic tree (figure 4) shows three clusters obtained based on the dissimilarity matrix of neighbour joining diversity tree obtained from DARwin version.

4.2.3 Population structure analysis

To understand Population structure analysis was carried out using STRUCTURE 2.3.3 (Pritchard *et al*. 2000; Hubisz *et al*. 2009). The admixture model was used with a burn in period of 50,000 and 5000 iterations for *K* populations ranging from 1 to 10. Five runs for each *K* value were performed and the *ad-hoc* statistic Δk was used to determine the optimum number of sub-groups (Evanno *et al*. 2005). The peak of delta K was observed at K = 2, suggesting clearly the presence of two main populations (clusters, Q1 and Q2) in the carrot panel consisting of 96 genotypes (Figure 5). Accessions with estimated memberships with Q value $\geq$ 0.50 were assigned to corresponding populations. A total of 96 accessions/cultivars (100%) were grouped to one of the two populations. The first cluster of 49 genotypes (51.04% of total carrot accessions) was grouped into Q1, the next 47 accessions (48.96% of total accessions) into Q2. Fifteen genotypes were considered to be admixtures from among the two populations, in which, ten genotypes were in the first population and remaining five

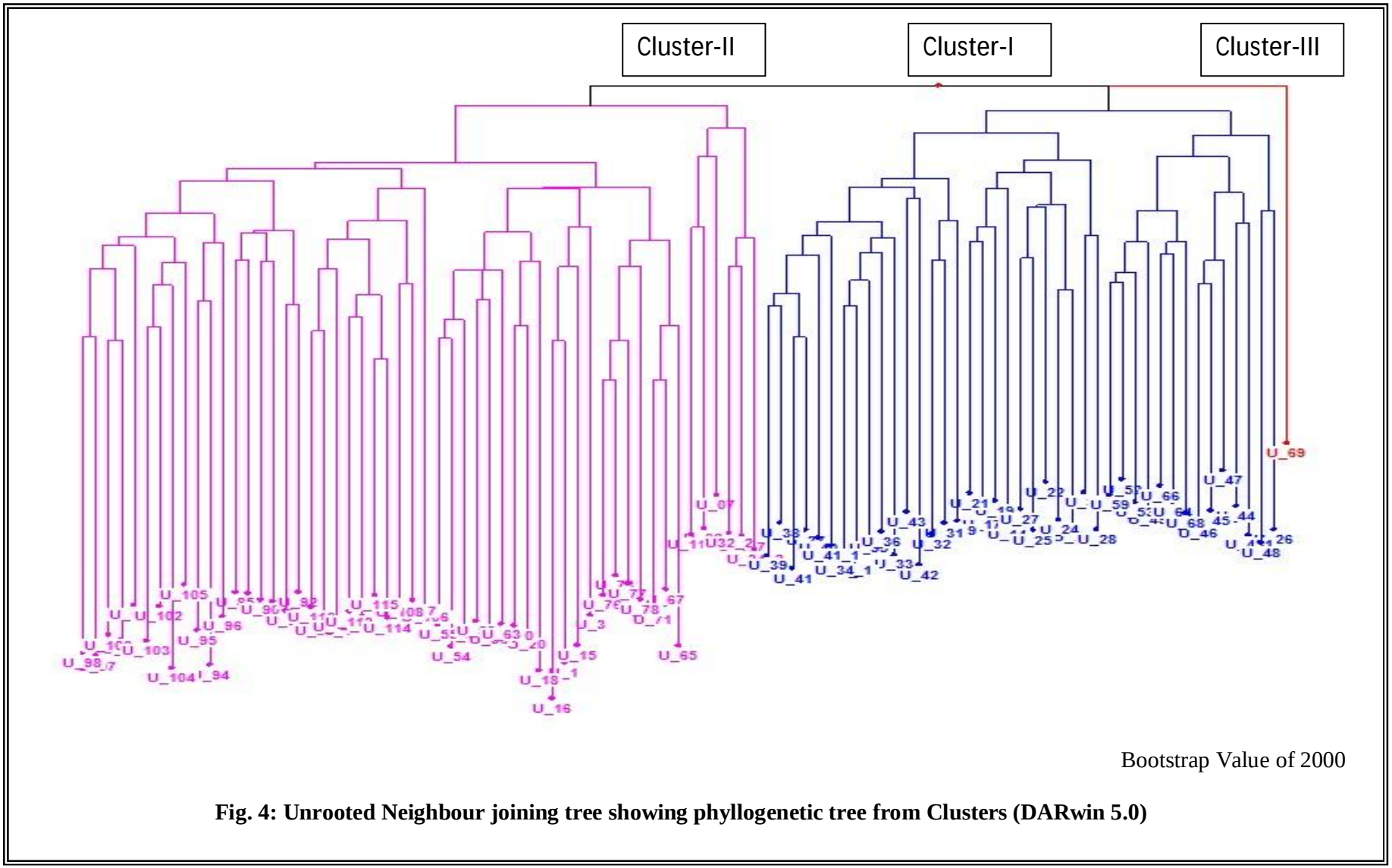


Fig. 4: Unrooted Neighbour joining tree showing phyllogenetic tree from Clusters (DARwin 5.0)

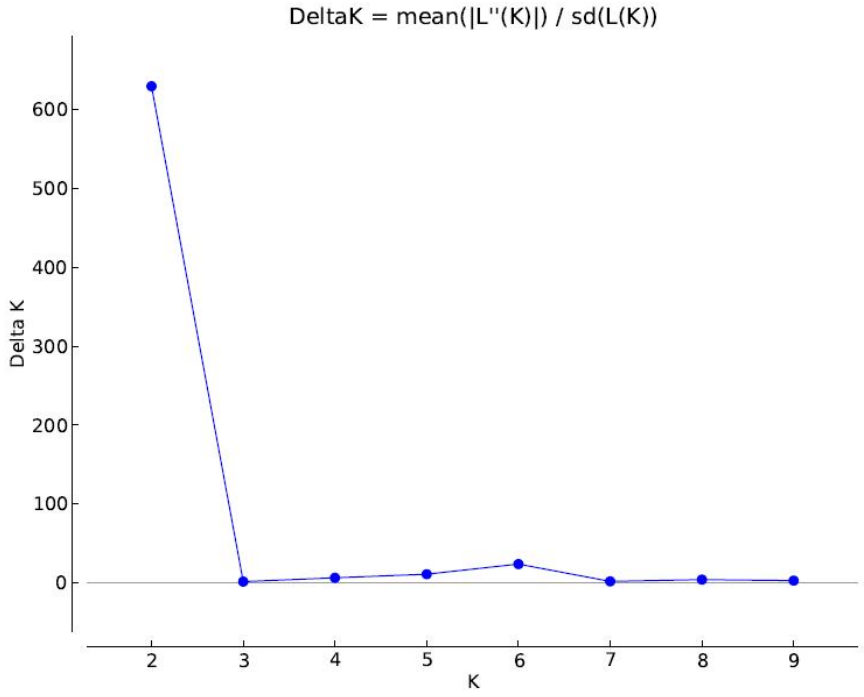


Fig. 5: Delta K value assumed for different number of populations assumed (K) in the STRUCTURE analysis extracted by STRUCTURE HARVESTOR

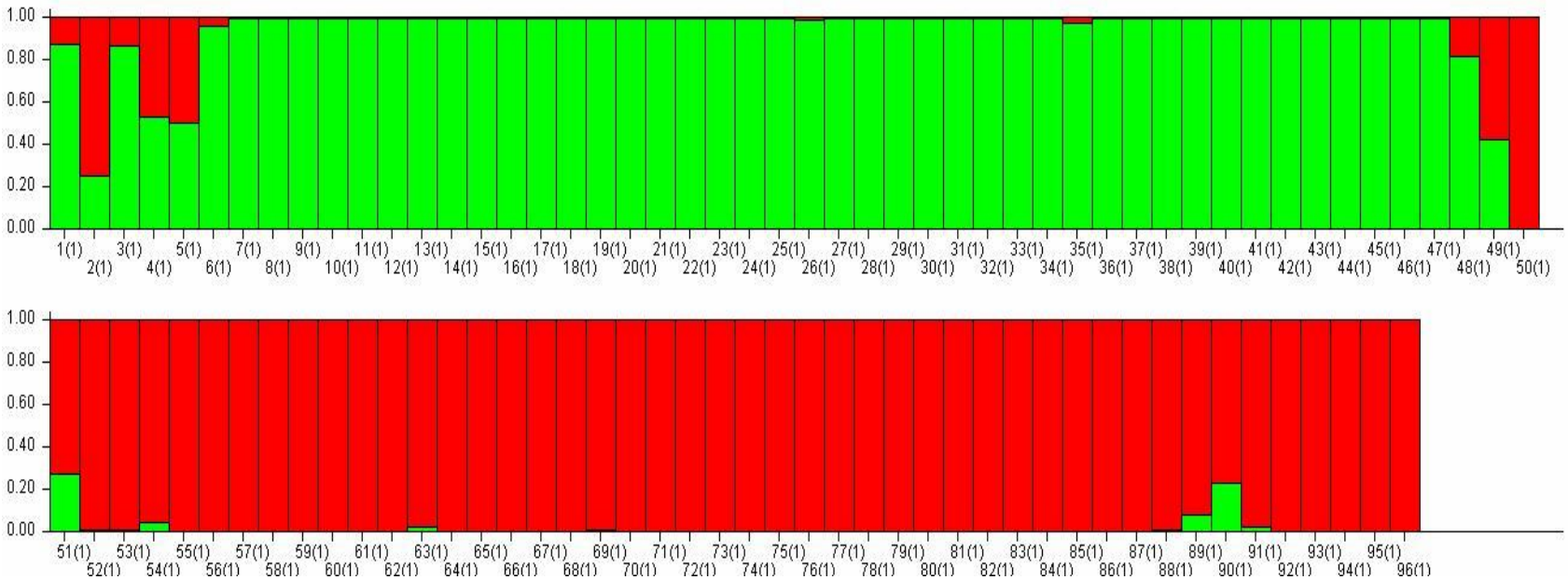


Fig. 6: Classification of 96 genotypes of carrot into 2 populations (K=2) allowing admixtures using STRUCTURE 2.3.4

were in the second population (Fig. 6). These admixtures were not considered for AMOVA as each of these genotypes were assigned to either of the populations based on Q value of K=2 populations structure file for further analysis of AMOVA.

To assess distinctiveness among and within the sub-populations, an Analysis of Molecular Variance (AMOVA) was carried out from the genotypes selected from two populations identified in the STRUCTURE analysis. Genotypes with Q value more than 0.5 were assigned to the respective populations 1 and 2 after the STRUCTURE analysis. So total of 49 genotypes were belonging to 1stpopulation and remaining 47 genotypes belonged to 2nd population. The contribution of estimated variance explained by different components is presented in Table 16a and figure 7a. AMOVA has clearly brought out significant differences among various genotypes evaluated. It was observed that greater variance (71%) was observed by individuals within population (Intra population), while between the two populations the extent of variation was comparatively less with 13.00% variation (inter populations) where as between the populations the extent of variance was 16.00%. the overall Fit statistics (0.842) indicates that the genotypes in the population are highly diverse in which partially the fixation index was due to the populations (Fst=0.129) and the major contribution was between the individuals within the populations (Fis=0.818).

The extent of genic differentiation among populations as measured by *F*-statistics, is in agreement with the diversity analysis table (Table 16a).Calculation of *F*-statistics revealed a *F*is (0.818) and *F* % (0.842) value. The mean *F*st value of indicates a level of genetic differentiation among the accessions. The pair wise fixation indices (Fst) between the two populations were 0.129, where as its Rst value was 0.071.

When the allelic patterns were compared between two populations to know the distinctness of each population with other (Table 17 and Fig. 7b), the average no of alleles for the 1st population (9.02) was more than the 2nd population (6.74) as well as the total population (7.88) indicating that more diversity is existed in the first population. The trend of the diversity and distinctness of 1st population over 2nd population was continued for other genetic parameters such as no .of effective alleles (4.98-1st population and 3.75 for 2nd population), Shannon's information index (I=1.63 for 1st population, I=1.34 for 2nd population) as well as observed heterozygosity (0.13 and 0.14 for 1st and 2nd population respectively). Number of private alleles unique to

Table 16a: Analysis of Molecular Variance (AMOVA) for the two populations identified by Structure analysis of Carrot

Source	df	SS	MS	Est. Var.	%	Fixation index
Among Populations	1	240.912	240.912	2.23**	13.00%	Fst=0.129
Among Individuals	94	2569.353	27.334	12.30**	71.00%	Fis=0.818
Within Individuals	96	262.500	2.734	2.73**	16.00%	Fit=0.842
Total	191	3072.766		17.260	100.00%	

Fst = AP / (WI + AI + AP) = AP / TOT, Fis = AI / (WI + AI), Fit = (AI + AP) / (WI + AI + AP) = (AI + AP) /TOT, Nm = [(1 / Fst) - 1] / 4
Key: AP = Est. Var. Among Pops, AI = Est. Var. Among Individuals, WI = Est. Var. Within Individuals

Table 16b: Pair wise Rst (Above diagonal) and Fst (below diagonal values)

Populations	Pop1	Pop2
Pop1	0.000	0.071
Pop2	0.129	0.000

Nm value depicting gene flow is 1.689 between the populations

Table 17: Details of distribution of markers, allelic patterns and trend of various genetic parameters across two populations in comparison with the total population

Pop		Na	Ne	I	Ho	He	F	Na Freq. >= 5%	I	No. Private Alleles	%P
Pop1	Mean	9.02	4.98	1.63	0.13	0.71	0.77	4.62	1.63	4.48	97.62
	SEm	0.82	0.51	0.10	0.03	0.030	0.06	0.28	0.10	0.74	
Pop2	Mean	6.74	3.75	1.34	0.14	0.63	0.77	3.83	1.34	2.19	100
	SEm	0.61	0.37	0.09	0.03	0.030	0.04	0.27	0.09	0.38	
Total	Mean	7.88	4.37	1.48	0.14	0.67	0.77	-	-	-	98.81
	SEm	0.52	0.32	0.07	0.02	0.02	0.035	-	-	-	1.19

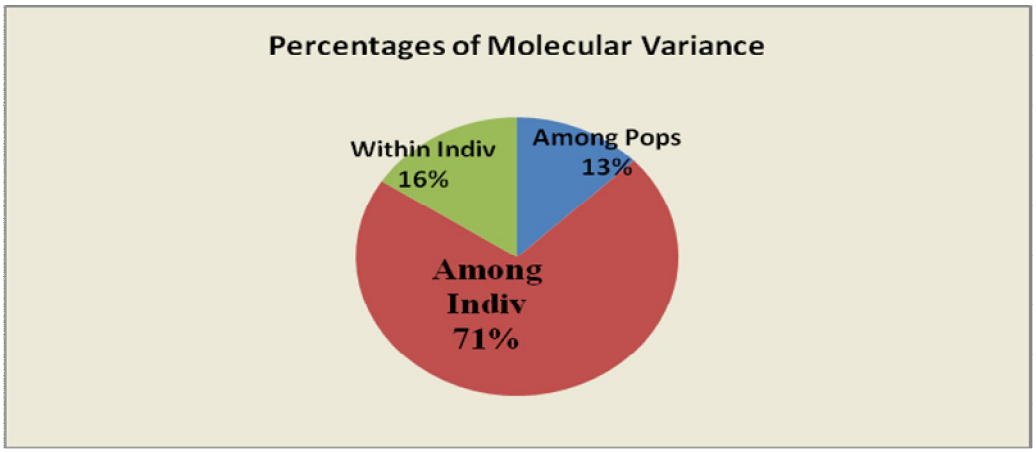
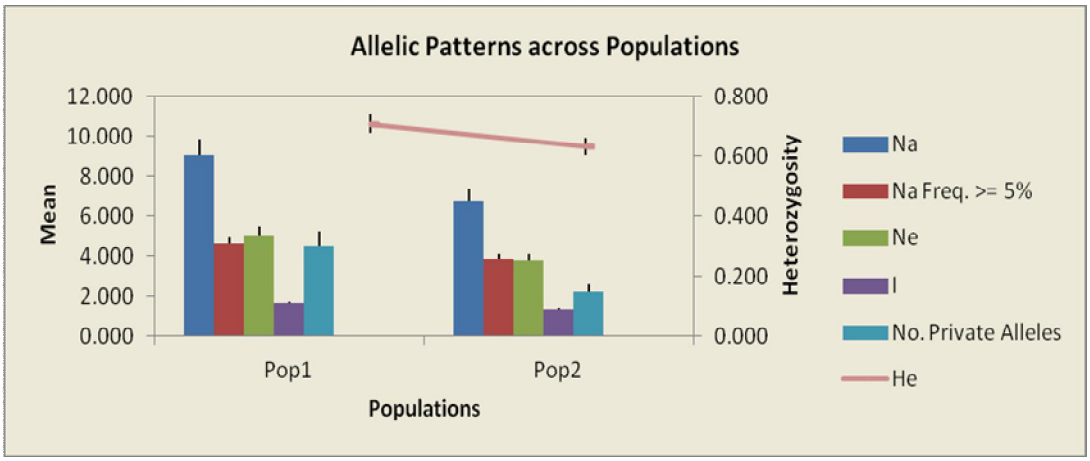


Figure 7a: Analysis of Molecular variance showing the contribution of various components total molecular variance from two populations



Na = No. of Different Alleles, Na (Freq >= 5%) = No. of Different Alleles with a Frequency >= 5%, Ne = No. of Effective Alleles = 1 / (Sum pi^2), I = Shannon's Information Index = -1* Sum (pi * Ln (pi)), No. Private Alleles = No. of Alleles Unique to a Single Population, He = Expected Heterozygosity = 1 - Sum pi^

Figure 7b: Allelic patterns showing the distribution of molecular marker loci, alleles, no of unique alleles, observed heterozygosity identified across the two populations of carrot

each population was also estimated, where in the 1st population showed more no of average private alleles (4.48) than the second population (2.19). But the average percent polymorphism was comparatively higher for the second population (100%) than the first population (97.62).

4.3 Marker-Trait Association Analysis by Generalized Linear Model

Association analysis was performed for all the 18 quantitative traits (including root morphological and biochemical) as well as 15 qualitative parameters using TASSEL 3.0. The analysis was performed by taking 42 markers genotypic data, 18 quantitative traits phenotypic data and population structure output file of Q values at K=2. The marker and the trait was declared as significant at P value <0.001. Since only two populations were obtained and hence only General linear model (GLM) was followed.

Out of 18 quantitative traits, only for seven traits, significant marker trait association was obtained from the present study (Table 18). Among 15 qualitative parameters, size traits showed significant marker trait association with very high R^2 value.

Among the quantitative traits, Harvest index was associated with two markers (BSSR94 and GSSR 97) with the R^2 value of 0.33 and 0.51 respectively. GSSR 19 and ESSR59 was linked with two traits, petiole length (0.62 & 0.65 R^2) and root length (0.81 & 0.71 R^2) with very high R^2 value. Another marker GSSR 98 was associated with four traits such as root length (0.34 R^2), root width (0.35 R^2), shoulder width (0.35 R^2) and xylem width (0.44 R^2). Among the biochemical traits, beta carotene was showed an association with BSSR76 and GSSR 97 with high R^2 value of 0.56 and 0.54.

Among the qualitative traits, GSSR111 (shoulder colour=0.34 R^2 and root texture=0.37 R^2) and OPK 9 (root hairiness = 0.42 R^2 and plant growth habit=0.47 R^2) were associated with two traits each. For cambium colour two markers (GSSR 16 and GSSR154), shoulder colour (GSSR111 and ESSR71) and root texture (GSSR111 and GSSR97) were associated with two markers each. Remaining three traits (Root hairiness, plant growth habit and root colour were associated with one marker each.

Table 18: Marker-Trait association by General linear model identified significantly associated markers for root traits (both quantitative and qualitative traits)

Trait	Marker	Marker F	Marker p	Perm p	markerR^2
Quantitative traits					
Trait	**Marker**	**marker F**	**Marker p**	**Perm p**	**markerR^2**
Harvest Index	BSSR94	4.06	0.00013	0.003	0.33
	GSSR97	2.55	0.00095	0.025	0.51
Petiole Length (cm)	ESSR59	3.20	0.00008	0.860	0.62
	GSSR19	2.81	0.00029	0.881	0.65
Root Length (cm)	GSSR19	6.53	0.00000	0.735	0.81
	ESSR59	5.13	0.00000	0.830	0.72
	GSSR98	3.46	0.00063	0.924	0.34
	GSSR6	2.98	0.00085	0.929	0.40
Root Width (cm)	GSSR98	3.73	0.00029	0.038	0.35
Shoulder Width (cm)	GSSR98	3.68	0.00033	0.011	0.35
Xylem Width (cm)	GSSR98	5.33	0.00000	0.001	0.44
β-Carotene	BSSR76	4.29	0.00000	0.003	0.56
	GSSR97	2.71	0.00049	0.112	0.54
Qualitative traits					
Cambium Colour	GSSR16	3.23	0.00088	0.501	0.34
	GSSR154	2.86	0.00042	0.366	0.49
Root Hairiness	OPK9	3.29	0.00015	0.002	0.42
Plant Growth Habit	OPK9	3.17	0.00023	0.007	0.47
Root Colour	GSSR153	3.79	0.00053	0.026	0.31
Shoulder Colour	GSSR111	3.11	0.00098	0.334	0.34
	ESSR71	3.39	0.00026	0.178	0.41
Root Texture	GSSR111	3.54	0.00025	0.008	0.37
	GSSR97	2.62	0.00071	0.037	0.60

Significance at marker_P<0.001

Finally, the 96 genotypes of the present study were subjected to various phenotypic and molecular profiling and also performed association analysis to identify the significantly linked markers. The present study is the first of its kind exploring the genotypes cultivated in India which comprised of Asiatic and European cultivars representing multiple coloured carrots. Greater diversify was seen for the morphological, biochemical and molecular markers. The identified superior genotypes in the present study suitable to tropical region will be selected for multi-location trials to assess the performance at different locations and the tropical suitable varieties will be released and also utilized as parents in hybridization program for combining one or few traits from these varieties into one. In this regard, identified molecular markers may also helps in assisting selection for the desirable traits of interest in this long durated biennial crop.

5. DISCUSSION

Crop improvement depends on the extent of genetic diversity in the gene pool. The knowledge of nature and extent of genetic variation available in the germplasm helps the breeders for planning sound breeding programs. The crosses involving diverse parents within compatible range could be done to obtain high heterotic expression or to recover desirable transgressive segregants in subsequent generations.

Considering the above facts, it is, therefore, essential to identify and classify the variability present among the genotypes based on various criteria such as morphology, geographical distribution and genetic diversity. In the present investigation, an attempt has been made to characterize 96 genotypes of carrot based on the proportion of variability, broad sense heritability, predicted genetic advance, GCV and PCV with respect important quantitative as well as the qualitative parameters. The associations among these characters have also been studied with Mahalanobis D^2 analysis and principal component analysis to assess genetic diversity among the genotypes and further focusing on the following population structure, estimating frequencies measures of molecular data, gene diversity and heterozygosity. Large set of molecular markers profiled in these 96 genotypes were further assessed for their association with the economic traits of carrot. The results presented in previous chapter are discussed in detail under the following broad sub headings:

5.1 Analysis for Phenotypic Variability and diversity

5.2 Analysis for Genetic/molecular diversity

5.6 Population STRUCTURE Analysis

5.7 Marker trait association

5.1 Analysis for Phenotypic Variability and diversity

Selection, is a basic tool to every breeding programme, operates only on variation which is of genetic nature (Johannsen, 1909) and the success would mainly depend upon the scientific management of this variability. Greater the diversity in germplasm that a breeder handles, better are the chances for selection of superior

genotypes (Vavilov, 1951). Most of the economic traits, which are of interest to plant breeders, are quantitative in nature and are highly influenced by the environment for their expression. According to Fisher (1918), these quantitative traits exhibiting continuous variation are under the control of both heritable and non-heritable factors. Response to selection however would depend upon the relative proportion of heritable portion of the continuous variation. Though it is difficult to assess the genotypes directly, but it is possible to do so through the assessment of phenotypic expression of the existing material. Thus, the study of phenotypic variability for yield and its associated traits is of utmost importance. Statistical analysis is the powerful tool which helps a breeder to select the important characters and important genotypes after assessing various parameters. Application of the experimental data to various statistical methods confirms the results. Hence, the phenotypic data obtained for 15 qualitative and 18 quantitative traits were subjected to various statistical analyses and discussed herein.

5.1.1 Frequency distribution for qualitative parameters

Study of qualitative traits such as shape, size colour is an important part o breeding activity as these qualitative traits add characteristic features like attractiveness, indirect improvement in the yield, consumer acceptance, nutritional quality *etc*. In the present investigation also, 96 genotypes of the carrot were subjected to 15 qualitative traits were recorded (Table 3) such as leaf type, root shape, root colour (internal and external), level of cracking, branching, hairiness and tapering in the roots etc. all these qualitative characters were recorded with the help of standard IPGRI descriptor (IPGRI, 1998) developed for carrot. As they were of non parametric traits, based on the scores, they could not be subjected to statistical analysis such as mean, range, SEm or correlation or other analyses as the main assumption for these type of analyses is the data must be quantitative in nature and must show continuous variation. Hence, these 15 qualitative parameters were subjected to frequency distribution graphs based on the frequency of various types of classes for respective traits from among the 96 genotypes and presented in Fig. 1.

Among the nature of plant habit, semi-erect types were more frequent than erect type or prostrate type. For top attachment with the carrot root, single type of attachment was more than multiple types. As the number of petioles (top) attachment to the root

increases, the metabolites will be more diverted to the petiole than for the development of the root and the quality of the root may be reduced. Among the leaf type, normal carrot type of leaves were more common in the population, however, fern and celery type of leaves were also observed in the present study mainly in the European type collections. Most of the Asiatic type collections were (temperate) were of either normal leaf or celery. The texture of the root is most important trait for the consumer acceptance and generally European types which grow under temperate condition have smooth textured than Asiatic types mainly grown under tropical or subtropical conditions. In the present study, course textured genotypes were more frequent than smooth or ridged or dimpled texture. The root texture although is a genetic trait, but highly influenced soil conditions and the climate in which the carrots are grown.

With respect to shoulder colour, since most of the genotypes did not show any shoulders especially in the temperate varieties, however in either local varieties or tropical germplasm collections, either green or pink or orange coloured shoulder was seen mostly which was in comparison with the carrot root colour. Moderate to higher percentage of hairiness, cracking, tapering was also observed in the present investigation as shown by the frequency distribution in the carrot germplasm panel.

Root colour (external and internal) is the most important qualitative parameter of carrot and most of the breeding efforts are mainly concentrated to improve the colour of the carrot for its orange colour. In the present investigation, maximum number of genotypes showed external colour as Orange, however the internal colour (xylem and cambium) mostly showed yellow colour more frequently than orange or red or pink etc. The phloem colour was mainly dark orange with few genotypes showing the yellow, red, orange or pink. In most of the genotypes of the selected population, pointed roots were seen than blunt types, cracking was more often than non-cracking due to hardy soil of the experimental site. Medium to deep position of root were seen in many of the genotypes.

5.1.2 Analysis of variance

The analysis of variance revealed that mean sum of square due to genotypes were significant for all the traits studied *viz.* five plants root weight/five plants, five plants vegetative weight, root length, root diameter, plant height, leaf length, number of

petioles per plant, days taken to marketable maturity, root width, shoulder width, xylem width, phloem width, harvest index, reducing sugars and carotene content, indicating considerable scope for improvement among genotypes for all these characters. These results are in consonance with the findings of Prasad and Prasad (1980), Brar and Sukhija (1981), Saini *et al.*(1981), Singh *et al*. (1987), Kalloo *et al.* (1991), Ahmed and Tanki (1992), Tewatia and Dudi (1999), Bhatia *et al.* (2002), Kaur *et al.* (2005), Verma and Gupta (2005), Gupta *et al.* (2006) and Yadav *et al.* (2009), who have also reported the existence of variability for various traits among the carrot genotypes.

5.1.3 Mean, Range and genetic variability parameters

Estimates of mean values for 96 genotypes with respect to different traits along with their ranges, standard errors, critical differences and coefficients of variation are given in Table.

In the present study, wider range of variation was available for almost all the quantitative traits studied indicating the presence of variation and scope for further improvement by breeding. Important root parameters are compared in the following subheadings

Root length

Genotypes differed significantly from each other with respect to root length (17.51 $\pm$ 0.56). The average root length for check1 (Ghataprabha local) was 15.36 cm, for check-2 (Pusa Vrishti) it was 14.33 cm and for the check-3 (Vigro Kuruda), the root length observed was 12.70 cm. Among the genotype panel, the range was 10.38 cm to as high as 59.34 cm. The highest root length of 59.34 cm was shown for UHSBC-96. More than fifty percent of the genotypes recorded significantly higher than all the selected checks *viz.* Ghataprabha and Vigro Kuruda, Pusa Vristi in the present study. In the remaining genotypes root lengths were intermediate. Wide range of variability observed for this trait in different genotypes of carrot in the present study is in broad agreement with the findings of Prasad and Prasad (1980), Brar and Sukhija (1981), Singh *et al*. (1987), Ahmed and Tanki (1992), Tewatia and Dudi (1999), Bhatia *et al.* (2002), Alves *et al.* (2006) and Yadav *et al.* (2009).

Root width (diameter) (mm)

The root diameter among the checks was highest for Ghataprabha local (21.82 mm) followed by Pusa Vrishti (18.48 mm). Among all the genotypes studied, UHSBC-24 recorded the highest root diameter (31.64 mm) followed by UHSBC-63 (25.04 mm). About eleven genotypes recorded significantly higher root diameters than all the check varieties. The lowest root diameter was observed in UHSBC-42 (11.56 mm). Many genotypes had intermediate root diameters. A significant variation for root diameter has also been reported by earlier workers (Prasad and Prasad1980; Saini *et al.* 1981; Singh *et al.* 1987; Tweatia *et al.* 1990; Tewatia and Dudi1999; Bhatia *et al*. 2002; Verma and Gupta 2005, and Gupta *et al.* 2006). Zdravkovic (1989) reported that root diameter is a varietal character, which is generally more in hybrids than in cultivar. In contrast, in the present investigation, one hybrid (UHSBC-98) used was showing lower root width of 13.87 mm, may be because of the non-adaptability of this hybrid to the tropical climates.

Petiole length (cm)

The average petiole length of the population was 13.70 cm with maximum length in UHSBC-100 (50.50 cm) and minimum in UHSBC-51 (6.50 cm). Among the checks, Ghataprabha (16.17 cm) had significantly longer leaves than Pusa Vristi (10.17 cm) and Vigro Kuruda (10.62 cm). Many genotypes in the population showed significantly longer leaves than the check varieties. These results are in close conformity to those of Brar and Sukhija (1980), Singh *et al.* (1987), Tweatia *et al.* (1990), Ahmed and Tanki (1992), Tewatia and Dudi (1999), Bhatia *et al.*(2002) and Gupta *et al.* (2006), who have also observed wide variation for leaf length in carrot genotypes.

Number of petioles per plant

The analysis of variance revealed highly significant differences for this trait. Mean no of petioles was approximately 11.0 (10.80) and it ranged from 6.60 to 28.80 with a population mean of 8.83.The highest number of petioles per plant was recorded in UHSBC-26 (28.80). Many genotypes showed significantly higher than rest of the check varieties. Pusa Vrishti had more number of leaves than Ghataprabha local and

Vigro Kuruda.These finding are inconsonance with those of earlier workers (Prasad and Prasad 1980; Brar and Sukhija 1981; Singh *et al.* 1987; Tewatia and Dudi 1999; Bhatia *et al.* 2002 and Alves *et al*. 2006), who have also reported significant differences in their breeding material for number of leaves per plant.

Root Yield parameters

Single plant yield, five plant weight and harvest index were recorded for each of the genotypes and checks in the augmented block design. The highest root weight/plant was recorded by Ghataprabha local (53.53g) followed by Pusa Vrishti (34.47 g) and Vigro Kuruda (27.30g). Among the genotypes tested, the highest root weight was recorded with 97.60g by the genotype UHSBC-71. The average root weight was ranging from 16.00 to 97.60 g. the trend was same for other root yield parameters like five plants root weight, root width, xylem and phloem width.

5.1.3 Genetic parameters of variability

The estimates of genetic parameters of variability *viz.* phenotypic, genotypic and environmental variances, phenotypic, genotypic and environmental coefficients of variation (PCV, GCV and ECV, respectively) along with heritability (h^2) in broad sense and genetic advance (GA) as percentage of mean for different traits are given in Table 7. The results and discussion pertaining to these parameters are briefly presented below.

Absolute variability in different characters cannot be considered as a critical factor for deciding as to which character is showing the highest degree of variability. The relative values of phenotypic and genotypic coefficients of variation, therefore, give an idea about the magnitude of variability present in a population since the estimate of genotypic variability, heritability and expected genetic advance are useful for crop improvement and the above values were estimated to know the scope of improvement in the carrot varieties. The measure of genotypic co-efficient of variation is necessary to understand the role of environmental influence on different traits. In the present investigation, the genotypes exhibited considerable amount of variability for all the eighteen quantitative traits studied.

The phenotypic co-efficient of variation was slightly higher than genotypic co-efficient of variation for all the characters indicating the presence of environmental influences to some extent in the expression of the characters. Moderate to higher GCV and PCV was observed in most of the characters except TSS content and reducing sugars where GCV was lower although PCV was moderate indicating the role of environment/soil conditions/growth conditions on the expression of these traits. Yield parameters like single plant root weight and five plants root weight, vegetative weight and harvest index showed higher GCV as well as PCV indicating the existence of greater variation among these characters in the genotype panel selected for the present investigation. Similar results were also found by Tewatia and Dudi (1999) in carrot, Rabbani *et al.* (1998) in radish and Rahman *et al.* (2003) in tomato.

Heritability and genetic advance

Genotypic co-efficient of variation does not give an idea of total variation that is heritable. Further, it may not be feasible to determine the amount of heritable variation and the relative degree to which a character is transmitted from parent to offspring, by the estimate of heritability. Heritability estimate in broad sense alone, does not serve as the true indicator of genetic potentiality of the genotype since the scope is restricted by their interaction with environment. Hence, it is advisable to consider the predicted genetic advance as per cent of mean along with heritability estimate as a reliable tool in selection programme (Johnson *et al.*, 1955). Hence, both heritability and genetic advance as per cent of mean are determined to get a clear picture of the scope of improvement in various characters through selection. The extent of heritability >60.0% (>0.60) is considered to be high and in the present investigation, few traits especially the plant morphological traits (except xylem width and five plants root weight) showed very high heritability. Although for the biochemical components, higher heritability is expected, but in the present investigation, these traits showed moderate heritability as depicted by their moderate PCV but lower GCV indicating that, the expression of these traits are highly influenced by temperature, soil conditions as well as other environmental factors. Among the 18 traits studied, except beta carotene content, reducing sugars and the root width, almost all the traits showed higher GAM, where as in the farmer mentioned traits, the GAM was lower.

In the present study, high heritability was observed for harvest index, number of petioles, petiole length, vegetative weight, root length and root diameter. High heritability in broad sense indicated that large proportion of phenotypic variance was attributable to the genotypic variance and that these character differences among the genotypes were real and showed that the abovementioned traits with high heritability values were less influenced by the environment. The above findings are in close association with those of Brar and Sukhija (1981) and Tewatia and Dudi (1999) who reported high heritability for leaf length and root weight. High heritability for the characters controlled by polygenes might be useful to plant breeder for making effective selection.

Genetic advance expressed as percentage of mean was relatively high for vegetative weight, harvest index, number of petioles and petiole length.Low heritability was observed for root length. Genetic advance expressed as percentage of mean was relatively low for the characters *viz.*, plant height, root length and inner core (xylem) diameter. The results are in line with the findings of Amin and Singla (2010) and Ullah *et al* (2010), Yadav *et al*. (2009) for root length. Since genetic coefficient of variability, phenotypic co-efficient of variability and heritability estimates determine the component of heritable variation and genetic advance measures the extent of its suitability under selection, all these parameters should be considered simultaneously so as to bring effective improvement in yield and other characters.

High heritability coupled with high genetic advance indicates the role of additive gene action (Panse 1957) and consequently a high genetic gain is expected from selection under such situation. Hence, the traits like harvest index, number of petioles, petiole length, vegetative weight which exhibited high heritability coupled with high to moderate genetic advance, are likely to respond better to selection. Shoulder width, phloem width, root weight had moderate heritability associated with moderate genetic advance indicating thereby, that the selection based on phenotypic performance could be effective for the improvement of these traits. Days to maturity, plant height, root length and root diameter had moderate heritability associated with low genetic advance, suggesting thereby that inheritance of these traits was controlled by epistatic interaction. Moderate heritability coupled with low genetic advance for root length and root diameter have also been reported by Brar and Sukhija (1981) and Saini *et al.* (1981),

respectively, which are same as the present findings. Low heritability in combination with low genetic advance was observed for total soluble solids, beta carotenoid, reducing sugars, these characters are more under the influence of non-additive gene action and environment and do not respond to selection.

5.1.4 Principal component analysis

Principal component analysis (PCA), basically a data reduction technique, initially floated by Pearson (1901) and later developed by Hotelling (1933) offers solution to the complex problem of large and unmanageable data by transforming the original set of variables into a smaller set of linear combinations that account for most of the variability of the original set. It is a useful tool for evaluation of germplasm. This technique is an ordination method often used to simultaneously describe the relationships between sets of variables. It tends to reduce the dimension of multivariate data by removing inter-correlation among variables and allows a multi-dimensional relationship to be plotted on two or three principal axes. As a result, PCA allows visualization of the differences among the individuals, identification of possible groups and finding relationships among individuals and variables. The first principal component absorbs and accounts for maximum proportion of total variability in the set of all variables and remaining components account for progressively lesser and lesser amount of variation. Same trend was observed in the present study. The six principal components, having Eigen values greater than one altogether explained77.71% of the total variation and were retained for further studies. The first principal component explained 32.71% of the total variation. The remaining six principal components explained respectively 17.73, 7.66, 6.86, 6.50, and 6.21 % of the total variance.

Characters which showed association in PC1 were vegetative weight, five plants vegetative weight, number of petioles, root width, plant height, five plants root weight accounting 32.71% of total variation. PC2 with harvest index, root weight, root width, phloem width were accounting for 17.73 % of total variation. With PC3, plant height, petiole length, shoulder width, xylem width,PC4 extracted was a combination of shoot length, root length and reducing sugar, PC5 consists of days to maturity, TSS and beta carotenoid. In the sixth PC, combination of beta carotenoid and reducing sugar were present.

In conclusion, indirect selection via traits having higher heritability relative to root yield especially in early generations and strongly associated traits such as root length, root width, xylem and phloem width with higher root yield is emphasized in this study for genetic improvement of carrots. Ramesh *et al.,* 2011 have reported similar results for breeding these important traits in carrot while studying the principle components involved in European germplasm characterization.

5.1.5 Correlation studies

Selection primarily based on better quality, high yield and high productivity which is mainly polygenic characters, hence, the direct improvement of these traits is cumbersome. To overcome this bottleneck, manipulation of yield contributing characters through efficient selection programs has been considered desirable. As several characters are of interest to a breeder, it is necessary to know the concurrent change that would result in the unselected economic characters when selection pressure is applied for the improvement of certain other traits. For this purpose, it is beneficial to know the inter-relationship amongst the various economically important traits.

This consideration becomes still imperfective when one visualizes yield (root weight) and quality traits (biochemical traits) as a complex trait and product of the roots will tend to reduce the marketable root yield (root weight) per plot. However, Bhatia *et al.* (2002) and Gupta and Verma (2007) reported positive correlation between these traits. This may be attributed to the differences in the genotypes included and environmental conditions especially the soils, under which the present study was conducted. Quality traits did not show any significant correlation with root weight, root length, root diameter, phloem width or xylem width at phenotypic level, though the association of plant height with total soluble solids was positive and fairly high and it approached the significance level at genotypic level for days to maturity indicating that TSS accumulates more at the later stages of plant growth. These results are in conformity with the findings of Sukhija and Nandpuri (1983) and Singh *et al.* (1989). With respect to the economic yield such as root weight/plant and root weight/five plants, they both were influenced by almost all the plant morphological characters as depicted by their strong correlation except with shoot length, days to maturity, harvest index and biochemical parameters.

5.1.6 D^2 Analysis

Genetic divergence was determined using Mahalanobis D^2statistics (Mahalanobis, 1936). Group constellation was formulated after computation of D^2values as suggested by Rao (1952).

Genetic diversity existing within and between groups of germplasm is important, and particularly, useful in proper choice of parents for realizing higher heterosis and obtaining useful recombinants. D^2 statistic is a useful tool for estimating the genetic divergence in plant breeding experiments. Based on the divergence study, 96 carrot genotypes involved in the present study were grouped into 12 clusters. Clustering pattern revealed the presence of considerable amount of genetic diversity in this material. In general, intra-cluster distances were relatively lower than inter cluster distances showing that genotypes included within a cluster were genetically less diverse than the genotypes included in different clusters. Cluster pattern revealed that cluster I was the largest one with 85 different genotypes and remaining other formed solitary clusters. Genotypes from different sources were grouped in the same cluster thereby, indicating that geographical diversity does not necessarily represent genetic diversity. These findings suggested that the pattern of clustering was independent to their geographical origin. The same findings of distribution of genotypes into different groups are independent to place of collection. This implied that genetic material from same geographical region may provide substantial diversity. These finding are in conformity with those of earlier workers Asima *et al.*, 2010, and Ramesh *et.al.*, 2014who have also reported significant differences in their breeding material for genetic diversity.

The inter cluster distance was found to be highest between cluster XII and X (D^2 value =671114.10), followed by cluster XII and I (D^2 value=456310.80), cluster XI and X (D^2 value =411913.10) indicating wide diversity between these two clusters, while the minimum inter-cluster distance with D^2 value of 3256.20 was observed between cluster V and III followed by cluster VI and II with D^2 value 3331.50 indicating their close relationship.

The genotypes collected and utilized in the present study consisting of larger genetic variation with broad genetic background including local cultivars, IIVR

germplasm collections which were least explored in earlier carrot breeding programs Germplasm panel also consisted of released varieties suitable to tropical and temperate conditions, Ooty, Kodaikenal (Karnataka), Mukteshwar (Sub-Himalayan Region) and other temperate climates of India. Despite this larger genetic background most of the genotypes (85) belonged to one cluster (1st) and remaining eleven genotypes diversified as eleven solitary clusters. Most of the genotypes in these solitary clusters are either IIVR collections or the local varieties indicating that these unexplored germplasm lines are highly diverse in nature and could be utilized in future breeding program concentrating on tropical region. One cluster consisted of hybrid Kuruda (Cluster-10) and the other released variety (Pusa Payasa).

It is also indicated that forces other than eco-geographical differentiation such as natural and human selection pressure would exert considerable influence on the genetic divergence. But to get more heterotic F1's and large number of desirable transgressive segergants, selection of parents for hybridization should be properly based on genetic diversity rather than geographic diversity. An effective hybridization program may be initiated involving the genotypes belonging to diverse clusters with high mean for almost all component characters. The cluster means for all the characters revealed considerable differences among them for most of the characters studied.

5.2 Analysis for Genetic/molecular diversity

5.2.1 Genetic diversity parameters

Diversity studies are very important (Rao and Hodgkin, 2002; Zeb *et al*., 2009). Present work was thus, carried out to study the genetic diversity and population structure among 96carrot genotypes, so that they can be utilized effectively by breeders in selection of diverse parents for future crossing programs. SSR markers were utilized in the study considering their high polymorphism, specificity (Pestova *et al*., 2000), reproducibility (Stachel *et al.*, 2000) and high variability (Brown *et al*., 1996). Few indels, SCARs were also utilized in the present study, which were specifically developed for carrot. Polymorphism of simple sequence repeats (SSR) is a marker system extensively used in the assessment of genetic diversity in many plant species (Kalia *et al*., 2011). Recently, new SSRs were identified in carrot (Niemann, 2001;

Cavagnaro *et al*., 2011) and were used for the evaluation of carrot genetic resources (Baranski *et al*., 2012).

Considering their high level of polymorphism, the chosen primers were very informative therefore SSR markers are utilized in present study. In the present study 42 SSR markers were used to estimate the genetic diversity among 96 carrot accessions collected from different parts of India. After evaluation of diversity statistics for all 96 individual genotypes, molecular markers profiling detected 471 alleles across 96 genotypes from 42 loci. The PIC values of the markers used from the enriched library ranged from 0.0782 to 0.927. There were many most informative markers which are listed in Table15. There seemed to be no strong correlation between the PIC value and the repeat motif or repeat length with these markers. The overall number of alleles per SSR locus across 96 individuals ranged from 2 to 24. The observed heterozygosity (Ho) in the total sample was 0.14, which deviated from the expected heterozygosity (He) (0.67). Molecular evidence for the existence of two carrot gene pools was postulated by Clotault *et al.* (2010), who screened 47 accessions. That was further extended and supported by SSR analyses of a larger collections of 88 accessions (Baranski *et al*., 2012) as well as by DArT (Grzebelus *et al*., 2013) and SNP analyses (Iorizzo *et al*., 2013). The genetic parameters clearly showed the diversity at the locus and allelic level.

5.2.2 Diversity measurements

Unrooted neighbor joining tree constructed using DARwin 5.0 broadly divided 96 genotypes into three major groups. Although both the 1st (48) and 2nd (47) clusters almost carried equal number of genotypes, the third cluster had only one accession (UHSBC-69), that may be a forced separation because in our present study only 42 polymorphic markers were used which do not cover whole genome. Hence, the diversity assessment for that particular accession need still more markers for assigning it to any one cluster. This minor group accession was found to be a admixture as revealed clearly by STRUCTURE (49th accession in the 1st cluster) as shown in structure figure 5.

Population of 96 genotypes were grouped in to 3 main clusters but only one genotype was separated as 3rd cluster (UHSBC-69) which belongs to Ooty collection, New Kuruda. The divergence of this single genotype as solitary cluster need to be further tested with new set of molecular markers. In the first cluster, there were two sub

cluster (sub cluster-I and sub cluster-II) with 26 and 22 genotypes respectively and in the second cluster there were again 2 sub clusters (I, II) with 30 and 17 genotypes respectively. The genotypes in the I-sub cluster of cluster-I was mainly of local cultivars, and also the temperate varieties collected from Tamilanadu. In the second sub cluster of I cluster, the genotypes were few IIVR collections and IARI released varieties. In the second main cluster (Cluster-II), both sub cluster I and II consisted mainly of IIVR collections with few local cultivars of Karnataka. Diversity analysis could not show any clear cut divergence of these genotypes based on the geographical origin or the colours. Maksylewicz and Baranski 2013, analysed 9–20 individuals per population to verify the previous assignments and to quantify genetic intra-population diversity in carrot. Their reports enabled us to see the diversity in Indian continent carrot and to see whether they belong to western gene pool or Asian gene pool.

5.3 Population STRUCTURE analysis

Population structure of 96 accessions was estimated using STRUCTURE *ver* 2.3.3 software. The number of subpopulations (K) was identified based on maximum likelihood and delta K (ΔK) values. The value of LnP (D) increased from 1 to 10, but showed a knee at a value of two which implied that there may be two subpopulations. Maximum percentage of cultivars was found in sub-population P1 with about 41.66 % (40) genotypes. It was followed by P2 with 42.71 % (41) and admixtures were 15.62% (15) number of genotypes in them. The number of admixtures in the population indicates the possibility of another cluster in the population which need to be confirmed further by screening with few more markers.

Analysis of molecular data was carried out using AMOVA and Bayesian statistics. The grouping obtained through UPGMA unrooted neighbour joining tree was found to be comparable to the Bayesian clusters obtained through STRUCTURE analysis. Since, the genotypes belong only to Indian sub-continent, clear distinction cannot be made about the distribution of genotypes in different groups on the basis of their region. There were 3 clusters in UPGMA unrooted neighbour joining tree and two populations present in STRUCTURE with the admixtures. When these three are compared distance-based cluster analysis is in accordance with the results obtained using STRUCTURE and AMOVA, with no clear geographical isolation of the

population studied, which is similar to previous reports using SSR markers in the carrot study (Irorizzo *et al.,* 2013). Ganesan *et al.,* 2014 revealed the same while working with genetic diversity and population structure study of drumstick (*Moringa oleifera* L.). Similar results were also found in accordance with Rajalakshmi *et al*., 2017 who screened 20 SSR markers in the 97 *Moringa* accessions.

The type of clustering pattern observed in the present study can be related to the spread of planting material and rate of gene flow, which is associated with the location of sample collection, that are in close proximity to each other. Similar observation had been reported in several other plant species by Singh *et. al*., 2014, while working with molecular diversity and SSR transferability studies in Vetiver grass.

On the other side, clustering of individuals from the same population in different clusters indicates high genetic variation within population which may be attributed to the use of seed sources, mutation or breeding system which is in agreement with the fact that it is predominantly an out-crossed plant.

5.3.1 Analysis of molecular variance (AMOVA)

Analysis of molecular variance in the entire population set showed a maximum of 71% variance within the population and 13% among the population. However, a high level of genetic divergence was observed within population indicating the existence of sufficient variation within the germplasm pool although less number of clusters are formed. This could be due to the sharing of allelic diversity between the genotypes of the carrot due to out crossing nature of carrot. The organization of genetic variation in carrot populations was examined using the F-statistics. These statistics have as a common focal point the fixation index, F, which represents the deviation from Hardy-Weinberg proportions due to the combined effects of finite population size, selection, inbreeding and other forces shaping the genetic makeup of the population.

F-statistics showed a mean *Fit* value of 0.842 (within individuals) indicating a high amount of genetic differentiation among accessions, with a *Fis* value (0.818) representing heterozygote excess or out-breeding of population which could be related to various factors such as genetic drift, gene flow, selection and mating system. The positive values of inbreeding coefficients and lower values of observed heterozygosity

than expected heterozygosity, indicate excess of alleles in the homozygous state. The excess of homozygous loci resulted most likely from two years sib pollination and repeated selfing during breeding programs before utilizing this material in the present investigation. The results are similar with Maksylewicz and Baranski 2013 in carrot.

The mean number of private alleles in population-I was found to be more (4.48) than population-II (2.19) indicating allelic richness in the 1st population. The results obtained support earlier opinion that cultivated carrot is largely genetically non-structured (Bradeen *et al*. (2002)). Carrot being an out-crossing species shows less intense population differentiation and more uniform distribution of genetic diversity than inbreeding species (Brown, 1989). High intra-population variation was observed in other allogamous species, too. The use of 56 ESSR and 4 GSSR markers allowed discrimination of 89 inbred and open pollinated bulb onion populations according to their heterozygosity, which was higher for landraces and cultivars in comparison to inbreed lines (McCallum *et al.*, 2008). They also differentiated accessions originating from temperate climate regions from those typical for tropical regions (McCallum *et al.*, 2008). An evaluation of intra-population diversity indicates that plant to plant variation in carrot is substantial and might be a source of different alleles even if phenotype variability is small as suggested by Simon *et al*. (2008). In the present study also, the intra population genetic diversity was high (71.0%) as depicted by AMOVA indicating the existence of more diversity within the population. However, only 2 populations from either diversity or structure analysis with only 42 markers indicates that few more loci need to be covered with many more molecular markers to get the true structure of the populations by localizing each genotype to the respective population.

High genetic variation may also have substantial impact on preservation strategies carried out in gene bank collections as they are supposed to preserve both common and rare alleles (Bradeen & Simon, 2007). The number of individuals required for the replenishment of seedstock of an accession partly depends on the variability of the accession (Le Clerc *et al.*, 2005). The higher homogeneity the fewer parental plants are required to maintain the allelic diversity (Gilbert *et al*., 1999). Conservation of rare alleles requires, however, larger mating population, particularly in allogamous species like carrot (Le Clerc *etal*., 2003). Thus, information on genetic diversity helps curators to rationalize the use of resources required forseed reproduction and to preserve allelic

variants (Gilbert *et al*., 1999). Our results indicated that carrot population genetic diversity varies depending on the accession, thus, identification and quantification of rare and private alleles in populations is of high importance for preservation strategy. Higher number of plants may be necessary to maintain gene diversity.

5.4 Marker-Trait Association Analysis by Generalized Linear Model

As an alternative to linkage mapping, association mapping strategy measures the correlation between genotypic and phenotypic variation based on the strength of the linkage disequilibrium (LD) across broad spectrum of germplasm lines. Number of studies have been conducted in various crops to identify the marker-traits association based on association mapping analysis. In the present study, 96 genotypes across 35 traits (including qualitative and quantitative traits) were subjected to marker-trait association analysis with the help of 42 various types of molecular markers using TASSEL 3.0 following GLM model. The analysis was performed by taking 42 markers genotypic data, 18 quantitative traits phenotypic data and population structure output file of Q values at K=2. The marker and the trait was declared as significant at P value <0.001. Since only two populations were obtained and hence only General linear model (GLM) was followed.

Out of 18 quantitative traits, only for seven traits, significant marker trait association was obtained from the present study. Among 15 qualitative parameters, size traits showed significant marker trait association with very high R^2 value.

Among the quantitative traits, Harvest index was associated with two markers (BSSR94 and GSSR 97) with the R^2 value of 0.33 and 0.51 respectively. GSSR 19 and ESSR59 was linked with two traits, petiole length (0.62 & 0.65 R^2) and root length (0.81 & 0.71 R^2) with very high R^2 value. Another marker GSSR 98 was associated with four traits such as root length (0.34 R^2), root width (0.35 R^2), shoulder width (0.35 R^2) and xylem width (0.44 R^2). Among the biochemical traits, beta carotene was showed an association with BSSR76 and GSSR 97 with high R^2 value of 0.56 and 0.54.

Among the qualitative traits, GSSR111 (shoulder colour=0.34 R^2 and root texture=0.37 R^2) and OPK 9 (root hairiness = 0.42 R^2 and plant growth habit=0.47 R^2) were associated with two traits each. For cambium colour two markers (GSSR 16 and

GSSR154), shoulder colour (GSSR111 and ESSR71) and root texture (GSSR111 and GSSR97) were associated with two markers each. Remaining three traits (Root hairiness, plant growth habit and root colour were associated with one marker each.

Finally, the 96 genotypes of the present study were subjected to various phenotypic and molecular profiling and also performed association analysis to identify the significantly linked markers. The present study is the first of its kind exploring the genotypes cultivated in India which comprised of Asiatic and European cultivars representing multiple colored carrots. Greater diversify was seen for the morphological, biochemical and molecular markers among the selected accessions.

Few significant molecular markers were associated with the root traits in the present study and these markers need to be further validated in a larger germplasm pool so that, these significantly associated markers could be further mapped and used in marker assisted breeding programs. The superior genotypes identified in the present study will be further evaluated in the multi-locations representing tropical conditions to release a variety suitable to respective regions.

6. SUMMARY AND CONCLUSIONS

The present investigation was carried out on "Genetic Diversity, Population Structure and marker-trait association for root traits by Association analysis in carrot (*Daucus carota* L.)" to assess the nature and magnitude of genetic variability and diversity present among various accessions of carrot at their genomic level for different horticultural as well as quality traits. The study also involved understanding of correlation and their causation among these traits. Ninety six genotypes of tropical/temperate carrot genotypes were sown in augmented block design with three replications at the experimental farm of College of Horticulture, Bagalkot during August-December, 2016. Observations were recorded on the 15 qualitative traits and 18 quantitative traits. The data were analysed as per standard statistical procedures.

In order to make the best utilization of genetic potential of genotypes for improvement of traits and for adaptation to various stress conditions, genetic study is very crucial. In this investigation, the level of diversity present in the carrot genotypes distributed across different agro-climatic regions of India is studied. Continuous genetic diversity assessment will help to maintain the diverse species for conservation and crop improvement. The results can also help the breeders so that they can effectively select the parents leading to progenies with high differentiation among them.

Analysis of variance revealed significant differences among the genotypes for all the traits studied indicating the existence of sufficient variation within the germplasm pool.

Study of qualitative traits such as shape, size colour is an important part of breeding activity, as these qualitative traits add characteristic features like attractiveness, indirect improvement in the yield, consumer acceptance, nutritional quality *etc*. In the present investigation also, 96 genotypes of the carrot were subjected to 15 qualitative traits such as leaf type, root shape, root colour (internal and external), level of cracking, branching, hairiness and tapering in the roots *etc* which were recorded with the help of standard IPGRI descriptor (IPGRI, 1998) developed for carrot. In the present investigation, maximum number of genotypes showed external colour as orange, however the internal colour (xylem and cambium) mostly showed yellow colour more

frequently than orange or red or pink or purple. The phloem colour was mainly dark orange with few genotypes showing the yellow, red, orange or pink. In most of the genotypes of the selected population, pointed roots were seen than blunt types, cracking was more often than non-cracking due to hardy soil of the experimental site representing the tropical region. Medium to deep position of root were seen in many of the genotypes.

The measure of genotypic co-efficient of variation is necessary to understand the role of environmental influence on different traits. In the present investigation, the genotypes exhibited considerable amount of variability for all the eighteen quantitative traits studied. The phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) were high for root weight, vegetative weight, and harvest index, TSS, carotenoid content. Sufficient variability existed in the material under study, which could be exploited either through selection or hybridization.

Genotypic co-efficient of variation do not give an idea of total variation that is heritable. Further, it may not be feasible to determine the amount of heritable variation and the relative degree to which a character is transmitted from parent to offspring, by the estimate of heritability. In the present study, high heritability was observed for harvest index, number of petioles, petiole length, vegetative weight, root length and root diameter indicating the effectiveness of selection for these traits.

Association studies revealed positive and significant correlations with respect to the economic yield such as root weight/plant and root weight/five plants. Both of these traits were influenced by almost all the plant morphological characters as depicted by their strong correlation except with shoot length, days to maturity, harvest index and biochemical parameters. Strong association of these traits revealed that the selection based on these traits would ultimately improve root yield and it is also suggested that hybridization of genotypes possessing combination of above characters is most useful for obtaining desirable high yielding genotypes.

Using Mahalanobis D^2 statistics, 96 genotypes were grouped into 12 clusters with cluster I having the highest number of genotypes (85), but remaining were solitary clusters. The results indicated that the distribution pattern of genotypes in different clusters was random and there was little association of genetic divergence with place of

origin of genotypes. The intra-cluster distance was recorded highest for cluster I as it contained 85 genotypes, which indicates the existence of maximum variability within this cluster. For remaining all other clusters the intra-cluster distance value was recorded zero as they were solitary clusters. Inter-cluster distance was maximum between cluster XII and X which indicates that the genotypes included in these clusters are genetically diverse and would be utilized in future breeding program. So, it is desirable to select accessions from the clusters having high inter-cluster distance in the recombination breeding programs. The minimum inter-cluster distance was observed between cluster V and III indicating their narrow diversity.

Principle component analysis is one of the powerful methods which reduce the number of variables into principle components based on the extent of variance of each variable. In the present study, the first six principal components had Eigen values greater than one and altogether explained 77.71% of total accumulated variability. The first principal component explained the maximum variance of 32.71% of the total variation. The remaining five principal components explained 17.73, 7.66, 6.86, 6.50, and 6.21 % of the total variance, respectively.

The cluster analysis by principal component analysis and Mahalanobis D^2 statistics for various morphological traits which were divided the accessions into different groups indicated the diversity among the investigated accessions.

Various types of molecular markers were utilized in the present investigation to screen these 96 germplasm lines of carrot collected all over the country. The overall number of alleles per SSR locus across 96 individuals ranged from 2 to 24. The observed heterozygosity (Ho) in the total sample was 0.14, which deviated from the expected heterozygosity (He) (0.67). Few markers were highly informative based on the Shannon's Informativeness (I) where in, the I value was >2.5 for markers such as ESSR59, ESSR61, GSSR 154, GSSR19, GSSR54, GSSR97 and OPK 9. Few markers showed maximum number of different alleles with >20 alleles for the markers such as BSSR76, ESSR59, ESSR61 GSSR154, GSSR19. This indicates that the gene diversity and allelic diversity is of good amount in the existing population.

Both diversity and population structure revealed only different clusters in the population despite greater genetic diversity seen within each population as shown by

AMOVA. This indicates that the markers need to be further increased to get the true clusters for the present population. Also, the population need to be increased with few more genotypes from the carrot germplasm pool. However, being a highly cross pollinated species like carrot, higher gene flow and migration of genes among the accessions could be another cause of lower diversity and population structure among the genotypes of the present investigation.

Unrooted neighbor joining tree constructed using DARwin 5.0 broadly divided 96 genotypes into three major groups. Although both the 1st (48) and 2nd (47) clusters almost carried equal number of genotypes, the third cluster had only one accession (UHSBC-69), that may be a forced separation because in the present study only 42 polymorphic markers are used which do not cover whole genome. The value of LnP (D) increased from 1 to 10, but showed a knee at a value of two which implied that there may be two subpopulations. Maximum percentage of cultivars was found in sub-population P1 with about 41.66 % (40) genotypes. It was followed by P2 with 42.71 % (41) and admixtures were 15.62% (15) number of genotypes in them. The number of admixtures in the population indicates the possibility of another cluster in the population which need to be confirmed further by screening with few more markers.

Although genotypes were collected from various parts of the country, with broad genetic background such as local varieties, IIVR collections, temperate varieties etc, but the diversity as population structure analysis could not separate these genotypes in to various clusters based on the geographical origin indicating the flow of genes due to its highly out crossing nature.

From the marker-trait association analysis, the present study could detect significant markers for important quantitative and qualitative traits with highly significant R^2 value as discussed in the earlier chapters. Few markers were showing association with more than one traits such as GSSR19 (Root length and Petiole length), GSSR 98 (root width, shoulder width and xylem width), GSSR111 (shoulder colour, root texture) and OPK 9 (root hairiness and plant growth habit). These markers would be the markers of choice if validated and confirmed in the mapping populations. The present study on marker traits association for root morphological traits with these

markers is the first of its kind in carrot especially in the carrot germplasm collected from the Indian continent.

Based on the results of the present investigation with the extensive information on the carrot genotypes both phenotypically and genotypically, the following future directions are put forth considering the breeders, farmers and consumers of carrot.

- 10-15 superior genotypes identified in the present investigation will be subjected to multi-location evaluation concentrating the tropical region.
- The population will be further increased with few more genotypes for further association mapping and Linkage disequilibrium analysis.
- The markers identified in the present investigation need to be confirmed in the mapping population contrasting for the respective traits before utilizing them in the marker-assisted breeding program.
- The superior genotypes identified in the present investigation are being used in the marker assisted recurrent selection program for development of superior varieties suitable to tropical region.
- Multiple coloured carrot genotypes evaluated in the present study are being further characterized for their nutritional traits, metabolites and other horticulturally important traits.

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GENETIC DIVERSITY, POPULATION STRUCTURE AND MARKER-TRAIT ASSOCIATION FOR ROOT TRAITS BY ASSOCIATION ANALYSIS IN CARROT (*Daucus carota* L.)

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ABSTRACT

A Research work was conducted at University of horticultural Sciences, Bagalkot during 2016-17 for 96 diverse genotypes of carrot in order to study diversity, population structure and the marker-trait association for the economic root traits. An Augmented block design comprising of 3 checks (Ghataprabha Local, Vigro Kuruda and Pusa Vrishti) having 6 blocks was utilized to screen these 96 genotypes comprising of European and Asiatic types with diverse colors. 33 root morphological traits (18 quantitative and 15 qualitative) and 42 various molecular markers (SCAR, Indels, EST-SSRs and G-SSRs) were studied.

Significant variation for all quantitative traits was observed based on ANOVA and variability estimates (GCV and PCV) indicated sufficient variability in the population. Higher heritability for harvest index, number of petioles, petiole length, vegetative weight, root length and root diameter indicated their effective phenotypic selection in breeding. Distinct classes were present for all 15 qualitative traits from frequency distribution.

PCA exhibited six principal components explaining 77.71 % of the total variation for all 18 quantiative characters. D^2 study classified genotypes into twelve major clusters wherein, I-Cluster contained 85 genotypes and remaining were solitary clusters. Diversity contribution of the root weight was maximum indicating the scope for selection of diverse parents for this trait. Correlation analysis indicated positive correlation of root weight with almost all the plant morphological characters.

Genotyping of 42 polymorphic markers for 96 genotypes revealed 471 alleles with PIC value ranging from 0.0782(Y-Indel) to 0.928 (ESSR59) with average of 2 to 28 alleles per locus. Unrooted Neigbour joining tree from DARWIN showed three diverse groups but a single genotype in 3rd cluster. STRCTURE analysis also revealed 2 populations. Greater genetic variation was observed within the individuals (71%) than among the populations (13%) as shown by AMOVA indicating the possible gene flow among the genotypes of the populations. Marker-trait analysis by TASSEL identified significant markers for 13 phenotypic traits with the R^2 value ranged from 0.31 (root colour-GSSR153) to 0.81 (root length-GSSR91) with the P value of <0.0001.

Present study identified 15 superior genotypes suitable to tropical conditions and the markers identified would be further confirmed and validated for their exploration in carrot crop improvement.

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96 ««z §tz Udj v½U½U 42 «©£v ¸Æa¸Äª ««z §UAiÄ ªiÁPðgU¼£Ä §¼¹ £ÆÃrzÁU 471 «©£ C°¯ïU½gÄªz£Ä vÆÃj¹zª. ¦.L.¹ ªiË®¦ 0.0782 (ª¦EAq¯ï) ¤Az 0.928 (EJ¸ïJ¸ïDgï59)g ªgU zÁR¯ÁzªÀ. ¥æ¯ÆÃP¸ïU C°¯ï ¸AS¦AiÄÄ 2 jAz 28 gµÄ ªÁ¦¹z¦ª. qÁ«ð£ï ¸Á¥ªÃgï MlÄ 96 v½U¼£Ä 3 «¨ÁUU¼ÁV «¨f¹vÄ. ¸¦Pgï 2 UÄA¥ÄU¼£Ä ga¹vÄ. CªÆÃªÁ CzÀiÄ£¢Az «¨ÁUU¼ ªÄz¦z «©£vVAv (13%) Cw ºa£ «©£vAiÄ£Ä ¥ÆÃP v½U¼° (71%) UªÄ¤¸¯Á¬ÄvÄ. mÁ¸¯ï ¸Á¥ªÃgï ªÄÆ®P ªiÁPðg-v½U¼ UÄt®Pt «±Ãµu CzÀiÄ£ª MlÄ 13 v½ ¸gÆ¦ UÄtU½U Cxð¥ÆtðªÁz Dgï² ªiË®¦ 0.31 (UdjAiÄ §t fJ¸ïJ¸ïDgï153)¤Az 0.81 (UdjAiÄ Gz¦ fJ¸ïJ¸ïDgï91) ªÄvÄ < 0.0001g ‘¦’ ªiË®¦ª£Ä UÄgÄw¸¯ÁVz.

¥ÇæP CzÀiÄ£ª Gµª®AiÄz ¥j¹wU½U ¸ÆPªÁz 15 ±Ãµ v½U¼£Ä UÄgÄw¹z ªÄvÄ UÄgÄw¹z ªiÁPðU¼£Ä Udj ¨¼U¼ ¸ÄzÁguUÁV z¦rÃPj¸¯ÁUÄvz.